

Dedication

To my parents, Naomi and Moshe Nussinovitch,
for their unconditional love, guidance, and support throughout my life.

THE HEART IN RHEUMATIC, AUTOIMMUNE AND INFLAMMATORY DISEASES

PATHOPHYSIOLOGY, CLINICAL ASPECTS AND THERAPEUTIC APPROACHES

Edited by

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The left upper panel of images exhibit giant coronary aneurysms, a possible result of coronary vasculitis (modified with permission from *van Beek et al*, *Neth Heart J* 2012;20:42–43) and mitral regurgitant flow (upper right image), a consequence of rheumatic heart disease (modified with permission, from *Huang et al*; *ECR* 2013. DOI:10.1594/ecr2013/C-1218). The lower left image demonstrates an example of nonbacterial endocardial involvement (modified with permission; http://www.wikidoc.org/index.php/Endocarditis_pathology) and the right lower image is a histologic example of Churg-Strauss vasculitis (modified with permission; http://commons.wikimedia.org/wiki/File:Churg-Strauss_syndrome_-_very_high_mag.jpg).

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Dr. Nussinovitch has mainly dedicated his research to cardiac autoimmunity and autoinflammation, cardiac manifestations of systemic diseases, cell- and gene-based cardiac therapies, and the modulation of the cardiac electrophysiologic substrate for therapeutic purposes. He has published articles in leading rheumatologic journals and has authored papers dealing with the cardiovascular system, including publications in *Nature* magazines. He has also published several

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Dr. Nussinovitch has been the recipient of several awards for his research including the J. Kellerman Award, the Noyfeld Award, the Lt. Grandir Award, and the Britain–Israel Research and Academic Exchange Partnership (BIRAX) Annual Award. He serves as a reviewer for leading rheumatologic and cardiac journals.

Dr. Nussinovitch carries out his clinical work at the Rambam Health Care Campus, a tertiary medical facility and leading referral center in northern Israel, academically affiliated with the Technion Institute of Technology, Israel. Some of his clinical research was conducted in collaboration with the Zabłudowicz Center for Autoimmune Diseases and the Heller Institute of Medical Research, at the Sheba Medical Center, Israel.

Preface

The prevalence of autoimmune diseases and rheumatic conditions is constantly increasing. Autoimmune diseases affect approximately 7–10% of the population of the United States, while more than 50,000,000 American adults suffer from some type of arthritis. Many of these clinical conditions are characterized by multiorgan involvement and systemic inflammation. Cardiac complications in rheumatic, autoimmune, or inflammatory conditions are major influences on the clinical outcome, quality of life, and overall prognosis of these common diseases, resulting in morbidity, recurrent hospitalizations, decreased quality of life, and early death. Actually, in many systemic and inflammatory illnesses (rheumatoid arthritis and others), cardiac disease has become the most common cause of death. Also, in other cases, unique cardiac manifestations may go undetected, mainly due to the lack of medical awareness, high index of suspicion, and occasionally the need for advanced diagnostic modalities.

Researchers have observed that autoimmune and inflammatory mechanisms play a pivotal role in atherosclerosis and ischemic cardiomyopathy. This interplay between immune mechanisms, inflammation, and cardiac diseases emerges as a distinct medical field affecting all age groups, genders, and populations. These mechanisms are of particular clinical importance in systemic rheumatic and autoimmune disease.

Other than a systemic proinflammatory state that advances the process of atherosclerosis, there are several main pathways in which cardiac injury may occur. Vascular inflammation may involve the coronary vascular tree, thus resulting in many life-threatening medical complications and distorted coronary anatomy. A directed antiheart autoimmune response may aggravate any nonspecific cardiac insult. Nonischemic cardiomyopathy, microvascular dysfunction, valvular damage, and cardiac dysrhythmias may also appear. Connective tissue disease and systemic vasculitis may affect all aspects of cardiac performance including cardiac perfusion, the contractile function, impulse propagation, induction of arrhythmias, inflammation of the pericardium, or involvement of the heart valves. A hypercoagulability state may also result from systemic inflammation and specific immune responses, and consequently may facilitate ischemic cardiac injury and the development of nonbacterial thrombotic endocarditis. Therapies used in the treatment of systemic autoimmune conditions may also

substantially contribute to cardiovascular risk factors, as well as harbor direct cardiac influences. Interestingly, there are several autoantigens shared by the joints and the heart tissue, which may give rise to the involvement of both these organs. In addition, although infrequent, direct tissue deposits of amyloid or crystals may ultimately manifest as cardiac disease in some chronic medical conditions.

In recent years, extensive medical research has expanded our understanding of the pathophysiological mechanisms that mediate cardiac illnesses in systemic and autoimmune diseases. Although a broad review in a medical textbook is needed, more than a decade has passed since this topic was systemically addressed in a scientific manuscript. The authors herein endeavored to fill this gap and provide a complete overview of the current knowledge relating to the role of the immune processes and inflammation in the pathogenesis of heart involvement in rheumatic, systemic autoimmune, and inflammatory diseases. It is hoped that this extensive collection of data will simplify the comprehension of these complex, yet clinically significant mechanisms.

The book is subdivided into three major sections. The first section focuses on molecular pathways, autoimmune mechanisms, and the pathogenesis of organ-specific, autoimmune-mediated, myocardial injury. A chapter will be devoted to atherosclerosis, within the context of systemic inflammation. Another chapter will describe the battery of cardiac tests that can be employed to identify the risks for an adverse cardiac outcome in patients with systemic diseases. This specific chapter will aim at furthering the understanding of the experimental results reviewed in the second section of the book (focusing on the specific diseases and medical entities). In addition, this chapter can be used as a guide for conducting a comprehensive cardiac study in patients with systemic diseases and suspected myocardial involvement.

In the second part of the book, various rheumatic, inflammatory, and autoimmune diseases will be included and reviewed in a systemic manner. Chapters are devoted to inflammatory arthritis (rheumatoid arthritis and juvenile idiopathic arthritis, spondyloarthritides, and polymyalgia rheumatica), autoimmune and connective tissue diseases (systemic lupus erythematosus, neonatal lupus, Sjögren's syndrome, systemic sclerosis, dermatomyositis, and polymyositis), and crystal-induced arthritis (gout).

Vasculitides will also be extensively discussed, including vasculitis affecting large arteries (giant-cell arteritis and Takayasu's arteritis), medium-size vessels (polyarteritis nodosa and Kawasaki disease), and small-size vessels (microscopic polyangiitis and Churg-Strauss syndrome, Wegener's granulomatosis). Other chapters will focus on postinfectious autoimmune cardiac diseases (rheumatic fever and Chagas heart disease) and cardiac manifestations of systemic autoinflammation found in familial Mediterranean fever.

In each chapter, the state-of the art knowledge of cardiac manifestations, pathogenesis, and therapeutic aspects is extensively reviewed and systemically discussed. The aforementioned include clinical topics, present epidemiological data, genetic basis, and diagnostic criteria. The main focus of the chapters is the pathophysiology of cardiac involvement, different clinical aspects, and the manifestations of cardiac diseases. The covered clinical topics will include endothelial dysfunction and detection of subclinical atherosclerosis, ischemic cardiac disease, coronary vasculitis, pericardial, myocardial and endocardial involvement, prevalence and markers of ventricular and supraventricular arrhythmias, cardiac amyloidosis, and the overall occurrence of cardiac-related adverse events and prognosis. Each chapter concludes with a discussion on disease-specific and cardiac-specific therapeutic options. The therapeutic approach for each clinical entity is presented with the level of evidence and strength of recommendation according to the following commonly accepted scale:

The third section of the book focuses solely on therapeutics by systemically describing the adverse and desirable cardiovascular effects of different therapeutic approaches used in the aforementioned indications and the novel emerging means of treatment and prevention of immune-mediated cardiac diseases and atherosclerosis. Specifically, anti-inflammatory agents, immunosuppressive drugs, cardiac immunomodulation approaches, and autoantibody-targeted therapies are described. Importantly, some exciting and recently emerged immuno-targeted therapies hold great promise and may revolutionize patient care.

It should be noted that autonomic nervous system abnormalities that may indicate, mediate, or facilitate to some extent the progression of cardiac diseases and the development of cardiac arrhythmias is beyond the scope of the current manuscript.

A comprehensive index was added that will aid in finding material of theoretical and practical importance within the book.

Insightful readers are encouraged to contact the editor with any suggestions or remarks as to the content of the book, via email, at:

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It is my sincere hope that this book will be a valuable reference for rheumatologists, cardiologists, immunologists, and medical practitioners in the continuous pursuit of improving patient care and medical research.

Level of Evidence		Strength of Recommendation	
A	More than one randomized controlled trial (RCT)/ <i>meta</i> -analysis	Class I	Conditions for which there is evidence and/or general agreement that a given procedure/therapy is useful and effective
B	A single RCT or well-designed nonrandomized trial, i.e., prospective observational registries (case controls, cohorts).	Class II	Conditions for which there is conflicting evidence and/or divergence of opinion regarding the usefulness/efficacy of performing the procedure/therapy
C	Expert consensus: Includes case reports and retrospective series; the expert decides based on his or her experience	Class IIa	Weight of evidence/opinion is in favor of usefulness/efficacy
		Class IIb	Usefulness/efficacy is less well established by evidence/opinion
		Class III	Conditions for which there is evidence and/or general agreement that a procedure/therapy is not useful/effective and in some cases may be harmful

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Pathophysiology of Autoimmunity and Immune-Mediated Mechanisms in Cardiovascular Diseases

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1. PATHOPHYSIOLOGY OF AUTOIMMUNITY

1.1 Etiology of Autoimmunity

A common etiology of virtually all autoimmune diseases is a dysregulated and uncontrolled self-reactive CD4 T-cell response [1]. Several factors are known to affect autoimmunity, including immunologic, infectious, and genetic predispositions. Researchers have acknowledged that many triggers may contribute to the development of autoimmune diseases in susceptible individuals [2]. In recent years we have acquired a better understanding of the pathogenesis of autoimmunity. Although in some cases, the link between specific triggers and an autoimmune disease has been established (ie, the poststreptococcal pharyngitis development of acute rheumatic fever (RF)), in most cases the etiology remains elusive and is considered multifactorial. Autoimmunity may be confined to a specific organ or be associated with a systemic disease with various different clinical manifestations. Importantly, there is an outstanding difference in the prevalence of different autoimmune diseases reported in different countries, further supporting the association between ethnogeographical factors (and presumably different exposure to infectious agents) and autoimmunity [3]. Diabetes mellitus (DM) type 1 is an example of an immune-mediated organ-specific disease that is characterized by a diverse global prevalence. For undetermined reasons, prevalence appears to be higher in North America, northern Europe, and Australia/New Zealand [4].

Herein, we will discuss the multifactorial etiology of autoimmune diseases.

1.1.1 Environmental Factors

Several environmental factors contribute to the development of autoimmune diseases, eg, occupational exposures, drugs, tobacco smoke, silica, organic solvents, dietary intake of certain elements, and exposure to UV light [5,6]. This association was validated in 2010 by an expert panel workshop of the National Institute of Environmental Health Sciences (NIEHS) deliberating on the role of the environment in the development of autoimmune disease [7].

Drugs are important environmental triggers of autoimmunity. The hallmark of drug-induced autoimmunity (DIA) is a systemic lupus erythematosus (SLE)-like phenotype. First described in 1945 in the context of sulfadiazine, it is now recognized that over 90 drugs can induce SLE and other autoimmune diseases, such as vasculitis and arthritis [8,9]. The highest risk for drug-induced lupus erythematosus (DILE) is attributed to procainamide and hydralazine, while quinidine and other drugs are associated with moderate and low risk, respectively [8]. Recently, biological treatments such as the tumor necrosis factor- α (TNF- α) inhibitors and interferon (IFN) have been identified as causing DILE in some patients [9]. As DILE is often reversible, once the offending drug has been removed, early diagnosis and distinguishing DILE from SLE is important [9,10]. However, currently, there are no diagnostic criteria for DILE [8,9]. Identification of a temporal relationship between

drug administration and symptom development, as well as symptom resolution after cessation of the drug, is required for diagnosis [10].

Exposure to silica and organic solvents are associated with systemic sclerosis (SSc). Silica may also be associated with the development of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and antineutrophil-cytoplasmic antibody (ANCA)-associated vasculitis [6,7]. Suggested mechanisms for silica-induced autoimmunity include adjuvant effects of apoptotic debris, dysregulation of apoptosis, altered CD4⁺/CD4⁺ CD25⁺ T-cell ratio, and induction of circulating autoantibodies (such as anti-dsDNA, anti-Ro/SSA, anti-La/SSB antibodies in silica associated SLE) [7]. Solvents were found to be associated with circulating SSc autoantibodies (anti-Scl-70), increased IFN- γ , and reduced interleukin (IL)-4 productions [7]. Other substances, such as aromatic amines, hydrazines, hair dyes, aliphatic chlorinated hydrocarbons (vinyl chloride, trichloroethylene, and perchloroethylene), environmental estrogens (such as bisphenol A), inorganic mercury, and the mineral oil component 2-, 6-, 10-, 14-tetramethylpentadecane (TMPD or pristane, which was found to induce acute inflammatory arthritis in rats), are acquired due to occupational exposure, which can also trigger autoimmunity in predisposed individuals [7,11,12]. One suggested mechanism for these substance-induced autoimmune responses is the estrogenic influences on the immune system, which use binding to self-antigens and the creation of neoantigens for which immune tolerance is lacking [3]. Tobacco smoking has been known to be associated with autoimmunity and connective tissue diseases. For instance, the odds ratio (OR) for developing SLE increases by 50% in smokers compared with nonsmokers. The association with RA is even higher (OR of 2.6–3.8 compared with nonsmokers). Conjugation of smoking and a specific genetic background (HLA-DR alleles) produces a synergistic effect and a higher risk of developing RA [13]. Grave's disease is also remarkably susceptible to smoking [14]. The exact mechanisms by which tobacco smoking affects autoimmunity are not fully understood but increased tissue damage, generation of free radicals, and increased systemic inflammation seems to mediate its unfavorable immune effects [5]. Suggested mechanisms for tobacco smoking-induced RA include post-translational modification of antigen citrullination and anti-cyclic citrullinated peptide (CCP) antibodies, T helper (Th)-17 activation by nicotine, and upregulation of heat shock gene expression and circulating autoantibodies (such as rheumatoid factor and antiheat shock protein 70 (HSP70)) [7].

Interestingly, among other factors, SLE is influenced and exacerbated by ultraviolet (UV) radiation [15]. Animal models suggest that exposure to UV radiation can also lead to the development of an SLE-like disease.

A possible mechanism is the inhibition of T-cell DNA methylation by UV radiation, which can convert normal antigen-specific CD4⁺ T lymphocytes into autoreactive, cytotoxic, proinflammatory cells [16]. The role of DNA methylation modulation in the pathogenesis of autoimmunity is further indicated by the presence of immune dysregulation in two rare congenital diseases: Russell-Silver and Beckwith-Wiedemann syndromes [7]. Other studies have suggested that UV light causes a clinically significant autoimmune disease only in individuals with a genetic predisposition to SLE [16]. UV radiation plays a role in the pathogenesis of cutaneous lupus erythematosus (CLE) by triggering keratinocyte apoptosis and externalization of nucleoprotein autoantigens, such as Ro/SSA, to the keratinocyte cell surface and exposing them to circulating autoantibodies [17,18]. Exposure to UV light is also known to be associated with the release of proinflammatory cytokines, thus contributing to the pathogenesis and progression of SLE. These cytokines include IFNs, TNF α , and IL-1, IL-6, IL-8, IL-10, and IL-17, among others [18].

Vitamin D, another solar exposure byproduct (due to its production in the skin and the kidneys in a biphasic process; although it may be externally derived from nutritional sources), is considered an important immune modulator. Interestingly, low levels of vitamin D were observed in various autoimmune conditions and were reported to be associated with more advanced disease stage and adverse clinical outcomes [19–22]. Other dietary exposures were also suggested to mitigate autoimmunity, such as the development of gluten-sensitive enteropathy, eosinophilia-myalgia syndrome, and toxic oil syndrome as a result of ingestion of gluten, L-Tryptophan and 1, 2-di-oleyl ester (DEPAP), and oleic anilide-contaminated rapeseed oil, respectively [7]. Furthermore, cow milk proteins introduced into the diet at a young age were reported to be linked to the development of DM. Excessive iodine consumption was found to be associated with autoimmune thyroiditis [7]. Similarly, animal meat, fat, sweets, and sugar were suggested as a trigger for inflammatory bowel disease (IBD), although the latter association has yet to be proven. Many of the environmental possible effectors have been linked to the Western lifestyle [4].

1.1.2 Infectious Agents

Molecular mimicry between human and pathogenic (bacterial, viral, parasitic, and fungal) antigens has been suggested as a possible trigger or aggravator of autoimmune diseases. Chagas heart disease (CHD) and RF are good examples of autoimmune diseases triggered by infectious agents, ie, *Trypanosoma cruzi* and group A *Streptococcus*, respectively. Both pathogens are related to cardiac damage, but as the former affects mostly the myocardial tissue, the latter may be associated with

pancarditis and the development of valvulopathy, which may result in progressive heart failure [23,24]. Molecular mimicry, epitope spreading, bystander activation, polyclonal activation, and other mechanisms of action were all found to induce autoimmunity following infections [25].

“Molecular mimicry” is the presence of cross-reactivity between infectious and self-epitopes. Subsequently, naïve autoreactive T cells recognize self-antigens and initiate or facilitate tissue damage. This mechanism may be proven by disease induction in animal models following immunization, or by disease induction following the transfer of sensitized T cells or autoantigens. “Epitope spreading” is a process in which a dominant epitope is metabolized and presented to the immune system as a neoepitope, thus eliciting an autoimmune response. This immune mechanism appears to play a pivotal role in the pathogenesis of many autoimmune diseases and cardiac-related immune damage [2]. “Bystander activation” is a term describing infection-mediated tissue damage (either direct or immune-mediated), thus eliciting exposure of self-antigens, an immune presentation, and an autoimmune response. Infection-mediated non-specific macrophage activation and cytokine secretion by specific T cells (directed against the infectious agent) also further facilitate the tissue damage induced by autoreactive T cells.

Many infectious agents are known to produce specific molecules, also known as pathogen-associated molecular patterns (PAMPs). PAMPs are able to bind to toll-like receptors (TLRs) and thereby activate T, B, and antigen presenting cells (APCs). Importantly, infections may result in cell apoptosis and a release of endogenous damage-associated molecular patterns (DAMPs), which may further induce immune activation via TLRs pathways [2]. Constant immune activation may result in immune-complex-mediated tissue damage, and eventually in organ dysfunction [25].

Notably, superantigens can be produced by certain infectious agents, thus resulting in nonspecific T-cell activation, with potential T-cell self-reactivity. Viruses may also induce TLRs activation via an overexpression of type 1 INF genes (INF and INF-related cytokines and chemokines), which may further contribute to the pathogenesis of autoimmunity. Certain infections promote IL-17 secretion and a Th17-mediated immune response, another important contributor to autoimmunity [2]. It has been suggested that in most patients not a single infection, but rather the “burden of infections” affecting an individual throughout his lifetime, advocate an autoimmune response. More than a few infectious agents have been identified in this context including the Epstein Barr virus (EBV), parvo B19, and the hepatitis C virus (HCV); all were reported as possible triggers of RA. EBV and *Chlamydia pneumonia* were also reported as

probable triggers of multiple sclerosis (MS). Coxsackie virus B (CVB), cytomegalovirus, and mumps were identified as triggers of type 1 DM, and CVB, EBV and hepatitis C virus (HCV) are likely to be triggers of Sjögren’s syndrome (SS) [6].

There are many experimental models in animals demonstrating an autoimmune response following exposure to infectious antigens. For instance, immunizing animals with EBV nuclear antigen 1 (EBNA-1) fragments were found to induce a serological response similarly found in SLE patients [25]. CVB3, a common viral cause of acute myocarditis, is another example. Mice models previously demonstrated that CVB3 can induce a chronic organ-specific immune response to cardiac myosin [26,27]. One study demonstrated the generation of myosin heavy chain (MyHC)- α -autoreactive CD4 T cells in myocarditis-susceptible mice infected with CVB3. This autoreactive T-cell repertoire appears to contain high levels of IL-17-producing cells capable of inducing myocarditis [28]. Importantly, infections were reported to affect the clinical presentation of systemic autoimmune and rheumatologic disease. For instance, in SLE patients who were also exposed to rubella (with high IgM), neuropsychiatric lupus usually developed.

In contrast, high titers of anti-EBV antibodies were reported to correlate with skin and joint manifestations [25]. Interestingly, Strachan et al. demonstrated an inverted correlation between exposure to certain infectious agents and development of autoimmunity. This negative association is also known as the “hygiene hypothesis” [29]. The correlation is mostly related with type 1 DM, IBD, and MS, which explains in part the increase in their incidence during the last century in the Western world. This hypothesis is also corroborated by the lower levels of antibodies against *Helicobacter pylori*, CMV, EBV, and toxoplasma in the serum of patients with type 1 DM [25]. It is not fully understood as to why certain infectious agents promote an autoimmune response and others produce the opposite immune-protective effect. It may be associated with specific characteristics of the infectious agents, or due to host response-related mechanisms and genetic predisposition. Certain viral and fungal infections are capable of promoting Treg cells (CD4⁺/CD25⁺ positive cells), which play a role in immune regulation and may suppress autoimmune mechanisms [2].

Atherosclerosis, an inflammatory process involving the vascular wall, was suggested as one of the multifactorial pathophysiological processes resulting in part from an infections-associated cumulating effect. Interestingly, certain infectious agents were found to be linked with accelerated atherosclerosis, including *Helicobacter pylori*, *Chlamydia pneumoniae*, herpes-simplex type 2, and CMV [30].

1.1.3 Hormonal Associations

For an undetermined reason, most autoimmune diseases are much more common in females than in males [31]. This prevalence exists in patients with MS, hyperthyroidism/Graves' disease, RA, Hashimoto's disease, and many other immune-mediated diseases (Table 1.1) [31]. Autoimmune diseases among males are characterized by a lower incidence rate and a more severe phenotype, as compared to females. A wide range of female-to-male prevalence has been reported in the medical literature for certain diseases, which may be attributed to various geoepidemiologic factors, prevalence of possible triggers, and possibly to diagnostic factors [32]. However, there are a few diseases where no gender-association was found (ie, DM and Beçhet's Disease, which occurs equally in both species) [4]. Furthermore, exacerbation of autoimmune diseases appears more commonly during puberty, pregnancy and postpartum periods, all life events that are characterized with gender-related hormonal alterations. Ovarian stimulation may be associated with increased expression of autoimmunity, as well as exogenous estrogen supplementation (oral contraceptive or hormonal replacement therapy) that may be associated with disease flare-ups [5]. Shoenfeld et al. suggested that the sex-related differences in prevalence and clinical

presentation of autoimmune diseases are attributed to sex hormones [33]. Sex hormones are known to affect the function of immune cells. Estrogens increase the Th2-type immune response (associated with increased secretion of IFN γ , tumor-necrosis factor TNF α , TGF β , and IL-1, 4, 5, and 10) and increases the autoreactive B-cell count, while androgen and progesterone may downregulate immune mechanisms via the Th1 immune response and decreased levels of proinflammatory cytokines [33]. Thus the balance between estrogen and progesterone can possibly influence presentation of autoimmune diseases [31].

Interestingly, patients with autoimmune diseases have no altered hormonal profile compared with healthy individuals of the same sex. There are several other gender-related immune differences. Females have increased IgM levels, higher CD4⁺ T-cell counts, and a tendency toward increased secretion of IL-4, IFN γ , and IL-1 compared with males [32].

Suggested mechanisms in SLE that account for the female predominance in this disorder are presented in Fig. 1.1 [31].

Specifically, in the context of immune mediated cardiac injury, sex hormones modulate postviral myocarditis tissue damage induced by anticardiac autoimmune responses. Unlike most systemic autoimmune and rheumatologic diseases, pure autoimmune cardiac damage is more common in males by 1.2–2 folds [35,36]. Males also tend to experience a more severe cardiac injury, more global cardiac involvement and a higher rate of fibrosis, resulting in a grave prognosis in some patients [44]. Some of the gender-related models associated with cardiac injury in a postmyocarditis mouse model are found in Table 1.2 [32]. Testosterone was reported to promote a cell-mediated immune response, whereas female mice demonstrated a dominant Th2 response manifested by B-cell predominance [32].

1.1.4 Psychological Effectors

Psychological mechanisms may produce profound physiological effects. Reports of exaggerated emotional stress preceding autoimmune disease onset is not uncommon and were reported in up to 80% of patients, according to some case series [5]. Furthermore, emotional stress was reported prior to disease exacerbation. Importantly, the disease itself may cause abundant physical and emotional distress, thus inducing a "vicious cycle" cumulating in disease aggravation. Stress may affect the neuroendocrine system, activate the hypothalamic-pituitary-adrenal axis, and induce the release of proinflammatory cytokines, such as IL-1, IL-6, IL-8, IL-18, TNF- α , and C-reactive protein (CRP) [45]. Therefore emotional stress management is considered an integral component of autoimmunity treatment and patient management [5].

Major depression (MD) is a hallmark for the bidirectional relationship between psychological diseases and

TABLE 1.1 Female-to-Male Prevalence Ratio in Several Immune-Mediated Disorders

Disease	F:M ratio	References
Ankylosing spondylitis	1:3	[34]
Autoimmune myocarditis	1:1.2–2	[35,36]
Beçhet's disease	1:1	[37]
Mixed Connective tissue Disease ^a	8:1	[35]
Polymyositis/dermatomyositis ^a	2:1	[38]
Rheumatoid arthritis ^a	2.7–4:1	[34,35,37–40]
Scleroderma ^a	2.7–4:1	[34,35,37–40]
Sjögren's syndrome ^a	4–20:1	[34,35,38,40]
Systemic lupus erythematosus ^a	7.4–9:1	[34,35,37–40]
Addison's disease ^a	12.3:1	[38]
Celiac disease ^a	1.8–3.3:1	[34,41,42]
Grave's disease ^a	3.5–7.2:1	[34,35,38]
Hashimoto thyroiditis ^a	5.2–50:1	[34,35,37,38]
Myasthenia gravis ^a	1.6–3:1	[34,35,39,43]
Primary biliary cirrhosis ^a	7.8–10:1	[34,35,38–40]

^aCharacteristic female predominance.

Adapted from Nussinovitch and Shoenfeld [32].

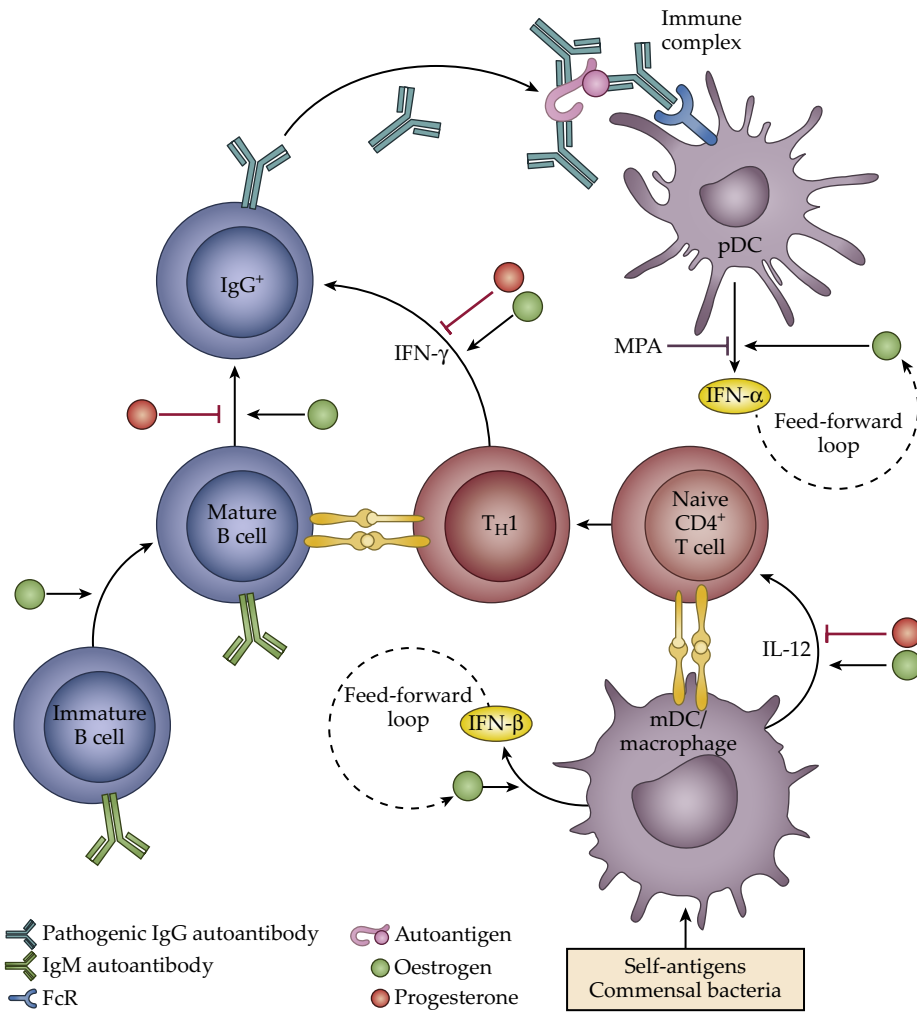


FIGURE 1.1 Potential mechanisms through which estrogen and progesterone might modulate the loss of immune tolerance and regulate the production of pathogenic autoimmune autoantibodies in SLE. Adapted from Hughes and Choubey [31].

TABLE 1.2 Hormonal influences in a CVB-3 mediated autoimmune myocarditis mouse model

Estrogen (dominant Th2 response)	Testosterone (dominant Th1/Th17 response)
Polarization of macrophages into M2 response and increased M2 response	Increased number of macrophages, neutrophils and cell-mediated autoimmunity
Upregulation of the M2 marker Arg1 and increased markers of M2 differentiation (Dectin-1, CD169, galcatin-3, CCL-2, CCL-7, CXCL-3)	Elevated levels of TLR4, caspase-1
Increased IL-1β, IL-4, IFNγ, TNFα, TGFβ, IL-5, and IL-10 secretion	Enhanced necrosis of cardiomyocytes
Greater percentage of B-cells and CD25, FOXP3, and Treg	Autoreactive cytolytic T cells in the spleen
Increased Tim-3 expression in mast cells, macrophages, and T cells	Increased CD8-cell activation
Increased fibrosis	Decreased levels of IL-1β, IL-4, IL-5, IL-6, and TNFα
Increased antibody-mediated and immune complex-associated tissue damage	Increased secretion of IL-10

Adapted from Nussinovitch and Shoenfeld [32].

autoimmunity. Two important factors found in this relationship are cytokine dysregulation and the presence of autoantibodies [46]. Cytokine dysregulation plays a role in patients with MD, as high levels of proinflammatory cytokines, including CRP, IL-6, and TNF- α , were detected [47]. This proinflammatory process appears to play a role in the pathogenesis of MD [46]. As a result, these patients may be at risk for autoimmune disorders, as was previously demonstrated in a large-scale epidemiological study [47]. Interestingly, this proinflammatory state may link MD with cardiovascular diseases (CVD) [48]. Several studies describing MD patients to have increased risk for hypertension and stroke, as well as studies that found MD to be an independent risk factor for CVD, support this notion [49].

Several studies have suggested that an autoimmune mechanism triggers the development of MD via the production of autoantibodies. In SLE, antiribosomal P antibodies (anti-P antibodies) were found associated with neuropsychiatric manifestations [50–53]. Moreover, they were linked to hepatic involvement and acceleration of glomerulonephritis in these patients [54,55]. However, it appears that anti-P antibodies may play a role in the development of MD as well. In one study, anti-P antibodies were injected intracerebra-ventricularly into mice, inducing a depressive-like behavior. Treatment with fluoxetine succeeded in relieving the symptoms [56]. This intriguing association between anti-P antibodies, SLE, and MD implies that the presence of MD may affect the development of autoimmune diseases. However, a causal relationship has yet to be proven.

1.2 Complement System and Autoimmunity

The complement system was discovered over 100 years ago [57]. At first, it was considered only to participate in the recognition and destruction of pathogens. At present, it is known to possess regulatory functions in adaptive [58], humoral [59], and T-cell immunity [57]. There are three complement-cascade activating pathways: the classical, lectin, and alternative pathways (Fig. 1.2 [57]). All three pathways lead to the cleavage of C3 into the opsonin C3b and the anaphylatoxins C3a, which activates the C5 with formation of a membrane attack complex (MAC) [60,61]. The classical pathway is triggered by the binding of immunoglobulin (Ig)-M and IgG to the C1 complex [60,62]. The binding of C1q to an antibody further activates C4 and C2 and induces the formation of the C4bC2a complex, a C3 convertase [60,63]. The lectin pathway facilitates recognition of microbial carbohydrate patterns either by a mannose-binding lectin (MBL) or ficolins. This leads to the activation of C2 and C4 through the MBL-associated serine proteases (MASP) and the formation of the C4bC2a complex, similar to that of the classic pathway [60,62]. Factor D, a serine

protease, is the main inducer of the alternative pathway. It cleaves to factor B, which forms a complex with the hydrolyzed iC3b, thus leading to the formation of Ba and Bb. Bb and C3b generate the C3 convertase of the alternative pathway, C3bBb [60,64]. Enzyme activation of C5 by C3bC4bC2a (lectin or classical pathway) or by C3bBbC3b (alternative pathway) leads to the formation of C5a and C5b, which in turn form the MAC C5b-9, thus causing cell lysis [60]. Further effects of the complement system include the MAC's activation of granulocytes and endothelium; increased opsonization and phagocytosis by the deposition of C3 fragments on the membranes; clearance of immune complex and apoptotic bodies; and alterations in immune cell signal transduction, adhesion activation, and cytokine production [60].

Defects in the complement system or in its regulatory proteins may contribute to the development of autoimmune diseases. Herein, we will explore the association between the complement system and autoimmunity.

1.2.1 Complement Compound Deficiency and Autoimmunity

The complement system's role in tissue repair may be responsible for autoimmune response [60]. Although hypocomplementemia was associated with primary SS and SSc [60], the hallmark autoimmune disease affected by complement deficiency is SLE [61]. Complement deficiency was in fact identified as the strongest genetic susceptibility factor in humans for SLE [65,66]. In particular, the deficiency of C1 (C1q, C1r, C1s), C4, and C2 were associated with high, moderate, and low risk for the development of SLE, respectively [60,61,65]. This may result from bypass mechanisms absent in C1q and C4 [67].

In the case of C3 deficiency, the clinical picture is different, rarely associated with SLE, and more commonly characterized by recurrent pyogenic infections, membranoproliferative glomerulonephritis, and rash [60]. Prevalence of SLE development in patients with C1q, C1r/C1s, C4, and C2 deficiencies is estimated at 93%, 57%, and 75%, respectively [65,68,69]. The exact estimation of SLE development in C2 deficiency is more difficult than in other complement deficiencies due to higher prevalence of heterozygosity in these patients. In one study it was found to be 30% [65]. These clinical observations were further confirmed by mice models deficient in C1q-, C4-, C3-, and CD35/CD21 (CR1/2) and were shown to predispose the subjects for autoimmune diseases [61].

In addition to complement compound deficiency anti-C1q autoantibodies also appear to play a role in the development of SLE. Present in up to one-third of SLE patients, these autoantibodies create immune complexes, which are correlated with more severe disease [65,70]. Anti-C1q antibodies may play a promising future role in the early detection and monitoring of SLE patients, especially in those with renal involvement [71].

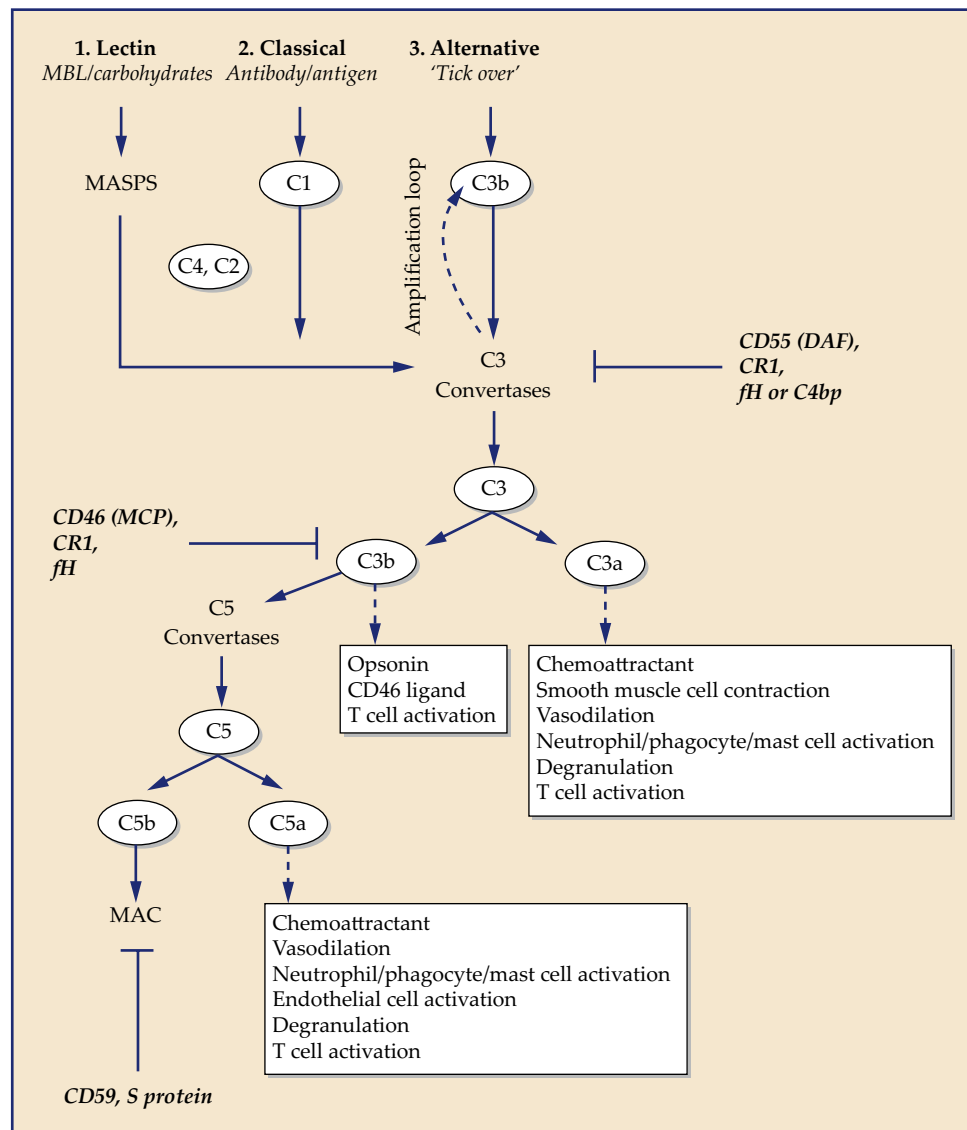


FIGURE 1.2 Complement system, regulation, and function. In red are the major effectors of C3a, C3b, and C5a. Adapted from Heeger and Kemper [57].

Three suggested hypotheses for the mechanism of SLE development in complement deficiency include impaired clearance of immune complexes, apoptotic cells, and abnormal B- and T-cell activation, which may cause a defective tolerance in self-antigens. However, none of these hypotheses have been completely proven [61,65].

1.2.2 Impaired Function of Regulatory Proteins of the Complement System

The complement system has over 30 membrane-bound regulator proteins that interact with different cells of the immune system [57,61]. Important regulators in the pathogenesis of autoimmunity include CD46 (membrane cofactor protein, MCP), CD55 (decay accelerating factor, DAF), CD59 (protectin), CD35 (complement

receptor 1, CR1), and the complement receptor 1-related gene/protein y (Crry) [72,73]. Other regulators involved in autoimmune diseases are membrane soluble and can be found in plasma or lymphatic fluid. These proteins include factor H (fH), C4b-binding protein (C4bp), vitronectin (S protein), clusterin (apolipoprotein J), and the C1 inhibitor (C1-INH) [72]. Herein, we will elaborate on five main regulator proteins: the CD55, CD59, CD46, Crry, and C1-INH.

1.2.2.1 CD55

This glycosylphosphatidylinositol (GPI)-anchored membrane protein inhibits the formation and accelerates the decay of C3 and C5 convertases in all pathways [73]. Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by susceptibility of platelets and erythrocytes to

a complement attack. It was previously demonstrated that CD55 deficiency in patients with PNH is responsible for the lack of inhibition of the complement system and thus to an autoreactive complement response [73].

1.2.2.2 CD59

Similar to CD55, CD59 is a GPI-anchored membrane protein [74], expressed on red blood cells (RBC). It regulates the terminal step in the complement system by inhibiting MAC formation [73,74]. Furthermore, recent studies have suggested a direct inhibitory role of CD59a on T-cell activation, as demonstrated by the increased *Vaccinia*-specific CD4⁺ T-cell responses in CD59-knockout mice [73,75]. In murine models of SLE, a CD59a deficiency was found to exacerbate skin autoimmune diseases, increase antichromatin autoantibody titers, and cause higher levels of proteinuria in male mice [74]. CD59 deficiency has also been associated with PNH, autoimmune-hemolytic anemia (AIHA), RA, MS, and SS [73,76–78].

1.2.2.3 CD46

CD46 is a transmembrane glycoprotein, which binds, inactivates C3b and C4b [79], and plays a major role in downregulating the Th1 response by substituting IFN γ +IL-10 - CD4⁺ T cells into IFN γ +IL-10 + cells [72,79]. This regulatory process is mediated by IL-2 and allows for an anti-inflammatory, IL-10-mediated, T-cell response [79]. Involvement of CD46 has been demonstrated in MS, RA, and SLE [72].

An interesting study suggests that in MS, errors in CD46 signaling may be associated with dendritic cells (DC) in IL-23 production, which in turn induces IL-17 production and thus accelerates an autoimmune response [72,80].

1.2.2.4 Crry

Crry includes both CD46 and CD55 functions [73]. In fact, it coexpresses with CD55 and thus differentiation between the two molecules' functions may be difficult [73]. Crry seems to play a special and critical role in fetomaternal tolerance [73]. SLE mice models treated with Crry were found to have reduced serum anti-dsDNA antibody levels and trends toward reduced glomerular IgG deposition levels [81,82]. Moreover, Crry improved survival and reduced proteinuria, glomerular C3 deposition, circulating immune complexes levels, the presence of skin lesions, lung bronchiolar, and vascular inflammation [82,83].

1.2.2.5 C1-INH

C1-INH is a glycosylated serine protease inhibitor displaying a regulatory role in the complement and contact systems as well as the intrinsic coagulation cascade [84]. A tendency toward autoimmunity was previously

demonstrated in patients with C1-INH deficiencies suffering from hereditary angioedema (HAE). Up to 12% of HAE patients suffer from autoimmune diseases [84]. An association between SLE and acquired C1-INH deficiency was previously reported, suggesting the presence of anti-C1-INH autoantibodies as a possible etiology [84]. However, SLE patients presenting with C1-INH deficiency and angioedema without circulating anti-C1-INH antibodies were also reported [85].

Other autoimmune diseases linked to HAE include SS, autoimmune thyroiditis, RA, DILE, pernicious anemia, IBD, celiac disease, MS-like syndrome and mixed connective tissue disease [84]. Suggested mechanism include the defective clearance of immune-complexes and apoptotic cells, which cause inflammatory damage and trigger autoimmune responses [84]. Moreover, HAE patients were found to have autoreactive B cells, which produce autoantibodies such as antinuclear, rheumatoid factor, anticardiolipin, antitissue transglutaminase, antiendomysial, anti-Saccharomyces cerevisiae, antithyroid, antineutrophil cytoplasmic antibodies and others [84,86–89]. Even anticholesterol autoantibodies were isolated in HAE patients, suggesting a major role of autoreactive B cells in HAE pathogenesis [90]. The presence of increased levels of TLR-9 in HAE patients may account for this phenomenon [86].

1.3 Autoantibodies and Autoantigens

Autoantibodies play a major role in the pathogenesis of immune diseases and are considered a hallmark of autoimmunity. In some instances, autoantibodies can be used as a marker for disease activity, may have prognostic significance, and may appear in the sera of unaffected patients' years prior to clinical presentation. Their positive predictive value may be near 100% according to some reports. For instance, rheumatoid factor (anti-IgG) and anti-CCP predict RA, whereas anti-SSA (Ro) and anti-SSB (La) are associated with SS. SLE occurrence is also associated with many preceding autoantibodies including antiphospholipids (PL), anti-Ro, anti-La, anti-dsDNA, anti-Sm, antinuclear ribonucleoprotein, anti-heparan sulfate (HS), antinucleosome, antihistone, and antiribosomal P protein [91].

In the context of heart diseases, dilated cardiomyopathy (DCM) is commonly mediated by autoimmune mechanisms (and may evolve in patients with systemic autoimmune-inflammatory conditions). Several antiheart antibodies have been found in cardiac diseases such as DCM, as well as in healthy individuals [92,93]. Autoantibodies can be reactive to all cardiac components including contractile proteins, receptors, intracellular antigens, and connective tissue elements [94,95]. However, their clinical significance and role in pathogenesis is a matter of debate and remains to be

explored. This topic is extensively covered in [Chapters 2 and 3](#) of this book.

1.4 T helper Balance and Autoimmunity

T-cell mediated adaptive immunity has classically been divided into Th1- and Th2-dominant responses [96,97]. While Th1 cells were found to be key players in immune protection against intracellular organisms and in autoimmunity pathogenesis, the Th2 response was found to protect against extracellular organisms and helminths and to participate in the pathogenesis of inflammation and allergy [96]. The two groups of CD4⁺ T cells produce different cytokines, comparable to Th1 cells, which produce IFN- γ , lymphotoxin- α (LT α) and IL-2 and the Th2 cells, which produce IL-4, IL-5, IL-9, IL-10, IL-13, IL-25, and amphiregulin [96]. These two parts of the adaptive immune system are assumed to interact in a delicate balance. Thus autoimmunity and allergy development were thought to be a result of a shift toward Th1- and Th2-dominant response, respectively [97]. However, it seems that the exact picture is more complex. It is now known that naive CD4⁺ T cells actually split into four subgroups: Th1, Th2, Th17, and induced Treg (iTreg) cells. Each lymphocyte subset has its own features, cytokine production, immunological pathways, and roles in disease pathogenesis (see [Fig. 1.3](#)) [96]. Similar to Th1, IL-17 producing Th17 cells also play a role in autoimmunity, although some studies suggest different clinical and histological phenotypes of autoimmune diseases in the two groups [98]. Induced by IL-23

production, IL-17 was found to be a disease mediator in MS, RA, SLE, IBD, EAE, psoriasis, DM type 1, SS, autoimmune uveitis, and autoimmune DCM, via production of IL-17, IL-22, and CCL20 [98–100]. Interestingly, some studies support the view of an overlap between the four CD4 subsets, since a group of CD4⁺ T cells, which produces both IFN- γ and IL-17 (Th1 and Th17 cytokines, respectively), was found [101,102].

Since the balance between the four CD4⁺ T-cell subsets has an important role in autoimmunity pathogenesis, its modulation may have therapeutic potential.

IL-4-producing Th2 cells and IL-10-producing CD4⁺ CD25⁺ forkhead box p3 (Foxp3⁺) iTreg cells inhibit both Th17 and Th1 cells [98].

It seems that a balance between IL-10 and IFN- γ regulates the Th1's normal/autoreactive response. IFN- γ is a key cytokine in Th1-mediated autoimmunity development [79,96]. On the other hand, Th1 autoreactivity was found to be attenuated by IL-10, which is produced by both iTreg and Th1 cells themselves, and inhibits Th1 proliferation and IL-2 production [79]. Regulation of this balance is complex and involves the CD46 regulatory protein of the complement system [79].

Th17 regulation is complex. Differentiation of Th17 is mediated by the transcription factor retinoid-related orphan receptor γ t (ROR γ t) and induced by TGF- β and IL-6 via the activation of a signal transducer and activator of transcription (STAT) 3 [101]. However, it was previously demonstrated that TGF- β induction of Th17 differentiation is a concentration-dependent process. At low concentrations, TGF- β induces Th17 differentiation

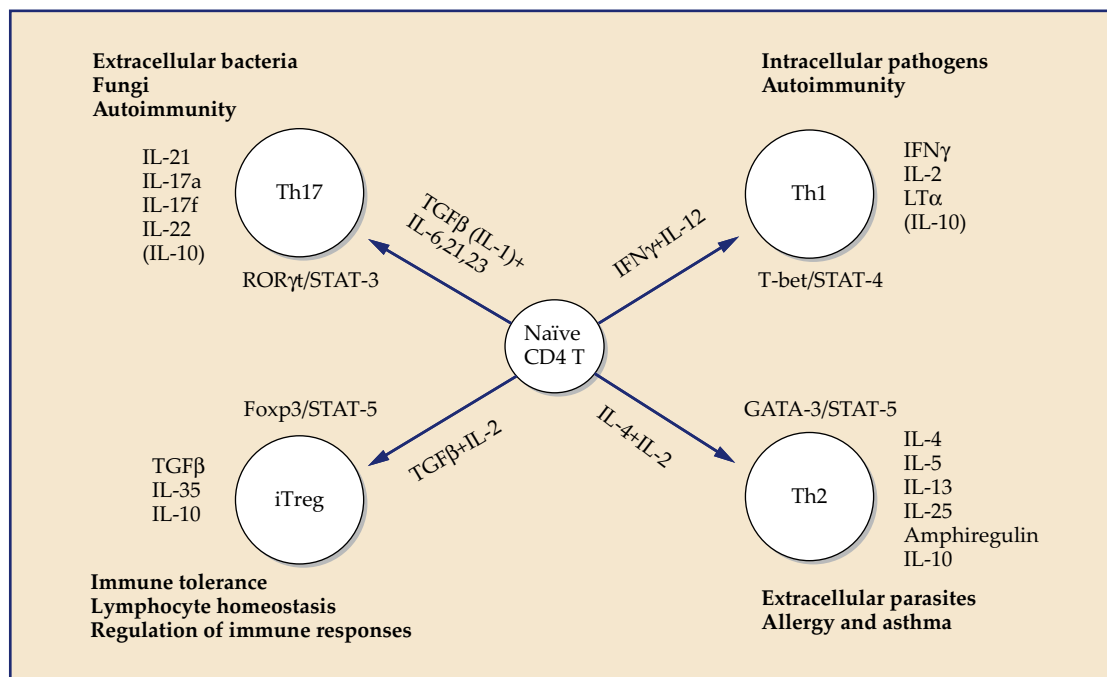


FIGURE 1.3 A summary of CD4⁺ T-cells subsets roles in adaptive immunity. Adapted from Zhu and Paul [96].

by synergy with IL-6 and IL-21 and upregulates the IL-23 receptor (IL-23r). On the other hand, high concentrations of TGF- β downregulates the IL-23r expression and thus induces Foxp3⁺ Treg cells by inhibiting Th17 differentiation [103]. Predicting autoimmunity development, as the end result of this balance between Th1, Th17, Th2, and iTreg, is difficult. Previous studies have targeted several molecules in Th1- and Th17-mediated responses in order to attenuate autoimmune disease pathogenesis. These attempts include blocking ROR γ t, transfer of polyclonal Treg cells, and inhibition of STAT3 by small interfering (si) RNA and of proinflammatory cytokines, such as IL-6 and IL-17A, using monoclonal antibodies [101]. However, in order to carefully evaluate the efficacy and safety of these therapeutic modalities, further studies are needed.

1.5 Diagnostic Criteria for Immune-Mediated Diseases

There has been an ongoing attempt to identify the diagnostic criteria for autoimmune diseases. In 1993, Rose et al. [1] suggested three types of evidence in order to establish an autoimmune pathogenesis: direct proof, indirect evidence, and circumstantial evidence. Direct evidence for autoimmunity can be found in disease induction following a transfusion of autoantibodies or autoreactive T cells [1]. On rare occasions, autoimmune diseases may also be transferred following bone marrow transplantation [104]. Direct proof for autoimmunity may also be found in the transplacental migration of pathogenic IgG, which are well known for their ability to induce many neonatal illnesses including myasthenia gravis and Graves' disease. Direct experimental proof for autoimmunity may be achieved by inducing a disease in animals following a transfusion of pathogenic autoantibodies or the patient's serum.

In cases of a T-cell mediated autoimmune disease, the pathogenic T cells can be transfused into rodents with severe combined immune deficiency (SCID). Indirect proof of autoimmunity may be achieved by disease induction following the immunization of animals with equivalent antigens known to induce disease in humans. Indirect proof of autoimmunity may also be generated from the isolation of autoantibodies or autoreactive T cells in the affected organ. Circumstantial evidence for autoimmunity includes factors such as the appearance of an autoimmune disease in a close family member or a statistical association with a particular MHC haplotype [1]. In most autoimmune diseases only some of the aforementioned criteria by Rose et al. can be found (Table 1.3).

A few years ago, we proposed a new criteria system for autoimmune-mediated DCM (Table 1.4) [92]. The system mandated echocardiographic evidence of DCM, and the exclusion of other nonimmune

mechanisms that underlay the pathogenesis. Based on the criteria of Rose et al., we suggest five minor criteria for the diagnosis of autoimmune DCM [1]. The criteria are either immunohistochemical histological data (mononuclear cell infiltrates with an abnormal human leukocyte antigen presentation; autoreactive lymphocytes and/or autoantibodies in cardiac tissue); serological (circulating antiheart autoantibodies or autoreactive lymphocytes); animal-model based (disease induction in animals following a transfusion of the patient's serum, antibodies, or lymphocytes); or clinical (clinical or echocardiographic improvement following immunoadsorption or immunosuppressive therapy). Several findings may provide supporting evidence but are inadequate as criteria, since one may find a clinical course of exacerbation and remissions, positive HLA DR4 (which was reported to be weakly associated with autoimmune DCM), and familial clustering of autoimmune diseases and/or family history of DCM [92].

2. IMMUNE-MEDIATED MECHANISMS OF CARDIOVASCULAR DISEASES

2.1 Systemic Inflammation and the Vascular Wall

The endothelium is a cell monolayer lining the blood vessels' lumens [107]. Several functions have been attributed to the endothelium such as participation in vascular metabolism, acting as a selective barrier, and maintaining and regulating vasoconstriction/vasodilation balance [107].

Triggers for vascular wall inflammation include aging, dyslipidemia, hypertension, hyperglycemia, obesity, oxidative stress, smoking, and other cardiovascular risk factors [107,108]. As inflammation occurs, the integrity and function of the endothelial cells become damaged. As a result, endothelial repair mechanisms including local replication of endothelial cells and the incorporation of circulating progenitor cells are activated [109]. However, chronic exposure to cardiovascular risk factors can eventually overwhelm this repair system, resulting in endothelial dysfunction (ED), damage to endothelial continuity, and the detachment of endothelial cells or microparticles into the circulation [107,109]. Once ED develops, progression to atherogenesis may occur [107,108]. Inflammation involvement in atherogenesis consists of three stages: leukocyte adhesion to the intima layer, penetration to the media layer, and plaque rupture and thrombus formation [108].

Inflammation of the vascular wall induces expression of several molecules promoting the binding of blood

TABLE 1.3 Evidence of Autoimmunity in Various Clinical Diseases

Disease	Direct proof			Indirect proof						
	Transfer of disease by Ab			Transfer of disease by cells to SCID mice	Induction of disease in animals by an autoantigen	Identification within lesions of:		Transfer of the disease by lymphocytes in experimental models	Genetic models	Autoantibodies or self-reactive T cells
	Experimental	Maternal	To animals			Ab	T cells			
Rheumatoid arthritis						X	X	X	X	X
Sjögren's syndrome									X	X
Systemic lupus erythematosus				X		X		X	X	X
Systemic sclerosis				X				X	X	X
Polymyositis										X
Myocarditis					X			X		X
Myasthenia gravis		X	X		X	X	X			X
Graves' disease		X		X		X	X			X
Type 1 Diabetes mellitus								X	X	X
Addison's disease										X

Adapted from Rose and Bona [1].

TABLE 1.4 Proposed Diagnostic Criteria for Autoimmune DCM^a

Major criteria	Minor criteria	Supporting evidence but not considered criteria
1. Fulfilment of accepted echocardiographic criteria of DCM [105,106]	1. Proven mononuclear cell infiltrate with abnormal human leukocyte antigen (HLA) presentation	1. Clinical course of exacerbation and remissions
2. Excluding secondary cardiac injury due to infections, alcohol, toxins or chemotherapeutic drugs, metabolic abnormalities, nutritional deficiencies, neuromuscular diseases, or collagen vascular disorders	2. Circulating antiheart autoantibodies or autoreactive lymphocytes in patients and in unaffected family members	2. Positive HLA DR4
3. Familial Clustering of autoimmune diseases and/or family history of DCM (two or more affected individuals or sudden cardiac death in a first-degree relative <35 years old)	3. In situ evidence of autoreactive lymphocytes and/or autoantibodies in cardiac tissue	
	4. Disease induction in animals following transfusion of the patient's serum, antibodies, or lymphocytes	
	5. Proven clinical or echocardiographic improvement following immunoadsorption or immunosuppressive therapy	

^aDiagnosis requires two major and at least one minor criterion.
Adapted from Nussinovitch and Shoenfeld [92].

leukocytes to the endothelium. This includes molecules that enable adhesion, rolling, and attachment of leukocytes to the vascular wall endothelium (ie, vascular cell adhesion molecule-1 (VCAM-1), selectins, and integrins, respectively) [108].

This is followed by leukocyte penetration into the media layer, the formation of macrophages foam cells, and replication of smooth muscle cells (SMC), all of which account for the propagation of the atherosclerotic plaque [108]. Several key molecules mediate this process, such as CXC chemokines (IFN-inducible protein 10 (IP-10), monokine induced by IFN- γ (Mig), and IFN-inducible T-cell α chemoattractant (I-TAC)) [110], monocyte chemoattractant protein-1 (MCP-1) [111], and macrophage colony-stimulating factor (M-CSF) [112]. Finally, the thinning of the fibrous cap and release of prothrombotic factors by macrophages foam cells all promote plaque rupture and thrombus formation [108]. Therefore it seems that restoring endothelial function and targeting vascular wall inflammation may hold the key for preventing atherosclerosis and CVD.

Lifestyle modification, including exercise and weight control, smoking cessation, lipid-lowering agents, such as statins, better glycemic control in diabetic patients, and other methods, reduce vascular wall inflammation, improve endothelial function, and prevent atherosclerosis [107]. Other therapeutic modalities shown to improve endothelial function involve the use of angiotensin-converting enzyme (ACE) inhibitors, renin antagonist, endothelin-1 antagonists, and β -blockers [107]. Development of new therapies for ED, including vitamin D administration, is under investigation [113].

Immune-mediated accelerated atherosclerosis is discussed in detail in [Chapter 4](#) of this book.

2.2 Pro- and Anti-inflammatory Cytokines Balance and Cardiovascular Diseases

Recent studies have evaluated the efficacy of cytokine modulation as a therapeutic modality to CVD, such as heart failure and myocardial infarction [114,115]. Balancing pro- and anti-inflammatory cytokines is a complicated process. Overlapping pro- and anti-inflammatory activities of certain cytokines such as IL-6 accounts for the difficulty in identifying the outcome [116].

[Fig. 1.4](#) demonstrates the balance between pro- and anti-inflammatory cytokines, whose effects on the cardiovascular system are discussed herein. Most cytokines have both cardioprotective and cardiotoxic functions. The net impact on cardiac tissue is often unpredictable and may be related to the duration of plasma level elevations [116,117]. Herein, we will explore and clarify the available data.

2.2.1 Anti-inflammatory Cytokines

Major anti-inflammatory cytokines involved in cardiovascular diseases (CVD) include IL-4, IL-10, IL-11, IL-13, and the transforming growth factor (TGF)- β [116].

2.2.1.1 IL-4

IL-4 is an STAT-6 signaling-dependent 20-kd glycoprotein, which shifts the Th balance toward a Th2-mediated response. Other activities include downregulation of IL-12 production, the Th1 suppression response, and

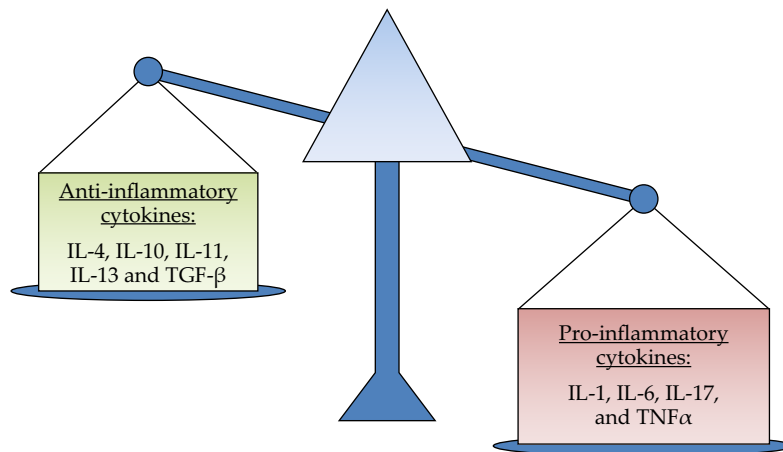


FIGURE 1.4 Pro- and anti-inflammatory cytokine balance. Adapted from the personal collection of the authors.

the suppression or blockage of the monocyte-derived cytokines including IL-1, TNF- α , IL-6, IL-8, and the macrophage inflammatory protein (MIP)-1 α . The Th2-mediated response of IL-4 includes recruitment of mast cells and induction of IgE secretion [116].

IL-4 involvement in CVD was previously suggested due to its profibrotic characteristics. IL-4 was found to be a mitogen of microvascular and a large-vessel endothelial wall [118]. However, whether IL-4 contributes to hypertension pathogenesis remains unclear, as serum concentrations of IL-4 do not differ between hypertensive patients and healthy subjects [119].

Profibrotic characteristics of IL-4 were also examined in the context of myocardial fibrosis and cardiac remodeling. One study demonstrated reduced cardiac interstitial collagen and normal cardiac architecture and function in IL-4 deficient mice, compared to controls. Moreover, IL-4 deficient mice had reduced angiotensin II-mediated interstitial myocardial fibrosis and DCM [120].

IL-4 may also play a role in pulmonary hypertension pathogenesis. IL-4-deficient mice were found to be protected from hypoxia-induced mitogenic factor, (FIZZ1/RELM α)-induced endothelial cell apoptosis, and pulmonary hypertension development compared to wild type mice [121]. IL-4 involvement in pulmonary vascular remodeling was suggested as a cause of schistosomiasis-induced vascular inflammation [122].

2.2.1.2 IL-10

IL-10 is a potent STAT-3 signaling-dependent, anti-inflammatory cytokine inhibiting the Th1 response and pro-inflammatory cytokines, including monocyte/macrophage-derived TNF- α , IL-1, IL-2, IFN- γ , IL-6, IL-8, IL-12, granulocyte colony-stimulating factor (G-CSF), MIP-1 α , and MIP-2 α . It also inhibits cell-surface expression of major histocompatibility complex (MHC) class II, CD14, and TNF receptors [116].

Several studies have demonstrated increased levels of proinflammatory cytokines, such as TNF- α , IL-1 β , and

IL-6 in the plasma, circulating leukocytes, atherosclerotic lesions, and myocardium of patients with heart failure [123]. Moreover, anti-inflammatory cytokines such as TGF β did not increase. Key players for this imbalance between pro- and anti-inflammatory cytokines in cardiac inflammation are thought to be TNF- α and IL-10, respectively [124].

IL-10 appears to be upregulated in the inflammatory response following myocardial infarction (MI). IL-10-deficient mice were shown to have neutrophil recruitment, elevated plasma levels of TNF- α , and higher tissue expression of ICAM-1 [125]. Moreover, IL-10 administration to post-MI mice reduced inflammation and improved cardiac remodeling [115,125]. However, it seems that it is mainly involved in controlling acute inflammation and is not a critical regulator of infarct healing and cardiac remodeling [125].

2.2.1.3 IL-11

IL-11 is a 178-amino acid nonglycosylated peptide serving both as a hematopoietic growth factor and an immune regulator. IL-11 is a Th2 cytokine, belonging to the IL-6 cytokine family. It inhibits IFN- γ , IL-2, IL-1, and TNF α synthesis and induces the release of IL-4 and inhibitory nuclear factor (NF)- κ B, which serves as a transcriptional activator for proinflammatory cytokines [116].

IL-11 performs an antifibrotic role in the heart following MI [126]. Administration of IL-11 to mice at the start of reperfusion, following an MI, reduces ischemia/reperfusion injury and preserves cardiac function [114]. This protective role of IL-11 seems to be STAT3 dependent, as cardiac-specific ablation of STAT3 results in attenuation of IL-11 protective activity and DCM [127].

IL-11 was found to protect endothelial cells from vascular injury in a mechanism that involves surviving expression induction [126,128]. Interestingly, it has also been found to play a role in the treatment of peripheral vascular disease (PVD), as its administration to mice

following femoral artery ligation results in the mobilization of CD34(+)/VEGFR2(+) mononuclear cells, increased collateral vessel growth, and perfusion recovery [129].

2.2.1.4 IL-13

IL-13 is a 132-amino-acid nonglycosylated cytokine that shares common features with IL-4. IL-13 inhibits TNF, IL-1, IL-8, and MIP-1a production, NF- κ B activation and CD14 and Fc γ receptor expression. It induces cell surface expression of b2 integrins and MHC class II antigens [116].

IL-13 appears to play a profibrotic role in the aging heart. One study found a 28% increase in IL-13 in 30-month-old mice compared to mice 3 months old. This elevation of IL-13 appears to be MCP-1-dependent, presenting a shift toward a Th2 response in the aging heart [130]. Elevation of IL-13 in the aging heart was found to be associated with periostin expression, an extracellular matrix (ECM) protein that plays an important role in cardiac fibrosis [131]. This profibrotic role of IL-13 was also demonstrated using a murine model of cardiac transplant rejection, as it was inhibited by blocking IL-13/TGF- β 1 interaction using IL-13R α 2 siRNA [132].

IL-13 was found to modify cardiac wound healing following an MI. IL-13-deficient mice experienced increased left ventricular dilatation and mortality following an MI. It seems that leukocyte infiltration reduction and M2-like monocyte/macrophage differentiation induction in the infarct zone may account for this feature [133].

2.2.1.5 TGF- β

TGF- β is a 25-kD homodimer with regulatory functions on cell proliferation, differentiation, and formulation of the ECM, inhibits the proliferation and differentiation of T and B cells, and reduces IL-2, IFN- γ , and TNF production [116].

Ayca et al. found that patients with hypertrophied cardiomyopathy (HCM) had higher TGF- β levels than the control group. For those with higher TGF- β levels, a larger left-atrial size, thicker interventricular septum, a higher NYHA class, and more hospitalizations were noted [134].

TGF- β 1 and TGF- β 2 were shown to be elevated in DCM patients as well [135]. It was found to play a key role in the pathogenesis of myocardial inflammation as well as atrial fibrillation [136]. Furthermore, it was demonstrated that TGF- β expression is upregulated in animal models of myocardial infarction [137].

In post-MI mice models, early and late upregulation of TGF was observed, as high levels of TGF- β 1, TGF- β 2, and TGF- β 3 were measured, respectively [137]. Interestingly, angiotensin-II appears to play a role in TGF- β -mediated inflammation, post-MI. Treatment of post-MI

rat models with angiotensin receptor blocker (ARB) or a combination therapy of ARB and ACE inhibitors normalized cardiac collagen as well as TGF- β 1 mRNA levels and inhibited macrophages and myofibroblasts in the infarct zone [138]. Angiotensin-II-TGF- β interaction in cardiac fibrosis was also demonstrated in another study, suggesting a regulatory function of angiotensin on Smad family proteins in post-MI heart failure [139].

TGF- β involvement in cardiovascular disease extends outside the heart. TGF- β was found to be a biomarker for fibrosis in adult cardiac patients with peripheral vascular disease (PVD) [140]. It is thought to play a role in the pathogenesis of atherosclerosis, since it was found elevated in diabetic patients. This may account for the accelerated atherosclerosis found in these patients [141].

One suggested mechanism for TGF- β 's role in atherosclerosis is the induction of Smad1/5 in the macrophages and the subsequent activation of the proatherogenic genes: Hepcidin antimicrobial peptide (HAMP), the plasminogen activator, and the Urokinase receptor (PLAUR). In contrast, it appears that Smad2/3 regulates the athero-protective effects of TGF- β . Thus a balance between Smad1/5 and Smad2/3 through activation of TGF- β is maintained, and TGF- β 's contribution to the development of atherosclerosis is probably a function of this balance [142].

2.2.2 Proinflammatory Cytokines

Major proinflammatory cytokines involved in cardiac diseases consist of TNF- α , IL-6, and cytokine families of IL-17 and IL-1.

2.2.2.1 TNF- α

TNF- α is a 157-amino acid cytokine, binding to two membrane bound receptors: 55kDa TNFR1 and 75kDa TNFR2. Cardiotoxic and cardioprotective effects are mediated by TNFR1 and TNFR2, respectively [117,143]. Cardioprotective roles of TNF- α have been previously reported. Pretreatment with TNF- α was found to inhibit the LDH release by hypoxic myocytes [144].

In desmin-deficient mice, which represent a genetic heart failure model, TNF- α induced an NF- κ B-mediated ectopic expression of keratin 8 and keratin 18 in myocytes. These proteins were cardioprotective through maintenance of a normal intercalated disc structure [145]. Furthermore, TNF- α cardioprotective features may be mediated through the induction of vascular cell adhesion molecule (VCAM)-1 secretion and increased cell adhesion ability, as was previously demonstrated in post-MI mice models [146].

Other cardioprotective features of TNF- α include antioxidant activity via a manganous superoxide dismutase (Mn-SOD) release and upregulation of heat-shock proteins [117]. The cardiotoxic role of TNF- α was previously studied. A recently published meta-analysis of 36

studies found that TNF- α G-308A polymorphism was associated with increased risk of ischemic heart disease (IHD) in the total population [147]. TNF- α involvement in CVD is extensive and includes cardiac fibrosis, contractile dysfunction, ECM remodeling, myocyte hypertrophy, and apoptosis [117]. Evidence of a TNF- α role in cardiac fibrosis is emerging [117]. It appears that there is a synergic interaction between TNF- α and angiotensin-II, resulting in cardiac fibrosis.

In one study, the administration of angiotensin-II to mice deficient in both TNF- α receptors failed to result in cardiac fibrosis, suggesting that TNF- α is required for angiotensin-II-induced fibroblast maturation from monocytes [143]. Furthermore, angiotensin-I receptor upregulation in cardiomyocytes was found to be induced by TNF- α and increase angiotensin II-mediated cardiac fibroblast responses [148]. TNF- α appears to induce cardiomyocytes apoptosis [149]. Mice with TNF overexpression were found to have progressive DCM and increased cardiomyocyte apoptosis [150].

Pretreatment of myocytes with anti-TNF α monoclonal antibodies improved left ventricular end diastolic function (LVEF) after cardiac microembolization and inhibited cardiomyocyte apoptosis and the expression of caspase-3 and caspase-8 [151]. Cardiac contractile dysfunction was observed in rat and dog hearts with normal perfusion following the administration of TNF- α [152].

A suggested mechanism is dysregulation of calcium homeostasis involving the induction of sphingosine by TNF- α and a subsequent reduction of a calcium release from the sarcoplasmic reticulum, inhibition of sarcoplasmic reticulum Ca-ATPases (SERCA2a) cell expression, and reduction of SERCA2a activity via β -adrenergic receptor coupling reduction [152]. Other mechanisms such as oxidation of myocardial contractile filaments by TNF- α -induced reactive oxygen species (ROS) have also been described [152]. Finally, TNF- α dysregulates collagen synthesis and is thus involved in cardiac ECM remodeling.

Culturing rat cardiac fibroblasts with TNF- α decreased the collagen synthesis and increased the matrix metalloproteinase (MMP) activity, which degraded the collagen, specifically MMP-13, MMP-2, and MMP-9 [153]. Inhibition of tissue inhibitors of MMP (TIMP) by TNF- α further decreased the collagen synthesis, accounting for the cardiac ECM remodeling [154].

2.2.2.2 IL-1

The IL-1 cytokine family consists of IL-1 α , IL-1 β , IL-18, IL-33, IL-36 α , IL-36 β , and IL-36 γ , three receptor antagonists (Ra; IL-1Ra, IL-36Ra, and IL-38), and the anti-inflammatory cytokine IL-37 [155]. It seems that pretreatment with IL-1 decreases cardiac ischemic/reperfusion injury [117,156].

In rat models of myocardial ischemic/reperfusion injury, IL-1 α preconditioning resulted in better LVEF, increased coronary flow, and reduced creatinine kinase (CK) release. Cardioprotective mechanisms include a release of HSP27 and increased activities of Copper/Zinc SOD, Mn-SOD, catalase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase (G6PD) [157].

Induction of Mn-SOD by IL-1 α in the context of myocardial reperfusion injuries was reported in another study [158]. IL-1 β was found to be involved in the regression of cardiac fibrosis by the expression of MMP-2 and MMP-9 [159]. In addition, IL-33 was found to have cardioprotective features including an inhibition of myocardial fibrosis, improvement of myocardial function, and reduced cardiomyocytes apoptosis and hypertrophy [160].

In contrast, IL-1 appears to play a proapoptotic role in cardiomyocytes. IL-1 β was found to induce cardiomyocyte apoptosis in neonatal mice via the activation of caspase 3 and the release of cytochrome c into the cytoplasm [161]. Another study found increased expression of nitric oxide (NO) and Bcl-2 associated with IL-1 β -induced cardiomyocytes apoptosis [162]. Furthermore, treatment of post-MI mice with anti-IL-1 β monoclonal antibodies inhibited cardiomyocyte apoptosis, reduced left ventricular enlargement, and improved systolic dysfunction without affecting the inflammasome formation or the caspase-1 activation [163].

Previous studies have found an increased expression of IL-1 β in pressure and volume-induced myocardial hypertrophy [117]. Pressure-induced cardiac hypertrophy is TLR2-mediated through IL-1 β upregulation via NF κ B activation [164]. Furthermore, IL-1 β was found to promote cardiomyopathy through the activation of the NOD-like receptor family, pyrin domain containing (Nlrp) three inflammasome and an intracellular danger-sensing pathway [165].

IL-18 has also been associated with cardiomyocytes hypertrophy. One suggested mechanism is through the phosphatidylinositol 3-kinase-phosphoinositin-dependent kinase-1-Akt-GATA4 signaling pathway [117]. Other cardiotoxic mechanisms of IL-1 include dysregulation of calcium homeostasis and increased ECM remodeling through decreased expression of procollagen and a degradation of collagen via the induction of MMP-2, 3, 9, and 13 [117].

2.2.2.3 IL-6

IL-6 and its related cytokine leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), and cardiotrophin-1 (CT-1) consists of a family of pleiotropic cytokines produced in various tissues and by virtually all cells in the immune system [117,166]. It mediates proliferation, growth, differentiation, survival, and apoptosis signals [117]. As in other cytokines, IL-6

appears to have both cardioprotective and cardiotoxic characteristics [117] thought to be associated with an acute and chronic increase in its levels found in plasma or tissues, respectively [166]. An acute elevation of IL-6 appears to have cardioprotective characteristics. This has previously been reported in the context of viral myocarditis [166–168]. Mice infected with the encephalomyocarditis virus (EMCV) and treated with human IL-6 were found to have a reduced viral titer and viral replication in the heart, reduced circulating TNF- α levels, decreased natural killer (NK)-cell activity, increased titer of anti-EMCV neutralizing antibodies, reduced rates of myocardial injury, and an increased percentage of macrophages in the heart and spleen [168].

On the other hand, IL-6, along with other cytokines, may mediate a Th17-induced progression of viral myocarditis to autoimmune myocarditis and subsequently to DCM (see Fig. 1.5) [166,169,100]. Thus the IL-6 cardioprotective role in myocarditis appears to be limited to the acute phase of the viral disease. Cardioprotective qualities of IL-6 are found in an ischemic/reperfusion injury. Pretreatment of post-MI mice with IL-6 induced phosphatidylinositol (PI) 3-kinase and nitric oxide (NO)-dependent cardiomyocyte protection were found to be correlated with an altered calcium homeostasis [170].

Exercise preconditioning against myocardial ischemic/reperfusion injury was also found to be IL-6-mediated [171]. Cardioprotection is most likely achieved through the Janus kinase (JAK)-STAT signaling pathway, upregulation of inducible nitric oxide synthase (iNOS), and cyclooxygenase (COX)-2 [172]. Furthermore, IL-6-induced upregulation of prohibitin via STAT3 phosphorylation was found to play a cardioprotective role against H₂O₂-induced cardiomyocyte injury [173].

Cardiotoxic effects of IL-6 were previously described. IL-6 levels were found to be elevated in patients with

HCM and associated with myocardial fibrosis, inflammation, and activation of NFkB [174]. The prohypertrophic effect of IL-6 on cardiomyocytes was demonstrated in another study and was found to be associated with cardiac fibrosis and inhibited by the addition of losartan, suggesting that angiotensin may be involved in the process [175].

Angiotensin involvement in IL-6-induced cardiomyocyte hypertrophy is associated with CT-1, as CT-1 was found to increase angiotensinogen mRNA levels in hypertrophied hearts of rats. Adding losartan succeeded in inhibiting this effect [176]. IL-6 was previously shown to be connected with increased cardiac fibrosis and reduced myocardial contractility [117]. Cardiomyocyte responsiveness to β -adrenergic stimuli also decreased in the presence of IL-6, thus contributing to decreased myocardial contractility [166]. One suggested mechanism is JAK2/STAT3-mediated IL-6-induced activation of iNOS [177]. This NO-cGMP-mediated pathway seems to involve transients of cardiomyocyte intracellular Ca²⁺ and thus contributes to reduced myocardial contractility [178].

2.2.2.4 IL-17

The IL-17 cytokine family consists of IL-17A, IL-17F, and IL-22 [100]. IL-17A is a major player in the inflammatory processes. First thought to be produced only by Th17 cells, it is now known to be produced by a wide variety of cells, including $\gamma\delta$ T cells, neutrophils, and CD8 T cells [179]. IL-17A plays a central role in cardiac remodeling, fibrosis, and DCM pathogenesis. IL-17A deficient mice with reduced IL-6 levels and increased numbers of cardiac MMP-2 and MMP-9 have to be protected from viral myocarditis-induced autoimmune DCM [100].

In another study, treatment of CVB3-induced chronic myocarditis mice with adenovirus containing IL-17

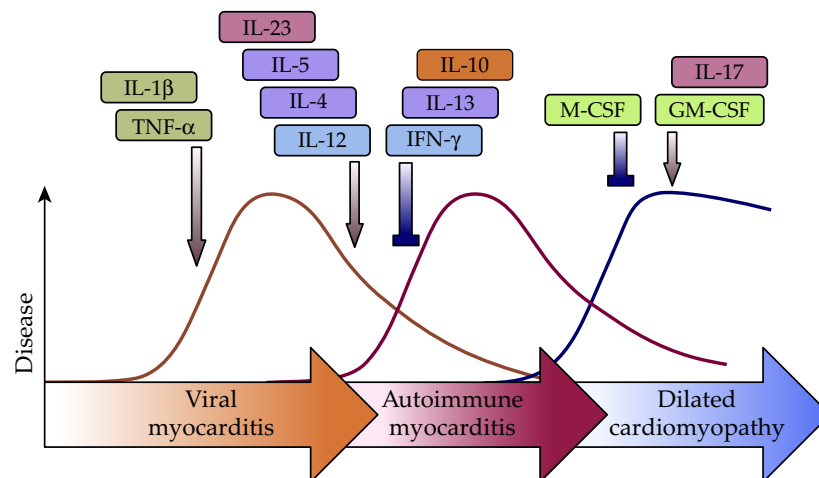


FIGURE 1.5 Cytokine involvement in the progression of viral myocarditis to autoimmune myocarditis and DCM. Adapted from Rose [100].

receptor A resulted in a reduction of Th17 cells production, cardiac fibroblasts, and reduced levels of IL-17A, TNF- α , IL-6, disintegrin, metalloprotease with thrombospondin type 1 motifs (ADAMTS-1), MMP-2, and collagen I and III [180]. Moreover, treatment of post-viral myocarditis mice with anti-IL-17A monoclonal antibodies was found to be protective against myocarditis-induced fibrosis, DCM development, and cardiac contractile dysfunction [181].

Targeting IL-17A may also prove beneficial in the treatment of MI. IL-17A was found to play a role in the pathogenesis of ischemic/reperfusion injury in post-MI mice by inducing cardiomyocyte apoptosis and neutrophil infiltration [182]. Furthermore, limited fibrosis, reduced DCM development, and improved survival were observed in tIL-17A deficient post-MI mice, suggesting its role in MI-induced-DCM pathogenesis [183,184].

3. CONCLUSIONS

Etiology for autoimmune diseases is multifactorial. Identifying all the risk factors for autoimmunity may help in defining high-risk groups and promote programs for primary prevention. However, at present, the complete picture is obscure and further studies in this field are needed. Such treatments could be developed, for example, by modulation of certain compounds of the complement system and its regulatory proteins, intervention in the imbalance between pro- and anti-inflammatory cytokines, or in the balance between Treg/Th2 and Th1/Th17 immune responses. Note that these topics are discussed in more detail in [Chapter 28](#) of this book. Special attention should be given to uncovering immune-mediated mechanisms of CVD, since understanding of the key cytokines such as IL-6 and IL-10 and their role in CVD pathogenesis may hold the solution for developing new specific-cytokine modulating drugs. Moreover, understanding that atherogenesis is an inflammatory process by nature is important for the future development of drugs aimed at stopping or reversing plaque formation. Future studies in the field of autoimmunity and CVD must consider all the above and a dialogue between clinical and basic immunologists, rheumatologists, and cardiologists should be promoted.

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Organ-Specific Autoimmune Myocardial Diseases: From Pathogenesis to Diagnosis and Management

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1. INTRODUCTION

1.1 Definition of Organ-Specific Autoimmune Disease

Autoimmune disease results from the loss of tolerance to self-antigens, which is maintained under physiological conditions. A disease must fulfill at least two of the major criteria proposed by Witebsky and later modified by Rose [1] to be classified as autoimmune. There are also minor criteria, some of which are common to all autoimmune conditions and others which are found in only a few of them (Table 2.1). Organ-specific autoimmune diseases develop as a result of both genetic predisposition and environmental factors. The genetic predisposition is responsible for both the fact that different autoimmune conditions may be associated in patients or in their family members, as well as for the well-known feature that single autoimmune diseases often run in families. The inheritance of susceptibility is usually polygenic. Organ-specific autoimmune diseases are commonly associated with specific human leukocyte antigens (HLA) class II antigens, although the mechanisms by which multiple HLA and non-HLA genes, often involved in immune regulatory pathways [3–6], may determine disease predisposition are still undefined [1,2].

Autoimmune disease is characterized by the presence of circulating autoantibodies, which are not always pathogenic but represent markers of ongoing tissue damage. In nonorgan-specific autoimmune disease the autoantibodies are against ubiquitous autoantigens (eg, nuclear antigens in systemic lupus erythematosus) and

tissue damage is generalized. In organ-specific autoimmune disease, immunopathology is restricted to one organ or apparatus within the body, and the autoimmune process, antibody and/or cell-mediated, is directed against autoantigens, which are unique to the affected organ (eg, thyroid peroxidase in Hashimoto's thyroiditis). The majority of organ-specific autoimmune diseases are chronic and apparently "idiopathic". The histological hallmark of organ-specific autoimmunity is an early mononuclear cell infiltrate in the affected organ, eg, insulitis in Type 1 insulin-dependent diabetes mellitus (IDDM), with inappropriate expression of HLA class II and of adhesion molecules. At a later stage inflammatory cells tend to disappear and the tissue undergoes profound fibrotic changes with end-stage atrophy and organ dysfunction (such as in Hashimoto's thyroiditis). In other instances organ-specific autoimmunity may lead to enhanced target organ function (eg, Basedow's disease).

Organ- and disease-specific antibodies are found in affected patients. These antibodies are also detected in family members even years before the development of disease, and thus identify asymptomatic relatives at risk [1]. Involvement of organ-specific autoimmunity has been suspected in the following cardiovascular diseases: post-pericardiotomy and post-myocardial infarction (Dressler) syndromes, rheumatic carditis, idiopathic forms of inflammatory cardiomyopathy, and of brady or tachyarrhythmias, systemic arterial hypertension. However, currently the Rose–Witebski criteria for organ-specific autoimmunity are entirely fulfilled in infection-negative biopsy-proven myocarditis and in

TABLE 2.1 Fulfilled Rose–Witebsky Autoimmune Criteria in Myocarditis/DCM: 2016 Update**Major**

- Mononuclear cell infiltration and abnormal HLA expression in the myocardium in the absence of infectious agents or other known causes: **yes**
- Circulating autoantibodies in patients and in unaffected family members: **yes**
- Autoantibody and/or autoreactive lymphocytes in situ within the myocardium: **yes**
- Identification and isolation of organ-specific autoantigen(s) involved: **yes**
- Disease induced in animals by immunization with relevant autoantigen, and/or passive transfer of serum, purified autoantibody, and/or lymphocytes: **yes**
- Efficacy of immunosuppression in proven autoimmune myocarditis: **yes**

Minor

- Findings relating to all autoimmune disorders
 - Middle-aged women most frequently affected: **No**
 - Familial aggregation: **yes**
 - HLA association: **yes**
 - Hyper- γ -globulinemia: **No**
 - Clinical course with hot phases and remissions: **yes**
 - Autoimmune diseases associated in the same patient or in family members: **yes**
- Findings of organ-specific autoimmune disorders
 - Autoantigens at low concentration: **Not known**
 - Autoantibodies against organ-specific autoantigens: **yes**
 - Immunopathology mediated by type II, IV, V, VI reactions: **yes**
 - Induction of antibodies induces an organ-specific disease/phenotype: **yes**
 - Transfer of autoantibodies also transfers the disease/phenotype: **yes**

inflammatory cardiomyopathy (eg, defined as myocarditis with myocardial dysfunction) that are covered in this chapter.

1.2 Definitions of Myocarditis and of Inflammatory Cardiomyopathy

The World Health Organization (WHO) classification of cardiomyopathies defines myocarditis as an inflammatory disease of the myocardium, which is diagnosed by endomyocardial biopsy (EMB) using established histological, immunological, and immunohistochemical criteria; in addition, myocarditis may be idiopathic, infectious, or autoimmune and may heal or evolve in dilated cardiomyopathy (DCM) (Table 2.2) [7–10]. More recently, however, the term “idiopathic” has been used only in the absence of complete microbiological work-up on EMB (Table 2.2).

DCM features include dilatation and impaired contraction of the left or both ventricles in the absence of known, specific causes of heart failure, including coronary artery disease; idiopathic, familial/genetic, viral, and/

TABLE 2.2 Definitions**Myocarditis (WHO/ISFC, ESC) [7–11,33]**

Inflammatory disease of the myocardium diagnosed by established histological, immunohistochemical** and immunological criteria***.*

*established histological Dallas criteria [9] defined as follows:

“histological evidence of inflammatory infiltrates within the myocardium associated with myocyte degeneration and necrosis of non-ischæmic origin.”

**immunohistochemical criteria, abnormal inflammatory infiltrate [3,11,12,33] defined as follows:

“ ≥ 14 leukocytes/mm² including up to 4 monocytes/mm² with the presence of CD 3 positive T-lymphocytes ≥ 7 cells/mm².”

***immunological criteria and myocarditis etiology defined as follows [9,33]:

- Viral: Histology (Hx) and immunoHx positive (pos), polymerase chain reaction (PCR) pos for \geq virus
- Autoimmune: Hx and immunoHx pos; viral PCR negative (neg); with or without positive cardiac autoantibodies (AABs); exclusion of other known inflammatory causes.
- Viral and immune[^]: Hx and immunoHx pos; viral PCR pos; cardiac AABs pos

[^]N.B. a follow-up EMB may identify persistent viral myocarditis, resolved myocarditis (Hx and virological), or persistent virus-negative myocarditis, eg, post-infectious autoimmune.

Inflammatory cardiomyopathy and dilated cardiomyopathy (DCM)(WHO/ISFC, ESC)**[7–9,33]**

Myocarditis in association with cardiac dysfunction.

**** involved in the pathogenesis of DCM, includes idiopathic, autoimmune and infectious subtypes.

DCM is a clinical diagnosis characterised by dilation and impaired contraction of the left or both ventricles that is not explained by abnormal loading conditions or coronary artery disease.

*****DCM includes idiopathic, familial/genetic, viral, and/or immune, alcoholic/toxic subtypes.

or immune are recognized DCM forms (Table 2.2) [7,8]. Histological EMB findings in DCM include myocyte loss, compensatory hypertrophy, fibrosis, and acute or chronic myocarditis in 30–40% of cases. Therefore in a patient subset, myocarditis and DCM represent two sides of the same coin, eg, an inflammatory disease of the myocardium, which can be infectious, post-infectious immune, or primarily organ-specific autoimmune (Table 2.2) [7–10].

2. AETIOPATHOGENESIS OF MYOCARDITIS

Causes of myocarditis are shown in Table 2.3 [7–16]. Viral infections are presumed to be the most common cause in the Western world, with a variable frequency of viral genomes on EMB of patients with myocarditis and DCM by molecular techniques, mainly reverse transcriptase (RT) polymerase chain reaction (PCR) [14–32]. Koch’s postulates are used to establish whether a microbe causes disease. While many viruses can be detected in the myocardium by PCR, fulfillment of Koch’s postulates in animal models is

TABLE 2.3 Aetiopathogenetic Agents Associated With Myocarditis/Inflammatory Cardiomyopathy**1. Infective Myocarditis**

Bacterial	Staphylococcus, Streptococcus, Pneumococcus, Meningococcus, Gonococcus, Salmonella, Corynebacterium diphtheriae, Haemophilus influenzae, Mycobacterium (tuberculosis), Mycoplasma pneumoniae, Brucella
Spirochetal	<i>Borrelia</i> (Lyme disease), <i>Leptospira</i> (Weil disease)
Fungal	<i>Aspergillus</i> , <i>Actinomyces</i> , <i>Blastomyces</i> , <i>Candida</i> , <i>Coccidioides</i> , <i>Cryptococcus</i> , <i>Histoplasma</i> , <i>Mucormycoses</i> , <i>Nocardia</i> , <i>Sporothrix</i>
Protozoal	<i>Trypanosoma cruzi</i> , <i>Toxoplasma gondii</i> , <i>Entamoeba</i> , <i>Leishmania</i>
Parasitic	<i>Trichinella spiralis</i> , <i>Echinococcus granulosus</i> , <i>Tenia solium</i>
Rickettsial	<i>Coxiella burnetii</i> (Q fever), <i>R. rickettsii</i> (Rocky Mountain spotted fever), <i>R. tsutsugamushi</i>
Viral	Coxsackievirus A and B, echovirus, poliovirus, hepatitis viruses, influenza A and B viruses, adenovirus, respiratory syncytial virus, mumps virus, measles virus, rubella virus, dengue virus, Chikungunya virus, yellow fever virus, Junin virus, Lassa fever virus, lymphocytic choriomeningitis virus, herpes simplex virus, varicella-zoster, human herpes virus-6, cytomegalovirus, Epstein–Barr virus, variola virus, vaccinia virus, parvovirus B19, rabies virus, human immunodeficiency virus-1

2. Immune-Mediated Myocarditis

Allergens	Tetanus toxoid, vaccines, serum sickness Drugs: Penicillin, cefaclor, colchicine, furosemide, isoniazid, lidocaine, tetracycline, sulfonamides, phenytoin, phenylbutazone, methyl dopa, thiazide diuretics, amitriptyline
Alloantigens	Heart transplant rejection
Autoantigens	Idiopathic: Virus-negative lymphocytic, virus-negative giant cell Associated with autoimmune or immune-oriented disorders: Systemic lupus erythematosus, rheumatoid arthritis, Churg–Strauss syndrome, Kawasaki’s disease, inflammatory bowel disease, scleroderma, polymyositis, myasthenia gravis, insulin-dependent diabetes mellitus, thyrotoxicosis, sarcoidosis, Wegener’s granulomatosis

3. Toxic Myocarditis

Drugs	Amphetamines, anthracyclines, cocaine, cyclophosphamide, ethanol, fluorouracil, lithium, catecholamines, hemetine, interleukin-2, trastuzumab, clozapine
Heavy Metals	Copper, iron, lead
Miscellaneous	Scorpion sting, snake, and spider bites, bee and wasp stings, carbon monoxide, inhalants, phosphorus, arsenic, sodium azide,
Hormones	Pheochromocytoma, vitamins: Beri-beri
Physical agents	Radiation, electric shock

Reprinted from Caforio et al. [33].

limited to infections by enterovirus and cytomegalovirus [14–35]. Claims made of the importance of other viruses are not supported by Koch’s postulates. More studies are therefore needed to clarify whether a growing list of viruses really cause myocarditis or are detected coincidentally as remnants of past benign infections or as experimental contamination and artifact (Table 2.3). At the state of current knowledge, myocarditis is defined as autoimmune if no infectious agents are identified on EMB and other known causes are excluded [2,33]. Autoimmune myocarditis may develop with exclusive (organ-specific) cardiac involvement or in the context of autoimmune disorders with predominant extracardiac organ involvement [2,16,33], eg, in systemic lupus erythematosus. Here, we focus on organ-specific myocarditis/inflammatory cardiomyopathy.

2.1 Pathogenesis of Organ-Specific Autoimmune Myocarditis/Inflammatory Cardiomyopathy

In several susceptible mouse strains viral genomic material and inflammation persist in the heart for weeks, triggering myocardial autoimmune reactions [2,33–35]. However, the same genetically predisposed mouse strains also develop autoimmune lymphocytic or giant cell myocarditis (and later on DCM) in the absence of a viral challenge, after immunization with specific cardiac autoantigens, eg, cardiac myosin, or spontaneously under control of both major histocompatibility complex (MHC) and non-MHC genes [33,34,36–45] (Fig. 2.1). It has been shown that some of these MHC genes predisposing to organ-specific autoimmune myocarditis are also

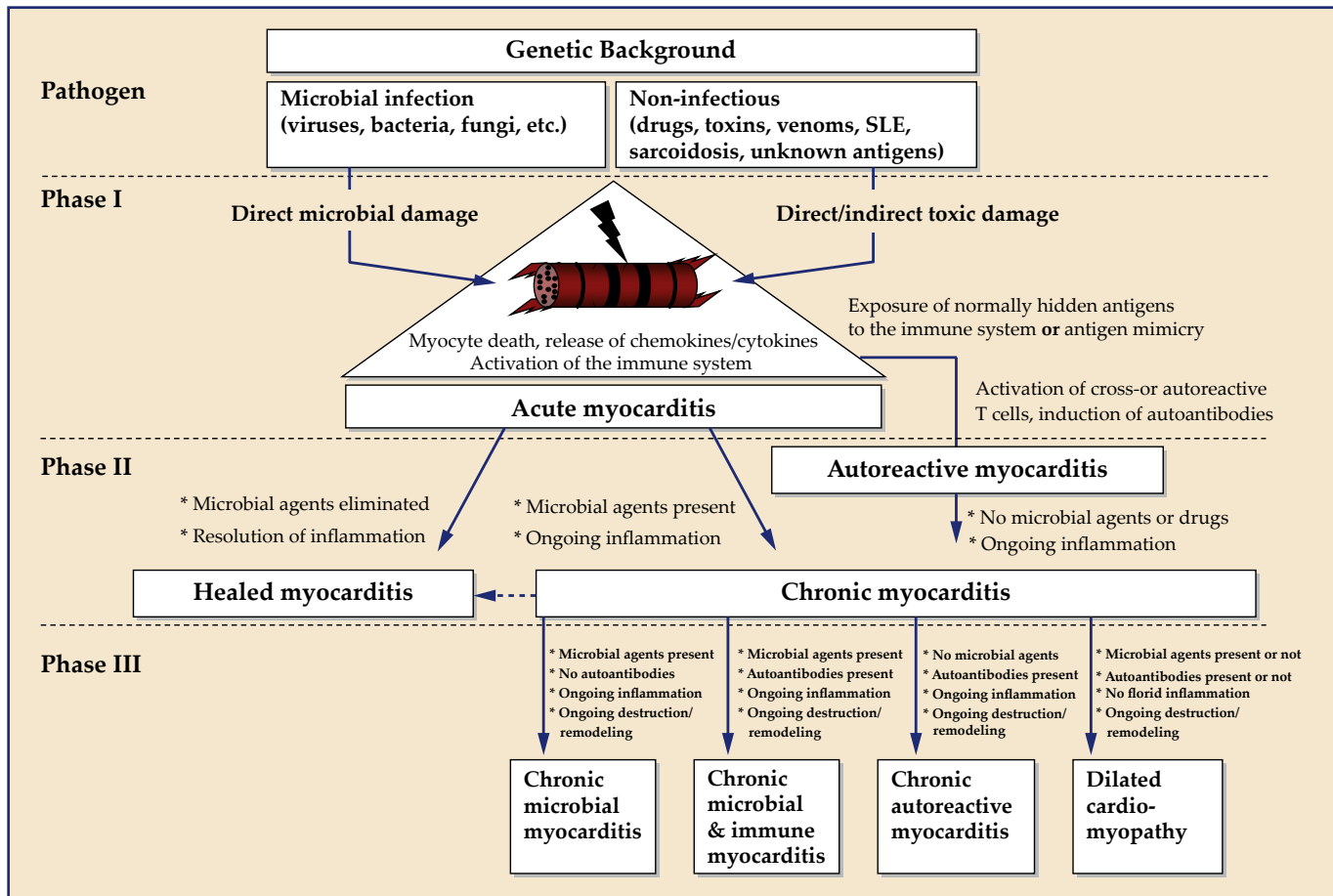


FIGURE 2.1 The image shows the pathogenetic mechanisms involved in myocarditis and progression to dilated cardiomyopathy. Adapted from Caforio et al. [33].

associated with Type 1 diabetes and other autoimmune diseases [34,43,45–47]. Both myocarditis and inflammatory DCM may be familial [48–53] and have been associated with HLA [54,55]. Importantly a recent landmark large multicenter European genome-wide association study reported that, without a prespecified hypothesis, HLA is a risk locus for DCM, in keeping with the autoimmune paradigm [55]. Biopsy-proven myocarditis has also been described in other cardiomyopathies, besides DCM [56,57], and in some channelopathies [58]. It remains to be established whether this reflects involvement of autoimmunity as a final common pathway of chronic cardiac damage in other genetically determined cardiomyopathies or a mere association of distinct diseases. More work should be focused on the identification of HLA and non-HLA immunogenetic basis of human myocarditis/DCM.

In keeping with Rose–Witebski postulates, in human myocarditis/DCM (Table 2.1) there are myocardial mononuclear cell infiltrates, abnormal expression of HLA class II, and/or adhesion molecules on cardiac endothelium in the absence of viral genomes (as assessed by PCR on EMB) in index patients and family members [59–61]; increased serum levels of cytokines and cardiac autoantibodies (AABs) in patients and relatives [16,33,48–54,

63–133]; experimentally induced models of myocarditis/DCM as a consequence of immunization with recognized autoantigen(s) [34,36–47]; and response to immunosuppression or immunomodulation in patients with giant cell myocarditis and with autoimmune DCM forms [22,32,33,64,100,101]. Many distinct cardiac AABs have been found in human myocarditis/DCM (Table 2.4). Several findings support a direct pathogenic role for some of these autoantibody specificities. Functional effects of cardiac AABs isolated from index patients have been shown in vitro [73,96,134]. Secondly, the cardiac abnormalities seen in human post-myocarditic DCM have been reproduced in experimental animals following immunization with defined autoantigens, eg, β 1-adrenergic or M2 muscarinic receptors, cardiac myosin, and cardiac troponin (cTNI) [38–41,77–80,83,91,92]. Thirdly, myocardial pathology has been produced by transfer of immune components from one experimental animal to another [79,80,83–86,93]. Last but not least, improved cardiac morphology and function has been achieved by specific removal of β 1-adrenoceptor AABs (β 1-AABs) by immunoadsorption (IA) in rabbits, or by specific scavenging of β 1-AABs by epitope-mimicking cyclic peptides in rats with autoimmune DCM [92,93]. It has been shown that

TABLE 2.4 The 2013 ESC Task Force Criteria of Clinically Suspected Myocarditis [33]**Clinical presentations [33] include ≥1 of the following:**

- *Acute coronary syndrome-like*, with or without normal global or regional left ventricular (LV) and/or right ventricular (RV) dysfunction on echocardiography or cardiovascular magnetic resonance (CMR), with or without increased troponin (Tn)I/TnI (that may have a time course similar to acute myocardial infarction or a prolonged and sustained release over several weeks or months).
- *New onset or worsening heart failure* in the absence of coronary artery disease (CAD) and known causes of heart failure.
- *Chronic heart failure*, with heart failure symptoms (with recurrent exacerbations) of **>3 months duration**, in the absence of CAD and known causes of heart failure).
- *Life-threatening condition* (including life-threatening arrhythmias and aborted sudden death, cardiogenic shock, severely impaired left ventricular function), in the absence of CAD and known causes of heart failure.

Diagnostic criteria [33] include ≥1 of the following features from categories I to IV:

1. *Electrocardiogram (ECG)/Holter/stress test features*
 - a. newly abnormal 12 lead ECG and/or Holter and/or stress testing, any of the following (see also Fig. 2.2): I To III degree atrioventricular block, or bundle branch block, ST/T wave change (ST elevation or non-ST elevation, T-wave inversion), sinus arrest, ventricular tachycardia or fibrillation and asystole, atrial fibrillation, reduced R-wave height, intraventricular conduction delay (widened QRS complex), abnormal Q waves, low voltage, frequent premature beats, supraventricular tachycardia
2. *Myocardiocytolysis markers (elevated cardiac troponins)*
3. *Functional and structural abnormalities on cardiac imaging (echo/angio/CMR)*
 - a. new, otherwise unexplained left ventricular (LV) and/or right ventricular (RV) structure and function abnormality (including incidental finding in apparently asymptomatic subjects): any of those in Fig. 2.2
4. *Tissue characterization by cardiovascular magnetic resonance (CMR)*
 - a. edema and/or late gadolinium enhancement (LGE) of classical myocarditic pattern (according to Lake-Louise criteria) [150].

AABs-induced endoplasmic reticulum stress induces cardiomyocyte apoptosis, which is potentiated by nor-epinephrine [95]. It is worth noting that anti-cardiac myosin AABs, induced by immunization of rats against cardiac myosin, cross-react with cardiac membrane β 1-adrenergic receptors and increase cAMP-dependent protein kinase A activity in myocytes [40]. Passive transfer of purified AABs from cardiac myosin-immunized rats leads to myocardial IgG deposits and increased myocyte apoptosis, leading to cardiomyopathy in recipients [40].

3. CLINICAL PRESENTATION AND DIAGNOSIS OF MYOCARDITIS

Clinical presentation in myocarditis is variable; cardiac signs and symptoms lack specificity, depending on the extent of myocardial inflammation

and ventricular dysfunction, and may be subtle [15,17,18,22,30–33,135–137].

Myocarditis is more common in the young with few coronary artery disease risk factors. These subjects, days or weeks after a presumed respiratory or gastrointestinal viral syndrome with or without increased systemic inflammatory markers and fever, may develop dyspnea or orthopnea, or palpitations, or effort intolerance/malaise, or heart failure, or chest pain (which may be pleuritic if concomitant pericarditis is present) with or without cardiac troponin I or T (cTNI or cTNT) release and unobstructed coronary arteries at coronary angiography [16,18,138,139]. Biopsy-proven myocarditis may also have an arrhythmia presentation (including any type of unexplained new-onset atrial or ventricular tachy- or bradiarrhythmias), with palpitation, syncope, or aborted sudden death [16]. Myocarditis may present with signs and symptoms of unexplained chronic heart failure, such as decreased exercise tolerance and dyspnea during exercise, with or without DCM [10,16,22,33,140,141], or of new-onset acute heart failure, such as dyspnea at rest and/or cardiogenic shock [16,61,140,141]. Myocarditis is also a differential diagnosis in patients with peripartum cardiomyopathy [33] or takotsubo cardiomyopathy [135]. Fulminant myocarditis has been described as having a distinct onset of unexplained heart failure, viral prodromes in the preceding 4 weeks, and a good prognosis, provided that some malignant causes, in particular giant cell myocarditis, are excluded [140]. Myocarditis can mimic many noninflammatory pathologies, thus any other cause (eg, valve heart disease, pericardial constriction, coronary artery disease) should be excluded; selective coronary angiography is recommended in the diagnostic work-up [33].

3.1 Electrocardiography (ECG) and Echocardiography

ECG findings are neither specific nor sensitive for myocarditis and include all types of “unexplained” atrial or ventricular tachy or bradyarrhythmias, P-Q segment depression, and/or repolarization changes [16,33,142,143]. ST-T segment elevation is more concave in myocarditis (convex in ischemia) and diffusely present over the precordial leads, without reciprocal changes. PR depression is common in pericarditis associated with myocarditis, but is rare in cardiac ischemia. Q waves are rare in myocarditis. It is worth noting that in myocarditis T-wave inversion generally occurs after complete ST-T normalization, but after myocardial infarction it usually takes place while the ST segment is still elevated. It has been described that in myocarditis QRS prolongation was an independent negative predictor [143].

Standard *trans*-thoracic echocardiography in myocarditis may show a normal examination, increased

wall thickening with normal cardiac dimensions, global systolic and/or diastolic dysfunction or segmental wall motion abnormalities, pericardial effusion, endocavitary thrombi, or DCM [144–146]. The left ventricle is generally nondilated, thickened, and hypocontractile in fulminant myocarditis [144]. Apical left ventricular aneurisms suggest Chagas's disease. Echocardiography may exclude some noninflammatory causes of cardiac signs and symptoms or associated conditions, eg, valve disease. It is also a useful noninvasive imaging tool; temporal changes in systolic or diastolic function, or chamber cavity dimensions and wall thickness, may occur very quickly during follow-up in myocarditis, requiring multiple examinations [33].

3.2 Nuclear Techniques and Cardiovascular Magnetic Resonance Imaging

Nuclear techniques in myocarditis are rarely used, because of limited availability, low diagnostic accuracy, radiation exposure, and delays in obtaining images. However, recent development of novel (molecular) nuclear tracers holds some promise, at least in animal models of myocarditis and/or acute myocardial infarction, for future noninvasive detection of inflammatory processes in the heart [146–148]. A relevant exception is Gallium-67 scintigraphy and positron emission tomography with fluorodeoxyglucose in the acute phase and in the follow-up of cardiac sarcoidosis [149].

Similar to echocardiography, cardiovascular magnetic resonance (CMR) provides noninvasive morphofunctional assessment of the heart, but in addition, specific tissue characterization sequences are available [17,139,146,150–154]. To get optimal information from CMR, the “International Consensus Group on CMR Diagnosis of Myocarditis” has suggested the combined use of three different CMR techniques, known as the Lake–Louise criteria; since correlation data with biopsy-proven myocarditis are still limited, diagnostic accuracy of CMR should be better defined in multicenter trials with standardized protocols comparing CMR to biopsy-proven criteria [150]. CMR features do not distinguish various causes of myocarditis, eg, infectious versus autoimmune or toxic forms, or the type of inflammatory infiltrate, eg, lymphocytic versus giant cell (Fig. 2.2). According to the 2013 European Society of Cardiology (ESC) Myocarditis Task Force EMB is the gold standard in the diagnosis of myocarditis [33]. CMR can refine the clinical suspicion of myocarditis and be used in the follow-up of infectious-negative forms, but a second EMB is required to check viral clearance from the myocardium; however, it should not delay EMB in life-threatening presentations [33].

3.3 Clinically Suspected Myocarditis: 2013 ESC Task Force Criteria

To aid the clinician in the identification of myocarditis, the ESC Myocarditis Task Force has introduced new strict criteria for clinically suspected myocarditis, using the combination of a plausible clinical presentation and of diagnostic criteria from different categories, as well as exclusion of known noninflammatory causes, eg, coronary artery disease that could explain the syndrome (Fig. 2.3, Tables 2.4 and 2.5) [33,155]. These criteria have been proposed to refine the clinical and noninvasive suspicion of myocarditis in centers that do not routinely perform EMB.

3.4 Definite (Biopsy-Proven) Myocarditis: 2013 ESC Task Force Criteria

In patients fulfilling the diagnostic criteria for clinically suspected myocarditis (Tables 2.4 and 2.5), the ESC Myocarditis Task Force recommends selective coronary angiography and EMB, including conventional histology, as well as immunohistochemistry and PCR detection of infectious agents (Fig. 2.3, Table 2.6) [33]. Based on standard histology (Dallas criteria), the first EMB may show active myocarditis, eg, inflammatory cell infiltrates associated with necrosis or degeneration of cardiomyocytes, borderline myocarditis in the presence of only inflammatory cells, or absence of myocarditis [9]. On the second or follow-up EMB, after comparison of the morphological findings with those observed in the preceding biopsy, persistent, resolving, or healed myocarditis may be present. On top of standard histology, immunohistochemistry is required using a panel of monoclonal and polyclonal antibodies, including anti-CD3, T lymphocytes; anti-CD68, macrophages; and anti HLA-DR, and a cut-off of <14 leukocytes/mm² with the presence of T lymphocytes <7 cells/mm² [11–13,33]. The detection of HLA-DR upregulation on EMB tissue sections is a marker of infectious-negative autoimmune myocarditis where immunosuppression may be considered [33,156]. Other immunofluorescence stains used to define humoral rejection in heart transplant EMB, such as C3d and C4d, may provide additional markers of immune activation in patients with inflammatory cardiomyopathy; a limitation of these stains is that they require frozen material. Immunohistochemical analysis together with molecular detection of viral genomic sequences increases the diagnostic accuracy of EMB [11,12,14,15] and provides diagnosis of infectious myocarditis, absence of infectious agents identifies immune-mediated myocarditis, either primary or post-infectious if an infectious agent had been identified on a previous EMB (Fig. 2.1). This recommendation also applies to patients with an acute

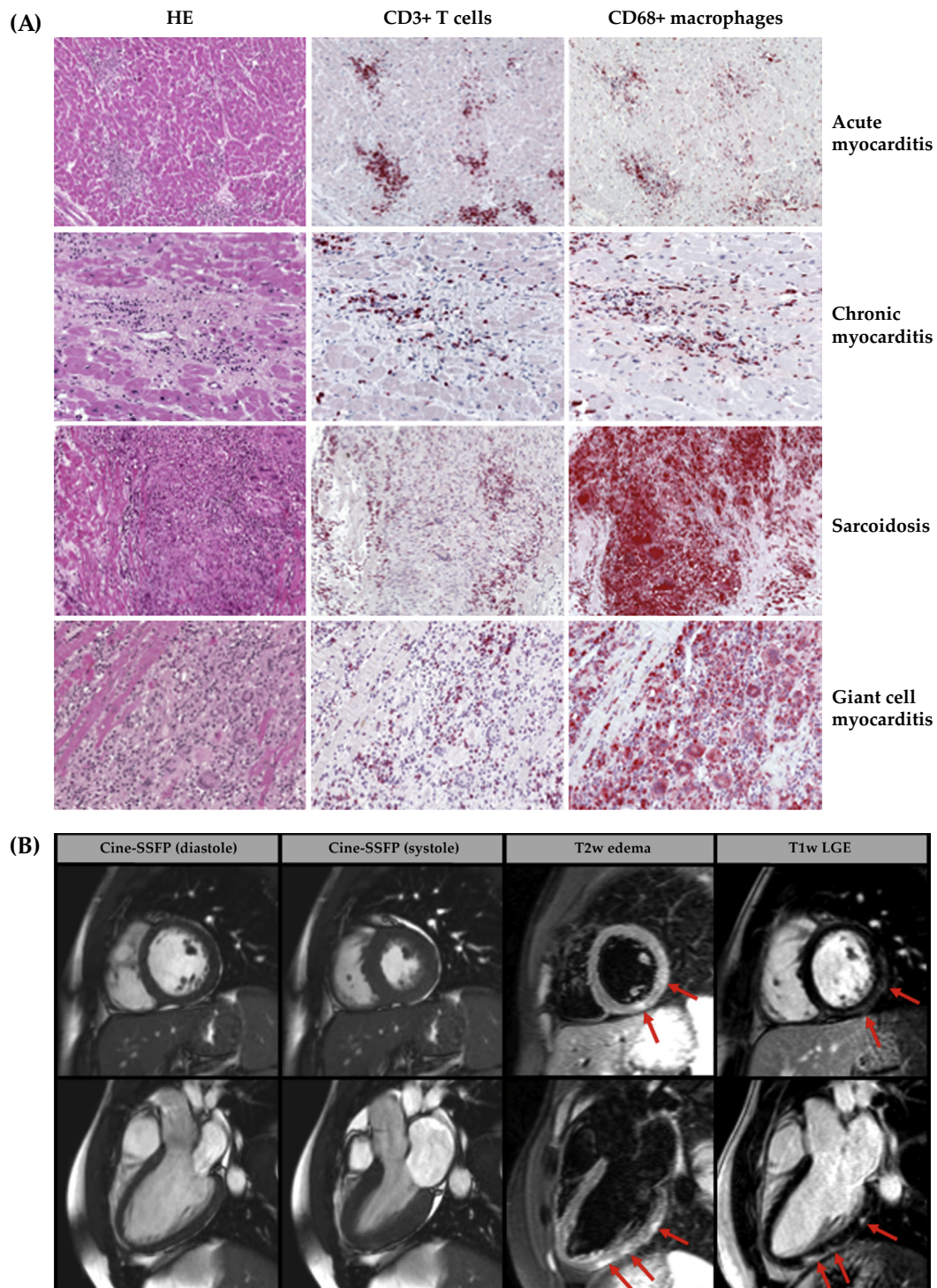


FIGURE 2.2 (A) Histopathology and immunopathology of acute lymphocytic myocarditis (first row, $\times 100$), chronic lymphocytic myocarditis (second row, $\times 200$), sarcoidosis (third row, $\times 100$) and giant cell myocarditis (fourth row, $\times 200$). Left column = hematoxylin-eosin (HE); middle column = staining with anti-CD3 antibody (pan T lymphocyte marker); right column = staining with anti-CD68 antibody (macrophage marker). (B) Short-axis (upper line) and long-axis (lower line) CMR images of a young patient with acute myocarditis. In the first two columns, cine-SSFP images are shown in diastole and systole and suggest absence of any wall motion abnormality. In the next column, T2-weighted edema images demonstrate presence of patchy focal edema in the subepicardium of the inferolateral wall (*red arrows*). In the last column, T1-weighted LGE images demonstrate presence of subepicardially distributed LGE (*red arrows*) which is typical for acute myocarditis. Adapted from Caforio et al. [33].

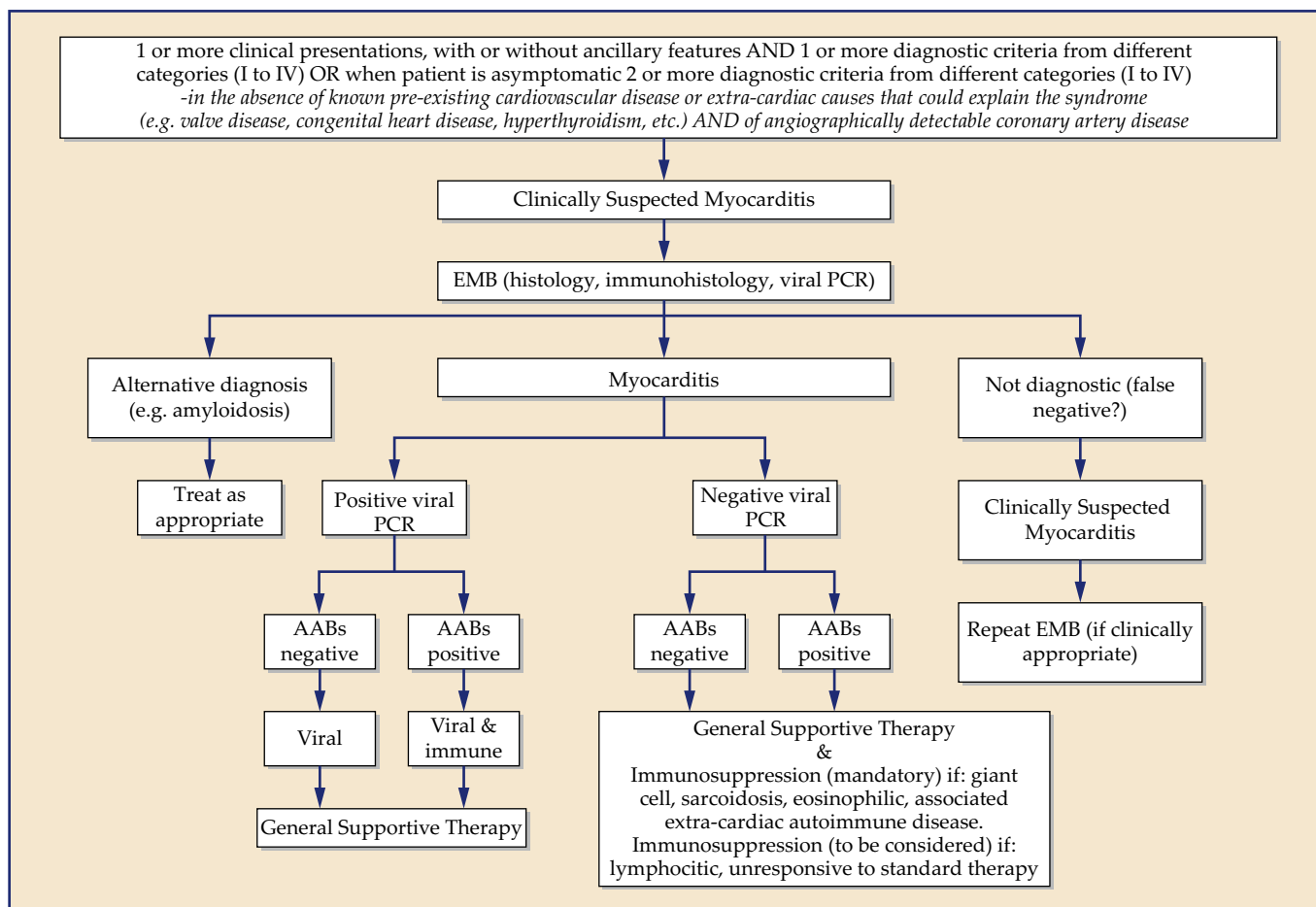


FIGURE 2.3 Diagnostic work-up and etiology-based management in myocarditis according to ESC 2013 Task Force criteria. **Myocarditis (Acute or chronic, lymphocytic, or other inflammatory infiltrate)**=Dallas criteria positive (active or borderline myocarditis) and/or immunohistology positive (see text), with positive or negative viral polymerase chain reaction (PCR) on endomyocardial biopsy (EMB), with or without a DCM clinical phenotype, with normal or depressed biventricular function. Specific myocarditis types would also be included in this definition according to standard histopathological diagnosis (eg, giant cell, eosinophilic, polymorphic, granulomatous myocarditis). **Not diagnostic**=Not diagnostic for myocarditis according to the Dallas histological criteria (or technically inadequate for histological diagnosis). A proportion of these cases may represent EMB false-negatives, thus clinical follow-up is recommended, and EMB may be repeated if clinically indicated. **No myocarditis (Alternative diagnosis)**=Histological diagnosis alternative to myocarditis or DCM, eg, cardiac amyloid, arrhythmogenic right ventricular cardiomyopathy, etc. This would reject the clinical suspicion of myocarditis and establish an alternative diagnosis. EMB, endomyocardial biopsy; PCR, polymerase chain reaction; AABs, cardiac autoantibodies. Adapted from Caforio et al. [155].

TABLE 2.5 Definition of Clinically Suspected Myocarditis by the 2013 ESC Task Force Criteria [33]

- **Clinically suspected myocarditis is defined by the presence of ≥ 1 clinical presentation (with or without ancillary findings) and ≥ 1 diagnostic criteria from different categories from Table 2.4, in the absence of:**
 - angiographically detectable coronary artery disease (coronary stenosis $\geq 50\%$).
 - known preexisting cardiovascular disease or extracardiac causes that could explain the syndrome (eg, valve disease, congenital heart disease, etc.). Suspicion is higher with higher number of fulfilled criteria.
 - If the patient is asymptomatic ≥ 2 diagnostic criteria should be met.
- **Medical history should focus on:**
 - family history of: DCM, other cardiomyopathy, sudden cardiac death, autoimmune disease
 - patient history: Recent (days to 2 weeks) upper respiratory or gastrointestinal suspected viral syndrome, allergy, other autoimmune diseases, previous clinically suspected or proven myocarditis, heavy alcohol intake, assumption of drugs and toxic substances (eg, cocaine), vaccines, travel to places where specific cardiotropic infection is possible or endemic (eg, Brazil, Argentina, and Chile for Chagas' disease), proximity with domestic animals, conventional coronary risk factors, etc. The aim is search as well as exclude possible treatable causes (eg, drug-related toxicity or hypersensitivity).

TABLE 2.6 Diagnosis of Definite (Biopsy-Proven) Myocarditis: Key Points From the 2013 ESC Task Force Consensus Document [33]

- EMB, including conventional histology (Dallas criteria), as well as immunohistochemistry and PCR detection of infectious agents is the gold standard for diagnosis of myocarditis
- Absence of infectious agents identifies immune-mediated myocarditis, either primary or post-infectious if an infectious agent had been identified on a previous EMB, and is the basis for safe (infection negative) immunosuppression
- EMB is essential to identify specific myocarditis types (eg, giant cell, eosinophilic, sarcoidosis), which imply different treatments and prognosis
- EMB provides differential diagnosis from diseases that may mimic myocarditis (arrhythmogenic right ventricular cardiomyopathy, Takotsubo cardiomyopathy, peripartum cardiomyopathy, infiltrative/storage disorders, cardiac masses)
- If EMB is performed in experienced centers, its complication rate is similar to that of standard coronary angiography
- EMB may be taken from the right or from the left ventricle according to center and operator preference and expertise. Antiplatelet or anticoagulation strategy for left ventricular EMB may be different among centers.

coronary syndrome-like presentation, not included in the American Heart Association/American College of Cardiology/Heart Failure Society of America (AHA/ACC/HFSA) scientific statement on EMB [157]. The Task Force gave this recommendation based on the following considerations (Table 2.6): (a) If EMB is performed by experienced teams, its complication rate is low and similar to that of standard coronary angiography (0–0.8) [11,13,16,158,159]; (b) EMB confirms the diagnosis of myocarditis and at present it is the only tool to identify the underlying etiology and the type of inflammation to determine treatment and prognosis [11–13,15–18,22,23,26–28,32,33]; and (c) EMB is the basis for safe (infection negative) immunosuppression [33,100].

3.5 Inflammatory Diagnostic Markers

Erythrocyte sedimentation rate and reactive C-protein levels are not raised in the majority of patients with biopsy-proven myocarditis [33], but are often increased in acute pericarditis [160].

3.6 Troponins and Natriuretic Peptides

Cardiac troponins do not differentiate ischemic from inflammatory myocyte injury, may be raised in several other conditions, and when normal do not exclude myocarditis [33]. The time course of cardiac troponin release in biopsy-proven myocarditis may be similar to that of an acute myocardial infarction or prolonged over weeks or months in the absence of angiographically detectable coronary artery disease. Cardiac hormones, such as brain natriuretic peptides, circulating cytokines, markers related to extracellular matrix degradation, and other new biomarkers, such as pentraxin 3, galectin 3, and growth differentiation factor 15 have not been shown to be specific for myocarditis [33].

3.7 Viral Serology

Positive viral serology is not direct proof of active myocardial infection according to Koch's postulates; the prevalence of circulatory IgG antibodies to cardiotropic viruses in the general population is high in the absence of viral heart disease and infection with noncardiotropic enteroviruses may cause an antibody response that is not different from the response to cardiotropic viruses [33]. Last but not least in a recent study there was no correlation between virus serology and EMB findings [161]. Serology for viruses and other infectious agents is not recommended by the ESC 2013 Task Force, except for hepatitis C, rickettsial phase 1 and phase 2, Lyme disease in endemic areas as well as human immunodeficiency

virus serologies in high-risk patients and in certain populations at high prevalence of infection [33].

3.8 Serum Cardiac Autoantibodies (AABs): Diagnostic and Clinical Relevance

In patients with myocarditis/DCM and of their symptom-free relatives serum heart-reactive AABs recognize multiple antigens (Table 2.7), some of which are only expressed in the myocardium (eg, organ-specific) and others in heart and skeletal muscle (eg, muscle-specific), as shown in Fig. 2.4. Each of these AABs has a different frequency in disease and normal control cohorts; the organ-specific and cross-reactive-1 types of anti-heart AABs shown in Fig. 2.4 are disease-specific for myocarditis/DCM (Table 2.7). AABs of the IgG class, if demonstrated to be cardiac and disease-specific for inflammatory heart muscle disease, can be used as autoimmune markers for identifying patients in whom immunosuppression and/or immunomodulation therapy may be beneficial and their relatives at risk [16,32,48–50,65,66]. Some AABs may have a functional role and/or, being associated with hot phases of the disease, represent negative prognostic markers [68,73,75,83,84,86–94,97,98,101,134,162]. The ESC Task Force recommended to assess sera with clinically suspected or definite myocarditis for cardiac AABs using one (or more) of the published tests (see the following), according to specific center expertise, preferably disease-specific AABs [33].

4. SPECIFIC AABs TESTS

4.1 Anti-heart AABs and Anti-intercalated Disk AABs by Standard Indirect Immunofluorescence (Fig. 2.4)

Using indirect standard indirect immunofluorescence (s-I IFL) on 4- μ m-thick unfixed fresh frozen cryostat sections of blood group O normal human heart and skeletal muscle, and absorption with human heart and skeletal muscle and rat liver, organ-specific IgG anti-heart AABs (AHA) giving a diffuse cytoplasmic staining pattern of myocytes, and a negative pattern on skeletal muscle (Fig. 2.4, panels A, B), were found in about 30–56% of myocarditis/DCM patients and their symptom-free family members, in 1–4% of patients with other cardiac disease, in 3% of normal subjects, and in 17% of patients without cardiac disease, but with autoimmune polyendocrinopathy (Table 2.7) [50,65,66,76]. AHA of the cross-reactive 1 type, partially cardiac-specific by absorption, gave a fine striational staining pattern on myocytes, but were negative or weakly stained skeletal muscle (Fig. 2.4, panels C, D), and were also more frequently detected in DCM/myocarditis than in controls. On the other hand,

TABLE 2.7 Circulating Cardiac Autoantibodies (AABs): Frequency in Myocarditis/DCM and in Control Groups [33]

Cardiac autoantibody (Ab)	% AABs positive		% Antibody positive		References
	Myoc	DCM	OCD	Normal	
Muscle-specific (AFA,IFA,AMLA)	28–59*	9–41*	NT	0–25	[105–108]
Cardiac-specific AHA	41–56*^	26–30*^	1–4	3	[16*^,65,66*^,49*^,50*^,99,66*^,137*^]
AIDA	17*^	16*^	2–4	0	
Anti-β1-AR	33	40–51^	13–55	0–13	[70,96,97,102,109–117,72,73,86,162]
	NT	35*^	16	7	
	73–96*^	29–95*^	8	0	
	NT	27–28	10	0	
Anti-β2-AR	NT	30–38^	33	15	[71,86,118^,119]
	NT	13–14			
	NT	30–75*^	37	18	
Antimuscarinic acetylcholine receptor-2	11	30–77§	23^–61	8–13	[90,102,112,113,115,117,120–124]
	NT	83§§			
Cardiodepressant (Fγ-γ-receptor 2a) Anti-Ky channel-interacting protein 2, KChIP2.6 – ELISA)	NT	64			[101,102,125,126,164,166]
	NT	14^	8	4	
Anti-α-MyHC (cardiac-specific) anti-β- MyHC (muscle-cross reactive)	17–37*^	20–46*^	4–16	0–2.5	[63,66–69*^,127*^,103]
Anti-MLC 1v	NT	17^–35	25	0–15	[67^,129]
Anti-tropomyosin	NT	55^	21	NT	[129]
Anti-non-myofibrillar	NT	46*^	17	0	[67]*^
Anti-MyHC	NT	67^	42	NT	[129]
Anti-actin	NT	71^	21	NT	[129]
Anti-troponin I,T	NT	1.7^–20^	0^–18	0–4	[102–104]
Anti-laminin	73	78	25–35	6	[130]
Anti-HSP60,70	NT	10–85^	1–42	3	[129,131]
Anti-s.Na/K-ATPase	26*		NT	2	[87]
Anti-ANT	91*^	57*^	0	0	[81,132,133*^]
Anti-M7	13*	31*	10	0	[88]
Anti-BCKD-E2	100	60	4	0	[89]

* $P < .05$ versus normal; ^ $P < .05$ versus OCD. *^ = cardiac and disease-specific for myocarditis/DCM; §77% (in Chagas-DCM); §§ (in selected ELISA-positive heart failure patients) ^^ (in atrial fibrillation patients) ‡ Increase L-type Ca^{2+} current; short term positive inotropic effects; Increase in cytoplasmic, cAMP and cAMP/FRET-activity. Abbreviations: AFA, antifibrillary Ab; AHA, Organ-specific and partially organ-specific anti-heart AABs; AIDA, Anti-intercalated disks-AABs; AMLA, anti-myolemmal AABs; ANT, adenine nucleotide translocator; AR, adrenergic receptor; ASA, anti-sarcolemmal AABs; BCKD, branched chain α -ketoacid dehydrogenase dihydrolipoyl transacylase; HSP, heat shock protein; IFA, anti-interfibrillary AABs; MLC1v, myosin light chain 1 ventricular; MyHC, Myosin heavy chain; Myoc, myocarditis; NT, not tested; OCD, other cardiac disease.

AHA of the cross-reactive 2 type, entirely skeletal muscle cross-reactive by absorption, gave a broad striational “myasthenic” pattern on heart and skeletal muscle (Fig. 2.4, panels E, F), and were found in similar proportions among groups [50,65,66,76]. Anti-intercalated disk AABs (AIDA), giving a linear staining on cardiomyocytes (Fig. 2.4, panel A), are associated with myocarditis/DCM and with idiopathic recurrent acute pericarditis [137].

4.1.1 Clinical and Prognostic Significance of AHA in Symptom-Free Subjects

In autoimmune disorders, circulating AABs identify symptom-free subjects at risk years before clinical presentation. So far, the clinical and prognostic significance of cardiac AABs has been prospectively assessed only for serum AHA in symptom-free relatives of DCM index patients, not for other published AABs.

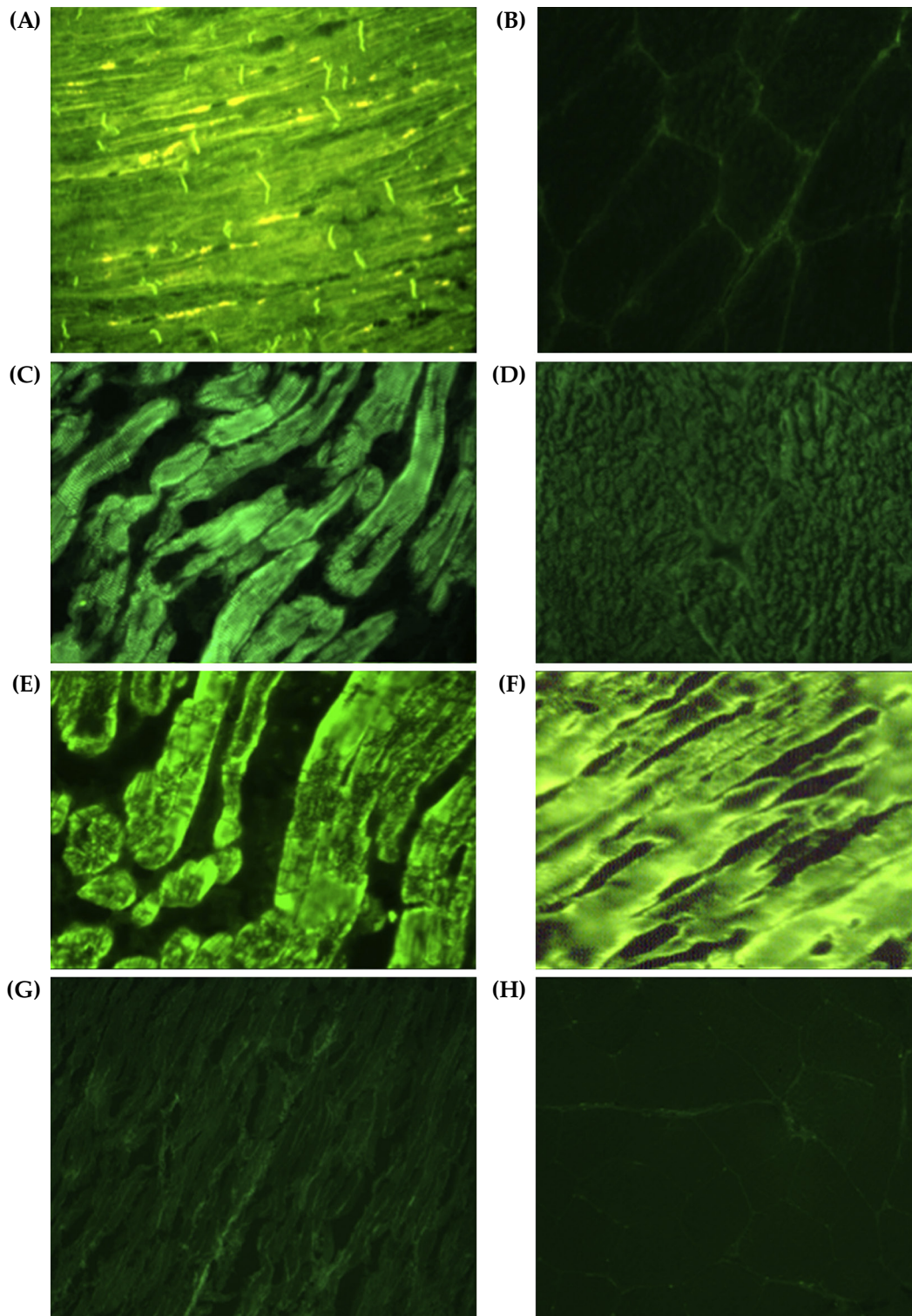


FIGURE 2.4 Anti-heart AABs (AHA) patterns by indirect immunofluorescence test. *Organ-specific AHA and AIDA pattern:* panel A on human heart tissue: cytoplasmic diffuse staining of cardiac myocytes (organ-specific AHA pattern) and linear staining of the intercalated disks (AIDA pattern) ($\times 400$); panel B ($\times 400$) on human skeletal muscle tissue: negative. *Partially organ-specific (or cross-reactive 1) AHA pattern:* panel C on human heart tissue: strongly positive fine striational pattern ($\times 400$), and panel D on human skeletal muscle: weak positive fine striational pattern ($\times 400$). *Entirely cross-reactive (or cross-reactive 2) AHA pattern:* panel E on human heart tissue: strong positive broad striational (myasthenic) pattern ($\times 400$), and panel F on human skeletal muscle: broad striational (myasthenic) pattern ($\times 400$). *Negative AHA control serum pattern:* panel G on human heart tissue: negative ($\times 400$), and panel H on human skeletal muscle: negative ($\times 400$). Adapted from Caforio et al. [48].

Healthy relatives of patients with DCM who have echocardiographic changes, including left ventricular enlargement (LVE) or depressed fractional shortening (dFS) at baseline, have increased medium-term risk for DCM development. Approximately one-third of relatives from both familial and nonfamilial pedigrees have serum AHA at baseline [49]. Prospective family studies have shown that AHA are independent predictors of DCM development in symptom-free relatives at 5-year follow-up [50]. In these studies, baseline evaluation, including electrocardiography, echocardiography, and AHA, was performed in 592 asymptomatic relatives of 169 consecutive DCM patients (291 males; mean age 36 ± 16 years). Relatives were classified in accordance with published echocardiographic criteria; those who did not have DCM were followed up on (median of 58 months). DCM among relatives was diagnosed by echocardiography at follow-up. Of the 592 individuals evaluated, 77% were assessed as normal, 4.4% as having DCM, and 19% as possibly affected on the basis of dFS without ventricular dilatation in 17 and LVE without systolic dysfunction in 94. Five-year follow-up of 311 relatives revealed that 26 had progressed (13 to DCM, 11 to LVE, and 2 to dFS). Relatives who developed DCM were more frequently AHA-positive than those who did not (69% vs 37%, $P = .02$). Five-year probability of progression to DCM, among normal or possibly affected relatives, was higher in AHA-positive cases ($P = .03$). By Cox regression, positive AHA at baseline was an independent predictor of progression (RR 2.26, CI 1 to 5.1, $P = .03$). Thus in this study it was suggested that LVE and dFS, represent early, preclinical DCM or asymptomatic left ventricular dysfunction in symptom-free relatives, similar to the first-phase insulin response (FPIR) to intravenous glucose in prediabetes [50]. Furthermore, AHA, similar to the multiple antibody markers in preclinical diabetes, preceded other diagnostic abnormalities of heart dysfunction. In keeping with this, positive AHA status alone had higher sensitivity (61%) than its combination with abnormal echocardiogram (sensitivity 27%) as a predictor of progression to DCM, LVE, or dFS. In other words, positive AHAs with normal echocardiogram identified a proportion of relatives at risk of progression to DCM, or of progression from normal to preclinical DCM (eg, LVE or dFS), which would not have been identified by echocardiography alone. In addition, the authors suggested that both techniques are necessary in DCM family screening and counseling. In fact, the positive predictive value (PPV) for progression to DCM was higher (18%) for both abnormal echocardiogram and positive AHA than for AHA alone (7%) or echocardiogram alone (10%). Similarly, the PPV for any progression (eg, DCM, LVE, or dFS) was higher (18%) or

both an abnormal echocardiogram and positive AHA than for AHA alone (13%) or echocardiogram alone (10%). The combination of abnormal echocardiography and positive AHA seemed to identify relatives at a more advanced stage of preclinical DCM, who need closer follow-up and could potentially benefit from therapeutic intervention to attenuate or prevent disease development. Finally, negative AHAs alone or in combination with a normal echocardiogram had a good negative predictive value (98%) and allowed the identification of the majority of subjects at low risk of progression at least up to 5 years [50]. An important issue that needs further study is the long-term outcome of these AHA-negative relatives with normal echocardiograms. In type 1 diabetes mellitus, a staging of preclinical disease has been proposed for siblings of affected children based on a combination of the initial number of antibodies and FPIR to intravenous glucose, by analogy, the authors proposed that, if the same applies to DCM, the staging could be as follows: no pre-DCM (negative AHAs, normal echocardiogram), early (positive AHAs, normal echocardiogram), advanced (AHA-positive and positivity for one or more of the other antibodies described in DCM), and late pre-DCM (at least one antibody marker and LVE or dFS). Although 98% of relatives with negative AHAs and normal echocardiograms did not progress up to 5 years, a long latency period and slow progression are features of organ-specific autoimmune disease, and therefore a proportion of them may develop AHA in the future, thus becoming at risk. Therefore the authors suggested that longer follow-up is needed to completely reassure these subjects, and it may be appropriate to provide echocardiographic and immunologic testing, although less frequently than for those with AHAs and/or abnormal echocardiography [50]. These recommendations have been incorporated in a recent expert consensus paper on DCM of the ESC working group on myocardial and pericardial diseases (Fig. 2.5) [163].

4.2 AABs to Myosin Heavy Chain

AABs to α and β myosin heavy chain (MyHC) have been detected by several groups and various techniques (Table 2.7), including enzyme-linked immunosorbent assay (ELISA) and immunoblotting. It has also been shown that α and β MyHC isoforms are two of the autoantigens recognized by the AHA detected by s-I IFL in DCM and in myocarditis [63,66–69,127]. The α isoform is exclusively expressed within the atrial myocardium, thus AABs to this molecule are organ-specific. In some studies the anti-myosin AABs were associated with deterioration of cardiac function [69] or with negative inotropic effect in vitro [63]. Myosin is an intracellular protein. The

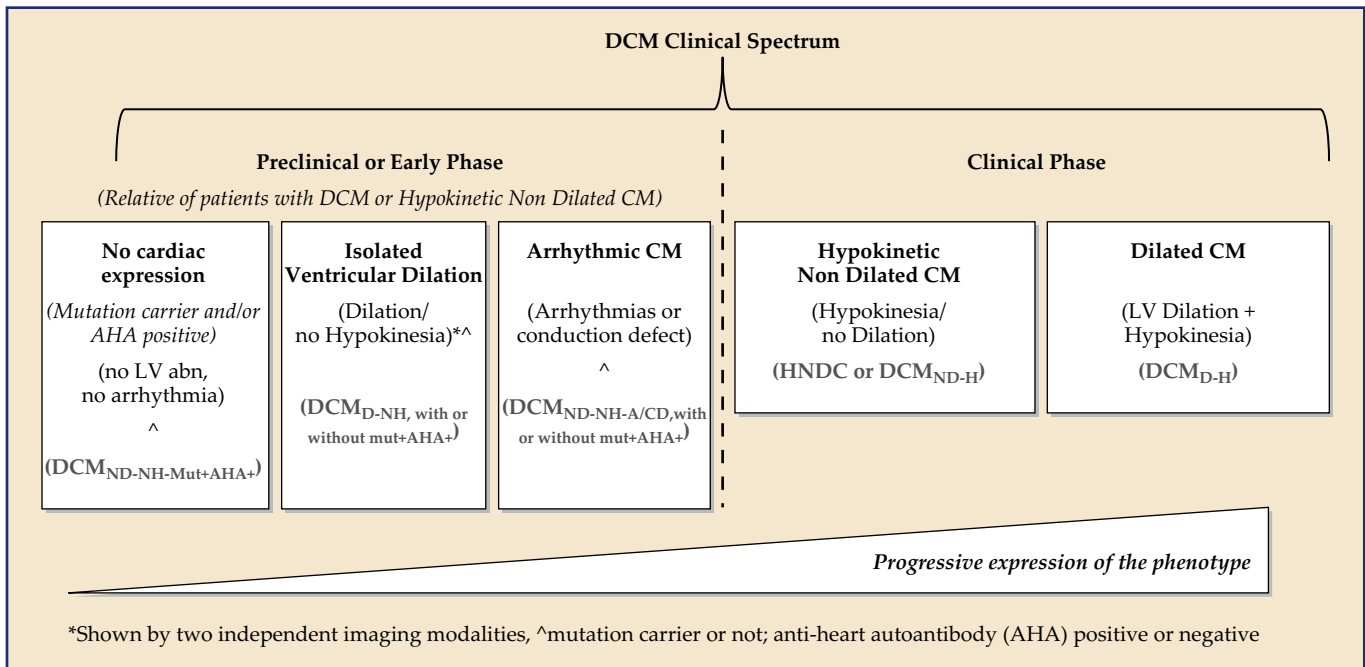


FIGURE 2.5 Description of the clinical spectrum of DCM. LV abn: left ventricle abnormality. DCM can be further classified as ND or D (non dilation/dilation) or NH or H (non hypokinetic/hypokinetic) or mut+ (mutation carrier) or AHA+ (anti-heart autoantibody positive) or A/CD (arrhythmia/conduction defect). Adapted from Pinto et al. [163].

major hypotheses to explain interruption of tolerance to myosin include: molecular mimicry, since cross-reactive epitopes between cardiac myosin and infectious agents have been found; myocyte necrosis due to viral infection or other noxae [2]; and cross-reactive mimicry between cardiac myosin and the β_1 -adrenergic receptor, resulting in apoptosis of cardiac myocytes [40]. In some murine strains, eg, Balb/c mice, Coxackie B3 virus-induced, or myosin-induced myocarditis is T-cell mediated [39], whereas in other strains, eg, DBA/2 mice, it is an antibody-mediated [94]. These data have led to the hypothesis that the anti-myosin AABs may be directly pathogenic in some, but not all patients with myocarditis/DCM according to different immunogenetic backgrounds, isotype [94], and/or subclass specificity of these AABs [69].

4.3 AABs to β -adrenergic and M2—Muscarinic Receptors

Fu et al. showed anti-M2 AABs in 39% of DCM sera and 7% of normal subjects by ELISA, using as antigen a synthetic peptide analogous to the 169–193 sequence of the second extracellular loop of human M2 muscarinic receptors [90].

A significant inhibitory activity, attributed to anti- β_1 -adrenoceptor IgG antibodies, was reported in 30–75% of DCM sera, 37% of disease controls, and 18% of sera from normal subjects by other groups, using a binding inhibition assay on rat cardiac membranes [70,71]. Magnusson

et al., using as antigens synthetic peptides analogous to the sequences of the second extracellular loop of β_1 - and β_2 adrenergic receptors by ELISA, found AABs in 31% of DCM patients, 12% of normal subjects, and in none of the disease controls [72].

Antibody-positive DCM sera [70] or the affinity-purified β_1 -receptor AABs [72] increased the beating frequency of isolated neonatal rat myocytes in vitro. β_1 -blocking drugs inhibited the effect of the AABs. Stimulating anti- β_1 -receptor AABs were described in 96% of myocarditis, 26–95% of DCM sera, 8–10% of controls with ischemic heart disease, and 0–19% of normal individuals (Table 2.3). Functional fluorescence resonance energy transfer (FRET) assay using novel cAMP-sensors, a recent, sensitive screening technique for the detection of functional β_1 -adrenoceptor AABs, is employed in the ongoing prospective Etiology, Titre-Course, and Survival (ETiCS) Study in patients with biopsy-proven myocarditis [96,98,164,165].

4.4 Cardiodepressant AABs

Functional cardiodepressant AABs have been described in DCM sera using an in vitro bioassay system and isolated rat cardiomyocytes as antibody targets [75,101]. The negative inotropic effect of these AABs, which may predict hemodynamic benefits from IA therapy in DCM [164], could be mediated by binding of their FC fragments to cardiac FC γ IIa receptors [166].

4.5 AABs to Other Sarcolemmal Autoantigens and Heat Shock Proteins

AABs to heat shock proteins (HSP)-60 and HSP-70 [129,131] and to troponin I and T have also been detected in DCM (Table 2.7) [102–104]. Cardiac troponin would be an organ-specific cardiac autoantigen, but the disease-specificity for myocarditis/DCM as compared to ischemic heart disease is not entirely clear [167]. Anti-Na-K-ATPase AABs were found by ELISA in 26% of DCM and in 2% of normal subjects, using porcine cerebral cortex sarcolemmal Na-K-ATPase as antigen, and were independently associated with cardiac sudden death [87]. On the basis of this association the authors hypothesized that the AABs may lead to electrical instability, because of abnormal Ca^{2+} handling by reduced Na-K-ATPase activity. A potential limitation of this work is that sarcolemmal Na-K-ATPase is not an organ-specific cardiac autoantigen [37].

4.6 AABs to Mitochondrial Antigens

AABs to several mitochondrial antigens were described, including the M7 [88], the adenine nucleotide translocator (ANT) [81,132,133], and the branched-chain α -ketoacid dehydrogenase dihydrolipoyl transacylase (BCKD-E2) [89]. The M7 antibodies of IgG class, assessed by ELISA using beef heart mitochondria as antigenic substrate, were found in 31% of DCM patients, 13% of those with myocarditis, 33% of controls with hypertrophic cardiomyopathy, and were absent in controls with other cardiac disease, immune-mediated disorders, or in normal subjects [88]. ANT, a protein of the internal mitochondrial membrane, was purified from beef heart, liver, and kidney and used as antigen in an indirect micro solid-phase radioimmunoassay (SPRIA); anti-ANT antibodies were found in 57–91% of myocarditis/DCM sera; and in no controls with ischemic heart disease, or in normal subjects [81,132,133]. Although mitochondrial antigens have generally been classified as nonorgan-specific, the heart specificity of the M7 AABs was shown by absorption studies. Experimentally induced affinity-purified anti-ANT antibodies cross-reacted with calcium channel complex proteins of rat cardiac myocytes, induced enhancement of transmembrane calcium current, and produced calcium-dependent cell lysis in the absence of complement [133]. Antibody-dependent cell lysis has not been reported using the AABs from patients' sera.

5. NATURAL HISTORY AND PROGNOSTIC FACTORS OF BIOPSY-PROVEN MYOCARDITIS

The evolution of biopsy-proven acute myocarditis ranges from spontaneous healing in about 50% of cases, to incomplete recovery in 25%, and death or

progression to end-stage DCM in the remainder [7,8,10,22,30,33,140,141]. Myocarditis may also relapse [16]. Prognosis is related to etiology, clinical and diagnostic features at presentation, and disease stage [16,33], eg, giantcell myocarditis [10,16,22,30,33,64,136], biventricular dysfunction at presentation [7,8,10,22,30,140,141], and immunohistological inflammation [61] were associated with poor prognosis [10,16,22,30,33,64,136]. Fulminant myocarditis was reported as having a favorable outcome in adults, but the cohort was relatively small and rare causes with a dismal prognosis, such as giant cell myocarditis, were excluded from the analysis, which may introduce some bias in the data [140]. The prognostic role of viral genome on EMB is controversial [33].

6. SPECIFIC FORMS OF IMMUNE-MEDIATED MYOCARDITIS

6.1 Chagas Myocarditis

Trypanozoma cruzi (Chagas' disease) is a common cause of myocarditis/DCM in South America [168]. Following an acute mild febrile disease, there is a prolonged (up to 30 years) symptom-free latent phase. A high proportion of chronically affected patients have been found to be affected by systolic and diastolic heart failure, ventricular aneurisms, arrhythmias, and cardiac autonomic dysfunction. AABs have been detected in chronic Chagas disease, in keeping with an autoimmune component, possibly triggered by molecular mimicry between parasitic and cardiac autoantigens, eg, MyHC, the M2 cholinergic, and the β -1 adrenergic receptors, as well as extracardiac autoantigens, eg, small nuclear ribonucleoproteins, nervous and skeletal muscle tissue [169]. Therefore the myocarditis is not exclusively organ-specific in Chagas disease [169]. *T. cruzi* DNA has been found in cardiac tissue from patients both in the indeterminate and the overt cardiomyopathy stages. Thus the pathogenetic roles of chronic parasite presence and autoimmunity in the development of cardiac pathology are still unclear.

6.2 Giant Cell Myocarditis, Sarcoidosis, and Myocarditis in Extracardiac Autoimmune Disease

Giant cell myocarditis, the prototype of autoimmune myocarditis, is a rare but severe disease. Ventricular tachycardia, heart block, and/or heart failure are possible presentations for other forms of myocarditis, but typically giant cell myocarditis has a bad, refractory downhill clinical course regardless of the institution of optimal medical care [10,22,30,64,136]. However, if the diagnosis is reached early and cardiac damage is not massive, since the disease is immune-mediated, giant cell myocarditis can be stabilized on immunosuppression.

Giant cell myocarditis may be associated with a variety of extracardiac autoimmune disorders and with up to a 25% rate of recurrence in the native heart following recovery and in the donor heart following transplantation; relapse in both conditions usually responds to intensified immunosuppression [41,42,64,136,170]. EMB features of giant cell myocarditis include a diffuse or multifocal infiltrate of lymphocytes, eosinophils, and multinucleated giant cells, associated with cardiomyocyte damage and fibrosis, in the absence of well-formed granulomas or specific etiology (Fig. 2.2) [156].

6.3 Cardiac Sarcoidosis and Other Systemic Immune-Mediated Diseases

Sarcoidosis is a systemic granulomatous disease of suspected immune-mediated pathogenesis. The clinical suspicion is higher if a patient with known extracardiac sarcoidosis develops cardiac signs and symptoms that do not differ from those developing in other forms of myocarditis. However, cardiac involvement may come first, or predominate in the clinical picture; the disease may be found at autopsy of sudden cardiac death victims or in cardiac arrest survivors. Cardiac sarcoidosis is a highly arrhythmogenic condition, in particular if granulomas are located in the ventricular septum and/or in conduction system involvement, mimicking arrhythmogenic cardiomyopathy; thus prophylactic use of an implantable cardioverter defibrillator is often indicated [149,156]. The histological hallmarks are nonnecrotizing granulomas, fibrosis, and few eosinophils, with no evidence of infection or other specific causes [156].

Myocarditis may occur in connective tissue diseases, as well as in other immune-mediated systemic diseases (Table 2.3), is thought to be more common in SLE and in dermatomyositis, and should be suspected using the ESC Task Force clinical and noninvasive diagnostic criteria [33,171–174]. However, there is a lack of controlled prospective studies including EMB in connective tissue diseases, as well as in other immune-mediated systemic diseases, thus the true frequency of biopsy-proven myocarditis is unclear. The EMB is essential to distinguish between SLE-related myocarditis and cardiotoxic effects of chloroquin [156], as well as to discriminate infectious from noninfectious forms and the need for reduced versus increased immunosuppression to control cardiac symptoms and the extent of pump dysfunction, arrhythmia, and troponin release [33]. In addition, coronary angiography should be considered to rule out coronary artery disease [33].

6.4 Hypersensitivity Myocarditis

Hypersensitivity myocarditis, the most common form of drug-induced cardiac toxicity, is not directly related to drug dosage and is therefore unpredictable. Nonspecific

skin rash, malaise, fever, and blood eosinophilia are its hallmarks, but may often be absent [156,175,176]. EMB features include a polymorphic inflammatory infiltrate, including variable numbers of eosinophils, lymphocytes, macrophages, giant cells, and neutrophils. Conversely, *direct cardiac toxicity*, which needs to be differentiated from hypersensitivity, is dose-dependent, with a cumulative effect. It may be reversible and is often potentiated by other antineoplastic treatments, such as chemotherapy and radiotherapy. The histopathology of cardiac toxicity is variable [156].

6.5 Myocarditis in Rheumatic Heart Disease

6.5.1 Clinical Diagnosis

Rheumatic fever (RF) is an inflammatory multisystem disease, occurring a few weeks to 6 months following group A streptococcal (GAS) infection of the tonsillopharynx. Its diagnosis dates back to the 1992 revised Jones' criteria [177]. Major criteria include:

- Carditis
- Polyarthritits
- Chorea
- Erythema marginatum
- Subcutaneous nodules

Minor criteria include: (1) fever; (2) arthralgia; (3) elevated erythrocyte sedimentation rate or C-reactive protein; and (4) prolonged P-R interval on standard 12-lead ECG. If supported by evidence of GAS, eg, positive throat culture or rapid streptococcal antigen test, high or rising streptococcal antibody titer, the presence of two major criteria (or one major and two minor) indicates high probability of acute RF.

The diagnosis of cardiac involvement during acute RF is based on unequivocal fulfillment of any of the following features: (1) new onset of nonfunctional cardiac murmurs; (2) cardiac enlargement; (3) sign and symptoms of heart failure; and (4) pericardial friction rubs or accumulation of pericardial fluid.

Although RF remains a leading world cause of acquired cardiac disease, well-conducted recent EMB-based studies are lacking since RF is rare in Europe and North America; most of the literature on this subject date back to the 1950s. It is unclear whether or not myocarditis in RF can be defined as infectious or post-infectious autoimmune according to the 2013 Task Force criteria [156,33]. The histological finding of Aschoff bodies is the hallmark of rheumatic inflammation in the heart [156,33]. Rheumatic myocarditis is thought to be a component of acute rheumatic carditis, which also includes pericarditis and valvulitis. However, in acute rheumatic myocarditis, histopathology reveals no necrosis and for this reason there are authors who have questioned whether rheumatic myocarditis exists [156,33]. Because acute rheumatic myocarditis is frequently asymptomatic, its

diagnosis is difficult and requires a high-suspicion rate [156,33,178]. Rheumatic patients with acute myocarditis often present mild symptoms such as tachycardia or mild worsening of heart failure, frequently attributed to worsening of the valvular heart disease or another cause of decompensation, such as volume or salt overload. The diagnosis of acute rheumatic myocarditis requires the use of multiple imaging techniques. An echocardiography can reveal mild-to-moderate pericardial effusion (rarely pericardial effusion or even pericardial tamponade). Transesophageal echocardiography can sometimes show small multiple vegetations on the edge of native valves, representing the rheumatic *verrucae* that characterize the acute phase of the disease. The 12-lead ECG may reveal a first-degree atrioventricular heart block that may reflect myocarditis, but this is not very sensitive. Imaging techniques that highlight inflammation in the heart may be useful, eg, Gallium-67 myocardium scintigraphy [156,33,178]. A good correlation has been shown between a positive scintigraphy and EMB for the diagnosis of active rheumatic myocarditis. Positron-emission scintigraphy associated with tomography (PET-CT) is being evaluated and appears to have better sensitivity than the Gallium scan [156,33,178]. In conclusion, acute rheumatic myocarditis is a difficult diagnosis that should be considered in any patient, including patients who have just undergone valve surgery, with rheumatic valvular heart disease who present with a sudden worsening of heart failure symptoms or rapid-onset ventricular dysfunction, particularly if the patient is not on secondary prophylaxis for RF.

6.5.2 Immune Pathogenesis of Rheumatic Carditis

Rheumatic carditis may represent an autoimmune disorder triggered by streptococcal infection via molecular mimicry. In acute RHD, histological analyses have shown the presence of dense valvular, mainly CD4⁺ inflammatory cell infiltrates and Aschoff nodules in the myocardium [156,33]. In addition, CD4⁺ and CD8⁺ infiltrating T-cell clones recognized streptococcal M peptides and cardiac tissue proteins, suggesting that molecular mimicry may be the mechanism responsible for post-infectious autoimmunity to heart structures [156,33]. Indeed, streptococci and heart tissue have several antigenic components for which molecular mimicry has been documented [179]. During RF, patients produce both anti-streptococcal antibodies and AHA, since rheumatogenic streptococci and heart tissue have shared antigens.

6.5.3 Anti-streptococcus Antibodies

The external parietal layer of group A streptococci contains distinct M protein types in different strains. More than 80 M protein types are known. M proteins of rheumatogenic streptococci share a long terminal

antigen domain and contain epitopes that are shared with human heart tissue, particularly sarcolemmal membrane proteins, cardiac myosin, and tropomyosin, as well as with skeletal muscle and smooth muscle, renal glomerular basement membrane [179–181]. In addition, shared epitopes have been reported between *N*-acetylglucosamine of the midparietal *streptococcus* cell layer and glycoproteins of mammalian cardiac valve tissue and another cross-reaction has been found between the streptococcal hyaluronate and protein polysaccharide of mammalian cartilage [179]. In addition, anti-streptococcal antibodies, cross-reactive with heart antigens and with thalamus and subthalamus components, have been found in some patients with carditis and chorea [182,183]. The heart cross-reactive anti-streptococcal antibodies, identified by a combination of immunofluorescent and precipitin-absorption techniques, were present in 55% and 58% of patients with active and inactive rheumatic heart disease, respectively, or with acute glomerulonephritis in 24% of patients with recent streptococcal infection and only rarely (2%) in disease controls without rheumatic heart disease or previous streptococcal infection, but the pathogenetic significance of these antibodies remains undefined [182,183].

6.5.4 Anti-heart AABs (AHA)

Several methods were used, in the earliest studies, for detection of circulating AHA in RF, including agglutination tests with collodion particles coated with saline extracts of the heart and other organs, complement fixation test (CFT) with saline extracts of the heart, liver, and spleen, tanned red cell hemagglutination test, and antiglobulin consumption with heart homogenate [182]. Subsequently, s-I IFL on cryostat-cut sections became the method of choice; however, tissue substrates varied, including human or rat myocardium, normal human myocardium obtained at autopsy, or at surgery in subjects with congenital heart defects, normal human myocardium of O blood group obtained at surgery in subjects with congenital heart defects [184,185]. Cryostat-cut sections were fixed in acetone or unfixed and then incubated with serum diluted at 1:5 or undiluted; sections were washed in PBS and then the autoantibody binding was revealed with fluoresceinated serum antihuman IgG [184]. In a more recent study, patient serum, at 1:10 dilution, was incubated on unfixed cryostat-cut sections and, after washing, the section was stained with fluoresceinated serum antihuman IgG, IgA, or IgM [185]. Clearly, the lack of an s-I IFL protocol makes it difficult to compare results from different studies, particularly in terms of AHA frequency in rheumatic heart disease.

Taking into account such limitations [184], circulating AHA have been found, s-I IFL, in sera in 25–87% of patients with active RF, 12–21% of those with inactive rheumatic disease, in 0–4% of normal subjects [182,184],

in 81% of patients with streptococcal infection, 80% of those with post-streptococcal nephritis, 87% with acute RF, 47% with rheumatic carditis, 100% with post-cardiotomy syndrome, and in none of the control subjects [186]. The AHA patterns observed in RF were similar to those described in Dressler's and in post-pericardiotomy syndromes, but the *sarcolemmal-sarcoplasmic* or *peripheral* pattern was found more frequently than the *diffuse* stain [184,187]. In rheumatic carditis anti-conductive tissue antibodies as detected by IFL on ox heart false tendon were also reported [184,188]. The AHA from rheumatic sera could be absorbed out by streptococcal membranes as well as myocardial extracts; conversely, those found in post-pericardiotomy sera reacted exclusively with myocardial extracts, suggesting that the antigenic determinants were different in the two cardiac conditions [186]. In terms of relations of AHA to clinical activity of rheumatic disease, overall the frequency and/or titer of AHA was higher in clinically active than in clinically inactive disease, in patients with more severe presentation, in those with than without carditis, and in those with a higher number of previous attacks of RF [182,184].

Regarding the time course of AHA detection, using IFL or antiglobulin consumption tests, it was shown that AHA was detected in some patients before and, in other patients, after the onset of clinical symptoms of RF [184,189]. In the majority of cases antibody was first detected within the first week of symptom onset, then it rose to maximal titer. Subsequently, antibody titer declined, in some cases rapidly, possibly in relation to steroid treatment; in other cases a more variable or slower pattern of decay was documented with persistence of low titer antibody up to 2–3 years after an attack of RF [182,184]. On the other hand, autoantibodies not detected using s-I IFL or antiglobulin consumption, and directed against aqueous and alcoholic heart tissue extracts, seemed unrelated to clinical findings, suggesting that some but not all antibody specificities produced in rheumatic heart disease may be pathogenetic markers and/or directly pathogenic [182,183]. As an indirect proof of potential pathogenetic relevance, bound immunoglobulin G and complement were also identified within the myocardial, as well as pericardial and valvular tissues in post-mortem rheumatic hearts and in surgical specimens of auricular appendages from patients with rheumatic heart disease [190].

7. MANAGEMENT

7.1 Symptomatic Treatment and Heart Transplantation

Symptomatic treatment of heart failure and of arrhythmia should be conducted according to guidelines,

although controlled trials in biopsy-proven myocarditis and its distinct etiopathogenetic forms are unavailable [33]. Thus patients with hemodynamically stable heart failure should be treated with diuretics, angiotensin-converting enzyme inhibitor, or angiotensin receptor blockade and β -adrenergic blockade. If heart failure persists despite optimal management, additional treatment with aldosterone antagonists should be considered. The timing of heart failure therapy discontinuation following recovery of ventricular function is undefined. Nonsteroidal anti-inflammatory drugs (NSAIDs), eg, acetylsalicylic acid and colchicine, are indicated for acute pericarditis, but there are no controlled clinical data on myocarditis, and NSAIDs have been associated with higher mortality in experimental animals [22,141,191]. In cardiogenic shock and severe ventricular dysfunction unresponsive to intravenous inotropes, ventricular assist devices or extracorporeal membrane oxygenation (ECMO) may be needed to provide a bridge to transplant or to recovery [192–194]. The 2013 ESC Task Force recommends that cardiac transplantation should be deferred in the acute phase but may be considered for hemodynamically unstable myocarditis patients if maximal intravenous pharmacologic support and mechanical assistance fail [33].

7.2 Physical Activity and Follow-Up

Intense physical activity should be avoided during myocarditis until resolution. Exercise ECG testing is contraindicated, because it may trigger life-threatening arrhythmia. Athletes should be temporarily excluded from sport activity. After clinical resolution (at least 6 months after the onset of the disease), reassessment is indicated before the athlete resumes sport activity [195,196]. In keeping with the 2013 ESC Task Force recommendations the same applies to all patients with myocarditis [33]. In addition myocarditis patients should undergo prospective clinical and noninvasive cardiac assessment, more frequently in the first year, then as indicated by symptoms [33]. Since natural history of the disease is ill-defined and risk stratification is poor, long-term follow-up is advisable [33].

7.3 Role of High-Dose Intravenous Immunoglobulin

High-dose intravenous immunoglobulin (IVIG) is used in several systemic AABs-mediated autoimmune diseases [197]. IVIG was associated with improved left ventricular ejection fraction in heart failure of various etiologies [198,199]. Conversely, the Intervention in Myocarditis and Acute Cardiomyopathy (IMAC) trial in recent onset DCM of whom only 15% of patients had biopsy-confirmed myocarditis of unknown etiology did

not demonstrate benefit [199]. The 2013 ESC Task Force experts concluded that, on the basis of current data, IVIG is not recommended, but since it has no major side effects, may be used in selected myocarditis patients, viral (eg, associated with parvovirus B19 infection) [13], or proven AABs-mediated autoimmune [33]. However, further study of IVIG with well-described immunoglobulin preparations in the context of biopsy-proven myocarditis of defined etiology is warranted.

7.4 Novel Approaches to AHA

Novel approaches to myocarditis/DCM [75,164], similar to other autoimmune disorders [200–204], include application of specific epitope-derived peptides as antibody scavengers, or direct targeting/suppression of AABs-producing B cells and/or plasma cells and IA of disease-causing AABs [93,94,96,205–210]. In small pilot studies and retrospective analyses, IA reduced the duration of subsequent hospitalization and improved hemodynamics, New York Heart Association (NYHA) class, exercise capacity [74,75,97,101,164], and myocardial inflammation in DCM [207,208]. The goal of IA is the removal of pathogenic AABs through the extracorporeal removal of patient immunoglobulin following plasmapheresis. IA is followed by supplemental administration of IVIG to prevent infection or antibody production rebound [207–209]. However, a substantial portion of patients do not respond to IA treatment, which has limited its use; in addition, the therapy is expensive and requires an invasive procedure. Therefore it is key to look for biomarkers that identify responders to IA before initiating such therapy. Following the observation that a subset of DCM patients with serum cardiodepressant AABs were responders to IA treatment [101,125], a recent study compared levels of cardiodepressant antibodies and myocardial gene expression profiles between responders and nonresponders [210]. The baseline presence of cardiodepressant antibodies in combination with myocardial gene expression profiles was found to accurately predict treatment outcome within these groups [210]. A larger phase IV controlled IA trial and a clinical phase II trial with the β 1-AABs-specific cyclic peptide COR-1 in postmyocarditic DCM are underway ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00558584) Identifier: NCT00558584 and EudraCT 2010-022579-68).

7.5 Current Indications to Immunosuppression

Overall the available immunosuppression trials show benefit mainly in chronic virus-negative myocarditis/DCM [32,100], in giant cell myocarditis [64], and in active myocarditis defined as autoimmune (eg, virus-negative and positive for cardiac AABs) [17], using a combination of azathioprine, steroids, and/or cyclosporine A. In contrast, immunosuppression had a neutral effect

in the Myocarditis Treatment Trial, where patients had myocarditis of unspecified etiology [31]. A major feature of autoimmune disease is its response to immunosuppressive therapy, and the accumulating evidence for the involvement of autoimmunity in myocarditis/DCM is compelling (Table 2.1). Therefore the 2013 ESC Task Force experts [33] concluded that immunosuppression is recommended in proven autoimmune myocarditis forms, such as giant cell myocarditis [64], cardiac sarcoidosis [208], and virus-negative myocarditis associated with known extracardiac autoimmune disease [171–174,211] (Fig. 2.3). In keeping with the ESC consensus document, steroids are indicated in cardiac sarcoidosis regardless of the degree of ventricular dysfunction, and in virus-negative eosinophilic or toxic myocarditis, with heart failure and arrhythmia [211,33]. Drugs causing hypersensitivity myocarditis should be identified and not reintroduced after recovery [211]. Finally, the ESC Task Force experts [33], on the basis of a recent, single-center controlled trial showing benefit of combined azathioprine and steroids in virus-negative myocarditis [100], concluded that immunosuppression may also be considered in virus-negative myocarditis refractory to standard therapy with no contraindications to immunosuppression [33] (Fig. 2.3).

8. FUTURE PERSPECTIVES: ROLE OF LYMPHOCYTIC SUBTYPES AND CYTOKINE NETWORKS

Evidence has been accumulated on the role of regulatory T cells (FOXP3, CD25, CD4⁺ cells) and Th17 cells in modulating development and evolution of experimentally induced myocarditis [212]: remission was achieved by passive transfer of Treg in a murine model of virus-induced myocarditis [213]; knock-out models of Th17 cell-induced myocarditis paved the way for the experimentation of anti-IL-17 monoclonal antibodies to cure experimental autoimmune myocarditis [214]. Much less is known regarding the role of these lymphocytic subtypes in human biopsy-proven myocarditis. A role for T cells in humans is indirectly supported by case reports of myocarditis occurring in patients treated with biologic agents to activate cellular immunity against malignancy [215]. Reports of biopsy-proven or clinically suspected autoimmune myocarditis in patients treated with anti-PD1, anti-CTLA-4, and anti-CD20 agents for refractory oncologic disease have appeared [216], including a case of biopsy-proven rituximab-induced myocarditis [217]. However, in the latter case, tissue PCR was positive for enteroviral myocardial infection, so it is plausible that myocarditis was facilitated by the immunosuppressive action of the anti-CD20 monoclonal antibody (MoAb) rather than by autoimmunity. On the other hand our

group recently reported a case of autoimmune giant cell myocarditis recurrence in a transplanted patient, refractory to standard immunosuppression, that was successfully treated with rituximab [218]. Therefore it seems mandatory that new immunosuppressive/immunomodulatory treatments be tested in clinical trials recruiting myocarditis patients with biopsy-proven autoimmune disease.

In principle, immune-modulatory agents could compromise general and organ-specific cytokine/lymphokine networks and be responsible for cardiac dysfunction even in the absence of myocardial inflammatory infiltrates [219]. Future studies should be aimed at better elucidating the immune network sustaining chronic myocardial inflammation (cytokine and cellular milieu), as well as the final effector mechanisms of myocyte necrosis and/or dysfunction in humans. In septic shock [220], Cushing's syndrome [221], pheochromocytoma, or Takotsubo cardiomyopathy [135,222], reversible severe ventricular dysfunction may be induced by cardio-depressant factors in the absence or with minimal myocyte necrosis, as revealed by high sensitivity troponins. For instance, we described a case of biopsy-proven virus-negative acute myocarditis presenting as transient apical ballooning, eg, Takotsubo cardiomyopathy [135]. In addition, several authors have advocated a pathogenic role for humoral autoimmunity in chronic DCM with circulating cardiac AABs, even in the absence of florid myocardial inflammation on EMB, supporting the rationale for strategies aimed at targeting functional AABs [40,62,63,68,70–75,78,82,83,86,91,92,94,97,101,102,110,114,116,125,128,133,134,164,166,208–210]. Thus we hypothesize that an “immune-mediated myocardial stunning/hibernation” takes place in acute or chronic myocarditis/DCM, which, if reversed by tailored immune therapies before the occurrence of irreversible myocardial damage, could be clinically beneficial.

In conclusion, given the complexity of the immune network in myocardial inflamed tissue, future clinical immunomodulatory trials should be designed using distinct patient subsets as defined by their specific cytokine and/or cellular immune biomarker profiles, eg, personalized immune intervention.

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Autoantibodies Directed Against G-Protein-Coupled Receptors in Cardiovascular Diseases: Basics and Diagnostics

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1. INTRODUCTION

Autoimmunity is increasingly accepted as the origin or amplifier of various diseases. Classic autoantibodies (AABs) induce immune responses resulting in destruction of the affected tissues. However, an additional class of AABs shows functional activity. Such AABs are directed against the G-protein-coupled receptors (GPCRs; GPCR-AABs) and affect the receptor function [1]. These AABs are thus named “functional AABs.” Consequently, classic autoimmune diseases must be supplemented by the new class of autoimmune diseases that are characterized by the presence of GPCR-AABs and evidence their disease-specific pathogenic activity. This class of autoimmune disease could be called “functional autoantibody disease.”

Knowledge of GPCR-AABs as pathogenic drivers in cardiovascular diseases has continuously increased over the last two decades [1–4]. Table 3.1 summarizes GPCR-AAB data, its activity, distribution, and prevalence in patients with cardiovascular diseases and/or with diseases associated with vascular alteration.

In 1976, Sterin–Borda et al. [5] discovered the agonistic activity of human chagasic sera in the isolated atrial preparation of rats. With this discovery, the story of GPCR-AABs in cardiovascular diseases began. A short time later, in 1980, Venter et al. [6] described AABs directed against the β 2-adrenergic receptor (β 2-AABs) in the sera of patients suffering from allergic asthma and rhinitis. In 1984, Borda et al. [7] attributed the primary finding of agonistic activity in chagasic sera to

circulating IgG, which binds to β -adrenoceptors of the myocardium for modulating the receptor activity. Due to the blocking of the positive inotropic and chronotropic effects of the IgG by the specific β 1-adrenergic receptor antagonist but not by the β 2-antagonist, the β 1-adrenergic receptor was classified as the target.

In view of other cardiomyopathies, Wallukat and Wollenberger [8] first reported the existence of AABs against the β 1-adrenergic receptor (β 1-AABs) in patients with idiopathic dilated cardiomyopathy (DCM) that target either the first (anti- β 1(I)-AABs) or second extracellular loop of the receptor (anti- β 1(II)-AABs) [9,10].

1.1 G-Protein-Coupled Receptors

GPCRs, the largest superfamily in the human genome, are essential elements in the regulation of nearly the entire body system, from sensory reception to the regulation of cell activity, movement, and death. Many of the neurotransmitters and hormones bind to GPCRs and transfer information this way.

GPCRs are integral membrane proteins. Their amino acid chain forms seven transmembrane regions, which result in the extracellular N-terminal, intracellular C-terminal domains, and three extracellular and three intracellular loops. Glycosidic moieties and disulfide bridges contribute to GPCR stability and functionality and are consequently involved in the regulation of receptor response to agonists and antagonists. After extracellular ligand binding, the receptor conformation shifts, which induces the cycle of G-protein activation

TABLE 3.1 G-Protein-Coupled Receptors Targeted by Functional Autoantibodies: Representative References for Prevalence Are Given; Measurement With Functional Assay (Prevalences in Red), FACS (Blue), and ELISA (Black); Not Determined (n.d.)

Receptor	Disease	Activity	Extra cellular loop	Prevalence (%)	References
β 1-adrenergic	Dilated cardiomyopathy	Agonistic	I. Or II.	70,80,80	[56,100,124]
β 1-adrenergic	Peripartum cardiomyopathy	Agonistic	II.	60,100	[60,61]
β 1-adrenergic	Myocarditis	Agonistic	II.	80,100	[10,43]
β 1-adrenergic	Chagas' cardiomyopathy	Agonistic	II.	100	[55]
β 1-adrenergic	Electric. Cardiac abnormalities	Agonistic	II.	35	[62]
β 1-adrenergic	Ventricular arrhythmia	Agonistic	II.	50	[62]
β 1-adrenergic	Atrial arrhythmia	Agonistic	II.	15	[62]
β 1-adrenergic	Periodontitis	Agonistic	II.	75	[65]
β 2-adrenergic	Allergic asthma	Inhibitory	III.	100	[76]
β 2-adrenergic	Chagas' cardiomyopathy	Agonistic	II.	90	[55]
β 2-adrenergic	Alzheimer's disease	Agonistic	I.	45	[86]
β 2-adrenergic	Open angle glaucoma	Agonistic	II	70	[72]
β 2-adrenergic	Regional pain syndrome	Agonistic	II.	55	[75]
β 2-adrenergic	Orthostatic hypotension	Agonistic	n.d.	30	[106]
β 3-adrenergic	Heart failure		II	40	[78]
α 1-adrenergic	Refractory hypertension	Agonistic	I. Or II.	60	[83]
α 1-adrenergic	Idiopathic pulmonary hypertension	Agonistic	II.	n.d.	[81,82]
α 1-adrenergic	Diabetes mellitus type II	Agonistic	II.	35	[85]
α 1-adrenergic	Alzheimer's disease	Agonistic	I. Or II.	55	[86]
α 1-adrenergic	Cancer after chemotherapy	Agonistic	II.	n.d.	[88]
Muscarinic M2	Chagas' disease	Agonistic	II.	100	[55]
Muscarinic M2	Dilated cardiomyopathy	Agonistic	II.	35,40	[99,100]
Muscarinic M2	Regional pain syndrome	Agonistic	II.	85	[75]
Muscarinic M3	Sjögren's syndrome	Agonistic	II.	n.d.	[103]
Muscarinic M3	Orthostatic hypotension	n.d.	n.d.	70	[106]
Angiotensin II AT1	Malignant hypertension	Agonistic	II.	15–30	[94]
Angiotensin II AT1	Preeclampsia	Agonistic	II.	90	[89]
Angiotensin II AT1	Vascular renal rejection	Agonistic	II.	50	[96]
Angiotensin II AT1	Scleroderma	Agonistic	n.d.	85	[97]
Angiotensin 1–7 Mas	Cancer after chemotherapy	Agonistic	II.	n.d.	[88]
Endothelin 1 ETA	Idiopathic pulmonary hypertension	Agonistic	II.	n.d.	[81,82]
Endothelin 1 ETA	Scleroderma	Agonistic	n.d.	85	[97]
Serotonergic 5HT4	Systemic lupus erythematosus	Antagonistic	II.	n.d.	[19,20]
Melanocortin-4	Obesity	Inverse agonistic		n.d.	[107,108]

and inactivation localized on the intracellular receptor side. In this way, the G-protein modulates the activity of enzymes and ion channels, regulating the formation and concentration of cytosolic second messengers. Consequently, GPCRs play an important role in the

regulation of signal transduction from the extracellular environment to the internal metabolic machinery. Disturbances in the regulation of these signal pathways can cause a shift of the metabolic balance and may induce pathologic conditions.

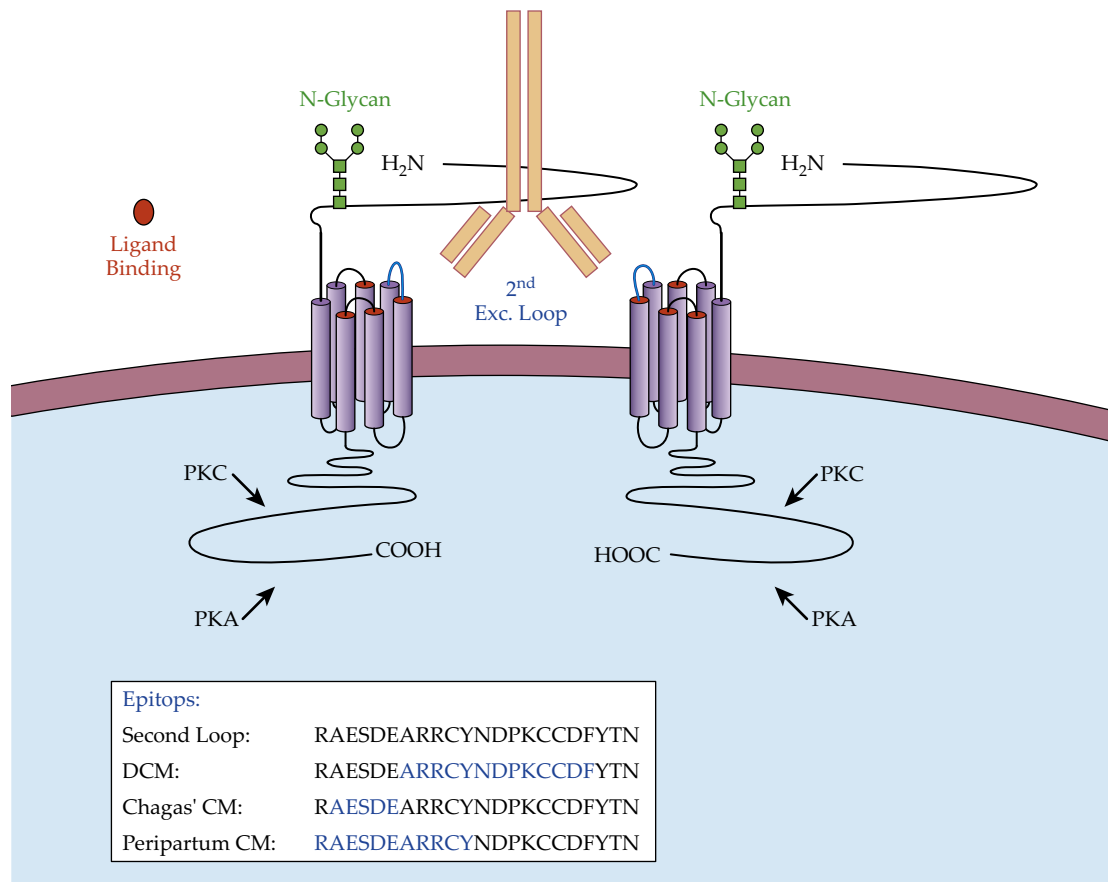


FIGURE 3.1 The human G-protein-coupled receptor targeted by the corresponding autoantibody. The N-terminal domain usually contains fewer than 50 amino acids and is located in the extracellular space, whereas the C-terminal part of the protein varies from 23 (muscarinic2 receptor) to about 100 amino acids (β 2-adrenergic receptor). The transmembrane (TM) regions usually contain between 23 and 24 amino acids, limited by the helical secondary structure and the thickness of the hydrophobic lipid bilayer. The homology varies throughout the entire superfamily. However, the homology is higher in the TM helices (from 35% to 90%) and functionally important side chains in the TM helices, and the loops are strongly conserved between different vertebrate species. Agonists bind to a hydrophobic cave formed by the TM helices. The indicated epitopes on the second extracellular loop are the targets of β 1-adrenergic receptor autoantibodies present in patients with idiopathic dilated cardiomyopathy (DCM), Chagas' cardiomyopathy (Chagas' CM), and peripartum cardiomyopathy (peripartum CM). *Reproduced from Munoz-Saravia et al. [11].*

In the case of physiological and pharmacological ligand binding, receptor downregulation and desensitization of signal transduction are important events to prevent overboarding receptor activation and to protect from disturbed metabolic balance and pathologic conditions. However, this clearly contradicts the case when GPCR-AABs bind to the receptor because comparable prevention mechanisms are lacking in this situation.

Fig. 3.1 shows the schematic GPCR structure which is representative of the β 1-adrenergic receptor and its communication with β 1-AABs directed to the second extracellular receptor loop. Depending on the disease (eg, DCM, Chagas' cardiomyopathy, peripartum cardiomyopathy), as depicted in the box, the disease-related anti- β 1(II)-AABs target different epitopes on the second extracellular receptor loop. For

the β 1-adrenergic receptor and other GPCRs, AABs directed against the first and third receptor loop were also found.

1.2 Autoantibodies Against G-Protein-Coupled Receptors

AABs that recognize epitopes of the first and second receptor loop exert mainly stimulatory effects similar to those of their corresponding physiologic agonists [8,12–14]. In contrast, inhibitory GPCR-AABs, targeting mainly the third extracellular loop, block the receptors and prevent their activation through the relevant agonists [15].

Despite years of research, the origin of GPCR-AABs is still far from clear, and none of the hypotheses discussed here claim exclusivity.

One of the hypotheses based on molecular mimicry was discussed in detail [16–18]. In molecular mimicry, the human immune response is directed against proteins, eg, presented by parasites, bacteria, and viruses affecting humans, which correspond to human proteins. Consequently, the immune system can crossreact with these proteins. In view of Chagas' cardiomyopathy, ribosomal proteins of the infectant *Trypanosoma cruzi* (*T. cruzi*) were found to be similar to human heart proteins such as GPCRs. This could explain the formation of β 1-AABs, as well as AABs directed against the β 2-adrenergic and muscarinic M2-receptor (β 2-AABs, M2-AABs). As summarized in [16,17], experiments in which animals generated GPCR-AABs and in parallel showed heart alteration after both immunization with *T. cruzi* antigens and transfer of *T. cruzi*-activated T cells support the mimicry hypothesis. In systemic lupus erythematosus anti-SSA/Ro52 autoantibodies are present that crossreact with the cardiac 5-HT₄ serotonergic receptor [19,20]. The mimicry hypothesis could possibly also fit for infectious myocarditis. In addition to molecular mimicry, bystander activation may be supported. In bystander activation, parasites or metabolic conditions produce pro-inflammatory conditions that result in host cardiovascular damage associated with the liberation of autoantigens and cryptic epitopes. These epitopes are normally inaccessible to the human immune system, but are now recognizable by autoreactive T cells that perpetuate the immune response against these structures. While the mimicry hypothesis could possibly also fit for infectious myocarditis, in the case of cardiovascular diseases such as idiopathic DCM with no clearly defined pathogen present, unspecific inflammation processes leading to bystander activation could probably better explain the generation GPCR-AABs than mimicry. However, molecular mimicry as a reason for GPCR-AABs generation in DCM should not be absolutely excluded if we remember the suggested virus hypothesis of DCM [21].

Classic agonists use hydrophobic pockets of the receptor for binding; GPCR-AABs, in contrast, bind to the extracellular receptor domains shifting their conformational states, which, as frequently demonstrated [22–25], stabilize the active receptor conformation. Therefore, and critical for the assay development, GPCR-AABs binding to linear representations or denatured variants of the GPCRs domain are strongly restricted. Based on our own investigation [26] and according to other authors [27], we suggest that the GPCR-AABs in relation to the bivalent structure of IgG are ideally suited for receptor crosslinking and could stabilize the receptor conformation in this way. Furthermore, specific predisposing conditions may favor the receptor epitope recognition by GPCR-AABs. While ischemic arteries responded to autoantibodies against the angiotensin II-angiotensin

receptor type I (AT1-AABs) with vasoconstriction, non-ischemic vessels did not [28]. In line with this, β 2-AABs realized their effects only in cultured cardiomyocytes that were partially undersupplied with oxygen but not in cells with optimal oxygen supply. However, the latter became β 2-AABs sensitive after the addition of lactate [29]. We postulate, therefore, that specific metabolic conditions, such as hypoxia, ischemia, and/or inflammation are conditions that support the GPCR-AABs' effects.

Among the downstream effects of GPCR-AABs, the following is evidenced for β 1-AABs: activation of the adenylate cyclase and consequently cAMP increase [30], activation of protein kinase A for phosphorylation of proteins [31], elongation of the action potential duration, increase in the L-type Ca⁺⁺ (ICa) current from the extracellular into the cytosolic compartment [32], mitochondrial structure and membrane potential changes [33], induction of apoptosis, and cell death [34–37], as well as influencing the maturation and degranulation of cardiac mast cells [38]. Via activation of the MAPK/ERK pathway in cardiomyocytes, β 1-AABs could promote the development of cardiac hypertrophy [39]. When β 1-AABs targeted their related receptor on T cells, changes in T-cell proliferation and secretion resulted from the activation of the β 1-AR/cAMP/PKA and p38 MAPK pathways [40]. The activation of the MAPK/ERK pathway as well as increased ROS production have been discussed for autoantibodies directed against the angiotensin II-angiotensin receptor type I (AT1-AABs) [41]. It has been hypothesized that the establishment of inflammatory and profibrotic conditions occurs when AT1-AABs and autoantibodies directed against the endothelin A receptor (ETA-AABs) target immune cells [42].

As already mentioned above, GPCR-AABs lack regulation mechanisms for the prevention of overboarding receptor stimulation such as the receptor desensitization and internalization seen with physiologic agonists. This lack has been observed for all GPCR-AABs and should play a key role in the pathogenesis of GPCR-AABs-associated diseases [43–45]. Recently in view of changes in the receptor conformation and receptor internalization, these effects were specifically substantiated for IgGs carrying β 1-AABs. Interestingly, the β 1-AABs effects have been seen for the β 1-AABs of the majority but not of all DCM patients [46].

Animal studies have clearly manifested the role of agonistic GPCR-AABs as pathogenic drivers. After immunization with peptides corresponding to the second extracellular loop of the β 1-adrenergic receptor, rabbits and rats developed β 1-AABs and then structural and functional heart alterations typical for heart failure [47,48]. By transferring the β 1-AABs from the immunized and diseased animals to healthy inbred rats of the same strain, the rats also developed signs of heart failure [48]. Comparable results

were found if preparations and/or lymphocytes from cardiomyopathic rabbits or DCM patients were transferred into mice [49,50]. Rats immunized for β 1-AABs generation presented cardiac arrhythmias [51].

However, evidence is most persuasive for the role of GPCR-AABs as a pathogenic driver, as the removal of β 1-AABs from the blood of DCM patients via immunoadsorption either stops disease progression or, in a considerable number of patients, causes disease regression [52]. The driving role of GPCR-AABs was supported by clinical studies that revealed the GPCR-AABs as a predictor of poor clinical outcome, which was derived from general cardiovascular mortality, more specifically from sudden death as well as from clinical parameters such as arrhythmia [53].

In summary, the role of GPCR-AABs as a pathogenic driver should, to the best of our knowledge, be indisputable in the future, although GPCR-AABs have been detected in a distinct number of healthy subjects as summarized in Ref. [54]. However, GPCR-AABs positivity in healthy subjects was preferentially detected using enzyme linked immunosorbent assay (ELISA) techniques for measurement without data validation with a bioassay. Using the bioassay of spontaneously beating neonatal rat cardiomyocytes, GPCR-AABs positivity was found much less frequently.

1.3 Autoantibodies Against G-Protein-Coupled Receptors in Various Diseases

1.3.1 Autoantibodies Against β -Adrenergic Receptors

The prevalence of β 1-AABs in the blood of patients with idiopathic DCM is 70–80%, as indicated in Table 3.1. Both anti- β 1(I)- and anti- β 1(II)-AABs were found, but with variable frequency depending on the patients' geographic origin. These data were based in general on measurements using the bioassay of cultured spontaneously beating neonatal rat cardiomyocytes [55] described in Section 2.2.1. With 70%, a similar prevalence was found by measurements with the FRET (Förster resonance energy transfer) technique [56]. A high frequency of β 1-AABs and additionally of M2-AABs was also demonstrated for patients with Chagas' cardiomyopathy [57]. Using the bioassay, nearly all patients with Chagas' cardiomyopathy carry anti- β 1(II)-AABs and M2-AABs (see Section 1.4.4). Anti- β 1(II)-AABs of idiopathic DCM patients target the middle part of the second extracellular receptor loop, whereas anti- β 1(II)-AABs of Chagas' patients target an epitope of the second extracellular loop, which is localized nearer to the N-terminus. The affinity of the chagasic β 1-AABs is in the μ M range [18,58,59].

Furthermore, β 1-AABs were also found in patients with peripartum cardiomyopathy [60,61], primary

electrical cardiac abnormalities, arrhythmias, ventricular tachycardia, and sudden death, as well as in patients with myocarditis [10,43,62–64]. β 1-AABs positivity has been also documented for patients with periodontitis, which could be in agreement with the relationship between periodontal infection and cardiovascular disease [65].

In addition to β 1- and M2-AABs, chagasic heart patients also present β 2-AABs but to a lower extent [55]. However, β 2-AABs were mostly dominant in Chagas' patients suffering from mega syndromes in the gastrointestinal tract. Nearly 100% of these patients also presented M2-AABs but only 40% presented β 1-AABs. Chagasic patients suffering from heart and gastrointestinal diseases frequently carried (nearly 100%) all the three AABs.

Among patients with Chagas' disease who are symptomless (asymptomatic Chagas' disease), 30% are carriers of β 1-, β 2-, and M2-AABs, which could indicate those patients who progress to the development of Chagas' cardiomyopathy and/or gastrointestinal mega syndromes [55]. Consequently, finding GPCR-AABs in asymptomatic Chagas' patients may indicate their risk for the life-threatening complications of Chagas' disease [66]. In correlation with the cardiomyopathy progression in chagasic patients, the β 1-AABs level increased from the asymptomatic patients being AABs positive to the patients with mild/moderate cardiomyopathy to those with severe cardiomyopathy [55]. However, even when considering all the presently available data, the pathogenic role of GPCR-AABs in Chagas disease is still confusing and is therefore controversially discussed [57,67,68]. Analyzing the chagasic patients' benefit after immunoadsorption of their GPCR-AABs would, in our view, clarify the autoantibodies' role. Unfortunately, related studies are still missing. To judge the pros and cons of the GPCR-AABs, role in Chagas' disease, in vivo neutralization of GPCR-AABs in chagasic animals using a new strategy [69–71] would also be helpful.

β 2-AABs were also found in patients with open-angle glaucoma [72] and with complex regional pain syndrome targeting the second extracellular loop, as well as in Alzheimer's patients presenting β 2-AABs directed against the first receptor loop [73–75]. β 2-AABs directed at the third extracellular loop presenting with antiadrenergic activity were found in patients with allergic asthma [76]. The orchestra of GPCR-AABs affecting the heart grew by the recent finding of β 3-AABs in heart failure patients. The prevalence was 30–40% but the patients were not classified in view of the disease origin. The β 3-AABs have negative inotropic and chronotropic activities and could be therefore—as recently demonstrated in rats—cardio-protective [77–79]. Further investigations must show the specific role the β 3-AABs play in the concert of all the other heart-affecting GPCR-AABs.

1.3.2 Autoantibodies Against the α 1-Adrenergic Receptor (α 1-AABs)

As indicated in Table 3.1, patients with malignant hypertension, refractory hypertension, essential, and idiopathic pulmonary hypertension [80–84] are positive for autoantibodies directed against the α 1-adrenergic receptor (α 1-AABs) for which it was suggested that the AABs influence the vessels' contractile apparatus and may, in this way, be involved in the pathogenesis of hypertension. Furthermore, α 1-AABs are also found in patients with Alzheimer's disease [74] and in those with Diabetes mellitus [85].

As demonstrated in a rat model, the application of α 1-AABs prepared from the sera of Alzheimer's patients induced swelling of the endothelial cells of the brain vessels combined with a reduction of the blood flow in these areas [86,87]. Therefore, GPCR-AABs could be responsible for the vascular component in Alzheimer's disease and could consequently be a treatment target. Following chemotherapy, a number of cancer patients develop the metabolic syndrome characterized, among others, with high blood pressure and Diabetes mellitus. For one such patient, α 1-AABs positivity was documented in a case study [88].

1.3.3 Autoantibodies Against the Angiotensin II-Angiotensin Receptor Type I

With a prevalence of nearly 90%, AT1-AABs targeting the second receptor loop were found in patients with preeclampsia [89] and were thought to be responsible for pathogenic events such as the activation of NF κ B, tissue factor promoter AP-1, and NADPH oxidase [90,91]. AT1-AABs induce the synthesis of the plasminogen activator inhibitor-I, which may contribute to hypercoagulation [2,14,92] and activate cardiac mast cells while inducing adhesion of monocytes to endothelial cells [93].

AT1-AABs carry nearly 35% of patients with secondary malignant hypertension, 18% with renovascular diseases, and 15% with malignant essential hypertension [94]. AT1-AABs are also present in patients with arterial occlusion disease [95].

A distinct number of kidney transplant patients developed AT1-AABs recognizing the second extracellular AT1-receptor loop, which is associated with allograft vasculopathy and considered as a reason for allograft rejection in these patients [96]. AT1-AABs have also been identified with high prevalence in patients with scleroderma [97,98] contributing to disease initiation and progression [42].

1.3.4 Autoantibodies Against the Muscarinic Receptor M2

In total, 30–40% of asymptomatic Chagas' patients and nearly all chagasic heart patients and those with

gastrointestinal mega syndromes carry M2-AABs [55]. These autoantibodies were also found in a distinct number of DCM patients (35–40%) [99,100]. The M2-AABs, recognizing an epitope on the second extracellular receptor loop [101], produce negative chronotropy as clearly evidenced in neonatal rat cardiomyocytes. M2-AABs were preferentially observed in patients who suffer from arrhythmic disorders as summarized in [54]. More recently, M2-AABs were also found in a distinct number of patients with peripartum cardiomyopathy [61]. Patients with complex regional pain syndrome are also positive for M2-AABs [75], whereas those with Sjögren's syndrome present M3-AABs [102–104], which were thought to contribute to the development of disturbances in secretory functions typical of Sjögren's syndrome. Consequently, any pathogenic role of anti-M3R antibodies in xerostomia has been suggested [105].

M3-AABs together with β 2-AABs are present in patients with orthostatic hypotension, where AABs are believed to induce vasodilation and may cause or exacerbate orthostatic hypotension [106].

1.3.5 Autoantibodies Against Endothelin1 Receptor Type A

Autoantibodies directed against the endothelin one receptor type A (ETA-AABs) recognize an epitope on the second extracellular receptor loop and are present in the blood of patients with pulmonary hypertension (PH) [81,82]. Due to amelioration of the clinical symptoms after ETA-AABs removal by immunoadsorption, probably resulting from reduced pulmonary resistance and dilatation of the right cardiac atrium and ventricle, we postulated that ETA-AABs may play a central role, probably in combination with the α 1-AABs mentioned previously, in the development and progression of PH. ETA-AABs are also present in patients with scleroderma [97] and arterial occlusion disease [95].

1.3.6 Autoantibodies Against the Angiotensin (1–7) Mas Receptor

In addition to the presence α 1-AABs [88], we found autoantibodies against the angiotensin (1–7) Mas receptor (Mas-AABs) in patient blood following cancer chemotherapy. In contrast to the α 1-AABs, Mas-AABs exert a negative chronotropic response.

1.3.7 Autoantibodies Against the 5-Hydroxytryptamine Receptor 4

Antibodies against the 5-hydroxytryptamine receptor 4 (5HT4-AABs) are present in the blood of patients with systemic lupus erythematosus, where they may contribute to the development of the typical lupus congenital heart block visible in lupus-affected neonates [19,20].

1.3.8 Autoantibodies Against the Melanocortin 4 Receptor

Autoantibodies against the melanocortin 4 receptor have been found in patients with obesity. As an inverse agonist, AABs receptor binding resulted in increased food consumption [107,108].

1.4 Conclusion and Outlook

With the finding of GPCR-AABs in diseased subjects—mainly those suffering from diseases of the cardiovascular system—a new class of autoimmune diseases was introduced. While autoantibodies in classic autoimmune diseases play a role in the destruction of their targets and target tissues, GPCR-AABs are so-called “functional autoantibodies” that affect their related receptors in a similar way as physiologic agonists or antagonists, but without controlling mechanisms such as, eg, receptor downregulation, which is well known with the physiologic receptor ligands. Due to these functional autoantibodies, the new class of autoimmune disease can be called “functional autoantibody disease.” Among the diseases presenting with GPCR-AABs, cardiomyopathies such as idiopathic-dilated cardiomyopathy (DCM), Chagas’ cardiomyopathy, and peripartum cardiomyopathy are the most prominent.

With the knowledge of GPCR-AABs as pathogenic drivers in cardiovascular diseases, and in diseases associated with vascular alterations, evidenced in vitro, in cell and animal investigations as well as in human studies supported by the detection of the exact receptor epitopes targeted by the cardiovascular specific GPCR-AABs, these autoantibodies have become therapeutic targets (see Chapter 28). However, the presently developed strategies for treatment of “functional autoantibody disease” are still preferentially focused on patients with DCM, but, in view of the proven benefit for DCM patients, may be transferred to other GPCR-AABs associated diseases.

2. DIAGNOSTICS OF AUTOANTIBODIES AGAINST G-PROTEIN COUPLED RECEPTORS

2.1 Introduction

Knowledge of GPCR-AABs as pathogenic drivers in cardiovascular diseases, as indicated previously, has continuously increased over the last two decades [1–4]. Consequently, treatment strategies directed at the removal or neutralization of GPCR-AABs have been developed and are presently being studied for patients, mainly those with cardiomyopathy (as documented in Chapter 28). However, for manifestation of the concept “functional

autoantibody disease” as a new class of autoimmune diseases characterized by GPCR-AABs presence and evidence for their disease-specific pathogenic activity and therefore needing specific treatments, existing inconsistencies must be removed concerning the actual prevalence and clinical impact of the GPCR-AABs.

Furthermore, and demonstrated for DCM patients vs. healthy subjects, diseased and healthy subjects are not only determined by the mere presence of $\beta 1$ -AABs, but also by the different impact on their autoantibodies’ function concerning the $\beta 1$ -receptor mediated signaling. For example, the presence of only functional active $\beta 1$ -AABs is clearly correlated with increased morbidity and mortality in DCM [109]. Consequently, the exact measurement of functionally active GPCR-AABs in patient blood would be essential to select patients who should profit from GPCR-AABs directed therapy and for their posttreatment monitoring.

Unfortunately, and despite the now nearly 40-year long story of GPCR-AABs, there are, among the several ELISA and indirect immune fluorescence measurements, no methods that sufficiently discriminate functionally active from inactive GPCR-AABs. Furthermore, to the best of our knowledge, there are no methods that fully meet the requirement of “good laboratory practice” for their applicability in laboratory medicine.

When looking for the reasons that have until now prevented ELISA from being the preferred tool for GPCR-AAB quantification, as it is for many other antigens in the human blood, we first see the difficult and complex mechanisms for receptor conformation changes that are necessary for GPCR-AABs receptor binding, especially of the functionally active GPCR-AABs. This only allows definite downstream signaling to produce the pathogenic effects of the GPCR-AABs.

While native receptors present various transient conformational epitopes during their activation/inactivation process, which can be targeted by the GPCR-AABs, in our view, such a conformational epitope can only be poorly represented by synthetic epitope mimics, which are in general destroyed by fixation. Based on this and focused on $\beta 1$ -AABs, the preferential GPCR-AABs presently under human study, it was recently stated that “...peptide-based immunoassays can currently not be accepted as a scientifically or diagnostically valid tool for the detection of clinically relevant $\beta 1$ -AABs in humans” [110].

This leads to the assumption that with future perspectives, GPCR-AABs can reliably be detected only by assays utilizing native GPCRs as the test antigens.

In agreement with this, correlations with clinical data suggest that results derived from native cell-based assays are more valid. Consequently, assays based on native (living) cells should be preferentially used tools in clinical and preclinical studies.

2.2 Current Approaches to Diagnostic Assays

Several approaches have aimed at developing a reliable test system to detect pathogenic GPCR-AABs. Although not all test systems introduced here directly target cardiopathogenic antibodies, the underlying test principles are convenient. The development of each test is dependent on the application of GPCR-AABs derived from patients and seemingly sensible concerning the quality of antibody preparations applied [3,46,111,112]. A two-tailed classification of all methods is possible: first, indirect assays, which means the detection of a second messenger signal that increases after the GPCR-AABs bind to their related cellular receptor presented by (living) cells; this could be global functional cell parameters such as pulsation and contraction rate as well as specific signaling molecules such as Ca^{2+} or cAMP formed downstream in the signal cascade. Second, the direct detection of the GPCR-AABs after binding to GPCR epitope mimics, which is still the major goal for anchoring the GPCR-AABs measurements in laboratory medicine, although there are some limitations, as previously highlighted.

2.2.1 Indirect Assays (Bioassays)

The first bioassay for GPCR-AABs was introduced as the key experiment for GPCR-AABs exploration in 1976 [5], and was later refined in 1984 [7]. Briefly, rat atria were prepared for the bioassay, attached to a glass holder, and immersed in a tissue chamber containing the various dilutions of sera and/or IgG (sampled from GPCR-AABs positive patients with Chagas' disease) in modified Krebs–Ringer solution. A

constant resting tension of 750 mg was applied to the atria and the activity of spontaneously beating atria was analyzed in terms of tension (mg) and frequency of contractions (number of contractile cycles per minute). The atria were allowed to function for 180 min. Tension and frequency changes were calculated, comparing values at 0 min and 60 min, respectively, and at 120 and 180 min. Another bioassay [8,113] introduced in the late 1980s and continuously refined [55,70,114] was used for GPCR-AABs activity measurements in the test system of cultured spontaneously beating neonatal rat cardiomyocytes and monitors changes in the beating rate of the cells after the application of GPCR-AABs.

In brief, as demonstrated schematically in Fig. 3.2, the procedure involves the preparation of fresh neonatal heart cells from 1- to 3-day-old rats. The cardiomyocytes are cultivated for 4 days. For GPCR-AABs testing, IgG from controls and patients was prepared, applied to the test system, and the change in beating rate monitored by microscopy. In cases where the IgG contained more than one GPCR-AABs, the monitored change in the beating rate represented the mix resulting from GPCR-AABs being positively chronotropic and those being negatively chronotropic. By intelligent and successive application of receptor antagonists, the chronotropic activity of each of the different GPCR-AABs can be measured. Based on this, the bioassay is qualified for the global measurement of GPCR-AABs.

Fig. 3.3 shows this exemplarily for the sample of one patient with Chagas' disease suffering from heart disease and megacolon presenting with β_1 -, β_2 -, and M2-AABs.

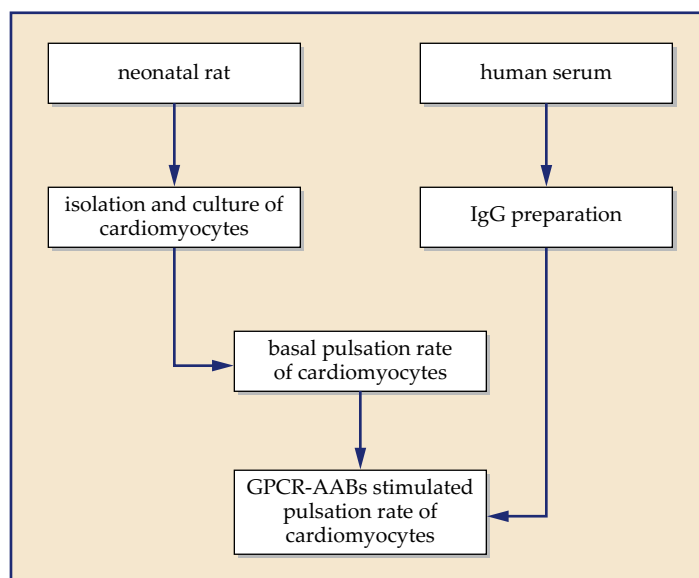


FIGURE 3.2 Chart of the bioassay using cultured spontaneously beating neonatal rat cardiomyocytes for measurement of autoantibodies directed against G-protein-coupled receptors. (IgG preparation from serum using usual procedures; cardiomyocyte basal beating rate: between 100 and 220 beats/min; GPCR-AABs stimulated beating rate: lower limit of detection ($\times \pm 3\text{SD}$) $< \delta \pm 4$ beats/min; cut off for clinical significance $\geq \delta \pm 8$ beats/min). Adapted from the personal collection of the authors.

Alternatively in our view, this assay could be performed with spontaneously beating human embryonic cardiomyocytes generated by in vitro differentiation from induced human adult stem cells. Both bioassays are instruments by which to depict the ultimate effect of GPCR-AABs binding, but are limited in their application for clinical diagnostics because they are constituted of primary cells that must be freshly prepared each time the assay is to be performed. Moreover, the readout is difficult to standardize, even when employing automated monitoring by microscopy using high-speed cameras or cell impedance [115]. However, this kind of bioassay is indispensable for research and must be available as a “gold standard” to validate newly developed assays, most of which are considered for diagnostic routine.

Another approach toward diagnostic testing is to measure key molecules formed downstream in a signal cascade after GPCR-AABs receptor binding. Measurement of downstream products can, in principle, also be applied to the bioassay using native cells prepared from neonatal rats; however, this technology is mainly directed at cells overexpressing the GPCRs of interest. Consequently, and in contrast to the rat cell bioassay, only the related GPCR-AABs can be measured in a single run. Therefore, separate tests must be developed for each of the GPCR-AABs.

In particular, second messengers of the signaling cascade, which undergo massive changes in their concentrations during signaling process, are suitable as sensitive targets. Among these, cAMP is the most prominent. Recently, an assay was composed based [56] on the detection of cAMP and utilizes an FRET cAMP sensor that depends on HEK293 cells stably overexpressing the β_1 -adrenergic receptor

transiently transfected with an EPAC (exchange protein activated by cAMP) protein. The cAMP binding EPAC protein is fused to an FRET construct with yellow and cyan fluorescent proteins (YFP and CFP) that change emission properties due to a conformational change in the molecule when the EPAC protein is bound to cAMP. The ratio of fluorescence changes is determined by fluorescence microscopy, which is a measure of cAMP concentration changes. For test validation, IgG preparations of DCM patients and healthy controls were measured. Here, 60% of the DCM patient samples were positive for β_1 -AABs, while no healthy sample had altered cAMP concentration. Seemingly, the results are very promising and the discrimination of patients and healthy individuals achieved. Unfortunately, FRET- and bioassay was not applied in parallel to the subject cohort.

For patients with Sjögren's syndrome who present autoantibodies against the muscarinic M3 acetylcholine receptor (M3-AABs), an assay using a bioluminescence resonance energy transfer (BRET)- Ca^{2+} sensor was developed for the detection of changes in Ca^{2+} concentrations after receptor activation by M3-AABs as a measure of their functional activity [116].

This BRET- Ca^{2+} assay is based on the cloning and transient cotransfection of CHO cells with muscarinic M3 acetylcholine receptor and a calcium-sensitive bioluminescence fusion protein consisting of aequorin and green fluorescent protein (GFP). The emitted fluorescence is quantified by measurements with a luminometer, allowing inference with Ca^{2+} concentrations.

Using the principle of the BRET- Ca^{2+} assay adapted to CHO cells with β_1 -adrenergic receptors, measurement of β_1 -AABs should be possible.

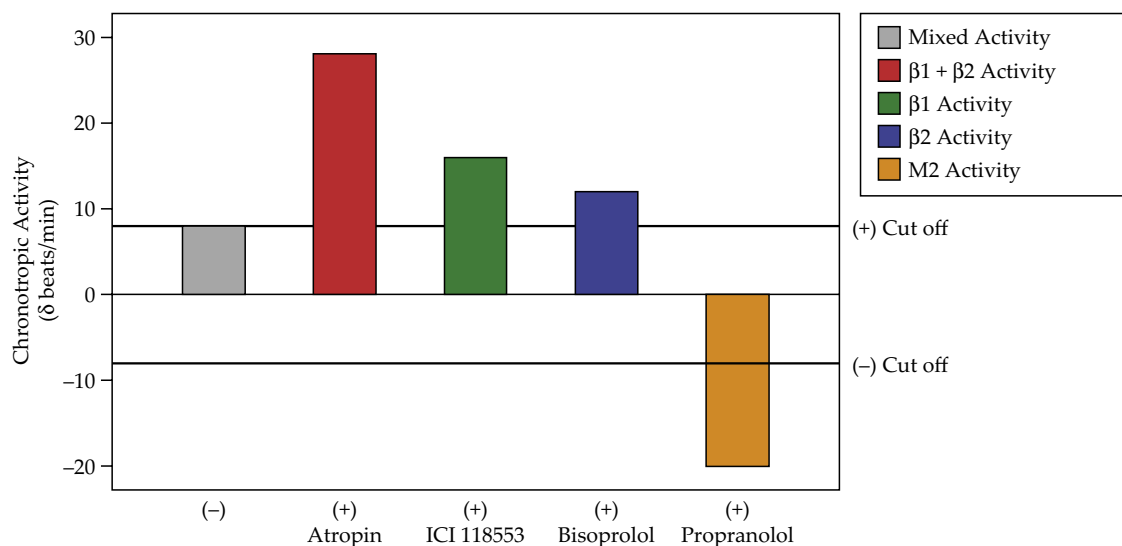


FIGURE 3.3 The bioassay using cultured spontaneously beating neonatal rat cardiomyocytes for the differentiation of autoantibodies directed against G-protein-coupled receptors by successive use of receptor antagonists; cutoff for clinical significance $\geq \delta \pm 8$ beats/min; ICI 118553 is a β_2 -blocker, for details see Ref. [55]. Adapted from the personal collection of the authors.

Recently a further cell-based assay, commercialized by DiscoverX (Fremont, CA) [117], was introduced, which, in principle, can be used for the measurement of all the interesting GPCR-AABs, in which the CHO cells overexpressed with the related GPCRs are available. One principle assay (cAMP Hunter eXpress GPCR Assay) was based on measurement of the cAMP formation after GPCR-AABs receptor binding, while the other (Path-Hunter β -arrestin technology) monitors GPCR activity by detecting the interaction of β -arrestin with the activated GPCRs using β -galactosidase enzyme fragment complementation. The β -arrestin recruitment occurs as a function of ligand activation of the target receptor. The assays using IgG preparations as samples have already been applied for the measurement of β 1-, β 2, and M2-AABs in patients with orthostatic hypotension [106] and postural tachycardia syndrome [118].

2.2.2 Direct Methods

Using immunoassays, it should be possible to quantify the receptor-bound GPCR-AABs directly. These assays are based on the idea that binding GPCR-AABs is sequence-specific rather than structure-dependent. The immunoassays use peptides representing the receptor epitopes targeted by the GPCR-AABs for autoantibody binding. In the future, it may be possible to supplement the peptides with aptamers [119,120]. For many of the disease-associated GPCR-AABs, immunoassays have been developed and applied in human studies. However, to the best of our knowledge, all of these assays are inhouse tests, and validation and standardization with respect to the requirement of laboratory medicine practice is missing. Consequently, all of the study data collected with immunoassays and the related conclusions should in our view be seen as preliminary. This is even truer for the β 1-AABs data collected with immunoassay. For example, an ELISA was composed for β 1-AABs measurements carrying the sequence pattern analog to the first and second extracellular loop of β 1-adrenergic receptor immobilized on a matrix. After β 1-AABs binding, the autoantibodies were visualized through the binding of secondary antibodies fused to a Horseradish Peroxidase (HRP) for catalyzing the colored detection reaction. In a case study of 2062 patients suffering from chronic heart failure, the prevalence for β 1-AABs was around 8% in DCM patients compared to healthy individuals (2.2%), which is not in agreement with the data collected using bioassays.

A lack of sensitivity of the β 1-AABs ELISA has also been confirmed by other studies [112], where a higher β 1-AABs prevalence was reported for the control group (35%) than for the patient group (29%). An advanced form of ELISA assays is the competitive ELISA [112] using PFA-fixed adherent SF-9 insect cells instead of peptides, which express the β -1 adrenergic receptor

and serve as a target for β 1-AABs. In this assay, β 1-AABs in the patient serum compete with monoclonal mouse antibodies for the binding site of the β 1-adrenergic receptor. The detection takes place via an antimouse HRP-coupled secondary antibody. In this test, the prevalence of β 1-AABs was 62% in a poorly clarified cohort of heart failure patients and 8% in healthy subjects.

Last but not least, living HEK 293 cells stably overexpressing the β 1-adrenergic receptor fused with YFP on their surface were seeded on a matrix, incubated with patient autoantibodies and subsequently fixed with acetone. Receptor-bound antibodies were detected by a fluorophore (Alexa647)-fused secondary antibody against human IgG using fluorescence microscopy. Thus this method allows visualization of the receptor antibody complex by colocalization of fluorescence signals. This method was tested using a probe set of 10 DCM patients and 10 healthy controls as the methodological approach and revealed a discrimination of 80–20% [46]. Automated digital fluorescence microscopy with modern pattern recognition algorithms could be a promising option for the standardization of such native cell-based assays in a clinical setting [121,122].

2.2.3 Limitations and Obstacles of Current Diagnostic Assays

The main challenges for the development of assays to detect GPCR-AABs relevant for diseases of the cardiovascular system are sensitivity and reproducibility in terms of discrimination between patients and healthy controls. At present, different assays exhibit a number of antibody-positive controls with a range reaching results up to 35% for the peptide ELISA assay. On the other hand, it is difficult to verify the sensitivity of assays as most studies fail to clarify how many of the investigated DCM patients suffer from autoantibody-induced DCM.

Furthermore, the introduced direct assays ensure measuring the true binding of the GPCR-AABs to the related receptor epitope mimics, but these assays do not supply any information about the functionality of GPCR-AABs. This effect can be determined using indirect assays, in which there is a hierarchy in the assays from the bioassay using the neonatal rat cardiomyocytes, which are seen as the global test to those assays measuring second messengers and applicable specifically for only one of the GPCR-AABs. Consequently, among the indirect assays, the greatest effort in standardization is necessary for the neonatal rat cardiomyocyte assay.

The other critical point is the very low GPCR-AABs serum concentration, which makes sample isolation and concentration necessary before measurement. There are various protocols to isolate the IgG out of the serum (e.g., caprylic acid or ammonium sulfate precipitation or purification with certain Protein A and G columns). It is clear

that different isolation methods lead to different quality and composition of the resulting GPCR-AABs preparations, which will affect assay results. Also, it appears to be extremely important to regenerate the pH and consider the fact that IgGs are very sensitive to freeze–thaw cycles. For a routine assay, it is absolutely necessary to specify standard operating procedures for the handling and storage of samples of blood, serum, and derived antibody preparations. This is a prerequisite to obtain reproducible results with low variation numbers.

This makes the development of standardized and valid routine assays for the detection of potentially cardiovascular-pathogenic GPCR-AABs in human blood samples difficult, which is even truer for β 1-AABs, which are presently the main focus.

2.2.4 Conclusion and Outlook

All of the indirect assays currently in use for the detection of GPCR-AABs and the determination of their pathogenic impact are excellent research tools and can be used for GPCR-AABs measurements in small cohorts of clinical studies; however, they are presently unfit for routine healthcare, because they employ primary cardiomyocytes or cell lines overexpressing the target receptor. The readout of such live cell reporter systems is difficult to standardize—even when automated. Additional problems concern the need for specific sample preparation (IgG), which is not highly automatable in general. However, the formulation of standards and calibrators that are representative of the pathogenic GPCR-AABs found in patients and probably also in some healthy subjects is imperative. Furthermore, partial automation, mainly of the readout systems, would even better qualify the indirect assays; in our view, preferentially, the assay using the spontaneously beating neonatal rat cardiomyocytes, as the “gold standard” for the measurement of different GPCR-AABs. Last but not least, definite “standard operating procedures” (SOPs) for the indirect assays, such as that which is presently under development for the neonatal rat bioassay, are the most important prerequisite for making these assays fit for the future.

In view of direct assays, semisynthetic targets for the GPCR-AABs that consist of the related native receptor solubilized from the cell membranes and reconstituted in synthetic lipid vesicles may offer a hopeful alternative to the present dilemma with the immunoassays. Assays have been developed and successfully used for the functional reconstitution of β 1-adrenergic signal transduction from purified components [123]. β 1-adrenergic receptors thus reconstituted in their native conformation are known to remain fully functional upon prolonged storage at -20°C and thus could be used in immune assays as a durable test antigen that presents the full scope of native conformational epitopes targeted

by β 1-AABs. Alternatively, application of the technology of a recently described “good laboratory praxis” conform assay for β 1-AABs measurement for the measurement of the other functional GPCR-AABs could close the diagnostic gap. This β 1-AABs assay is based on fluorescence-activated cell sorting (FACS) of native cells overexpressing YFP-fused human β 1-AABs for autoantibody binding. The sensitivity and specificity of the assay for discrimination of patients with DCM positive for β 1-AABs relative to healthy subjects were $>90\%$ [124]. In regards to the bioassay of spontaneously beating neonatal rat cardiomyocytes used as the “gold standard” for β 1-AABs measurement, a reasonable quantitative correlation existed [125].

However, only the future will tell whether the concept of immunoassay development will solve the imminent specific test problems discussed here for the currently available indirect and direct assays for GPCR-AABs as well as global problems like the standardization of test circumstances.

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Immune-Mediated Accelerated Atherosclerosis

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1. ATHEROSCLEROSIS

1.1 The Pathomechanism of Atherogenesis

Cardiovascular disease (CVD) is the leading cause of death worldwide [1,2]. The main underlying pathology of CVD is atherosclerosis, which is a chronic inflammatory disease of the artery wall that leads to formation of plaques. Rupture of such plaques causes atherothrombotic events such as heart attacks and strokes. Several nonmodifiable and modifiable risk factors for atherosclerotic CVD have been identified, including family history, ethnicity, and age as well as smoking, hypertension, obesity, type II diabetes, and hypercholesterolemia [3,4]. High LDL cholesterol levels and low HDL cholesterol are key risk factors in the pathogenesis of atherosclerotic plaques. High plasma LDL can be a result of an autosomal dominant disorder, called familial hypercholesterolemia (FH), which is caused by genetic mutations in the LDL receptor (LDLR) or apolipoprotein B100 (apoB100) [5]. In addition, gain of function mutations in the enzyme proprotein convertase subtilisin/kexin type 9 (PCSK9), which degrades LDL receptors, can also result in hypercholesterolemia [5]. However, in a majority of cases high LDL cholesterol levels are the result of a combination of polygenic and lifestyle factors. Of note, genome-wide association studies (GWAS) do not support a causal role for HDL cholesterol levels in cardiovascular disease [6].

When LDL enters the artery wall, it becomes trapped in the subendothelial space, where it undergoes several types of modifications including sequential steps of oxidation [7–9]. The accumulation of oxidized LDL (OxLDL) in the intima and the associated activation of arterial endothelial cells represent initiating steps of atherogenesis. Infiltrating macrophages take up modified LDL particles via scavenger receptors, leading to the

generation of lipid-laden foam cells that are hallmark cells of atherosclerotic lesions of all stages [10]. In the initial stages of plaque formation lesions appear as so-called “fatty streaks,” which can then further progress into more complex plaques. Lesions of all stages display signs of inflammatory activation and are characterized by the recruitment of T lymphocytes secreting several proinflammatory cytokines (eg, IFN- γ).

HDL on the other hand, exerts a variety of other anti-atherogenic properties such as the inhibition of lipid oxidation, restoration of endothelial function as well as other anti-inflammatory effects [6]. Indeed, HDL has been shown to dampen OxLDL-induced expression of cytokines and to modulate the recruitment and adhesion of monocytes. In addition, HDL has been shown to inhibit antigen presentation as well as lymphocyte proliferation. Thus, HDL seems to have effects on both innate and adaptive immune responses, which needs to be elucidated in future studies [11,12]. Moreover, it is critically involved in controlling the cellular cholesterol levels by promoting the reverse cholesterol transport from peripheral tissues, such as the vasculature, to the liver. The ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) on macrophages mediate the efflux of cellular cholesterol onto HDL and its major protein ApoA-I [13]. However, if HDL becomes dysfunctional due to chemical and structural changes, it can also become proinflammatory, and even propagate oxidation of LDL, enhance vascular inflammation, and impair cholesterol efflux [6]. Indeed, at a certain stage macrophage foam cells become unable to maintain cellular cholesterol homeostasis by mediating HDL-dependent cholesterol-efflux, and undergo apoptosis and/or necrosis, which consequently result in the development of an acellular necrotic core inside the lesion [7,14,15].

These advanced lesions are further marked by proliferation of smooth muscle cells that promote plaque stabilization by the production of extracellular matrix and the secretion of collagen to form a fibrous cap covering acellular necrotic areas. In part triggered by inflammatory stimuli and the secretion of matrix metalloproteinases, fibrous caps become increasingly thin and prone to rupture, ultimately resulting in the release of procoagulant lipids and tissue factor into the circulation that trigger thrombosis. This results in typical adverse clinical effects, such as myocardial infarction (MI) and stroke [16]. Interestingly, recent studies have indicated that an increasing number of cases of myocardial infarction are nowadays caused by plaque erosion rather than plaque rupture [17]. Plaque erosion occurs in the absence of plaque rupture and arises in lesions without a large lipid core and few inflammatory cell infiltrates [18].

1.2 Atherosclerosis and Inflammation

It is now evident that inflammation plays a pivotal role in the development of atherosclerosis and that inflammation and disturbed lipid metabolism both feed into this chronic inflammatory process in the vascular wall [16,19–21]. This specific interplay is thought to mediate both the persistence of initiating triggers as well as an impaired resolution of inflammatory responses. Moreover, a variety of innate and adaptive immune functions have been identified as modulating factors that influence the inflammatory process via several cellular as well as noncellular players [9,22].

The prominent role of inflammation in the initiation and progression of atherosclerotic lesions as well as in—the clinically most relevant—plaque rupture is well established [23–25]. Besides clear histopathological evidence showing infiltration of inflammatory cells, which are activated and produce cytokines, many epidemiological studies as well as data from GWAS support a causal role for inflammation in atherosclerosis. For example, more than 20 prospective cohort studies have revealed clinical evidence for highly sensitive measurements of the acute phase reactant C-reactive protein (CRP), which is a pentraxin elevated in response to inflammatory triggers and is independently associated with cardiovascular risk. For example, high sensitivity C-reactive protein (hsCRP) levels are elevated in more than 65% patients suffering from unstable angina [21,26,27]. Similarly, levels of serum IL-6, which induces CRP-expression, have also been directly associated with CVD in epidemiological studies [28,29]. Moreover, Mendelian randomization analyses of 40 studies including up to 133,449 individuals have identified that a polymorphism in the IL-6 receptor signaling pathway (rs7529229), which results in reduced plasma CRP

levels, is associated with a decreased risk for coronary heart disease events [29,30]. Besides CRP and IL-6 as circulating inflammatory biomarkers, pentraxin 3 (PTX3) has also been implicated in CVD, and it has been suggested that levels of PTX3 reflect local inflammation in atherosclerotic lesions more accurately than CRP [31]. Furthermore, increased serum concentration of serum amyloid A (SAA), IL-1R antagonist as well as soluble adhesion molecules have been identified as independent predictors of coronary heart disease [21,32].

Importantly, GWAS have identified the chromosome region 9p21 as a hotspot locus for CVD risk [33,34] and recently, in another large-scale study cyclin-dependent kinase inhibitor 2B (CDKN2B) within the 9p21 region was identified as the gene with the highest association with CVD risk [35]. CDKN2B was investigated by Kojima et al. with respect to its role in atherogenesis. Interestingly, deficiency of CDKN2B in high-fat diet-fed ApoE^{-/-} mice resulted in increased atherosclerosis with more complex plaques as a result of impaired apoptotic cell clearance [36]. Another candidate gene relevant to the inflammatory process of atherogenesis that was identified by GWAS encodes for the chemokine C-X-C motif ligand 12 (CXCL12), which is the ligand for C-X-C chemokine receptor type 4 (CXCR4) and is involved in neutrophil egress from bone marrow but also regulates neutrophil recruitment to atherosclerotic lesions [37]. However, identified SNPs have been associated with reduced as well as increased plasma levels of CXCL12, therefore suggesting both pro- and anti-atherogenic roles for this chemokine.

Major insights into the molecular and cellular events that drive the inflammatory response in atherosclerosis come from a plethora of experimental studies in recent years regarding the involvement of innate and adaptive immunity in atherosclerosis [8,9,38,39]. These studies suggest the recognition of disease-specific antigens for the involvement of both arms of immunity as critical modulators of atherogenesis.

1.2.1 Antigens in Atherosclerosis

A number of antigens have been identified as targets of disease-relevant immune-inflammatory responses. These include bacterial and viral antigens, though little experimental support exists for a causative involvement of infectious agents [40,41]. Other potential antigens are products of tissue injury, such as heat shock proteins, β 2glycoprotein I (β 2GPI), which is a cofactor for cardiolipin as autoantigen, and advanced glycation-end (AGE) products [9,19]. Major efforts have focused on the identification of antigens derived from LDL and its major protein component, ApoB-100, and several ApoB-100 peptides have been described as triggers of innate

immune responses and potential antigens for B and T cells [42–44]. These peptides, such as the recently identified ApoBDS-1, may act in concert with OxLDL as key mediators of inflammation in atherosclerosis.

Most evidence supports a role for epitopes that are generated when LDL is oxidized. Both enzymatic and nonenzymatic oxidation of LDL results in the generation of structural changes that lead to the formation of neoepitopes on the surface of OxLDL [45]. For example, when the oxidation-prone sn-2 polyunsaturated fatty acid of phosphatidylcholine undergoes oxidation, several highly reactive breakdown products, such as malondialdehyde (MDA) with its many complex condensation products, 4-hydroxynonenal (4-HNE), and the remaining “core aldehyde” 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC) are formed [46]. In turn, these aldehydes form covalent adducts with the amino groups of proteins and lipids resulting in the formation of oxidation-specific epitopes (OSE) that are recognized by specific immune responses in a hapten-specific manner [47]. Notably, OSE are also formed on the surface of dying cells and microparticles, which are circulating extracellular vesicles shed from dying or activated cells [48]. In this latter context OSE act as tags that identify cellular waste to housekeeping functions of immunity, eg, the clearance of dying cells. As both OxLDL and cellular debris accumulate in atherosclerotic lesions, the presence of the same OSE on both moieties also offers an explanation for the involvement of these specific immune responses in atherosclerosis.

1.3 Role of Innate Immunity in Atherosclerosis

Innate immunity mediates fast and blunt responses to protect the host by providing the first line of defense against instigating stimuli. Its responses are directed at the recognition of specific molecular patterns that signal “danger” to the host. In the case of microbes, these are termed pathogen-associated molecular patterns (PAMPs) [49]. However, endogenous self-molecules can also become dangerous to the host, eg, subsequent to tissue damage and/or excessive cell death, and also need to be sensed. Such “altered” self-molecules are recognized as damage-associated molecular patterns (DAMPs) by components of innate immunity [49]. Their recognition ensures swift responses to eliminate either microbial or endogenous antigens in order to prevent the negative consequences of tissue damage [50]. Innate immune responses involve a variety of humoral and cellular germline encoded pattern recognition receptors (PRRs), which are highly conserved and limited in their variability and specifically recognize PAMPs and DAMPs [51,52]. One prototypical example for a PAMP represents lipopolysaccharide (LPS), which binds to the toll-like receptor-4 (TLR-4), a prototypic cell surface PRR. Of note, PRRs also

exist in soluble form, such as CRP, which binds to PC conjugated to the lipoteichoic acid present on capsular polysaccharides of *S. pneumoniae* [51]. Representative for many PRRs, TLRs sense not only microbial PAMPs, such as LPS, but also DAMPs, including heat-shock proteins (HSP) and heparan sulfate [49,53]. Once they bind their cognate antigens, PRRs signal or phagocytose, and thereby play a major role in initiating the host response against dangerous stimuli [49]. Upon engagement of TLRs with their specific PAMP or DAMP, the inflammatory response gets amplified by the activation of transcription factors, such as NF- κ B, which results in the secretion of proinflammatory cytokines and induction of costimulatory molecules [54]. Thus, together with the chemokines and cytokines that are released when PAMPs or DAMPs are sensed, PRRs orchestrate an inflammatory response to eliminate the inciting trigger until complete resolution. Persistent stimulation and/or impaired resolution of innate immune responses will result in chronic inflammation, as observed in atherosclerosis [55].

Several PRRs have been implicated in the development of atherosclerotic lesions, and many of them specifically recognize OSE, which have been identified as major DAMPs of innate immunity [56,57]. OSE have been shown to be proinflammatory by activating endothelial cells leading to the upregulation of adhesion molecules and by inducing chemokine and cytokine secretion by monocytes/macrophages [58]. It is well established that OxPL upregulates the expression of a plethora of chemokines, such as MCP-1, IL-8, IL-6, and MIP-1 α [58]. Notably, the chemokines MCP-1 (CCL2) and IL-8 (KC) have been shown to play major roles in promoting atherosclerosis [59–61]. In addition, we recently demonstrated that a subset of circulating microparticles, which have been shown to be elevated in CVD patients [62], carry OSE that have the capacity to induce IL-8 chemokine secretion by monocytes [48]. Thus, OSE play an important role in the initiation phase of atherosclerosis.

Lesional macrophages sense DAMPs via TLRs, and experimental studies in TLR-deficient atherosclerosis-prone mice have shown that TLRs play an essential role in the progression of atherosclerosis [63–65]. Initially, the involvement of TLRs in atherosclerosis stems from studies using mice deficient in MyD88, which is the intracellular adaptor protein for TLR signal transduction. MyD88-deficiency in Apolipoprotein E-deficient (ApoE^{-/-}) mice resulted in diminished atherosclerosis [66]. TLR-2 has been shown to play a pro-atherogenic role, as deficiency of TLR-2 in low-density lipoprotein receptor-deficient (Ldlr^{-/-}) mice on cells, although not of bone marrow origin, led to reduced atherosclerosis [67]. This indicates the influence of an unknown endogenous TLR-2 agonist, which contributes to atherogenesis by the activation of TLR-2 in non-bone marrow cells, which likely include endothelial cells. However,

the exact ligand that is recognized by TLR-2 in atherosclerotic lesions remains elusive. Deficiency of TLR-4 also results in reduced atherosclerosis in cholesterol-fed ApoE^{-/-} mice [66,68]. The involvement of TLR-4 in lesion progression is explained by its ability to mediate pro-atherogenic responses of endothelial cells and macrophages to various OSE [69,70]. For example, oxidized cholesterol-esters that are enriched in minimally modified LDL as well as the oxidized phospholipid oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (OxPAPC) activate macrophages via TLR-4 to secrete proinflammatory chemokines and cytokines, such as CXCL2 and IL-6, respectively [71,72]. Notably, TLR-4 has been shown to heterodimerize with TLR-6 in the response to OxLDL [73]. Both the OxLDL-induced activation and chemokine expression of Gro1 α , MIP-2 and RANTES of macrophages have been shown to require a heterodimer of TLR-4/TLR-6 in cooperation with the scavenger receptor CD36, which mediates binding and uptake of OxLDL [73]. Interestingly, epidemiological studies in humans have identified that a polymorphism in TLR-4 (Asp299Gly), which is associated with decreased levels of proinflammatory cytokines as well as acute phase proteins and soluble adhesion molecules, is associated with a reduced risk of carotid artery disease (CAD) [74]. However, this could not be confirmed by another study [75].

Other TLRs have also been implicated in the inflammatory response in atherosclerosis. For example, TLR-3, which is located in the endosome and senses dsRNA, as well as TLR-7, which senses ssRNA, have both been implicated as atheroprotective in studies using the ApoE^{-/-} mouse model [76,77]. Recently, a study in TLR-9^{-/-} ApoE^{-/-} mice has revealed an atheroprotective role for TLR-9, which is located in the endosome and recognizes bacterial DNA via CpG [78]. TLR-9-deficient ApoE^{-/-} mice fed an atherogenic diet showed accelerated atherosclerosis, which is mediated by CD4⁺ T cells as depletion of those cells resulted in attenuation of atherosclerosis.

In conclusion, TLRs are critically involved in the inflammatory response in the artery wall that is characterized by the secretion of specific cytokines and chemokines, which is reviewed in detail elsewhere [8].

Another family of PRRs, scavenger receptors (SRs), have a key function in atherosclerosis, as they are responsible for the uncontrolled uptake of OxLDL, but not native LDL, resulting in the formation of foam cells – a critical pathological event in atherogenesis. A variety of SRs have been identified, such as CD36, scavenger receptor A (SRA)-1 and SRA-2, SR-BI, MARCO, and LOX-1 [56,79,80]. Of these, CD36 and SRA-1 have been found to be responsible for nearly 90% of OxLDL-uptake by macrophages through the recognition of different OSE in OxLDL [81,82]. In the case of CD36, oxidized

phospholipids mediate binding of OxLDL via two different motifs—the PC head group on the one hand and the oxidized sn-2 fatty acid on the other hand [83,84]. Among all SRs, CD36 and SRA are most prominently investigated in foam cell formation. Initial studies have shown that both CD36 and SRA are important for the formation of foam cells, as peritoneal macrophages isolated from SRA- and CD36-deficient mice account for 90% of modified LDL uptake [85]. This would suggest that genetic deletion of these SRs in mice should ameliorate atherosclerosis. However, *in vivo* studies showed mixed results. Initial studies by Febbraio et al. revealed a pro-atherogenic role, as CD36^{-/-} ApoE^{-/-} mice were protected from atherosclerosis [86]. A bone marrow transplantation study of CD36-deficient macrophages into ApoE^{-/-} mice performed by the same authors also resulted in markedly diminished atherosclerosis, thereby establishing a proatherogenic role of CD36 on macrophages [87]. Another study investigating high-fat diet-fed ApoE^{-/-} mice deficient in CD36 and SRA showed increased atherosclerosis despite a severe reduction of foam cells in peritoneal macrophages *in vivo*, thereby suggesting a different mechanism of lipid deposition in the artery wall [88]. Moreover, combined deficiency of both CD36 and SRA resulted in no further reduction of atherosclerosis compared to CD36^{-/-} ApoE^{-/-} control mice, suggesting a limited role of SRA in atherogenesis [89]. Taken together, the role of SRs in atherosclerosis is sometimes not clearly observed in animal studies using genetic knockout models [79,88,89]. However, as several SRs are responsible for uptake of OxLDL, the effect of one genetically targeted SR may be compensated by other scavenger receptors that may be expressed at different levels in different tissues. Finally, besides their role in foam cell formation, SRs have been shown to be involved in lesional macrophage proliferation [90] and in mediating proinflammatory responses by TLRs (see above).

One of the consequences of OxLDL uptake and foam cell formation by macrophages is the intracellular accumulation of cholesterol crystals [91]. Cholesterol crystals are found at all stages of atherosclerosis and are in particular enriched in advanced lesions, where they are thought to trigger the physical rupture of the fibrous cap [92]. However, they have recently been found to also be involved in the inflammatory response in atherogenesis through the engagement of intracellular sensing mechanism of the inflammasome. The inflammasome, which consists of an adaptor protein apoptosis-associated speck-like protein containing CARD (ASC), as a sensor molecule, connected to caspase-1, as a molecular platform for the activation of the proinflammatory protease, and caspase-1 that leads to the release of proinflammatory IL-1 β and IL-18 [93]. Duewell et al. showed that following lysosomal damage, cholesterol crystals can activate the NLRP3 inflammasome in macrophage foam cells, which leads to the activation of caspase-1 and the secretion of IL-1 β in atherosclerotic

lesions [91]. Consequently, atherosclerosis-prone mice that were reconstituted with the bone marrow of NLRP3-deficient donors develop decreased atherosclerosis when fed an atherogenic diet [91]. As activation of the NLRP3 inflammasome by cholesterol crystals leads to the generation of IL-18, these mice also exhibited reduced IL-18 levels. Data on the role of IL-18, which is a promoter of Th1 differentiation, in atherosclerosis points toward a pro-atherogenic role. IL-18-deficient ApoE^{-/-} mice showed diminished expression of IFN- γ as well as increased IgG levels and reduced atherosclerosis compared to controls [94]. Of note, IL-18^{-/-}ApoE^{-/-} mice showed increased serum cholesterol and triglycerides compared to controls, indicating that IL-18 also has effects on lipid metabolism. On the other hand, administration of IL-18 to ApoE^{-/-} mice led to increased atherosclerosis and lesional inflammation, and these effects were found to be dependent on IFN- γ [95]. Recently, an additional receptor for IL-18 has been identified, which is the NaCl cotransporter (NCC or SLC12A3) [96]. The authors have demonstrated that loss of the IL-18 receptor in ApoE^{-/-} mice alone does not have any effect in atherosclerosis, whereas codepletion of NCC reduces atherosclerosis. These data suggest that the NaCl cotransporter has the capacity to mediate the full pro-atherogenic effect of IL-18. Rajamäki et al. have shown that the NLRP3 activation pathway is also functional in cholesterol crystals-exposed human macrophages, thereby linking cholesterol metabolism with inflammation in the context of atherosclerosis [97]. Taken together, experimental studies in mice identify cholesterol crystal-induced inflammasome activation and products thereof as inflammatory components in atherogenesis. CD36-mediated binding of OxLDL has been found to coordinate these responses via priming which is TLR-4/6-dependent and expression of pro-IL-1 β , which are the required signals for the full activation of the NLRP3 inflammasome in macrophages, as well as via endocytic uptake of OxLDL resulting in cholesterol crystal accumulation [98].

Thus, OSE represent proinflammatory DAMPs that engage several PRRs to promote inflammatory responses in atherosclerosis. In addition to their presence on OxLDL, OSE on microparticles and dying cells can also contribute to inflammatory responses [99]. In particular, later stages of atherosclerosis with excessive accumulation of OxLDL are also associated with increased foam cell apoptosis [55]. In turn, uptake of apoptotic foam cells also contributes to cholesterol uptake by macrophages, promoting a vicious cycle of disease promotion. Furthermore, impaired clearance of apoptotic foam cells results in their accumulation, which further renders them proinflammatory and contributes to the chronic inflammatory process of atherosclerosis [55]. Resolution of inflammation depends on the efficient clearance of apoptotic cells, a mechanism called efferocytosis. Efficient apoptotic cell clearance would prevent secondary necrosis thereby

limiting inflammation. In fact, the swift uptake of apoptotic cells has been shown to induce anti-inflammatory cytokines, such as TGF- β and IL-10, which may diminish plaque progression in certain settings, such as early stages of lesion development [55,100]. Several studies have identified a critical role for apoptotic cell accumulation in atherogenesis. In this regard, MFGE8 (milk fat globulin E8) expressed by macrophages has been identified as a bridging molecule, which promotes the phagocytosis of apoptotic thymocytes via the interaction of phosphatidylserine on apoptotic cells and α v β 3 integrin on phagocytes [101]. Ait-Oufella et al. reconstituted irradiated Ldlr^{-/-} mice with Mfge8^{-/-} bone marrow, which were then fed an atherogenic diet and displayed systemic as well as lesional accumulation of apoptotic cells [102]. Furthermore, these mice had reduced splenic IL-10 as well as increased splenic and lesional IFN- γ accompanied by changes in suppressive functions of Tregs dependent on dendritic cells (DCs). Interestingly, mice reconstituted with Mfge8^{-/-} bone marrow also displayed increased levels of circulating microparticles. Thus Mfge8 plays a role in controlling the accumulation of apoptotic cells within atherosclerotic plaques, consequently reducing atherosclerotic lesion development. Another protein that is implicated in apoptotic cell clearance represents mer receptor tyrosine kinase (MertK). Its role in atherosclerosis has been addressed by two independent studies. One study investigated high-fat diet-fed MertK-deficient ApoE^{-/-} mice and observed increased atherosclerosis accompanied by more lesional apoptotic cells and larger necrotic cores [103]. On the other hand, Ait-Oufella et al. reconstituted Ldlr^{-/-} mice with mertk-deficient bone marrow and observed increased apoptotic cell accumulation, as well as an inflammatory plaque phenotype and enhanced atherosclerosis [104]. The classical way of complement activation has also been shown to promote the clearance of apoptotic cells. In this regard, Ldlr^{-/-} mice deficient in C1q have increased lesional apoptotic cells and display larger atherosclerotic lesions [105]. Furthermore, deficiency of transglutaminase-2 (TG2), which is a regulator of protein crosslinking, in bone marrow transplanted Ldlr^{-/-} mice fed an atherogenic diet has been associated with increased apoptotic cells inside lesions [106]. Collectively, these data implicate different ways of apoptotic cell disposal in the context of atherosclerosis. Notably, both TLR-4 as well as scavenger receptors have been also implicated in the recognition of microparticles and apoptotic cells [107,108].

1.4 Role of the Adaptive Immune System in Atherosclerosis

Adaptive immunity is represented by the activation of T and B cells that recognize antigens via B cell receptors (BCR; membrane bound Ig) and T cell receptors

(TCRs), respectively. In contrast to innate PRRs, these receptors are a product of somatic recombination and give rise to a nearly unlimited repertoire of specificities. Following antigen encounter different responses are effected by T and B cells to eliminate antigens. These responses are under tight control by several mechanisms that prevent exaggerated or inappropriate responses, including elimination of self-reactive clones as well as the downregulation of initiated responses. Accumulating evidence now suggests that the balance and the tight regulation are lost in atherosclerosis, resulting in proinflammatory responses and a failure of resolution. Histological evidence for the presence of both activated CD4⁺ and CD8⁺ T cells and antigen presenting cells as well as immunoglobulins in atherosclerotic plaques initiated decades of research investigating the role of adaptive immune functions in atherosclerosis [109]. In fact, clonal expansion of T cells has been demonstrated in human lesions by TCR spectratyping from coronary artery specimen as well as in lesions at different stages in ApoE^{-/-} mice [110,111], indicating the occurrence of antigen-specific reactions inside the lesions. Moreover, plaque T cells were found to have a restricted usage of TCRs indicative of an oligoclonal expansion [112]. Moreover, T cells isolated from lesions were found to specifically proliferate in response to OxLDL when presented in an MHC-II restricted manner and immunoglobulins eluted from lesions were found to be complexed with OxLDL [112,113]. This is complemented by an increasing number of clinical studies demonstrating a significant association of antibody titers to OxLDL and HSP60 as well as frequencies of specific T and B cell subsets with CVD [114–116].

For example, an increased frequency of CD8⁺ T cells has been identified as associated with a higher incidence of coronary events [117]. In addition, higher circulating levels of CD4⁺CD28^{null} cells are associated with poor prognosis and recurrence of acute coronary syndrome [118]. Regarding the role of circulating Tregs, clinical studies have revealed conflicting results. One study investigated circulating Tregs in patients with carotid or coronary atherosclerosis and did not find a correlation with CAD [119]. This is in contrast to previous studies, which reported that patients with acute coronary syndrome show a decreased frequency of circulating Tregs in their blood [120–122]. We may speculate that discrepancies of these analyses result from differences in the assessment of these T cell subpopulations. In contrast to T cells, only a few B cells are present inside the plaques. Nevertheless, systematic analyses of GWAS as well as of gene expression data of peripheral blood from the Framingham Heart Study have causally linked B cell immune responses in CVD [123].

Thus several lines of evidence suggest involvement of adaptive immunity in human atherosclerosis, and the profoundly increased cardiovascular risk in patients with autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), provides additional support to this notion.

Critical evidence for the involvement of adaptive immunity comes from models of experimental atherosclerosis. This is best documented by the fact that Ldlr^{-/-} or ApoE^{-/-} mice rendered lymphocyte-deficient (through deficiency in Rag1^{-/-}, Rag2^{-/-} or SCID) develop significantly less atherosclerosis, suggesting a net pro-atherogenic effect of adaptive immunity [124–126]. However, even in the absence of adaptive immunity mice develop atherosclerotic lesions and when cholesterol levels are exceedingly high no effect of lymphocyte deficiency is observed [127]. Thus, adaptive immune functions are not required for lesion formation, but are important modulators of the disease process. Moreover, the complete absence of both T and B cells may result in the elimination of both pro- and anti-atherogenic subsets that possess different activities in atherosclerosis. Thus the dissection of the individual functions of different T and B cell subsets have been the focus in recent years in an effort to understand the complex interplay of adaptive immune responses in atherosclerosis as well as mechanisms by which these can affect disease acceleration in certain conditions.

1.4.1 T Cells in Atherosclerosis

CD4⁺ Th cells have been identified as a prominent subset of atherosclerotic lesions of all stages of disease [128] (see Fig. 4.1). Based on the cytokines they secrete they can polarize into different subsets exhibiting different effector functions. Th1 cells, which produce proinflammatory cytokines, such as IFN- γ , are critical involved in the initiation and progression of atherosclerosis [129]. IFN- γ exerts pro-atherogenic activities, including the induction of adhesion molecules as well as chemokine expression by endothelial cells, the stimulation of proinflammatory cytokines and chemokines by macrophages, the production of reactive oxygen species as well as matrix metalloproteinases by macrophages, the inhibition of cholesterol efflux from foam cells as well as preventing collagen synthesis by vascular smooth muscle cells [130]. In addition, IFN- γ exerts its functions as activator of monocytes/macrophages as well as dendritic cells, consequently resulting in an ongoing pathogenic Th1 response [32]. Administration of IFN- γ accelerated atherosclerosis in ApoE^{-/-} mice [131], whereas ApoE^{-/-} or Ldlr^{-/-} mice deficient in IFN- γ display reduced atherosclerosis [132,133]. In these settings, T cell-independent IFN- γ secreted by macrophages, natural killer cells as well as vascular cells were found to be responsible for disease progression. All of these

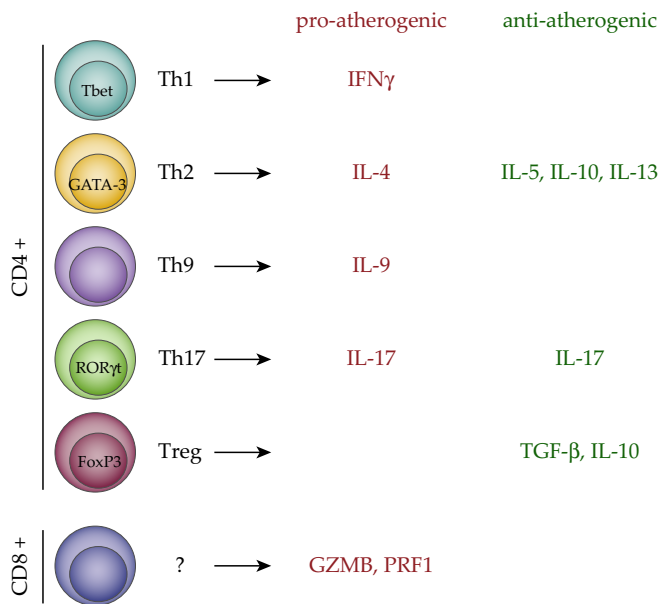


FIGURE 4.1 The role of T cells in atherosclerosis. Th1 cells, which differentiate under the control of the transcription factor Tbet, secrete IFN- γ and promote atherosclerosis. Th2 cells are the producer of IL-4, IL-5, IL-10 and IL-13, which have shown to possess different roles in atherosclerosis. The recently identified Th9 cells produce IL-9, which is suggested to play a pro-atherogenic role. However, a direct implication of IL-9 as well as Th9 cells in atherosclerosis has to be elucidated. Th17 cells produce IL-17, but the exact role in the context of atherosclerosis is controversial. Tregs are the producers of the cytokines IL-10 and TGF- β , which are atheroprotective. CD8 $^{+}$ T cells have been suggested to be pro-atherogenic via a mechanism involving perforin- and granzyme-B-mediated apoptosis. Adapted from the personal collection of the authors.

experimental studies performed point towards the role of IFN- γ in lesion promotion. Indeed, IFN- γ secreting T cells have been isolated from human plaques [134]. The prominent role of Th1 responses in atherogenesis is strongly supported by a number of studies demonstrating that atherosclerosis is promoted by IFN- γ and cytokines, such as IL-12 and IL-18, which induce Th1 cell differentiation and IFN- γ secretion [135]. Moreover, impaired Th1 differentiation in mice lacking the Th1 lineage-specific transcription factor Tbet results in reduced atherosclerosis [136]. On the other hand, the role of type I interferons (including IFN- α and IFN- β) in atherosclerosis remains to be explored, as different studies have revealed both pro- as well as anti-inflammatory functions [137]. For example, IFN- α produced by aortic plasmacytoid dendritic cells (pDC) has been shown to several-fold upregulate TNF-related apoptosis-inducing ligand (TRAIL) on CD4 $^{+}$ T cells, consequently inducing the cytolytic functions for smooth muscle cells [138]. Moreover, IFN- α has been shown to promote the uptake of OxLDL as well as foam cell formation via the upregulation of the expression of SRA [139]. Recently, more light was shed on the role of pDCs in atherosclerosis. Ldlr $^{-/-}$ mice selectively deficient for the transcription

factor Tcf4, which results in loss of pDCs, were protected from atherosclerosis and displayed reduced Th1 cells [140]. Moreover, treatment of Ldlr $^{-/-}$ and ApoE $^{-/-}$ mice with IFN- β results in increased atherosclerosis by enhancing macrophage adhesion to sites of atherosclerotic plaque formation in a chemokine-dependent manner [141]. It is speculated that upregulated chemokines as well as their receptors play a role in accelerated atherosclerosis in this model. Interestingly, IFN- β treated mice exhibited more lesional macrophages, despite no differences in total cholesterol accompanied by increased IL-10 production compared to controls. The same authors also investigated the role of the major receptor for type I IFN, which is called IFNAR1, by transplanting IFNAR1 $^{-/-}$ bone marrow cells into Ldlr $^{-/-}$ mice [141]. As a result, mice exhibited markedly reduced atherosclerosis as well as decreased necrotic cores inside the lesions.

Th17 cells are the main producers of IL-17, but their role in atherosclerosis remains controversial, as both anti- as well as pro-atherogenic effects for IL-17 have been found in experimental atherosclerosis [142,143]. However, experimental studies using IL-17 blocking antibodies or genetic deletion of IL-17A in Ldlr $^{-/-}$ as well as ApoE $^{-/-}$ mice favor a pro-atherogenic role of Th17 cells and IL-17, as this resulted in decreased atherosclerotic lesion formation, reduced numbers of lesional macrophages and ROS generation, and diminished expression of proinflammatory cytokines, such as TNF- α and IL-6 [144–146]. In contrast, administration of IL-17 has been shown to decrease atherosclerosis [143,147] and IL-17 deficiency accelerated atherogenesis [147], thereby also identifying atheroprotective roles for IL-17. Thus experimental studies of the role of Th17 cells and IL-17 in atherosclerosis require further investigation [148].

Similarly, data on the function of Th2 cells, which are characterized by the secretion of IL-4, IL-5, and IL-13, and downregulate Th1-mediated responses, stimulate antibody production by B cells, and promote alternative macrophages activation [149] provide mixed results. IL-4 has been suggested to be pro-atherogenic, although Ldlr $^{-/-}$ mice deficient in IL-4 did not show any effect in atherosclerosis in one study [150], previous work by the same group has indeed demonstrated decreased atherosclerosis [151,152]. In contrast, a protective role for IL-5 and IL-13 has been established in experimental atherosclerosis [153]. We have shown that IL-5 possesses the capacity to stimulate atheroprotective OxLDL-specific natural IgM and that cholesterol-fed Ldlr $^{-/-}$ mice reconstituted with IL-5-deficient bone marrow develop increased atherosclerosis [154]. On the other hand, IL-13 protects from atherosclerosis through the promotion of a favorable plaque phenotype and the induction of anti-inflammatory alternatively activated macrophages [155]. However, these protective Th2 cytokines are also secreted by type 2 innate lymphoid cells [156], suggesting that these effects are independent of Th2 cells.

Although CD8⁺ cytotoxic T cells are less abundant in atherosclerotic plaques, they may have a role particularly in advanced atherosclerosis. Depletion studies using monoclonal antibodies for CD8 α and CD8 β in high-fat diet fed ApoE^{-/-} mice have shown that CD8⁺ cells mediate proinflammatory pro-atherogenic effects [157]. To gain mechanistic insights, Kyaw et al. performed adoptive transfer experiments of CD8⁺ T cells deficient in the cytolytic enzymes perforin and granzyme-B into lymphocyte-deficient ApoE^{-/-} mice, which did not increase atherosclerosis. These enzymes mediate target cell lysis upon the activation of CD8⁺ T cells into cytotoxic T cells and therefore promote apoptosis-induced inflammation and necrosis [158]. In addition, the authors also showed that TNF- α is implicated mechanistically, as CD8⁺ T cells are potent producers of this cytokine, which is an important inflammation modulator. These data provide experimental evidence for a pro-atherogenic role for CD8⁺ T cells via perforin and granzyme-B mediated cytotoxicity as well as TNF- α promoted inflammatory mechanisms.

T-cell responses are tightly regulated by regulatory T cells (Tregs)—most prominently Foxp3 expressing CD4⁺CD25⁺ $\alpha\beta$ TCR⁺ cells. Notably, a series of studies have identified a clear atheroprotective role for Tregs, which suppress and counterbalance immune responses by regulating costimulation or secreting specific cytokines, including the atheroprotective cytokines IL-10 and TGF- β . Depletion studies by anti-CD25 [159] or immunization with Foxp3-transfected DCs [160] have demonstrated an atheroprotective role of Tregs. Moreover, direct transfer experiments of Tregs have demonstrated that these can limit T-cell responses and lead to diminished atherosclerosis [161,162]. In addition, blocking or deficiency of TGF- β and IL-10 has been shown to increase atherosclerosis [163–166]. It can be speculated that enhancing the suppressive functions of Treg may provide an attractive method to inhibit pro-atherogenic responses of both CD4⁺ and CD8⁺ T cells.

1.4.2 B Cells in Atherosclerosis

Several studies over the past 15 years have shown that B cells are key modulators of hypercholesterolemia-induced inflammatory milieu [191], despite the fact that they are present in low numbers in both murine and human atheromas [167–169]. GWAS data support a role of B cells in human atherosclerosis by involving proliferation and activation status of B cells as important factors in CVD risk [123]. In agreement with this, increased numbers of activated CD19⁺CD86⁺ B cells were found to be associated with increased risk for stroke [170].

In support of the human data, a large set of experimental studies has investigated the role of B cells in atherosclerosis. Caligiuri et al. was the first to address how B cells affect experimental atherosclerosis by investigating the effect of adoptive transfers of B cells into

splenectomized atherosclerosis-prone ApoE^{-/-} mice [171]. The authors reported that B cells isolated either from wild type or ApoE^{-/-} donors reversed the splenectomy-induced accelerated atherosclerosis. Notable, the protective effect was even more profound in the splenectomized recipients that received B cells from ApoE^{-/-} donors. These data were in line with a study showing that lethally irradiated Ldlr^{-/-} mice that were injected with bone marrow isolated from B-cell deficient (μ MT) donors developed increased atherosclerosis compared to recipients that received wild type bone marrow [172].

Although these studies indicate an atheroprotective role of B cells, one has to keep in mind that B cells are a very heterogeneous population including several different subsets with sharply different properties with respect to their activation, immunoglobulin profile secretion as well as differentiation pathways. B cells are divided in two large subsets: the conventional B2 and B1 populations. For example, B2 cells (which include follicular (FO) and marginal zone B (MZB) cells) are short lived, they are derived from the bone marrow, and they become activated mainly in a T-cell dependent manner. On the other hand, B-1 cells have fetal liver origin and they produce pre-existing, germline-encoded natural IgM antibodies in the absence of T cell help [173,174]. Thus dissecting the role of individual not only subsets but also activation pathways is important in order to translate these findings into the clinic (see Fig. 4.2).

Along these lines, treatment of hypercholesterolemic ApoE^{-/-} and Ldlr^{-/-} mice with the B-cell depleting anti-CD20 antibody, which preferentially depletes B2 cells and results in a strong reduction of total IgG titers, while it largely preserves B-1a cells and natural IgM, confers an atheroprotective effect [175,176]. The atheroprotective effect of anti-CD20 treatment was attributed to enhanced Th17 responses as anti-IL-17 antibody treatment abrogated the atheroprotective effect of anti-CD20 injections. These data suggest that B2 cells exhibit a pro-atherogenic effect. This conclusion is supported by the finding that adoptive transfer of splenic B2 cells into lymphocyte-deficient Rag2^{-/-} γ -chain^{-/-}ApoE^{-/-} or μ MT/ApoE^{-/-} recipients enhanced atherosclerosis [176]. It is important to note that the latter data imply that B2 cells are also able to aggravate atherosclerosis in absence of T cells; however, the mechanisms by which they do so require further investigation. Furthermore, studies that investigate the role of the B cell activating factor (BAFF) system in atherosclerosis also suggest a pro-atherogenic role for B2 cells. BAFF binds to BAFF receptors (BAFFR) and thereby facilitates the survival of B2 cells (note that this pathway is indispensable for the survival of B-1 cells) [177]. Therefore BAFFR-deficient animals lack B2 cells [178]. In line with this, Baffr^{-/-}ApoE^{-/-} mice, lethally irradiated Ldlr^{-/-} that were transplanted with BAFFR-deficient bone marrow, and ApoE^{-/-} mice treated with

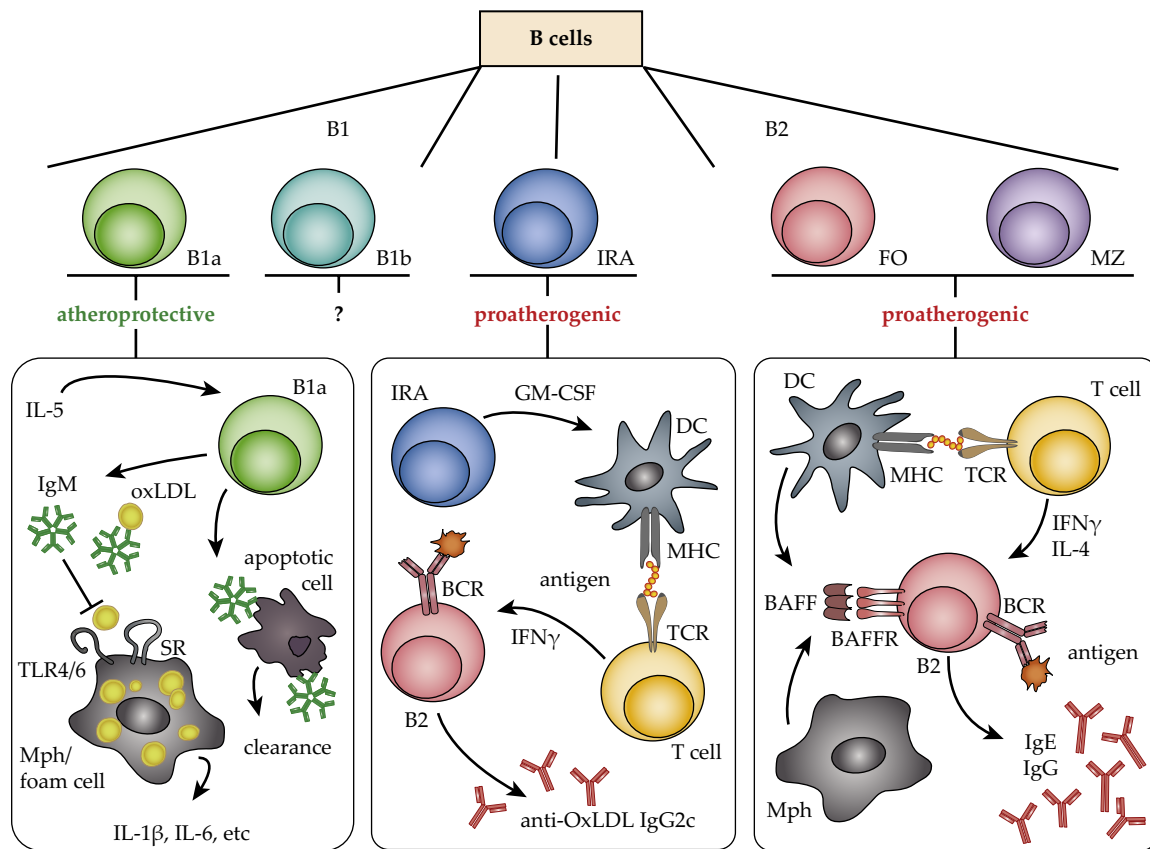


FIGURE 4.2 B-cell subsets and their role in atherosclerosis. B cells can be subdivided into the major subsets B-1 and B2. B-1 cells can be further distinguished into B-1a and B-1b subsets, of which both secrete natural IgM and have been shown to be atheroprotective. In contrast to this, B2 cells comprising FO and MZB cells have been suggested to be pro-atherogenic – however, the mechanisms are not yet clear. IRA B cells, which are a subset of B-1a cells and secrete GM-CSF, have been shown to promote atherosclerosis via the expansion of anti-OxLDL IgG_{2c} antibodies. BAFFR, B-cell activating factor receptor; BCR, B-cell receptor; GM-CSF, granulocyte macrophage colony-stimulating factor; MHC, major histocompatibility complex; Mph, macrophage. Adapted from Tsiantoulas et al. [191].

an anti-BAFFR blocking antibody lack B2 cells and develop decreased atherosclerosis [179–181]. However, the pro-atherogenic role of B2 cells has been challenged by Doran et al., who observed decreased atherosclerosis in μ MT/ApoE^{-/-} mice upon adoptive transfer of splenic B2 cells isolated from ApoE^{-/-} donors [182]. Thus further studies are required to pinpoint the exact role of B2 cells in atherosclerosis.

In contrast, the data on the role of B-1 cells suggest a protective role in atherosclerosis [191]. B-1 cells predominantly localize in the peritoneum and pleural cavities and are divided as B-1a and B-1b cells [173]. Evidence regarding the atheroprotective role of B-1a cells comes from Kyaw et al., who showed that adoptive transfer of natural IgM secreting B-1a cells (in contrast to sIgM^{-/-} B-1a cells) into splenectomized ApoE^{-/-} mice, which develop accelerated atherosclerosis and display 50% reduced plasma IgM, reversed the splenectomy-induced accelerated atherosclerosis [183]. Moreover, a recent report by Rosenfeld et al. demonstrated that adoptive transfer of B-1b cells into Rag1^{-/-} ApoE^{-/-} mice resulted

in decreased lesion formation compared to PBS injected controls [184]. The limitation in both these studies is that they address the role of the B-1 cell subsets in specific contexts (splenectomy and lymphocyte deficiency) and do not allow conclusions for the role of these cells in intact conditions. For example, a recently identified B-1a derived cell subset, the innate response activator (IRA) B cells [185], have been shown to expand in the spleen of hypercholesterolemic mice and interestingly they aggravate atherosclerosis, presumably via enhancing Th1 responses and anti-OxLDL IgG_{2c} production [186]. These data suggest that increasing the B-1a numbers could result in enhanced IRA B-cell generation and thus more atherosclerosis. Therefore studies addressing the role of B-1 cells in an intact immune system are of great interest in order to gain confidence regarding their atheroprotective role.

Finally, the potential role of B regulatory cells (Breg) in atherosclerosis has recently been investigated. Breg are characterized by their increased production of the atheroprotective cytokine IL-10 [187,188]. Strom et al.

recently demonstrated that Breg increased in the lymph nodes of hypercholesterolemic ApoE^{-/-} mice. Interestingly adoptive transfer of lymph node-derived B cells into ApoE^{-/-} recipients resulted in an atheroprotective effect that was dependent on the ability of these cells to produce IL-10 [189]. In line with this, the authors also reported that purified Breg (defined as CD21^{high}CD23^{high}CD24^{high}) from the lymph nodes of ApoE^{-/-} donors also resulted in atheroprotection. While these data suggest that Breg confer atheroprotection via IL-10 secretion, Sage et al. reported that B-cell derived IL-10 does not affect atherosclerotic lesion formation [190]. Taken together, further studies are also required to elucidate the role of Breg in atherosclerosis.

1.4.3 Humoral Immunity in Atherosclerosis

An important role for immunoglobulins in atherosclerosis is supported by both epidemiological and experimental studies [192]. Epidemiological studies—though not consistently—have reported a positive association of anti-OxLDL or anti-Hsp65 IgG antibodies and CVD adverse effects [153,193]. Experimental studies mainly addressing the effect of immunization against prominent antigens of atheromas such as Hsp65 and OxLDL have overall suggested a pro-atherogenic role for IgG (see Fig. 4.3). For example, immunization of normocholesterolemic rabbits or chow diet-fed Ldlr^{-/-} mice with Hsp65 accelerated atherosclerosis [194]. These data are in line with a study by George et al. who infused IgG preparations from Hsp65-immunized mice into chow-fed Ldlr^{-/-} mice, which resulted in enhancement of fatty-streak formation [195]. On the other hand, immunization of Ldlr^{-/-} WHHL rabbits with homologous MDA-LDL resulted in the strong induction of MDA-LDL specific IgG titers and decreased atherosclerosis compared to controls [196]. Similar data were obtained from ApoE^{-/-} or Ldlr^{-/-} mice that were immunized with MDA-LDL [154,197–200].

An explanation for these discrepancies could be that IgG antibodies consist of different subclasses (humans: IgG1, IgG2, IgG3, IgG4; mice: IgG1, IgG2a/c, IgG2b, IgG3) and therefore activate different families of Fcγ receptors (activating or inhibitory) [201,202]. For example, ApoE^{-/-} mice lacking the Fcγ-chain and thus lack all activating receptors, but still express the inhibitory receptor FcγRIIB, develop decreased atherosclerosis [203]. In line with this, ApoE^{-/-} mice that lack the inhibitory FcγRIIB receptor display increased atherosclerosis [204].

Despite its particularly low abundance in plasma, IgE seems to possess an important role in the development of atherosclerosis (see Fig. 4.3). Epidemiological studies have reported a positive association between plasma IgE levels and CVD risk [192,205]. Importantly, IgE antibodies have recently shown to promote and correlate with active SLE [206]. The latter is particularly important as

SLE patients are characterized by premature atherosclerosis development and high prevalence of myocardial infarction [207]. Recently, a study by Wang et al. suggested a disease-promoting role for IgE in experimental atherosclerosis by examining atherosclerosis-prone mice that lack the high affinity receptor for IgE (FcεRI). These mice developed significantly reduced both plaque size and complexity. Moreover, the authors reported that IgE were able to promote macrophage and smooth muscle cell activation and/or death [208]. However, it remains unknown what the endogenous antigens that IgE recognize are and if antigen binding is required.

A major protective role in atherosclerosis has been attributed to IgM antibodies (see Fig. 4.3). The secreted form of IgM antibodies—in contrast to IgG and IgE—consist of a pentameric structure. The vast majority of IgM antibodies (~80%) are produced in the absence of any T cell or cognate help. These natural antibodies are germline encoded and appear very early in life [173,192,209]. We have previously shown that >35% of IgM recognize different OSE [210]. Previous studies have shown that OSE-specific natural IgM have the capacity to block OxLDL uptake and promote the clearance of apoptotic cells [211]. For example, the T15/E06 clone, which binds to PC of oxidized phospholipids, has been shown to block the recognition of POVPC by the scavenger receptor CD36 [212]. Moreover, the OSE-specific clone NA-17, which recognizes MDA, has been shown to promote uptake of apoptotic cells by macrophages [210,213]. These properties of natural IgM are particularly important in counteracting atherosclerosis progression. In line with this, we have shown that cholesterol-fed atherosclerosis-prone mice immunized with heat-killed *S. pneumoniae* developed strongly increased T15/E06 antibodies and decreased atherosclerosis [214]. These results were in agreement with a study by Faria-Neto et al., who reported reduced vein graft atherosclerosis in ApoE^{-/-} mice that received T15/E06 IgM antibodies [215]. Further evidence of the atheroprotective role of T15/E06 comes from our studies in which immunized mice with MDA-LDL developed high titers of T15/E06 antibodies in an IL-5 dependent manner and decreased atherosclerosis [154]. These data are also supported by epidemiological studies showing an inverse correlation of anti-OxLDL IgM plasma levels and CVD risk [192]. Of great interest, similar data were reported for SLE patients. For example, it has been shown that SLE patients display an inverse association between low anti-PC IgM levels (even when normalized to total IgM) and atherosclerotic plaque incident [216,217]. These studies are particularly interesting considering the premature atherosclerosis development in SLE patients and point to an important immunomodulatory role of anti-OxLDL IgM. Therefore identifying the crucial epitopes on OxLDL as well as the right vaccination approach that favors the expansion of such atheroprotective IgM would be of particular interest in the prevention of atherosclerosis.

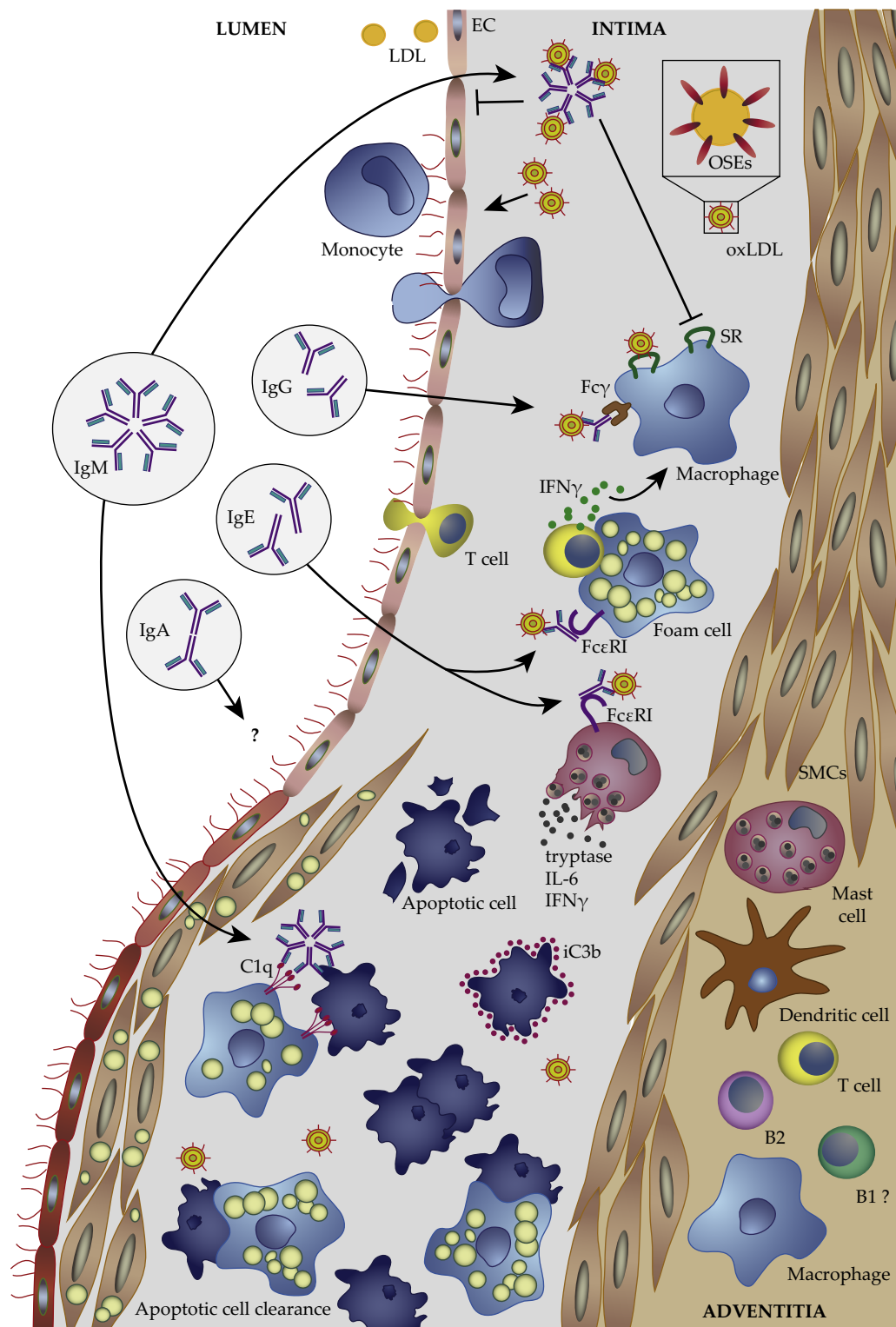


FIGURE 4.3 Immunoglobulins and their role in atherosclerosis. A variety of immunoglobulins are present in atherosclerotic plaques. For example, HSPs and OxLDL are prominent antigens for these immunoglobulins. IgM antibodies exert a variety of atheroprotective functions, such as the neutralization of proinflammatory properties of OxLDL, inhibition of the uptake of OxLDL by macrophages, and promotion of the clearance of apoptotic cells. IgG antibodies specific for OxLDL have been suggested to be pro-atherogenic via the activation of macrophage Fcγ-receptors. IgE antibodies have been shown to promote plaque destabilization via the activation of mast cells and macrophages via FcεRI engagement. The role of IgA antibodies in atherosclerosis remains elusive. Adapted from Tsiantoulas et al. [192].

For example, we recently identified peptide mimotopes of MDA that are recognized by specific human IgM antibodies [218].

The studies above suggest a protective role of increased (above physiological) levels of IgM. These protective effects are mediated via preventing foam cell formation and promotion of apoptotic cell clearance. Interestingly, reduced levels of IgM result in aggravated atherosclerosis. Lewis et al. has shown that mice deficient in secreted IgM develop accelerated atherosclerosis [219]. In agreement with this, Kyaw et al. reported that the accelerated atherosclerosis in ApoE^{-/-} mice upon splenectomy, which also results in 50% reduced plasma IgM, is entirely reversed by adoptive transfer of IgM secreting B-1a cells (reconstitution of plasma IgM) compared to mice that received sIgM^{-/-} B-1a cells [183]. However, both studies provide no evidence that the IgM deficiency or the protective effect of IgM normalization is attributed to changes in apoptotic cell or OxLDL clearance. These data suggest that the pro-atherogenic effect of deficiency or reduced levels is mediated via different—currently unknown—mechanisms. Notably, secreted IgM deficiency in a lupus-prone mouse model has been shown to accelerate autoantibody formation and enhanced glomerulonephritis and decreased survival [220]. Taken together, we may speculate that low IgM levels could also be a risk factor for CVD in SLE patients.

2. ASSOCIATION WITH AUTOIMMUNE AND INFLAMMATORY DISEASES

From the data reviewed above it is clear that alterations of specific immune functions can have a profound effect on the initiation and progression of atherosclerotic lesions, including plaque rupture and thrombosis, which lead to the clinical manifestations of atherosclerosis. Thus it is not surprising that certain chronic inflammatory and autoimmune diseases that are characterized by dysregulation of different layers of immunity are epidemiologically linked with accelerated atherosclerosis. In addition to the profound impact of inflammation on atherogenesis, dysfunctional B and T cell responses as well as the pathological accumulation of apoptotic cells may provide a critical link between atherosclerosis and autoimmune disorders. For example, both rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are associated with a sharply increased cardiovascular risk and as underlying pathologies represent a major risk factor for the development of atherosclerosis. Both chronic inflammation as well as the specific immune responses present in these diseases contribute to the premature manifestation of atherosclerosis in these settings [221]. Therefore RA and SLE have a profound impact on cardiovascular morbidity as well as mortality [222].

2.1 Rheumatoid Arthritis

RA, which affects approximately 1% of the general population, is a destructive disorder affecting the joints of hands and feet and resulting from chronic inflammation and immune dysregulation [223]. RA patients exhibit a higher prevalence and earlier onset of atherosclerosis compared to the general population [224–226]. Thus RA itself represents a significant independent risk factor for early atherosclerosis. A higher and earlier plaque burden as well as a more unstable/vulnerable plaque phenotype has been reported in RA patients [227–230]. In turn, because traditional CVD risk factors, such as smoking, diabetes, dyslipidemia, hypertension, and metabolic syndrome, are known to promote chronic inflammation, they also have the potential to influence the inflammatory response in RA [231].

A number of GWAS studies identified genetic risk factors for both RA and CVD. For example, a polymorphism in the human leukocyte antigen (HLA-DRB1) gene has been implicated to increase the risk for CVD by increasing systemic inflammation as reflected by elevated hsCRP levels [232]. Thus identifying the key epitope in this setting and developing so-called peptide-mimotopes that would block the interaction of this HLA complex with the respective antigen-specific T cells would be an attractive therapeutic strategy. Other polymorphisms that have in part been shown to be also associated with CVD include genes of the NFκB signaling pathway, MHC2TA coding for MHC expression, and IRF5 coding for an interferon regulating factor [29,232–238].

However, very little is known about the pathogenesis of accelerated atherosclerosis in the context of RA. Most likely multiple factors, including chronic inflammation as well as genetic factors, act in concert with traditional risk factors for CVD to mediate premature atherosclerosis in RA patients (Fig. 4.4). Interestingly, both RA and atherosclerosis share similar inflammatory pathways [231]. CRP is elevated in

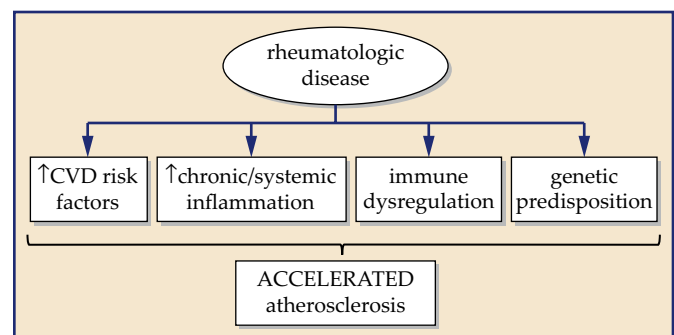


FIGURE 4.4 An interplay between different factors leads to enhanced atherosclerosis. Rheumatologic diseases are thought to result from an interplay of different factors, involving increased CVD risk factors, increased chronic and/or systemic inflammation, dysregulation of the immune system, and genetic predisposition. All of these place individuals at a higher risk for developing accelerated atherosclerosis. Adapted from the personal collection of the authors.

RA patients and has been identified as independent risk factor for CVD [239]. High levels of proinflammatory cytokines in RA have been associated with the premature development of atherosclerosis [240]. For example, the proinflammatory cytokines TNF- α , IL-6, and IL-17 are considered key drivers for joint damage in RA, and all three have been implicated to various extents in the pathogenesis of atherosclerosis as discussed above. Thus TNF- α and IL-6 expression in RA-affected joints may also act on distant tissues such as the vasculature and promote atherosclerosis. The systemic inflammation in RA can also contribute to endothelial dysfunction as well as oxidative stress, thereby promoting atherogenesis [226]. Increased reactive oxygen species (ROS) as well as oxidative stress have been shown to play a role in the pathogenesis of RA [241].

The fact that disease activity of RA is associated with an increased CVD risk suggests that the severity and duration of inflammation-causing damage and impairment of the joints also favors the onset and progression of atherosclerosis [242–247]. Further support for a role of chronic inflammation or the predisposition for it as underlying mechanisms for accelerated atherosclerosis is provided by the identification of periodontitis as a common risk factor for both diseases [248–250].

Interestingly, active inflammation in RA has been shown to result in a reduction in both LDL and HDL cholesterol levels and increased triglycerides [251]—a phenomenon that has been termed “lipid paradox,” as low cholesterol levels are associated with higher risk for developing CVD in these patients. In addition, the atheroprotective HDL cholesterol in RA patients has in some instances been found to correlate with CVD [252,253]. A possible explanation for this discrepancy to conventional CVD is that RA-associated inflammation can result in the modifications of lipoproteins, including HDL, thereby altering their structure and function and rendering them pro-atherogenic [254]. For example, modified HDL is rendered dysfunctional and may lose its anti-inflammatory potential [6]. This might further promote the oxidation of LDL, enhance foam cell formation, and impair cholesterol efflux, thereby further contributing to CVD risk [255,256].

Cellular immunity has also been implicated to contribute to an increased risk of CVD in RA, as the frequency of CD4⁺CD28^{null} T cells were found to be increased in RA patients with preclinical atherosclerosis [257]. This unusual subset of T cells represents TNF- α and IFN- γ producing effector T cells with cytotoxic functions [258] and has been described in patients with RA as well as in patients with unstable angina [259,260]. This suggests that dysregulated T cell responses in RA could contribute to accelerated atherosclerosis in these patients. Notably, blockage of TNF- α —a common treatment for RA—restored CD28 expression, thereby suggesting an additional mechanism by which anti-TNF therapy impacts on CVD [257]. Although little is known about the role of B cells in RA-associated

accelerated atherosclerosis, anti-CD20 B cell depletion strategies that represent another therapy in RA have been shown to decrease lesion formation in atherosclerosis-prone mice. On the other hand, atheroprotective B cells, such as B-1 cells, may also play a role in RA development. In this regard it is of interest that infusion of the B-1 cell derived OxLDL-specific T15/E06 natural IgM antibody has been shown to suppress disease in collagen-induced arthritis (CIA) models in mice [261].

2.2 Antiphospholipid Syndrome

The antiphospholipid syndrome (APS) is characterized by the presence of arterial or venous thrombotic events and/or pregnancy morbidity in patients positive for antiphospholipid antibodies (aPL), such as anticardiolipin (aCL) or anti- β 2-glycoprotein I (anti- β 2GPI) antibodies, or the lupus anticoagulant (LA) [262]. The major cause of morbidity and mortality in APS are thrombotic events, resulting from recurrent thrombocytopenia and a procoagulant state. Other manifestations of this disease include deep vein thrombosis, pulmonary thromboembolism, stroke, transient ischemic attack, and coronary artery disease [263].

Some studies suggest that risk factors for premature atherosclerosis are an additional clinical feature of APS. For example, the presence of high levels of aCL antibodies, which are the most abundant autoantibodies in APS, has been found to be associated with an increased risk of myocardial infarction in a prospective study of middle-aged dyslipidemic men [264]. Another study found elevated levels of aCL and anti- β 2GPI antibodies as well as anti-OxLDL antibodies in patients with coronary heart disease compared to controls [265]. Interestingly, some antiphospholipid antibodies have been identified as antibodies directed against oxidized phospholipids, as human APS patient aCL bound only to oxidized cardiolipin but not cardiolipin unable to undergo oxidation [266]. Moreover, β 2GPI has been shown to be recognized by some antibodies from APS patients only when bound to oxidized cardiolipin but not to unoxidized cardiolipin [267,268]. This indicates that many aCL are directed toward neopeptides generated after oxidation of cardiolipin.

Of note, β 2GPI is found to be abundantly present and to colocalize with CD4⁺ T cells in specimens of human atherosclerotic plaques [269], suggesting β 2GPI as a potential antigen for immune responses that promote atherosclerotic lesion progression. Interestingly, stimulation of macrophages with OxLDL in the presence of β 2GPI and aCL antibodies has been shown to enhance binding and uptake of OxLDL [270]. Nevertheless, the exact role of these IgG antibodies in foam cell formation remains elusive.

Experimental animal models also support an association between APS and accelerated atherosclerosis. For example, immunization of Ldlr^{-/-} mice with human

aCL IgG preparations from an APS patient induced high titers of aCL antibodies and led to aggravated atherosclerosis [271]. Furthermore, immunization of both *Ldlr*^{-/-} and *ApoE*^{-/-} mice with β 2GPI induced high levels of anti- β 2GPI antibodies and also resulted in increased atherosclerosis compared to nonimmunized controls or mice immunized with ovalbumin [272,273]. The aggravated atherosclerosis is thought to be mediated in part by specific T cells, as adoptive transfer of β 2GPI-reactive lymphocytes isolated from immunized mice increased atherosclerosis in recipient *Ldlr*^{-/-} mice [274]. These data suggest that autoimmune responses associated with APS accelerate atherosclerosis and identify β 2GPI as the potential antigen responsible for it.

2.3 Systemic Lupus Erythematosus

SLE is a potentially fatal autoimmune disease characterized by acute and chronic inflammation of various tissues of the body [275]. It is caused by autoimmune dysregulation and predominantly occurs in young women. The reported prevalence of CVD in SLE patients ranges from 6% to 10% in a variety of cohorts [276–278]. Interestingly, premature atherosclerosis already develops at an early stage of SLE, which makes older age at diagnosis an even stronger predictive factor of atherosclerosis in SLE [278]. Although traditional CVD risk factors, such as high blood pressure, high cholesterol, diabetes, and smoking account for an increased risk of atherosclerosis in SLE patients, they are not sufficient to explain the increased prevalence of atherosclerosis and its manifestations, such as myocardial infarction (MI) and stroke, in SLE patients [279]. Notably, after controlling for traditional risk factors, patients with SLE still have a substantial increased risk of cardiovascular disease [279]. This suggests that additional SLE-specific risk factors exist, and that the high risk for atherosclerosis in SLE patients results from a combination of both traditional and SLE-specific risk factors. In this regard, novel biomarkers, such as autoantibodies against apoA-I, the major protein of HDL, have been found to be associated with accelerated atherosclerosis in SLE [280]. Nevertheless, autoimmune disease itself may be the strongest risk factor for cardiovascular disease in SLE. The etiology of the increased risk for atherosclerosis in SLE is likely multifactorial, resulting from a complex interplay between traditional CVD risk factors and SLE-driven inflammation.

Several potential mechanisms for accelerated atherosclerosis in SLE have been investigated, and much evidence exists for the fact that dysregulation or activation of specific immune functions result in an acceleration of atherosclerosis in mouse models of lupus when crossed onto atherosclerosis-susceptible backgrounds. Among others, increased activation of CD4⁺ T cells and macrophages,

production of type I interferons, as well as impaired apoptotic cell clearance have been investigated in atherosclerosis-prone mouse models of lupus that are typically characterized by anti-dsDNA antibodies and glomerulonephritis to varying degrees [281–284] (Table 4.1).

For example, increasing evidence points to an important role for impaired efferocytosis in atherosclerosis progression [14,55], and excessive generation or impaired clearance of apoptotic cells is considered a key contributing factor to SLE, as it can result in the activation of autoimmune responses and the production of pathogenic autoantibodies against self-antigens. Indeed, mice with inactivating mutations in the proapoptotic Fas (*lpr*) or FasL (*gld*) present lupus-like autoimmunity compared to human disease. The *lpr* mutation leads to inactivation of the Fas receptor, consequently resulting in autoreactive T and B cells characterized by anti-ssDNA antibodies as well as lymphoproliferation [285]. *Lpr* mice crossed onto an autoimmune background, such as MRL, develop accelerated lupus [286]. The *gld* mutation represents a point mutation in a gene-encoding murine FasL and results in the development of a mild form of spontaneous autoimmune disease in mice on a C57BL/6 genetic background. Furthermore, these mice are characterized by the production of low levels of autoantibodies and mild splenomegaly, lymphadenopathy, and glomerulonephritis [286–289]. The combination of the *gld* mutation and *ApoE*-deficiency (*gld.ApoE*^{-/-} mice) has been shown to result in more severe autoimmunity than is seen with the *gld* mutation alone and more severe atherosclerosis than is seen with *ApoE*-deficiency alone [281]. The increased atherosclerosis in these mice is explained by the defective clearance of apoptotic cells directly, as well as an increased accumulation of proinflammatory OxLDL, which in part competes for the same clearance mechanisms, thereby enhancing the inflammatory response as well as the accumulation of apoptotic cells. Similarly, *ApoE*^{-/-}*Fas*^{-/-} mice that exhibit increased levels of apoptotic cells have also been shown to develop accelerated atherosclerosis in the context of lupus-like disease despite lower cholesterol levels [290,291]. Accelerated atherosclerosis was also accompanied by increased levels of antibodies to OxPL in these mice. Ma et al. also evaluated atherosclerosis in MRL/*lpr* *ApoE*^{-/-} mice, which developed higher cholesterol levels and also increased atherosclerosis [291]. To obtain additional mechanistic insights, Gautier et al. reconstituted irradiated *Ldlr*^{-/-} mice with the bone marrow of *gld* mice and fed them an atherogenic diet for 12 weeks [283]. Despite similar plasma lipid levels with a comparable lipoprotein profile and preserved renal function, recipients of *gld* bone marrow developed increased atherosclerosis, demonstrating that FasL deficiency in the hematopoietic compartment is sufficient to accelerate lesion formation. Consistent with a defect in apoptotic cell clearance, these mice displayed

TABLE 4.1 Summary of Mouse Models to Study the Mechanisms of Accelerated Atherosclerosis in SLE

Mouse	Mouse model lupus	Mouse model atherosclerosis	Description of mutation/method	Diet	Total cholesterol	Atherosclerosis	Other characteristics	References
gld.ApoE ^{-/-}	gld	ApoE ^{-/-}	Inactivating mutation in FasL	12 weeks Western diet	↓	Increased	Impaired apoptotic cell clearance, lymphadenopathy, splenomegaly, autoantibodies	Aprahamian et al. [281]
BMT: Sle1.2.3→Ldlr ^{-/-}	Sle	Ldlr ^{-/-}	Mutation in lupus susceptibility genes	8 weeks Western diet	↓	Increased	Increased activation of CD4 ⁺ T and B cells, higher antibody levels to OxLDL and cardiolipin	Stanic et al. [282]
Ldlr.Sle	Sle	Ldlr ^{-/-}	Mutation in lupus susceptibility genes	8 weeks Chow diet	↓	Increased	Inflammatory cell accumulation in lesions	Braun et al. [303]
ApoE ^{-/-} Fas ^{-/-}	Fas (lpr)	ApoE ^{-/-}	Inactivating mutation in Fas	5 months Chow diet	↓	Increased	Increased autoantibodies, increased apoptotic cells in renal and vascular lesions, osteopenia	Feng et al. [290]
BMT: gld→Ldlr ^{-/-}	gld	Ldlr ^{-/-}	Inactivating mutation in FasL	12 weeks Western diet	No difference	Increased	Aortic apoptotic cell accumulation, autoantibodies, increased CD4 ⁺ T cell activation	Gautier et al. [283]
MRL/lpr.ApoE ^{-/-}	MRL/lpr	ApoE ^{-/-}	ApoE ^{-/-} crossed onto MRL/lpr	24 weeks Chow diet	↑	Increased	Increase in antibody titers	Ma et al. [291]
Irf5 ^{-/-} .gld.ApoE ^{-/-}	gld	ApoE ^{-/-}	Gain-of-function polymorphisms in IFN regulatory factor 5	12 weeks Western diet	↑	Increased	Reduced lupus, metabolic dysregulation: Hyperlipidemia, increased adiposity insulin resistance	Watkins et al. [298]
BMT: Irf5 ^{-/-} .gld.ApoE ^{-/-}	gld	ApoE ^{-/-}		6–9 weeks Western diet	↑	Increased		Watkins et al. [298]
BMT: CD4 ⁺ T cells (B6.Sle)→Ldlr ^{-/-} Rag ^{-/-}	Sle	Ldlr ^{-/-} Rag ^{-/-}	Effects of B6.Sle CD4 ⁺ T cells in atherosclerosis	10 weeks Western diet	No difference	Increased	Dysregulation of IL-17 production, decreased IL-10R expression of B6.Sle Treg	Wilhelm et al. [304]

an accumulation of apoptotic cells in the aortic plaques. Moreover, accelerated atherosclerosis was also found to be associated with increased recruitment of monocytes and lymphocytes, enhanced generation of autoantibodies, and CD4⁺ T cell activation [283].

Type-I interferons play a central role in SLE pathogenesis, as they play a major role in sustaining the activation of autoreactive T and B cells, which in turn are responsible for the production of pathogenic autoantibodies [292]. Interestingly, expression profiling studies of peripheral blood cells of SLE patients revealed a spontaneous expression of type I IFN-induced genes, thereby identifying type I IFNs as central in the development of SLE [293–295]. Moreover, gain-of-function polymorphisms in IFN regulatory factor 5 (IRF5) have been associated with an increased risk for developing lupus [296]. In line with this, deficiency of IRF5 in mouse models of lupus has been shown to improve disease outcome. Although IFN- γ is considered to be one of the major proinflammatory cytokines in atherosclerosis, a detrimental role for type I IFNs (IFN- α and - β) has also been established, as discussed above [297]. Thus type I IFNs may play an important role in accelerated atherosclerosis associated with SLE [284]. Watkins et al. recently evaluated the effect of IRF5 deficiency on lupus-accelerated atherosclerosis by breeding IRF5^{-/-} mice with gld.ApoE^{-/-} mice and feeding them a Western diet for 12 weeks. Interestingly, they made the surprising observation that mice developed more atherosclerosis due to increased plasma cholesterol levels, despite reduced severity of lupus [298]. Moreover, bone-marrow chimeric gld.ApoE^{-/-} mice lacking IRF5 in the hematopoietic compartment developed a similar metabolic phenotype (hypertriglyceridemia and hypercholesterolemia) and also displayed increased atherosclerotic lesion formation [298]. These data identify a protective role for IRF5 in lupus-associated atherosclerosis and suggest that these atheroprotective effects are independent of the promotion of proinflammatory Th1-dependent immune responses that aggravate lupus. As possible mechanism for atheroprotective functions of IRF5, TLR-7, and TLR-9-dependent IL-10 secretion has been suggested, as reduced IL-10 production in several immune cells as well as diminished aortic IL-10 expression was observed in IRF5-deficient gld.ApoE^{-/-} mice. Indeed, several studies demonstrate robust anti-atherogenic properties for IL-10.

Another model that provided insights into the pathogenesis of accelerated atherosclerosis in lupus has been the exploitation of the lupus-susceptible mouse strain NZM2410 that has been derived onto a C57BL/6 background to generate B6.Sle mice. Three major chromosome regions, ie, Sle1, Sle2, and Sle3, that harbor lupus susceptibility have been identified in the NZM2410 mouse strain [299]. Studies of mice with combined or single congenity for these loci identified Sle1 as a mediator of loss of tolerance to nuclear antigens [300], Sle2 as responsible for lowering the activation threshold of

B cells resulting in an expansion of B-1 cells as well as IgM [301], and Sle3 for reducing the activation threshold of T cells and increasing cell-dependent polyclonal IgG as well as reducing activation-induced cell death [302]. Cholesterol-fed Ldlr^{-/-} mice that were reconstituted with bone marrow of B6.Sle mice developed anti-dsDNA autoantibodies, and lupus nephritis [282]. Importantly, despite lower plasma cholesterol levels recipients of B6.Sle bone marrow exhibited significantly accelerated atherosclerosis with a three-fold higher T cell content in lesions consistent with increased numbers of splenic CD4⁺ cells. Moreover, both IgG₁ and IgG_{2a} antibodies to OxLDL and cardiolipin were increased in B6.Sle bone marrow chimeric Ldlr^{-/-} mice compared to recipients of wild type bone marrow. Interestingly, despite no difference in total IgM, aCL IgM antibodies were robustly elevated in Ldlr.Sle mice. Of note, atherosclerosis in bone marrow chimeric Ldlr.Sle mice was found equally accelerated when mice were fed a regular chow diet, suggesting that immune dysregulation in these mice is dominant and increased atherogenesis independent of cholesterol levels [303]. Moreover, using adoptive transfer experiments of CD4⁺ T cells from B6.Sle mice into Ldlr^{-/-}Rag^{-/-} mice, Major and colleagues showed that the effector T cells are sufficient to mediate the pro-atherogenic effect of B6.Sle [304]. T cells exhibited an increased frequency of IL-17 producing cells and decreased expression of the IL-10 receptors, rendering them less responsive to Treg-mediated suppression. This finding is in line with a study showing that SLE patients with atherosclerosis exhibit increased levels of IL-17 in the serum compared to healthy controls without atherosclerosis, thereby confirming a potential pro-atherogenic role for IL-17 in SLE [305].

Wade et al. also investigated whether the gene interval Sle3 that is associated with increased T cell activation and autoantibody production is responsible for accelerated atherosclerosis mediated by this chromosome region by generating bone marrow chimeric Ldlr^{-/-} mice with bone marrow from B6.Sle3 mice and feeding them an atherogenic diet for 8 weeks [306]. Interestingly, despite increased autoantibody titers to dsDNA and dysregulated T cell response characterized by polyclonal IgG antibodies and a decrease in activation-induced cell death in CD4⁺ T cells, no difference in atherosclerotic lesion formation was found when mice were fed an atherogenic diet. Although decreased plasma cholesterol levels in these mice could be in part responsible for these results, the data suggest that Sle3-associated dysregulation of T cells alone is not sufficient to promote atherosclerosis.

Thus a series of experimental models support the epidemiological link of increased CVD in SLE. In particular, models with defects in apoptotic cell clearance due to mutations in the Fas/FasL system have clearly demonstrated an impact on accelerated lesion formation.

Moreover, pathological T cell activation has also been associated with accelerated lesion formation in some lupus models. However, it remains to be shown whether these are directly responsible or whether they only reflect increased disease activity in these models.

3. STRATEGIES TO MINIMIZE THE RISK OF ATHEROSCLEROSIS IN AUTOIMMUNITY

As mentioned above, the premature atherosclerosis and the high CVD risk of SLE and RA patients cannot be explained by classical Framingham risk factors such as total cholesterol, low HDL, age, and smoking [207]. This suggests that an altered immunity in these patients directly influences atherosclerosis development. Consequently, the development of therapeutic approaches that target different arms of immunity is of great interest for the treatment of the accelerated CVD risk in these patients.

Although there is an overwhelming number of experimental studies that clearly demonstrate a crucial role of inflammation and the immune system, the therapeutic value of these findings has not been investigated in humans yet. However, two ongoing large phase III clinical trials, the Cardiovascular Inflammation Reduction Trial (CIRT) [307] and the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) [308], aim to provide more insight into the causal role of inflammation in human atherothrombosis. The CIRT trial includes 7000 MI patients who suffer from type 2 diabetes or metabolic syndrome and are randomly treated with either low-dose methotrexate or placebo. The CANTOS trial investigates whether neutralization of using a human monoclonal anti-IL-1 β antibody (Canakinumab) results in reduction of CVD clinical events. The CANTOS trial includes >17,000 MI patients with elevated CRP levels (>2 mg/L) that will receive either placebo or three different doses of Canakinumab (50, 100, or 300 mg) every three months. Interestingly, low-dose methotrexate and Canakinumab is a common treatment or being validated in clinical trials, respectively, for RA patients.

T cells exhibit both protective as well as pathological properties in rheumatologic diseases [257,282,283,304,306]. Thus the development of therapeutics that modulate T cell responses appears to be an attractive option. For example, Abatacept, which is the decoy form of the CTLA4 (cytotoxic T lymphocyte associated four) receptor fused onto an Ig backbone (CTLA4-Ig), is used for the treatment of RA patients. CTLA4 prevents T cell activation by blocking the interaction between the costimulatory molecules CD80/86, which are present on antigen presenting cells, and the costimulatory receptor CD28 on the T cell surface [309]. The role of the CD80/86 pathway in atherosclerosis is

not clear as experimental studies have yielded opposite results [310]. However, it has been recently shown that treatment of Apoe^{-/-} mice with CTLA4-Ig decreased homocysteine-accelerated atherosclerosis [311]. Thus it would be worthwhile to investigate whether CTLA4-Ig treatment also confers a protective effect against the premature atherosclerosis in RA patients.

Because B cells are major players in promoting the pathology of several autoimmune diseases, including SLE and RA, via the production of autoantibodies [312], the development of therapeutic agents that target B cells has gained a lot of attention. For example, Rituximab, an anti-CD20 monoclonal antibody that results in an Fc γ -mediated B cell elimination [313], was the first B cell-targeting biological approved for RA patients. As treatment of atherosclerosis-prone mice with anti-CD20 resulted in decreased atherosclerotic plaque size, CVD risk in patients treated with Rituximab may also be reduced. Thus it will be of considerable interest to monitor atherosclerosis and CVD risk in this group of RA patients.

In line with this, therapeutic targeting of the BAFF–APRIL (B-cell activating factor—a proliferation inducing ligand) system has been investigated in SLE [314]. The BAFF–APRIL system consists of two ligands, BAFF and APRIL, which are expressed in soluble and membrane bound form, and three receptors BAFFR, the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and B cell maturation antigen (BCMA). These receptors are expressed predominately on B cells and play an important role in cell survival and antibody class switching. For example, BAFFR or BCMA activation facilitates B2 and plasma cell survival, respectively. TACI is involved in antibody class switching [177]. Belimumab, a blocking antisoluble BAFF antibody, was the first drug approved for SLE in 50 years [314]. Belimumab blocks the binding of BAFF to BAFFR leading to B cell depletion and consequently to a strong decrease in both total and anti-dsDNA immunoglobulin titers [315]. Although the effect of anti-BAFF treatment in atherosclerosis has not yet been investigated, BAFFR-deficient or mice treated with a blocking anti-BAFFR antibody, which depletes B2 cells but preserves the atheroprotective B-1 cell subset, exhibit reduced atherosclerosis. This suggests BAFF blockage with Belimumab might have a beneficial effect in premature atherosclerosis of SLE patients. It is important to mention that although Rituximab is by far more efficient in depleting B cells than Belimumab, it has failed to show any benefit for SLE in two independent double-blind phase II/III clinical trials [316]. These data challenge the current paradigm that the protective effect of Belimumab in SLE is mediated via its net B cell depleting effect and raises the question of whether BAFF affects the responses of other cell types besides B cells.

In addition to the biologicals mentioned above, Atacicept, which is decoy form of the TACI receptor fused onto an Ig backbone (TACI-Ig), has been tested in a phase II/III clinical trial for SLE [317]. Atacicept neutralizes both BAFF and APRIL and results in mature B cells as well as plasma cell depletion and a robust decrease in total antibody levels [318,319]. The results of the phase II/III clinical trial indicate that Atacicept might have a protective effect when administered in a high dose. However, further investigation is required as the recruitment of patients in the high-dose group was completed prematurely due to two unexpected deaths [317]. Similar to anti-BAFF, the effect of TACI-Ig in atherosclerosis remains elusive. It is tempting to speculate that TACI-Ig might confer an atheroprotective effect due to B2 cell depletion. On the other hand, the strong decrease in total immunoglobulins including the atheroprotective IgM upon TACI-Ig treatment, may mask its atheroprotective B cell depleting properties.

Taken together, studying patients with rheumatologic diseases that receive T and B cell modulating therapies would extend our knowledge on the role of adaptive immunity and will allow the development of more precise therapeutic options in atherosclerosis.

4. CONCLUSION

Autoimmune rheumatic diseases are characterized by premature atherosclerosis and a robustly increased CVD risk. However, the underlying mechanisms for this remain elusive. As traditional risk factors cannot entirely explain the premature atherosclerosis, it is tempting to hypothesize that altered immunity of these patients is responsible for this. This is particularly interesting as despite the success of LDL-lowering drugs to decrease CVD adverse effects in the general population, a highly significant risk still remains and thus novel therapeutic approaches are needed. The immune component of atherosclerosis appears to be currently the most attractive target. As mentioned above, several immunomodulatory treatments are available in the clinic and notably are used for the treatment of patients with rheumatic diseases. Thus addressing the effect of immunomodulation in atherosclerosis in these patients presents an excellent opportunity, and such studies may be of great interest as they would generate conclusive evidence regarding the potential therapeutic benefit of targeting the immune system in atherosclerosis and CVD.

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A Study of Cardiac Function, Atherosclerosis, and Arrhythmogenicity

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1. INTRODUCTION

Some, if not the vast majority of adults, develop a degree of coronary atherosclerosis throughout their life-time. In certain patients, this process may be asymptomatic, while in others cardiovascular related morbidity and mortality may develop including cases when this happens at a young age [1]. Atherosclerosis is a slow yet progressive multifactorial process involving the arterial wall. It is influenced by genetic, immune, and metabolic factors, diet, physical exercise, drugs, and systemic diseases. The process usually begins in childhood and progresses into adulthood where clinical complications may occur. Early in the course of atherosclerosis, endothelial dysfunction appears. The endothelium plays a pivotal role in regulating the vascular tone. It affects also cell adhesiveness, vascular growth, and the regulation of the coagulation cascades. Abnormal endothelial function in the presence of an established vascular plaque also increases plaque vulnerability and the risk for ischemic vascular events [2].

The presence of endothelial dysfunction can be evaluated by measuring the degree of brachial artery vasodilation induced by endogenous nitric oxide (NO) secretion. The most commonly applied technique is known as flow-mediated dilatation (FMD). Nitroglycerin-mediated dilation (NMD) is a different method for evaluating smooth muscle integrity in response to exogenous NO supplementation. A more advanced disease of the vessel wall can be diagnosed based on the presence of increased subintimal thickness (also known as increased intima-media thickness; IMT) and the presence of vascular atherosclerotic plaque (commonly visualized in the carotid artery). Further progression of the atherosclerosis

lesion is characterized by plaque calcification, which can be detected on coronary CT angiography and quantified with a coronary calcium score (CCS). The progressive nature of the atherosclerotic process and the different tests that may be used for its quantification is illustrated in Fig. 5.1 [3]. Importantly, arterial-wall disease may manifest by increased arterial-wall stiffness. These changes in the arterial-wall properties can be quantified by calculating arterial pulse-wave velocity (PWV) and arterial-wall distensibility.

Ischemic heart disease may also predispose to ventricular arrhythmias. Attempting to predict the occurrence of ventricular and supraventricular arrhythmias, many non-invasive electrocardiographic screening tools have been proposed including T-wave abnormality, QT variability index (QTVI), heart-rate turbulence (HRT), signal-averaged electrocardiogram (ECG), and T-wave alternans. A diagnosis of cardiac ischemia may advocate the need for invasive coronary interventions. Therefore various diagnostic tools have been developed in an attempt to quantify myocardial perfusion, coronary anatomy, and the degree of coronary artery stenosis. It is generally acknowledged by most physicians that clinical integration of the information generated from different diagnostic modalities is necessary to provide the clinician with a solid evidence-based clinical decision-making process. Furthermore, available tests differ in sensitivity and specificity in various patient groups and therefore use of different diagnostic techniques is considered necessary for patient screening (Table 5.1) [4]. Some tests remain experimental (like FMD, QTVI, etc.), while others are commonly used in clinical practice. More than a few practical clinical guidelines are available to assist the clinician in deciding which tests are

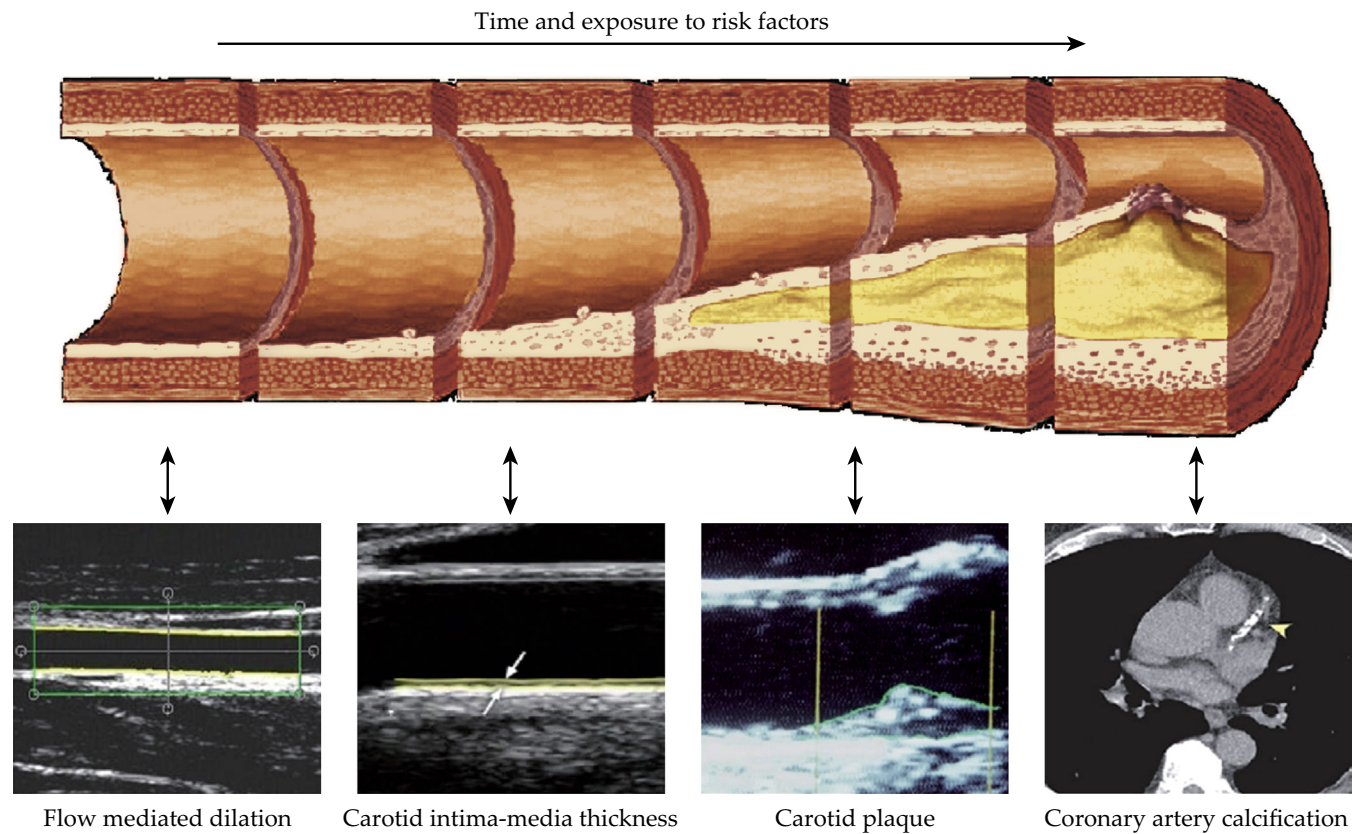


FIGURE 5.1 The progressive nature of atherosclerosis, as manifested in different diagnostic modalities. Adapted from Peters and Bots [3].

TABLE 5.1 Sensitivity and Specificity of Different Diagnostic Tests for the Identification of Coronary Artery Disease

Test	Sensitivity (%)	Specificity (%)
Exercise ECG	45–50	85–90
Exercise stress echocardiography	80–85	80–88
Exercise stress SPECT	73–92	63–87
Dobutamine stress echocardiography	79–83	82–86
Dobutamine stress MRI	79–88	81–91
Vasodilator stress echocardiography	72–79	92–95
Vasodilator stress SPECT	90–91	75–84
Vasodilator stress MRI	67–94	61–85
Coronary CT angiography	95–99	64–83
Vasodilator stress PET	81–97	74–91

PET, positron emission tomography; SPECT, single-photon emission computed tomography.
Adapted from Montalescot et al. [7].

more suitable for each patient. These are published frequently by the American College of Cardiology (ACC) [5] and the European Society of Cardiology (ESC) [6]. While asymptomatic patients without comorbidities usually

require little screening, the presence of cardiovascular risk factors (not only the well-known physical risk factors such as hypertension or increased body weight but also socioeconomic and lifestyle risk factors such as persistent stress or smoking) and a systemic proinflammatory state might predispose for complications, which merits further clinical investigation and patient screening.

The aim of the current chapter is to review a battery of selected cardiac tests developed for evaluating coronary disease and detecting risk of arrhythmias. The chapter can be used as a concise guide for the clinician who wishes to utilize toolkits for cardiac testing of patients with systemic diseases. This chapter will also aid in understanding the other chapters in this book, where different cardiac tests are described for patients with rheumatic immune and systemic inflammatory conditions.

2. ENDOTHELIUM

The endothelium consists of a continuous group of endothelial cells located on the basal lamina, with each cell being about 1–2µm thick and 10–20µm in diameter (Fig. 5.2). Endothelial cells overlap each other and form a tight seal on the vascular wall, and this is perhaps why they were historically considered as simple barriers between the blood and vascular-wall interface.

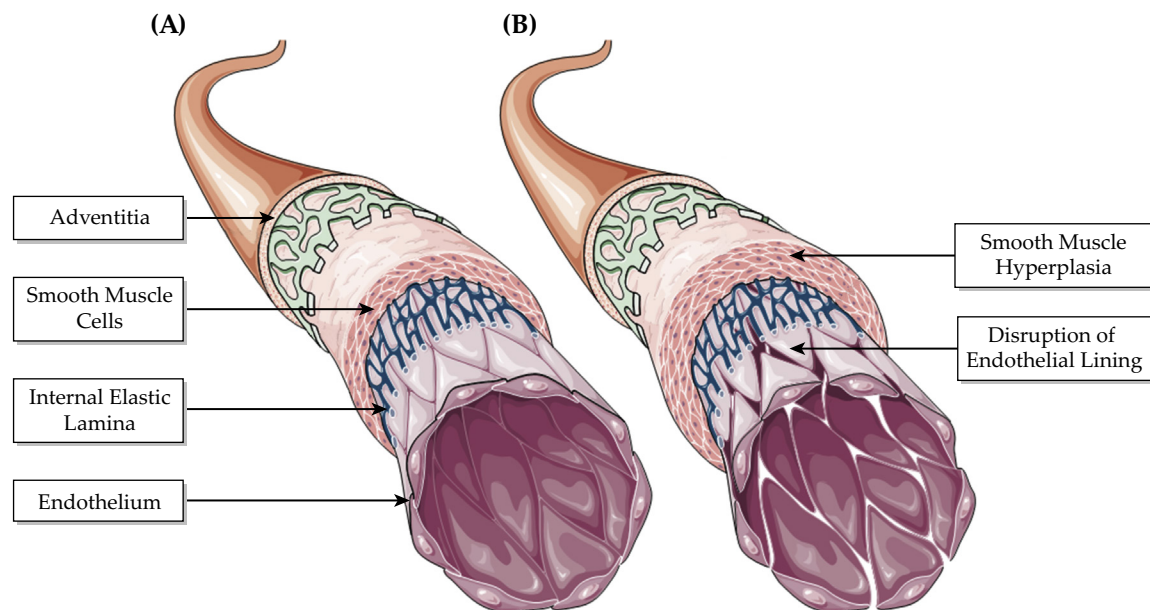


FIGURE 5.2 The vascular wall is made up of three layers; the intima (endothelial cells located on internal elastic lamina), the tunica media (smooth muscle cells), and the tunica external (adventitia). A healthy vessel contains endothelial cells that are tightly sealed and impermeable to damaging stimuli (A). Disruption of the endothelial lining from injurious stimuli allows gaps to form between endothelial cells causing proliferation of smooth muscle cells (B). *Adapted from the personal collection of Dr. A. Sandoo, Bangor University, Bangor, Wales, UK.*

However, it is now known that endothelial cells contain pinocytotic vesicles allowing passage of small molecules, water, and proteins from the blood into the deeper layers of the vessel as well as specific surface receptors that facilitate endocytosis of different molecules including lipids, growth factors, and antibodies [8]. The most notable advancement in understanding the function of the endothelium came from pioneering research studies conducted almost 40 years ago that revealed that endothelial cells release various vasoactive factors that directly regulate CV function (including angiogenesis), vascular homeostasis (platelet aggregation and immune response), and vascular tone (vasodilatation and vasoconstriction) [9,10].

Vascular homeostasis is dependent on the balanced production of these vasoactive factors that help to maintain an atheroprotective environment. However, damage to the endothelium either physically from scratching of the cell surface or from injurious stimuli (such as free radicals), results in the *tight seal* around the cells being disrupted (Fig. 5.2). This increases endothelial cell permeability and allows harmful molecules to advance into and damage the underlying smooth muscle of the vessel, a process known as endothelial dysfunction [11].

2.1 How Does the Endothelium Regulate Vascular Tone?

As stated previously, endothelial cells continuously release various vasoactive factors that help to maintain basal vasodilator tone by inducing vasodilation

(NO, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF)), or cause vasoconstriction (thromboxane and endothelin-1 (ET-1)). Since NO is the most important factor in preventing atherosclerosis, and its activity can be measured using noninvasive assessments of the vasculature, only this factor is discussed in the rest of this chapter. For a detailed review about other vasoactive factors, see Sandoo et al. [11].

2.2 Nitric Oxide and Its Vasorelaxant Effects

Furchgott and Zawadzki were the first to identify that NO is an endothelium-derived relaxing factor of the underlying smooth muscle [12], with subsequent studies revealing that it is important in regulating basal vasodilator tone [13]. The production of NO is dependent on the enzyme nitric oxide synthase (NOS), which converts the amino acid L-arginine to NO [14].

Three isoforms of NOS exist: neuronal isoform (nNOS), which produces NO to act as a neuronal messenger that regulates synaptic neurotransmitter release [15]; macrophage or inducible isoform (iNOS), which is only expressed in cells that have been exposed to inflammatory mediators or other injurious stimuli that activate the macrophages [16]; and endothelial NOS (eNOS), which produces nitric oxide in the vasculature [17]. The isoforms are classified by the cells they were originally found in, although it is now known that expression of these isoforms also occurs in other cells, such as cardiac myocytes [18], skeletal muscle, blood platelets, and the hippocampus [19]. Inactive eNOS is generally located in small

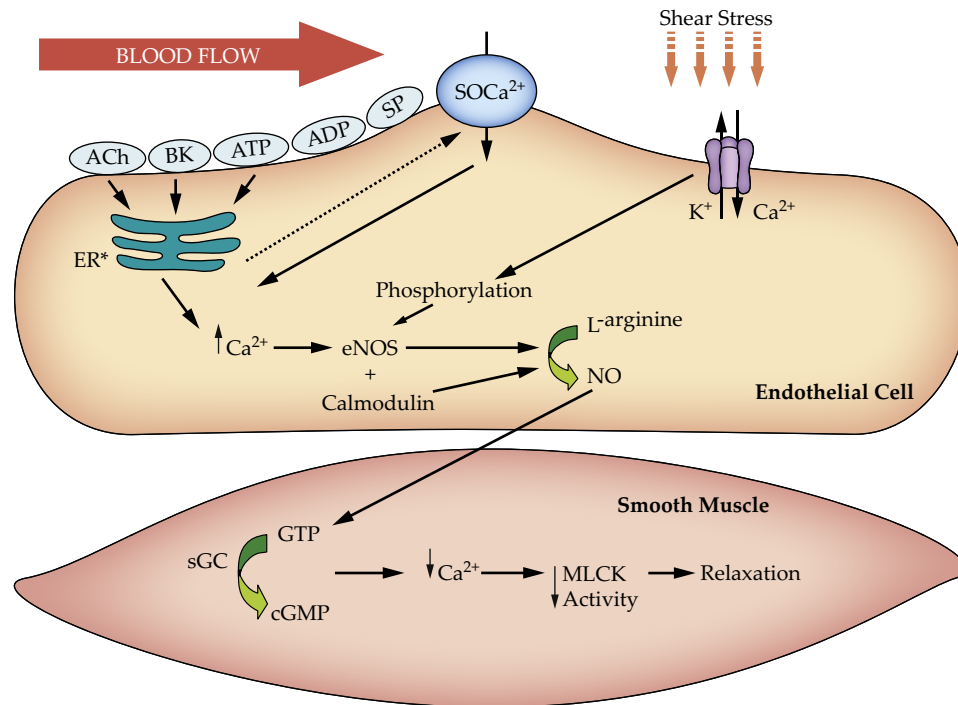


FIGURE 5.3 Endothelial nitric oxide production and its actions in the vascular smooth muscle cell. *ACh*, acetylcholine; *ADP*, adenosine diphosphate; *ATP*, adenosine triphosphate; *BK*, bradykinin; *cGMP*, cyclic guanosine-3', 5-monophosphate; *ER*, endoplasmic reticulum; *MLCK*, myosin light chain kinase; *NO*, nitric oxide; *sGC*, soluble guanylyl cyclase; *SOCa²⁺*, store-operated Ca^{2+} channel; *SP*, substance P. *When Ca^{2+} stores of the endoplasmic reticulum are depleted a signal is sent to the *SOCa²⁺* channel that allows extracellular Ca^{2+} into the endothelial cell. Adapted from Sandoo et al. [11].

invaginations in the cell membrane called caveolae, where it is bound to a protein called caveolin [20]. Activation of eNOS depends on an increase in intracellular Ca^{2+} levels that help eNOS detach from caveolin [20]. This process can be aided by specific NO agonists including bradykinin (BK), acetylcholine (ACh), adenosine tri-phosphate (ATP), adenosine di-phosphate (ADP), substance P, and thrombin [21], which all facilitate eNOS-caveolin detachment by promoting the release of Ca^{2+} from the endoplasmic reticulum (Fig. 5.3) [22].

Once intracellular Ca^{2+} stores are depleted, a signal, which as yet is unidentified, is sent to the membrane receptors to open Ca^{2+} channels allowing extracellular Ca^{2+} into the cell [23,24]. This process of Ca^{2+} regulation is known as store-operated Ca^{2+} entry or capacitative Ca^{2+} entry [25]. Ca^{2+} attaches to the protein calmodulin in the cytoplasm of the cell, after which it undergoes structural changes that allow it to bind to eNOS [26]. The activated eNOS then converts L-arginine to NO with the aid of the NO cofactor tetrahydrobiopterin (BH_4). BH_4 helps to transfer electrons produced at the eNOS site to an oxidase domain, causing activation of oxygen, which subsequently couples with L-arginine to produce NO [27]. eNOS is inactivated when Ca^{2+} levels are reduced causing the calcium-calmodulin complex to dissociate from eNOS [26]. In summary, the levels of intracellular Ca^{2+} in the endoplasmic reticulum as well as Ca^{2+} that

diffuses into the cell from extracellular stores is integral for the activation of eNOS and subsequent production of NO. This pathway of NO production is depicted in Fig. 5.3.

2.3 Phosphorylation of eNOS and the Role of Shear Stress

The intracellular Ca^{2+} stores contribute mainly to the short-term increase in NO, as when Ca^{2+} stores are depleted, another mechanism involving the phosphorylation of eNOS helps synthesize NO [28]. The phosphorylation of eNOS is dependent on the activity of various protein kinases including protein kinase A [22] and cyclic guanosine-3', 5-monophosphate (cGMP) protein kinase-dependent II [28]. Many of these kinases are stimulated by shear stress, the dragging frictional force exerted on the endothelium from laminar blood flow, with strong evidence for protein kinase A and protein kinase B (Akt) interacting with each other to increase eNOS activity [28,29].

Shear stress can also stimulate specific endothelial cell receptors by facilitating the transfer of bloodborne agonists to attach to specific endothelial cell receptors and increase intracellular Ca^{2+} [30]. In particular, shear stress activates specialized Ca^{2+} -activated K^+ channels on the endothelial cell surface, causing K^+ efflux and

Ca^{2+} influx into the cell (Fig. 5.3) [31]. The duration of shear stress is important in determining the contribution of Ca^{2+} release and eNOS phosphorylation on NO production. Short shear-stress durations usually elicit intracellular Ca^{2+} release [32], whereas longer durations (>30 min) deplete intracellular Ca^{2+} stores and cause eNOS phosphorylation [33].

2.4 Effects of NO on Vascular Smooth-Muscle Cells

Once synthesized, NO diffuses across the endothelial cell into the adjacent smooth muscle, where it binds to the enzyme soluble guanylyl cyclase (sGC) [34] and increases the conversion rate of guanosine triphosphate (GTP) to cGMP, thereby decreasing smooth-muscle tension (Fig. 5.3) [35]. In addition, cGMP promotes Ca^{2+} uptake into the sarcoplasmic reticulum and prevents any further Ca^{2+} release into the cell [36,37]. Both actions reduce smooth-muscle cell contraction by reducing myosin light-chain kinase activity [35]. Interestingly, the administration of nitroglycerin (NTG) can cause smooth-muscle relaxation despite the absence of an intact endothelium [13]. The mechanism by which NTG causes vasodilatation is still not clear. Some studies suggest that NTG undergoes bio-conversion to NO [38–40], while others report that NTG

causes vasodilatation via other mechanisms unrelated to increases in NO [41]. Further research that identifies the precise mechanisms will enable clinicians to understand why some patients develop tolerance to NTG.

2.5 Techniques to Examine Endothelial Function

In recent years, a number of noninvasive techniques have been used to examine endothelial function as an easier and safer alternative to direct assessment of the coronary arteries (depicted in Fig. 5.4) [42]. These assessments are conducted in the peripheral circulation, as several studies have revealed that peripheral vascular function correlates with the coronary circulation [43–45], associates with classical CV risk factors [46], and predicts future CV events in healthy older individuals [47] and in patients with CV [48–51] or PAD [52].

The loss of endogenous NO increase is one of the earliest signs of endothelial dysfunction and is characterized by a reduction in vasodilatation. Examination of peripheral endothelial function allows quantification of the vasodilatory response to a specific stimulus (pharmacological or physiological), with an attenuation of the dilatory response indicative of endothelial dysfunction [11]. If endothelial dysfunction is not halted, vascular

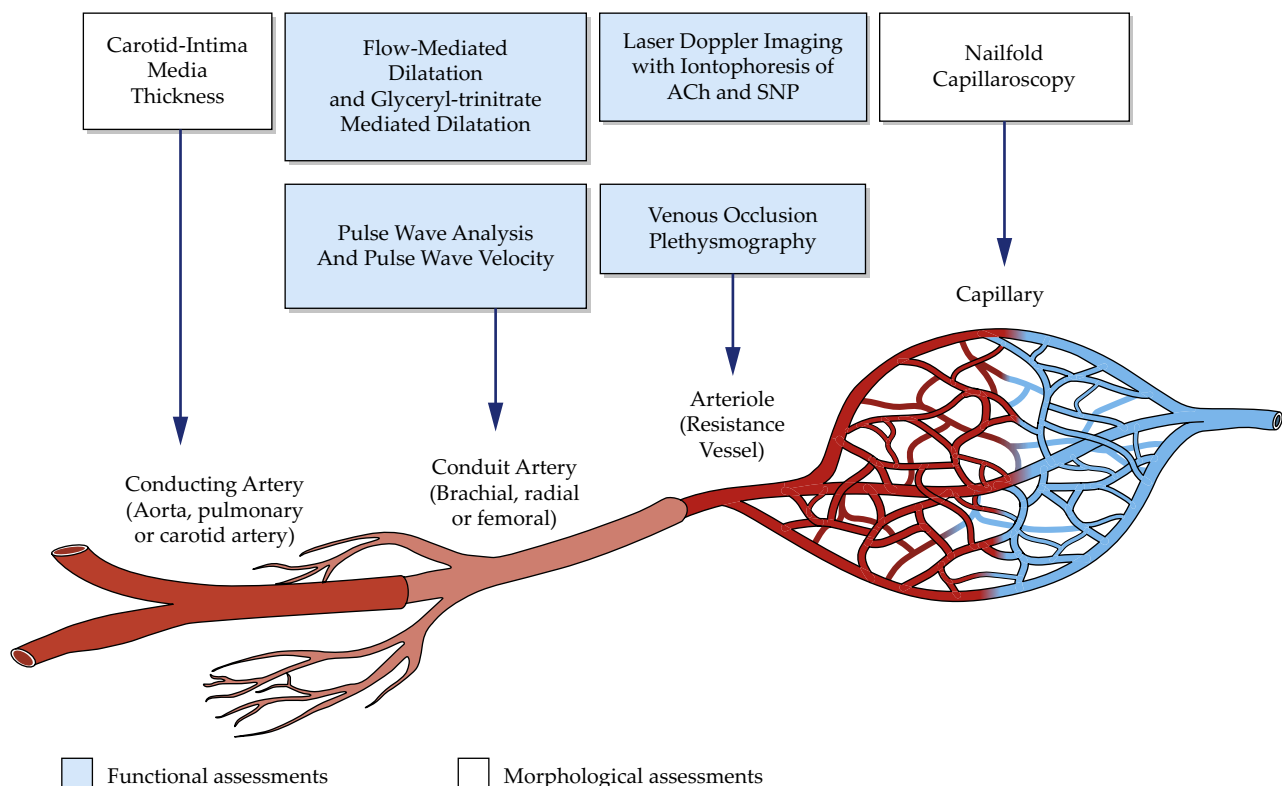


FIGURE 5.4 An overview of the assessments for endothelial function and vascular structure performed in different vascular beds. ACh, acetylcholine; SNP, sodium nitroprusside. Adapted from Sandoo et al. [54].

inflammation can degrade the internal elastic lamina and deposit collagen in the intima causing a reduction in vessel distensibility. This intermediate stage of atherosclerosis can be measured using pulse-wave analysis (PWA) and PWV, which provide an index for arterial stiffness. Advanced (but subclinical) atherosclerosis is often characterized using carotid ultrasound with a specific focus on the thickness of the intima-media and identification of carotid plaque as well as type of plaque (vulnerable vs stable) [53].

The endothelium is a diverse organ with different structures and phenotypes depending on the vessel type [55]. The structural differences may also impact on function, as heterogeneous responses to *in vitro* stimulation are apparent in different vascular territories and in different segments of the same vascular territory [56–58]. Thus, it is likely that endothelial dysfunction also occurs in selective vascular beds [58]. Indeed, studies in rheumatoid arthritis (RA) patients (a group characterized by excessive risk for cardiovascular disease) have revealed that microvascular and large-vessel endothelial function as well as functional and morphological assessments are independent of each other [59]. This suggests that assessments of endothelial function should encompass examination of functional and morphological changes in the microvessels and in the large vessels (Fig. 5.4).

The following section details several noninvasive assessments of vascular function and morphology according to well-established protocols that incorporate appropriate methodological and technical guidelines. It is hoped that the reader can use the information when developing protocols for use in their own laboratories. A video providing step-by-step demonstration of the techniques can be found in the paper by Sandoo and Kitas [53].

3. MARKERS FOR ATHEROSCLEROSIS AND ENDOTHELIAL DYSFUNCTION

3.1 Laser Doppler Imaging With Iontophoresis

In the microcirculation, laser Doppler flowmetry (LDF) and laser Doppler imaging (LDI) with iontophoresis of vasodilator agonists are commonly used to examine microvascular perfusion [60]. Both techniques measure the Doppler shift created by scattered light from moving red blood cells. Microvascular perfusion is represented as blood flux (average red blood cell velocity and concentration) rather than blood flow (mL/min), as measurement of blood flux linearly associates with blood flow (Fig. 5.5) [61]. The assessment of LDI offers considerable advantages over LDF, because the perfusion is mapped over a larger area (rather than a single point as with LDF) and can improve reproducibility by

limiting the effect of heterogeneous skin blood flow to different areas [62].

The stimulus to increase NO bioavailability is achieved using a technique called iontophoresis, which administers a small electrical current to pass negatively and positively charged vasoactive agents through the skin into the underlying resistance vessels [63]. For examination of endothelium-dependent and endothelium-independent function, the vasodilator agonists acetylcholine (ACh) and sodium nitroprusside (SNP) are used, respectively [64]. Iontophoresis of ACh allows activation of endothelial-cell muscarinic receptors releasing NO from the mechanisms described earlier. Sodium nitroprusside causes maximum vasodilatation (and perfusion) by activating smooth-muscle cell receptors and allows for examination of smooth muscle integrity [64]. It is worth noting that ACh may stimulate non-NO pathways such as cyclooxygenase-mediated pathways [60], but previous studies in RA patients (a group likely to have reduced NO bioavailability) have revealed impaired microvascular responses to ACh and SNP [54]. Further, exercise interventions known to improve NO bioavailability also improve ACh-mediated blood flux using LDI in patients with RA [65]. Acetylcholine and SNP are usually suspended in sodium chloride or deionized water (known as vehicles) [62,66], and while some vehicles may increase skin perfusion, this can be limited by using 0.5% sodium chloride [62]. It is also necessary to control for external factors such as diet, time of day, and menstrual cycle, as these all affect microvascular blood flow [67,68]. Therefore, it is important to follow established guidelines when conducting the tests [69].

The quantification of microvascular endothelial function can be expressed in a number of ways with cutaneous vascular conductance, a product of flux divided by arterial pressure, used in studies where blood pressure may change over the study duration (ie, during exercise or antihypertensive treatment) [60]. Other methods include the calculation of the area under the curve (AUC) for blood flux, or percentage change in perfusion from baseline. It is noteworthy that while there is no official consensus for presenting data, investigators should use an approach that shows good reproducibility.

3.2 Forearm Blood Flow and Venous Occlusion Plethysmography

Another method for examining microvascular endothelial function includes the use of venous occlusion plethysmography (VOP) [70]. In VOP, a blood-pressure cuff placed around the forearm is inflated to just below diastolic blood pressure (the cuff pressure is typically 40 mmHg) for 10 s, followed by 5 s of cuff deflation. This stops venous return from the forearm, but allows arterial inflow (ie, blood can enter the forearm but cannot return

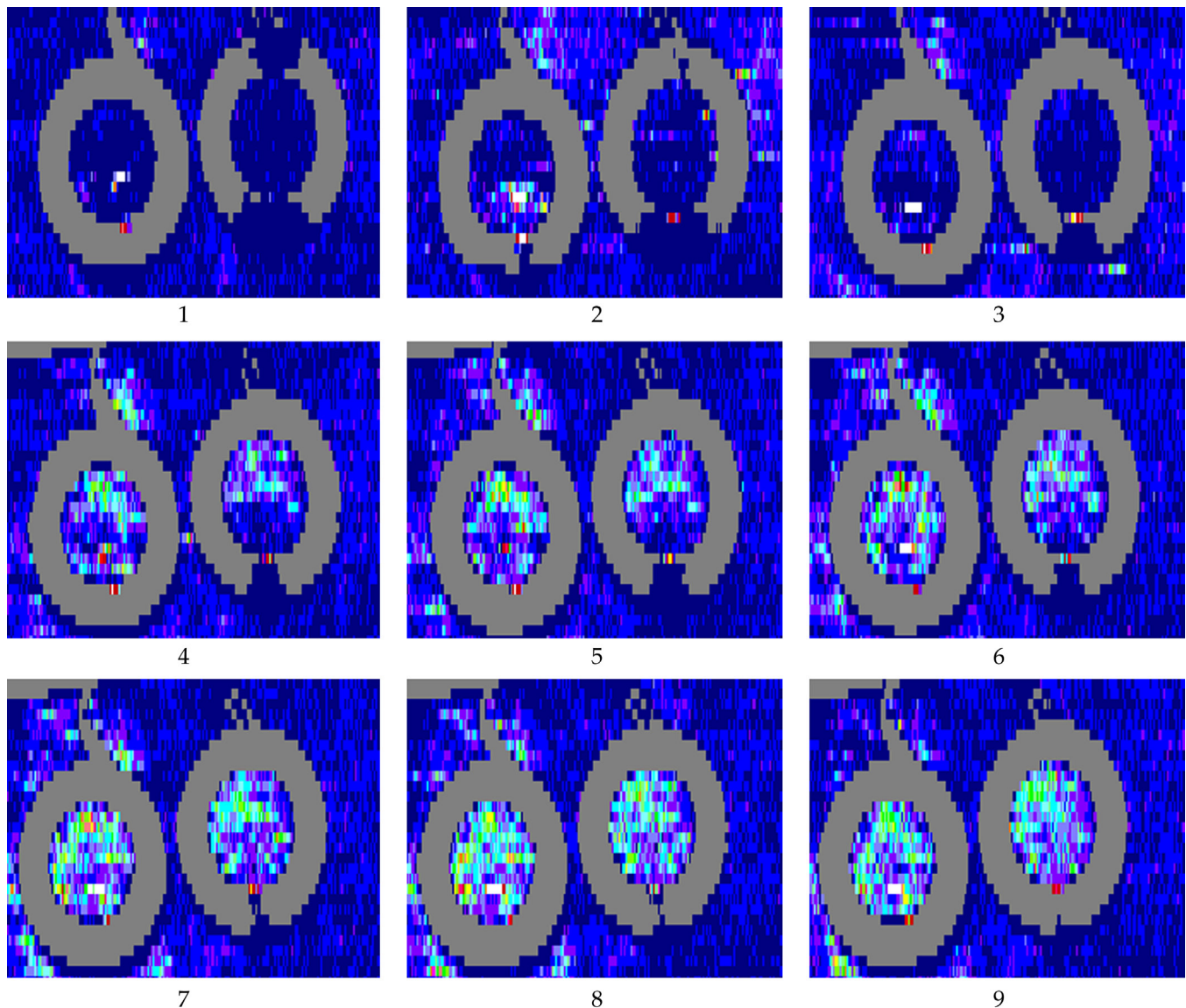


FIGURE 5.5 A series of repeat laser Doppler imaging scans while acetylcholine (first chamber) and sodium nitroprusside (second chamber) are simultaneously iontophoresed into the skin microvessels. The change in color represents an increase in blood flux as the vessels dilate in response to the two agents. *Adapted from the personal collection of Dr. A. Sandoo, Bangor University, Bangor, Wales, UK.*

back to the heart) resulting in a linear increase in forearm volume with time, which is proportionate to arterial blood flow [70]. To reduce variation in blood volume due to temperature variations of the resistance vessels of the hand, the hand is excluded from the measurement by inflating a wrist cuff to suprasystolic pressures. Automatic cuff inflators/deflators are used to precisely control blood flow in the forearm, while a strain-gauge plethysmograph is placed around the widest part of the forearm. Any increase in the length of the strain gauge is detected by a change in electrical resistance and is quantified as an increase in forearm blood flow (FBF). Assessments of FBF are often made in the contralateral arm so that time-dependent changes in basal blood flow due to arterial pressure fluctuations can be accounted for [70]. Administration of vasoactive agonists (ACh, substance

P, BK) or inhibitors of NO (L-NG^g-monomethyl Arginine citrate) can be used to examine biological mechanisms associated with microvascular blood flow [71].

3.3 Nailfold Capillaroscopy

Morphological abnormalities (specifically capillary structure) in the microcirculation can be examined using nailfold capillaroscopy [72]. Immersion oil is applied to the nailfold epidermis of all 10 fingers, which are then placed under a microscope for characterization of capillary size, number, and structural characteristics. Capillaroscopic abnormalities can be classified into three stages (early, active, and late), with the earliest change relating to an enlargement in capillary size. A reduction in capillary number and

structural impairments are seen in the active and later stages of microangiopathy [72].

3.4 Flow-Mediated Dilatation

Flow-mediated dilation (FMD) is an accepted method commonly used in evaluating endothelial function in large conduit vessels such as the brachial artery. This is mainly due to its relative simplicity, low cost, and good reproducibility when compared with other techniques developed for the evaluation of endothelial function [2]. However, its routine clinical implementation is thus far limited. It is well established that an intact endothelium can secrete NO in response to different stimuli, thus regulating vascular tone. This fundamental process of regulating endothelial function is impaired early in the course of atherosclerosis and may predispose the patient to future adverse cardiac events. Early studies of endothelial dysfunction have evaluated the coronary artery response to intracoronary acetylcholine infusion during coronary artery catheterization, but this approach is

limited by its invasiveness and inaccessibility for most caregivers. In 1992, Celermajer et al. described an alternative noninvasive method for quantifying endothelial function, currently known as FMD, which was subsequently enthusiastically adopted for cardiovascular research. This approach is an appealing noninvasive alternative to the quantification of coronary vasodilation using pharmacological stimuli [73], as studies show an elimination of the FMD response following administration of the NO inhibitor, L-N^G-monomethyl Arginine citrate [74]. The FMD protocol involves a 2 min Doppler ultrasound scan of the brachial artery (using a 5–7 MHz linear array transducer placed in a mechanical clamp and positioned proximal to the elbow) to determine baseline arterial diameter (Fig. 5.6). It is worth noting that the angle of insonation should be perpendicular so that the lumen-intima interface can be visualized on the near (anterior) and far (posterior) walls [75]. Following this, a pneumatic tourniquet is placed around the wrist and is inflated to >50 mmHg above the patients systolic blood pressure for 5 min. This results in tissue ischemia and

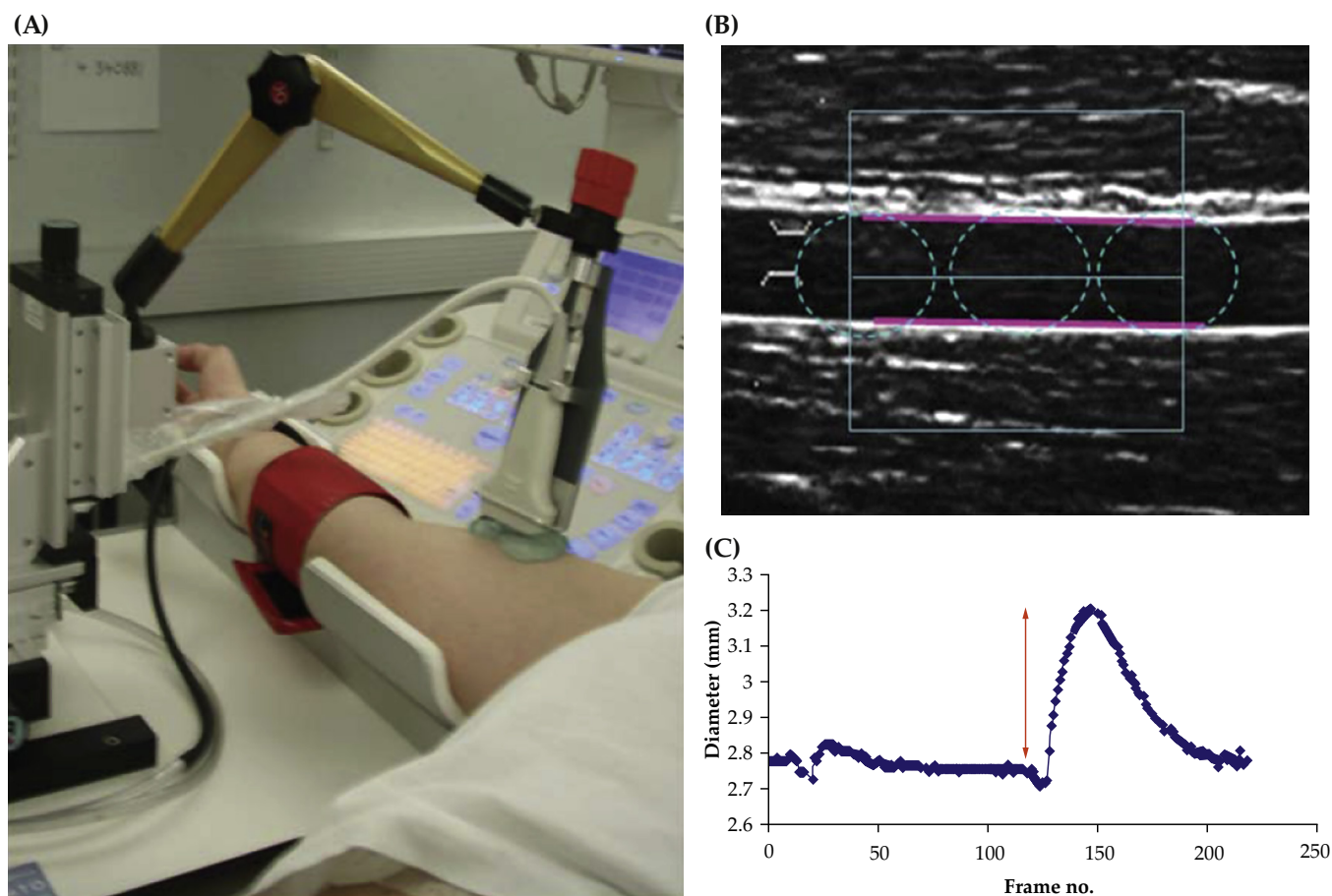


FIGURE 5.6 Technical aspects and setup for FMD evaluation: (A) Note the accepted positioning of the sphygmomanometer cuff (distal to the elbow) and the ultrasound probe (proximal to the elbow). (B) The “region of interest box” is used for automated analysis of the brachial artery diameter, preferably by using designated semiautomated FMD calculation software. (C) A graph showing the raw time series data of brachial artery diameter changes. Adapted from Charakida et al. [2].

dilation of downstream resistance vessels via auto-regulatory mechanisms. Deflation of the blood-pressure cuff evokes a sudden increase in blood flow (reactive hyperaemia) through the brachial artery, resulting in shear stress-mediated dilatation of the vessel [73]. The change in vessel diameter following cuff deflation and at baseline can be determined manually (using caliper placement) [73] or using automated edge-detection software [76]. The measurement of peak dilatation following cuff release should be performed over a protracted period of time because measurement of peak diameter within the first 60 s following cuff deflation can underestimate FMD by 25–40% [77]. Recent evidence suggests that a period of 180 s is required to capture the true peak diameter, with most peak values occurring within the first 120 s [77]. Flow-mediated dilation is usually expressed as the percentage increase in postcuff-release vessel diameter relative to the baseline diameter (Fig. 5.6). Recently the accuracy of this calculation has come under scrutiny due to interindividual variations in baseline diameter impacting on the FMD value (Fig. 5.7) [78,79]. This issue can be resolved by using appropriate allometric scaling that involves reporting the difference in absolute diameters, with baseline diameter entered as covariate in logarithmic-linked analysis of covariance [78,79]. Other variables that can be quantified include time to maximum dilatation, duration of vascular dilatation, and the area under the dilatation curve. It is also important to consider characterizing the shear stress stimulus on the brachial artery. This can be achieved using information from the pulse-wave velocity signal that allows calculation of the shear rate (velocity/diameter) [80]. If the sample volume for flow encompasses the entire lumen of the vessel a numerator of eight should be used to account for the slower blood flow (ie, $8 \times (\text{velocity/diameter})$). If only the center of the vessel is sampled, then a numerator

of four is sufficient to account for the higher velocities. Shear rate is reported as AUC until peak diameter and is measured using the trapezoidal method [81]. The principal stimulus for the FMD technique is the production of shear stress, which activates specific endothelial receptors to release NO [82], but shear stress can also stimulate the release of other vasoactive factors [83]. Thus the correct characterization of FMD requires careful consideration of a number of methodological principles. For example, the duration of cuff inflation is recommended to be 5 min as this primarily elicits NO-mediated dilatation (since longer cuff occlusion results in non-NO mediated dilatation) [84]. In addition, NO-mediated dilatation is dependent on the occluding cuff being placed around the wrist and distal from the ultrasound probe, as placement of the cuff proximal to the probe causes vasodilatation from factors other than NO (possibly due to a larger ischemic territory altering the shear stress stimulus) [85].

Flow-mediated dilation is affected by several cardiovascular risk factors known to affect coronary artery endothelial-dependent vasodilatation, and these should be accounted for when reporting the analysis [2,75]. Importantly, the FMD response can be affected by external factors such as sleep deprivation [86], hyperhomocysteinemia [87], caffeine [88], smoking [89], antioxidant therapy [90], menstrual cycle [91], and time of day [92]. Therefore, it is important to control these factors [75]. It is recommended that the test should be performed in a temperature-controlled environment following at least 20 min of complete rest, with patients asked to fast for 8–12 h, and avoiding exposure to tobacco smoke for at least 4–6 h prior to the examination. Interestingly, the FMD technique has been utilized in several research studies examining the impact of various interventions such as weight loss, physical activity, and medication use, with these studies reporting improved FMD following each intervention [2]. Thus despite some limitations, FMD can be used effectively to characterize the health of the large vessels.

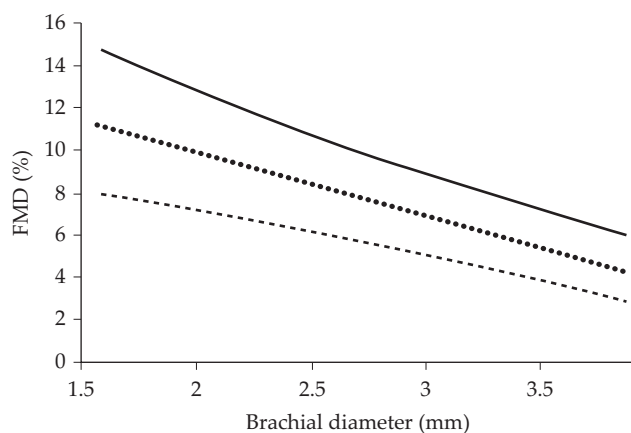


FIGURE 5.7 Nomogram for FMD and its correlation to baseline vessel diameter. The black line shows the 75th percentile, the circle dashed line the 50th percentile, and the rectangular dashed line, the 25th percentile of the population. Adapted from Charakida et al. [2].

3.5 Nitroglycerin-Mediated Dilation

Nitroglycerin-mediated dilation (NMD) is used to quantify the endothelium-independent maximal vascular dilatation following exogenous NTG supplementation and helps determine if impairments in vasodilatation are due to a loss in smooth-muscle cell integrity, or the inability of endothelial cells to release NO [93]. Nitroglycerin-mediated dilation is measured following at least 10 min of complete rest and is commonly performed after completion of the FMD assessment. The technical measurement settings remain similar to those used for FMD quantification, however, in NMD, the sphygmomanometer cuff inflation and deflation is replaced by a single high-dose (0.4 mg) intake of sublingual NTG spray or tablet. The peak vasodilatation is usually achieved 3–4 min

following administration of NTG [93]. Similar to FMD, NMD is expressed as a percentage increase in dilation relative to the baseline diameter, and this ratio should be allometrically scaled in accordance with recent statistical guidelines [78,79]. Nitroglycerin-mediated dilation is less affected by diseases compared to FMD, although it has been suggested that reactive-oxygen species (important features of various cardiovascular diseases and atherosclerosis) might cause NO inactivation and thereby affect NMD values [75].

3.6 Intima-Media Thickness and the Presence of Carotid Plaques

Thickening of the intima-media reflects several atherosclerotic processes that are initiated by a reduction in NO bioavailability and an increase in ET-1 levels, leading to activation of proinflammatory cytokines, diapedesis of leukocytes through the damaged endothelium, activation of thrombotic factors, smooth-muscle cell proliferation, and formation of lipid-rich plaques [94]. In addition, abnormal shear forces applied on the vessel wall may also increase IMT values [95]. The concept of IMT measurement was first described by Pignoli et al. [96]. The authors demonstrated that B-mode ultrasound measurements yielded similar results to those measured from pathological cross-sectioning of the investigated vessel. Carotid ultrasound, if performed correctly, accurately depicts the two lines of Pignoli, with the first

echogenic line representing the lumen-intima interface and the second line representing the media-adventitia interface (Fig. 5.8) [96]. The assessment is able to predict arterial structure better than other techniques such as magnetic resonance imaging or radiographic assessments [97]. Assessments of IMT typically integrate measurements from the common carotid artery, internal carotid artery, and carotid bifurcation points [98], as the vascular wall at each site may vary in size, and readings from one anatomical site might not represent values at other sites [95]. Indeed, measurement of the vessel wall from two or more angles and from various sites appears to have a clinical advantage, especially in light of the asymmetrical-wall involvement [3]. It is worth highlighting that the common carotid artery is more easily visualized than other sites due to its anatomical location, but measurement of the common carotid artery alone may not suffice for cardiovascular risk evaluation and for monitoring the response to treatments [3].

Examination of IMT requires the participant to lay supine with their head rotated to the opposite side of the transducer. The anatomical position of the artery can be affected by the degree of head tilt [99], so it is important to ensure that the participant hyperextends the neck slightly and that the head position is adjusted while scanning to further optimize the image [99]. The ultrasonographer should sit behind the participant and start by scanning the common carotid artery along its entire section using a 10MHz linear array transducer.

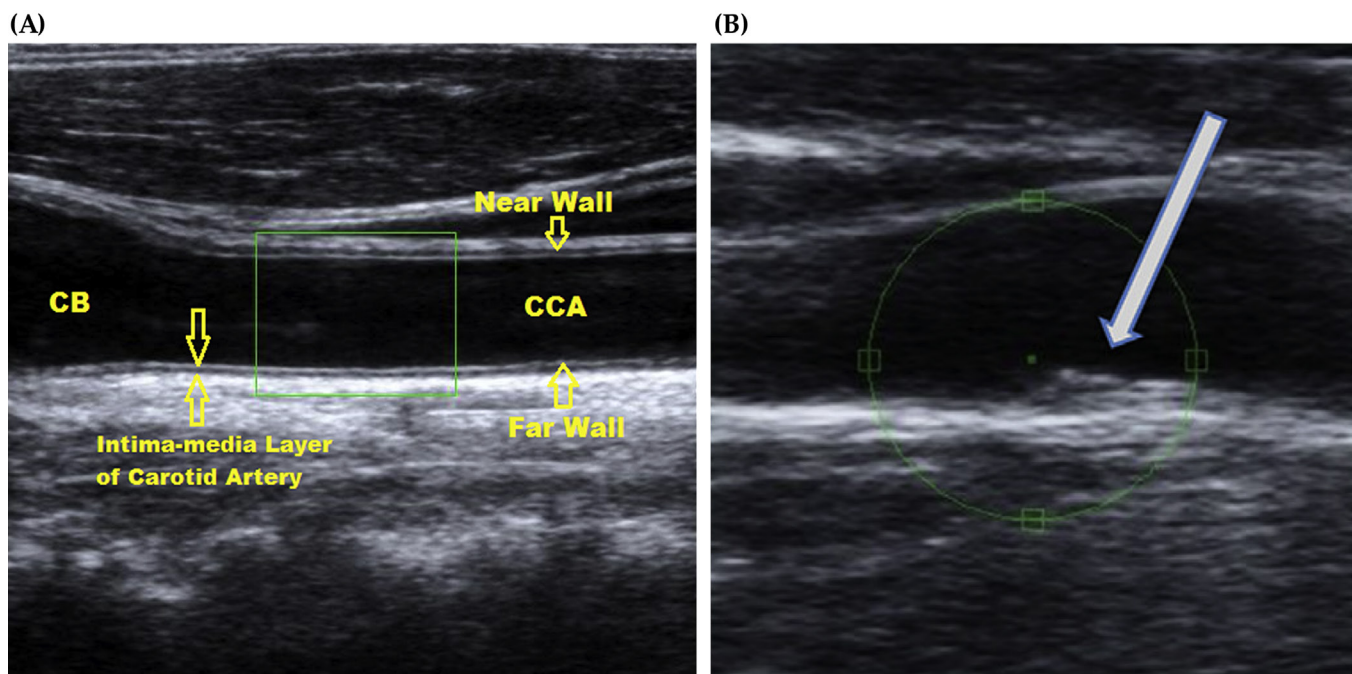


FIGURE 5.8 Ultrasonographic evaluation of the carotid artery for IMT: (A) Doppler ultrasound scan of the common carotid artery (CCA) and carotid bulb (CB). Measurement of the carotid intima-media thickness is taken in the far wall 1 cm from the CB, demonstrating the characteristic finding of the “double echo” marking the intima-media complex. Adapted from the personal collection of Dr. A. Sandoo, Bangor University, Bangor, Wales, UK. Occasionally, a vascular atherosclerotic plaque is found, marking more progressive vascular involvement (B, arrow). Adapted from Dalla Pozza et al. [102].

The transducer should then be moved along the artery to image the carotid bifurcation (also known as the carotid bulb) as well as the internal and external carotid arteries (Fig. 5.8). To optimize the image, the depth, focus, and gain must be adjusted according to recommendations from the most recent technical guidelines [53,97,99]. It is important to note that the anatomy of the carotid bifurcation is heterogeneous between individuals as its structure depends on the nature of the artery diameter, curvatures, and angles, all of which impact on shear-stress forces remodeling the vascular wall [97].

It remains unclear as to which vascular wall (near or far) IMT readings should be obtained from, but it appears that the averaged results of measurements taken from the near and far vascular walls may have some advantage over the far wall alone [100]. The most recent technical guidelines suggest that still images of the far wall 1 cm from the carotid bifurcation point in the left and right common carotid artery should be acquired at the peak of the R-wave (from a three-lead electrocardiogram) and should clearly show two perpendicular echogenic lines [97]. The images can then be analyzed offline using specialist automated edge-detection software [101]. The software involves marking out a specific region of interest that displays the IMT and lumen diameter. The analysis should be performed on three images from each side, and the readings from both sides should then be averaged to give the overall IMT [100].

Increased IMT is a marker of atherosclerosis burden and is associated with increased risk of myocardial infarction and overall rate of cardiovascular diseases. In particular, and dependent on the measurement site, values >0.9 – 1.0 mm were found to be associated with increased cardiovascular risk [100]. In children, lower values were reported to be of clinical importance (0.38 – 0.59 mm in different studies). The authors also stated that nomograms should probably be developed for different vessel diameters and body surface area (BSA) [102]. Occasionally carotid plaques can be observed throughout the test, demonstrating more advanced atherosclerotic disease (Fig. 5.8) [3,102]. According to the 2013 European Heart Society (EHS) guidelines, the strength of recommendation for IMT measurement for patients with suspected stable coronary vascular disease without known atherosclerotic disease and in asymptomatic patients at intermediate cardiovascular risk is IIa, and the level of evidence is C and B, respectively [7].

4. MARKERS OF AORTIC ABNORMALITIES

Increased aortic stiffness and decreased aortic conduction of blood flow are the late sequela of arterial aging, ongoing endothelial dysfunction, and the progression of

atherosclerotic disease. The measurement of aortic indices is used as a marker of vascular health. Aortic stiffness can be assessed by various methods such as pulse-wave velocity and by calculating aortic distensibility. The arterial stiffness is influenced by aging and cardiovascular risk factors, thus representing structural and functional changes in the arterial wall. These changes are not unique to the aorta and are thought to correspond to changes affecting the overall (or at least proximal) vascular tree. Changes in arterial stiffness were found to be associated with cardiovascular diseases [103]. Aortic elasticity is greater in the proximal areas and its alterations are easily quantified in a noninvasive way. Changes in elasticity are mainly induced by increased degradation of elastin and increased synthesis (as well as a reduced degradation) of collagen [103].

4.1 Aortic Distensibility

Aortic distensibility represents the change in the aortic diameter induced by the ventricular systole and is correlated with a degree of arterial stiffness. The distensibility coefficient can be calculated by dividing the change in the arterial diameter with the systolic pulse pressure. Alternatively, the cross-sectional area change in response to the change of pressure (also known as cross-sectional compliance) can be measured [104]. The aortic diameter is visualized using a B-mode ultrasound. Lower arterial compliance manifested as lower aortic distensibility was found correlated with an increased risk of cardiovascular disease. At age 25, the distensibility coefficient is approximately 30×10^{-3} /kPa and gradually decreases to about 18×10^{-3} /kPa at age 60 [104].

4.2 Pulse-Wave Analysis and Pulse-Wave Velocity

Each left ventricular contraction forces blood into the large conduit arteries to be stored during systole and then released toward the tissues during diastole. This process is aided by arterial compliance, which dampens the forward-traveling oscillometric pressure waves created by the left ventricle and ensures smooth blood flow [105]. When the pulse-pressure waves reach the microcirculation, approximately 80% of the forward-traveling waves are reflected back towards the heart [105]. The summation of the forward-traveling and reflected wave can be measured using PWA and PWV, which utilize a mathematical transfer function to derive the central aortic-pressure waveform (Fig. 5.9A) [106].

In healthy individuals, the pulse-pressure wave is usually reflected back to the heart slowly during diastole to aid filling of the coronary vessels, but in individuals with reduced arterial elasticity, the pressure wave returns quickly to the heart arriving during systole, thus augmenting afterload (the pressure the ventricle has to

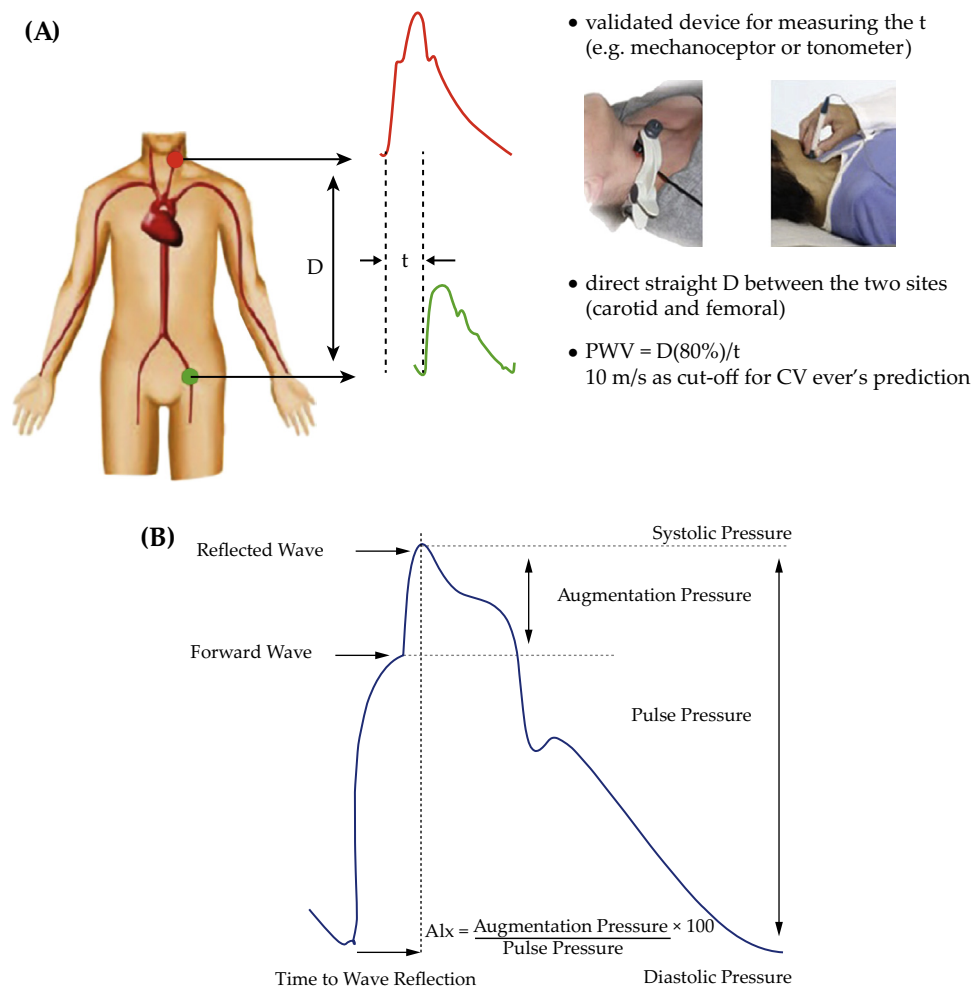


FIGURE 5.9 (A) A measurement technique and calculation method for evaluating aPWV. t , pulse-wave travel time; D , distance. Adapted from Bruno et al. [103]. *Cardiovasc Ultrasound* 2014;12:34. (B) The pulse wave is derived from arterial tonometry and provides information on incident and reflected waves that summate to create augmentation pressure. Augmentation index (AIx) is calculated by dividing the augmentation pressure by the pulse pressure and is expressed as a percentage of the pulse pressure. In arterial stiffness, the augmentation pressure is increased, which subsequently increases AIx. Adapted from the personal collection of Dr. A. Sandoo, Bangor University, Bangor, Wales, UK.

exceed to open the aortic valve) [107]. Importantly, the microcirculation plays a crucial role in regulating the reflection of the pressure wave as sympathetic activation of the arterioles can alter their diameter (and therefore resistance), impacting on the velocity of wave reflection [108]. Indeed, in conditions characterized by increased sympathetic activation such as hypertension, an increase in arterial stiffness can be attributed to increased systemic vascular resistance, as well as a reduction in arterial compliance [108]. Further, assessments of the pulse-pressure wave in the peripheral circulation are associated with coronary microvascular endothelial function [109]. Within the vessel, reductions in NO bioavailability and smooth-muscle tone contribute to arterial stiffness, with other changes including breakdown of elastic fibers and collagen deposition [110,111]. Thus measurement of the pulse-pressure wave represents both functional and structural changes to the vessel, which often overlap and occur at an intermediate stage of atherosclerosis.

Pulse-wave analysis is performed by placing a tonometer on the radial artery with moderate pressure to record the pulse-pressure waveform. The waveforms are calibrated against the standard brachial blood pressure and provide the maximum (systolic) and minimum (diastolic) points of the pressure curve. Mathematical transformation of the pressure wave allows for the generation of a central aortic waveform, which contains the first and second systolic peaks and displays the augmentation index (AIx) (Fig. 5.9B). The AIx is the difference between the second and first systolic peaks and is expressed as a percentage of the pulse pressure, with a high value indicating greater arterial stiffness [112]. Importantly, AIx is dependent on the length of the cardiac cycle and is often standardized to a heart rate of 75bpm [113]. The PWA technique primarily represents the reflection of the pressure wave from the microvessels rather than the velocity of the wave [108].

Measurement of carotid-femoral (also known as aortic pulse-wave velocity (aPWV)) provides detailed information regarding wave-transit time and can predict cardiovascular events and mortality independently of other known cardiovascular risk factors and is considered the gold standard for evaluating aortic stiffness [114]. The method is simple, noninvasive, reproducible, and inversely correlates with artery distensibility and compliance. Aortic PWV is also intercorrelated with the extent of carotid and aortic atherosclerosis, as measured by different diagnostic modalities [115]. The measurements are conducted using mechano-transducers, monometers, oscillometers, volume plethysmographic, or photo-plethysmographic devices (Fig. 5.9A). Alternatively, echocardiographic measurements of the two elected sites can be used for detecting distension waves and calculating aPWV. This tool was reported to have clinical importance, especially in younger individuals with intermediate cardiovascular risk [114]. Information regarding wave velocity is derived using the Moens-Korteweg equation, $PWV = \sqrt{(Eh \div 2r\rho)}$, where Eh is the incremental Young's elastic modulus of the artery wall, h is wall thickness, r is artery radius at end-diastole, and ρ is blood density [108]. It is worth noting that the arterial wall does not strictly follow the Young's Modulus as it is composed of nonelastic components such as water and the proportion of elastic fibers differ according to vessel type. Nevertheless, the equation mentioned above is still useful for calculating wave velocity. The measurement is performed by deriving the arterial pressure waveforms simultaneously from two arteries: the common carotid artery (proximal artery) and the femoral artery (distal artery). These arteries are selected because they are superficial and allow easy application of the tonometer, but measurements can also be recorded from the brachial artery and the ankle. The distance between the two arterial sites is measured and the time difference in wave velocity is taken by measuring the foot of the pressure wave at each arterial site at end diastole (R-wave on the electrocardiogram). Pulse-wave analysis is then calculated using the equation: distance/ Δ_{time} [108,113]. Increased PWV represents faster wave reflection toward the heart and indicates greater arterial stiffness [116]. Ben-Shlomo et al. reported that a 1m/s increase in aPWV was associated with a 7% increased risk of a cardiovascular event for specific, presumably, low-intermediate risk individuals [114]. An absolute value of 10m/s is the acceptable threshold for predicting cardiovascular events [103]. In a meta-analysis of 18 studies (8169 subjects), high brachial-ankle PWV values were found to be associated with higher risk of overall cardiovascular events, associated mortality, and general mortality (by 3-, 5-, and 2.5-fold, respectively) [117].

Finally, there are a number of commercial devices facilitating the automated evaluation of aPWV that are expected to further advance their adaptation into

clinical use. Not surprisingly, aPWV measurements were included in the latest European Society of Hypertension/European Society of Cardiology guidelines [118]. Yet, other organizations, for instance, the American Heart Association, still do not recommend PWV evaluation as a screening tool for asymptomatic patients [119].

4.3 Ankle Brachial Pressure Index

Ankle brachial pressure index (ABPI) is a method for the quantification of peripheral vascular disease that results from advanced atherosclerosis. It is therefore assumed that ABPI reflects a degree of cardiac atherosclerosis. Ankle brachial pressure index is calculated by dividing the ankle (posterior tibial and dorsalis pedis arteries) by brachial blood-pressure values as measured by an oscillometer. It can also be calculated as a ratio of blood-pressure indices, as measured by a continuous-wave Doppler ultrasound. Although the two methods yield significantly intercorrelated results, they are not interchangeable ($r=0.6$ in nondiabetics and 0.49 in diabetic patients; $p<.001$ for both correlations) [120]. Alternatively, ABPI can be measured using photoplethysmography (PPG) [121].

Ankle brachial pressure index is mostly used to evaluate the presence of peripheral vascular disease and indirect quantification of atherosclerosis. It is also used as an indirect marker for increased cardiovascular risk. Nevertheless, no reliable association was found between ABPI results and angiographic coronary anatomy, further emphasizing that atherosclerosis is not a homogenous vascular process [122]. Pressure value/index could be measured from two separate sites and the results averaged (although in some studies a single measurement technique was used and considered sufficient by some researchers) [123]. There is also no consensus as to whether blood pressure should be *simultaneously* measured in the upper and lower limbs. In addition, the specific patient position in which measurements should be undertaken also lacks standardization. Finally, it seems that either excessive or low blood-pressure values might influence the results. Any interpretations should be made within that context [121].

The normal reference values reported in the medical literature are >0.9 (although according to other references a threshold of >0.95 should be used), while lower values possibly reflect peripheral vascular disease. Sensitivity and specificity of 79% and 96%, respectively, has been reporting as detecting $\geq 50\%$ decrease in lumen diameter using the ABPI approach [120] as well as $\sim 90\%$ and $\sim 98\%$, respectively, according to others [123]. These differences may stem from dissimilar measurement techniques and different clinical characteristics of the recruited patients. Accordingly, sensitivity was reported to be lower in certain populations of interest such as diabetic patients and the elderly [121]. These patients commonly have relatively incompressible calcified vessels

that may yield false high ABPI values, especially when an oscillometer is used for ABPI analysis [124].

A meta-analysis of several large cohorts reported that $ABPI \leq 0.9$ was associated with a significant increase in all-cause mortality, with a relative risk (RR) of 2.35 (95% CI 1.66–3.32; $p < .001$) and cardiovascular mortality with a RR of 3.34 (95% CI 2.12–5.28; $p = .002$). Multivariate analysis and adjustment to multiple risk factors demonstrated a weaker association that nonetheless remained significant (all-cause RR 1.6, cardiovascular RR 1.96) [123]. Since ABPI seems to decrease with aging, low ABPI values measured at a younger age are even more suggestive of peripheral vascular disease and increased cardiovascular risk [123]. Ankle brachial pressure index measurement is simple and quick, and is therefore a clinically relevant research and diagnostic tool. Yet, further investigation is needed to determine the clinical benefits of a wide population-based screening, as to whether ABPI is sufficiently sensitive for the detection of seemingly insignificant vascular involvements, and whether exclusion of peripheral vascular disease is sufficiently associated with the absence of coronary-related vascular risk in the general population and in specific patients. Ankle brachial pressure index should be considered in asymptomatic patients at intermediate cardiovascular risk (strength of recommendation IIa, the level of evidence B) [7].

5. EVALUATION OF VENTRICULAR GEOMETRY AND PERFORMANCE

5.1 Echocardiographic Study of the Heart

Echocardiography epitomizes one of the mainstay methods of evaluating cardiac structures and performance

and is one of the most commonly used diagnostic cardiac techniques. Measurements can be performed in M-mode, 2D or 3D modes (using a matrix array ultrasound probe). Echocardiography is used for diagnosing and follow-up of cardiac patients. This method can be performed in a trans-thoracic manner (the most commonly applied technique) or through a trans-esophageal approach (requiring sedation, yet providing higher resolution images). The Doppler echocardiogram is also capable of evaluating the blood-flow patterns within myocardial structures and is specifically efficient in detecting abnormal blood flow throughout the cardiac valves. An ultrasound contrast agent is usually constructed from tiny gas microbubbles and a protein shell. Definity and Optison are commonly used commercial examples of such agents. Using an echocardiographic contrast agent enables improved imaging of the LV borders, thus improving the quantification of global and regional systolic function and facilitating the identification of intra-cardiac lesions (thrombi, tumors, etc.), as well as evaluating blood perfusion through the myocardial tissue.

Tissue Doppler imaging analyzes the wall motion. Echocardiography can also be performed following physical exertion (treadmill walking or running or another exercise modality), searching for exercise related wall-motion abnormalities or inadequate cardiac perfusion. This clinical tool can also be used to identify abnormal cardiac structures such as the prolapse of cardiac valves (Fig. 5.10A), more commonly encountered in patients with connective tissue diseases, in the presence of other structural cardiac anomalies, or in aneurysm of the left ventricle (Fig. 5.10B).

Symptomatic heart failure in the presence of a preserved ejection fraction might indicate the presence of diastolic dysfunction. The process of chamber distension is mediated through a passive distensibility of the cardiac tissue

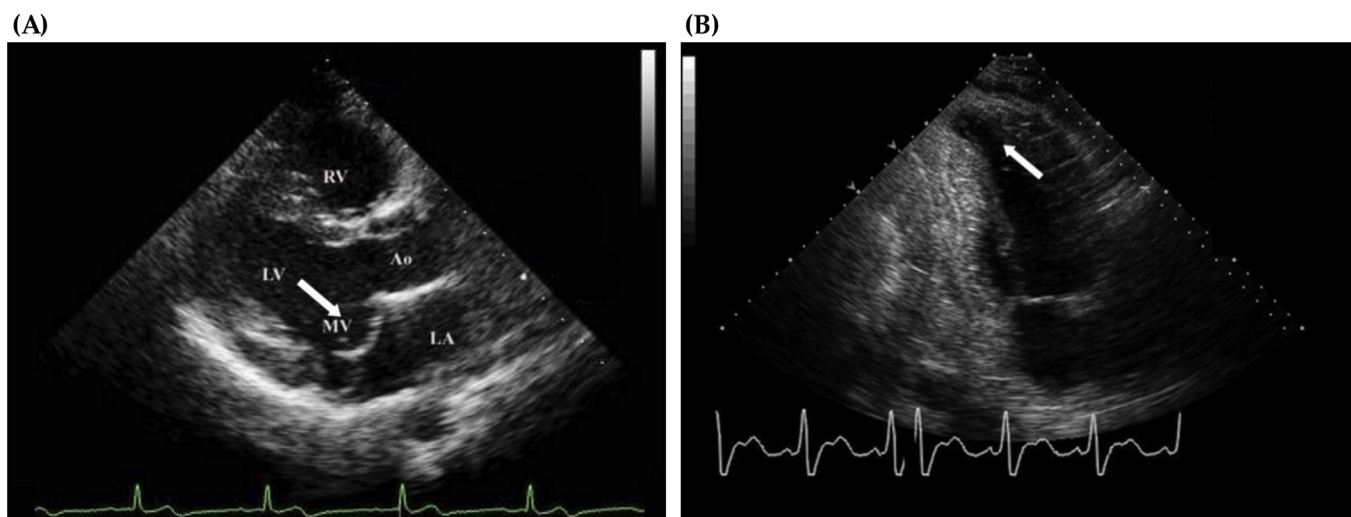


FIGURE 5.10 Examples of cardiac anatomic and functional abnormalities such as mitral valve prolapse (A; arrow; long axis parasternal view) and an LV aneurysm (B; arrow). Adapted from Janiec et al. [125] and Kasirye et al. [126], respectively.

and the process of active shift from cardiac contraction to the cardiac relaxation phases. Evaluation of LV diastolic dysfunction is usually conducted by measuring the blood through the mitral valve. Yet, this measurement is also affected by left atrial pressure, age, heart rate, and other factors. The quantified parameters usually include amplitude of the early (E) and late (A) filling waves and their ratio (E/A ratio), the deceleration time of the E-wave (DT), intraventricular relaxation time (IVRT, ie, the time elapsed between the closure of the aortic valve and the opening of the mitral valve), and the duration of the A-wave (A-dur) (Fig. 5.11A). Notably, diastolic abnormalities might lead to increased left atrial pressure resulting in a pseudonormal E/A ratio (Fig. 5.11B) [127].

Increased left ventricular mass and low ejection fraction are well-acknowledged risk factors for developing arrhythmias and an overall adverse cardiac prognosis [128]. Left ventricular mass is calculated by subtracting the LV cavity dimension from the maximal area filled by the epicardium. The results are then multiplied by the

known density of the cardiac tissue (1.05 g/mL). Overall, the following equation is used:

$$\text{LVM} = 0.8 \times [1.05 \times (\text{IVST} + \text{LVID} + \text{PWT})^3 - (\text{LVID})^3] + 0.8 \text{ g}$$

where LVM=LV mass; IVST=interventricular septum thickness; LVID=LV internal dimension; PWT=posterior-wall thickness. It is acceptable to have the LVM indexed to the body scales. Although different scaling methods have been used, the body surface area (BSA) is the most common scaling reference ($\text{BSA} = 0.007184 \times \text{weight [Kg]}^{0.425} \times \text{height [cm]}^{0.725}$). Yet, this approach is not free of limitations, since it appears to underestimate the rate of LV hypertrophy in obese patients [129]. Resting echocardiography is highly recommended in all cases of stable coronary artery disease in order to quantify ventricular performance (strength of recommendation I, level of evidence C) [7].

5.2 Evaluation of Cardiac Systolic Function

At present, evaluating cardiac systolic function is of major clinical importance. There are prognostic implications that may affect treatment choice. Different diagnostic modalities such as angiographic ventriculography, echocardiography, radionuclide ventriculography, cardiac computed tomographic angiography, cardiac SPECT, and cardiac magnetic resonance imaging (CMR) have been developed to assess cardiac performance. These techniques differ in sensitivity, possible side effects, costs, and accessibility.

5.2.1 Echocardiographic Measurements of Cardiac Systolic Function

Among other modalities, echocardiography is the best available tool for measuring cardiac systolic function. Fractional shortening (FS) is calculated from an M-mode measurement of the LV end-diastolic dimension (LVEDD) and end-systolic dimension (LVESD), according to the following equation:

$$\text{FS} (\%) = \frac{\text{LVEDD} - \text{LVESD}}{\text{LVEDD}} \times 100$$

Measurements should be taken from a parasternal long-axis or short-axis view (Fig. 5.12A). Normal values are considered within the range of 25–40%. Fractional-shortening calculation is especially effective in evaluating contractile function in the absence of asymmetric-wall motion or LV shape. Practically, FS is not commonly used for the evaluation of LV function, although it is sometimes applied for the measurements of the right ventricular performance.

This simple quantification technique of the LV function is considered to be unsatisfactory since it is influenced

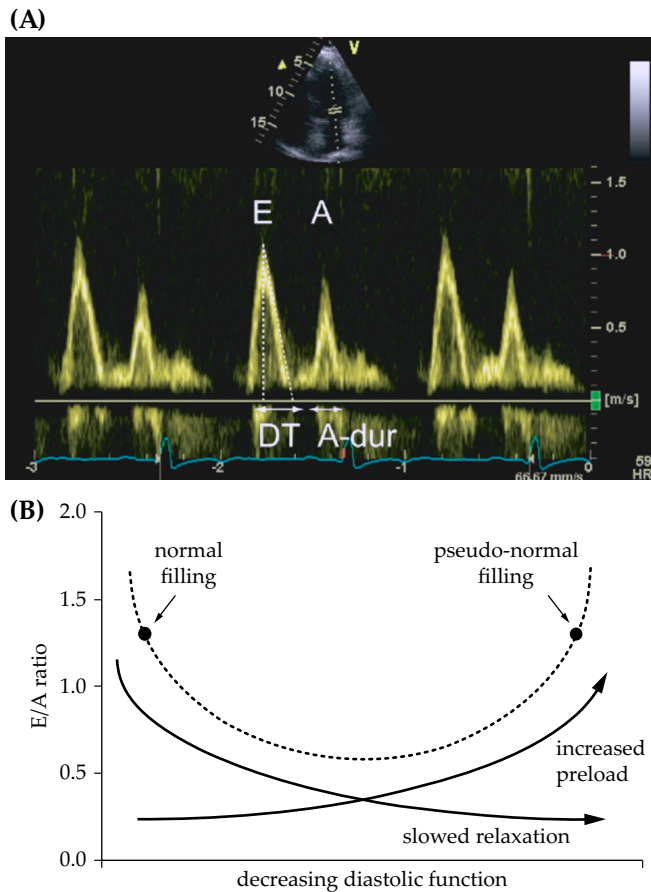


FIGURE 5.11 An example of normal transmitral blood flow velocity measured with a pulse-wave Doppler system. Note the peak amplitude of the E and A waves, DT and A-dur (panel A). The E/A ratio is used for evaluating diastolic abnormalities, when pseudonormal results might be the result of increased left atrial pressure due to progressive diastolic dysfunction (panel B). Adapted from Claessens *et al.* [127].

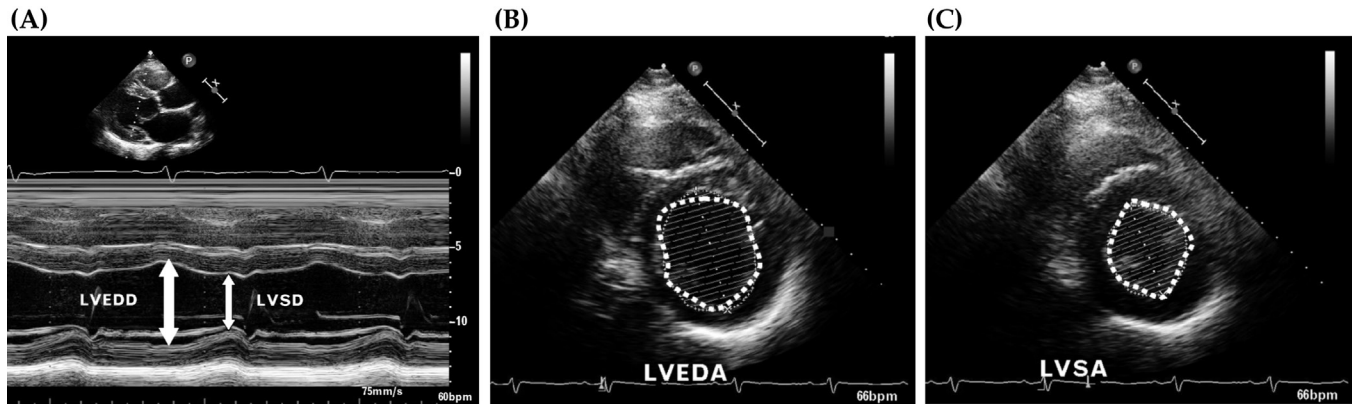


FIGURE 5.12 The method for calculating fractional shortening based on the measurements of LVEDD and LVESD (A) and fractional shortening following the measurement of LVEDA (B) and LVSA (C). Adapted from Slama and Maizel [130].

by preload, afterload, heart rate, and its limited ability to correct for wall-motion abnormalities. Therefore an index known as velocity of circumferential fiber shortening (Vcf) was developed and calculated by dividing FS with ejection time (ET). The correctional method limits the effects of preload on the calculated index, yet the Vcf does not reveal wall-motion abnormalities [131]. Velocity of circumferential fiber shortening could also correct the heart rate by dividing its value to the square root of the RR interval. Changes in the area of the LV (as measured at the level of the papillary muscle) may also assist in the quantification of the LV function. Fractional area change is calculated based on the measurements of the LV end-diastolic area (LVEDA; Fig. 5.12B) and LV end-systolic area (LVSA; Fig. 5.12C) according to the following equation [130]:

$$\text{Fractional area change (\%)} = \frac{\text{LVEDA} - \text{LVSA}}{\text{LVEDA}} \times 100$$

Nonetheless, indexes of contractile function, generated from a 2D echocardiography study, did not correlate well with other seemingly more accurate diagnostic modalities such as a cardiac MRI scan [132]. Other proposed echocardiographic techniques for evaluating LV contractile performances are based on Doppler measurements of the peak and mean aortic flow acceleration (dv/dt). Measurement of time velocity integral (TVI), obtained from across the aortic valve and the calculation of the orifice cross-sectional area (CSA), also enables the calculation of LV stroke volume (SV = TVI × CSA). The SV could be multiplied by the heart rate, thus yielding an estimate of the cardiac output (CO). Although this approach is appealing due to its relative simplicity, it was found to be an inaccurate manifestation of cardiac function compared with invasive measurements. Therefore the latter technique is not generally being used. Using 2D cardiac imaging,

a volumetric analysis of the ejection fraction (EF) can be performed while still taking into account wall-motion abnormalities [131].

$$\text{EF (\%)} = \frac{\text{LVEDV} - \text{LVESV}}{\text{LVEDV}} \times 100$$

The accepted normal range of the EF is 55–75%. Accuracy is increased when applying 3D reconstruction algorithms based on multiplane measurements, available in new echocardiography machines [130].

5.2.2 Angiographic Evaluation of Left Ventricular Performance

Angiographic ventriculography is used to grossly estimate the LV function during cardiac catheterization by an intraventricular injection of a contrast agent (usually 30–40 mL during 3–4 s). A catheter is inserted retrograde through the aortic valve and fluoroscopic imaging captured during different cardiac cycle phases, usually by using the right anterior oblique projection (Fig. 5.13) [133]. During the 1970s, prior to the wide acceptance of cardiac echocardiography, the use of ventriculography was considered as the gold standard for evaluating the LV functions. Currently, it is usually conducted within the context of concomitant coronary angiography.

The clinical information generated from this study is important where cardiac performance was not recently evaluated (usually in the setting of emergent cardiac interventions). It may also provide added information when transthoracic echocardiography exhibits low-resolution figures (as seen in obese patients). Nevertheless, some researchers argue that its use should be limited and replaced with other noninvasive imaging techniques. Moreover, the 2012 taskforce on the appropriate use criteria for diagnostic catheterization suggested that the decision as to whether or not left ventriculography should be conducted should be made by the invasive cardiologist performing the procedure based on the

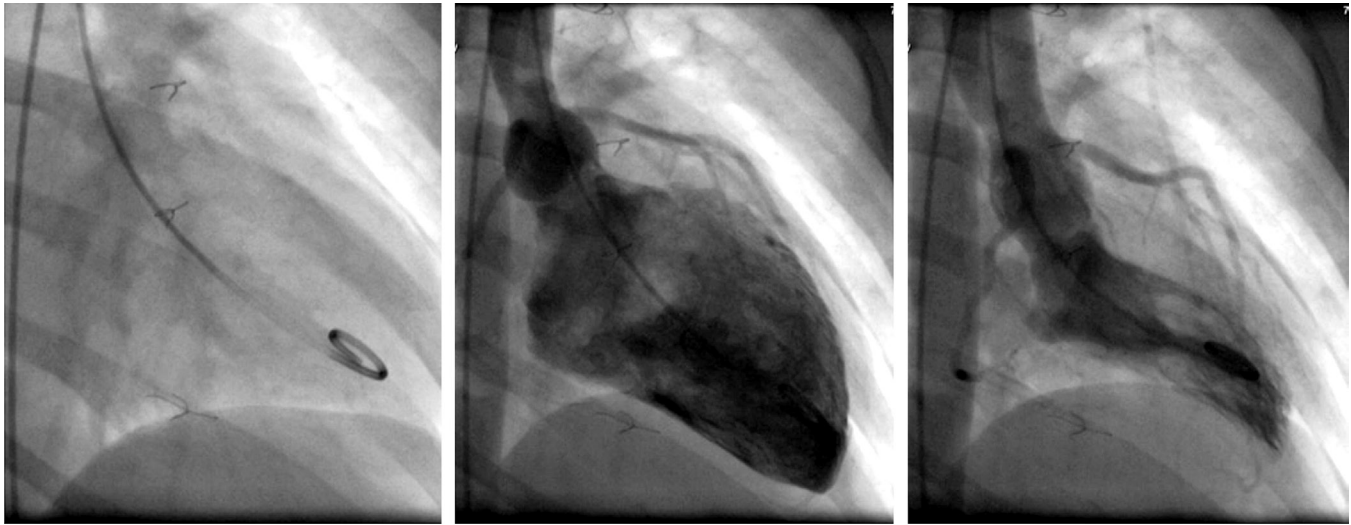


FIGURE 5.13 Left ventriculography conducted following a retrograde ventricular access through the aortic valve (*left*). 30–40 mL of contrast agent is being injected into the ventricular space, thus demonstrating its volume and shape during diastole (*middle*) and systole (*right panel*). Adapted from Witteles et al. [133].

clinical scenario [134]. A lack of consensus regarding the use of cardiac ventriculography has resulted in major variability among different medical facilities.

Gigliotti et al. suggested that ventriculography should be considered when LV function is unknown, abnormalities are suspected, and the results may influence treatment choice [135]. Biplane ventriculography is superior to single-plane ventriculography in assessing cardiac function, but is not widely applied due to the increased complexity of the procedure, increased costs, and higher radiation exposure. Left ventriculography could be used to assess the EF (in qualitative and a semiquantitative ways), detect intraventricular objects, and investigate the response to various stimuli (ie, infusion of inotropic or vaso-dilating agents). Yet, its application remains limited as a sole diagnostic technique due to the wide availability of different and more affective diagnostic approaches [135]. Today this approach appears to be overused due to its limited added value compared with other diagnostic modalities [133].

5.3 Radionuclide Ventriculography

Radionuclide ventriculography (RNV) utilizes Technetium-99m (or less frequently, thallium-201) for red blood cell labeling or labeling human-serum albumin. Following an intravenous injection, the circulatory flow is captured by a γ -ray camera. Acquisition may be performed by a first-transit study of 8–10 cardiac cycles or by ECG-gated blood-pool imaging executed over 5–10 min. The latter approach is also known as a multiple-gated acquisition (MUGA) study. First transit studies are ideal for evaluating the contractile function of the

left ventricle and are also sensitive to the visualization of intracardiac shunts. In contrast, in a MUGA study, multiple images are taken during the same cardiac phase. The latter approach yields higher resolution and is very efficient in evaluating cardiac-contraction indexes and wall-motion abnormalities. Measurements may be taken at rest or following physical exercise. Importantly, this approach does not depend on any geometric assumptions. Although this technique is considered highly accurate, even more so than echocardiographic studies, the associated exposure to radiation and high costs limits its use. It is more commonly used in treating oncologic patients where cardiac function influences the dose and type of chemotherapy [135].

5.4 Gated Cardiac Computed Tomographic Angiography

CT scans may be utilized in evaluating coronary anatomy by using contrast agents (usually eight phases of cardiac cycles). These gated CT images may also be used in measuring LV volume and EF, since the contrast agent can easily demonstrate the endocardial borders, thus making multiple diagnostic studies unnecessary. Commercial software enables automated endocardial edge detection that is crucial for volumetric quantification (Fig. 5.14) [136]. By using a contrasted cardiac CT scan, which is superior to cardiac ventriculography and echocardiography studies, rapid assessment of LV volume can be ascertained. Due to its high spatial resolution, this scan is the preferred diagnostic modality in patients with complex LV geometry [135]. Yet, temporal resolution may be lower than with a cardiac MRI. Despite its possible advantages, the associated radiation exposure, the need for an intravenous

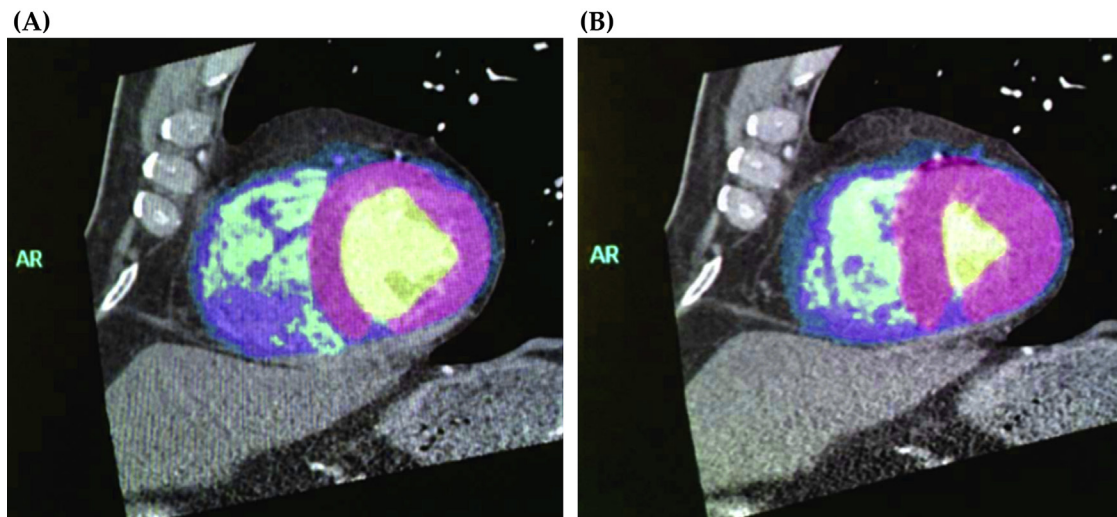


FIGURE 5.14 Automatic recognition of the LV endocardial borders and thus the ventricular lumen using a commercial software and cardiac CT angiography. End-diastolic measurements (A) and end-systolic measurements (B) enable the computation of the EF. *Adapted from Singh et al. [136].*

contrast agent, and the high costs makes this test unsuitable for cardiac function, unless a noninvasive evaluation of the cardiac coronary anatomy is vital [136].

5.5 Gated Cardiac Single-Photon Emission Computed Tomography

The data gathered during SPECT cardiac perfusion studies can also be used for quantification of EF following analysis of the gated images. The use of gated cardiac SPECT in evaluating cardiac performance, preferably in scans with Technetium-99m (and not thallium-201), was used to produce images of higher quality [137]. Measuring both cardiac perfusion and function in a single study has many clinical advantages, due to the high conformities with echocardiographic measurements. Nevertheless, in certain patient groups, such as those with smaller hearts, inaccurate evaluation of end-systolic volume may result in EF overestimation [138].

5.6 Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance imaging (CMR) is acknowledged by many to be the gold standard of a cardiac contractile function evaluation, providing both detailed anatomic data and dynamic assessment of the cardiac function. This diagnostic modality appears to be superior in terms of sensitivity, specificity, and reliability in detecting cardiac disorders associated with abnormal structure and function than other diagnostic techniques and does not involve exposure to ionizing radiation [132]. In particular, a cardiac MRI is able to detect cardiac inflammation and myocardial fibrosis using late gadolinium enhancement (LGE). The presence of myocardial

ischemia can also be detected following an infusion of low-dose dobutamine, while perfusion imaging following administration of a vasodilator provides additional functional information [139]. Yet, due to its high cost, limited accessibility, and the fact that it's very time-consuming, a cardiac MRI is often not performed. Although both cardiac echocardiography and cardiac MRI yield intercorrelated values, the LVM values yielded from each technique were reported to be different and therefore not interchangeable [129]. In comparison to other diagnostic techniques evaluating the LV's contractile function, a cardiac CT scan and 3D echocardiography also demonstrated small conformity (Fig. 5.15) [132]. Importantly, a recent meta-analysis demonstrated that CMR findings were able to predict adverse cardiovascular outcome in patients with ischemic heart disease [139]. The presence of LGE in patients with nonischemic cardiomyopathy also provides an additional powerful predictive tool.

6. MARKERS FOR CARDIAC ISCHEMIA AND ADVANCED CORONARY ATHEROSCLEROSIS

6.1 Resting Electrocardiograph

Resting electrocardiograph (ECG) is a commonly applied screening cardiac test. Its clinical utility was initiated by Willem Einthoven at the beginning of the 20th century by using a string galvanometer. For this discovery and for laying the foundations of modern electrophysiology he was awarded a Nobel Prize in Medicine at 1924 [140]. This clinical tool quickly gained popularity and today it is routinely being performed in many medical visits. It is applicable both for the diagnosis of

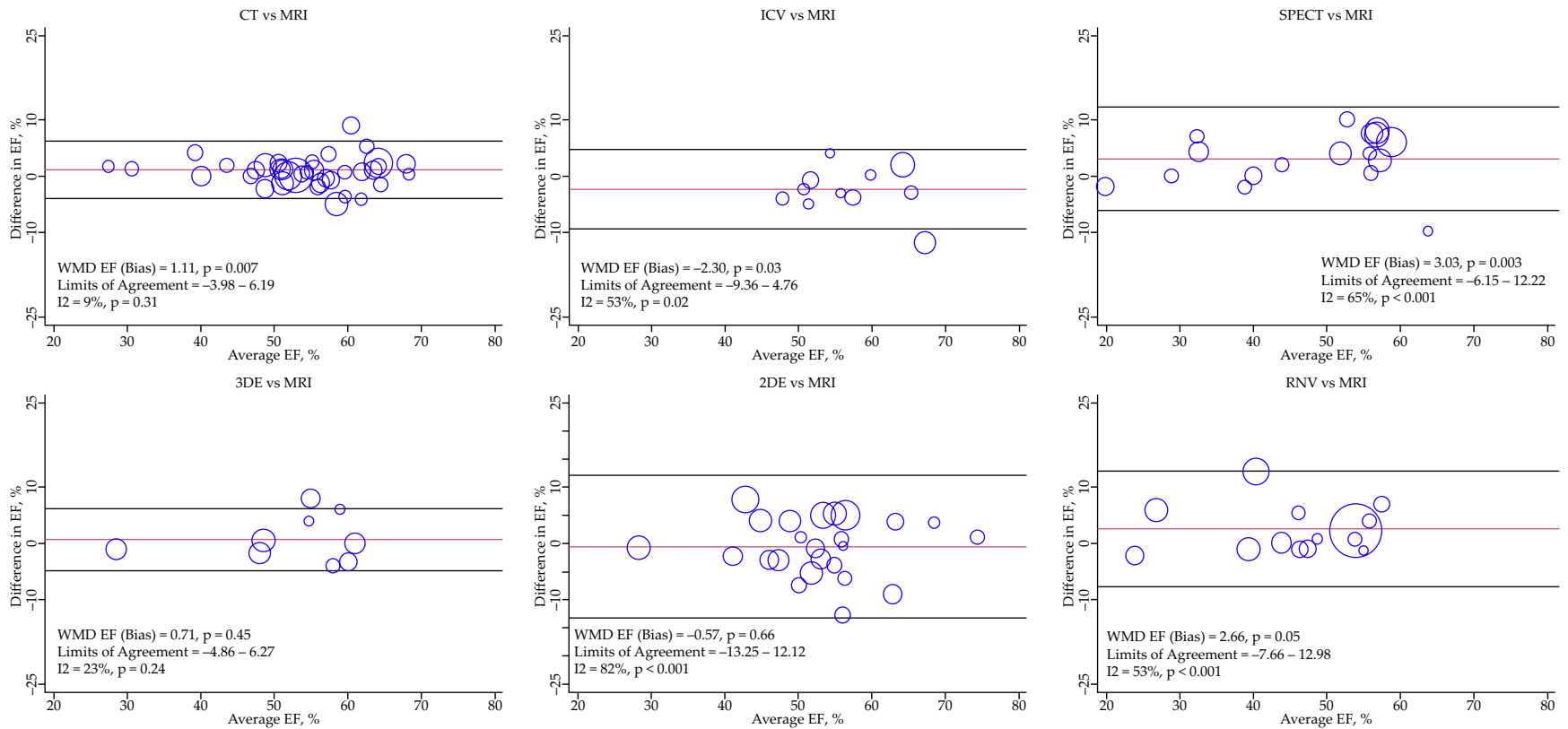


FIGURE 5.15 Modified forest plots comparing cardiac MRI to other diagnostic modalities in evaluating EF. The meta-analyzed data shows mean weighted difference and limit of agreement. 2DE and 3DE, 2D or 3D echocardiography; CT, cardiac computed tomography; ICV, invasive cardiac cine ventriculography; MRI, cardiac magnetic resonance imaging; RNV, radionuclide ventriculography; SPECT, cardiac single-photon emission computed tomography; WMD, mean weighted difference. Adapted from Pickett et al. [132].

acute disorders, the detection of previous myocardial insults, evaluation of rhythm disorders, and identification of congenital or acquired predisposition for ventricular arrhythmias. Abnormal ECG usually mandate further diagnostic workup by other stress test, anatomic evaluation, or electrophysiological study, depending on the family history, clinical symptoms, and signs. Chest pain is a class I indication for the conduction of a resting ECG study, but it should also highly be considered in patients with traditional cardiovascular risk factors, such as hypertension and diabetes mellitus (strength of recommendation IIa, level of evidence C) [7].

6.2 Exercise Stress Test

This commonly used test is aimed to demonstrate clinical symptoms of angina and ECG changes during a structured exercise protocol as a result of increased cardiac workload and increased oxygen demand. Ischemic changes in the ECG tracing and typical complaints or the development of arrhythmias may suggest insufficient coronary blood supply or anatomic cardiac substrate for arrhythmias. The Bruce's multistage protocol used for treadmill exercise testing was first reported on in 1963, and it has commonly been used for cardiac screening ever since. The complete test lasts 21 min and contains seven exercise stages (which differ in slope speed). A modified Bruce protocol starts with two additional stages with a lower workload, hence is more appropriate for elderly patients and patients with limited exercise capacity. Various other medical parameters are being monitored during the test including heart-rate and blood-pressure response. Despite its efficacy for risk stratification for many patient populations [141], sensitivity may be insufficient in specific populations of interest. Stress test is recommended as the first diagnostic modality for stable coronary artery disease in symptomatic patients with intermediate pretest probability (strength of recommendation I, level of evidence B) [7].

6.3 Stress Echocardiography

Echocardiographic evaluation prior and following a physical (ie, exercise on a treadmill or bicycle ergometer) or pharmacological stress test (ie, Dobutamine infusion) has increased sensitivity and specificity compared with ECG for the detection of cardiac ischemia [142]. It is aimed to identify wall-motion abnormalities as a sign for myocardial perfusion, which does not meet myocardial oxygen demand. Pharmacological provocation is especially applicable in patients who cannot exercise. Patients with interventricular conduction defects also cannot be evaluated for ECG changes and hence would benefit from other imaging modality. Stress echocardiography has a very high negative

predictive value and hence is highly efficient in the exclusion of coronary artery disease. In contrast, wall-motion abnormalities involving three or more wall segments (out of 17) is considered as a marker for increased future cardiovascular events (>3% annual mortality) [7].

6.4 Coronary Flow Reserve

Invasive coronary angiography is indicated in patients with severe stable angina or in those who are found to have an increased risk for cardiovascular events according to other diagnostic modalities (strength of recommendation I, level of evidence C) [7]. While coronary angiography provides important anatomical data as to macrovascular-coronary circulation, it reveals only a small portion of the coronary vascular tree. The presence of vascular narrowing is not always significant in a manner that merits intervention, especially in borderline lesions associated with 40–70% lumen stenosis. Therefore a specialized provocation test, also known as coronary flow reserve (CFR), was developed in an attempt to offer the interventional cardiologist further functional information on the coronary blood flow. Crucial life-supporting organs such as the heart is found to have autoregulatory maintenance of perfusion pressure and therefore the local autoregulatory process distal to any significant coronary vascular stenosis results in vasodilation. In such a case, the overwhelmed compensatory response does not facilitate a further increase of blood flow and unification with increased myocardial oxygen consumption, resulting in cardiac ischemia.

These physiological conditions underlie the principles for CFR calculation. Coronary flow reserve may be described in terms of absolute flow reserve (AFR), relative flow reserve (RFR), and fractional flow reserve (FFR). To this end, a flow wire catheter is placed distal to the vascular narrowing, continuously measuring average peak velocity at rest and following an adenosine provocation. Absolute flow reserve represents the ratio between hyperemic flow (due to adenosine infusion) and baseline flow. Values within the range of two to three are considered normal (ie, reflecting on a nonoverwhelmed cardiac arteriolar vascular response). Notably, the results of AFR may be affected by microvascular disease rather than macrovascular coronary abnormalities. Relative flow reserve stands for the ratio between hyperemic flow in a normal vessel compared to the narrowed vessel. This approach does not mandate evaluation of baseline flow. Fractional flow reserve represents the ratio between the maximal hyperemic flow in the presence of vascular stenosis and the expected measured flow if the vessel is unobstructed. In the maximal vasodilation state, FFR can be calculated by dividing the pressure measured distal to the obstruction

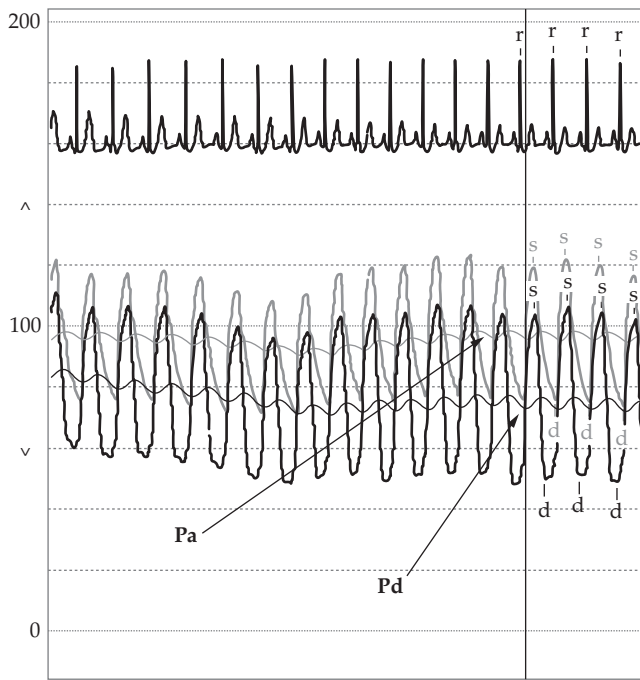


FIGURE 5.16 Measurements of intracoronary pressures proximal (Pa) and distal (Pd) to the vascular narrowing used for FFR calculation. Measurements were taken during adenosine-induced hyperemia. Adapted from El-Ahdab and Ragosta [143].

(Pd) and then to the pressure measured proximally to the obstructive lesion (Pa; Fig. 5.16). This estimate is based on the hypothesis that coronary venous pressure is negligible and that coronary vascular resistance is constant and uniform during the hyperemia phase. These assumptions simplify FFR calculations but may cause FFR underestimation, which may be particularly important in borderline cases. Fractional flow reserve values >0.8 are considered normal, values within the range of 0.75 – 0.8 are considered borderline, and values <0.75 are known to be associated with ischemia. It should be emphasized that although pressure wires are commonly used in clinical practice (for FFR) calculations, flow wires for the measurements of flow reserves are used mostly as a research tool.

Maximal hyperemia is achieved approximately 10 s following an intracoronary injection of adenosine (30 – $40\mu\text{g}$ to the right coronary artery and 80 – $100\mu\text{g}$ to the left coronary artery) [143]. Importantly, measurements of AFR and FFR were reported to correlate with the risk of coronary events [1]. Despite the wide implementation of FFR in the decision-making process of coronary intervention, this tool is highly limited in some patients. For instance, diffuse coronary disease may yield an FFR >0.8 despite myocardial ischemia, while in other cases a discrete lesion with FFR <0.75 may be accompanied by microvascular patency and cellular-level physiological remodeling of the cardiac tissue, thus resulting in the absence of actual tissue ischemia [1].

6.5 Myocardial Perfusion Single-Photon Emission Tomography

Single-photon emission computed tomography (SPECT) imaging can be used for evaluating myocardial perfusion, also known as myocardial perfusion SPECT (MPS). It is assumed that the myocardial uptake of different cations is proportional to the myocardial blood flow, the cellular integrity of cardiomyocytes, and regional potassium levels. Therefore radioisotopes of potassium and thallium were conjugated with SPECT imaging for the purpose of evaluating cardiac perfusion and well-being [144]. Thallium-201 is a radioactive potassium analog, transported into cardiomyocytes via the Na^+/K^+ adenosine triphosphate transport system. It is also effective in detecting limited myocardial flow and abnormal myocardial uptake (especially 5–10 min following an intravenous injection during an exercise stress test). A hypoperfused area will not reuptake thallium at the acute phase following a radioisotope injection, but rather a few hours later.

In contrast, a scar will appear as a fixed-perfusion defect. The use of thallium is limited by attenuation artifacts, low-resolution imaging in some patients, and prolonged half-life of the isotope [144]. Technetium-99m is an appealing alternative to thallium, due to its shorter half-life, thus enabling administration of higher doses, which yield higher image quality. Yet, its sensitivity to low-grade ischemia is lower than Thallium-201. Technetium is passively transported through the cell membrane where it attaches only to the mitochondria of viable cells. Unlike thallium, which has several distribution phases, the use of Technetium requires a separate injection at rest and during a stress test [144].

Different studies evaluated the correlation between FFR and MPS and found a moderate correlation [4]. SPECT imaging may be limited by artifacts, low spatial resolution, and a lack of quantitative data [1], but it is commonly used for detecting myocardial ischemia, especially in ambiguous cases of chest pain. The reported sensitivity and specificity of myocardial SPECT were reported as 87% and 73%, respectively [144]. The use of myocardial SPECT is limited by the considerable radiation exposure.

6.6 Positron Emission Tomography

Myocardial perfusion imaging by positron emission tomography (PET) supplies a very high resolution of myocardial perfusion imaging. Infusion of Rubidium-82 results in high sensitivity and specificity (82% and 90%, respectively) in detecting myocardial hypoperfusion and is associated with a decreased exposure to radiation compared with other techniques [144]. Measurements are conducted both at rest and following a stress

myocardial event, yielding data that enables computation of relative CFR, which is regarded as an equivalent to the data extracted from FFR [1]. It has been reported that normal global CFR, measured using PET, is highly predictive in discovering absent high-risk coronary artery disease on angiography [145]. Also, unlike SPECT imaging, PET scans have improved resolution and correction for attenuation [144].

6.7 Coronary CT Angiography

Multislice computed tomography, also known as coronary CT angiography (coronary CTA), has rapidly developed during the last two decades, yielding a high-resolution clinical tool for noninvasive evaluation of the coronary anatomy. Despite the fact that coronary angiography remains the gold standard for coronary artery anatomical evaluation, coronary CTA plays an important role in the diagnostic milieu of patient evaluation due to its relatively lower costs, noninvasiveness, and high diagnostic accuracy (an illustrative example of abnormal CTA result is found in Fig. 5.17). Sensitivity and specificity of CTA have been reported as high as 85–90% and 90–97%, respectively [146], but another study reported CTA sensitivity of 94–99% and specificity of 64–83% in detecting coronary stenosis [147]. Importantly, CTA is characterized with an extremely high negative predictive value, reaching 99% according to some studies. Therefore it is especially useful in patients with

low-to-intermediate pretest possibilities of significant coronary artery disease or when other diagnostic modalities yield inconclusive results [148].

The major limitation of coronary CTA is overestimation of coronary disease, with a reported low positive predictive value of ~50% according to some cohorts [147]. The diagnosis accuracy of coronary CTA is dependent on vessel diameter and may be overestimated in the presence of heavy calcification vessels. Importantly, coronary CTA may be used to some extent for evaluating plaque composition [146]. This diagnostic tool is also very efficient in visualizing other anatomical abnormalities of the coronary arteries, including coronary aneurysms [148].

Coronary calcium score, an established estimator for future cardiovascular events, can be obtained from non-contrasted cardiac CT images or from contrast-enhanced coronary CT angiography [149]. Coronary calcium score was found to be an independent predictor of death and coronary events following multivariate analysis in various studies in specific patients [150]. Coronary CTA is considered as a good alternative for stress imaging for ruling out stable CAD in patients with lower intermediate pretest probability for CAD. Coronary CTA is particularly valuable when stress could not be performed, or stress imaging yielded nonconclusive results (strength of recommendation IIa, level of evidence C). Yet, this test is not recommended for screening of asymptomatic patients when coronary disease is not suspected [7].

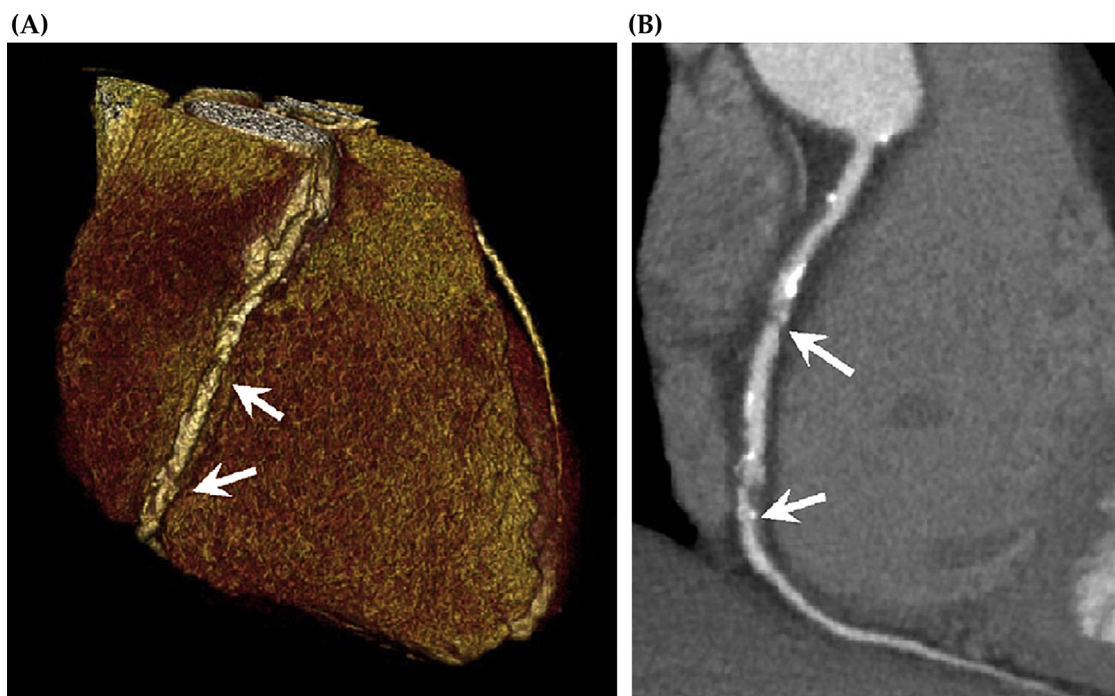


FIGURE 5.17 Coronary artery disease detected by CTA in a 68-year-old patient who presented with suspected acute coronary syndrome. Reconstruction of the right coronary artery exhibits significant coronary lesions (A) and coronary calcifications (B; arrows). Adapted from de Graaf et al. [146].

7. MARKERS OF INCREASED RISK FOR VENTRICULAR ARRHYTHMIAS

7.1 Late Ventricular Potentials and Wavelet-Decomposition Analysis

Both late ventricular potentials (LPs) and wavelet-decomposition analysis (WDA) are electrocardiographic methods designed to detect regions of delayed myocardial depolarization and abnormal myocardial impulse propagation. Late ventricular potentials are low-amplitude electrical signals that appear at the end of the QRS complex. These small electrical signals are believed to be generated by slow conduction circuits in the diseased myocardium, predisposing to reentrant arrhythmias. The micropotentials can be detected by signal averaging of some 300 ECG complexes using a signal-averaged ECG (SAECG) technique, while maintaining a low-measurement noise level. Several parameters (and agreed cutoffs distinguishing normal from abnormal results) were developed for LPs quantification: filtered QRS complex duration (fQRS) <114 ms; root-mean square voltage during the last 40 ms of the QRS (ie, RMS) >20 μ V; and a terminal filtered QRS complex <40 μ V (LAS) <38 ms (Fig. 5.18).

The coexistence of at least two abnormally defined parameters qualifies as abnormal LPs and thus an increased risk factor for developing ventricular arrhythmias [151]. Although these aforementioned criteria are widely acknowledged by most researchers, others have

suggested that cutoffs depend on gender and the ECG device used (Table 5.2) [152]. Also, at the time of frequent clinical use of LPs, a consensus also emerged that the normality limits also to some extent depend on recording circumstances and thus need to be adjusted by each electrocardiographic laboratory.

Although detection of slow ventricular conduction appears significant especially in patients who have experienced an MI [153], it has also been reported in various other cardiac conditions including cardiomyopathies [154], myocarditis, Kawasaki disease [155], inherited channelopathies [156], and other clinical conditions. The presence of LPs may suggest either slow conduction in the presence of a cardiac scar or abnormal intercellular function of myocardial gap-junctions, which could result from other circumstances. While the negative predictive values of LPs are considered high, the presence of abnormal results have a low-positive predictive value of only 8–29%. Before the modern advances of acute care, LPs appeared useful in risk stratification in patients who survived an acute MI. With the development of an acute treatment for ischemic episodes (eg, introduction of instrumental revascularization procedures in patients suffering from acute myocardial infarcts), the extent of myocardial scars has been substantially decreased. Consequently, standard LP analyses have largely lost their clinical values given the reduced prognostic power of LPs in the vascular reperfusion era [151,157]. Nevertheless, it is also possible to use different data analytical procedures and investigate the abnormalities within the single-averaged QRS complex, establishing the signal-averaged ECGs suitable for assessing QRS fractionations and other similar abnormalities. Further research in this field is still crucial.

There are several alternatives to the traditional LPs measurement (a time-domain analysis parametric system), such as WDA. Wavelet analysis endeavors to identify the small and transient perturbations of the analyzed signal usually hidden within the larger amplitudes. It's been reported that WDA is more effective than LPs measurement in identifying patients with ischemic cardiomyopathy, who are more prone to develop

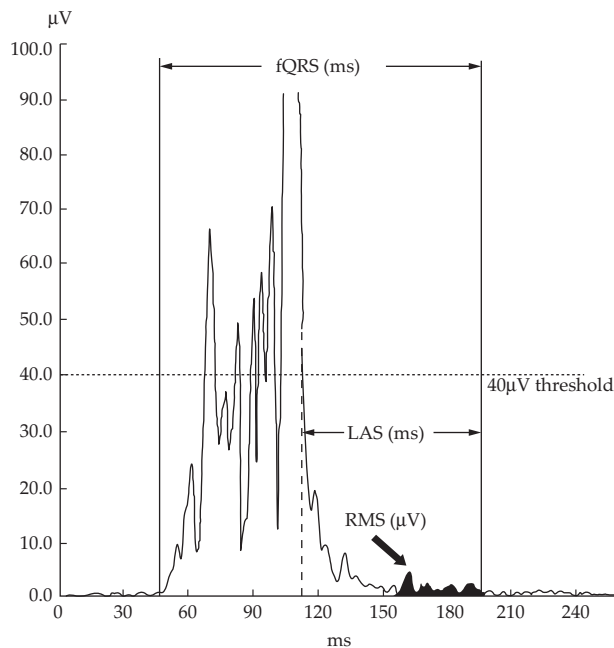
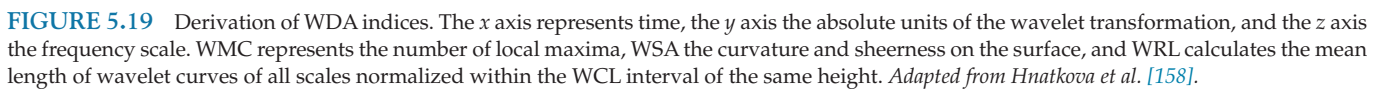


FIGURE 5.18 LPs evaluation by measuring the filtered QRS complex duration (fQRS), root-mean square voltage during the last 40 ms of the QRS (ie, RMS), and a terminal filtered QRS complex <40 μ V (LAS). Adapted from Santangeli et al. [151].

TABLE 5.2 Suggested Normal Signal-Averaged ECG Values Demonstrating Gender and Device-Based Influences on the Cutoffs Used to Determine Abnormal LPs

Device	Gender	fQRS (ms)	LAS (ms)	RMS (μ V)
Marquette (GE)	M	≤ 124	≤ 42	≥ 16
	F	≤ 116	≤ 42	≥ 15
ART (Corozonix)	M	≤ 115	≤ 47	≥ 11
	F	≤ 107	≤ 43	≥ 13

Adapted from Marcus, et al. [152].



An illustrative explanation of WDA indices derivation is shown in Fig. 5.19. Although WDA was found to successfully predict an adverse cardiac outcome in certain cardiac indications, both time-domain parameters and WDA were found to have a lower predictive value in patients with nonischemic cardiomyopathy, thus limiting the use of these clinical tools to specific patient subsets [160,161].

The QT interval reflects the overall repolarization time and is influenced by the heart rate. Numerous equations have been suggested for QT correction, of which Bazett remains the most popular, due to its simplicity, although it is the least precise. When a T-wave shape becomes abnormal, the QT interval measured from each ECG lead separately may yield different durations. For some years, it was speculated that each lead represented localized repolarization time and that the increased difference between the longest and the shortest QT intervals (also known as QT dispersion; QTd) represented heterogeneous repolarization and an increased risk for malignant ventricular arrhythmias [162,163]. These assumptions, and in particular, the possible association between lead measurements and regional repolarization are controversial and have been abandoned by some researchers. Others studies have shown that a 12-lead ECG constructed from a Frank lead system can also result in repolarization dispersion,

despite the lack of regional information yielded from such ECG tracings [164,165]. QTd measurement appears extremely simplistic and may not be prolonged even in cases of evident repolarization abnormalities (Fig. 5.20). Also, the more abnormal the T-wave morphology, the more difficult it becomes to determine the T-wave offset, thereby possibly resulting in seemingly higher QTd values [166]. Hence, presently, researchers have acknowledged that this tool has a low measurement reproducibility, low

sensitivity and overspecificity and therefore is of no practical value for clinical use [167]. Also, there is no concurrence as to the particular QTd values that might possibly differentiate low- and high-risk patients. Fig. 5.21 clearly demonstrates the high range of QTd values reported in various clinical conditions and in healthy individuals and the overlapping between health and disease in some studies. These results further emphasize the inability to set a threshold for abnormal QTd values, which further

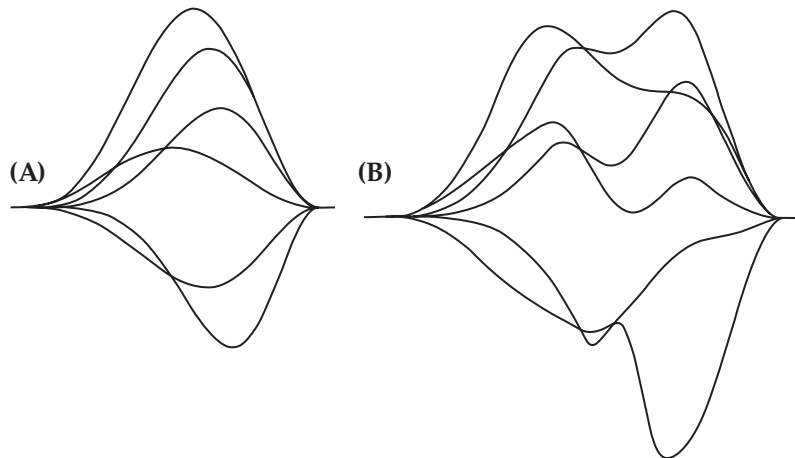


FIGURE 5.20 An example of normal (A) and abnormal (B) T waves yielding the same repolarization dispersion and QTd values further demonstrating the limitations of QTd evaluation for arrhythmogenicity quantification. Adapted from Malik and Batchvarov [210].

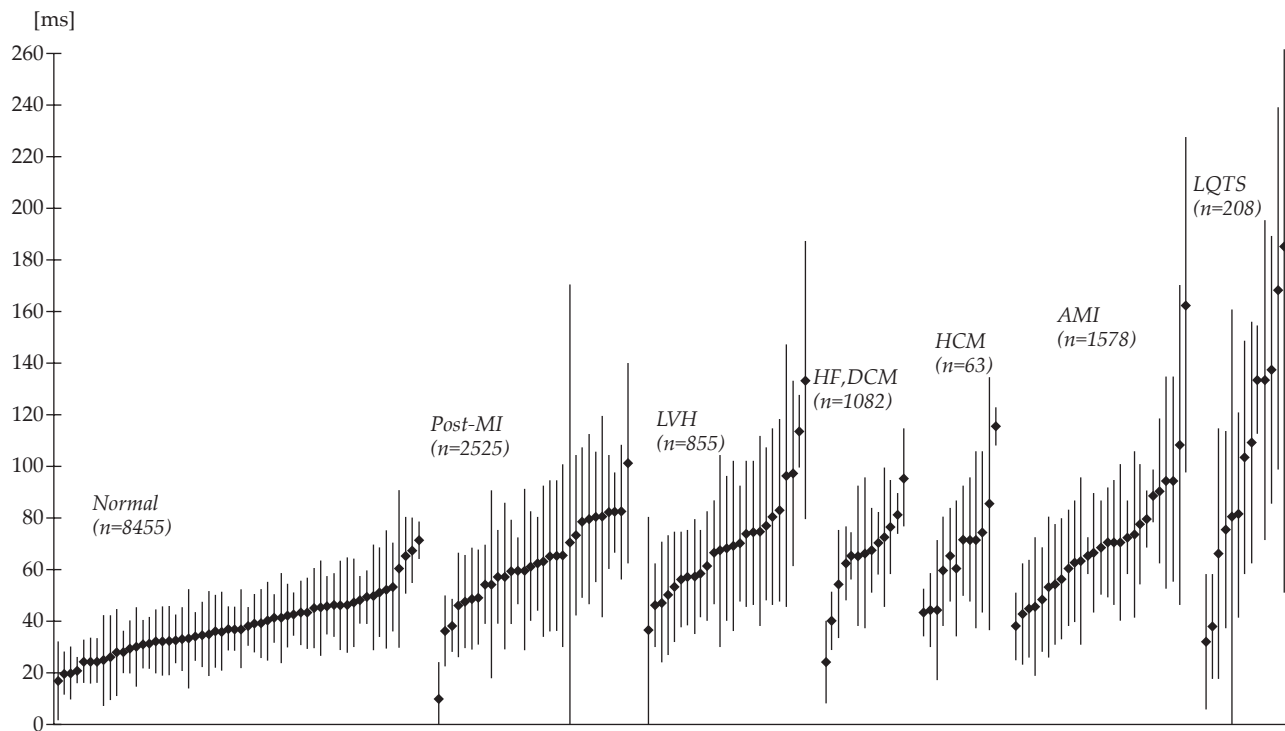


FIGURE 5.21 Several large-scale published QTd values (mean \pm SD) in various patient groups: healthy individuals, patients who survived an MI (post-MI) or acute MI (AMI), left ventricular hypertrophy (LVH), patients with heart failure (HF), dilated and hypertrophic cardiomyopathy (DCM and HCM, respectively), and long QT syndrome (LQTS). Note that a high range of QTd values were reported in health and disease and therefore no cutoff was established for identifying high-risk patients. Adapted from Malik and Batchvarov [166].

prevents the implementation of QTd measurement into clinical practice [166].

Given the major limitations of QTd measurements and interpretation, researchers have continued to explore novel electrocardiographic markers for abnormal repolarization in various patient groups that may possess a prognostic value. In 1999, Acar et al. proposed several new methods for quantifying the variations in T-wave morphologies and found a relationship between the depolarization and repolarization patterns, also known as total cosine R to T (TCRT) or ventricular gradient (VG) angle [168,169]. T-wave morphologic variables such as the VC angle appear to be influenced by multiple variables such as heart rate, gender, and perhaps autonomic regulation and technical issues, ie, respiration. Most important, both the TCRT and the T-wave loop dispersion (the variation of the interlead relations among the domain of interlead relations spanned by the ECG vector) were found to be related to adverse clinical outcomes in patients who survived an MI [170].

Another promising marker is known as T-wave residuum (TWR), nondipolar components contained within the ECG but that are not manifested in the global reconstructed T-wave vector. It was demonstrated that a lower relative TWR and lower absolute TWR values were associated with an improved prognostic outcome in a large patient cohort [171]. Since the 3D orientation of the QRS complex may be hard to define due to its complexity even in healthy individuals, implementation of these electrocardiographic tools into clinical practice is currently halted and reserved mostly for research purposes [169].

7.3 QT Variability Index

Similar to RR interval, the QT interval is erratic albeit to a much lesser degree. As noted earlier, the

interrelationship between the QT duration, changes in heart rate, and different “correction” methods were described in the 1920s. Among these correction methods, Bazett [172] and Fridericia’s techniques [173] were most popular due to their simplistic nature, with Fridericia’s formula more accurate than Bazett’s correction.

In 1997, Berger et al. [174] proposed a method of quantifying the changes of QT interval over time and suggested that these abnormal beat-to-beat changes in ventricular repolarization may be associated with increased arrhythmogenicity. Specifically, they reported on alternation of the QT interval and the lack of heart-rate changes in patients with dilated cardiomyopathy (DCM) but not in healthy controls (Fig. 5.22). The operator defined the template of a QT interval. A duration of QT interval for other beats is determined by calculating how much each beat must be stretched or compressed in time to best match the template.

QT variability index was computed by a log ratio between the QT-interval and heart-rate variability, following normalization by the squared mean of the respective time series according to the following equation:

$$QTVI_{(RR)} = \log_{10} \left[\frac{QT_v / QT_m^2}{RR_v / RR_m^2} \right]$$

The heart-rate mean (HR_m) and variance (HR_v) and QT-interval mean (QT_m) and variance (QT_v) were computed from a 456-s-long time series [174]. Normalized QT variability (QTVN), unlike QTVI, is not adjusted either by heart-rate variability or RR-interval changes. QTVN is calculated in the following manner:

$$QTVN = \frac{QT_v}{QT_m^2}$$

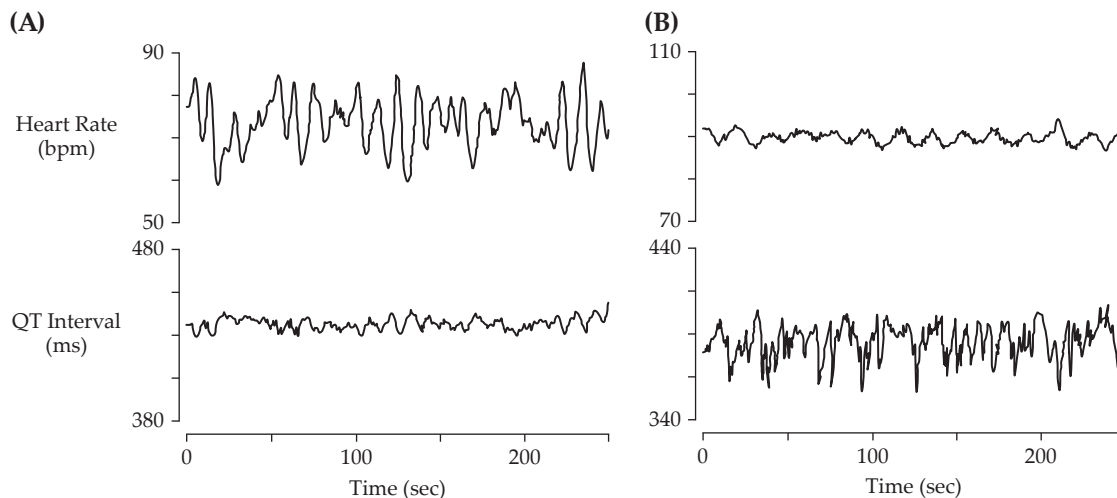


FIGURE 5.22 Example of heart-rate and QT-interval changes over time in a control subject (A) and in a DCM patient (B) demonstrating abnormal changes in heart rate and significant changes in QT-interval duration in DCM. Adapted from Berger et al. [174].

Haigney et al. [175] investigated the QTVI and QTVN values in Multicenter Automatic Defibrillator Implantation Trial II patients (inclusion: history of myocardial infarction and ejection fraction <30%) and reported significantly increased values of both parameters in patients who developed malignant ventricular arrhythmias. In the multivariate Cox proportional-hazards regression model, QTVN in the high-risk quartile was associated with an HR of 2.20 (95% CI 1.36–3.56, $p=.001$) [175]. While Berger's et al.'s [174] method for the quantification of QT variability gained the most popularity, other methods for the quantification of QT variability have been proposed [176–178]. Interestingly, a wide range of QTVI values were reported in healthy individuals within the range of -0.97 to -2.23 . In contrast, much higher values were found in various cardiac diseases (-0.9 to 0.22) [179]. The distinctive range differentiating so-called “normal” from “abnormal” results suggests that a certain cutoff could be used in defining a high arrhythmic risk according to QTVI. Indeed, several values have been proposed by various research groups, such as -0.52 [175,180], -0.47 [181], -0.11 [182], -0.84 [183], and -1.19 [184]. Although definitive values have yet to be determined, it seems safe to say that values higher than -0.47 should be considered proarrhythmic.

It was suggested that QTVI reflects also on ventricular autonomic regulation. Nevertheless, no association between norepinephrine spillover (which is used as a “gold standard” of cardiac sympathetic activity) and QTVI makes the hypothesis implausible [185]. To some extent, due to chest-wall movement during respiration, there is some change in the orientation of the T-wave loop in comparison with the ECG electrodes, thus influencing QT measurements and QTVI quantification. In most circumstances, technical issues with ECG measurements and low levels of signal-to-noise associated with quantification of milliseconds-long changes may also hamper true QTVI quantification [186]. Further studies into the predictive value of QTVI and QTVN are needed before this electrocardiographic marker can be implemented into clinical practice.

Consensus is still lacking on whether it is suitable to relate the QT-interval variability to the RR-interval variability in a linear fashion as proposed by Berger et al.'s seminal study [187]. In recordings of a stable heart rate (ie, with less RR-interval variability) simple measurements of QT-interval variability (measured, for instance, by standard deviation of QT-interval durations) also seem to predict arrhythmic substrate in patients suffering from ischemic heart disease.

7.4 T-Wave Alternans

The concept of dynamic repolarization manifested as beat-to-beat alterations in T-wave morphology were observed more than a century ago by Mines who reported on an “alteration in form and in sign of the

final ventricular variation” [198]. Changes in repolarization can be attributed to abnormal intracellular calcium handling, which in turn may predispose to arrhythmias. The microvolt changes in the T-wave amplitude (ie, T-wave alternans; TWA) are clearly associated with an increased risk of cardiac-related death in patients who have survived an MI, cardiomyopathies, and heart failure. In contrast, factors known to have some protective role against the development of arrhythmias, ie, beta-blockers and sympathetic denervation, were found associated with a decreased TWA [199]. This phenomenon is confirmed by increased heart rates and therefore necessitate an exercise stress test or prolonged Holter monitoring, although the efficacy of the latter for TWA analysis are considered controversial by some cardiologists [200,201].

There are two main acceptable approaches for analyzing TWD: the spectral and modified moving average (MMA) methods [202]. The spectral approach requires maintaining a heart rate of 105–110bpm, use of special electrodes, and employs the fast Fourier transform, calculating the average TWA value across the ST-T wave. The main drawbacks of the spectral method are the technical challenges and consequently its nonapplicability or nondiagnostics in some patients.

A positive test is determined by the presence of spectral TWA levels $\geq 1.9\mu\text{V}$ with a signal-to-noise ratio of $K=3$, maintained for 2min. The MMA system in contrast utilizes data drawn from either Holter monitoring or an exercise stress test and conducts a noise-rejection principle of recursive averaging for calculating the peak TWA value. The technical advantage of MMA compared to the spectral method for TWA analysis is the absence of a targeted sustained heart rate. Using the MMA method, the cutoff for abnormal TWA results was suggested as $\geq 47\mu\text{V}$ and $\geq 60\mu\text{V}$ (for abnormal and severely abnormal results, respectively) [199].

It has been argued that the negative predictive value of this marker is high (about 95%), while the positive predictive value remains very low (<30%) [201]. Regardless of the method used, higher TWA magnitudes are considered a higher risk for arrhythmias [199]. Most recently, it was proposed that the observed difference in TWA results were caused by differences in pretest patient handling. Therefore presently it has been proposed that patients on beta-blockers should not discontinue their medication in preparation for the test. This is expected to eliminate the previously observed discordant results.

7.5 Baroreflex Sensitivity

The baroreflex system (BRS) plays a pivotal role in the regulation of blood pressure and heart rate. Its malfunction is associated with adverse cardiovascular outcomes. Several interventions known to improve the

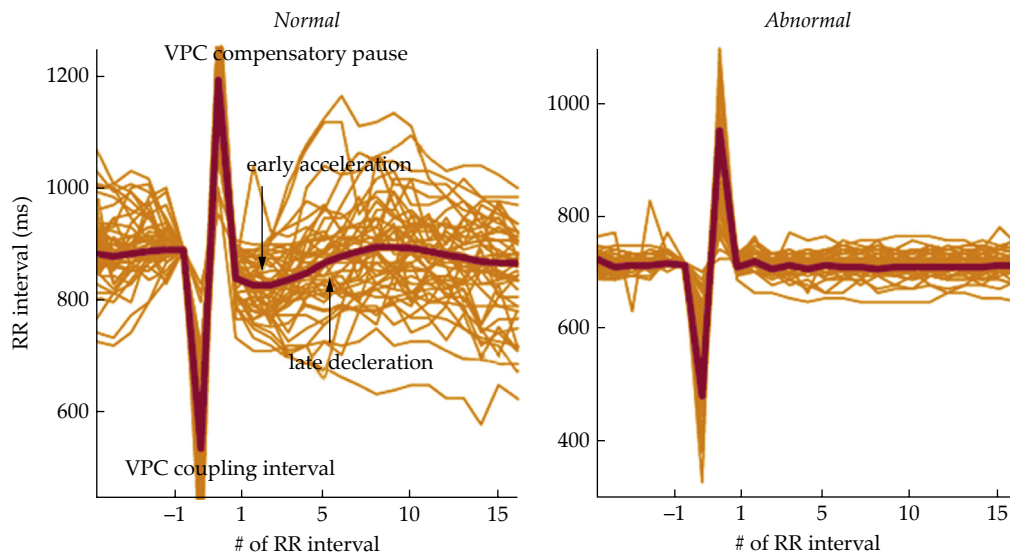


FIGURE 5.23 Changes in the RR interval following a VPC. Early acceleration of heart rate is followed by a late deceleration in healthy individuals (left; bold tracing represents a mean value). This phenomenon is obliterated in various clinical conditions (right). Adapted from Bauer et al. [192].

BRS (ie, physical activity and β -adrenergic antagonists) may also improve prognosis. There are many different ways to trigger a baroreflex response including carotid sinus massage or electrical stimulation of carotid sinus nerves, the Valsalva maneuver and head-up tilting, lower body negative pressure application, intravenous bolus injection of vasoactive agents, and other techniques [188]. Different approaches include the application of increased or reduced neck pressure using a neck chamber device.

Phenylephrine is an example of a vasopressor (α -adrenergic) that is clinically used to elicit the BRS due to its limited direct cardiac effects. It has replaced the infusion of angiotensin II used during the 1960s. Heart-rate and blood-pressure values are measured simultaneously and the BRS is calculated based on the ratio between average blood-pressure changes and the average change of the RR interval (assuming a linear regression) during a prolonged infusion of phenylephrine (50–200 μ g) or sodium nitroprusside (as a vasodepressor) [189]. An increase of systolic blood pressure by 15–40 mmHg is obligatory in order to perform the test [190].

7.6 Heart-Rate Turbulence

Heart-rate turbulence (HRT) is used to quantify short-term cyclic changes in the heart rate following a ventricular premature contraction (VPC). The concept was first introduced by Schmidt et al. in 1999, and the physiological process associated with the phenomenon has been well established [191]. It is recognized that the VPC initiates a baroreflex-mediated response of the sinus node to the transient change in blood pressure, thus providing

an understanding of the autonomic regulation of the heart rate.

In individuals with preserved baroreflex reactions, a VPC is followed by a brief acceleration and subsequent deceleration of the heart rate in comparison with the mean baseline rate prior to the VPC. The initial phase of the phenomenon is assumed to be caused by transient vagal denervation (due to ineffective ventricular contraction induced by the premature beat and insufficient baroreflex stimulation). Thereafter, relative sympathetic overactivity causes an increase in arterial blood pressure and secondary vagal tone recruitment, which is responsible for heart-rate deceleration. Since heart-rate variability may influence heart-rate changes prior and following a VPC, it is acceptable to compute HRT from a 24-h Holter recording. At least five RR intervals prior to the VPC and 15 following the VPC and an average tracing of at least five VPCs are required to construct a tachogram from which HRT can be computed (Fig. 5.23) [192]. Included VPCs for an HRT analysis should have a prematurity of $\geq 20\%$ and a compensatory pause of $\geq 120\%$ compared to the mean RR interval of the last five sinus beats prior to the VPC.

In order to quantify HRT, two variables are used: turbulence onset (TO) and turbulence slope (TS). Turbulence onset is calculated according to the following equation, where RR_{-1} and RR_{-2} represent the two RR intervals prior to the VPC. RR_1 and RR_2 stand for the two RR intervals following the VPC.

$$TO = \frac{(RR_1 + RR_2) - (RR_{-1} + RR_{-2})}{(RR_{-1} + RR_{-2})} \times 100 \text{ [\%]}$$

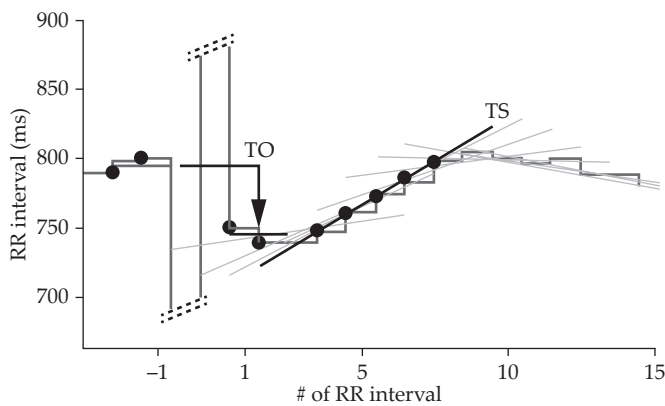


FIGURE 5.24 Calculation of turbulence onset (TO) and turbulence slope (TS) from the RR-interval changes following a VPC. Adapted from Bauer et al. [192].

Turbulence slope is calculated from the average tachogram as the maximum positive slope fitted over 5 mean consecutive sinus rhythm RR intervals, within the first 15 sinus rhythm RR intervals after the VPC (Fig. 5.24).

The TO values observed in normal individuals were reported within the range of -2.7% to -2.3% . The TS was reported to be $11.0\text{--}19.2\text{ms/RR interval}$ [193–196]. Nevertheless, values of $\text{TO} > 0\%$ and $\text{TS} > 2.5\text{ms/RR interval}$ are often considered normal [191,197]. Having either an abnormal TS or abnormal TO is associated with an intermediate risk for cardiovascular mortality; while having both abnormal TS and abnormal TO is considered to be associated with high risk. Among other markers, HRT appears to have a very high predictive value, especially in post-MI patients [192]. While this clinical tool seems to be well established and cutoff values differentiating different risk levels were predefined, its widespread use is hampered by the need for prolonged Holter monitoring.

8. MARKERS OF INCREASED RISK FOR SUPRAVENTRICULAR ARRHYTHMIAS

Atrial fibrillation (AF) is the most common arrhythmia, affecting million of patients worldwide. The risk for developing AF significantly increases in older patients and may affect approximately 9% of patients in their 80s [203]. Atrial fibrillation is a common cause of medical complications such as stroke and a leading cause of hospitalizations. While various cardiac illness may increase the risk for developing AF, it may appear unprovoked in some patients [203].

8.1 P-Wave Dispersion

The same logic that governs the calculation and use of QTd underlies the development of P-wave dispersion (Pd) and its evaluation. As noted earlier, although

highly controversial and abandoned by many research groups, some researchers believe that each of the ECG leads contain regional information and that differences in P-wave durations as measured from multiple ECG leads suggest heterogeneous activation of the atriums and increased risk for supraventricular arrhythmias. Nevertheless, similar to the debate on QTd, it was suggested that the P-wave loop projection on different ECG leads is responsible for the phenomenon, rather than the actual regional data. P-wave dispersions calculated by subtracting the shortest from the longest P-wave durations, measured simultaneously from a 12-lead ECG tracing. A cutoff of 40ms to differentiate low- from high-risk patients has been suggested by several researchers [204]. Nonetheless, a very high range of values has been reported for healthy individuals in the medical literature, ranging from $7 \pm 2.7\text{ms}$ to $58.6 \pm 16.2\text{ms}$ [205].

Both autonomic nervous system dysfunction and cardiac ischemia have been suggested as possible contributors to the phenomenon of increased Pd. Yet, lack of clinical standardization of the measurement method and the high inter- and intraobserver variability associated with manual measurements has limited the widespread use of this clinical tool. Overall, similar to QT dispersion, P-wave dispersion measurements have now largely been discarded.

8.2 P-Wave Averaging

As noted earlier, SAECC can be used for evaluating low-amplitude potentials that may suggest an anatomical substrate for reentrant ventricular arrhythmias. The averaged ECG for assessing P-waves necessitates three bipolar leads arranged in orthogonal planes. Similarly, prolongation of the filtered P-wave signal (by using the SAECC method) was suggested as a method for identifying atrial conduction abnormalities and an increased risk of developing AF. Prolongation of the P wave may indicate delayed atrial conduction, which may predispose for supraventricular arrhythmias [206]. Various p-wave duration (PWD) values have been suggested as possible markers for increased AF risk, within the range of $>110\text{ms}$ to $>155\text{ms}$. Accordingly, different positive and negative predictive values were reported in different patient populations [203,207]. Lower root mean-square voltage of the last 20ms of the P wave (RMS20) was also suggested to correlate with increased risk of AF (Fig. 5.25).

Budeus et al. [206] reported that $\text{PWD} \geq 126\text{ms}$ and cooccurrence of $\text{RMS20} \leq 3.1\mu\text{V}$ had produced a 61% sensitivity, an 84% specificity, and 80% positive predictive value in forecasting AF relapse following cardioversion. Interestingly, PWD was found correlated with the size of the atria [208] and with age [204], both of which are established risk factors for the development and recurrence

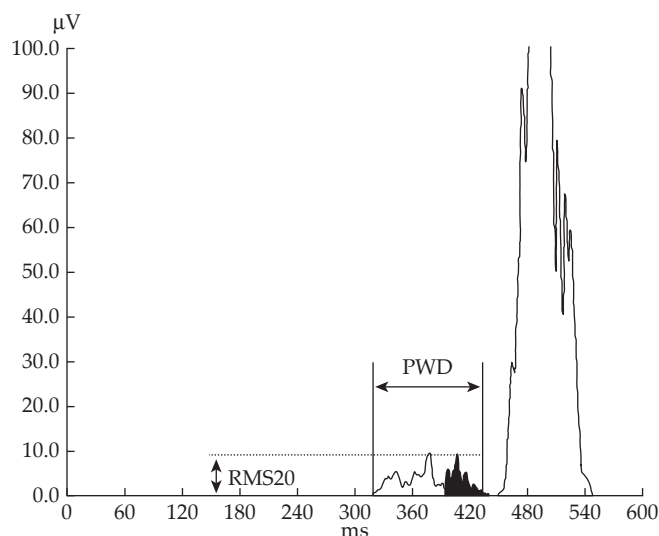


FIGURE 5.25 SAECG measurement of PWD and RMS20 performed in an attempt to identify high-risk patients who would develop AF. Adapted from Budeus et al. [206].

of AF. Although filtered P-wave duration was found to be of high reproducibility, voltage parameters were of lower reproducibility, and any interpretation of signal-averaged P-wave parameters should be performed with caution [209].

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Rheumatoid Arthritis

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1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic progressive systemic inflammatory disease affecting the joints and other parts of the body. The clinical course of RA is generally one of exacerbations and remissions with disease activity and burden varying greatly from person to person. As a result, long-term outcomes are highly variable: some patients experience a relatively self-limited disease, whereas others have a chronic progressive illness leading to physical disability with significant psychosocial and financial consequences [1]. Although the precise etiology is not known yet, RA, like other systemic autoimmune disorders, appears to have a multifactorial pathogenesis with a combination of environmental (eg, cigarette smoking, infection, or trauma) and hormonal factors activating the immune system in genetically susceptible individuals leading to synovial hypertrophy, chronic joint inflammation and destruction, along with the potential for extra-articular manifestations [2].

Synovitis is the major characteristic of the disease and synovial membrane has a dominant role in joint inflammation and destruction. From a histopathological standpoint, RA is characterized by synovial lining layer hyperplasia, neovascularization, and inflammatory infiltration of the synovial membrane, which is gradually transformed to the hyperplastic “aggressive” tissue called pannus [3]. Pannus invades cartilage and bone,

resulting in nonreversible joint damage and deformities. Typically RA presents as chronic symmetric inflammatory polyarthropathy affecting predominantly the small joints of the hands and feet, but all joints can potentially be damaged in various patterns with the exception of distal interphalangeal joints and the lower spine. In addition to joint pain, stiffness, and swelling, constitutional symptoms such as fever, malaise, and weight loss as well as extra-articular involvement including cardiopulmonary disease and today more rarely vasculitis and eye inflammation can represent initial manifestations of the disease. Immunological abnormalities, namely rheumatoid factor (RF) and anticitrullinated protein (anti-CCP) antibody positivity, are common in RA patients and more importantly they hold significant diagnostic and prognostic value as their presence usually relates to more aggressive and severe disease [4,5].

The diagnosis of RA is based on patients' medical history, clinical, radiological, and laboratory findings. In 1987 the American College of Rheumatology (ACR) established recommendations for the classification of patients with RA (Table 6.1) [6]. Given that over the last decade biologic drugs revolutionized treatment strategies and outcomes in inflammatory arthropathies—including RA—a joint working group of the ACR and the European League Against Rheumatism (EULAR) developed a new approach for classification of RA in order to facilitate the study of people at earlier stages of the disease (Table 6.2) [7]. Currently both

TABLE 6.1 The 1987 ACR Revised Criteria for the Classification of RA by ACR [6]

Criterion	Description
Morning stiffness	Morning joint stiffness lasting at least for an hour before improvement
Arthritis of three or more joints	At least three joint areas (out of 14 possible)
Arthritis of hand joints	Swelling of wrist, metacarpophalangeal (MCP) or proximal interphalangeal (PIP) joints for at least 6 weeks
Symmetric arthritis	Simultaneous involvement of the same joint areas (defined in 2) on both sides of the body (bilateral involvement of PIP, MCP, or metatarsophalangeal [MTP] joints is acceptable without absolute symmetry) for at least 6 weeks
Rheumatoid nodules	Subcutaneous nodules over bony prominences, extensor surfaces or in juxta-articular regions, observed by a physician
Rheumatoid factor (RF)	Detected by a method positive in fewer than 5% of normal controls
Radiographic changes	Typical of RA on posteroanterior hand and wrist radiographs; it must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis [OA] changes alone do not qualify); feet erosions

classifications are employed, with the former being more applicable to the routine clinical setting and the latter being used in academic projects and clinical trials.

Besides joint destruction and damage, chronic systemic nature of inflammatory process in RA has various effects on internal organs and the vasculature leading to a number of comorbidities such as osteoporosis, infections, lymphomas, and cancers as well as premature death [8]. Despite remarkable advances in the management of joint inflammation and functional disability over the last two to three decades the mortality gap between RA patients and the general population is not closing, with cardiovascular (CV) mortality representing one of the leading causes of reduced life expectancy in RA [9].

1.1 Epidemiology of CV Disease and Mortality in RA

1.1.1 CV Mortality in Rheumatoid Arthritis

Patients with RA have been shown to have higher mortality rates than the general population [10]. A *meta-analysis* examining 24 studies of mortality in RA comprising a total of 111,758 patients found that CV mortality was increased by 50% in RA patients [*meta-stan-*

TABLE 6.2 American College of Rheumatology/European League Against Rheumatism 2010 Criteria for Classification of Early RA [7]

Joint involvement (0–5)	
1 medium/large joint	0
2–10 medium/large joints	1
1–3 small joints	2
10–10 small joints	4
>10 joints (at least one small)	5
Serology (0–3)	
Neither RF or APCA	0
At least one test low positive	2
At least one test high positive	3
Duration of synovitis (0–1)	
<6 weeks	0
>6 weeks	1
Acute-phase reactants (0–1)	
Neither ESR nor CRP abnormal	0
Either ESR or CRP abnormal	1

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; APCA, anticitrullinated protein antibody. The criteria are used for classification of patients presenting with at least one joint with definite clinical synovitis that is not better explained by another disease. A score of ≥ 6 fulfills requirements for definite RA.

dardized mortality ratio (SMR) 1.5, 95% CI 1.39–1.61]. The weighted combined *meta*-SMR for ischemic heart disease (IHD) and cerebrovascular accidents (CVAs) were 1.59 (95% CI 1.46–1.73) and 1.52 (95% CI 1.40–1.67), respectively [11]. This *meta-analysis* also found that patients with a short duration of disease may have a lower risk of CV mortality than those with long disease duration; interestingly no significant increase in CV mortality risk was identified in inception cohorts (*meta*-SMR 1.19, 95% CI 0.86–1.68), ie, patients identified early in the course of their disease and at a uniform time point. Unrecognized coronary heart disease and sudden death have been shown in a population-based cohort study to be almost twice as likely in RA patients compared with the general population (HR 1.94, 95% CI 1.06–3.55) [12].

1.1.2 Atherosclerotic Cardiovascular Disease in RA

The risk of CV events (CVEs) is increased in RA. A *meta-analysis* of 14 studies from inception to 2011 including 41,490 patients found a 48% increased risk of incident CVE in RA patients compared with controls [13]. Ten of these studies examined risk of myocardial infarction (MI) and found this was increased by 68% in RA patients (pooled RR 1.68, 95% CI 1.40–2.03).

A case control study comprising nearly 12,000 RA patients found that the prevalence of ischemic heart

disease was significantly higher in RA patients than in controls, 16.6 versus 12.8%, respectively, $p < .001$ [14].

Myocardial infarction may occur earlier in RA than in the general population. A nationwide study including just over 10,000 RA patients reported the risk of MI in RA to be similar to the risk in non-RA subjects who were a decade older [15]. There is also evidence that this increased CV risk is present even before the diagnosis of RA is made: one study found that at time of diagnosis of RA, patients were more than three times as likely to have had a prior MI compared with non-RA patients [13].

Compared with MI, less is known about the epidemiology of stroke in RA and study results have been conflicting. However, evidence exists that the risk of stroke is also increased in RA. The *meta-analysis* described earlier found a 41% increased risk of cerebrovascular accidents in RA patients compared with controls [14]. This risk was present in both males and females.

With respect to CVEs in RA, research has focused mainly on IHD and CVA and less is known about the relationship between RA and peripheral arterial disease (PAD). Peripheral arterial disease is a marker of systemic atherosclerosis and is considered to be a CV risk equivalent. A nationwide population-based study in Taiwan involving approximately 30,000 RA patients found that the incidence of PAD was significantly higher in RA patients compared with non-RA controls (HR 1.73, 95% CI 1.57–1.91) [16]. Risk of PAD was highest in younger RA patients and those with comorbidities such as diabetes and hypertension.

Heart failure is a significant contributor to the excess CV risk associated with RA [17,18]. A population-based cohort study found that the risk of developing heart failure in RA patients was almost twice that of non-RA patients (HR 1.87, 95% CI 1.47–2.39) [19]. This risk was more pronounced in RA patients who were rheumatoid factor-positive compared to rheumatoid factor-negative RA patients.

The precise determinants of increased CV risk in RA have not yet been completely understood. It has been suggested that disease-specific factors including systemic inflammation and autoimmune activation trigger pathways of atherosclerosis and/or thrombosis and, combined with traditional risk factors, lead to increased CV morbidity and mortality [20,21] (Fig. 6.1). In the following we discuss the pathogenesis of different aspects of cardiac complications in RA, review current recommendations, and focus on practical issues regarding management and prevention of CV disease in this population.

2. CARDIAC INVOLVEMENT IN RA

2.1 Presentation

The appreciation that CV complications with underlying ischemic pathology—predominantly coronary artery disease and myocardial infarction—are more common and confer the greatest mortality risk should not underestimate the role of other syndromes, which may also present in RA patients. Additional, nonatherosclerotic mechanisms such as impairment of microvascular circulation due to small-vessel disease, pulmonary vasculature involvement, myocardial inflammation, and fibrosis also considerably add to excess CV risk [22]. As a result, clinical phenotypes of heart involvement cover a wide spectrum of different conditions including pericarditis, myocarditis, pulmonary hypertension, and cardiomyopathy accompanied by reduced myocardial contractility and performance [23] (Table 6.3). Today amyloidosis and severe valve disease are very rare with almost unremarkable clinical significance [24]. In recent years cardiac autonomic neuropathy and conduction defects have been recognized as emerging, novel contributors to CV morbidity and sudden deaths [25,26]. The prevalence of each of these varies greatly and

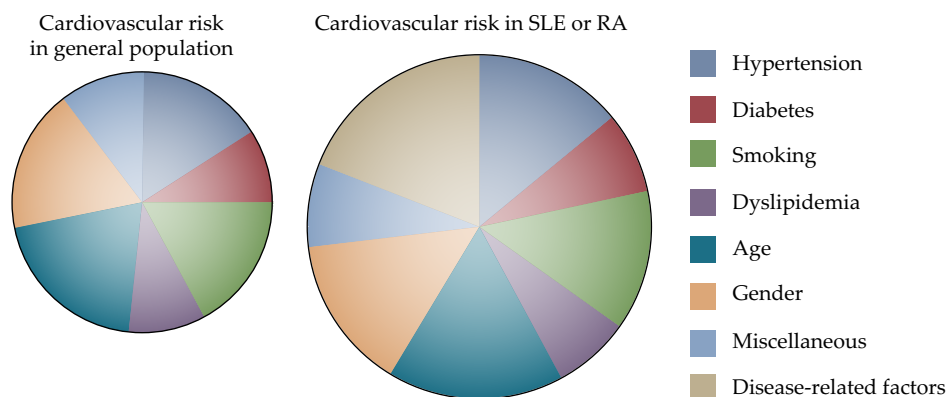


FIGURE 6.1 Distribution of classic CV risk factors in general population, RA, and SLE patients. Rheumatoid arthritis confers an overall higher CV risk than in the general population mainly due to the contribution of disease-related factors, namely systemic inflammation and autoimmune activation. Traditional CV risk factors contribute to the excess risk albeit in a different way or to a lesser extent to RA in than in the general population. CV, cardiovascular; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus. Adapted from Symmons and Gabriel [39].

TABLE 6.3 Cardiac Manifestations of Rheumatoid Arthritis

Atherosclerotic heart disease	Coronary artery disease
	Myocardial infarction
Heart failure	Ischemic heart disease
	Diastolic heart failure
	Reduced contractility due to myocardial fibrosis
Pericarditis	Usually asymptomatic
	Cardiac tamponade (rare)
Myocarditis	Usually asymptomatic common during active disease
Cardiomyopathy	Myocardial fibrosis
	Amyloidosis (rare)
	Drug-induced hydroxychloroquine (rare)
Conduction system abnormalities	Varying degrees including complete heart block (uncommon)
Valve disease	Mitral and aortic regurgitation predominance
Coronary arteritis	Small-vessel disease
Pulmonary hypertension	Pulmonary arterial hypertension (rare)
	Hypoxia induced pulmonary hypertension (interstitial pulmonary fibrosis)

whether they significantly impact on mortality needs further investigation. A *meta*-analysis of 10 case-control studies published between 1975 and 2010 on valvular and pericardial involvement in patients with RA found that the risk of pericardial effusion and valvular nodules was more than 10 times higher in RA patients than in controls (pooled OR 10.7, 95% CI 5.0–23.0 and OR 12.5, 95% CI 2.8–55.4, respectively). The odds ratio for valvular insufficiency and stenosis was also increased significantly in RA patients (OR 4.3, 95% CI 2.3–8) [27].

Clinical symptoms of cardiac disease are subtle and nonspecific and may overlap with those of other comorbidities such as anemia, musculoskeletal pain, or lung involvement. For example, new onset or worsening of dyspnea and tiredness in a middle-aged patient with suboptimally controlled RA can be easily attributed to RA itself, to anemia of chronic disease, or to drug toxicity (eg, methotrexate) rather than to impairment of cardiac function. On the other hand, the sedentary lifestyle adopted by RA individuals due to reduced functional status masks signs such as chest pain and dyspnea on exertion, which may raise the suspicion of evolving heart disease. Last but not least, clinical features such as ankle swelling and reflective shoulder or upper limb pain can be misinterpreted as symptoms of RA rather than indications of progressive heart failure and coronary ischemia,

respectively. Coronary artery disease in particular has an atypical presentation and usually is unrecognized clinically [28]. Similar to what occurs in diabetes mellitus, RA patients are less likely to suffer from chest pain or angina. Acute coronary syndromes are clinically silent and present with collapse or dyspnea [12] and myocardial infarction is associated with higher fatality rates and outcomes than in the general population [29,30]. According to a Danish nationwide study [15] and a prospective study of a Dutch RA cohort [31], the magnitude of CV risk in RA is analogous to that of diabetes mellitus—the prototypic disease carrying excessive risk for CV events—with these observations being in line with studies demonstrating a similar rate of atherosclerosis in these two conditions [32]. The pathophysiology of heart failure is also different in RA—consisting mainly of diastolic dysfunction with well-preserved left ventricular ejection fraction [33]—with fewer classic symptoms than in patients without arthritis; when symptoms eventually become clinically apparent they may be indicators of severe advanced disease. The prognosis is also unfavorable as the risk for death in the first period after the clinical manifestations of heart failure appears to be heightened among RA individuals than in controls [34].

However, the occult nature of clinical symptoms is not the only barrier in the early diagnosis and management of CV disease in this population. Currently used routine diagnostic methods lack sensitivity to capture abnormalities of cardiac function in subclinical stages [35]. For example, conventional echocardiography does not detect structural and functional changes of left ventricle myocardial remodeling such as hypertrophy and fibrosis associated with the effect of chronic inflammation on cardiac muscle in preclinical stages [36]. Given the high mortality rates of CV complications within the RA population, the establishment and validation of noninvasive methods to identify patients at higher risk and initiate cardioprotective treatment promptly remains an unmet need. The development of novel imaging modalities—in particular, cardiac magnetic resonance—may allow the determination of subclinical myocardial involvement and detection of myocardial fibrosis and other structural changes in asymptomatic patients [37,38] and lead to the implementation of CV prevention strategies, which may diminish the number of CV events and improve the course of the disease.

2.2 Traditional CV Risk Factors

The impact of conventional risk factors on the development of CV disease in the general population as well as in people with RA is well described. The prevalence of such factors among RA individuals appears to be higher than in subjects without arthritis, although ethnic differences should be taken into account [39]. However, traditional CV risk factors are not sufficient to explain the increased

CV risk on their own as the absolute risk for CV events and deaths for RA patients remains higher than in the general population, even after controlling for hypertension, dyslipidemia, body mass index, cigarette smoking, and insulin resistance [40]. It seems that known CV risk factors operate differently in the RA population as specific factors including male gender, smoking, and personal cardiac history appear to confer significantly “less” risk for the development of CV disease in RA patients than in age- and sex-matched non-RA subjects [41]. However, a *meta-analysis* revealed that traditional risk factors independently increase the risk of CV morbidity in RA: hypertension (RR 2.24, 95% CI 1.42, 3.06), hypercholesterolemia (RR 1.73, 95% CI 1.03, 2.44), diabetes mellitus (RR 1.94, 95% CI 1.58, 2.30), smoking (RR 1.50, 95% CI 1.15, 1.84), and obesity (RR 1.16, 95% CI 1.03, 1.29) [16]. The influence of novel CV risk factors such as hyperhomocysteinemia and hypercoagulation [24], the cardiotoxic effects of antirheumatic treatment as well as the positive modulation of systemic inflammation to the effects of established CV risk factors on vascular homeostasis may provide additional explanations to the complex interrelations between several different mechanisms involved in the development and promotion of accelerated atherosclerosis in RA (Fig. 6.2).

2.2.1 Hypertension

Hypertension is a major risk factor for CV disease in the general population worldwide, affecting almost 30% of the adult population in the United States [42]. Hypertension significantly contributes to CV disease in RA as it is associated with preclinical atherosclerosis [43,44] and has been demonstrated as one of the most significant independent predictors of CV [45] and target-organ (eg, renal) damage [46]. The reported incidence of hypertension within RA patients varies from 4% to 73% depending

on study design, population assessed, and definition used for the characterization of hypertensive patients [47]. The results of the international COMORbidities in Rheumatoid Arthritis (COMORA) study showed a prevalence of 40%, but these findings should be considered against the background of the lack of a control population in this study [48]. Whether hypertension is more common in RA than in healthy individuals remains an issue of debate. A *meta-analysis* included seven case-control studies and showed that no significant differences existed on the prevalence of hypertension among 2956 RA subjects and 3713 controls (OR 1.09, 95% 0.91–1.13) [49]. On the contrary, other studies have demonstrated that hypertension is more prevalent in RA, which may be associated with the higher rates of underdiagnosis and undertreatment of hypertension especially among younger patients [50,51].

The pathophysiology of increased blood pressure in RA encompasses various mechanisms. Disease-related factors, predominantly the adverse effects of proinflammatory cytokines on endothelium, lead to diminished nitric oxide bioavailability and subsequently to reduced arterial wall pliability, increased stiffness, and vascular resistance [52]. Physical inactivity and the presence of specific genetic polymorphisms represent additional factors contributing to the development of hypertension [53,54]. Last but not least several antirheumatic drugs affect blood pressure positively or negatively, with the majority of them such as Leflunomide, cyclosporine, steroids, and nonsteroid anti-inflammatory drugs resulting in new onset or worsening of preexisting hypertension [55] (Fig. 6.3). The impact of novel biologic therapies on blood pressure remains controversial as data from the Behandel-Strategieën (BEST) trial suggests that tumor necrosis factor- α inhibitors (TNF α) can reduce blood pressure [56], while a recent *meta-analysis* of a total of 6321 subjects with RA from 11 randomized clinical

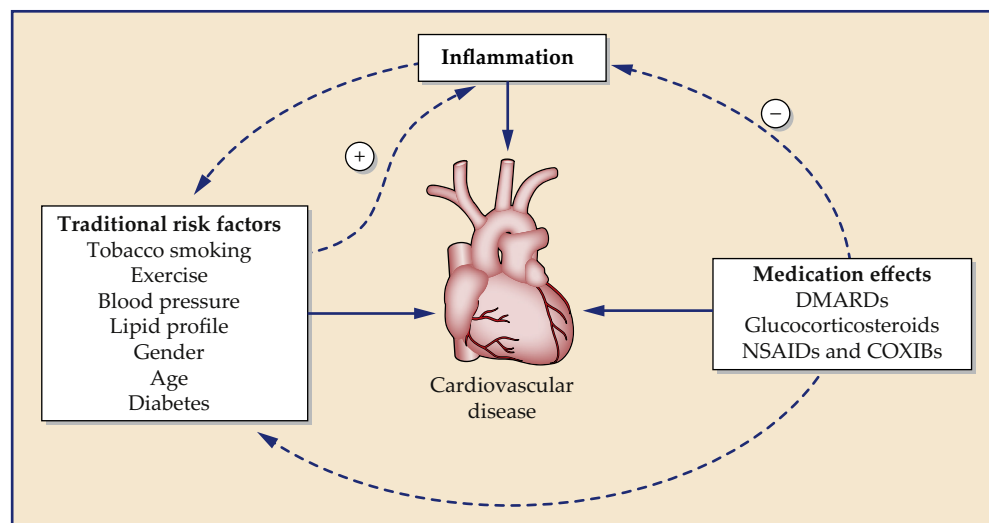


FIGURE 6.2 The different components of CV disease in patients with RA. Traditional and disease-related factors form a complex puzzle with numerous interrelations between each other. Adapted from Nurmohamed et al. [24].

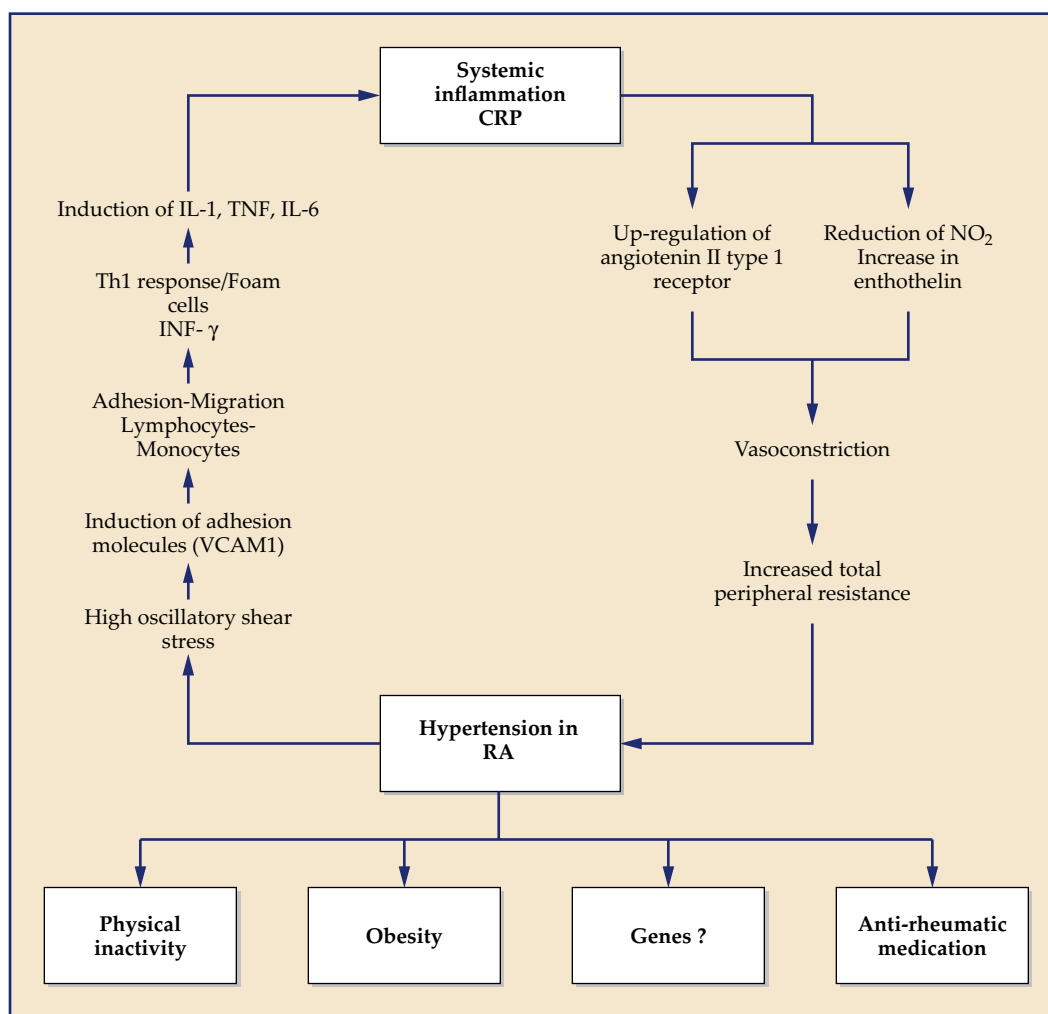


FIGURE 6.3 The interplays between RA-related factors and derangement of vascular physiology leading to hypertension. Vascular and systemic inflammation affects each other resulting in endothelial dysfunction, vasoconstriction, and high blood pressure. Sedentary lifestyle also contributes to hypertension; as well as several immunosuppressive regimens. *CRP*, C-reactive protein; *IL-1*, interleukin-1; *IL-6*, interleukin-6; *NO*, nitric oxide; *VCAM*, vascular cell adhesion molecule; *Th*, T-helper lymphocytes. Adapted from Panoulas et al. [47].

trials demonstrated that administration of such regimens imparts a significantly increased risk of developing hypertension (OR 1.8896, 95% CI 1.35–2.65) [57].

2.2.2 Lipids

Dyslipidemia is common in RA, affecting between 55% and 65% of patients [58]. In contrast to the general population in whom increased lipid levels hold a higher risk for CV disease, in the RA population it is the lower cholesterol levels that are associated with worse CV disease profile [59,60]. It is hypothesized that chronic inflammation results in suppression of total and low-density lipoprotein (LDL) with a proportionately greater suppression of high-density lipoprotein (HDL), yielding an unfavorable atherogenic index. Retrospective studies have reported abnormal lipoprotein pattern even 10 years prior to the onset of RA [61], suggesting that abnormal lipid metabolism may render people more susceptible to the future development of RA. Toms et al.

[62] reported an association between genes associated with RA susceptibility and dysregulated lipid profile, reinforcing the hypothesis that genetic background contributes to atherogenic profile in RA patients. Thus it is tempting to speculate that chronic persistent RA-related inflammation may have a greater and/or different effect on genetically predisposed individuals by modifying the transcription of specific genes, leading to dyslipidemia and vascular disease (Fig. 6.4). Importantly several studies have shown that successful treatment of the inflammatory component of RA leads to a substantial increase of lipid levels [63].

The paradoxical inversion of the common relationship between lipid levels and CV risk—known as the lipid paradox—has also been noted in other chronic inflammatory disorders such as sepsis [64] and cancer [65]. Such observations underpin the crucial role of inflammatory burden not only in quantitative changes but also in qualitative structural and functional alterations of lipid

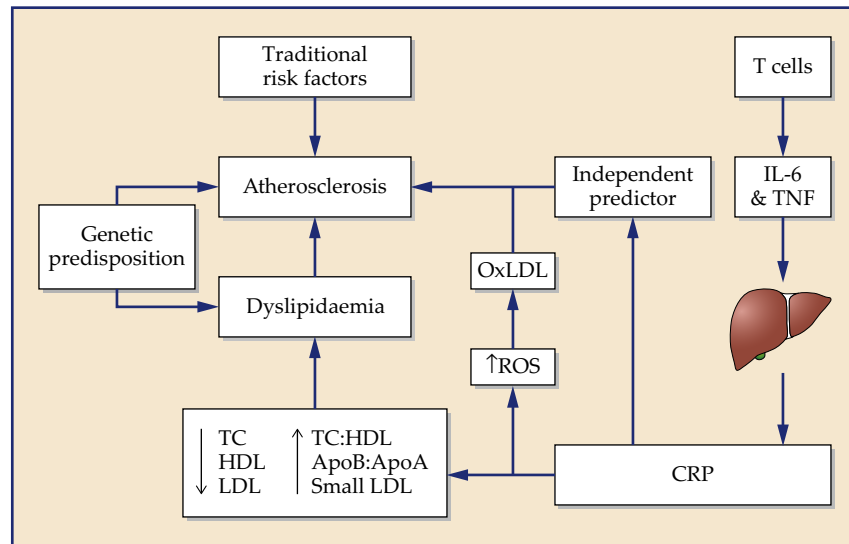


FIGURE 6.4 Schematic overview of the atherogenic effects of abnormal lipid metabolism in RA. Systemic inflammation influences lipid metabolism in liver and adipose tissue, leading to an unfavorable TC/HDL ratio. *IL-6*, Interleukin-6; *TNF*, tumor necrosis factor; *CRP*, C-reactive protein; *ROS*, reactive oxygen species; *OxLDL*, oxidized low-density lipoproteins; *TC*, total cholesterol; *HDL*, high-density lipoproteins; *LDL*, low-density lipoproteins; *ApoB*, apolipoproteinB; *ApoA*, apolipoproteinA; *TC/HDL*, total cholesterol/high-density lipoprotein. Adapted from Toms *et al.* [58].

molecules. The former is the result of suppressed cholesterol liver synthesis and increased rates of LDL cholesterol uptake by macrophages and hepatocytes mediated by C-reactive protein (CRP) [66]. An alternative explanation suggests that enhanced cholesterol ester fractional catabolic rates driven by active inflammation account for lower lipid levels, a process inhibited by treatment with the Janus-kinase inhibitor Tofacitinib, resulting in restoration of HDL and LDL in pretreatment levels [67]. On the other hand, systemic inflammatory burden modifies HDL subfraction composition converting it to a prooxidant, atherogenic molecule by depleting its anti-inflammatory and atheroprotective properties [68]. Other quantitative changes include small dense LDL cholesterol molecules, which are more atherogenic and antigenic and are seen in greater proportion in patients with RA [69]. Collectively the confounding effect of inflammation on lipid profile in RA patients implies that the traditional interpretation of cholesterol levels for predicting CV risk in other conditions associated with increased CV risk may not be applicable to RA and underlines the necessity for the incorporation of guidelines in the routine examination and follow-up of these individuals.

2.2.3 Obesity, Physical Inactivity, Rheumatoid Cachexia

2.2.3.1 Body Composition

Rheumatoid arthritis is characterized by a systemic manifestation called rheumatoid cachexia, a “syndrome” identified by Roubenoff and colleagues during the 1990s [70,71]. While maintaining the same weight, patients RA undergo significant body composition (ie, adiposity/

muscle content) changes, characterized by significantly increased adiposity and reduced muscle mass [72] with a significantly elevated resting metabolic rate [73]. As a result of this, studies in the literature suggest that the BMI cut-off points for the RA population should be reduced by 2 kg/m², specifically 23 for overweightness and 28 for obesity [74]. The adverse body composition changes in RA may impact on different outcomes, both for disease-related as well as systemic manifestations.

2.2.3.2 Reduced Muscle Mass

The molecular pathways that may describe reduced muscle mass in RA are potentially driven by inflammation, particularly the kinetics of TNF α . In specific, there is evidence to suggest that the progression of proteinolysis (muscle-mass breakdown) seems to occur via the ubiquitin-proteasome pathway [75,76], potentially stimulated by TNF α . TNF α binds to receptors and activates the transcriptional NF- κ B pathway, which in turn promotes the conjugation of ubiquitin to muscle proteins, an event known to promote protein degradation/catabolism in inflammatory environments [77,78]. Another potential mechanism is that TNF α may reduce the action of insulin by interfering with the insulin receptor signaling pathways [79]; insulin is a biomarker associated with intracellular enzyme activity that leads to protein synthesis. Apart from these specific pathways, lack of physical activity is also believed to be an important factor that contributes to this unfavorable loss of muscle mass in RA patients [80] (Fig. 6.5). Irrespective of the mechanism, reduced muscle mass has significant detrimental effects on the health of a patient, such as increased frailty and

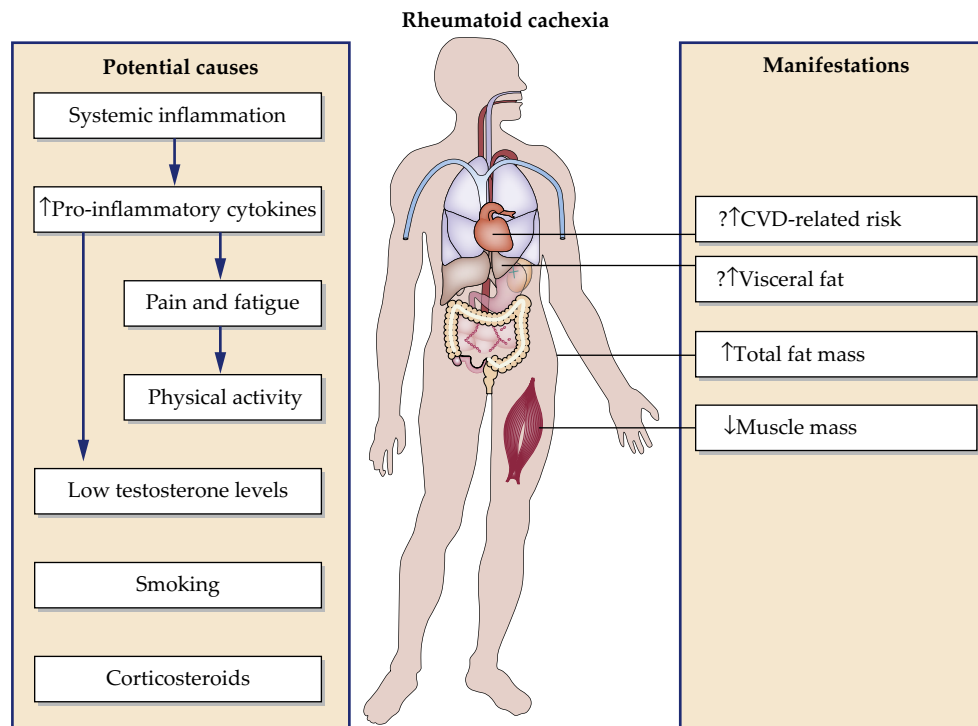


FIGURE 6.5 Rheumatoid cachexia and CV disease in RA. The effects of chronic systemic inflammation in multiple organs and tissues lead to altered body composition and further precipitated by reduced physical activity and immunosuppressive treatment especially steroids. CV: cardiovascular disease. *Adapted from Summers et al. [80].*

loss of independence [81] as well as increased mortality [82]. In RA, reduced muscle mass is associated with an increased number of swollen joints [71] and inflammatory markers [83,84] as well as extra-articular disease [85], thus suggesting that maintenance of healthy muscle mass may be important in this patient population.

2.2.3.3 Increased Adiposity

Obesity, a condition characterized by increased adiposity, has nearly doubled from 1980 to 2008, with more than half of European and US populations being overweight or obese, a fact that puts the financially overstretched National Health Services worldwide under additional pressure. For example, in England statistics demonstrate that the obesity cost for the National Health Service was almost £500 million in 1998 but £4.2 billion in 2007 [86], while in the United States the estimates for treating obesity range from \$147 billion to nearly \$210 billion per year [87]. Apart from these costs, obesity is also a risk factor for developing many different chronic diseases, such as CV disease [88], cancer [89] as well as RA [90], while it increases the likelihood of all-cause mortality [91]. As such, it is not surprising that obesity is considered a world pandemic.

The adipose tissue is now considered to be a key endocrine organ, altering metabolic functions via secretion of adipokines (or adipocytokines), including TNF α and IL-6, key mediators of the inflammatory response

and progression of RA. The prevalence of obesity in RA ranges between 18% and 31%, but it is reported that more than 60% of RA patients are overweight [92,93]. Obesity in RA can affect the RA disease phenotype both in terms of activity and severity. In particular, deteriorated disease activity and severity has been reported in RA patients [94,95], a phenomenon potentially occurring due to adiposity-induced production of inflammatory cytokines. In addition, obesity is associated not only with individual risk factors for developing CV, such as insulin resistance [96] and hypertension [97], but also with the incident of a wide range of comorbidities [98].

Obesity is a well-established risk factor for CV in the general population as it appears to coexist with other CV risk factors such as hypertension, insulin resistance, and dyslipidemia [99]. Although obesity is associated with the presence of other traditional CV risk factors in RA [100], a complex and in some cases paradoxical interrelation between body composition and CV disease has been described in this condition. Patients with RA adapt an inactive lifestyle with significantly reduced physical activity due to a number of reasons including joint pain and stiffness, psychological disturbances, or even fear of aggravating their disease, which apparently worsens CV risk profile and leads to higher body mass index [101]. However, even a low body mass index ($<20\text{ kg/m}^2$) confers a higher risk for CV disease among patients with RA than in individuals without arthritis or the general

population [102]. In particular, low body mass index at RA diagnosis was associated with a three-fold increased risk of CV death compared with that in non-RA subjects with normal body mass index. A possible explanation for these paradoxical effects may be alterations in body composition observed in RA subjects as chronic high-grade inflammation triggers metabolic pathways leading to increasing degradation of muscle tissue. In combination with reduction in energy expended on physical activity but normal energy intake these changes result in a tendency to store body fat with stable or slightly increased body weight [80]. This condition, which is characterized by the involuntary loss of skeletal mass with progressive increase of fat mass, is known as rheumatoid cachexia and can be easily missed on routine clinical examination as the increase of fat mass usually masks the reduction of muscle tissue. Given that a clear relationship between low body mass index and worse disease activity, severity, and quality of life has been described in several studies [70,71] it is most likely that rheumatoid cachexia reflects uncontrolled active disease, which provides further support to the hypothesis that chronic systemic inflammation plays a key role in the development of atherosclerosis and CV in RA. Another paradoxical association of altered body composition reported within RA individuals is a potential protective effect of obesity on joint damage [103]. Additionally high body mass index appears to prevent or retard the evolution of undifferentiated arthritis to typical RA [104].

It is possible that the alterations in body composition and obesity have a significant effect on CV mortality and morbidity in RA. Central obesity occurs in 20–57% of women and in 80–69% of men with RA [105], and there is some evidence that abdominal fat may be distributed differently in this population, with visceral adiposity having an additional adverse impact on cardiometabolic indices [106]. In the same study different patterns of abdominal obesity were attributed to a number of inflammatory and noninflammatory factors including factors that are modifiable within the context of RA disease management (eg, limiting cumulative exposure to glucocorticoids). Such observations further support the notion that body mass index measurements and definitions of obesity utilized in the general population may not be sufficient to determine the specific alterations in body composition among RA patients [74], suggesting that other assessments—for example, waist-to-hip ratio—or even more sophisticated assessments—such as bioelectrical impedance, DXA, or even MRI—may be warranted to accurately demonstrate lean and fat tissue changes in this population.

2.2.4 Insulin Resistance and Metabolic Syndrome

Epidemiological data suggests a strong link between insulin resistance, metabolic syndrome, and RA [107,108], although a direct association between RA and

diabetes mellitus is lacking [109]. According to published data, the occurrence of diabetes mellitus among RA individuals ranges between 7% and 35% [110–112]; however, only a couple of studies have found a higher prevalence of diabetes mellitus in RA patients than in non-RA controls [113,114] (Table 6.4). From a pathophysiological standpoint, insulin resistance and RA share common proatherogenic characteristics such as aberrant lipid metabolism, acute-phase response, and endothelial dysfunction [115]. Impaired insulin sensitivity has been reported as an independent predictor of the presence of subclinical atherosclerosis determined by carotid intima-media thickness (cIMT) in RA patients without traditional CV risk factors [116] and such relationships appear to be driven by the systemic inflammatory load as several studies have demonstrated correlations between insulin resistance, disease activity, and inflammatory markers [117,118]. The association between cardiometabolic syndrome and RA may be reciprocal, with chronic low-grade inflammation characterizing insulin resistance and high-grade RA-related systemic inflammatory activity reinforcing each other, resulting in a vicious circle, promoting oxidative stress and vascular damage [119]. The bidirectional influence between RA and impaired glucose tolerance is reflected in published data investigating the role of dimethylarginine as markers of endothelial dysfunction and atherosclerosis in this population [120,121]. We have reported that asymmetric dimethylarginine—the most potent endogenous inhibitor of nitric oxide synthase and an emerging biomarker of CV disease and mortality [122]—is significantly associated with both cumulative inflammatory burden [118] and indices of insulin resistance [123] in well-characterized and treated cohorts of RA patients. These findings on the one hand underline the importance of nitric oxide metabolism derangement as a common pathogenetic mechanism of endothelial dysfunction in both conditions, and on the other, underscore once more the close relationship between RA, metabolic syndrome, and inflammation, which further heightens CV risk in RA individuals [107,108].

2.2.5 Cigarette Smoking

Smoke from cigarettes is a mixture of 4000 toxic substances including nicotine, carcinogens (polycyclic aromatic hydrocarbons), organic compounds (unsaturated aldehydes such as acrolein), solvents, gas substances (carbon monoxide), and free radicals [124]. Some of these compounds are known to alter normal physiological function, and thus it should be considered that different compounds contained in cigarette smoke may have different effects on different mechanisms related to either the development and or progression of RA. However, in general there is overwhelming evidence demonstrating that smoking can be a risk factor for the development of

TABLE 6.4 Summary of Studies Assessing the Prevalence of Diabetes Mellitus and Metabolic Syndrome in RA

Study	Patients/Subjects	Study Type	Parameter Assessed	Results
Maradit-Kremers et al. [40]	603 RA	Population-based cohort Study of RA patients (Rochester epidemiology Project)	DM	DM 7.3%
Gonzalez et al. [41]	603 RA/603 controls	Longitudinal, population-based case-control study	DM	Prevalence DM similar in both groups
Han et al. [110]	28,208 RA/112,872 controls	Case-control study	DM	Prevalence ratio for diabetes 1.4
Simard J and Mittleman [111]	144 RA patients	Cross-sectional population-based cohort study (Nhanes III)	DM	Odds ratio between RA and T2DM not statistically significant
Solomon et al. [112]	287 RA/87,019 non-RA	Prospective longitudinal Case-control study	DM	No significant difference in prevalence of T2DM between RA women and control
Solomon et al. [113]	10 156 RA	Longitudinal cohort (CORRONA study)	DM	DM 7.1%
Chung et al. [114]	154 RA/85 controls	Case-control cohort	Metabolic syndrome	56 (36%) RA vs. 9 (11%) controls ($p = .03$)
Chung et al. [51]	197 RA/274 controls	Case-control study of RA patients ESCAPE RA and MESA cohort	DM	14 (7%) RA vs. 26 (9%) controls ($p = .41$)
Da Cunha [107]	283 RA/226 controls	Cross-sectional	Metabolic syndrome	39.2% RA vs. 19.5% controls ($p < .001$)

CORONA, comorbidities in rheumatoid arthritis; DM, diabetes mellitus; MESA, multi-ethnic study of atherosclerosis; RA, rheumatoid arthritis.

RA possibly due to the triggering of the immune system against citrullinated proteins antigens [125]. A 2010 *meta-analysis* of observational studies revealed that smoking could potentially increase by 40% the risk of developing RA, particularly in RF+RA men and heavy smokers [126]. A more recent 2014 dose-response *meta-analysis* demonstrated that lifelong smoking is associated positively with the risk of developing RA even among smokers with low lifelong exposure, but this risk does not further elevate with an exposure of more than 20 pack/year [127].

Not only is smoking potentially implicated in the development of RA, it may also impact significantly on the outcomes of this chronic inflammatory condition. In particular, associations between smoking and IgA RF levels account for more severe disease outcomes [128], which has been confirmed in RA populations with different ethnical backgrounds [129]. Notably, smoking ≥ 25 pack/year is significantly associated with RF positivity, radiographic erosions, and nodules [130], while another

study revealed that smoking ≥ 20 pack/year is associated with higher disease severity (via the Health Assessment Questionnaire) and more radiological joint damage [128]. Cross-sectional data also suggest that smoking is associated with a gradual increase in disease activity with never, former, and current smokers presented with a progressively higher number of swollen and tender joints and pain (via a visual analog scale), without however any associations with radiological progression [131]. The latter was also the case in a 2008 study by Westhoff et al. However, this study demonstrated that RA smokers required higher doses of disease-modifying antirheumatic drugs (DMARDs), a finding that may suggest reduced potency of DMARDs due to smoking [132]. Moreover, early-RA patients who are smokers are less likely to have a good response to methotrexate or biological medication (TNF α inhibitors) than RA patients who have never smoked or are ex-smokers [133]. Despite that collectively it can be suggested that smoking may negatively impact on disease severity and activity, the

earlier result should be treated with caution since these are cross-sectional observations and are limited by methodological constraints, which does not allow a definitive conclusion about the effects smoking on these outcomes.

While the effects of smoking are not quite clear in RA manifestations, smoking can influence aspects of human physiology associated with ill health. For example, patients with RA are presented with comorbidities that can be affected by smoking, namely hypertension [47,50], dyslipidemia [62,134], rheumatoid cachexia [80,135], insulin resistance [115,136], and vascular dysfunction [137,138]. Moreover, a study by Tureson and colleagues, implicates smoking as an independent factor for the risk of developing extra-articular (ie, pericarditis, pleuritis, major cutaneous vasculitis, Felty's syndrome, neuropathy, ophthalmological manifestations, glomerulonephritis, and other types of vasculitis) manifestations in RA [139].

2.3 Inflammation as a Novel Risk Factor for CV Disease in RA

2.3.1 Pathogenesis and Epidemiology

Over time patients with RA are exposed to chronically raised levels of inflammation, which is thought to contribute to increased CV risk [140]. Atherosclerotic vascular disease accounts for the largest burden of CV disease in RA. In the general population, atherosclerosis is now accepted to be a chronic inflammatory disease [141]. Inflammation is implicated in both the development and progression of atherosclerotic plaques in the general population. A large histological study was undertaken in which more than 500 carotid endarterectomy specimens were examined [142]. A high prevalence of dense macrophage infiltration was found in symptomatic plaques, which was significantly associated with recent stroke and also with fibrous cap rupture and thrombus formation (the key triggers for clinical stroke). Macrophage infiltration of plaque is thought to precipitate fibrous cap erosion, possibly through the release of matrix metalloproteinases [141]. Thus plaque inflammation is recognized as an important trigger for plaque destabilization and clinical events.

Population studies also support the link between inflammation and CV disease in the general population. A large *meta*-analysis evaluated the association between key inflammatory cytokines including TNF and IL-6 with incident CV events. Following adjustment for traditional risk factors, circulating levels for both TNF and IL-6 were significantly and independently associated with subsequent CV events (hazard ratios [95%] per 1 standard deviation increase: 1.17 [1.09, 1.25], 1.25 [1.19, 1.32] for TNF and IL-6, respectively) [143]. A further *meta*-analysis evaluated genetic markers of the IL-6 receptor pathway, along with functional studies provided evidence of a causal link between IL-6 and CV risk

[144]. In the RA population, IL-6 and TNF are key cytokines implicated in disease pathogenesis and circulating levels are increased in patients with active disease.

Observational studies in RA have demonstrated an association between levels of inflammation and CV events independent of traditional CV risk factors. Goodson et al. evaluated the association of CRP levels in patients with early inflammatory polyarthritis and subsequent risk of CV mortality over 10 years of follow-up [145]. The authors found that a CRP of >5 mg/L at baseline was an independent predictor of CV death at follow-up. Patients with a raised CRP at baseline were almost four times more likely to have died of CV disease within 10 years than those with normal CRP levels. Even when other factors were accounted for including rheumatoid factor status, age, gender, and smoking status, CRP was still associated with more than a three-fold increased risk of CV death. Burden of joint disease is also known to be associated with increased risk of CV disease. Those with higher cumulative disease activity (measured using the DAS28 score, a composite score of tender, swollen joints, patient score of disease activity, and serum inflammation) and those with more frequent flares in their arthritis have been shown to be at higher risk [146]. Furthermore, early remission of joint disease is associated with improved survival, highlighting that effective treatment of inflammation may improve CV outcomes for patients [147].

Inflammation is not only thought to lead to accelerated atherosclerosis in RA but may also lead to a more unstable, inflamed plaque phenotype [140]. Aubrey et al. compared coronary lesions in an autopsy study of RA patients and age- and sex-matched controls with a history of CV disease. Despite having a lower prevalence of severe stenosis (7% vs. 54%, $p = .023$), patients were more likely to have vulnerable and inflamed plaques [148]. A second study by Hollan et al. also demonstrated that patients with inflammatory rheumatic diseases, such as RA, were more likely to have inflammatory cell infiltration in the aorta compared to age- and sex-matched controls [149]. The clinical phenotype of CV disease in RA would also support the hypothesis that patients have more unstable lesions. Rheumatoid arthritis patients have fewer warning symptoms prior to a major event and have a higher case fatality following stroke and acute MI, suggesting more unstable plaques, which are prone to sudden rupture [150–152].

2.3.2 How Does Excess Inflammation Lead to Increased Risk of CV Disease?

While the link between chronic inflammation and increased CV risk in RA is clear, the underlying mechanisms are still poorly understood. It is likely that inflammation leads to increased risk by direct effects on the artery wall, through alteration in traditional risk factors, and also possibly through medications used to treat

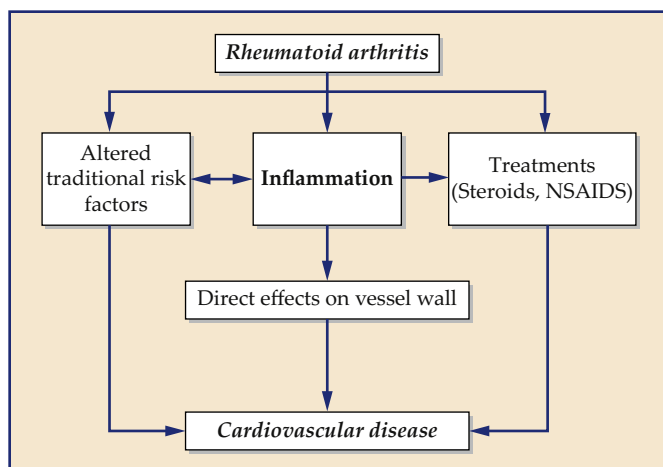


FIGURE 6.6 The interplay between RA, inflammation, and CV disease. Adapted from the personal collection of the authors.

inflammation (Fig. 6.6). As discussed earlier, a complex relationship between some traditional risk factors and inflammation exists, in particular with dyslipidemia and insulin resistance. The adverse effect of inflammation on these traditional risk factors is likely to significantly contribute to increased CV risk in RA.

2.3.3 Direct Effects on the Vessel Wall

Disease-activity measurements including CRP are associated with increased prevalence of subclinical CV disease [153]. Nagat-Sakurai et al. compared the rate of progression of intima-media layer thickening in the carotid artery wall in RA patients and controls [154]. A faster rate of progression was noted in the RA group, which was associated with CRP levels. This supports the hypothesis that inflammation promotes accelerated CV disease in RA. Maki-Petaja et al. employed positron emission tomography (PET) to evaluate aortic inflammation in a cohort of RA patients before and after treatment and compared findings to a cohort of non-RA patients with clinical CV disease [155]. Patients were found to have higher levels of inflammation within the aorta compared with patients with clinical CV disease, while vascular inflammation significantly reduced following treatment with anti-TNF therapy. Of note patients still had higher levels of vascular inflammation even after treatment compared to controls, suggesting low-grade inflammation may persist despite effective treatment for joint disease. This study is the first to demonstrate active vascular inflammation in RA patients, which significantly improves with antiinflammatory therapy. However, one question does arise from this study: does the aortic inflammation represent low-grade vasculitis or atherosclerotic plaque inflammation? Although this could not be answered fully in the study, sections of the aorta were noted to be particularly inflamed in a pattern that would suggest atherosclerosis rather than the uniform

inflammation seen in large-vessel vasculitis. A further study of 13 patients with active arthritis used PET to specifically to evaluate atherosclerotic plaque inflammation within the carotid artery [156]. This study found evidence of plaque inflammation, which was significantly associated with CRP levels in all 13 patients. In 5 out of 13 cases there was significantly higher inflammation within the plaque than in a portion on nonatheromatous artery wall, which suggests that the inflammation measured in the plaque is not merely a reflection of generalized vasculitis but in some cases represents preferential metabolic activity within the plaque due to an active atherosclerotic process.

While the Maki-Petaja et al. study demonstrated a link between systemic and vascular inflammation in RA, what is still not clear is whether inflammatory mediators generated within the joint travel through circulation and have a secondary effect on the vessels or alternatively a simultaneous pathological process affects both the joints and blood vessels. A number of pathophysiological features of inflamed synovium in RA and atherosclerotic plaque are similar, including the prominent role of inflammatory cells, neovascularization with relative tissue hypoxia, and matrix metalloproteinases activity [157]. Further research is required to better understand the link between joint and vascular inflammation.

3. ASSESSEMENT OF CARDIOVASCULAR RISK IN RA: NONINVASIVE ASSESSMENTS OF VASCULAR HEALTH

The majority of the evidence suggesting that the process of atherosclerosis is accelerated in patients with RA derives from studies assessing noninvasively the structure and function of the endothelium. The diagnostic tests that were developed for the evaluation atherosclerosis and endothelial dysfunction are elaborated on in Chapter 5. It is therefore of great importance to rheumatology healthcare professionals (1) to understand the biological processes that underpin adverse changes in the vasculature, (2) to appreciate how such changes can be detected using noninvasive assessments of vascular health, and (3) appraise critically the evidence that has arisen from such studies and how, if at all, they may relate to routine clinical practice.

3.1 Markers for Microvascular Endothelial Function in RA

3.1.1 Microvascular Endothelial Function in RA

Only a small number of studies of highly variable quality (from 22–96%, average: 64%) have examined microvascular endothelial function in RA. Studies comparing

RA patients with healthy control participants reveal mild abnormalities in nailfold capillaroscopy and reduced forearm blood flow in response to vasoactive agents [138]. The impact of RA disease-related inflammation on the microvasculature is unclear, as some studies report associations between CRP and microvascular endothelial function assessed using laser Doppler imaging (LDI), while other studies using laser Doppler flowmetry (LDF) do not [138]. Interestingly, very little attention has been given to the role of classical CV disease risk factors and microvascular function, with one prospective study revealing associations between several classical CV disease risk factors and microvascular endothelial function assessed using LDI, but not with large-vessel endothelium-dependent function [44]. Only a small number of low-to-medium quality (25–72%) studies have examined the effect of antiinflammatory medications on microvascular endothelial function and report improvements in endothelium-dependent function after follow-up periods ranging from 2 days to 6 months [138]. Crucially, a 6-month exercise intervention study in patients with RA reported improved microvascular endothelial function at follow-up [158]. At present, the small numbers of studies examining microvascular endothelial function incorporate a variety of methods, resulting in heterogeneous findings. Further large-scale prospective studies are needed to help determine the impact of RA (and its sequela) on the microvasculature.

3.1.2 Large-Vessel Endothelial Function in RA

A greater number of studies have examined large-vessel endothelial function in RA using the flow-mediated dilation (FMD) technique, with the 13 studies included into the systematic review having quality index scores ranging from 32–96% (average 64%) [138]. The majority of studies report poorer endothelial function in RA patients relative to healthy control participants, with no difference in endothelium-independent responses (ie, the response to nitroglycerin (GTN) administration) [138]. When examining whether inflammation is associated with large-vessel endothelial function, the majority of studies (quality index 54–96%) do not find such associations. The few studies that do report such associations are of largely good quality (75–77%), but reveal selective associations with CRP but not ESR in a similar cohort of patients [138]. Although there is a dearth of studies examining the impact of classical CV disease risk factors on large-vessel endothelial function, a study by Sandoo et al. revealed that several classical CV disease risk factors are associated with endothelium-independent responses to GTN [44]. Furthermore, neither current nor cumulative ESR and CRP (recorded over a 6-year period) were associated with any of the vascular parameters [44]. In another study, large-vessel endothelial function assessed using FMD was not different between RA and type II diabetic patients, even though CRP was greater in RA patients

[32]. Studies examining the longitudinal effects of medication (in particular, antitumor necrosis factor α) reveal transient (few hours) and long-term (18 months) improvements in endothelial function [138]. The quality index for these studies range from 23–88%, (average 58%).

In summary, it is clear that large-vessel endothelial function is impaired in RA patients relative to healthy control participants. However, while inflammation has been postulated to contribute to the impairments, it does not consistently associate with these vascular measures. It is worth noting that significant improvements in large-vessel endothelial function have been reported following a 6-month aerobic exercise-training program in patients with RA [158]. Thus future research needs to delineate the effect of classical CV disease risk factors and inflammation on large-vessel endothelial function.

3.1.3 Arterial Stiffness in RA

The majority of studies that have examined arterial stiffness are of good quality (average 70%, range 36–100%) and reveal greater arterial stiffness in RA patients relative to healthy control participants. However, evidence for an association between arterial stiffness and disease-related inflammation is not strong [138]. The effects of antiinflammatory medication on arterial stiffness yield mixed findings, and there is no clear consensus on whether antiinflammatory treatment can actually reverse arterial stiffness.

3.1.4 Carotid Plaque in RA

The characterization of carotid plaque and its susceptibility to rupture is an important consideration when examining CV risk, particularly in patients with RA [148]. Characterization of carotid plaque is usually performed by scanning the common carotid arteries as well as the internal and external carotid arteries using B-mode ultrasound. Plaques are defined when they meet the following criteria: a focal structure, which encroaches into the arterial lumen by at least 0.5 mm, or 50% of the surrounding IMT value, or IMT value greater than 1.5 mm [159]. Interestingly, a recent study conducted in ~1000 patients presenting with RA, human immunodeficiency virus, or type II diabetes revealed that examination of plaques in the femoral artery may have added prognostic value when examined together with carotid plaques [160]. Indeed, femoral plaques are able to predict future CV risk in middle-aged males [161], which may be due to similarities in vascular wall properties between femoral and coronary arteries [162,163]. Identification of the type of plaque can be performed using specialist automated edge-detection software, which characterizes the relative echogenicity of the plaques [164].

3.1.5 Carotid Atherosclerosis in RA

Carotid atherosclerosis has been widely examined in RA, and from the 30 studies examining cIMT between RA

and healthy controls (average quality index 63%, range 29–90%), 80% of studies reported increased cIMT in RA patients [138]. Although most of these case–control studies matched populations for age and gender, statistical adjustment for factors that might affect cIMT were not carried out. In particular, global CV risk scores and its individual components may impact on the vasculature and are important to consider when devising statistical models. Increased cIMT is evident in patients with early RA, but at present, it is unclear whether cIMT increases with disease duration, and if it does, whether this is due to increased age (a major contributor to elevated cIMT in the general population) [138]. As with the other vascular assessments, RA disease-related inflammation does not appear to be associated with cIMT, and evidence supporting whether cIMT can be reversed following antiinflammatory treatment is scarce. Of the studies included in the systematic review, some reported regression of atherosclerosis following treatment with antitumor necrosis factor- α , with others reporting no change following treatment [138]. The concept of *accelerated atherosclerosis* is often attributed to RA, yet there is no convincing evidence for this. In the study by Nagata–Sakurai et al. [154], IMT was greater in RA patients than in age- and sex-matched healthy controls, but disease activity was only assessed at baseline, and the relationship between change in inflammation and IMT could therefore not be characterized. Longitudinal studies are necessary to explore the concept of accelerated atherosclerosis further.

3.2 Summary and Future Research Direction

Systematic examination of the literature provides strong evidence that RA patients have impaired vascular function and morphology relative to healthy controls. However, at present, the literature does not strongly support a relationship between RA disease-related inflammation and vascular function and morphology. Moreover, studies examining the impact of antiinflammatory medication often yield inconsistent findings. Unfortunately, with the exception of some recent studies, there is very little research examining the impact of classical CV risk factors on vascular health, particularly in the microvasculature. Thus long-term prospective studies that examine the impact of lifestyle interventions known to reduce CV risk on vascular health are required in patients with RA.

There is strong evidence that microvessel and large-vessel endothelial function measured at baseline are good predictors of atherosclerotic progression and future cardiac events in patients with suspected and those with established CV [165]. However, to date, only one study has examined the predictive ability of cIMT for the development of cardiac events in RA, and found that patients who experienced a cardiac event over a 5-year follow-up

period had greater cIMT at baseline than patients who did not experience a cardiac event [166]. The findings must be interpreted with caution due to the small sample size ($N=47$), significantly older age of the patients that had a cardiac event, and a lack of follow-up examination for cIMT [166]. To understand whether assessments of vascular health are predictive for cardiovascular events in RA, prospective assessments, which include assessments of function and structure in the microvessels and large vessels and account for multiple determining factors, should be conducted. Finally, RA patients who present with clear epicardial coronary arteries often have evidence of impaired coronary microvascular perfusion [167], which can be reversed with antiinflammatory treatment [168]. In addition, the measurement of subendocardial viability ratio—a surrogate measure of myocardial ischemia reflecting microvascular dysfunction—is adversely affected by RA disease-related inflammation and classical CV risk factors [25]. Interestingly, RA has a similar CV risk burden and vascular profile to diabetes [32], a condition in which microvascular disease may contribute to large-vessel disease [169]. Given that the microvessels represent a large proportion of the vasculature and that peripheral microvessel and large-vessel endothelial function are heterogeneous in RA [137], it seems plausible that the assessment of microvascular endothelial function may offer more information about the development of CV in this population. However, further long-term prospective studies examining whether microvascular endothelial function predicts future cardiac events are warranted.

3.3 CV and Biochemical Markers in RA

The high burden of CV disease, the asymptomatic and silent presentation of myocardial ischemia as well as the inaccuracy of simple, cheap, easy, reproducible, and noninvasive methods to detect promptly cardiac involvement in RA have prompted rheumatologists to evaluate several molecules as potent biomarkers of CV disease. Although blood tests are not routinely used in the diagnostic workup of vascular dysfunction an increasing number of biomarkers have been described to be associated with (sub)clinical atherosclerosis, heart dysfunction, and CV outcomes and may help clinicians in CV risk stratification and assessment of disease severity (Fig. 6.7). For example, CRP—besides its role as a marker of systemic inflammatory activity—has been linked with accelerated atherosclerosis [172] and CV events [173], reinforcing the hypothesis that CV disease and inflammation in RA are tightly related. Endothelium-derived biomarkers such as angiotensin-2 [174] as well as other serological indicators of CV risk such as prothrombotic factors [175] and uric acid have also been assessed as markers of endothelial function, activation, or both [176,177]. Another interesting observation is the

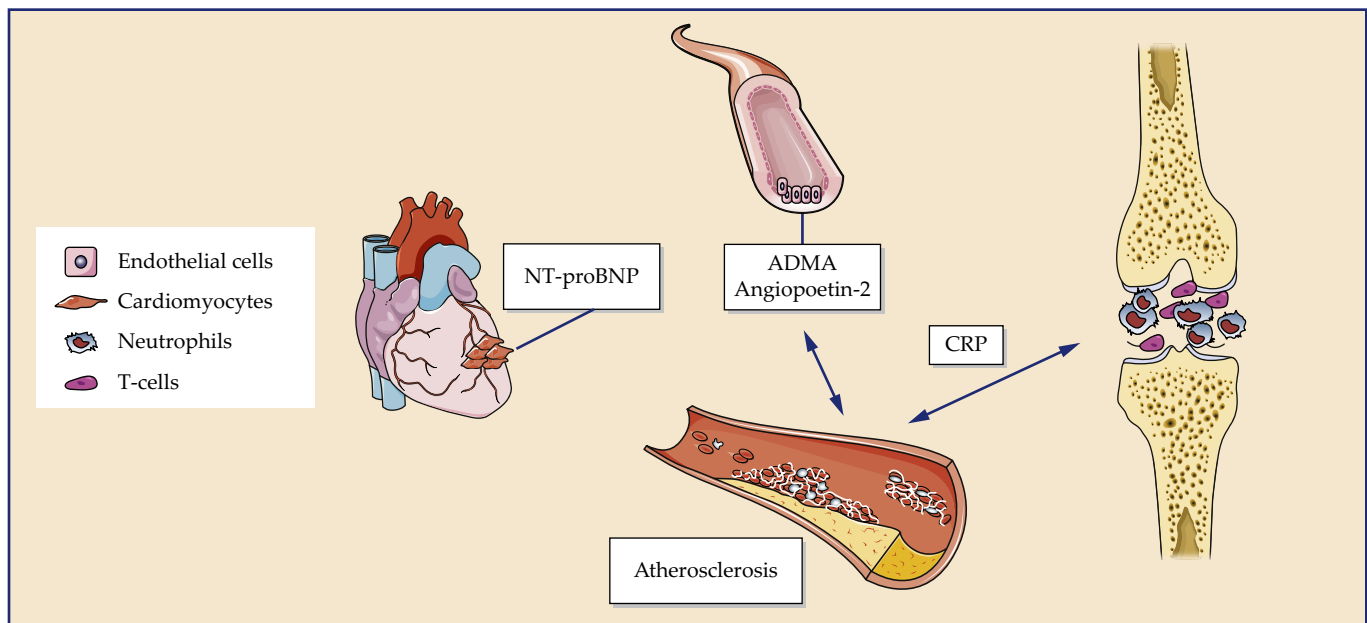


FIGURE 6.7 Sources of various biomarkers associated with CV disease in RA. Synovial and systemic inflammation are tightly linked with the initiation of endothelial injury and CRP levels are associated with morphological markers of early atherosclerosis. Increased formation and release of nitric oxide inhibitors—triggered among others by inflammatory load—precipitates the effects of systemic inflammation on vascular wall and mediates vascular disease. Activated and/or dysfunctional endothelial cells produce vasoactive agents such as angiopoietin-2 and adhesion molecules, contributing to the derangement of endothelium homeostasis and the development of atherosclerosis. Increasing release of natriuretic peptides by cardiac tissue reflects myocardial stress due to the impact of systemic inflammatory milieu on cardiac myocytes from one side and myocardial strain associated with—clinically manifested or not—heart failure on the other. *ADMA*, Asymmetric dimethylarginine; *CRP*, C-reactive protein; *NT-proBNP*, N-terminal b-type natriuretic peptide. *Adapted from the personal collection of the authors.*

association between osteoprotegerin—a decoy receptor for receptor activator of nuclear factor B ligand—not only with bone erosions and radiologic progression in RA [178] but also with CV disease on both the clinical [179] and genetic levels [180].

B-type natriuretic peptide is a well-established marker of cardiac dysfunction, reflecting myocardial strain due to various etiologies. Increased levels of natriuretic peptides have been demonstrated in RA independent of CV risk factors [181] even in patients without clinically overt heart failure or coronary artery disease [182,183]. Several lines of evidence suggest that RA-related inflammation itself may upregulate the neurohormonal system of the heart indicating a possible direct effect of inflammatory milieu on cardiac muscle as a cause of myocardial stress [184]. This concurs with observations showing a reduction of N-terminal probrain natriuretic peptide levels after effective control of disease activity with biologic drugs [185]. However, it remains unknown whether such changes are associated with a real reduction in CV risk or represent epiphenomena following suppression of systemic inflammation.

Derangement of nitric oxide metabolism is considered one of the most important pathogenic pathways through which systemic inflammation exhibits deleterious effects on endothelial function and promotes atherosclerosis. In that respect, dimethylarginines—L-arginine

analogues acting as endogenous inhibitors of nitric oxide synthase—have been extensively studied as biomarkers and predictors of CV disease in different disease settings related with CV morbidity and mortality as well as in the general population [186,187]. Asymmetric dimethylarginine in particular has emerged as a novel surrogate marker of adverse CV outcomes in patients suffering from coronary artery disease [188], heart failure [189], or stroke [190], conditions accounting for the majority of CV deaths within RA individuals. In the context of RA, a growing number of studies report that asymmetric dimethylarginine synthesis is enhanced [191,192] but higher asymmetric dimethylarginine levels were correlated with structural markers of endothelial dysfunction [193] and vascular injury in only a few studies [194], while others failed to establish such relationships [191]. Systemic inflammation is probably involved in the upregulation of dimethylarginine as indicated by published data demonstrating positive correlations with asymmetric dimethylarginine and cumulative inflammatory burden [195], making the interpretation of these findings in the daily clinical setting more complicated.

The utility of such biomarkers in CV risk stratification in RA patients should be consistently demonstrated in large prospective studies, which are currently lacking. In addition, the assessment of CV risk in RA is subject to multiple confounding parameters such as

systemic inflammation, autoimmune activation, classic CV disease factors, genetic background, and antirheumatic treatment, which makes the validation of any biomarker problematic. On the other hand, it should be taken into account that such molecules have pleiotropic biological effects on different cell types, which may also vary during the course of the disease depending on RA activity, type, and effectiveness of immunosuppressive regimens, concomitant conditions, and nonmodifiable factors such as age and gender. Consequently further studies with specific endpoints are warranted to provide evidence for causal associations between specific biomarkers and CV disease in RA and to evaluate their value to predict future CV events and mortality in this population.

3.4 CV Imaging in Rheumatoid Arthritis

CV imaging studies have allowed a better understanding of the burden of subclinical CV disease in RA and the effects of inflammation. The application of the main CV imaging modalities in RA will be reviewed in this section.

3.4.1 Echocardiography

Echocardiography is a well-validated, reproducible imaging method that does not involve ionizing radiation. This imaging technique can be used to evaluate pericardial disease, cardiac structure, and function. More recent advances including speckle tracking echocardiography and stress echocardiography allow assessment of myocardial strain and inducible ischemia. In RA, echocardiography has been used to examine the prevalence of cardiac abnormalities.

3.4.1.1 Pericardial Disease

Pericarditis is quite common in RA, with a number of studies having demonstrated a prevalence ranging between 20% and 40% [196]. However, severe pericardial effusions are unlikely and most of the data reports small and mild asymptomatic collection of fluid detected in echocardiography [197].

3.4.1.2 Valvular Heart Disease

A *meta*-analysis by Carroa [198] evaluated the risk of valvular heart disease in RA compared with controls. Increased prevalence of most valvular lesions was found. Rheumatoid arthritis patients were 12.5 times more likely to have valvular nodules than controls. Interestingly, while there was significant increased prevalence of mitral insufficiency (3.4-fold increase), mitral and aortic calcification (4.8- and 5.2-fold respectively), the greatest increase noted was for tricuspid insufficiency, which is likely secondary to pulmonary involvement in RA. This highlights the value of echocardiography in not only the

evaluation of primary CV disease but also in identifying signs of pulmonary disease.

3.4.1.3 Left Ventricular Morphology and Function

Changes in left ventricular morphology are known to be associated with increased risk of CV mortality. Rheumatoid arthritis patients present with significant structural alterations in left ventricular morphology as they are more likely to have a higher relative wall thickness and absolute LV mass [199]. In a study which enrolled RA patients with active arthritis, a significant association between LV relative wall thickness and disease activity within the joints was reported, highlighting the link with between myocardial remodeling inflammation [200].

The risk of diastolic dysfunction in RA patients is well established and is thought to be due to a combination of macrovascular and microvascular disease. Rarely other causes such as amyloidosis can be the underlying cause [197]. Diastolic dysfunction is characterized by reduction in ventricular distensibility and relaxation, both of which can be significantly abnormal despite no obvious change in systolic function. There are five measures routinely used to evaluate diastolic dysfunction. These are the E/A ratio, which is a measurement of pressure changes across the mitral valve in early diastolic filling and late diastole; the isovolumetric relaxation time; the early and late peak filling velocity; and the mitral valve deceleration time. Although no validated weighting of these measurements is available, they are used in combination to grade diastolic dysfunction.

Aslam et al. performed a systemic review and *meta*-analysis of echocardiography studies evaluating the prevalence of diastolic dysfunction in patients with RA [33]. Twenty-five papers were included and 22 found that RA patients had a significantly higher prevalence of diastolic dysfunction. There was up to nine-fold increased prevalence of some measures of diastolic dysfunction in RA including isovolumetric relaxation time. An increase in left ventricular mass, left atrial dimension, and increased pulmonary artery pressure was also noted in the RA group but a significant difference in LV function was not demonstrated. Some but not all of the studies included in the *meta*-analysis demonstrated an association between disease activity, disease duration, and diastolic dysfunction. However, most studies to date have been carried out in patients with established arthritis and the prevalence of myocardial dysfunction in early arthritis is not well described.

Stress echocardiography can be used to evaluate reversible ischemia and is performed by evaluating the heart following exercise or pharmacological stress. Toutouzas et al. evaluated echocardiography changes in RA patients without diabetes, non-RA type 2 diabetic patients, and non-RA, nondiabetic controls using dobutamine stress echocardiography [167]. Rheumatoid

arthritis patients had higher rates of inducible ischemia than controls and equivalent rates compared with type 2 diabetic patients (67% vs. 31%, $p=.19\%$ and 67% vs. 71%, $p=.71$, respectively). Eight of the RA patients with positive stress test underwent angiography and four out of eight had significant coronary disease. The fact that only 50% of those with inducible ischemia had evidence of macrovascular disease would support the hypothesis that microvascular disease is a significant contributing factor to CV risk in RA. Interestingly, higher levels of circulating inflammatory markers were associated with positive stress echocardiograms.

There is very little literature on the use of speckle tracking echocardiography to evaluate cardiac strain in RA. However, one study of 59 patients with RA and 59 matched controls by Fine et al. found that patients were more likely to have impaired left and right ventricular strain patterns [201]. Patterns were associated with a measure of RA disease severity but did not correlate significantly with circulating levels of inflammation or joint-disease activity.

A number of studies using echocardiography have demonstrated improvement in diastolic dysfunction and left ventricular morphology following treatment with biologic agents [202,203]. Daian et al. [203] compared left ventricular mass in patients undergoing treatment with etanercept, an anti-TNF drug, or traditional disease-modifying antirheumatic drugs, using echocardiography. At 6-month follow-up LV mass in the etanercept-treated patients had reduced by a mean (SD) of 14.2 (9.3) g/m² compared with -6.3 (7.6) g/m² in the control group ($p<.001$). It must be noted that those in the etanercept group had a longer disease duration and higher prevalence of antibody positivity, factors associated with heart failure in RA. However, there was a significant correlation between improvement in RA disease activity and left ventricular mass at 6 months across the groups ($r=0.63$, $p<.001$) suggesting that controlling disease improves cardiac morphology. Anti-TNF therapies are effective treatments for rheumatoid arthritis but are contra-indicated in patients with severe heart failure. The early use of echocardiography may allow detection of milder, reversible cardiac dysfunction for targeted therapy before more established heart failure occurs and anti-TNF therapies becomes contra-indicated. However further studies would be required to investigate this.

3.4.2 Coronary Artery CT

Coronary artery computerized tomography (CT) can be employed to perform noninvasive coronary angiography and coronary calcium quantification. Coronary calcification scores are measured using electron beam CT or more recently 64 slice multidetector CT. Coronary calcium is a well-validated predictor of CV risk and is employed particularly in low-risk chest pain cohorts due

to the high negative predictive value [204]. In RA, higher coronary calcium scores have been observed in a number of studies. Chung et al. undertook electron beam CT in 71 patients with established RA, 70 with early RA, and 86 controls [205]. Patients with established RA had higher coronary calcium scores than those with early or no RA disease (median [IQR] 40.2 [0–85], 0 [0–42.5], and 1 [1–19.0], $p=.016$). Patients with established RA were also more than three times more likely to have severe coronary calcification than the other two groups even after adjustment for traditional risk factors. Calcium scores were also associated with circulating levels of inflammation in the RA patients, emphasizing again the association between inflammation and CV disease in RA. Other studies have also demonstrated the association between coronary calcification in RA and circulating markers of inflammation and traditional risk factors such as smoking and insulin resistance [206,207]. While coronary artery calcification scoring on CT is well validated in terms of outcomes in the general population, no prospective study evaluating their ability to predict events in the RA population has been undertaken.

Recent developments in coronary CT allow evaluation of plaque phenotype in addition to degree of vessel stenosis. Karpouzas et al. compared CT angiography findings in 150 patients and matched controls [208]. A higher prevalence of plaque was confirmed in the RA group (71% vs. 45%, $p<.001$) but patients also had more affected coronary artery segments (13.5% vs. 6% of segments). On comparison of plaque phenotype, patients had a higher prevalence of more severely stenosed lesions and more “high-risk” lesions, ie, mixed or calcified plaques, than in controls. This study supports the hypothesis that patients with RA may have more severe unstable plaque phenotype than patients without RA. However, further studies are required using this modality to validate these findings and evaluate the association between CT findings and outcomes in the RA population.

3.4.3 Cardiac MRI

Cardiac MRI is the gold standard for measuring cardiac morphology and function including myocardial strain [22]. With the addition of gadolinium, inflammation and fibrosis patterns can be evaluated. Increased enhancement with gadolinium occurs when an increased volume of distribution occurs within the tissue, leading to altered pharmacokinetics of the contrast agent. These changes can occur due to infarction, fibrosis, infiltration, or inflammation, and multiweighted dual early and delayed imaging is required to differentiate pathology. Recent cardiac MRI techniques now allow estimation of diffuse myocardial fibrosis. Extracellular volume estimation using T1 mapping of cardiac tissue is known to correlate with myocardial fibrosis [37]. T2-weighted sequences can also be used to estimate myocardial

edema. These techniques are particularly valuable in the diagnosis of myocarditis and amyloidosis in RA, although both conditions rarely present clinically. Cardiac MRI is providing insight into the subclinical cardiac pathology seen in RA.

3.4.3.1 Morphology and Left Ventricular Function

There are only a few studies comparing findings in RA patients and controls on MRI. The largest study conducted by Giles et al. compared morphology in a prospective case controls study of 75 RA patients and 225 frequency matched controls [209]. Converse to findings in the *meta*-analysis of LV mass using echocardiography, patients had significantly lower LV mass than controls, even after adjustment for traditional risk factors. Only a minimal but statistically significant reduction in ejection fraction was noted in the RA group. While the authors acknowledged the findings were unexpected, it was hypothesized that the lower LV mass could be related to myocardial fibrosis and myocyte death secondary to myocarditis or hypoperfusion. The conflicting results from echocardiography and MRI studies could to some degree be related to differences in the cohorts studied; could also be related to differences in the cohorts studied; the MRI study excluded all those with a history of CV disease thus may select out those with more advanced cardiac disease. In addition, the use of different imaging techniques may also have contributed. MRI is known to be superior in the measurement of LV morphology compared with echocardiography [22]. One further study also found a reduced LV mass in RA using cardiac MRI [210]; thus further studies using cardiac MRI in RA are required to further investigate the conflicting findings.

3.4.3.2 Cardiac Inflammation and Fibrosis

The ability to evaluate inflammation and fibrosis using MRI has provided further clues into the mechanisms of cardiac disease in RA [211]. Puntmann et al. undertook a study using MRI to evaluate differences in cardiac morphology in patients with clinical myocarditis, RA patients, and healthy volunteers [212]. Each group was matched for age, sex, and CV risk factors and the whole study included 94 participants. In addition to finding lower LV mass, lower ejection fraction, and higher end diastolic volumes in the RA group, a significant increase in edema signal measured on a T2-weighted sequence and delayed enhancement was noted, consistent with diffuse myocardial inflammation. Although cross-sectional, this study exemplifies the range and extent of cardiac pathology in RA and the important role MRI is likely to play in the future.

Finally a study by Ntusi et al. combined all the previously described MRI methodologies in RA patients with no history of CV disease and age- and sex-matched controls [37]. Rheumatoid arthritis patients had evidence of both

diffuse and focal areas of fibrosis in addition to myocardial inflammation compared with controls. The diffuse pattern of fibrosis is consistent with microvascular disease. Indeed, diffuse fibrosis as measured on T1 mapping and measurement of extracellular volume was associated with left ventricular strain and also circulating measures of inflammation. This study allowed the linking of pathophysiological patterns with cardiac morphology and function, thus begins to unravel the mechanisms of cardiac disease in RA.

Cardiac MRI studies have not only demonstrated an increased burden of disease but also can assist in the understanding of distinct pathophysiological mechanisms contributing to increased CV morbidity and mortality. For example, Mavrogeni et al. identified four different imaging patterns of heart failure—namely, myocardial inflammation, MI, dilated cardiomyopathy, and diffuse subendocardial fibrosis—observed in RA patients (Fig. 6.8) [38]. These findings suggest that further studies using cardiac MRI are likely to shed more light on mechanisms of disease and improve our ability to manage and prevent CV risk in this population.

3.4.4 Nuclear Imaging

Cardiac single photon emission computerized tomography (SPECT) is used as an established method of evaluating cardiac ischemia in clinical practice. Cardiac SPECT studies of RA patients have confirmed the findings using other modalities that patients have significant subclinical microvascular disease, which is associated with circulating markers of inflammation [213,214]. The use of cardiac positron emission tomography (PET) has further improved the accuracy of evaluating myocardial perfusion and reversible ischemia [22]. However, the technology has yet to be applied in the RA population.

PET can also be used to evaluate vascular inflammation. Fludeoxyglucose (FDG) is a glucose analogue that is taken up into active cells and trapped. When radioactively labeled it emits a decay signal, which can be picked up by a row of γ cameras on a PET scanner. Metabolically active cells, specifically macrophages, have higher glucose requirements and thus take up more FDG. FDG-PET can be used to evaluate vascular wall inflammation and is used in clinical practice to diagnose large-vessel vasculitis. In addition, atherosclerotic plaques, which are vulnerable, have higher levels of inflammatory, metabolically active cells and have been shown to exhibit higher signal on FDG-PET. As discussed in an earlier section FDG-PET was used to evaluate vascular inflammation and in a case-control study of RA patients demonstrated significantly higher vascular inflammation than in non-RA controls with improvement following biologic therapy [155]. In addition, one study used FDG-PET to evaluate carotid plaque inflammation in patients with active arthritis. FDG uptake within plaque, signifying inflammation, was seen in all subjects and in

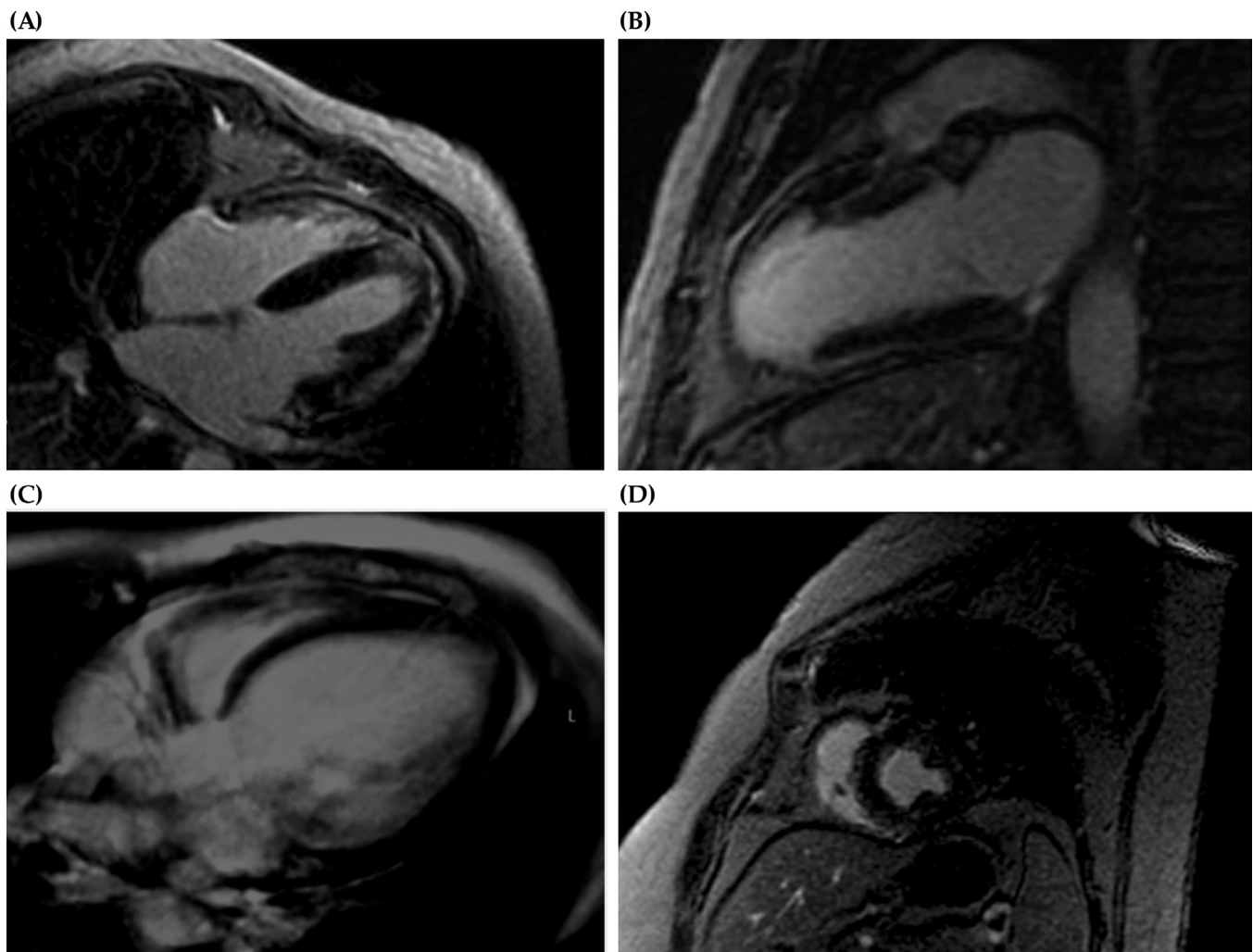


FIGURE 6.8 Different patterns of cardiac MRI imaging in RA. (A) Myocardial inflammation. (B) Myocardial ischemia due to MI. (C) Dilated cardiomyopathy. (D) Diffuse subendocardial fibrosis. *Adapted from Mavrogeni et al. [38].*

5 out of 13 cases plaques had significantly higher uptake within plaque than in the nonatheromatous wall [156]. Although only a pilot, this study highlights the role PET imaging may be able to play in understanding the mechanisms of CV disease in RA, in particular the role of vascular inflammation.

3.4.5 CV Imaging in Clinical Practice

There are currently no guidelines for enhanced screening in the RA population using CV imaging. Imaging studies clearly show a high burden of subclinical CV disease in RA compared with the general population, which may be reversible with antiinflammatory therapy. In the future there may be a case for screening subgroups of patients in order to identify those with CV inflammation or vascular disease for targeted therapy. However, further research is required to validate these techniques in the RA population and provide evidence that enhanced screening may improve outcomes before they become established in clinical practice (Table 6.5).

3.5 Use of CV Risk Algorithms in RA

3.5.1 Assessing CV Risk in RA With Risk Algorithms

It is important to be able to assess an individual's risk of future CV events. Composite CV risk scores allow an individual's absolute risk to be calculated. This assists the clinician with risk stratification of their patients so people can be prioritized for risk reducing interventions. In the general population a number of validated CV composite risk scores are available including the Framingham risk score (FRS) and the Reynolds risk score (RRS) used widely in the United States, the 2013 ACC/AHA 10-year atherosclerotic CV disease risk index, the Systematic Coronary Risk Evaluation (SCORE), used in Europe, and the QRISK2 used in the United Kingdom. The RRS includes CRP as one of its composite measurements, while the QRISK2 includes a diagnosis of RA in its calculation.

Evidence shows that these calculators underestimate future CV events in RA.

TABLE 6.5 Key Findings in CV Imaging Studies

Imaging Modality	Findings in RA Population
Echocardiography	Increased prevalence of pericardial effusion
	Increased prevalence of valvular nodules, calcification, and insufficiency
	Increased prevalence of tricuspid regurgitation and raised pulmonary artery pressures
	Increased LV mass
	Increased prevalence of diastolic dysfunction
	Abnormal strain cardiac patterns
CT coronary arteries	Increased prevalence of inducible ischemia
	Increased coronary calcification scores
	Increased prevalence of significant stenosis
Cardiac MRI	Increased prevalence of mixed or noncalcified plaque (high-risk lesions)
	Lower LV mass (converse to echocardiography studies)
	Increased LV strain and dysfunction
	Diffuse myocardial fibrosis correlated with LV strain
Nuclear medicine	Myocardial inflammation
	Reversible ischemia associated with inflammation
	Low-grade vasculitis
	Carotid plaque inflammation

LV, Left ventricle.

A study from the Netherlands, including 1050 RA patients, found that the FRS, RRS, and SCORE all underestimated CV risk and the QRISK2 tended to overestimate risk [215]. A population-based inception cohort of over 500 RA patients followed up for a mean of 8.4 years found that the observed CV risk was higher than predicted with both the FRS and RRS [216].

Whether addition of RA disease-related variables such as disease activity, disease duration, and use of certain medications, such as corticosteroids, added to a traditional CV risk model will improve CV prediction rates is under investigation. A study involving 23,605 RA patients from the Consortium of Rheumatology Researchers of North America registry found that inclusion of RA-related factors in a CV risk prediction score improved the classification of CV risk compared to a risk prediction score with traditional CV risk factors only [217]. However, as the authors acknowledged, this requires external validation and long-term follow-up. The RA population is a very heterogeneous group across nations, which makes it

difficult to create a single RA-specific CV risk calculator applicable to everyone.

In addition to tailoring CV composite risk scores for RA patients, use of markers of subclinical atherosclerosis such as carotid ultrasound to identify the presence of plaque may help to improve CV risk prediction in this group. Carotid artery ultrasound performed to identify atherosclerosis has been shown to reclassify RA patients into a more appropriate CV risk group [218]. Rheumatoid arthritis disease activity and disease duration have been shown to be associated with plaque vulnerability and size [219,220]. Recommendations on CV prevention in clinical practice published by the European Society of Cardiology suggest the use of carotid ultrasound to screen for atherosclerosis in patients with moderate CV risk [221].

3.5.2 Implementation of Risk Assessment in the Clinical Setting

EULAR recommends that RA patients have an annual CV risk assessment. As part of this assessment they advise the use of national guidelines or if these do not exist, the SCORE should be used to calculate risk [222]. As a method to include RA-related risk factors in CV risk prediction, EULAR recommends a multiplication factor of 1.5 to the CV risk score model for RA patients who have two out of three of the following criteria: RF or anti-CCP antibodies positivity; greater than 10 years disease duration; and the presence of extra-articular features. EULAR also advises measurement of total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-c) to calculate the TC/HDL-c ratio. High-density lipoprotein-cholesterol levels may fluctuate with disease activity, with lower HDL-c concentration reported in RA patients with active disease, resulting in a more unfavorable TC/HDL-c ratio [223,224]. These recommendations have recently been updated but the relevant paper has not yet been published.

There is some evidence that even after modification of SCORE with multiplication of 1.5, it may still underestimate CV risk in RA [225,226]. However, without access to a validated RA-specific CV risk calculator at the present time, use of available composite risk scores in RA patients in conjunction with early control of disease activity and management of traditional risk factors are important elements to be included in targeting CV risk in RA.

4. MANAGEMENT OF CV RISK IN RA

4.1 Lifestyle Interventions

Apart from controlling and ameliorating disease-related manifestations through pharmaceutical

interventions, research findings currently suggest that lifestyle interventions are equally important, particularly for RA patients. As such, current clinical practice should involve different lifestyle factors for improving patient care, and specifically physical activity, weight management, nutrition, and smoking cessation.

4.1.1 Physical Activity

Rheumatoid arthritis is a chronic inflammatory disease where the inflammatory responses are controlled by the acute-phase response. This occurs due to a disturbance of the homeostasis of the organism, which is directly linked to RA playing both a major adaptive and defensive role with regard to tissue injury and infection. It is an intrinsic response initiated after the first few days following an inducing stimulus and is associated with a vast number of changes, both at the systemic and metabolic level [227]. During the acute-phase response, acute-phase reactants are produced in the liver, helping to promote the immune response while having the ability to influence one or more stages of inflammation, healing, or adaptation to a noxious stimulus [228]. Normally, the acute-phase response lasts only a few days. However, in cases of chronic or recurring inflammation such as in RA, an aberrant continuation of some aspects of the acute-phase response may contribute to the underlying tissue damage that accompanies the disease. Two major acute-phase proteins in humans are the CRP and the serum amyloid α . Normally present in trace amounts in the plasma, an adverse stimulus can result in a dramatic increase in their rate of synthesis as well as a subsequent increase in their concentration [229]. C-reactive protein, stimulated by interleukin 6 (IL-6), is a biomarker extensively studied as a response to inflammation [230] or in response to physical activity or RA, as it is expressed rapidly with a noticeable increase in its concentration in response to infection or tissue injury [231]. Furthermore, compared to other plasma proteins, CRP has a half-life of approximately 19–20 h [232–234], with elevated circulating levels decreasing by up to 50% per day with the resolution of the acute-phase stimulus [235]. In turn, it plays a prominent role in the restoration of normal structure/function by clearing apoptotic and necrotic host cells of injured tissues [231].

Physical activity is defined as any movement by the human body produced by the skeletal muscles and as a result that increases energy expenditure above resting levels. Exercise is a part of physical activity that is planned, structured, and repetitive with the aim to improve human health and/or performance (athletic populations). There have been significant advances in the last 20 years about the current understanding of how physical activity can benefit RA, with good-quality studies, systematic reviews, and *meta*-analyses. Collectively, research findings agree that physical activity can have significant beneficial effects on both the disease-related

and systemic manifestations of this chronic inflammatory disease, while at the same time improving psychosocial wellbeing.

The disease-related manifestations in RA can be partly alleviated via exercise, possibly due to exercise-induced antiinflammatory effects. In diseased populations where the disease is characterized by low-grade inflammation, a *meta*-analysis of 42 randomized controlled trials with 2808 participants [236] showed that physical activity significantly reduced CRP. Although the inflammatory response is expressed by a CRP overexpression triggered by IL-6 in the acute-phase response and despite the fact that IL-6 may elevate in response to physical activity, it is proposed that IL-6 increases as a result of exercise-induced reductions in intramuscular glycogen most likely to act as a messenger for initiating hepatic glycogenolysis and lipolysis in the adipose tissue [237]. Thus it is suggested that exercise-induced IL-6 overexpression is not inflammatory, but may play an antiinflammatory and immunosuppressive role [238]. Specifically, during acute structured (exercise) physical activity, IL-6 increases significantly and is dependent on the intensity, duration as well as the type of exercise. Exercise that can cause muscle damage—ie, damage in the structure/function of the sarcomere—such as high-intensity eccentric exercise can result in an inflammatory response, but physical activity (such as walking, cycling, gardening) or nondamaging exercise (such as concentric strength training, or intense aerobic training) does not result in inflammation [239].

Early studies suggested that IL-6 was derived by leukocytes in response to local muscle damage of the exercising muscles after eccentric exercise [240]. However, exercise-induced IL-6 kinetics demonstrated that this cytokine returned to resting values postexercise, whereas muscle damage biomarkers and specifically creatine kinase and myoglobin concentrations remained elevated [241]. In addition to that, in the inflammatory response IL-6 triggers the increase of adhesion molecules on leukocytes as well as endothelial cells, and thus if IL-6 exerted an inflammatory response during exercise, increased expression of adhesion molecules would also be expected to occur, which in fact is not the case either after nondamaging eccentric or maximal exercise [242,243].

Although the exact source as well as molecular mechanisms of exercise-induced IL-6 is still an area of ongoing research (for extensive reading see the narrative review by Reihmane and Del [239]), studies collectively agree that exercise has long-term antiinflammatory effects, which can be explained as follows:

- Exercise increases antiinflammatory mediators: the inflammatory environment that characterizes autoimmune diseases, such as RA, presented with

a high-grade inflammatory response is markedly different from that occurring during physical activity. In the former we have continuous production of proinflammatory cytokines whereas in the latter this is not the case. Rheumatoid arthritis patients have significantly higher levels of TNF α and IL-1 β (proinflammatory cytokines that interplay with IL-6 inducing the acute-phase response) than in the normal population, with both these cytokines being targeted as part of relevant pharmaceutical interventions. In contrast, although IL-6 increases during physical activity, this is not the case for TNF α or IL-1 β [244,245]. In addition, antiinflammatory cytokines, namely IL-1 receptor antagonist and IL-10, are overexpressed postexercise, inducing an antiinflammatory environment [246].

- Reductions in adiposity: research studies reveal that obesity is associated with higher CRP, IL-6, and TNF α production compared to the normal population, suggesting that obesity is a low-grade inflammatory condition [247]. The expression of these inflammatory proteins decreases in response to increased physical activity [248], a fact also confirmed by a *meta-analysis* in diabetes [238]. In RA, these findings were confirmed in cross-sectional studies [94] but also in a controlled trial that investigated the effects of a 6-month combined aerobic and resistance exercise program [158,249]. This study demonstrated that compared to patients that receive usual care, those who exercised three times per week significantly reduced their body fat, which accounted for the observed concomitant reductions in inflammation.

Apart from their antiinflammatory effects, physical activity and/or exercise result in physiological adaptations that may significantly alleviate other important symptoms of this disease. Increased physical activity in RA is associated with improved cardiorespiratory fitness [249], musculoskeletal strength [250], function [251], fatigue [252], CV profile [101], and vascular function [158] and thus it seems reasonable to suggest that these adaptations may be partly responsible for the better clinical presentation of the more physically active RA patients than in those who are physically inactive. It is also important to note that currently there is no single study reporting adverse effects of physical activity and/or exercise, even if the intensity of the physical activities is high [253].

Given all these promising literature findings, patients with RA unfortunately still remain physically inactive. From the patients' perspective, specific barriers can prohibit participation in physical activity despite its multiple benefits. Specifically, lack of appropriate RA-specific physical activity and/or exercise programs, lack

of support from healthcare professionals as well as lack of knowledge about the benefits of physical activity are in the forefront of the mostly identified barriers collectively reported in the literature [254]. This necessitates the development of relevant interventions to address these barriers and facilitate uptake of physical activity in order to optimize healthcare for RA patients. On the other hand, currently there seems to be a lack of provision from relevant official national and international bodies to promote physical activity (including EULAR). There is also lack of sufficient information for health practitioners and patients to facilitate the development of effective and tailored exercise prescriptions. The only available resource that provides adequate information and detailed guidance is the European Musculoskeletal Conditions Surveillance and Information Network, which details information for incorporating aerobic physical activities in the management of RA, although with a very conservative approach for resistance exercise, which brings about different physiological adaptations that may have different health benefits for this population compared to aerobic exercise [251,255,256].

Studies that have utilized physical activity and/or exercise interventions in RA report overall average to high attendance to such exercise programs for the duration of the intervention [256]; however, it must be noted that these are predominantly supervised interventions, which require participants to attend facilities for exercising. Even nonsupervised Internet-based physical activity interventions may be able to increase physical activity levels for the duration of an intervention [257]. However, the matter that currently stands as a truly societal health issue is the long-term adherence to a physically active lifestyle, ie, maintaining increased physical activity for at least 12 months postintervention. The World Health Organization recognizes physical inactivity as the fourth leading cause of overall mortality, whereas epidemiological studies reveal that physical inactivity and low cardiorespiratory fitness levels have stronger associations with all-cause mortality than smoking, obesity, or hypertension [258]. These results are also confirmed by cross-sectional observations in RA, where low physical activity levels as well as low cardiorespiratory fitness are both associated with a significantly deteriorated CV profile, increased 10-year CV risk, and even a higher rate of hospital admission [101,259,260]. Thus it can be suggested that developing tailored interventions may be key in promoting physical activity in RA, whether this is based on community-based initiatives or even utilizing existing infrastructure, such as cardiac rehabilitation centers; it is also important to tailor the program to the patient's functional ability and preferences in order to optimize healthcare. A detailed approach on how to develop safe

and tailored exercise programs has been recently published [261,262].

To promote long-term adherence to a physically active lifestyle, approaches that are supported by a theoretical background may be necessary. A recent systematic review revealed that new studies are indeed emerging in this field and consist of either behavior change interventions or combined practical physical activity and behavior change interventions, which utilize a large variety of different relevant techniques with varied success [263]. Combining a motivation- and action-focused intervention [264] or alternatively interventions that target the modification of beliefs and attitudes about physical activity [265] can both improve physical activity levels. However, despite these attempts, the available studies seem to lack a strong and detailed theoretical underpinning, often not assessing and/or taking into account the theoretical constructs that are important to understand what caused the beneficial changes in behavior [263]. Thus the exact behavioral constructs that may improve physical activity in RA require further attention and research. Moreover, educational interventions that aim to increase the awareness of RA patients in terms of healthy lifestyle show that although awareness of the benefits of physical activity improves, this does not change the patients' behavior toward being more physically active [266]. New studies with a clearer focus on the theoretical underpinning of physical activity behavioral changes (ie, self-determination theory) in RA are currently ongoing [267].

International physical activity initiatives also focus on promoting physical activity in diseased populations during routine clinical visits, given that the relationship between the health-practitioner and patient is a key-stone in healthcare. Recently, the *Journal for the American Medical Association* published a manuscript that aims to promote physical activity in all patient encounters with their healthcare professional and/or clinic staff but also provides means for effective integration of this approach into busy clinical settings [268]. It suggests that healthcare professionals:

- Focus on making physical activity a “vital sign” for every routine visit
- Incorporate in the clinical routine visit a question about exercising habits: if the patient exercises the health professional should ask how many minutes and how often, if the patient does not exercise, they should be asked if they are willing to start
- Discuss briefly the benefits of exercise in reducing the incidence of many different chronic diseases including CV disease and cancer
- Produce an agreed daily physical activity prescription, working up to at least 30 min of walking or any other physical activity of moderate intensity

- Encourage the use of a pedometer and advise recording daily physical activity
- Recognize success when patient engages or encourage reluctant adopters

Studies that have adopted such approaches to increase physical activity report excellent physical activity-induced improvements in various different health-related outcomes. In specific, US trials reveal that when clinicians in primary care are trained to deliver brief (3- to 4-min) interventions during routine patients' visits, this results in increased levels of physical activity over a 2-year follow-up, but importantly it promotes significant improvements in cardiorespiratory fitness [269]. In addition, the PREMIER trial [270] provided strong evidence that brief lifestyle and physical activity counseling among adults with prehypertension or stage 1 hypertension (highly prevalent in RA) [50] resulted in significant reduction in CV risk (12–14% relative reduction in the 10-year Framingham Coronary Heart Disease Risk Score), which was maintained at 18 months. Despite these very promising results and current recommendations to increase physical activity in this patient population, no studies to date have addressed the involvement of clinicians in increasing physical activity in RA.

4.1.2 Diet

Diet interventions and manipulations have been frequently utilized and are used today by patients diagnosed with RA in the hope that they will ameliorate disease-related symptoms. There are a variety of diet interventions available in the literature with varied effectiveness for disease-related symptoms, necessitating further investigation into this research topic.

Collectively the dietary interventions that have been utilized in the literature are:

- Fasting followed by vegetarian diet: 7–10 days of subtotal fasting approximating between 800 and 1260 kJ per day, followed by 1 year of vegetarian diet, resulted in significant improvement in many different RA-related outcomes, including—among others—the number of tender and swollen joints and pain and duration of morning stiffness even after 4 weeks; these improvements were maintained after 1 year [271]. In addition, another study, which has a high risk of bias, reported that 7 to 10 days of fasting followed by 9 weeks of a lacto-vegetarian eating diet did not improve any of the studied outcomes in RA patients [272].
- Fasting followed by vegan diet: Despite the fact that some relevant studies exist in this field their results are conflicting or show no effects on RA-related outcomes [272–274]. It is important to note that the methodological quality of the studies still does not sufficiently assess their effectiveness.

- Mediterranean diet: The nutrients contained in a Mediterranean diet are frequently characterized in the literature as antiinflammatory. This diet is based primarily on plant-based foods, such as fruits and vegetables, whole grains, and nuts, as well as fish and omega-3 polyunsaturated fatty acid, olive oil as the main source of fat, and spices instead of salt for flavoring the foods; this diet also keeps dairy products and red meat to a minimum. A 2007 systematic review and *meta-analysis* or randomized controlled trials concluded that omega-3 polyunsaturated fatty acids (highly expressed in this diet) could possibly be an attractive adjunctive specifically for the treatment of joint pain in RA [275]. Moreover, a randomized controlled trial investigating the effects of the Mediterranean diet against an ordinary Western diet revealed improvements in inflammatory activity, an increase in physical function, and improved vitality in RA [276].
- Exclusion diet: A double-blinded, placebo-controlled study, which utilized an exclusion diet, where patients underwent a washout period from all previous therapy and then followed an exclusion phase for 1 week in which only foods that were nonallergenic, revealed beneficial effects on important clinical outcomes including in joint pain, stiffness, and inflammation [277]. However, in general, it seems that the overall results of these diets are contradictory in RA, with studies supporting either beneficial or no effects [278].

A systematic review and *meta-analysis* on the effects of these different diets on RA-related outcomes concluded that their effects are still unclear due to the existence of small trials with moderate and high risk of bias, while potential adverse effects should not be ignored [279].

4.1.3 Interventions for Reversing Rheumatoid Cachexia

Given the potential effects of TNF α on enhancing muscle catabolism, it was hypothesized that blocking TNF α via biological medication could potentially reverse rheumatoid cachexia. However, two studies in this field reveal that body composition remains unchanged after anti-TNF α medication in RA, either acutely (2 weeks) or long term (6 months) [280,281]. In contrast, when physical activity interventions incorporate resistance training, significant effects are observed in reversing these phenomena. Specifically, combining aerobic and resistance training significantly reduces body weight and fat mass at 6 months [249]. Moreover, 24 weeks of progressive high-intensity resistance (but not aerobic) training can restore muscle mass and

function in RA patients without exacerbating any RA-related symptoms [249], but these benefits are lost at 3-year postintervention [282], suggesting that adherence to resistance training is key to reversing rheumatoid cachexia. Importantly, acute resistance training in combination with whey protein intake (0.5 g/kg lean body mass) can stimulate muscle protein synthesis and muscle gene expression in a similar manner to both RA patients and age-gender-BMI-activity matched healthy controls [283]. These studies collectively suggest that when physical activity incorporates aerobic exercise alongside resistance training (as per the current physical activity guidelines promoting resistance training at least twice/week), is a more effective intervention than pharmacological interventions to induce beneficial body composition changes (ie, increase in muscle mass and reduction in adiposity) for the RA patient.

Contemporary clinical care should focus on identifying effective interventions to help patients with RA quit smoking. This is particularly important since current smoking prevalence in RA ranges between 21% and 35%, while 50–69% RA patients are either past or current smokers [284,285]. Moreover, a relevant 2014 international survey revealed that one-fourth of the surveyed rheumatology departments provided advice for smoking cessation or had a specific relevant protocol they were utilizing in their clinical practice [286]. As such, it seems that specific barriers pertaining to clinical practice and protocols may inhibit the facilitation of smoking cessation in RA patients. Smoking cessation therefore may be a problem that is very difficult to overcome particularly given that research on smoking cessation reveals that 70% of smokers report that they want to quit but only 4–7% succeed in doing so [287].

Until recently studies addressing barriers for smoking cessation in RA or specifically smoking cessation via relevant interventions were lacking. A pilot study that investigated means of modifying lifestyle factors (including smoking) in RA revealed that none of the patients quit smoking at the end of the intervention [288]. Moreover, a randomized controlled trial utilized an 8-week cognitive behavioral patient education intervention to induce behavioral changes about modifiable CV disease risk factors in RA patients, including smoking cessation, physical activity, and healthy eating [266]. Despite the higher awareness achieved at 6 months of the intervention about modifiable CV disease risk factors, none of the patients reported an intention to quit smoking. The findings of these studies suggest that targeting smoking cessation as part of a multicomponent interventional approach may not be effective for smoking cessation in RA and thus specifically designed and

trials with a specific focus on smoking cessation may be necessary to achieve this.

The only available study targeting smoking cessation in patients with arthritis, which included 55 patients with RA, utilized face-to-face verbal and written advice by a rheumatologist with a subsequent visit at 3 months for reinforcement and receiving of relevant pharmacological treatment to help arthritis patients quit smoking [289]. At 3 months, 11.8% of the patients quit smoking a percentage, which improved at 12 months to 15.9%; it is also important to note that >50% of patients reduced smoking at the end of the intervention. Specifically for RA, only recently a study investigated—using a mixed-methods approach (interviews and questionnaires)—the barriers to smoking cessation in patients with RA [290] and identified the following five barriers:

- Patients with RA lack the awareness of the links between smoking and their disease and therefore did not consider this as a reason for quitting
- Smoking is used as a distraction from pain
- Lack of awareness about exercise, a consistent barrier in the literature for RA [254], meant that RA patients were unable to use exercise as an alternative distraction from pain
- Smoking was utilized as a coping mechanism for the frustrations of living with RA
- RA smokers felt unsupported and isolated from other RA patients

The same group of researchers developed a tailored smoking cessation intervention that is to be delivered by trained arthritis health educators using components such as nicotine replacement therapy, telephone or face-to-face interviews to identify specific barriers combined with activities to overcome these barriers; this promising trial is currently ongoing and no results are available at present (only the protocol of this trial has been currently published) [291].

4.2 Antirheumatic Therapy and CV Risk

4.2.1 Introduction

Antirheumatic drug therapies have substantially improved the quality of life and increased the lifespan of patients with RA over the past few decades. One of the powerful mechanisms implicated in the improved outcomes of the therapies seems to be associated with dampening inflammation and slowing the progressive course of accelerated, inflammation-induced atherosclerosis [292]. By suppressing systemic inflammation and disrupting autoimmune and inflammatory pathways in synovial joints, methotrexate as the frontline disease-modifying antirheumatic drug (DMARD) improved CV

morbidity and mortality across large cohorts of patients with RA [293]. In a systematic review of 10 observational studies of methotrexate effects in RA and other inflammatory polyarthritides the use of the drug was associated with a 21% lower risk of total CV disease and an 18% lower risk of MI [294]. That study even justified the notorious CV Inflammation Reduction Trial (CIRT), which may even support the use of methotrexate for secondary CV prevention in non-RA cohorts [295].

Anti-TNF α agents, which have been widely used in RA over the past two decades, suppress inflammation more potently than methotrexate and other nonbiologic DMARDs and seem to be effective in retarding the course of atherosclerosis. Although hard CV endpoint studies in RA are limited and not always supportive of the beneficial effects of anti-TNF α agents, a recent systematic review of 23 studies focusing on surrogate markers of atherosclerosis and arterial stiffness in RA and other inflammatory arthritides demonstrated that anti-TNF α therapy prevents and even reverses the increase of carotid intima-media thickness [296]. The suppression of systemic inflammation may have a certain nonspecific positive role in improving CV outcomes in RA by anti-TNF therapies. But there may also be other mechanisms associated with direct effects on T and B lymphocytes, lipid profiles, platelet function, endothelial function, coagulation, and thrombosis. Pilot, short-term studies of anti-B-cell therapies in RA, and particularly for rituximab, have demonstrated that potent antiinflammatory effects are primarily implicated in the improved course of atherosclerosis in RA [297]. Six-month rituximab therapy decreases carotid intima-media and atherogenic index in patients with RA [298]. But it remains uncertain whether such beneficial CV effects of rituximab translate into better CV morbidity and mortality profiles.

4.2.2 Glucocorticoids

Despite the promising results of the observational studies and systematic reviews, there are many issues for the practitioners managing patients with RA to consider. One of the most common challenges is the use of glucocorticoids, which are included in combined therapies along with biologic and nonbiologic therapies. The use of glucocorticoids at variable doses helps to rapidly and potently suppress systemic inflammation and improve immune responses controlled by the hypothalamic–pituitary–adrenal axis. It also stimulates antiinflammatory mechanisms in various organ systems due to permissive action on a wide of range endocrine hormones [299]. Not surprisingly, the latest evidence-based recommendations of the EULAR consider low-dose glucocorticoid therapies (7.5mg prednisone) as part of the initial 6-month combined treatment strategy in RA [300]. In a UK-based, follow-up drug-utilization study a total of 7777 patients with RA (47%) received more than one glucocorticoid

prescription [301]. Of them, more than 50% were on doses above 10 mg/daily. High prescribers were mostly elderly patients and those with hypertension. Early tapering of glucocorticoid therapies is thus desirable but not always achievable, which is why adverse effects of glucocorticoid therapies are often reported globally. Adverse effects are mostly reported in patients with high disease activity, receiving high-dose and long-term therapies, leading to osteoporosis, glucose intolerance, diabetes, hypertension, and CV events [302]. Unfavorable results of the therapy may be also encountered in patients with the so-called glucocorticoid resistance due to glucocorticoid receptor and glucocorticoid-induced transcript 1 gene polymorphisms [303]. A large follow-up study of the relationship between glucocorticoids and mortality in 779 patients with RA revealed a dose-dependent increase of all-cause and CV mortality rates in subjects treated with daily doses above 8 mg [304]. Adjusted for the propensity to receive glucocorticoids hazard ratios (HR) of CV mortality were 2.27 (95% CI 1.36–3.79) in patients exposed to 8–15 mg daily doses and 3.21 (95% CI 1.14–8.97) in those exposed to doses above 15 mg. Similarly a *meta*-analysis conducted by Roubille et al. included 14 studies and evaluated the association between oral glucocorticoids use and cardiovascular events [305]. It was found that patients who had been treated with oral glucocorticoids had a 47% higher risk of having an event. Inflammation is likely to be a significant confounder in the relationship between steroids and cardiovascular risk, as those with more severe disease have higher levels of inflammation and are therefore more likely to be treated with oral glucocorticoids. Avina-Zubieta et al. aimed to unpick this complex relationship in a large mediation database study. The relationship between current or previous glucocorticoid use was evaluated in a cohort of 8384 RA patients with over 50,000 person-years follow-up [306]. The authors made adjustments for traditional risk factors based on reported comorbidities and medication use, within the administrative database and also for disease severity based on the number of visits to the rheumatologist and type and number of antirheumatic medications used. A significant association with both current dose (14% increased risk per 5 mg prednisolone) and cumulative dose (6% increased risk per gram of prednisolone) and cardiovascular events was found despite adjustment of these factors. As this was a large medication database study there was no measurement of disease activity, antibody status, or circulating inflammation, which are known to be factors associated with glucocorticoid use and also increased risk of CV disease. However, the authors attempted to address this by conducting a sensitivity analysis where adjustment was made for a large hypothetical confounder. Even with this adjustment the association persisted. It is likely that the use of glucocorticoid to treat inflammation contributes to the increased cardiovascular risk seen in RA.

4.2.3 Nonsteroidal Antiinflammatory Drugs

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used in patients with RA for symptomatic management of pain. Uncontrolled use of NSAIDs, and particularly in elderly patients with established CV comorbidities, may increase the risk of CV adverse effects [307]. A cautious approach is particularly required in the case of multiple drug interactions. Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) blockers have been marketed for many decades, and their gastrointestinal and CV safety profiles are well explored. Acetylsalicylic acid has been used at low doses to suppress platelet function and prevent thrombotic events across cohorts of patients with RA and other rheumatic diseases. However, its antiplatelet action vanishes when combinations with other NSAIDs are prescribed, leading to the so-called aspirin resistance and exposing patients with rheumatic disorders to the risks of cardio- and cerebrovascular events [308]. Evidence derived from a Cochrane systematic review suggests that concurrent use of NSAIDs and methotrexate in patients with inflammatory arthritis is generally safe provided that antiinflammatory doses of aspirin are avoided [309]. Selective COX-2 inhibitors are relatively new NSAIDs, with Rofecoxib being the notorious representative with the worst CV profile, which was withdrawn from the market. Among COX-2 inhibitors Etoricoxib is currently viewed as a relatively safe option for relieving pain in patients with RA. Patients with RA on 90 mg/daily Etoricoxib on top of DMARDs reach the American College of Rheumatology 20% (ACR20) response while lower doses have much less efficacy in terms of pain management [310]. However, the relatively high dose (90 mg) adversely affects renal function and leads to arterial hypertension.

4.2.4 Anti-TNF α agents

All currently approved anti-TNF α agents are recommended for patients with RA with poorly controlled systemic inflammation, even at early stages of the disease [300]. Although head-to-head powered trials are lacking, efficacy and safety of all anti-TNF α agents are believed to be similar. Importantly, a retrospective study of 110 patients with RA who were on biological therapy with Etanercept, Adalimumab, or Infliximab demonstrated a glucocorticoid-sparing effect of these agents with 76% of patients reducing dose and 15% discontinuing oral prednisolone within 3 months [311]. Anti-TNF α agents may increase levels of atherogenic lipid complexes, but clinical significance of such an effect is not significant since the atherogenic index usually remains unchanged [312].

In a large, Swedish cohort of early RA ($n=6000$) no any increase in the risk of acute coronary syndrome was

observed in those exposed to anti-TNF agents within 6 months of the treatment (OR 1.5, 95% CI 0.3–6.9) [313]. Longer duration of anti-TNF α therapy in RA was also reported to be safe and protective against CV events in another large retrospective cohort of 113,677 patients with RA. With increasing duration of the therapy from 1 to 2 and 3 years CV event risks decrease by 21%, 38%, and 51%, respectively [314].

Anti-TNF α therapy has been tested across non-RA cohorts of patients with chronic heart failure, and results of the trials have been discouraging. High doses of Infliximab, for example, have shown to worsen heart failure and increase the risk of cardiac events [315]. The infusion of Infliximab (3–5 mg/kg) in patients with RA without overt heart failure led to negative inotropic effect by reducing cardiac output and increasing peripheral arterial resistance [316]. A systematic review of 20 studies examining the effects on anti-TNF α agent on cardiac events and heart failure in RA cohorts demonstrated a reduced likelihood of adverse effects of the therapy [317]. In a large retrospective study comparing RA cohorts switching from methotrexate therapy to anti-TNF α or nonbiological DMARDs there was no increase of heart failure risk in both large arms [318]. However, subgroup analysis revealed that concurrent use oral glucocorticoids was associated with the risk of heart failure in a dose-dependent manner.

Based on the currently available evidence, anti-TNF α agents are not recommended for patients with overt heart failure (III–IV functional classes by New York Heart Association) [319]. A cautious approach is also advisable for patients with established CV comorbidities [55].

4.2.5 Tocilizumab

Tocilizumab (TCZ) is a humanized monoclonal antibody against membrane-bound and soluble interleukin-6 (IL-6) receptors. It has favorable effects on serum levels of IL-6, fibrinolytic activity [320], and left ventricular systolic function in patients with RA [321]. Patients unresponsive to methotrexate and biologic agents, and particularly those at risk of inflammatory amyloidosis, may benefit from repeated injections of TCZ [322].

Headache and hypertension are frequently reported adverse reactions in trials of TCZ [323]. A comparative, 8-week study of the effects of TCZ and adalimumab demonstrated more pronounced increase of low-density lipoproteins in response to targeted IL-6 blockade [324]. At the same time, TCZ therapy had a more pronounced effect on decreasing serum amyloid A, phospholipase A2, and lipoprotein (a). Major CV events ($n=50$) developing during 24 weeks of TCZ therapy were analyzed in a large retrospective posthoc study with 3986 patients with RA [325]. In that study higher disease activity but not changes in lipid profiles were associated with CV events. Finally, in a large postmarketing study with a total of 64,000 patient-years of TCZ

exposure no difference in terms of serious MIs and cardiac deaths were reported in comparison with anti-TNF α agents [326].

4.2.6 Abatacept

Abatacept is a fully human fusion protein composed of the cytotoxic T-lymphocyte associated antigen-4 (CTLA-4, CD152) immunoglobulin and a fragment of the Fc domain of human IgG1. It inhibits T-cell costimulation and subsequent activation of B-lymphocytes and macrophages. Abatacept is used in patients with moderate and severe RA in the case of unsuccessful therapies with one or more anti-TNF α agents. The results of the ACTION (AbataCepT In rOutiNe clinical practice) cohort study (1009 patients with RA) demonstrated that abatacept therapy over 24 months allowed to decrease glucocorticoids in 41% of patients, and particularly from >5 mg/day to <5 mg/day in almost one-third of cases [327]. Such a positive effect of abatacept may help prevent glucocorticoid-induced hypertension, glucose intolerance, diabetes and obesity in a sizable percentage of patients. In fact, a 6-month longitudinal study with 15 patients with RA treated with abatacept proved its beneficial effect on insulin sensitivity with a decrease of blood glucose, insulin, and glycated hemoglobin levels [328].

In a pooled analysis of eight trials of intravenous abatacept therapy in 3173 patients with RA there was a consistency between short- (1 year) and long-term (8 years) adverse effects, with cardiac disorders being the most frequent cause of death in the long term (26 cases) [329]. During the follow-up period the incidence rate for serious CV events was 1.22/100 patients-years and for MI 0.27/100 patient-years. A study of the effect of age on the risk–benefit balance of abatacept in RA ($n=1017$) did not report any serious CV adverse effects despite poor antiinflammatory response with increasing age at 2-year follow-up [330].

4.2.7 Rituximab

Rituximab (RTX) is a chimeric human-murine monoclonal antibody directed at the CD20 antigen of B cells. It is often used in patients with RA unresponsive to anti-TNF α agents. The latest US-based cohort study of patients with RA proved that CV safety of RTX and anti-TNF α is comparable [331]. Rituximab's targeted action on B cells has beneficial long-term implications. The same action, coupled with CV and other risk factors, may also increase the risk of adverse events. The drug's infusion-related adverse effects are well known (eg, hypotension, life-threatening arrhythmias, MI), and there are preliminary data suggesting that such effects are more frequent in patients with RA than in those with lupus due to the lower doses of glucocorticoids administered during the week prior to the infusion in RA [332]. The risk of adverse CV events in RA is high within 24 h after the RTX infusion. Several case

studies have described the occurrence of acute coronary events following RTX infusion [333]. However, a large follow-up study ($n=3194$, up to 17 infusions over 9.5 years) proved that the long-term safety of RTX in terms of cardio- and cerebrovascular events is comparable to that of the general RA population [334].

4.2.8 Conclusion

Overall, CV safety of the currently widely used anti-rheumatic agents is largely acceptable. The combined use of biologic agents has not been tested in RA, but it seems that such a therapeutic strategy should be avoided to minimize the likelihood of undesirable immune and CV effects. There are also no prospective head-to-head studies comparing CV safety of biologic agents. In real clinical practice, and to achieve tight control of disease activity, biologic agents are often used in combination with numerous antiinflammatory and immunosuppressive drugs that are more likely to predispose to CV events than the biologic agents themselves. In fact, glucocorticoids are major confounders diminishing cardioprotective effects of other antiinflammatory agents in RA and therefore their use in the short term and at minimal doses should be reserved for a small group of patients without CV risk factors. Nonsteroidal antiinflammatory agents are frequently prescribed to alleviate pain across RA cohorts. These agents are known to affect renal function and predispose to arterial hypertension, a common clinical condition in RA [50]. The likelihood of further CV events is increased three-fold in patients with RA and established CV disease who are exposed to Diclofenac and Etoricoxib [335].

Although most evidence-based data are derived from retrospective or short-term prospective studies with some inconsistencies within and between the studies, recommendations of learned associations favor the use of biologic agents in combination with nonbiologic antirheumatic drugs to suppress uncontrolled systemic inflammation and preserve vascular function across organ systems. Nonetheless, the absence of solid evidence of adverse CV events is not evidence of absence of such events. Most studies have inherent limitations due to the careful selection of patients with RA with mild-moderate disease activity and without established CV disease. It is unclear how patients with excessive burden of CV risk factors, comorbidities, and overt heart failure respond to biologic therapies on top of methotrexate or other nonbiologic agents. Older patients are often overlooked across cohorts and clinical trials. However, a recent study of 47,193 patients with RA with mean age of 64 specifically comparing CVCV effects of anti-TNF α agents (Etanercept and Infliximab) and Abatacept pointed to the increased risk of acute MI in those exposed to anti-TNF α therapies [adjusted (HR) 1.3, 95% CI 1.0–1.6] [336].

In the “evidence vacuum” and unavailability of specific tools for CV risk assessment, experts recommend

a cautious approach with recording risk factors, interventions aimed at smoking cessation, weight and blood pressure control, lipid-lowering therapy, and referral to and follow-up by cardiologists [337]. Extra efforts are also needed to minimize or exclude the exposure to glucocorticoids and nonsteroidal antiinflammatory agents in patients with CV comorbidities.

5. OVERALL SUMMARY AND CONCLUSIONS

Cardiovascular disease is a main comorbidity and important cause of mortality in patients with rheumatoid arthritis. A combination of overexpression of classical CV risk factors with significant contribution of direct and indirect effects of systemic inflammation on cardiac, vascular, and metabolic pathways are the most likely culprits. Recent studies suggest significant improvements in CV mortality, most likely due to the different and more aggressive therapeutic approaches, which lead to more rapid and successful control of systemic inflammation and its effects on the vasculature. Despite this, a differential still remains with the general population, which needs to be addressed. Important challenges remain and should define the future research agenda including timely and accurate risk stratification, monitoring of CV status ideally with validated biomarkers and noninvasive assessments, delineation of specific mechanisms particularly with respect to antirheumatic drug effects and top quality, and clinical trial-based evidence for relevant therapeutic interventions, including both pharmacological (cardiological and antirheumatic) and nonpharmacological lifestyle changes (Table 6.6).

TABLE 6.6 Recommendations for Managing CVD Risk in RA

Recommendations	Levels of Evidence	Strength of Recommendations
CV risk stratification and screening in all RA patients	C	I
Treatment for traditional CV risk factors (eg, lipid-lowering tablets—antihypertensives) should be administered in accordance with the available recommendations for the general population	B	I
Tight control of systemic and synovial inflammation	B	I
Lifestyle changes (weight loss, physical activity, tailored exercise program, smoking cessation) in order to reduce CV risk	B	I

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Juvenile Idiopathic Arthritis

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1. DEFINITION

The term juvenile idiopathic arthritis (JIA) embraces all forms of arthritis that begin before the age of 16 years, persist for more than 6 weeks, and are of unknown etiology [1–3]. It encompasses a heterogeneous group of conditions, all presenting with joint inflammation, but with distinctive clinical manifestation, course, and outcome and, perhaps, genetic background and pathophysiology.

2. EPIDEMIOLOGY

JIA is the most common chronic rheumatic disease of childhood and a leading cause of short- and long-term disability. Studies in European and North American populations have shown an incidence and prevalence ranging from 2 to 20 and from 16 to 150 per 100,000, respectively [1]. However, remarkable and still unexplained differences in the frequency of JIA subtypes have been observed in diverse geographical areas or ethnic groups [2–6]. Distinct distributions of age at onset and sex characterize each onset type. The overall frequency, sex distribution, and age at onset of the International League of Associations for Rheumatology (ILAR) categories of JIA are presented in Table 7.1.

3. GENETICS

As JIA is a complex disease and displays both autoimmune and inflammatory features, it is probable that multiple genetic risk factors are involved [7]. The importance of genetic factors has been demonstrated by the observation of familial aggregation of JIA, with a sibling recurrence risk similar to that of type 1 diabetes [8–10]. More recent estimates obtained with probabilistic record-linking analysis in a large cohort ($n=862$) of JIA patients

matched with around 7 million individuals in the Utah Population Database indicate that approximately 13% of cases of JIA can be attributed to familial factors [11]. As for most complex diseases, many genomic regions probably contribute relatively small amounts to the overall disease risk [12]. Several associations between specific disease subsets and particular HLA alleles have been described [8,13–15]. The closest is that between HLA-B27 and the enthesitis-related arthritis (ERA) category [15]. However, a large number of new JIA susceptibility loci in non-HLA regions including cytokine and other immune genes are being discovered [16,17].

4. ETIOLOGY AND PATHOGENESIS

The etiopathogenesis of JIA is still poorly understood. It is currently assumed that a genetically susceptible individual could develop an uncontrolled and harmful immune response toward a self-antigen on exposure to an unknown environmental trigger. This response would generate a self-perpetuating loop of activation of both innate and adaptive immunity that causes tissue inflammation and damage [18].

Several immunological abnormalities have been noticed in JIA [2,3,19], some of which are characteristic of certain disease subtypes. The synovial inflammation is similar to that observed in adult rheumatoid arthritis. There is marked hyperplasia of the synovium lining layer and extensive infiltration of the sub-lining layer by mononuclear cells, including T cells, B cells, macrophages, dendritic cells, and plasma cells [20,21]. Various investigations have measured blood and synovial cytokine levels in children with the different forms of JIA, but the results have been frequently variable [21,22]. The strong therapeutic effect exerted by medications that inhibit tumor necrosis factor (TNF) in many patients suggests that this cytokine plays a major pathogenic role.

Rheumatoid factor (RF)-positive polyarthritis in children is considered clinically and pathogenetically equivalent to adult RF-positive RA [23]. As in adults, a sizeable proportion of children with this disease possess antibodies to cyclic citrullinated peptide (CCP) [24]. It is widely agreed that ERA is part of the spectrum of spondyloarthropathies. Because this condition bears genetic, epidemiologic, and clinical similarities with reactive arthritis, which is related to an enteric or genitourinary tract infection, an infectious etiology has been hypothesized, although none has been proved. Recent lines of evidence have led to postulate a pathogenetic relationship with inflammation of the gastrointestinal tract [25,26] or a disturbance in the gut microbiome [27]. Whether HLA-B27 is involved in disease pathogenesis is still unclear. A molecular mimicry between the B27 molecule, or individual peptides that it presents, and a microbial antigen has been proposed as a mechanism eliciting a CD8⁺ T cell or crossreacting antibody response, which would result in an inflammatory reaction [28].

Antinuclear antibodies (ANA), which are seen most commonly in early-onset oligoarthritis, have been found to react against various nuclear components, none of which is specific for JIA [2]. Recent experimental data suggest that immunoregulatory mechanisms, particularly the regulatory T-cell compartment, can be involved in the induction of the self-limited course pursued by many patients with persistent oligoarthritis, as opposed to those with extended oligoarthritis or polyarthritis.

The prominent extra-articular features, the absence of autoantibodies, and the lack of association with HLA

alleles have led to the suggestion that systemic arthritis is a separate disease entity, with more similarities with autoinflammatory syndromes than with classic autoimmune diseases [29–31]. Furthermore, there is compelling evidence that IL-6, IL-1, and IL-18 are the cytokines that have the greater pathogenetic relevance, whereas the contribution of TNF is less prominent [30,32]. These observations have provided the rationale for the successful treatment with therapeutic agents that antagonize IL-1 and IL-6 (see below).

5. CLASSIFICATION

The current International League of Associations of Rheumatology (ILAR) classification of JIA, outlines seven disease categories: systemic arthritis, RF-positive polyarthritis, RF-negative polyarthritis, oligoarthritis, psoriatic arthritis, ERA, and undifferentiated arthritis (Table 7.2). The primary objective of this classification

TABLE 7.2 The International League of Associations for Rheumatology Classification of Juvenile Idiopathic Arthritis (Second Revision)

Systemic arthritis

Arthritis with, or preceded by, daily fever of at least 2 weeks' duration that is documented to be quotidian for at least 3 days, and accompanied by one or more of the following:

- Evanescent, nonfixed, erythematous rash
- Generalized lymph node enlargement
- Hepatomegaly and/or splenomegaly
- Serositis

Exclusions: a,b,c,d (see below)

Oligoarthritis

Arthritis affecting 1–4 joints during the first 6 months of disease.

Two subcategories are recognized:

- persistent oligoarthritis: Affects no more than four joints throughout the disease course
- extended oligoarthritis: Affects a total of more than four joints after the first 6 months of disease

Exclusions: a,b,c,d,e (see below)

Polyarthritis (RF-negative)

Arthritis affecting five or more joints during the first 6 months of disease: Tests for RF are negative

Exclusions: a,b,c,d,e (see below)

Polyarthritis (RF-positive)

Arthritis affecting five or more joints during the first 6 months of disease: Tests for RF are positive

Exclusions: a,b,c,e (see below)

Psoriatic arthritis

- arthritis and psoriasis or
- arthritis and at least two of the following:
 - dactylitis
 - nail pitting or onycholysis
 - psoriasis in a first degree relative

Exclusions: b,c,d,e (see below)

TABLE 7.1 Frequency, Age at Onset, and Sex Distribution of the ILAR Categories of JIA

ILAR category	Frequency (%)	Onset age	Sex ratio
Systemic arthritis	4–17	Throughout childhood	F = M
Oligoarthritis	27–56	Early childhood; peak at 2–4 years	F >>> M
RF-positive polyarthritis	2–7	Late childhood or early adolescence	F >> M
RF-negative polyarthritis	11–28	Early peak at 2–4 years and late peak at 6–12 years	F >> M
Enthesitis related arthritis	3–11	Late childhood or adolescence	M >> F
Psoriatic arthritis	2–11	Early peak in preschool years and late peak at 9–11 years	F > M
Undifferentiated arthritis	11–21	–	–

RF, Rheumatoid factor.

Adapted from Ravelli and Martini [1].

TABLE 7.2 The International League of Associations for Rheumatology Classification of Juvenile Idiopathic Arthritis (Second Revision)—cont'd

Enthesitis-related arthritis

- arthritis and enthesitis
- arthritis or enthesitis with at least two of the following:
 - sacroiliac joint tenderness and/or inflammatory lumbosacral pain
 - presence of HLA-B27
 - onset of arthritis in a male after 6 years of age
 - ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative

Exclusions: a,d,e (see below)

Undifferentiated arthritis

Arthritis that does not fulfil inclusion criteria for any category, or is excluded by fulfilling criteria for more than one category

Exclusion criteria for the classification of JIA

- a. Psoriasis in the patient or a first-degree relative
- b. Arthritis in an HLA-B27-positive male with arthritis onset after 6 years of age
- c. Ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative
- d. Presence of IgM rheumatoid factor on at least two occasions more than 3 months apart
- e. Presence of systemic arthritis

HLA, Human leukocyte antigen; JIA, juvenile idiopathic arthritis; RF, rheumatoid factor. Adapted from Petty et al. [33].

is to identify homogeneous, mutually exclusive disease subsets based on clinical and laboratory features seen in the first 6 months of illness [33]. In recent years, the ILAR criteria have been subject to several criticisms, and many suggestions for their revision have been offered [34–45]. In particular, Martini proposed some refinements that would enable better identification of clinically homogeneous entities [31,43], and has recently promoted a multinational collaborative effort aimed at revising the JIA classification and nomenclature, which is underway.

6. CLINICAL MANIFESTATIONS

6.1 Systemic Arthritis

Systemic arthritis accounts for 5–15% of all cases of JIA seen in Western countries and is quite different from the other categories [4,46]. Its diagnosis by the ILAR criteria requires the presence of arthritis, accompanied or preceded by a documented quotidian fever of at least 2 weeks' duration, together with at least one of the following: typical rash, generalized lymphadenopathy, enlargement of liver or spleen, or serositis [33]. The fever is characteristically intermittent, with one or two daily spikes, during which the temperature reaches 39°C or more, followed by quick



FIGURE 7.1 Typical erythematous macular rash of systemic arthritis. Adapted from the personal collection of the authors.

TABLE 7.3 Differential Diagnosis of Systemic Juvenile Idiopathic Arthritis

Infections:

- Septicemia
- Bacterial endocarditis
- Brucellosis
- Typhoid fever
- Leishmaniasis
- Viral infections

Malignancy:

- Leukemia
- Lymphoma
- Neuroblastoma

Acute rheumatic fever

Connective tissue diseases:

- Systemic lupus erythematosus
- Kawasaki syndrome
- Systemic vasculitides

Inflammatory bowel disease

Castleman's disease

Sarcoidosis

Autoinflammatory syndromes

normalization. The rash usually accompanies the fever spikes and is erythematous, salmon pink, macular, and distinctly evanescent (Fig. 7.1). Arthritis is frequently symmetrical and polyarticular, and may be absent at presentation and occur during the disease course weeks, months, or, rarely, years after the onset of extra-articular symptoms. When articular manifestations are not yet present, the differential diagnosis is broad and can be challenging (Table 7.3). Laboratory studies reveal leukocytosis (with neutrophilia), increased erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), and thrombocytosis. Anemia is common and sometimes profound. High levels of fibrinogen and moderately augmented ferritin and D-dimer often coexist with systemic inflammatory activity.

6.2 Rheumatoid Factor-Negative Polyarthritis

Rheumatoid factor (RF)-negative polyarthritis is defined as an arthritis that affects five or more joints during the first 6 months of disease in the absence of IgM RF [33]. This category is regarded as the most heterogeneous of all JIA subsets, as it includes at least three distinct subgroups [1,3,47]. The first is very similar to early-onset oligoarthritis, except for the number of joints involved in the first 6 months of disease. Indeed, it is characterized by asymmetric arthritis, early age of onset, strong female prevalence, frequently positive ANA, increased risk of iridocyclitis, and association with human leukocyte antigen (HLA)-DRB1*0801. Patients with the second form have a clinical phenotype comparable to that of adults with RF-negative rheumatoid arthritis, and have symmetric arthritis of large and small joints, onset at a later age, increased ESR, negative ANA, and variable outcome (Fig. 7.2). The third condition, known as “dry synovitis,” displays scarce joint swelling but prominent stiffness, flexion contractures, and normal or slightly elevated ESR [48,49]. These patients often respond poorly to treatment and may experience a destructive course.

6.3 Rheumatoid Factor-Positive Polyarthritis

RF-positive polyarthritis is defined as arthritis cumulatively affecting five or more joints during the first 6 months of disease, in the presence of least two positive tests for IgM RF performed at least 3 months apart [33]. As stated above, this disease is the same as adult RF-positive rheumatoid arthritis [3,47]. The most typical extra-articular



FIGURE 7.2 Arthritis of the proximal interphalangeal joints in a boy with rheumatoid-factor negative polyarthritis. Adapted from the personal collection of the authors.

feature of this category is the rheumatoid nodules. They are usually located in the board of the olecranon and at other bony prominences and pressure areas, or flexor tendon sheaths, Achilles tendon, and soles of the feet, and are seen in around 30% of patients in the first year of disease.

6.4 Oligoarthritis

Oligoarthritis accounts for 50% to 80% of all children with JIA in Caucasian populations [50]. It predominantly involves the joints of the lower extremities, with the knee being most frequently affected, followed by the ankles (Fig. 7.3). It is defined as an arthritis that affects four or fewer joints during the first 6 months of disease. This subset is further categorized as either persistent, if arthritis remains restricted to four or fewer joints during the entire disease course, or extended, if arthritis spreads to more than four joints after the first 6 months of illness [33]. In the ILAR classification, children who fulfill these criteria are excluded from the oligoarthritis category if they have psoriasis, a family history of psoriasis, an HLA-B27-associated disease in a first degree relative, a positive RF test, or if they are boys older than 6 years [33]. Although oligoarthritis is likely heterogeneous, most patients with this condition have a well-defined illness, which is exclusive of children (as it is not seen in adults) and is characterized by asymmetric arthritis, an early onset (before 6 years of age), female predilection, high frequency of positive ANA, and elevated risk of iridocyclitis. The homogeneity of this subgroup of patients is reinforced by the strict association with particular HLA alleles [43].

Approximately 30% to 50% of cases present with monoarthritis, generally of the knee. The small joints of the fingers and toes as well as the wrists, elbows, and temporomandibular joints are affected in a few patients, whereas involvement of the hips and shoulders is rare. Except for chronic uveitis, extra-articular manifestations are distinctly unusual. Ocular involvement may occur in

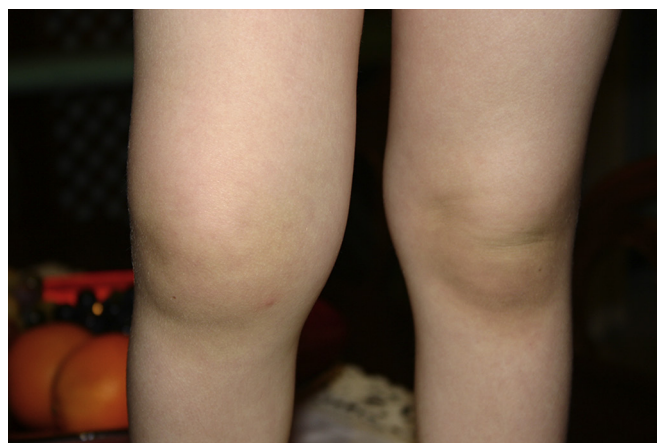


FIGURE 7.3 Swelling of the right knee in a girl with oligoarthritis. Adapted from the personal collection of the authors.

20% to 30% of cases and may cause irreversible damage, if not diagnosed or timely treated (Fig. 7.4). Acute phase reactants are frequently normal or slightly elevated, although ESR can be occasionally very high. ANA are detected in about 70% to 80% of patients, and constitute a risk factor for iridocyclitis.

6.5 Enthesitis-Related Arthritis

ERA mostly affects boys older than 6 years, and is characterized by the association of enthesitis and arthritis [33,51]. It is strongly linked to HLA-B27, whereas RF and ANA are absent. About half of the patients have an oligoarticular pattern of arthritis throughout the whole course of the illness. However, in many instances the disease eventually affects the joints of the axial skeleton, namely the sacroiliac and spinal joints, thus leading to the clinical picture of juvenile ankylosing spondylitis (JAS) (Fig. 7.5). Unlike ERA, radiographic demonstration of bilateral inflammation of the sacroiliac joints is needed for a definite diagnosis of this illness. However, although ERA in children is characterized by a greater frequency of involvement of nonaxial joints and enthesitis than adult-onset undifferentiated spondyloarthritis, it is thought to belong to the spectrum of spondyloarthritis [31].

6.6 Psoriatic Arthritis

The diagnosis of juvenile psoriatic arthritis (PsA) by the ILAR criteria requires the simultaneous presence of arthritis and a classic psoriatic rash (Fig. 7.6) or, if a rash is absent, the coexistence of arthritis and any two of the following: family history of psoriasis in a first-degree relative, dactylitis (sausage-like swelling of individual digits that extends beyond the joint margins), and nail pitting or onycholysis [33]. It was established that the diagnosis could be made in the presence of features suggesting a psoriatic diathesis,

when classic skin eruption is absent, due to the notion that skin disease lags behind arthritis in about half of children with psoriatic arthritis, sometimes by a decade or more [52]. Nowadays, it is increasingly argued that juvenile psoriatic arthritis does not constitute a homogeneous category and should not be treated as a single disease entity, as done in current classification schemes [53].

6.7 Undifferentiated Arthritis

Undifferentiated arthritis incorporates patients who do not meet the criteria for any category, or who fit the criteria for more than one. However, several analyses have shown that many patients who actually have a definite disease subtype could fall into this category [42,54]. Several proposals for revision of exclusion criteria to decrease the number of patients placed in the undifferentiated category have been presented [42,54,55], some of which have been integrated into the second revision of the ILAR classification [33].

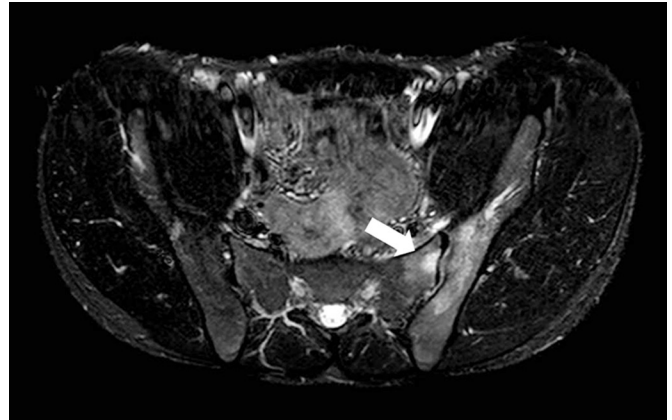


FIGURE 7.5 T1-weighted magnetic resonance image in a patient with juvenile spondyloarthritis showing active inflammation in the left sacroiliac joint (arrow). Adapted from the personal collection of the authors.

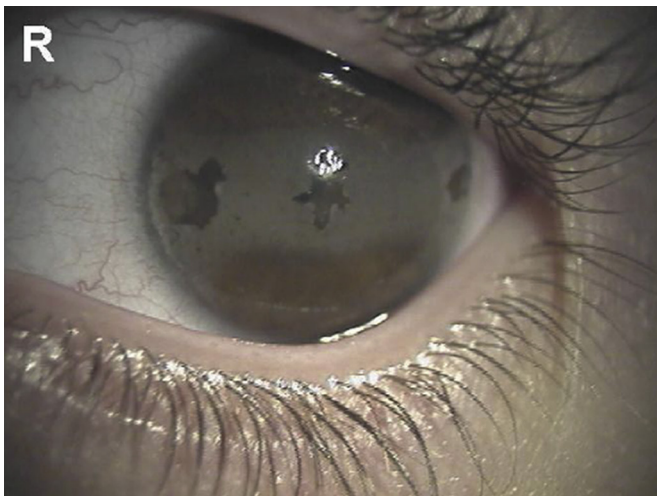


FIGURE 7.4 Band keratopathy in a girl with treatment-resistant chronic anterior uveitis. Adapted from the personal collection of the authors.



FIGURE 7.6 Psoriatic lesion in a boy with psoriatic arthritis. Adapted from the personal collection of the authors.

6.8 Macrophage Activation Syndrome

MAS is a serious and potentially fatal hyperinflammatory complication of systemic JIA [56–60]. It may occur in the absence of an identifiable trigger, generally in the course of active underlying disease, or be incited by an infection, a therapeutic modification, or a toxic effect of medications, including biologic response modifiers [58,60]. The syndrome is overt in about 10% of children with systemic JIA, but may occur subclinically in another 30–40% of cases [61,62].

The hallmark of MAS is an uncontrolled and dysfunctional immune response involving the unrestricted activation and expansion of T lymphocytes and macrophages, which leads to massive hypersecretion of proinflammatory cytokines [63,64]. The clinical picture is characterized by unremitting fever, pancytopenia, enlarged liver, spleen and lymph nodes, elevated liver enzymes, neurologic dysfunction, coagulation abnormalities, and a sharp increase in ferritin levels [65,66]. A characteristic feature is seen on bone marrow examination, which discloses, though not always, abundant morphologically benign macrophages exhibiting hemophagocytic activity (Fig. 7.7). Such cells may disseminate in many other organs, including lymph nodes, liver, and spleen. The sickest patients with MAS may develop multiorgan failure that may require admission to the intensive care unit and a very aggressive treatment. Table 7.4 shows the main clinical and laboratory features of MAS in systemic JIA. An international collaborative effort aimed at developing new classification criteria for the syndrome has been accomplished recently (Table 7.5) [67,68].

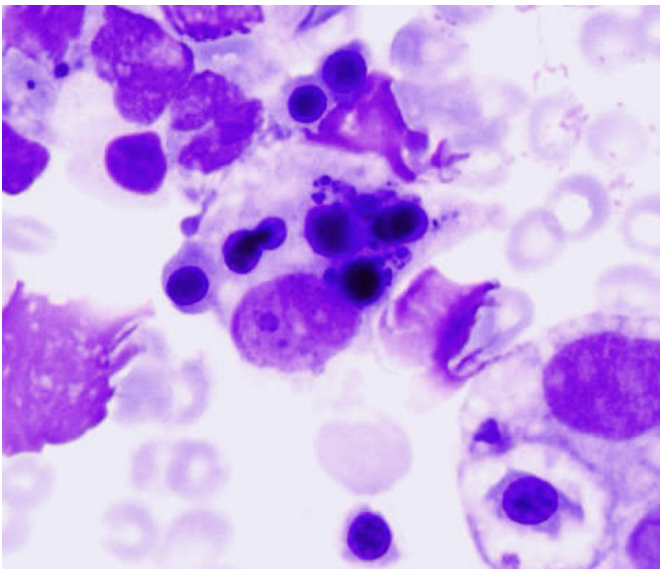


FIGURE 7.7 Bone marrow specimen showing macrophage hemophagocytosis in a patient with systemic arthritis and macrophage activation syndrome. Adapted from the personal collection of the authors.

TABLE 7.4 Main Features of Macrophage Activation Syndrome

Clinical features	
Unremitting fever	
Hepatomegaly	
Splenomegaly	
Lymphadenopathy	
Hemorrhages	
Central nervous system dysfunction	
Multiorgan failure	
Laboratory features	
Fall in white blood cell and platelet counts	
Abnormal liver function tests	
Decreased erythrocyte sedimentation rate	
Hypertriglyceridemia	
Hyponatremia	
Hypoalbuminemia	
Fall in fibrinogen	
Elevated D-dimers	
Prolonged prothrombin time and partial thromboplastin time	
Hyperferritinemia	
Elevated soluble CD25 and soluble CD163	
Decreased NK cell function	
Histopathological features	
Macrophage hemophagocytosis in the bone marrow	
Increased CD163 staining of the bone marrow	

TABLE 7.5 The Classification Criteria for MAS in Systemic JIA

A febrile patient with known or suspected systemic juvenile idiopathic arthritis is classified as having macrophage activation syndrome if the following criteria are met:	
Ferritin >684 ng/mL	
and any 2 of the following:	
Platelet count $\leq 181 \times 10^9/L$	
Aspartate aminotransferase >48 units/L	
Triglycerides >156 mg/dL	
Fibrinogen ≤ 360 mg/dL	

Laboratory abnormalities should not be otherwise explained by the patient’s condition, such as concomitant immune-mediated thrombocytopenia, infectious hepatitis, visceral leishmaniasis, or familial hyperlipidemia. Adapted from Ravelli et al. [68].

7. CARDIAC INVOLVEMENT IN JIA

Cardiac involvement in the form of pericarditis, pericardial effusion, myocarditis, and valvular disease is well documented in JIA. Additionally, increased blood pressure, heart rate, left ventricular dimensions and decreased left ventricular systolic, and diastolic function have been described. However, the frequency of overt heart disease is widely variable across subtypes: it is most common in systemic arthritis and its major complication, MAS, can occur occasionally in RF-positive polyarthritis, ERA, and JAS, but is seen only exceptionally in the other disease categories.

7.1 Pericarditis and Myocarditis

Pericarditis, with or without pleural effusion, is a characteristic of systemic arthritis. Although the estimated overall prevalence of pericardial involvement in JIA is 3–9%, this complication is seen almost exclusively in the systemic form [69]. Pericardial disease tends to occur in older children, but it is not related to sex, age at onset, or severity of joint disease [4]. It may precede occurrence of arthritis or develop at any time during the disease course, and is usually accompanied by systemic exacerbation. Most pericardial effusions are mild and asymptomatic. An enlarged retropericardial space, detected by echocardiography, is often the only feature of pericardial inflammation. Occasionally, however, the pericardial effusion is large (Figs. 7.8 and 7.9) and symptomatic, requiring corticosteroid therapy for its control. Pericardial tamponade requiring drainage occurs rarely. Chronic constrictive pericarditis is exceptional. The presence of pericarditis does not represent a

poor prognostic sign, as children with this complication do not have a worse outcome than others.

Myocarditis is much less common than pericarditis, although the two conditions can coexist. It generally responds well to corticosteroids. Myocardial disease causing progressive heart failure is a very uncommon life-threatening complication [70–72]. A small number of cases of endocarditis leading to aortic insufficiency have been reported in patients with systemic JIA, typically occurring years after the onset of the underlying disease [73,74]. A good outcome was reported after placement of a prosthetic valve in one of these patients.

Cardiac involvement was diagnosed in 15 of 320 cases of JIA (4.7%) by Svantesson et al. [75] and was most common in the systemic subtype. Ten children had pericarditis, two had myocarditis, two had perimyocarditis, and one had aortic valvulitis. Heart disease was found to occur most frequently in the first 3 years after disease onset. The prognosis was good for patients with pericarditis, as none developed cardiac tamponade or constrictive pericarditis and all had normal cardiac function on echocardiography at follow-up. Patients with myocarditis and perimyocarditis had a worse outlook, as two of four had dilated left ventricle and one died.

In a retrospective study of 172 patients with JIA, Goldenberg and coworkers [70] found that symptomatic cardiac involvement occurred in 13 (7.6%) patients, 11 with systemic arthritis, and 2 with polyarthritis. Seven patients had pericarditis, four had perimyocarditis, and two had myocarditis. Cardiac involvement was associated with worse prognosis as four of the 13 children with symptomatic carditis died versus only one child in the group without cardiac disease. Of the four children with cardiac involvement and fatal outcome, one had pericarditis with cardiac tamponade and three had myocarditis.

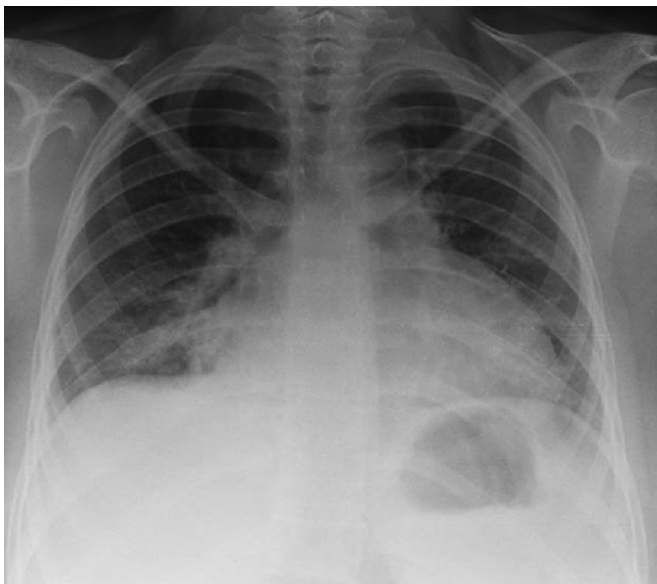


FIGURE 7.8 Chest radiograph showing enlarged cardiac silhouette in a patient with systemic arthritis and pericarditis. Adapted from the personal collection of the authors.

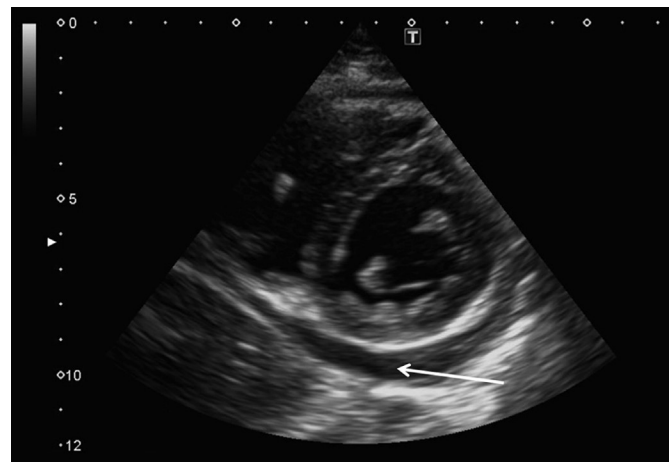


FIGURE 7.9 Pericardial effusion (arrow) on echocardiography in a patient with systemic arthritis. Adapted from the personal collection of the authors.

Myocarditis was seen in the background of severe, active systemic disease in all three children reported by Miller and French [71]. The complication presented with development of cardiomegaly or congestive heart failure or both in the absence of significant pericardiac effusion or extra-cardiac cause. High-dose corticosteroid therapy was rapidly effective in controlling the acute phase, but one child died after two months of treatment. A 7-year-old boy with systemic JIA who had dilated cardiomyopathy and was successfully treated in the acute phase with a combination of prednisone, methotrexate, and digitalis has been reported [76].

A favorable outcome of pericarditis with cardiac tamponade was described by Yancey and associates [77] in three patients, all of whom with systemic JIA. Pericardiocentesis was performed in two cases. Treatment was based on short-acting anti-inflammatory drugs and/or prednisone and no patient had significant morbidity after a mean follow-up of 2 years. In a 9-year-old boy with systemic JIA and pericarditis, the rapid worsening of cardiac tamponade in spite of corticosteroid treatment and pericardiocentesis required the emergency placement of a pericardial window [78].

7.2 Valvular Heart Disease

A few pediatric patients with RF-positive polyarthritis who developed valvular heart disease have been described [79–81]. Aortic insufficiency was observed most frequently. Patients with this complication may present with abrupt onset of congestive heart failure or experience sudden worsening after a variable period of stability following the detection of a cardiac murmur. Valve replacement is almost always required. Cardiac involvement may be discovered at intervals varying from 4 to 17 years from onset of JIA. However, pathologic murmurs may be noticed as early as 1 year after disease presentation. Excised aortic valves of children with this complication are grossly thickened. Granulomatous, nodular lesions are often found on the valve cusps. Histological findings include destruction of the normal architecture of the valve, granulomas that are histopathologically similar to rheumatoid nodules, nonspecific inflammatory changes, and fibrosis [47]. These reports indicate that all patients with RF-positive JIA who have organic cardiac murmurs should be evaluated for valvular insufficiency and monitored carefully.

Aubert and coworkers [82] reported a girl with polyarticular JIA since the age of 8 years who underwent surgery for severe mitral valve insufficiency 16 years later. At operation, fibrotic endocarditis involving mainly the posterior valvular leaflet and granulomatous vegetations associated with a large thrombus filling the left ventricular apex were found. After valvular repair, a flare of systemic

inflammation occurred, which was accompanied by a marked pericardial effusion that required surgical drainage. The follow-up was uneventful, with no cardiac symptoms and normal valve on echocardiography 8 years later.

In adult patients with ankylosing spondylitis, a variety of cardiovascular manifestations have been described, including aortitis and subvalvular bumps, aortic regurgitation, left ventricular outflow tract irregularities, diastolic left ventricular myocardial dysfunction, and bradyarrhythmias due to conduction disturbances [83]. Overall, cardiac disease develops in approximately 5% of adults with ankylosing spondylitis an average of 15 years after disease onset. Rarely, cardiac disease precedes occurrence of sacroiliac involvement [51]. Although cardiovascular disease has been reported much less frequently in children with spondyloarthropathies, perhaps due to the generally shorter follow-up than in adults, it can occasionally be severe. Marked aortic insufficiency has been described in at least seven patients with JAS [51]. In addition, the occurrence of heart valvular involvement in children with B27-positive peripheral arthritis enthesitis without back pain or radiographic evidence of sacroiliitis (ie, meeting the ILAR criteria for ERA) has been observed [84]. By comparing 40 patients with HLA-B27-positive JIA (only one of whom with ankylosing spondylitis) and age- and sex-matched HLA-B27-negative controls, Huppertz and coworkers [83] found that four patients and no control subjects had echocardiographic evidence of inflammatory aortic regurgitation. In addition, late diastolic flow velocity was significantly increased in patients at the termination of a bicycle exercise. Thus careful cardiac surveillance is mandatory in pediatric patients meeting the criteria for either ERA or ankylosing spondylitis.

However, clinically significant heart disease in ERA should be considered rare as in a recent study none of the 101 Indian patients with this condition was found to have symptomatic cardiac involvement. This finding led the authors to conclude that a modification of the extra-articular section of the Juvenile Arthritis Damage Index [85,86] to include evaluation of cardiac damage may not result in additional advantage for use of this tool in children with ERA [87]. In another study, none of 36 consecutive patients with JAS who were monitored for a mean of 4.3 years had symptoms related to the cardiovascular system, and only one developed the murmur of aortic regurgitation. Ecocardiography revealed no structural cardiac abnormalities and electrocardiography no conduction defects, but in a few patients color Doppler assessment showed mitral or aortic regurgitation, which was, however, mild in all cases. Systolic ventricular function was impaired in one patient [88].

Individual cases of valvular heart disease, leading to either aortic or mitral insufficiency, have been reported

in children with oligoarthritis [89,90] or RF-negative polyarthritis [91]. Although a coincidental association between the two conditions or a previously unnoticed rheumatic carditis could not be excluded, the absence of clinical or pathologic evidence or risk factors for endocarditis or rheumatic fever, the lack of prior clinical signs or symptoms consistent with antecedent structural valve disease, the histologic findings, and the rapid progression of valvular insufficiency suggested that valvular involvement was related to the JIA process.

7.3 Coronary Artery Abnormalities

The development of coronary artery abnormalities is the most worrisome complication of Kawasaki disease (KD). The detection of signs of coronary artery involvement on echocardiography in a child with a febrile inflammatory illness would typically favor the diagnosis of KD over that of systemic JIA. However, several patients with classical systemic JIA who had coronary artery dilation similar to that observed for children with KD have been reported. Some of these patients were initially misdiagnosed as KD [92,93]. These data suggest that the finding of coronary artery dilation on echocardiograms in a febrile child do not exclude the diagnosis of systemic JIA.

7.4 Cardiac Involvement in MAS

The characteristics of a large multinational series of patients with systemic JIA-associated MAS have recently been described [65,66]. Cardiac manifestations were reported in 90/353 (25.5%) of patients. The most common form of heart disease was pericardial involvement (16.1%), followed by arrhythmia (1.4%), heart failure (1.1%), and cardiomegaly (1.1%).

Recently, Kimura and coworkers [94] described the characteristics of 25 patients with systemic JIA who developed pulmonary arterial hypertension and other potentially fatal pulmonary complications (interstitial lung disease and alveolar proteinosis). Twenty of these patients (80%) developed MAS during their disease course and 15 (60%) had MAS at pulmonary diagnosis. Seventeen patients (68%) were taking or had recently (<1 month) discontinued a biologic agent at pulmonary symptom onset, and 12 patients (48%) were receiving anti-IL-1 therapy, primarily with anakinra. The mortality rate was 68%. The authors concluded that pulmonary arterial hypertension and other observed pulmonary complications are under-recognized complications of systemic JIA that are frequently fatal. The strong relation of these manifestations with MAS and administration or discontinuation of biologic medications led them to suggest that they could result from severe uncontrolled disease and be influenced by medication exposure.

8. STUDIES OF VENTRICULAR FUNCTION

In recent years, a number of studies have investigated systolic and diastolic ventricular function in children with JIA. Using M-mode and pulsed-wave Doppler echocardiography, Oguz and colleagues [95] found that JIA patients without cardiac symptoms had larger left ventricular end-systolic diameter and volume and decreased ejection fraction and fractional shortening compared to sex- and age-matched healthy controls. These findings, together with the observation of increase in heart rate, were interpreted as potential initial signs of myocardial functional deterioration. The JIA group also had increased late flow velocity, decreased early flow velocity, and prolonged isovolumic relaxation time, reflecting an abnormal relaxation form of diastolic dysfunction. The authors speculated that these diastolic changes could be due to a myocardial fibrotic process.

In another study, Alkady and coworkers [96] reported that children with chronic arthritis and no cardiac symptom had increased systolic and diastolic blood pressure, resting heart rate, and left ventricle systolic size and volume. On Doppler and tissue Doppler echocardiography, the JIA sample was found to have lower peak early filling velocity (E), higher peak atrial filling velocity (A), and prolonged diastolic E and A waves deceleration times and isovolumic relaxation time compared to healthy controls. These findings confirmed that asymptomatic children with JIA had significant systolic and diastolic functional abnormalities.

Koca et al. [97] did not find differences in QT dispersion, a simple noninvasive arrhythmogenic marker that enables evaluating the homogeneity of cardiac repolarization between JIA patients and healthy children. Unlike above studies, they did not observe clear signs of systolic dysfunction of the left ventricle. However, the JIA cohort had serious abnormalities in diastolic function, with decreased E and increased A, lower E/A ratio, and prolonged isovolumic relaxation time. The presence of impairment of diastolic function of the left ventricle, assessed by both conventional Doppler echocardiography and tissue Doppler imaging, in the absence of clinical signs of cardiac disease, was subsequently confirmed by the same group of investigators in 45 children with active JIA [98].

The function of the right ventricle was the focus of the analysis of Abul and associates [99]. By means of conventional echocardiography and Doppler imaging measurements, they found that peak systolic, early, and late diastolic tricuspid annular velocities were significantly lower in JIA patients than in control population. Isovolumetric acceleration, an indicator of myocardial acceleration during isovolumic contraction of the right ventricle, was also diminished in

the JIA sample. These findings added to the previous observations of left ventricular dysfunction by showing that the right ventricle may also be affected.

P-wave dispersion and its relation with diastolic dysfunction of the left ventricle were investigated by means of electrocardiography and Doppler echocardiography in JIA patients and healthy controls. No difference was seen in minimum, maximum P-wave duration, and P-wave dispersion between patients and controls. Augmented late flow velocity, decreased early flow velocity and prolonged isovolumic relaxation time suggested the presence of diastolic dysfunction in JIA patients. Because no case of supraventricular arrhythmia was seen in patients with diastolic dysfunction and patients with such dysfunction had normal atrial conduction parameters, the observed data did not indicate that JIA patients have an increased risk of atrial fibrillation [100].

Bharti and associates [101] found evidence of significant systolic and diastolic functional abnormalities in patients with JIA, despite an asymptomatic cardiac status. By means of two-dimensional and Doppler echocardiography, they assessed left ventricular systolic and diastolic function in 35 patients with JIA and an equal sample of age- and sex-matched controls. JIA patients had higher systolic and diastolic blood pressure, resting heart rates, left ventricular systolic and diastolic size and volumes as compared to controls. Ejection fraction and fractional shortening (FS) were normal in JIA patients, but lower than in controls. Doppler studies revealed lower peak E velocity, higher peak A velocity, higher A wave velocity time integral (A VTI), and more prolonged isovolumetric relaxation time (IVRT) in the JIA population. A VTI and IVRT were higher in males than in females. Diastolic and systolic dimension was larger and FS was lower in patients with longer disease duration, and E and A VTI were higher in the polyarticular disease subset than in the systemic and polyarticular categories.

Aulie and coworkers [102] investigated cardiac function in 85 adults with JIA who had a median disease duration of 29 years and had been in an active disease status for at least 15 years. The influence of inflammation, disease severity, and antirheumatic drug exposure was also evaluated. Patient findings were compared with those obtained in 46 matched controls. Study investigations included echocardiography, including tissue Doppler imaging and longitudinal peak-systolic global strain, and 12-channel electrocardiography. Patients had a thicker interventricular septum and altered diastolic function compared to controls. Abnormal diastolic function was characterized by lower mitral E-wave deceleration time, higher surrogate marker of left ventricular pressure, and larger left atrial area. Systolic and diastolic blood pressure were higher in patients, whereas QT corrected interval was similar in patients and controls. Worse clinical and laboratory disease severity parameters were associated with higher lateral E/e'.

In a similar patient and control population, the same investigators measured arterial hemodynamics by assessing the arterial stiffness markers pulse wave velocity (PWW) and augmentation index (Aix) through sphygmocor, and searched for coronary calcification using CT scan. Significantly higher PWW and systolic and diastolic blood pressure was observed in patients compared to controls. In spite of higher numerical value in patients, this parameter was not statistically different between patients and controls. Coronary calcification was seen in 26% of patients. Patients also had higher insulin resistance than controls. Disease-related variables as well as traditional cardiovascular risk factors were associated with higher Aix, diastolic blood pressure, and presence of coronary calcification. However, disease-related variables were not among the determinants of PWW. This parameter was mostly associated with increased diastolic blood pressure [103].

Altogether the results of these studies may lead to conclude that the impairment in the systolic and diastolic function of the left and right ventricles places clinically asymptomatic children with JIA at risk for ischemic heart disease or cardiomyopathy in the future. However, the significance of these findings is unclear as there is currently no evidence that JIA patients have an increased prevalence of such complications compared to the general pediatric population. Note that some caveats should be taken into account when interpreting the above observations. For example, all studies are cross-sectional and children were seldom stratified by onset subtype, age, sex, disease duration, disease activity or severity, and ongoing therapy. Well-designed longitudinal investigations are needed to ascertain the long-term impact of the reported abnormalities of ventricular function in children, adolescents, and young adults with JIA.

The findings of the studies of systolic and diastolic ventricular function in children with JIA are summarized in Table 7.6. The values of systolic and diastolic blood pressure and heart rate are reported in Table 7.7.

9. STUDIES OF ARTERIAL STRUCTURE AND FUNCTION AND ATHEROSCLEROTIC BIOMARKERS

Atherosclerosis is considered an inflammatory disorder starting in childhood. It has been suggested that chronic inflammation plays a major role in the development of early atherosclerosis, which may lead to an increased risk of acute cardiovascular events in young adulthood [104–106]. In adult patients with rheumatoid arthritis, cardiovascular disease is more frequent and is diagnosed earlier than in the general population [107,108]. Recent studies have shown that these patients have increased aortic stiffness, which is correlated with

markers of inflammation and aortic inflammation in the absence of overt signs of cardiovascular disease [109,110].

Because JIA is a chronic inflammatory disease, it has long-term effects on many organs and systems, including the cardiovascular system. As a result, children with this disease may be at risk of experiencing premature cardiovascular accidents [111]. In the past few years, a few studies of vascular function and structure and atherosclerosis-related biomarkers have been conducted in patients with JIA.

Vlahos and coworkers [112] evaluated endothelial function by means of brachial artery flow-mediated dilation (FMD), carotid intima-media thickness (IMT), and arterial stiffness in 30 patients with JIA and 33 age- and sex-matched controls. They found that JIA patients as a whole had decreased FMD compared to controls, independent of age, and that patients with systemic disease had greater IMT than patients with oligoarthritis, polyarthritis, or controls. The latter finding was attributed to the effects of the following risk factors: age, body mass index, blood pressure, disease activity, and corticosteroid use. There was no difference in arterial stiffness

between JIA patients and controls or between patients with systemic versus nonsystemic disease.

Inflammatory biomarkers, proinflammatory cytokines, lipid profile, and antioxidant status as well as carotid IMT and blood pressure were measured by Breda et al. [113] in 38 prepubertal children with JIA and 40 healthy controls. All parameters were reassessed in JIA patients after 1 year. At baseline, JIA patients had higher levels of inflammatory biomarkers, proinflammatory cytokines, total and LDL cholesterol, and urinary isoprostanes. Carotid IMT and blood pressure were also increased compared to controls. After 1 year of treatment with nonsteroidal anti-inflammatory drugs, conventional disease-modifying antirheumatic drugs (DMARDs), or etanercept, a significant reduction of all laboratory parameters and a decrease in blood pressure and carotid IMT were detected.

Satija and colleagues [114] assessed endothelial function (through flow-mediated and glyceryl trinitrate-mediated dilation of the brachial artery), arterial stiffness (by deriving the following arterial wall mechanic parameters: cross-sectional compliance and distensibility, shear stress and elastic modulus), and carotid IMT in 31 children with JIA and a similar number of controls. A tendency toward reduced cross-sectional compliance and distensibility and increased diastolic wall shear stress and elastic modulus was documented in patients versus controls, whereas endothelial function and carotid IMT were comparable. Cross-sectional compliance was significantly lower in patients with systemic arthritis than in controls.

To assess changes in the variables of aortic elasticity, phase contrast MR was performed in 31 patients with JIA (18 with oligoarthritis, 7 with systemic arthritis, and 6 with polyarthritis) and 28 healthy subjects. Aortic distensibility was lower and pulse-wave velocity (PWV) was higher in patients than in controls. In both patients and controls, PWV was positively correlated with age, whereas distensibility was negatively correlated with age. There was no association between distensibility and PWV and metabolic and disease activity parameters, and no differences were found between the three JIA subtypes [115].

Ilsson and associates [116] determined carotid IMT, carotid-femoral pulse wave velocity and adjusted augmentation index in 39 newly diagnosed JIA patients and 27 healthy controls. In addition, they measured the serum

TABLE 7.6 Summary of Studies of Systolic and Diastolic Ventricular Function in Children With JIA^a

First author, year, reference	Oguz, 2000 [95]	Alkady, 2012 [96]	Koca, 2012 [97]	Koca, 2014 [98]
LVEDD (cm)	4.0±0.6	4.3±0.7	3.9±0.6	–
LVEDS (cm)	2.6±0.5	2.8±0.4	2.4±4.4	–
EF (%)	64.6±7.4	58.6±4.9	64.5±7.2	69.9±5.1
FS (%)	34.9±5.3	31.2±2.4	34.8±5.2	38.6±3.9
LVEDV (mL)	71.6±24.0	92.7±17.7	–	–
LVESV (mL)	26.0±13.0	33.4±6.7	–	–
IVRT (ms)	58.0±18.0	93.5±15.0	94.0±15.2	62.7±11.5
E (m/s)	0.9±0.1	0.9±0.1	0.9±0.1	0.8±0.2
A (m/s)	0.7±0.1	0.7±0.1	0.7±0.1	0.8±0.2

LVEDD, Left ventricular end diastolic diameter; LVEDS, left ventricular end systolic diameter; EF, ejection fraction; FS, fractional shortening; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume; IVRT, isovolumic relaxation time; E, peak early diastolic flow velocity; A, peak late diastolic flow velocity.

^aValues are the mean ± standard deviation.

TABLE 7.7 Systolic and Diastolic Blood Pressure and Heart Rate in Studies of Children With JIA^a

First author, year, reference	Vlahos, 2011 [112]	Breda, 2013 [113]	Ilsson, 2015 [116]	Oguz, 2000 [95]	Alkady, 2012 [96]	Koca, 2012 [97]	Koca, 2015 [98]
Systolic BP (mmHg)	109.0±11.0	105.1±6.9	112.2±8.7	104.3±7.0	113.5±8.3	103.3±12.5	110.0±12.0
Diastolic BP (mmHg)	58.0±7.0	66.0±4.6	57.6±5.0	64.1±12.2	72.8±7.0	69.2±8.8	73.0±7.5
Heart rate (beats/min)				99.8±14.7	85.3±9.2	89.5±17.7	78.9±11.6

^aValues are the mean ± standard deviation.

levels of atherosclerosis-related biomarkers, including asymmetric dimethylarginine, myeloperoxidase, and adiponectin. Patients had increased mean IMT and myeloperoxidase levels compared to controls. Furthermore, the negative correlation between adiponectin and adjusted augmentation index suggested that the levels of this biomarker may influence arterial subclinical stiffening.

A study of microcirculation by means of nailfold videocapillaroscopy with computer-associated image analysis in 43 children with JIA and 20 healthy children showed wider and longer capillaries in JIA patients than in healthy controls. Children with JIA also had more commonly irregular capillaries and dilated subpapillary venous plexus. Serum levels of soluble intracellular adhesion molecule (sICAM) and vascular endothelial growth factor (VEGF) were significantly higher in JIA patients with capillary abnormalities than in those with normal capillaroscopy. These findings indicate that children with JIA have structural changes in the microcirculation, which may reflect endothelial injury. Whether capillaroscopic changes predict the risk of early atherosclerosis remains unclear [117].

Glowinska-Olzevska and coworkers [118] investigated the influence of obesity on the early, subclinical changes in the cardiovascular system. Thirteen (22%) of their 58 JIA patients were obese and had increased systolic and diastolic blood pressure, cholesterol, triglycerides, insulin, insulin resistance, hsCRP, and IL-6 compared to nonobese patients and 36 healthy controls. Obese JIA patients also had decreased flow-mediated dilation and increased IMT, accompanied by augmented left ventricle mass index. TNF α , standard deviation score-body mass index, and systolic blood pressure were independent predictors of early cardiovascular changes in the JIA cohort. The authors concluded that obesity in JIA is associated with insulin resistance, dyslipidemia, impaired endothelial function, and increased levels of inflammatory parameters, which supports the evidence of subclinical changes in the cardiovascular system predisposing to early development of clinically symptomatic atherosclerosis. Thus, the coexistence of JIA and obesity may considerably increase the cardiovascular risk.

Taken together, the results of these studies point toward the presence of endothelial dysfunction and of alterations in vascular function and structure in children with JIA, even at earlier disease stages and prepubertal age. These findings imply that this disease may be associated with an increased risk for premature atherosclerosis and early cardiovascular events. However, a cautionary note should be introduced as the carotid IMT, which is regarded as a highly sensitive and accurate indicator of subclinical atherosclerosis [119,120], was not found to be thicker than in controls in all studies, and in the study of Vlahos and coworkers [112] it was increased only in the sample with systemic arthritis. It is, nevertheless, reassuring that Breda et al. [113] reported an improvement of the IMT as well as of the atherosclerotic biomarkers after 1 year of effective

TABLE 7.8 Values of Atherosclerosis-Related Parameters in Children With JIA^a

First author, year, reference	Vlahos, 2011 [112]	Breda, 2013 [113]	Illisson, 2015 [116]
IMT (mm)	0.56 \pm 0.03	0.38 \pm 0.05	0.46 \pm 0.04
FMD (%)	7.14 \pm 2.35	–	–
Total cholesterol (mg/dL)	169.5 \pm 27.6	164.0 \pm 29.0	–
HDL cholesterol (mg/dL)	50.5 \pm 10.5	51.0 \pm 12.0	–
LDL cholesterol (mg/dL)	104.9 \pm 23.2	98.0 \pm 28.0	–
Triglycerides (mg/dL)	70.7 \pm 23.9	71.0 \pm 22.0	–
ESR (mm/h)	19.0 \pm 21.0	43.0 \pm 20.0	–

IMT, Intima media thickness; FMD, Flow-mediated dilatation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ESR, erythrocyte sedimentation rate.

^aValues are the mean \pm standard deviation.

therapy. This observation underscores the importance of early and adequate treatment to control the possibility of future cardiovascular events. Further investigations are needed to clarify the risk of early atherosclerosis related to JIA and to identify children who deserve a closer monitoring. Nevertheless, the findings of the above studies underscore the importance of identifying and managing the modifiable cardiovascular risk factors (dyslipidemia, hypertension, obesity, smoking, and low levels of physical exercise) in daily clinical practice. The evidence regarding the potential risk of early atherosclerosis in patients with JIA has recently been reviewed [121].

Table 7.8 shows the values of atherosclerosis-related parameters found in the reported studies.

10. CARDIOPULMONARY EXERCISE TESTING

Children with JIA are considered less physically fit than their healthy peers. Reduced aerobic exercise capacity has been documented in pediatric patients with chronic arthritides [122,123]. This phenomenon may contribute, together with chronic joint pain, stiffness, deformity, and muscle hypotrophy, to impair physical activity and may lead to an unsatisfactory lifestyle.

Metin et al. [124] assessed aerobic fitness, determined by measuring peak power and peak oxygen uptake (VO_{2peak}) during an incremental cycling test, in 24 patients with JIA and 21 healthy sedentary volunteers. All subjects tolerated maximal exercise well, but the JIA cohort had lower anaerobic fitness than controls. There was no difference in cardiopulmonary measures among JIA categories, but the ERA group had higher aerobic capacity than the other subgroups. The exercise capacity of patients with active disease or remission was comparable, suggesting that physical activity and exercise should be encouraged irrespective of the stage of disease activity.

A case-comparison study of the response to bicycle ergometer exercise between 30 children with JIA and 30 age-, sex-, and body surface area-matched healthy controls showed that JIA patients had lower VO_{2peak} , highest work load completed, exercise duration and peak heart rate, and higher submaximal heart rate than their healthy peers. No difference was seen between the two groups in resting heart rate, and there was no relationship between VO_{2peak} and severity of joint disease among children with JIA. These results suggest that aerobic conditioning programs may be indicated soon after diagnosis in children with chronic arthritis, regardless of the severity of their articular disease [125].

In 18 children with JIA, the physiologic response of the 6-min walk test was found to be, on average, 80% and 85% of the peak values of heart rate and oxygen uptake (VO_{2peak}) during the maximal exercise test, except for the minute ventilation, which had a mean percentage of 68.5%. Height and distance walked were the best predictors of VO_{2peak} during cycling on backward regression analysis. These findings indicate that the physiologic response of the 6-min walk test is at submaximal, intense level of exercise. Normative values of the test for children should be established to define the thresholds of functional exercise capacity that should be aimed for by treatment interventions [126].

11. GENERAL MANAGEMENT OF JIA

The management of juvenile idiopathic arthritis (JIA) is centered on a combination of pharmacologic interventions, physical and occupational therapy, and psychosocial support [1–3]. The objective of treatment should be to achieve disease remission, relieve pain, promote normal nutrition and growth, preserve the quality of life, and prevent long-term damage due to the disease or its therapy. Although medications that are able to cure the disease do not exist, prognosis has markedly improved in recent years due to important progress in therapy. These advances have increased the expectation for disease control [127–130]. It is increasingly realized that there is a window of opportunity to most effectively treat JIA, and accumulating evidence supports the advantage of early aggressive therapy [46,131–134].

The management of a child with JIA mandates the creation of a multidisciplinary team including a pediatric rheumatologist, ophthalmologist, orthopedic surgeon, specialist nurse, physical therapist, occupational therapist, and psychologist [1]. Patient and family education and involvement of parents and children in decision making are fundamental to fostering adherence to therapeutic prescriptions and to ensuring the success of management [135].

Because JIA is not a single disease, treatment regimens must take into account the diversities of subtypes [136–138]. However, a rational therapeutic strategy is

hindered by the incapacity to differentiate early in the disease course children who will pursue a benign and self-remitting course from children who will experience continuous disease activity with high risk of joint damage and functional disability.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have historically represented the cornerstone of treatment for all JIA subsets. Their role remains important, and most children are started on one of these medications. A 4- to 6-week course of a particular NSAID is necessary to evaluate its efficacy. However, their use as monotherapy for more than 2 months is discouraged if arthritis does not remit [139]. As NSAIDs are not disease modifying, they are administered to treat pain, stiffness, and the fever of systemic arthritis. Only a small number of NSAIDs are approved for use in children: the most commonly prescribed are naproxen (*Class I; Level of Evidence A*), ibuprofen (*Class I; Level of Evidence B*), and indomethacin (*Class I; Level of Evidence B*). Experience with cyclo-oxygenase (COX)-2 inhibitors in the pediatric age-range is limited [140,141].

Intra-articular corticosteroid (IAC) injections are widely performed to obtain quick improvement of inflammatory symptoms, to restore function, and to obviate the need for continued systemic therapy [142–144]. In patients with monoarthritis or oligoarthritis they can be used with, or in substitution of, NSAIDs. Although potentially efficacious on all forms of JIA, IAC injections are used most frequently for the management of oligoarthritis. This therapy may prevent some peculiar musculoskeletal abnormalities seen in this JIA subset, namely flexion contractures, valgus deformity, and leg-length inequality. In addition, IAC therapy has been shown to allow discontinuation of oral medications, heal Baker's cysts, and improve tenosynovitis [145].

The strategy of performing multiple IAC injections is advocated by some pediatric rheumatologists in children with polyarthritis to achieve rapid and generalized remission of synovitis, while concurrently starting systemic therapy with DMARDs and/or a biologic agent [146,147]. This intervention is viewed as an alternative to systemic corticosteroids to aim for the so-called bridge effect, which means to attain a quick resolution of inflammatory symptoms while waiting for the complete therapeutic action of a DMARD or biologic medication. Triamcinolone hexacetonide (TH), the least soluble agent, is regarded as the medication of choice for intra-articular therapy in JIA (*Class I; Level of Evidence A*).

Systemic corticosteroids are essentially reserved for the management of the extra-articular manifestations of systemic arthritis, particularly fever, anemia, pericarditis or myocarditis, and MAS (*Class I; Level of Evidence C*). In the nonsystemic subtypes of JIA, these drugs should be used with discernment because their potential side effects, particularly growth failure and osteoporosis, may prevail over any benefits to articular disease.

A brief course of low-dose prednisone (eg, 0.5 mg/kg/day) may be considered for improving pain and stiffness in patients with severe polyarthritis resistant to other therapies or while awaiting the full therapeutic effect of a recently initiated DMARD or biologic agent (*Class IIa; Level of Evidence A*). There is no evidence that systemic corticosteroids have a disease-modifying effect in childhood arthritis. The indications of corticosteroid therapy in JIA have been recently reviewed [148].

Methotrexate (MTX) is the most widely used synthetic DMARD in the management of JIA due to its satisfactory effectiveness and acceptable safety profile (*Class I; Level of Evidence A*) [149,150]. Therapeutic response is usually seen after 6–12 weeks. The efficacy of MTX was established in a randomized trial in 1992 at a dose of 10 mg/m² per week given orally [151]. A subsequent controlled study showed that the maximum benefit is obtained with the parenteral administration of 15 mg/m² per week. No additional advantage was seen when higher doses, up to 30 mg/m²/week, were used [152]. MTX can be given both orally and subcutaneously. The supplementation of folic or folinic acid may help prevent the occurrence of liver enzyme abnormalities, oral ulcerations, and nausea [153].

Leflunomide may have comparable efficacy and safety as MTX and is, thus, an alternative option for patients refractory or intolerant to MTX (*Class I; Level of Evidence B*) [154]. Current guidelines support the use of sulfasalazine in ERA, but not in other categories of JIA (*Class IIa; Level of Evidence B*) [139,155]. No controlled studies of cyclosporin A in JIA are available. Anecdotal experience suggests that this medication can be effective in patients who are resistant to MTX [156,157]. In systemic JIA, cyclosporin A may be more beneficial for controlling fever than for the treatment of arthritis and may facilitate corticosteroid tapering (*Class IIb; Level of Evidence C*). Thalidomide has been found to be efficacious in unresponsive systemic arthritis, both for systemic features and arthritis (*Class IIb; Level of Evidence C*) [158]. No significant toxicities were registered. However, use of this medication requires careful surveillance of the teratogenic effect and constant monitoring of development of peripheral neuropathy.

In the last 15 years, the management of JIA has been revolutionized by the introduction of biologic agents, which have offered a powerful therapeutic option for the treatment of patients who do not respond to traditional antirheumatic drugs, particularly MTX or sulfasalazine [159]. These compounds have been designed to target proinflammatory cytokines implicated in the pathogenesis of the disease, including tumor necrosis factor (TNF), interleukin (IL)-1, and IL-6 as well as signaling molecules involved in the regulation of B-cell and T-cell responses. Recent controlled clinical trials, most of which were conducted by means of the randomized

withdrawal design, have demonstrated the effectiveness and safety of the TNF inhibitors etanercept (*Class I; Level of Evidence A*) [160] and adalimumab (*Class I; Level of Evidence A*) [161], the T-cell activation blocker abatacept (*Class I; Level of Evidence B*) [162], and the IL-6 receptor inhibitor tocilizumab (*Class I; Level of Evidence B*) [163] in children with nonsystemic forms of JIA.

Several studies have shown that the anti-TNF agents have a lesser efficacy in children with systemic arthritis than in those with other forms of JIA [164–166]. These insufficient therapeutic results are probably due to differences in the pathophysiology of the inflammatory process. As stated above, there is mounting evidence that the key cytokines in the pathogenesis of systemic JIA are IL-6 and IL-1, rather than TNF. These findings have provided the rationale for the successful treatment of systemic JIA with the IL-1 inhibitors anakinra (*Class I; Level of Evidence B*) [167,168], canakinumab (*Class I; Level of Evidence B*) [169] and rilonacept (*Class I; Level of Evidence B*) [170], and with the IL-6 antagonist tocilizumab (*Class I; Level of Evidence A*) [171,172].

After more than a decade of extensive use, the safety of anti-TNF agents in children with JIA is well defined. Information for the other biologics is more restricted and mainly limited to the randomized controlled trials and follow-up analyses of patients enrolled in the trials. The potential of anti-TNF agents to induce malignancy is still uncertain. A large-scale effort aimed at collecting safety data related to biologic medication in a multinational sample of JIA patients is underway. This project is supported by the European Union (EU) and is coordinated by the Paediatric Rheumatology European Society (PRES) and the Pediatric Rheumatology International Trials Organization (PRINTO) [173]. In clinical practice, it is important to consider that the administration of anti-TNF agents has been associated with an increased risk of TB infection onset or reactivation. For this reason, accurate screening for TB at baseline and vigilant monitoring for the whole duration of treatment are mandatory [174].

The biologic medications currently used in the management of JIA are listed in Table 7.9.

TABLE 7.9 Biologic Medications Used in JIA

Category	Name
TNF antagonists	Etanercept, infliximab, adalimumab, golimumab
IL-1 inhibitors	Anakinra, canakinumab, rilonacept
Monoclonal antibody to IL-6 receptor	Tocilizumab
Inhibitor of T-lymphocyte activation	Abatacept

TNF, Tumor necrosis factor; IL, interleukin.

12. CARDIAC SIDE EFFECTS OF BIOLOGIC THERAPIES

A variety of side effects have been associated with the administration of biologic medications. However, little information is available on their potential for cardiac toxicity. A recent study evaluated the long-term cardiac function during administration with TNF inhibitors. Twenty-five patients with polyarticular-course disease and 22 healthy controls underwent conventional and tissue Doppler echocardiography, together with cardiac biomarker measurements, at treatment baseline. Twenty-one JIA patients were assessed longitudinally over 2 years. At treatment initiation, isovolumetric relaxation time of left ventricle, ventricular septum, E-wave, and VS S-wave velocity were all significantly reduced in JIA patients compared to controls. During anti-TNF therapy, no patient had heart failure or alteration of ejection fraction or other parameters, and only one patient developed mild pulmonary hypertension. Cardiac biomarkers remained in the normal range throughout the study, except in one patient who experienced mild troponin T elevation. The authors concluded that long-term administration of TNF blockers is safe in spite of the observed subclinical diastolic abnormalities [175].

Zeft and coworkers [176] reported a 10-year-old child with systemic JIA who died unexpectedly while receiving the IL-1 receptor antagonist anakinra. On autopsy, microscopic findings were consistent with inflammatory myocarditis without pericarditis (Figs. 7.10 and 7.11). However, whether this complication was due to the medication itself, an infectious process, or an incompletely controlled and clinically undetected myocarditis remains unclear.

In the registrative clinical trials of biologic medications conducted in the past 15 years [160–163,169–172,177], cardiovascular side effects were seldom observed. Of the 39 serious adverse events that were recorded in the TENDER trial of tocilizumab in systemic JIA [172], one involved cardiac failure and one pulmonary veno-occlusive disease leading to pulmonary hypertension. Cardiac failure related to the veno-occlusive disease was reported in the latter patient, who died 13 months after withdrawing from the study. Another patient withdrew after 48 weeks due to lack of efficacy and died from pulmonary hypertension 6 months later while receiving other biologic agents for persistently active systemic JIA. In the trial of infliximab in polyarticular-course JIA [177], one patient died approximately 10 days after the placebo infusion at week two due to cardiac failure during hospitalization for septic shock. A second patient, with systemic-onset JIA, died after study participation. This child, who withdrew from the study during participation in the open-label extension, experienced a severe disease flare 3 months after the final infusion of infliximab and died in the hospital of cardiac arrest. The child had been placed on the stem-cell transplant list because of JIA severity.

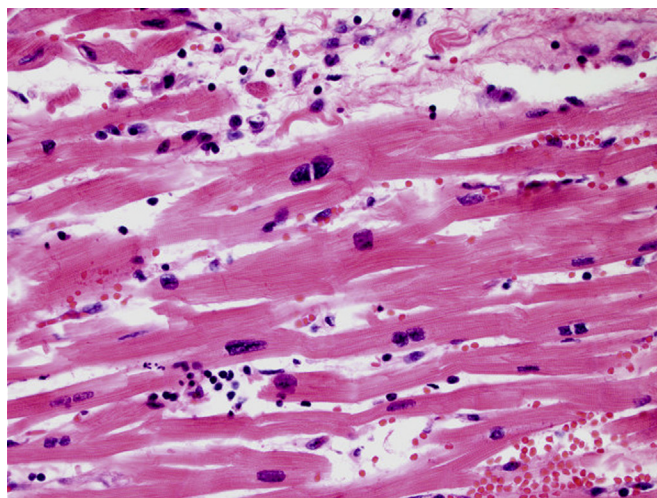


FIGURE 7.10 Myocarditis with predominantly lymphocytic inflammation. Photomicrograph (H&E, ×400) showing interstitial inflammatory cells consisting mostly of small lymphocytes with round nuclei and scanty cytoplasm, though rare granulocytes are also seen. This image also shows several binucleated myocytes, a feature of myocyte hypertrophy, though this was not a consistent finding elsewhere. Reproduced with permission from Zeft et al. [176].

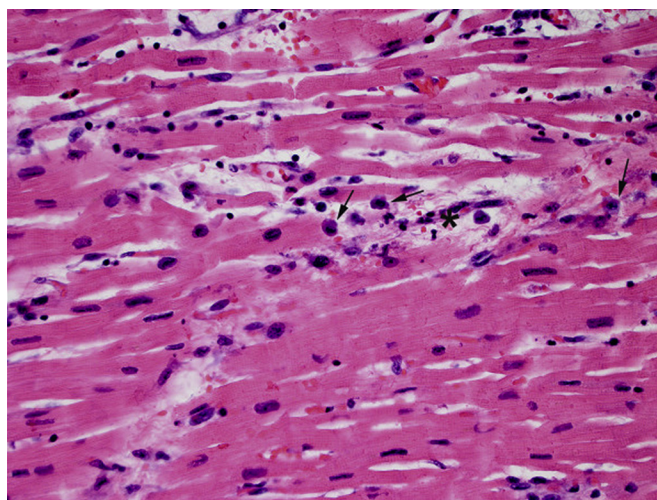


FIGURE 7.11 Myocarditis with focal myocyte injury and macrophage infiltration. Photomicrograph (H&E, ×400) showing a focus of myocyte injury. A necrotic myocyte is highlighted (asterisk) with surrounding macrophages (arrows) having more abundant cytoplasm. Reproduced with permission from Zeft et al. [176].

13. MANAGEMENT OF MAS

No information exists on the treatment of cardiac involvement in MAS specifically. We, therefore, provide herein a brief summary of the general therapeutic approach to this condition. As no controlled studies are available, the management of MAS is essentially based on anecdotal experience or expert advice. The mainstay of the therapy

is traditionally based on the parenteral administration of high-doses of corticosteroids (*Class I; Level of Evidence C*). In the most serious instances or in cases refractory to corticosteroids, oral or intravenous cyclosporine is added [178,179] (*Class I; Level of Evidence C*) [177,178]. The role of intravenous immunoglobulin (*Class IIb; Level of Evidence C*), cyclophosphamide (*Class IIb; Level of Evidence C*), plasma-exchange (*Class IIb; Level of Evidence C*), and etoposide (*Class IIb; Level of Evidence C*) is doubtful and debated. Etoposide is part of the therapeutic protocol for primary hemophagocytic lymphohistiocytosis (HLH) [180]. However, this protocol carries a significant risk of mortality and may not be suitable as a first-line therapy for MAS in systemic JIA [63]. Whether or not the use of lower doses of etoposide will be beneficial, remains unclear.

With the recent advent and use of a variety of biologic agents, novel therapeutic approaches are being scrutinized for MAS. The experience gained so far with TNF inhibitors has led us to assume that this therapy may not be ideal for MAS in the context of systemic JIA [63]. Recently, several patients with MAS who ameliorated dramatically with the administration of the IL-1 inhibitor anakinra, after insufficient response to corticosteroids and cyclosporine, were described [181–183]. However, there have been reports of MAS triggered by anakinra in children with systemic JIA [184,185], although the cause–effect relationship is difficult to establish. Therefore although the published experience is positive overall, more information is needed to define the role of IL-1 antagonists in the treatment of sJIA-associated MAS (*Class IIa; Level of Evidence C*). Similar to IL-1 inhibition, IL-6 blockade through the anti-IL-6 receptor monoclonal antibody tocilizumab has proven highly efficacious in treating systemic JIA (see above). Whether tocilizumab will be similarly helpful in treating MAS is currently unclear, as there has been a case of MAS attributed to anti-IL-6 therapy [186]. A more aggressive approach using antithymocyte globulin (ATG) has been tried with good results in two patients with probable MAS (*Class IIb; Level of Evidence C*) [187]. However, use of ATG is associated with significant risk of serious infection and mortality [187]. The B-cell depleting anti-CD20 antibody rituximab has recently been found to lead to remission in a substantial proportion of children with refractory systemic JIA (*Class IIb; Level of Evidence C*) [188]. Furthermore, this medication has anecdotally been used to effectively treat EBV-associated HLH/MAS [189]. However, to date no cases of MAS treated with rituximab have been reported.

14. MANAGEMENT OF PERICARDITIS AND MYOCARDITIS IN JIA

Because pericarditis and myocarditis usually occur in the context of active systemic JIA, at disease onset or during a disease exacerbation, and in conjunction with

other extra-articular manifestations, such as fever, rash, anemia, and occasionally MAS, their control mandates the administration of high-dose intravenous corticosteroids. The medication of choice is methylprednisolone, which can be administered at conventional doses (eg, 2–4 mg/kg/day in two to four daily doses) or with intravenous pulses at 10–30 mg/kg/day to a maximum of 1 g/day for one to three consecutive days (*Class I; Level of Evidence C*) [148]. A retrospective analysis [132] and a prospective study [133] have suggested that introduction of anti-IL-1 therapy early in the course of systemic JIA may help to prevent refractory arthritis and to obtain a quick and sustained disease control (*Class IIb; Level of Evidence C*). However, no specific data are available on the effectiveness of IL-1 and IL-6 blockers on cardiac manifestations.

A 20-year-old pregnant woman with a history of JIA who presented with myocarditis, associated with severe cardiomyopathy and severe systemic inflammation, 6 weeks after the change of her therapy from anakinra to etanercept was described by Movva et al. [190]. Therapeutic replacement was due to insurance coverage issues. After discontinuation of etanercept and in spite of high-dose pulse corticosteroid administration the clinical condition did not improve, with dyspnea, intermittent fever, persistent tachycardia, and hypotension requiring inotropic therapy. Dramatic improvement in symptoms was seen within 24 h after restart of anakinra. This report underscores the potential effectiveness of IL-1 inhibitors in patients with rheumatic illnesses who develop cardiac inflammation.

15. CONCLUSIONS

Cardiac involvement is well documented in JIA, although its prevalence is widely variable across subtypes. Pericarditis with or without pleural effusion is a characteristic feature of systemic arthritis. Myocarditis is much less common than pericarditis, although the two conditions can coexist. Cardiac failure can develop in the most severe forms of MAS, and frequently occurs in the context of multiorgan failure. Recently, patients with systemic JIA who developed pulmonary arterial hypertension and other potentially fatal pulmonary complications, often in association with MAS, have been described. A few patients with rheumatoid factor-positive polyarthritis and juvenile ankylosing spondylitis who developed valvular heart disease, most frequently aortic insufficiency, have been reported. Valvular involvement was also seen in children with B27-positive peripheral arthritis and enthesitis without back pain or radiographic evidence of sacroiliitis. In spite of increasing and widespread use, little information is available on the potential for cardiac toxicity of the novel biologic

medications as well on their specific effectiveness on cardiac inflammation. Although recent studies have shown impairment in the systolic and diastolic function of the left and right ventricles in children with JIA, the significance of these findings is unclear as there is currently no evidence of an increased risk for ischemic heart disease or cardiomyopathy. The finding of endothelial dysfunction and alterations in vascular function and structure may imply that JIA is associated with an increased risk for premature atherosclerosis and early cardiovascular events. However, the carotid IMT, which is considered a highly sensitive and accurate indicator of subclinical atherosclerosis, was not found to be thicker than in healthy controls in every study. Reassuringly, improvement of the IMT after 1 year of effective therapy was reported. Although children with JIA were found to have decreased anaerobic fitness than healthy pairs, the exercise capacity of patients with active disease or remission was comparable, suggesting that physical activity and exercise should be encouraged irrespective of the stage of disease activity.

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Spondyloarthritides: Ankylosing Spondylitis, Psoriatic Arthritis, and Reactive Arthritis

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1. INTRODUCTION TO SPONDYLOARTHRITIS

The concept of seronegative spondyloarthritides, which means the absence of rheumatoid factor, now called spondyloarthritides (SpA), dates back to 1974 when it was introduced by Moll and Wright for this partly heterogenous group of diseases. The term traditionally covers ankylosing spondylitis (AS), reactive arthritis (ReA), arthritis/spondylitis associated with psoriasis (PsA), and arthritis/spondylitis associated with inflammatory bowel disease (IBD). Ankylosing spondylitis has long been considered as the prototype of the SpA. But today the term AS is used less frequently because the spectrum has been broadened by the introduction of new classification criteria for SpA, especially those for the form with predominant axial involvement (axSpA) but also for peripheral SpA [1,2].

The SpA are genetically linked [3] and share characteristic clinical features such as inflammatory back pain (IBP) due to sacroiliitis and spondylitis [4] and others such as enthesitis, arthritis, anterior uveitis, and other organ manifestations such as psoriasis and chronic inflammatory bowel disease [5,6]. In addition to clinical findings, imaging (mainly radiography and magnetic resonance imaging (MRI)), and laboratory data (mainly HLA B27 and CRP) are important diagnostic tools for SpA [7–9]. The publication of the ASAS (Assessment in SpondyloArthritis International Society) classification criteria for axSpA has widened the spectrum of this field [1,2], which previously had largely been determined by the 1984 classification criteria for AS [10]. The latter constitutes the established part of axSpA that has definite

structural changes in the sacroiliac joints (SIJ) [10], in addition to what has now been termed nonradiographic axSpA (nr-axSpA)—the subset in which no such changes are present. The main argument for developing new criteria was the considerable delay in diagnosing AS using the old criteria [11]. Since imaging plays an important role in all criteria sets, ASAS recently organized expert consensus groups to agree on definitions for inflammatory changes in the SIJ [12] and the spine [13]. Patients with nr-axSpA, who seem to have somewhat less signs of inflammation compared to established AS, may represent axSpA in early disease stages who will develop structural changes and AS in the near future or especially female patients who may never develop such changes [14]. The term undifferentiated SpA [15] is therefore no longer used for patients with nr-axSpA (see later). However, it is still sometimes used for patients with peripheral SpA who do not have psoriasis, IBD, or a preceding infection.

In the past the term “undifferentiated SpA” was often used for patients who had clinical manifestations suggestive of SpA but who did not meet any of the currently available classification criteria for AS [10] and/or for SpA in general such as the 1990 European Spondyloarthropathy Study Group (ESSG) and the Amor criteria [16,17], which already considered the whole spectrum of SpA as one disease. The ESSG criteria actually split the whole group of SpA for the first time into the two subsets “predominant axial SpA” (dominated by back pain) and “predominant peripheral SpA” (dominated by affection of peripheral joints and or entheses), defined not by the accompanying disease but rather by the main location of the affected joints. Subsequently,

ASAS developed classification criteria for axial [1] and peripheral [2] SpA further with the inclusion of MRI and HLA-B27 to facilitate the recognition of the disease and to conduct clinical research studies.

Thus the term axSpA covers both patients who already have radiographic changes in the sacroiliac joints (radiographic sacroiliitis=AS) and patients who do not have such changes; this subgroup is now called nonradiographic axSpA (nr-axSpA). AxSpA is more common in men than in women in the case of established AS, while in the case of nr-axSpA there is a female predominance. The diagnosis of axSpA is often delayed as symptoms can be similar to other more common but usually less serious disorders such as nonspecific low back pain, a frequent complaint that makes patients visit general practitioners, orthopedists, and physiatrists. In addition, typical radiological changes of the sacroiliac joints may become visible only after many years, even in cases with persistent inflammation. However, 20–30% of patients with axSpA develop structural changes already in the first 2 years of disease. The main clinical symptoms of patients with axSpA are pain and stiffness of the back, predominantly of the lower back and the pelvis, but any part of the spine can be involved. The most common symptom of axSpA is IBP, which is only clinically defined, not by laboratory tests such as C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR). Patients with axSpA complain about morning stiffness of the back, with improvement on exercise but not by rest. In addition, or alternatively, they report awakening at night, mostly in the second half of the night, because of back pain, which improves on getting up and moving around.

According to the modified New York criteria, which are still widely used, the hallmark for a diagnosis of AS is the detection of sacroiliitis by conventional radiographs, based on a grading system 0–4. The ASAS has proposed new classification criteria that have been designed to cover all patients with axSpA based on their predominant axial involvement, but irrespective of the presence or absence of radiographic changes in the sacroiliac joints [1,2]. Thus magnetic resonance imaging (MRI) and HLA B27 have been included in the criteria, which also include other classical features of SpA such as enthesitis and extraarticular manifestations such as psoriasis [1,2].

There are several recommendations for the management of patients with axSpA, and the important ones come from ASAS and ASAS/EULAR. Next to physiotherapy, nonsteroidal antiinflammatory agents (NSAIDs) and biologics such as TNF blockers are frequently used, while conventional DMARDs are less efficacious for axial disease. Ustekinumab and Secukinumab may also work in axSpA.

The prevalence for the whole group of SpA has been estimated to be close to the prevalence of rheumatoid arthritis and is dependent on the local HLA-B27 prevalence.

Susceptibility to AS has been estimated to be genetically determined by more than 90%, with HLA-B27 being the strongest genetic factor associated with the disease, but new genetic associations have been described recently with potential functional and therapeutic relevance. Treatment of axial SpA has experienced major changes over the last decade, with TNF blockers now being the main treatment for those patients who fail conventional treatment with NSAIDs. However, other new treatment targets have come up recently. Thus there have been major advances in the different fields of axial SpA.

This chapter focuses on cardiac involvement in AS, PsA, and ReA—especially since involvement of the heart is rare and almost no data are available for the other subtypes.

2. SPONDYLOARTHRITIS—AN OVERVIEW

2.1 Pathogenesis of Axial Spondyloarthritis

A major breakthrough in research on the pathogenesis of AS and related SpA was the reported strong association of the disease with HLA-B27 in 1973 [18]. However, intensive research over more than three decades has not clarified the functional role of the HLA-B27 molecule in the pathogenic process. The center of the discussion on the pathogenesis of SpA has long been the interaction between bacteria and HLA-B27, as a result of known triggering bacteria in ReA, which occurs after preceding bacterial infections of the urogenital or gastrointestinal tract and the association with IBD; in the latter the immune system may interact with local gut bacteria because of a damaged mucosa [19]. Between 10% and 50% of HLA-B27-positive patients with ReA or IBD develop AS over the years. These findings have supported a central role for an interaction between bacteria and HLA-B27 in the pathogenesis; however, in the majority of patients with AS, no bacterial exposure can be detected, but subclinical bacterial infections or gut inflammation may contribute. The strong association of HLA B27 and other genes such as ERAP-1 and IL-23R suggests that a cellular immune response plays a role in the pathogenesis of axSpA (see later) [1].

Many recent MRI studies and older pathological investigations suggest that the primary target of the immune response is at the cartilage/bone interface, including the insertion of tendon and ligaments at the bone—the enthesis [20]. Since the presence of mononuclear cell infiltrates and osteoclasts seem to depend on the presence of cartilage on the joint surface of patients with coxitis due to AS [21]. Although there is currently no evidence that bacteria or bacterial antigens persist in the cartilage or close to the cartilage of spine and joints bacteria may trigger an autoimmune response against cartilage-derived antigens, such as proteoglycan or

collagen, possibly mediated somehow through HLA-B27. Of interest, these antigens are also present in other organs such as the aorta or the uvea [22]. However, this hypothesis has not yet been proven. A third and necessary triggering component could be microtrauma(s) of cartilage/bone because weight-bearing parts of the skeleton are almost exclusively affected in axSpA. Elevated expression of the cytokines interleukin (IL)-17 [23] and IL-23 [24] have been found in the subchondral bone marrow of patients with AS. These cytokines have recently gained interest as treatment targets, and antibodies targeting these cytokines, secukinumab and ustekinumab, have been successfully used for treating patients with AS (see chapter on treatment). Finally, IL-23 has been found to play a crucial role in the pathogenesis of enthesitis in an animal model with features resembling SpA [25].

The histopathological features of the inflammation in the aorta of AS patients were reported more than 40 years ago. Focal destruction of muscular and elastic structures of the media, thickening of intima, and adventitia and obliterative vessel disease, similar to syphilitic aortitis, were described. The typical valvular changes in AS have been described as fibrotic, thickened, and retracted cusps with rolled edges [26].

2.2 Genetics of Axial Spondyloarthritis

As already indicated, there is a strong genetic association of axial SpA with HLA-B27. HLA-B27 positivity is found in 80–90% of AS patients versus 6–10% in the total Caucasian population. More than 150 subtypes of HLA B27 have now been described. Detailed genetic analyses have revealed that, with the exception of HLA-B27*06 and HLA-B27*09, most common subtypes are associated with increased susceptibility to AS [27]. The pivotal functional role of HLA-B27 has been substantiated by the spontaneous development of a multiorgan inflammatory disease phenocopying human SpA in rats overexpressing human HLA-B27 [28]. However, how HLA-B27 mechanistically contributes to the disease has remained unclear. Based on the potential role of bacterial infections in triggering disease, this MHC class I molecule may be involved in the presentation of peptides, presumably of microbial antigens, to CD8⁺ T cells, which may then recirculate and be reactivated in the joint by, e.g., cross-reacting cartilage antigens. This “arthritogenic peptide” hypothesis is supported by the identification of CD8⁺ T-cell responses to both microbial and self-antigens in SpA patients [29,30] as well as by the gene–gene interaction between HLA-B27 and ERAP-1, an endoplasmic aminopeptidase trimming peptides for antigen-presentation, in AS [31].

An alternative hypothesis is based on the propensity of HLA-B27 to assemble abnormally due to cysteine residues in its β pocket. Intracellular misfolding of HLA-B27 can lead to endoplasmic reticulum stress and the activation of

an unfolded protein response [32]. When unresolved, this UPR induces massive IL-23 production by myeloid cells [33,34], thus providing an autoinflammatory link between HLA-B27, intracellular stress, and inflammation. While it was demonstrated in the HLA-B27 tg rat model, it remains a challenge to demonstrate HLA-B27-induced UPR in human SpA [35,36]. Alternatively, HLA-B27 can also form β -2 microglobulin-free heavy-chain homodimers on the cell surface [37]. These homodimers can directly activate NK and T-cell subsets by triggering the KIR2DL3 receptor *in vitro* and *in vivo* [38,39].

A final mechanism by which HLA-B27 may contribute to SpA is by modulating the microbiome. There is increasing evidence that the microbiome plays a crucial role in determining the development of acquired immune responses and thereby may predispose to immune-mediated inflammatory diseases [40,41]. The role of HLA-B27 in shaping the gut and skin microbiome is currently under sharp scrutiny [41].

It is important to realize that the aforementioned mechanisms are not mutually exclusive. It is even conceivable that they differentially contribute to the different disease manifestations of SpA.

2.3 Epidemiology of Axial Spondyloarthritis

Axial spondyloarthritis is a disease that starts normally in the third decade of life, with about 80% of patients developing the first symptoms before the age of 30 and less than 5% of patients being older than 45 at the start of the disease. Up to 20% of patients are even younger than 20 years when they experience their first symptoms [42]. Patients who are positive for HLA-B27 are about 10 years younger than HLA-B27-negative patients when the disease starts [43]. Men with AS are slightly more affected than are women, with a ratio of about 2:1, but women are equally affected compared to men in the nr-axSpA stage. Indeed, women generally develop chronic radiographic changes of the sacroiliac joints and the spine later than men, a possible explanation for the frequent underdiagnosis of AS in women in the past, resulting in a much higher male:female ratio than previously thought [43].

There is a correlation between the prevalence of HLA-B27 and the prevalence of AS in a given population: the higher the HLA-B27 prevalence, the higher the AS prevalence. HLA-B27 is present throughout the world with a wide ethnic and geographical variation. It is most prevalent in northern countries and some tribes (Tables 8.1 and 8.2) [44–48]. Overall, estimations on the prevalence of AS are between 0.1% and 1.4%, and most data are from Europe. Here a prevalence of 0.3–0.5% for AS and of 1–2% for the whole SpA group is likely. Recent studies from France, the United States, and Lithuania indicate that SpA is at least as common as rheumatoid arthritis (Table 8.1), strongly suggesting

TABLE 8.1 Prevalence of Ankylosing Spondylitis [44–48]

	AS prevalence (%)	HLA-B27 prevalence (%)
United States	1.0–1.5	8
Netherlands	0.1	8
Germany	0.55	9
Norway	1.1–1.4	14
Haida Indians	6.1	50

TABLE 8.2 Prevalence of HLA-B27 [49–52]

	SpA	RA
France	0.31	0.31
Lithuania	0.64	0.92
United States	1.31	0.6

that the SpA are of similar importance among the chronic inflammatory rheumatic diseases [49–53]. This has been recently confirmed by a study from the United States, which reported an overall prevalence of axSpA of about 1% [54].

HLA-B27 is positive in 90–95% of patients with AS. This percentage goes down to about 60% in patients with AS who also have psoriasis or IBD. In predominant peripheral SpA, less than 50% of patients are positive for HLA-B27. Patients with nonradiographic axSpA also have a somewhat lower percentage of HLA B27.

2.4 Ankylosing Spondylitis—Clinical Features

Ankylosing spondylitis is a chronic inflammatory rheumatic disease that is now part of the broader family of axial spondyloarthritis (Table 8.1) [5]. Ankylosing spondylitis is strongly associated with the HLA class I molecule, HLA-B27, which is prevalent in approximately 90% of AS patients compared to about 8% in the general population [1,55]. The disease mainly affects the sacroiliac (SI) joints and the axial skeleton by inflammation (Fig. 8.3) which, later, in the majority of patients leads to erosions, sclerosis, ankylosis and potentially fusion of the SI joints (Fig. 8.2) and other structures in the axial skeleton such as the vertebral bodies (Fig. 8.1). Structural changes and ankyloses of the axial skeleton are often painful, and eventually leading to spinal immobility [5]. The most common symptom of AS is inflammatory lower back pain that is relieved during movement and exercise. The diagnosis of AS is thus a combination of SI joint sclerosis (either grade 3–4 out of 4 unilateral, or 2 out of 4 bilateral) assessed with X-ray, inflammatory back pain for more than 3 months, and limitation of motion of the lumbar spine, or limitation of chest expansion (Table 8.3). The disease affects predominantly young, male patients. First symptoms often occur in the

**FIGURE 8.1** Typical lumbar spine radiographs (lateral and a.p.) of a 35-year-old male patient with ankylosing spondylitis and disease duration 10 years. Adapted from the personal collection of the authors.**TABLE 8.3** Criteria for Ankylosing Spondylitis and Axial SpA

(A) MODIFIED NEW YORK CRITERIA FOR ANKYLOSING SPONDYLITIS [10]

Clinical criteria	Low back pain and stiffness for more than 3 months that improves with exercise but is not relieved by rest
	Limitation of motion of the lumbar spine in both the sagittal and frontal planes
	Limitation of chest expansion relative to normal values corrected for age and sex
Radiographic criteria	Sacroiliitis grade ≥ 2 bilaterally or sacroiliitis grade 3–4 unilaterally

(B) ASAS CLASSIFICATION CRITERIA FOR AXIAL SPA [1]

	SpA features:
Sacroiliitis on imaging plus ≥ 1 SpA feature, or	<ul style="list-style-type: none"> • Inflammatory back pain • Arthritis • Enthesitis • Uveitis
HLA-B27 plus ≥ 2 other SpA features	<ul style="list-style-type: none"> • Dactylitis • Psoriasis • Crohn's disease/ulcerative colitis • Good response to NSAIDs • Family history for SpA • HLA-B27 • Elevated CRP

SACROILIITIS ON IMAGING

- Active inflammation on MRI is highly suggestive of sacroiliitis associated with SpA
- Definitive radiographic sacroiliitis according to modified New York criteria



FIGURE 8.2 Structural changes in the sacroiliac joints in a pelvic X-ray of a patient with ankylosing spondylitis. *Adapted from the personal collection of the authors.*



FIGURE 8.3 Inflammatory changes in the right sacroiliac joint of a patient with axial spondyloarthritis as assessed by MRI, STIR technique. *Adapted from the personal collection of the authors.*

second or third decade of life, but may occur in childhood. Next to spinal changes, other organ systems are often involved. Involvement of the anterior uvea, causing anterior uveitis in approximately 25% of patients, is well known in AS [5]. Furthermore, manifestations have been reported in the kidneys and the lungs [56–58]. Importantly, the heart of AS patients can be affected by the disease. This chapter focuses on the cardiac pathology of AS. In AS, there are mainly three anatomic sites in the heart commonly involved [57–60], namely the region around the aortic root [61], the conduction system [62], and the myocardium [57]. Affliction of these sites may lead to aortic disease, aortic valve dysfunction, conduction disorders, and ventricular dysfunction [57,60–62]. In addition, patients with AS have increased comorbidity [63] and mortality [64] due to atherosclerotic cardiovascular disease.

2.5 Mortality in Ankylosing Spondylitis

Mortality in AS is increased compared to the general population. Historically, the rate of cancer was increased in AS due to radiological treatment of the spinal disease. As radiological treatment was abandoned in the 1960s, the risk of cancer has been greatly reduced [66]. However, studies still estimate that the risk of death is increased by approximately 60% compared to the general population [64,67]. The major cause of death of AS patients in Europe is of circulatory origin, with an increased risk of atherosclerotic disease including myocardial infarction and stroke [63,64,67]. A large study conducted in Canada was comprised of 21,473 AS patients that were matched with 86,606 age-, gender-, and location-matched controls. Overall, adjusted hazard ratios (HRs) for vascular death in AS were 1.36 (95% CI 1.13–1.65), with a higher risk for males compared to females [63]. A large study from Sweden, comprised of 8600 AS patients and 40,460 controls revealed an age- and sex-adjusted HR of 1.60 (95% CI 1.44–1.77) for AS patients, with a higher risk for females [67]. In a study from Norway, comprised of 677 AS patients matched to 2031 controls from the general population, the standardized mortality rate (SMR) was 1.61 (95% CI 1.29–1.93), with an increased risk for males compared to females. The main cause of death in this study was of circulatory origin (40%) with coronary heart disease as the most frequent. Increased levels of CRP and infrequent use of NSAIDs were independent predictors of increased mortality. In a study from Hong Kong, comprised of 2154 AS patients, an age- and sex-adjusted SMR of 1.87 (95% CI 1.61–2.13) was reported compared to the general population. In this study cardiovascular death was the third as cause of death after infection and cancer [68].

2.6 The Heart in Ankylosing Spondylitis

2.6.1 Cardiovascular Morbidity

Increased mortality in AS is related to increased cardiovascular complications, including myocardial infarction and stroke. This increased atherosclerotic cardiovascular risk in AS is only partially explained by increased traditional cardiovascular risk factors such as hypertension [69–76]. A study from Sweden of 88 AS patients matched with 351 controls showed no increased prevalence of hypertension in AS [77]. However, other studies do show increased prevalence of hypertension [71,74,78,79]. Inflammation plays an important role in atherosclerotic disease in general [80], in other rheumatic diseases [81], and presumably also in AS [82]. In addition, AS-specific cardiac manifestations are probably also caused by excess inflammatory activity [83]. Antiinflammatory therapy, including the use of tumor necrosis factor- α blockers, might therefore decrease the

Patients with chronic back pain ≥ 3 months and aged <45 years

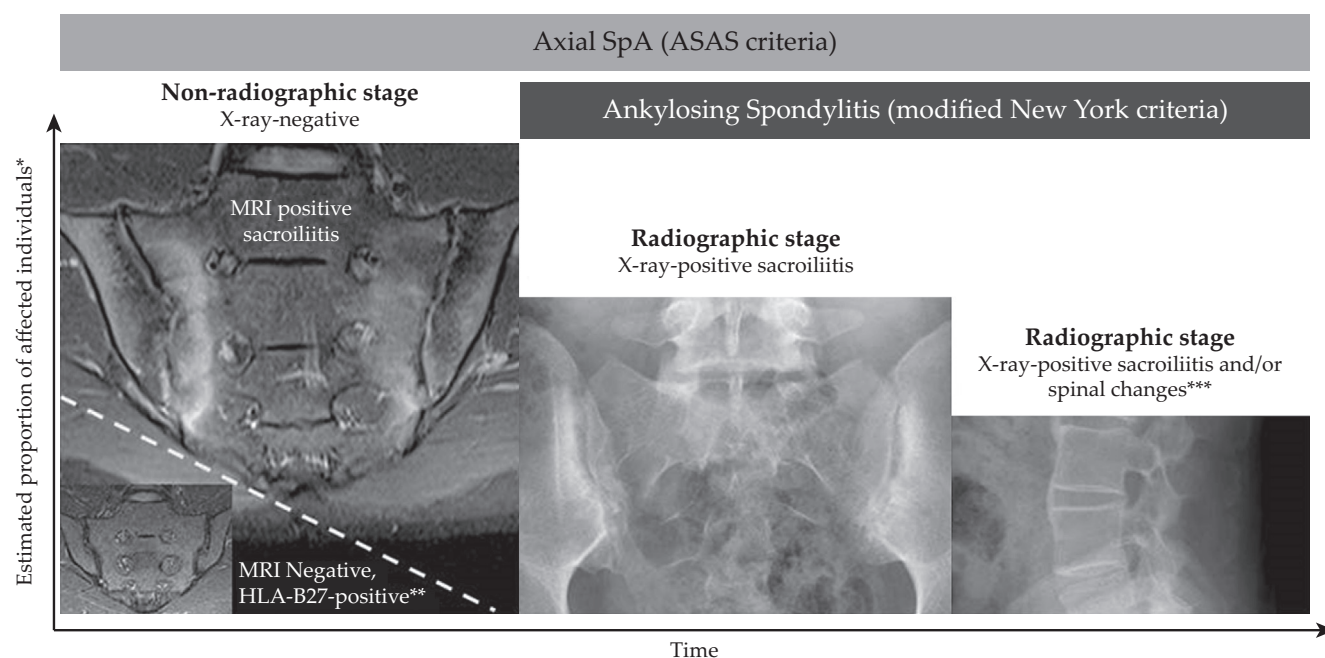


FIGURE 8.4 Possible course of axial spondyloarthritis. Adapted from Sieper and van der Heijde [65].

occurrence of AS-specific cardiac manifestations and atherosclerotic cardiovascular risk in AS [84], but there is currently no evidence to support this hypothesis. It should be noted that, cardiovascular risk management, as recommended by the EULAR guidelines, might help to decrease the increased atherosclerotic risk in patients with AS or SpA in the future [85].

2.6.2 Involvement of the Aorta and Related Structures

Pathoanatomically, in AS, there is evidence of involvement of the aorta ascendens, mainly the aortic root, but also of subaortic structures such as the membranous part of the interventricular septum and the basis of the anterior mitral leaflet. This can lead to conduction disturbances and mitral regurgitation, which are described separately [61]. Aortitis must be seen in connection with aortic (valve) disease due to the close proximity of these structures [26].

2.6.3 Aortitis

Historically, aortitis has been noted as a major factor in the occurrence of cardiac complications in AS patients. The exact contemporary prevalence of aortitis in AS is unknown, but diagnosis today is very rare. However, aortitis is a major and potentially deadly complication [86]. In AS, most information on aortitis is derived from case reports or case series [86–89]. Interestingly, in some patients, aortitis is discovered before symptoms of AS occur, or before a diagnosis of AS is made. In 1973, the histopathological features of aortic inflammation of eight AS patients were reported [26]. Focal destruction of muscular and elastic structures

of the media, thickening of intima and adventitia, and obliterate vessel disease, with some similarities to syphilitic aortitis, were described [26]. Thickening of the aortic valve cusps and aorta were seen. Fibrosis extending below the aortic valve caused a so-called fibrous ridge or “subaortic bump.” This extended fibrosis could lead to mitral valve dysfunction [88]. Both the aortic and mitral valve can be affected in AS [90]. Typical valvular changes in AS patients are fibrotic, thickened, and retracted cusps with rolled edges [26]. These changes may occur early in the disease [91] and can lead to valvular insufficiency over time (Fig. 8.5) [90]. A study from Sweden of 187 AS patients revealed an increased risk of aortic regurgitation compared to population data, with aortic regurgitation in 18% (95%CI 12–24%) of patients, ranging from mild to severe. In this study, HLA-B27 was equal among patients with and without aortic valve regurgitation. Other studies do reveal an association with HLA-B27 as a risk factor for the occurrence of aortic valvular disease [92,93]. Aortic insufficiency is reported in $<10\%$ of AS patients and increases with age, disease duration, and the presence of peripheral arthritis [94]. In a study from Korea, a group of 70 AS patients, of which 50 with less than 10-year of diagnosis, and 25 healthy controls, were investigated with transthoracic and transesophageal echocardiography revealing increased aortic valve thickness in the AS group, but the prevalence of aortic and mitral valve regurgitation was low and equal to the control group [91]. Interestingly, aortic valvular dysfunction might be symptomatic before symptoms of AS [95].

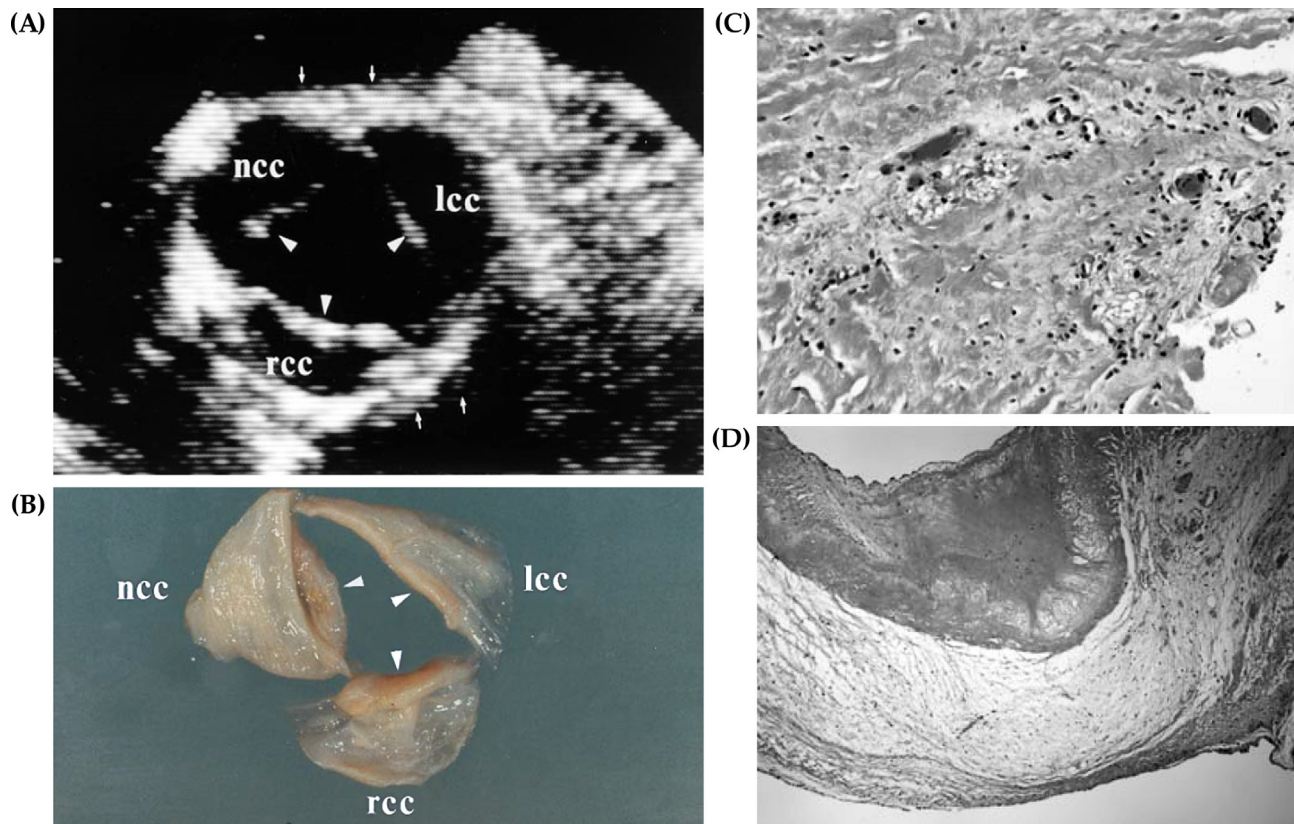


FIGURE 8.5 (A) This preoperative short-axis transesophageal echocardiography view of the aortic valve in a 34-year-old patient with AS and severe aortic regurgitation demonstrates thickening of the aortic root walls (*small arrows*) and irregular thickening of (B) retracted, rolled noncoronary cusp (ncc) and right coronary cusp (rcc), and irregularly thickened margins of the three aortic cusps (*arrowheads*). A central regurgitant orifice was confirmed. *lcc*, left coronary cusp. Adapted from Roldan et al. [90]. Histopathological images of an excised aortic valve from another patient with AS demonstrates evidence valvular fibrosis and neovascularization (C) as well as myxoid change (D). Adapted from Luckie et al. [100].

2.6.4 Mitral Valve Disease

The terminus “subaortic bump” refers to fibrosis of the basis of the anterior mitral leaflet in AS and has been described as a specific indicator of affection of subaortic structures in AS [26,90,96]. This fibrosis may lead to malalignment of the mitral valve cups and subsequent mitral valve dysfunction [26,97]. The prevalence of mitral valve dysfunction in AS is not precisely known. The previously mentioned Swedish study of 187 AS patients reported mild mitral regurgitation in 73% and moderate in 1% of patients, with no severe mitral dysfunction [98]. A study from Korea revealed no differences in prevalence of mitral valve regurgitation between AS patients and controls.

2.6.5 Complications

Ankylosing spondylitis patients are at increased risk of developing congestive heart failure and valve replacement due to aortic or mitral valve dysfunction [99]. In the study from Sweden, aortic regurgitation was associated with age, symptom and diagnosis duration, BASMI, BASFI, MSASSS, and erythrocyte sedimentation rate and anterior uveitis [98]. Interestingly, it was suggested that aortic root and valve disease might spontaneously resolve over time [99].

2.6.6 Conduction Abnormalities

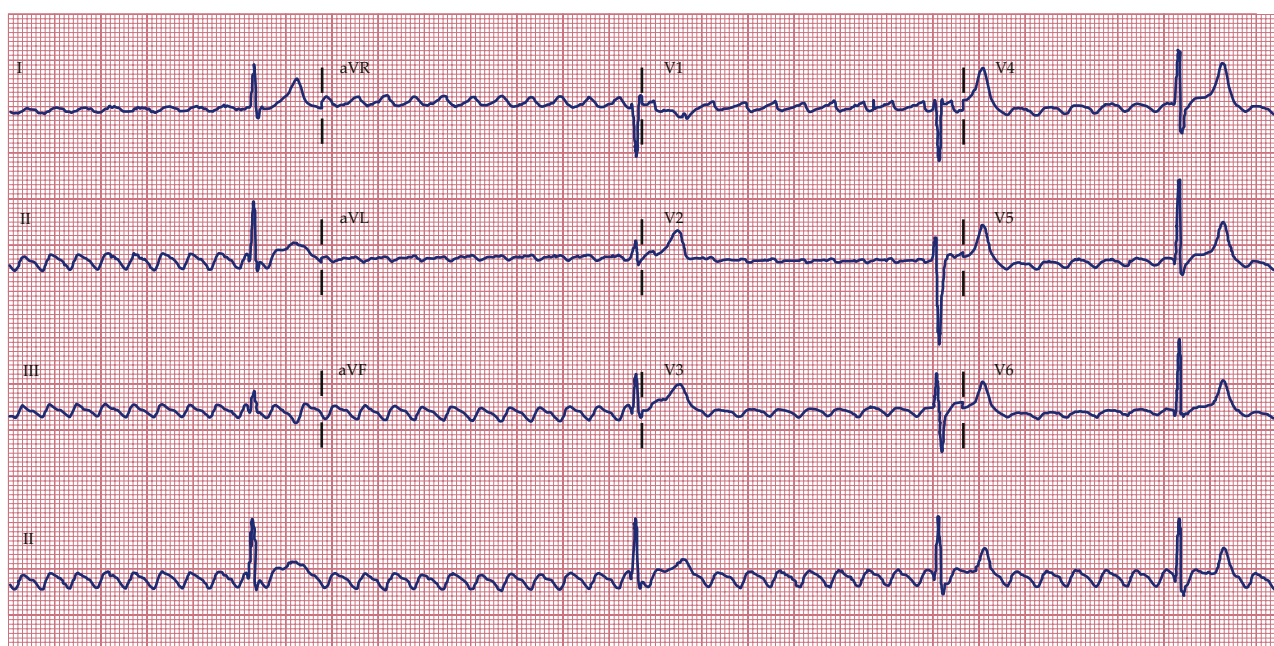
In AS cardiac conduction abnormalities occur more frequent than in the general population, predominantly due to involvement of the atrioventricular (AV) node [101]. Presumably, local inflammation affects the AV node, sometimes in such manner that complete heart block occurs and pacemaker implantation is required [26,94]. In AS, variable degrees of AV or bundle-branch block occur in approximately 5% of patients (Table 8.4; Fig. 8.6) [83]. The incidence of AV or bundle-branch block is increased among the HLA-B27-positive population, independently of the diagnosis of AS [60].

2.6.6.1 Localization

The first indication of an association of conduction abnormalities with HLA-B27 and AS was noted in 1966 [103]. Later, the relation between conduction disturbances and involvement of the membranous part of the interventricular septum was recognized [93]. Occasionally, extension of inflammation or aortitis into the interventricular septum results in second- or third-degree AV or fascicular blocks [96]. The anatomic site of the AV conduction problems in AS is mainly localized in the suprahisian region [101]. In HLA-B27-positive

TABLE 8.4 Conduction Disorders in Patients With AS

Studies on prevalence (From 1984 upwards)					
Author, year	AS patients/ controls	Exclusion criteria	Method	Conduction abnormalities including AV blocks and bundle-branch blocks, atrial fibrillation, syndromes	Notes
Forsblad, 2013 [94]	210/NA	Psoriasis, IBD, dementia, pregnancy, other severe disease, difficulty in answering questions	ECG	10%	
Park, 2012 [91]	70/25	History of cardiac disease, >50 years old, other rheumatologic disease	ECG	0% vs. N/A	
Rodrigues, 2012 [115]	1472/NA	None	N/A	3%	
Ho, 2012 [116]	641/NA	Psoriatic spondyloarthropathy, reactive arthritis, enteropathic arthropathy	ECG	9%	Retrospective study: ECGs made for medical reasons
Dik, 2010 [83]	131/NA	Brugada syndrome	ECG	15%	
Gunes, 2009 [117]	35/25	Hypertension, coronary artery disease, hypo/hyperthyroidism, COPD, DMII, renal failure	ECG	9% vs. N/A	
Kazmierczak, 2008 [110]	31/22	Cardiac disease, systemic hypertension, diabetes mellitus, thyroid disease	ECG	10% vs. 9%	
Brunner, 2006 [118]	100/NA	AS <15 year, female, manifest psoriasis, psoriatic arthritis, or Reiter's syndrome	ECG	17%	
Yildirim, 2001 [113]	88/31	History of cardiac disease, systemic hypertension, diabetes mellitus, other rheumatic diseases	ECG	8% vs. 0%	
Sukenik, 1987 [69]	40/40	History of rheumatic fever or serological evidence of syphilis	ECG	10 vs. 0%	

**FIGURE 8.6** High-grade AV block and atrial flutter presented in a patient with HLA-B27-associated spondyloarthropathy. Adapted from Tolat et al. [102].

patients, complete third-degree AV blocks are located within the AV node in 95% rather than in the expected 20% of cases [57].

2.6.6.2 Prevalence and Complications

In a review from 1997, Bergfeldt et al. noted that the frequency of HLA-B27 among men but not women with implanted pacemakers is significantly increased as compared to the general population [59]. Similar to the established prevalence of HLA-B27 in AS and Reiter's syndrome, a cardiac syndrome that consists of severe conduction system abnormalities plus aortic regurgitation associated with HLA-B27 was found to be present in 67–88% of patients with both of these clinical findings. Both cardiac conduction abnormalities and aortic regurgitation occur in patients with various HLA-B27-related extracardiac disorders, regardless of the severity of the latter. The mortality among pacemaker patients in comparison to the general population, also studied by Bergfeldt [104], showed no influence on mortality associated with HLA-B27 or with HLA-B27-associated rheumatic disorders.

In a population of 223 men with permanently implanted pacemakers, the prevalence of AS was assessed by screening pelvic radiographs [105]. Sacroiliitis was found in 19 men (8.5%), 15 of whom fulfilled the diagnostic criteria for AS (6.7%). These prevalence rates differed significantly from frequencies found in general Caucasian populations of 0.1–2%, respectively [105]. Thus a 15-fold increase in the prevalence of AS in this population with permanent cardiac pacemakers was found. In an extension study, 28 patients (12.6% (95% CI: 8.2–17%)) fulfilled SpA criteria [106] including 15 with AS (85% HLA B27-positive). Of interest, the majority of these patients had not been previously diagnosed with AS. Thus patients with severe bradyarrhythmias associated with SpA were found to constitute a large proportion of this population of men with permanent pacemakers [105,106].

In another study [107], the frequency of HLA-B27 was determined in 83 permanently paced men with complete heart block in whom the presence of radiological or clinical signs of a HLA-B27-associated rheumatic disease had, at this point in time, been excluded and 84 healthy subjects. HLA-B27 was found in 17% of the patients but in only 6% of the controls. This significant difference needs to be discussed in the context of other Scandinavian studies in which higher frequencies of HLA-B27 in populations living further north were reported [108]. Nevertheless, Bergfeldt and colleagues concluded that the development of heart block might be HLA-B27-associated in a subgroup of patients [105].

The prevalence of SpA among 35 patients with a pacemaker and heart block of unknown cause, selected from a total group of 350 men with pacemakers who

were still alive at the time of the study, was assessed in a Dutch study [109]. One of these 35 male patients had AS and another two patients had asymptomatic sacroiliitis, but all three were HLA-B27 negative. In contrast, HLA-B27 was present in 5 (14%) pacemaker patients—a significantly higher prevalence than in healthy controls (17/292=6%). The authors concluded that for the pathogenesis of heart block there must be more critical factors than HLA-B27 that cause the disease.

Through Holter monitoring, ventricular extrasystoles were present more often in 31 AS patients (55%) than in 22 healthy controls (28%), while supraventricular extrasystoles were similarly frequent (94% and 100%, respectively). Significant differences were found in heart-rate variability: ultralow-frequency power and root mean-square recessive difference (r-MSSD) were lower in the AS group [110]. Holter parameters did not correlate with the incidence of VESs, age, gender, clinical features, and duration of AS.

In another study searching for conduction abnormalities, ambulatory 24-h Holter monitoring revealed such changes in 12 of 48 HLA-B27-positive AS patients in whom the frequency of AV blocks and atrial tachycardia was reported to be higher than in healthy controls [111]. In another ECG-based study [112] using records of 99 AS patients and 132 of their adult first-degree relatives a similar distribution of P-R intervals was reported, in which only 4 cases of first-degree AV block were detected, one of whom had aortic valve insufficiency. One single case of pronounced conduction delay (P-R interval 0.42s) was recorded in an otherwise healthy HLA-B27-positive relative. The authors concluded that cardiac conduction disturbance was not more frequent in AS patients or in their relatives.

To assess the repolarization heterogeneity in AS, the QT dispersion (QTd) was examined by 24-h Holter monitoring in 88 AS patients and controls [113]. The QTd was found to be significantly greater in AS patients. This was correlated to disease duration and to the frequency of ventricular extrasystoles. Early application of this technique might identify AS patients at risk for myocardial involvement. However, these findings need to be confirmed [70].

The involvement of the autonomic nervous system (ANS) was analyzed by power spectral analysis of the heart-rate variability in AS patients. While one group [113] could not demonstrate any evidence of ANS involvement in 94 AS patients, another group [114] reported changes in ANS function in 18 AS patients including decreased parasympathetic activity, as evidenced by higher HR and lower baroreflex slope. Since these deviances were mainly observed in patients with active disease the authors concluded that these changes were more likely related to the inflammatory process than to the disease itself.

2.6.7 Left Ventricular Dysfunction

Myocardial involvement may occur as a complication in AS, in particular left ventricular dysfunction. Left ventricular dilatation and a poorly contracting left ventricle have been reported in AS [119] and were also observed in a necropsy study of the myocardium [62].

2.6.7.1 Prevalence

Cardiac function was investigated in 74 male AS patients (aged 21–65 years) who had no cardiorespiratory symptoms or known abnormalities of heart or lungs [62]. Chest radiographs and standard ECGs were normal in 73 subjects. In the echocardiographs of 30 men, left atrial size and left ventricular cavity size and wall thickness were normal. Minor abnormalities in the valve roots were present in three older men. However, early diastolic abnormalities of the left ventricle were demonstrated in 16 of 30 subjects. This finding was confirmed by repetition of the echocardiography 1 year later in 15 subjects and by comparison of 11 probands with their healthy brothers. In addition, myocardial tissue obtained at necropsy from 28 AS patients without ischemic or valvular heart disease or hypertension was studied. A mild, diffuse increase of interstitial connective tissue was seen, but there was no inflammatory change or amyloid. Computerized image analysis showed 31% interstitial reticulin compared with 18% in age- and sex-matched controls. The authors concluded that more than half of patients with AS had evidence of diastolic dysfunction and that most AS patients had a mild increase of interstitial connective tissue in the myocardium [62].

A comparison of the cardiac abnormalities as assessed by two-dimensional TTE detected in 20 juvenile onset (JOAS) and 31 adult onset patients with AS (AOAS) with 20 healthy controls [120] revealed a strikingly higher frequency of cardiomyopathy in AOAS (32%) and JOAS (25%) than in controls (0%). The disease duration was 15–20 years without differences between groups. The difference in HLA-B27-positivity between groups was unexpected: 90% in the JOAS vs. only 51% of AOAS patients were HLA-B27-positive.

In another study [121], the prevalence of both systolic and diastolic left ventricular (LV) dysfunction and other cardiac abnormalities in AS patients without clinical cardiac manifestations was assessed ($n=59$ patients; 49 men, mean age 42; mean disease duration 17 years). By echocardiography, abnormal diastolic LV function was detected in 12 patients (20%). Prolonged isovolumic relaxation time, prolonged deceleration time, reduced rate of descent of flow velocity in early diastole (EF slope), and reversal of the early and late peak transmitral diastolic flow velocities (E/A ratio) were noted in nine patients. Mild aortic regurgitation and mitral regurgitation was seen in one and

three patients, respectively. There was no correlation between the presence of LV diastolic dysfunction and age, disease severity, disease duration, and the presence of extraarticular manifestations.

The cardiac function at rest was assessed in 21 AS patients and 20 age-, sex-, height-, and weight-matched healthy controls using echocardiography, and, in addition, at rest and during supine bicycle exercise, using radionuclide angiography in the left anterior oblique position following equilibration with 740 MBq of technetium-99. No echocardiographic differences between patients and controls were detected. However, the global nuclide left ventricular function did show some differences: the peak filling rate during exercise and also the time to reach peak filling during exercise was significantly lower in AS patients. Importantly, most differences detected in AS patients were related to diastolic LV function [82,122–124].

The prevalence of diastolic LV dysfunction is increased in patients with AS, although the reported prevalence varies among studies [62,82,121–124]. A study of 40 AS patients and 42 controls reported diastolic LV dysfunction in 30% of AS patients compared to 12% in controls [125]. Another small case-control study of 49 AS patients and 33 controls reported diastolic LV dysfunction in (45%) of patients compared to 18% in controls [126]. In summary, up to 45% of AS patients might have diastolic LV dysfunction [124,126]. In AS patients without any cardiac history, systolic LV function seems to remain unaffected.

2.6.7.2 Pathogenesis, Treatment, and Complications

Pathogenically, inflammation and hypertension are likely causes of diastolic LV dysfunction in AS [124]. To date, there are no studies investigating the effects of treatment on ventricular dysfunction in AS. Only severe diastolic LV dysfunction leads to heart failure with subsequent symptoms. As moderate-to-severe diastolic LV dysfunction is associated with increased mortality [127], preventing diastolic LV dysfunction is important in AS patients, and hence regular blood pressure assessment is recommended.

2.6.8 Ischemic Heart Disease

Until approximately 15 years ago, the overall prevalence of ischemic heart disease in AS was not precisely known. However, accumulating evidence indicated a significantly increased prevalence [63,71–76,128]. Myocardial infarction is considered a major complication of AS, and the risk is increased up to 60% [129]. The prevalence of ischemic heart disease in AS varies from 18% to 40%, depending on the investigated population [69]. The origin of this increased risk of myocardial infarction appears multifactorial. Inflammation, medication use, increased prevalence of hypertension and dyslipidemia,

and decreased physical functioning may all contribute to this risk, mainly through the effects on atherogenesis. However, the individual contribution of these factors is unknown. This also holds for the effects of cardiovascular risk management.

More than 15 studies report the incidence of myocardial infarction (MI) in AS patients [129]. A large study of 8618 AS patients reported prevalence ratios compared to the general population. The risk of ischemic heart disease was 1.37 (95% 1.31–1.44) compared to the general population [73]. Interestingly, a study from the UK of 1686 AS patients showed no increased HR for MI compared to the general population: 1.28 (95% CI 0.93–1.74) [130]. A study from Taiwan of 2895 AS patients compared to 11,580 controls showed a significant higher incidence of coronary heart disease in AS patients [131]. Another study from Taiwan of 4794 AS patients aged between 18 and 45 years compared to 23,970 matched controls showed a higher incidence of ischemic heart disease in AS patients over time: 1.47 (95% CI 1.13–1.92) [76]. In a third study from Taiwan of 6262 AS patients and 235,048 non-AS controls the adjusted hazard rate of acute coronary syndrome was 1.36 (95% CI 1.16–1.59) [79].

In a study from the Netherlands, 3809 AS patients were matched with 26,197 controls [75]. In total, 4.3% of the patients had ischemic heart disease and 1.8% had acute myocardial infarction compared with 3.4% and 1.4% of the controls. The age–gender adjusted HR for developing IHD was 1.20 (95% CI 0.97–1.48), and for AMI 0.91 (95% CI 0.65–1.28) compared to controls. Results were different in a study from Taiwan of 10,763 new AS patients. In this study AS patients had increased risk of cardiovascular disease (OR 1.68 95% CI 1.57–1.80) compared to matched controls. Frequent use of NSAIDs was associated with a lower risk of cardiovascular disease. However, this might be due inclusion bias as patients with CVD are less often prescribed NSAIDs due to their unfavorable cardiovascular effects [132].

2.6.9 Therapeutic Approaches

Antiinflammatory therapy—Because cardiovascular disease in AS is multifactorial in origin, treatment of cardiovascular complications embraces several different areas. The EULAR (EUropean League against Rheumatism) published guidelines for cardiovascular risk management in patients with inflammatory diseases, including rheumatoid arthritis, AS, and PsA [133]. Although more evidence on cardiovascular disease in AS is continuously emerging, treatment advice remains generic due to lack of larger trials. Nevertheless, a key point is screening and management of traditional cardiovascular risk factors. The other key point in cardiovascular risk management in AS is to aim for

an as-low-as-possible disease, and thus inflammatory, activity. Currently, NSAIDs and TNF- α inhibitors are the primary options for treatment of AS. In general, treatment is initiated with NSAIDs, with no specific preference. NSAIDs are a double-edged sword in the treatment of AS. While NSAIDs decrease disease activity and inflammatory activity, they may also increase blood pressure through renal and vascular effects. There are contradictory reports on the effect of NSAIDs on cardiovascular risk in AS [132].

If disease activity is inadequately controlled by two consecutive treatment periods with NSAIDs, treatment with TNF- α inhibitors may be considered. All TNF- α inhibitors are strong antiinflammatory drugs, controlling inflammation adequately in the majority of AS patients. Overall, TNF- α inhibitors lower cardiovascular risk in inflammatory diseases [134], and although this has not yet been specifically reported in AS, this probably also holds for AS as well. However, the effects of TNF- α inhibitors on cardiac function and the presence of cardiac complications in AS are presently unknown.

Cardiovascular risk management—Due to the increased cardiovascular risk, cardiovascular risk management is very important in AS. At this moment, there is no clear evidence that cardiovascular risk management should be initiated earlier in AS than in the general population, or that treatment thresholds should be different. Cardiovascular screening of AS patients should be performed at least every 5 years according to the new EULAR recommendations [133,210], and should be reevaluated in case of major changes in disease activity or medical treatment.

Nonmedical treatment—Nonmedical treatment of AS includes physical training. Currently, this is the cornerstone of treatment of AS, but the effects of physical training on both cardiac functioning or cardiovascular risk are not known.

2.7 Discussion and Summary

There is significant involvement of the heart in AS, both atherosclerotic and nonatherosclerotic in origin. The prevalence of ischemic heart disease and stroke is especially increased in AS (Fig. 8.7). Other often affected cardiac structures in AS are the aorta and aortic valve. Furthermore, the myocardium and conduction system, especially the AV node, can be affected. Aortitis and HLA-B27-associated AV blocks may occur without any prior rheumatic disease manifestations. In general, clinical symptoms are related to the severity of the organ affection. This cardiac involvement causes increased morbidity and mortality in AS compared to the general population and therefore cardiovascular risk management is important in this disease.

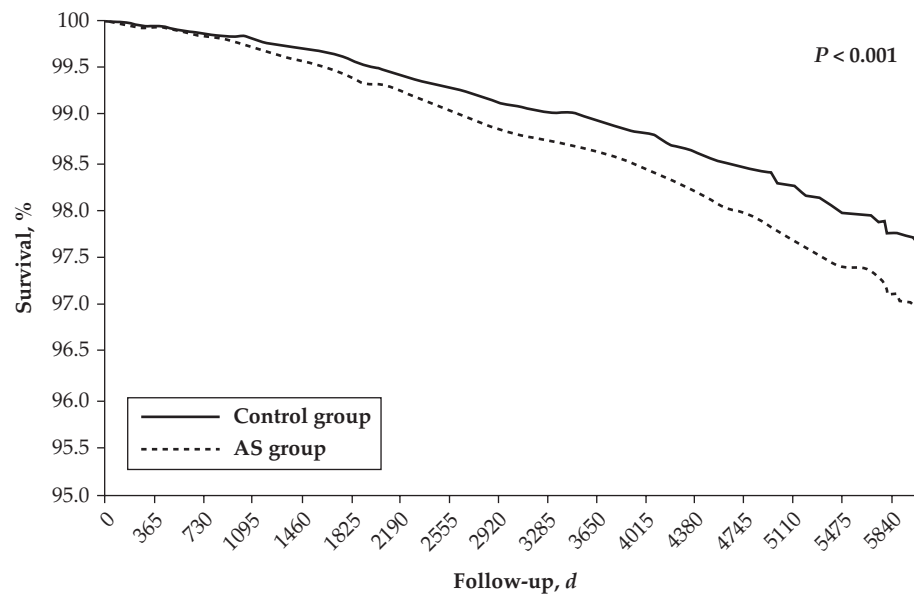


FIGURE 8.7 Kaplan–Meier survival curve of AS patients compared to non-AS controls after correcting for baseline variables. ankylosing spondylitis is found to be associated with increased vascular mortality. Adapted from Haroon et al. [63].

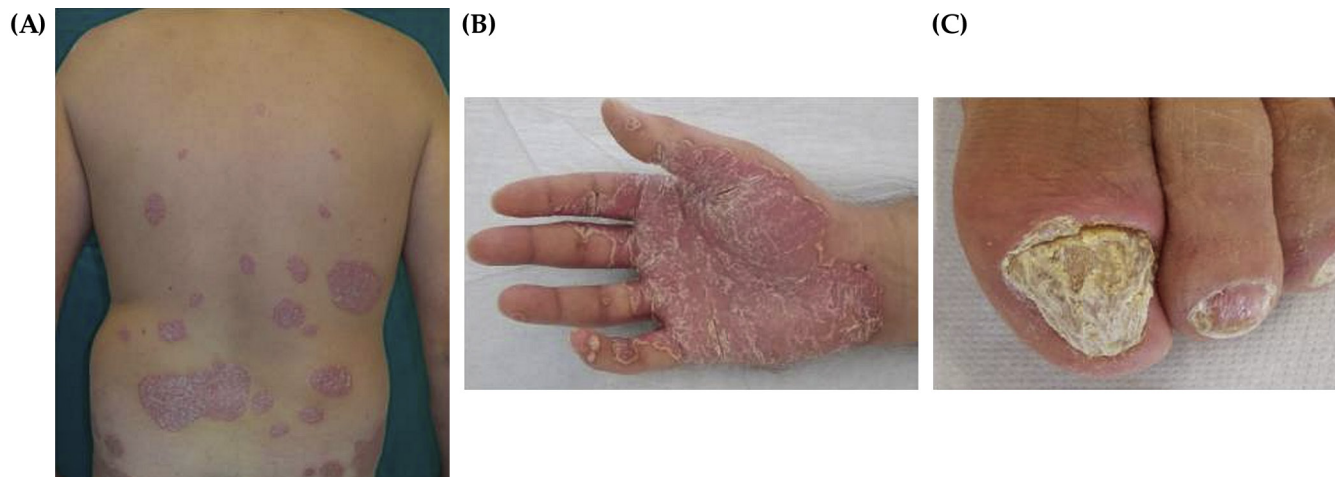


FIGURE 8.8 Different forms of psoriasis. (A) Plaque psoriasis, characterized by patches of inflamed skin with silvery scales. These lesions are mostly found on the elbows, knees, and trunk. (B) Hyperkeratotic psoriatic plaque in palmar psoriasis. (C) Thickened nail lamina in severe nail psoriasis. Adapted from Gisondi and Girolomoni [138].

3. THE HEART IN PSORIATIC ARTHRITIS

3.1 Clinical Features

Psoriasis is a very frequent disease in Europe—up to 3% of the population may be affected. The two major subtypes are plaque psoriasis and pustulosis palmaris et plantaris (Fig. 8.8). Psoriatic arthritis (PsA) is, as ankylosing spondylitis, part of the broader family of spondyloarthritis. Up to 1% of the population suffers from this disease, and there is no difference in prevalence between males and females [135]. The major symptoms of PsA are

an oligoarticular asymmetrical arthritis, in particular of the distal joints of the hands, alongside psoriasis. Common related symptoms are dactylitis, enthesitis, and axial joint inflammation. Of all patients with psoriasis, between 6% and 42% also have arthritis. There are classification criteria for PsA, which are currently used in clinical studies (Table 8.5). However, about 15% of patients with PsA have been reported who develop the disease according to their clinical symptoms who do currently or historically not have psoriasis. Furthermore, the vast majority of patients develop skin symptoms first while a minority develops arthritis earlier than skin disease. Patients with nail disease are clearly more likely to develop PsA.

TABLE 8.5 CASPAR (CIASsification criteria for Psoriatic ARthritis) Criteria for PsA

Inflammatory musculoskeletal disease (joint, spine, or enthesal) with three or more of the following:

1. Current psoriasis	Psoriatic skin or scalp disease present today as judged by a rheumatologist
2. Personal history of psoriasis (if current psoriasis not present)	A history of psoriasis that may be obtained from patient, family doctor, dermatologist, or rheumatologist
3. Family history of psoriasis (if personal history of psoriasis or current psoriasis is not present)	A history of psoriasis in a first- or second-degree relative according to patient report
4. Psoriatic nail dystrophy	Typical psoriatic nail dystrophy including onycholysis, pitting, and hyperkeratosis observed on current physical examination
5. A negative test for rheumatoid factor	By any method except latex but preferably by ELISA or nephelometry, according to the local laboratory reference range
6. Current dactylitis	Swelling of an entire digit
7. History of dactylitis (if current dactylitis is not present)	A history of dactylitis recorded by a rheumatologist
8. Radiological evidence of juxta-articular new bone formation	Ill-defined ossification near joint margins (but excluding osteophyte formation) on plain X-rays of hand or foot

Adapted from Taylor et al. [139].

Psoriatic arthritis is a multifaceted disease that can present as different clinical phenotypes: peripheral arthritis, axial disease, skin and nail disease, dactylitis, and enthesitis. According to an older proposal [136] there are five subtypes of PsA: those with involvement of distal interphalangeal joints only, asymmetrical oligoarthritis, polyarthritis, spondylitis, and arthritis mutilans. However, it is well known that these subtypes may well change and overlap over time, and patients starting with oligoarthritis may well develop polyarthritis after some years. Several years later a special subtype was named SAPHO (synovitis, arthritis, pustulosis, hyperostosis, and osteitis) syndrome [137].

3.2 Mortality

Just like other inflammatory joint diseases, mortality is increased in PsA compared to the general population [68,140,141]. An early study from 1997 of 428 PsA Canadian PsA patients showed an elevated SMR for both females [1.59 (95% CI 1.04–2.33)] and males [1.65 (95% CI 1.09–2.40)] [142]. A later study on this same cohort revealed an SMR of 1.36 (95% CI 1.12–1.64), suggesting a decrease in cardiovascular mortality in PsA [140]. In a Danish cohort study, 607 PsA patients had a relative risk of cardiovascular death of 1.84 (1.11–3.06) compared to control [143]. A study from Hong Kong showed age and gender adjusted a standardized mortality ratio of 1.59 (95% CI 1.16–2.03) for PsA patients [68]. In a study from 2000, no increased mortality was found in a study of 66 PsA patients compared to the general population [144]. Buckley et al. showed an SMR of 0.82 (95% CI 0.58–1.13), thus no difference from the general population

[141]. In an incidence cohort of 147 PsA patients, no increased mortality was observed [145].

3.3 Cardiac Involvement in PsA

The overall cardiovascular risk is increased in PsA, with enhanced prevalence of ischemic events and increased mortality. This increased risk is presumably due to accelerated atherosclerosis as a result of excess inflammatory activity. Unlike AS, there is currently no firm evidence for involvement of specific cardiac structures in PsA [146]. In a cohort of PsA patients aortic regurgitation (10%), tricuspid regurgitation (10%), and mitral regurgitation (16%) were not different than controls [146]. The pulmonary artery systolic pressure also did not differ between the groups. However, several reports do show increased ventricular dysfunction and conduction abnormalities in patients with PsA. Whether this is due to disease activity or other risk factors such as hypertension is unknown.

3.3.1 Heart Failure and Left Ventricular Function

Left ventricular dysfunction, as estimated with transthoracic-echocardiographic techniques and tissue Doppler imaging in 94 PsA patients, is increased in PsA compared to controls [147]. Another technique, i.e., two-dimensional speckle tracking imaging, showed subclinical impaired myocardial deformation in PsA patients, which was associated with disease activity [148]. In a study of 21 PsA patients, the end-systolic and end-diastolic parameters were statistically different than controls. Diastolic LV dysfunction was related to arthropathy and duration of psoriasis [149]. Another study of 22 PsA patients revealed

significantly reduced coronary flow reserve in PsA patients [150].

3.3.2 Arrhythmia

In a study on extraarticular manifestations in PsA, 387 PsA patients were included. In total, 7% had conduction disturbances, including atrioventricular blocks (46%), intraventricular blocks (23%), and bundle-branch blocks (31%) [151]. Arrhythmia was investigated in a study from Taiwan, in which 40,637 psoriasis patients were studied. In this study, the PsA patients had an HR for arrhythmia of 1.46 (95% CI 1.22–1.74) [152]. The underlying cause of arrhythmia was not further studied in detail in this study. A study using electrocardiography in 92 PsA patients compared to 92 age- and sex-matched controls showed no differences in conduction disorders. However, the PR interval was significantly longer in PsA patients [153].

3.3.3 Valvular Disease

A retrospective study of 387 PsA patients showed cardiovascular manifestations in 14% of all patients. Of these, 15 (4%) had aortic insufficiency [151]. In a study of 25 PsA patients, no aortic valvular pathology was found, but 14 patients (56%) suffered from mitral valve prolapse [154]. This was more than in a control group of psoriatic patients (6%). In the study from Gonzalez et al., no differences in valvular pathology were found [146].

3.3.4 Atherosclerotic Cardiovascular Disease

Psoriatic arthritis is associated with increased risk of cardiovascular disease, in particular myocardial infarction, ischemic heart disease, and angina pectoris (Fig. 8.9) [155–157]. In total, depending on the population

investigated, the prevalence of coronary heart disease is approximately 9% [155]. Among 196 Canadian PsA patients, 43% with early and 57% with established PsA, the prevalence of coronary heart disease (CHD) was 8.7% with no significant relation with disease duration [155]. In another study, cerebrovascular or coronary heart disease was found in 14% of PsA patients [156]. In a study from Canada 1091 PsA patients were followed for 35 years. In this period there were 104 cardiovascular events. Hypertension, diabetes, and the number of dactylitic digits were independent predictors of cardiovascular events [158]. A study from Norway of 338 PsA patients and 50,468 controls showed increased prevalence of angina pectoris and percutaneous coronary intervention [159]. In a longitudinal cohort study of 16 years the risk of a major adverse cardiovascular event was higher after adjustment for traditional risk factors in patients with PsA that did not receive DMARD treatment 1.24 (95% CI 1.03–1.49) [160]. In another study an increased HR was found for nonfatal cardiovascular disease [161]. A questionnaire study from The Netherlands showed that the risk of cardiovascular disease was equal to that in rheumatoid arthritis [162]. In another Canadian study of 648 PsA patients, the standardized prevalence ratio for myocardial infarction was 2.57 (95% CI 1.73–3.80) [156]. In a study of 3066 PsA patients, the prevalence ratio of ischemic heart disease was 1.3 (95% CI 1.1–1.5) [71]. In a study of 581 psoriasis patients, including 98 with certain PsA, the odds ratio for coronary heart disease in the PsA patients was 1.5 95% CI (0.4–5.2) [163].

3.3.5 Increased Prevalence of Cardiovascular Risk Factors

Patients with PsA more often have more cardiovascular risk factors such as smoking, hypertension,

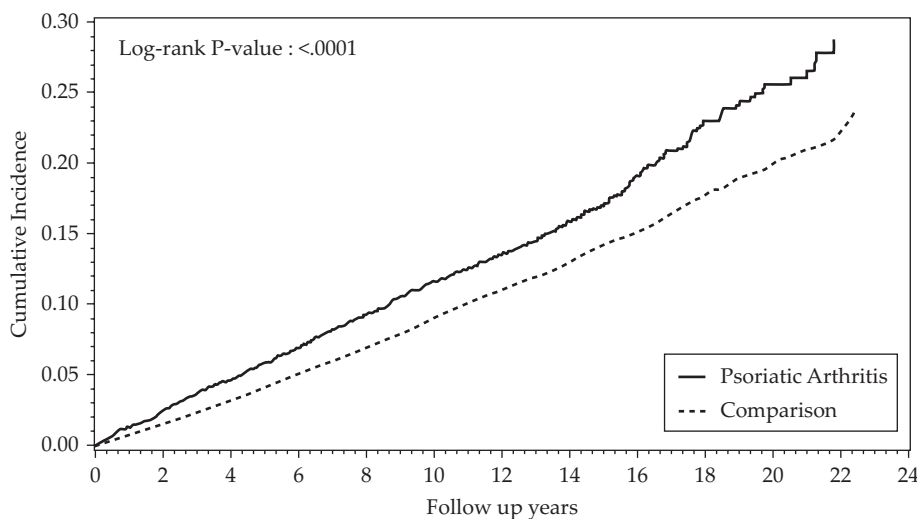


FIGURE 8.9 Cumulative incidence of cardiovascular diseases in PsA and non-PsA cohorts during prolonged follow-up. Adapted from Li et al. [164].

and metabolic syndrome than the general population [156,165]. In PsA patients who had cardiovascular disease compared to those without cardiovascular disease, the prevalence of hypertension (95% vs. 45%), dyslipidemia (75% vs. 27.7%), and diabetes (60% vs. 19%) was significantly higher (OR 21.0 for hypertension and 5.4 for DM) [166]. More patients with PsA have metabolic syndrome, which is associated with disease severity [167]. In a study in 1952 of PsA patients, hypertension was more prevalent compared to psoriasis patients [168]. Smoking was associated with cardiovascular disease in PsA patients [151].

3.4 Therapeutic Approaches

Psoriatic arthritis patients who are not treated with DMARDs are at increased risk of major adverse cardiac events (HR 1.2 95% CI 1.0–1.5) compared to untreated controls [160]. This is higher than in patients with only psoriasis not treated with DMARDs (HR 1.1, 95% CI 1.0–1.2), but lower than patients with RA not treated with DMARDs (HR 1.4, 95% CI 1.3–1.5). The use of methotrexate in PsA is associated with decreased risk of cardiovascular disease [169,170]. Among the biologics, data for tumor necrosis factor inhibitors suggest an overall reduction in cardiovascular events [171]. Other biologics that are now approved are ustekinumab (targeting IL-12/IL-23) and secukinumab (targeting IL-17A). Whether these agents also act on cardiac or cardiovascular disease has not been well studied to date.

4. THE HEART IN REACTIVE ARTHRITIS

4.1 Introduction

The term reactive arthritis (ReA) was introduced in 1969 [172] and is now commonly used instead of Reiter's syndrome (RS), a term that has been largely abandoned [173]. Despite some historical controversies there is some agreement about which factors matter when making a diagnosis [174,175]. Reactive arthritis is conventionally defined as an arthritis that arises following an infection, although the pathogens cannot be cultured from the affected joints. It is generally regarded as a form of SpA. The original definition did not specify the pathogens that were accepted as causes of ReA, but in 1999 a list of gastrointestinal and urogenital pathogens considered causative was published [175]. These included *Chlamydia trachomatis*, *Yersinia*, *Salmonella*, *Shigella*, and *Campylobacter*, and others have been added subsequently such as *Escherichia coli*, *Clostridium difficile*, and *Chlamydia pneumoniae*.

Two major clinical features that characterize ReA were identified:

- An interval ranging from several days to weeks between the antecedent infection and arthritis.
- A typically mono- or oligoarticular pattern of the arthritis, often involving the lower extremities, and sometimes associated with dactylitis and enthesitis.

Patients suspected of having ReA whose features initially or subsequently satisfy the Assessment of ASAS criteria for axial or peripheral SpA are also considered as having a form of SpA. A preceding episode of genital or gastrointestinal infection is included among the ASAS criteria that may support a diagnosis of peripheral SpA and the inclusion of a patient with ReA [1].

The literature search on "ReA and heart" revealed 318 hits, most of which were due to RS and almost none for ReA—with the exception of poststreptococcal ReA, which has been considered as a forme fruste of rheumatic fever (ARF) but this is not clear. The incidence of ARF, which is not subject of this chapter, is clearly decreasing and poststreptococcal ReA has become more prevalent [176].

4.2 Involvement of the Heart in Patients Previously Been Diagnosed With Reiter's Syndrome

This paragraph is mostly historical as severe forms of RS are rarely seen today. Even in 2003, it was noted that use of the term RS was decreasing [177]. However, the cases of heart involvement of RS reported since the introduction of the term RS in the Anglo-American literature in 1942 [178,179] closely resemble what has been reported for AS. The first case of this disease was published in 1946 describing an association of the disease with prolonged auriculoventricular conduction [180]. More cases on conduction abnormalities in patients with RS followed [181–187]. In parallel, several cases on aortic insufficiency in patients with RS were published [179,188–193], one with coronary artery stenosis [194] and one on a black woman who was HLA-B27-negative [193]. In conclusion, heart involvement in RS no longer plays an important role in the management of rheumatic diseases and documented earlier cases resemble those described in AS.

4.3 Involvement of the Heart in Reactive Arthritis

A study from Finland consisted of 18 consecutive adult patients with acute ReA, which were studied by the use of two-dimensionally guided M-mode and Doppler echocardiography. Aortic regurgitation was detected in one

patient [195]. Another patient had mild mitral regurgitation and left atrial dilatation. In addition, only two cases of heart involvement in ReA have been reported, both patients suffering from aortic insufficiency [196,197]. There have also been reports on aortic root thickening and subaortic fibrous ridging in ReA (Fig. 8.10) [198].

(A)



(B)



(C)

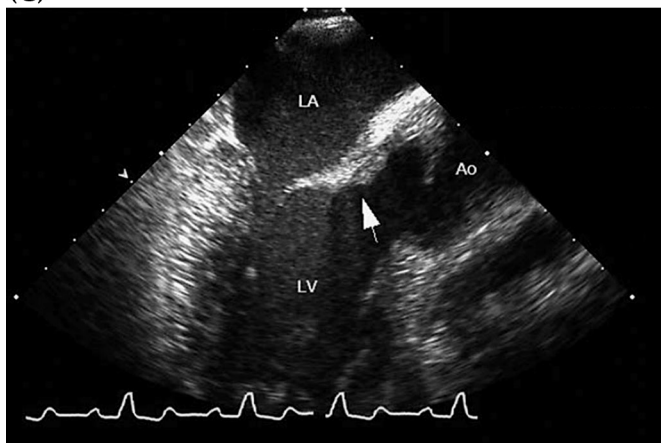


FIGURE 8.10 Transesophageal echocardiogram of a patient with ReA showing the aortic valve in short-axis, demonstrating diffuse thickening of the aortic wall (A; arrows) and aortic-mitral curtain (B; arrow), the so-called subaortic bump. In this particular patient, a subsequent study conducted 4 months later demonstrated massive thickening of the aortic-mitral curtain, extending to the body of the anterior mitral leaflet (C; arrow), while sparing the posterior mitral leaflet. Adapted from Novaro et al. [198].

In a large retrospective study performed in Brazil with 1472 patients diagnosed with SpA there were almost no cases with ReA and heart involvement [115]. In detail, 963 had AS, 271 PsA, 49 ReA, 48 arthritis associated with IBD, 98 uSpA, and 43 juvenile SpA. Cardiac involvement was reported in 44 patients (3.0%), of which only one was diagnosed with ReA. Pulmonary involvement was reported in 19 (1.3%), renal involvement in 17 (1.2%), and neurological involvement in 13 patients (0.9%). Most patients with visceral involvement had AS or PsA, and the mixed (axial + peripheral) and/or predominantly axial clinical form [115].

Due to all available data it seems clear that cardiac involvement in ReA is a rare event.

4.4 Poststreptococcal Reactive Arthritis

Poststreptococcal reactive arthritis (PSReA) is a recognized inflammatory articular syndrome that follows group A streptococcal infection in people not fulfilling the Jones criteria for the diagnosis of acute rheumatic fever (ARF). Characteristic features include nonmigratory arthritis, lack of response to aspirin or nonsteroidal antiinflammatory agents, and the presence of extra-articular manifestations, including vasculitis and glomerulonephritis.

Accordingly, in a recent editorial [176], ARF and PSReA were described to present differently—probably based on a different pathogenesis, but this is as yet unclear. Poststreptococcal reactive arthritis patients are generally older, have a shorter interval between infection and arthritis onset, and respond less dramatically to salicylates than ARF patients. The course of ARF may be complicated by carditis and valvular heart disease, but the course of PSReA is not. The course of PSReA is characterized by arthritis that, in contrast to ARF, is additive, nonmigratory, and is frequently chronic.

In a study with 17 adult patients, all with low socioeconomic status and severe arthritis starting shortly after a sore throat episode, extraarticular involvement including tenosynovitis, vasculitis, and glomerulonephritis was relatively common (29). More importantly, none exhibited clinical and/or echocardiographic evidence of cardiac involvement, and long-term antibiotic therapy was not given. In several other studies on children there was also no or very limited evidence of cardiac involvement, all without clinical significance [199–202].

In conclusion, echocardiographic studies in Caucasian adults and children with PSReA have revealed no clinically significant increase in valvular heart disease. There appears no indication for prophylactic antibiotic therapy.

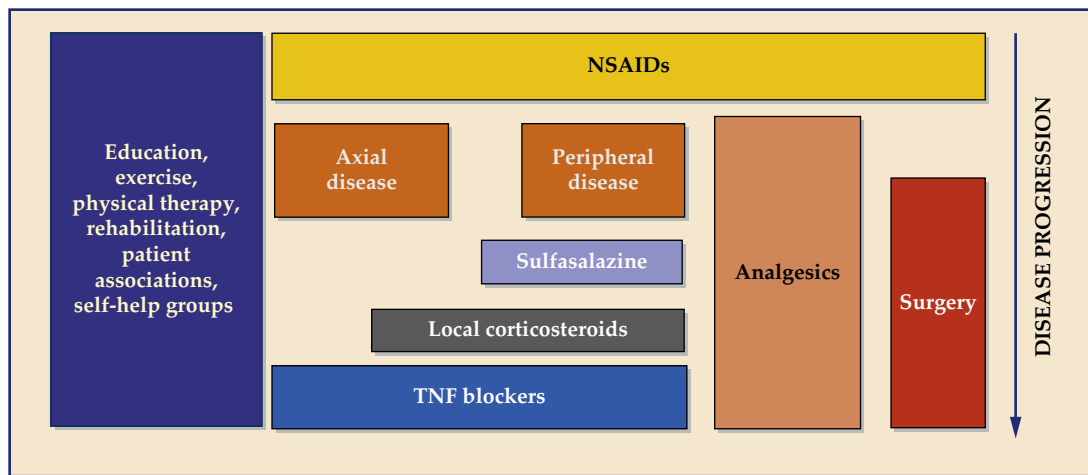


FIGURE 8.11 ASAS/EULAR recommendations for the management of AS. Adapted from Braun et al. [203].

4.5 Treatment of Spondyloarthritides

There are differences in the treatment of axial and peripheral spondyloarthritis including psoriatic arthritis.

For patients with axSpA there are recommendations from ASAS and EULAR [203,204] (Fig. 8.11) and for PsA there are recent EULAR recommendations and proposals made by the GRAPPA group [205,206]. In addition, there are treat-to-target recommendations for SpA [207].

Basically, there are NSAIDs, glucocorticoids, and conventional and biological DMARDs. Whether any of these treatments affect cardiac manifestations of SpA is not clear. However, it seems very likely that the suppression of inflammation will reduce the incidence of cardiovascular events [207]. Several datasets suggest that the antiinflammatory effects of TNF inhibitors are much more beneficial than glucocorticoids in patients who are at risk of CVD [171]. The use of NSAIDs in patients at risk should also be possibly avoided, but the use of naproxen may be safer in patients at risk [208]. However, this has not been found in other *meta*-analyses such as the one performed in patients with SpA [209]. An important factor to be considered in trials and *meta*-analyses is age, since SpA patients are often much younger than patients with osteoarthritis and many studies on NSAIDs have been performed in this population.

4.6 Treatment of Cardiac Disease

Treatment for coronary heart disease (CHD) is usually the same for both women and men. Treatment may include lifestyle changes, medicines, medical and surgical procedures, and cardiac rehabilitation.

The goals of treatment are to relieve symptoms, reduce risk factors in an effort to slow, stop, or reverse

the buildup of plaque, lower the risk of blood clots forming, widen or bypass plaque-clogged coronary (heart) arteries, and prevent CHD complications.

Cholesterol has been identified as a major risk factor for the development and progression of CHD. By blocking an enzyme in the formation of low-density lipoproteins (LDL), statins reduce levels of LDL in the blood and diminish the accumulation of lipids in arteries. Statins may exert a beneficial effect by decreasing inflammation and oxidative stress, both of which contribute to CHD and myocardial infarction.

Antihypertensive drugs include blockers of enzymes, receptors, or hormones and vascular channels such as angiotensin-converting enzyme (ACE) inhibitors, blockers of the adrenergic nervous system (β and α adrenergic blockers), calcium-channel blockers, and angiotensin-receptor blockers (ARBs). Diuretic agents have been strongly recommended for routine use in hypertension by the NIH (1). However, most patients with hypertension require multiple antihypertensive drugs for optimal blood pressure control. As with the reduction in LDL targets over time, the “normal” and “ideal” values for blood pressure have been progressively lowered as data support the finding that such blood-pressure reduction lowers the incidence of cardiovascular events.

A major breakthrough that proved lifesaving in the management of CHD was the advent of thrombolytic drugs and the use of aspirin. Thrombolytics dissolve clots in arteries, while aspirin prevents platelets from forming new clots. Several studies have identified a non-selective thrombolytic agent, streptokinase, as effective and generally safe for clot dissolution in acute situations such as myocardial infarction.

The EULAR has recommended assessing the annual cardiovascular risk in all patients with RA, AS, and PsA. Any cardiovascular risk factors identified should be managed according to local (national) guidelines. If no

such guidelines are available, cardiovascular risk management should be carried out according to the SCORE function*. In addition to appropriate cardiovascular risk management, aggressive suppression of the inflammatory process is recommended to further lower the cardiovascular risk.

Finally, the management of conduction disturbances and aortic valve disease is no different than diseases unrelated to SpA. It seems unlikely that antiinflammatory therapies will work when tissue degeneration such as fibrosis has already occurred.

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Polymyalgia Rheumatica

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1. INTRODUCTION

1.1 Epidemiology

First described in 1888 in subjects with “acute senile cases” belonging to “a different category from gout and rheumatism on the one hand and rheumatoid arthritis on the other” [1] and recognized in 1957 as a distinct disease [2], Polymyalgia rheumatica (PMR) represents the second most common inflammatory condition in the United States. The estimated annual incidence is 58.7 per 100,000 people over 50 years old with a prevalence of 711,000 people affected by the disease in the United States [3,4]. The disease incidence varies by geographical areas. Several studies have reported a higher incidence in Scandinavian countries and in Northern Europe; however, a lower incidence, ranging from 12.7 to 18.7 per 100,000 people, is seen in southern European countries such as Spain and Italy [5–7]. The incidence of PMR increases progressively with age, affecting only subjects aged more than 50 years with a reported mean age at diagnosis of 73 years [3,4,6]. Finally, the disease is more common in women, with a reported female to male ratio of 2–3:1. In the United States, the lifetime risk of developing the disease has been estimated at 2.43% for women and 1.66% for men [8].

1.2 Genetics, Etiology, and Pathogenesis

The etiology of PMR is unknown. Epidemiological studies support that a close interplay between genetic and environmental factors may have a role in disease pathogenesis and, although rare, a familial aggregation has also been described [9]. Specific genetic polymorphisms in human leukocyte antigen (HLA) genes and other genes related to immune system regulation have been reported

to be associated with PMR susceptibility. In subjects from northwestern Spain, tumor necrosis factor (TNF) microsatellite polymorphism b3 has been significantly associated with higher risk of disease appearance [10]. On the other hand, the association between HLA-DRB1 and isolated PMR is controversial, and genotype susceptibility to disease varies from one population to another. Some studies have described a significant association between HLA-DRB1*04 alleles and isolated PMR, while a weak but not significant association with the same alleles has been observed in other series [11]. In particular, isolated PMR was associated with HLADRB1*13/14 alleles in Northwest Spain and with increased frequency of HLA-DRB1*01 in native French patients [11].

The ethnic preponderance, seasonal variations, and cyclic pattern observed in the incidence, differences in geographical distribution within the same country, and the reported occurrence of the disease in married couples suggest a possible infectious or environmental trigger underlying disease pathogenesis or clinical appearance [12]. However, although higher incidence of disease appears to occur during *Mycoplasma pneumoniae*, Parvovirus B19, and *Chlamydia pneumoniae* infections or following influenza vaccination, to date no infectious etiology has been definitively confirmed [13–15].

Given the involvement of cytokines in the pathogenesis of the disease and the detection of proinflammatory cytokines in the interstitium of proximal limb muscles in PMR patients [16], several studies have been conducted to investigate whether cytokine gene polymorphisms may be associated with disease genetic susceptibility. In this setting, interleukin-10, -12, and -13 gene polymorphisms do not appear to be associated with disease susceptibility, phenotype, or severity in large population-based studies [17–19]. Similarly, there is insufficient evidence supporting a role

of Toll-like receptor (TLR) polymorphisms in disease susceptibility. It is well known that TLRs play a pivotal role in the activation and regulation of the innate and acquired immune responses. Moreover, TLR polymorphisms and activation have been implicated in the development of autoimmunity and chronic inflammation, including giant cell arteritis (GCA), a systemic inflammatory vasculopathy that may coexist with PMR. However, recent studies did not observe an association between certain TLR gene polymorphisms, including TLR4 and TLR9 variants, and disease susceptibility, inflammatory response, or clinical features [20,21].

Of note, recent findings suggest that, as with other chronic autoimmune conditions, an altered balance between immunosuppressive T-regulatory (Treg) lymphocytes and proinflammatory T-helper 17 (Th17) cells may characterize disease pathogenesis. In comparison to healthy subjects, patients with PMR display a significantly reduced frequency of circulating Treg cells and a significant increase in Th17 cells, suggesting that the shift in Th17 cell/Treg cell balance toward an increased Th17 cell response may be associated with the inflammatory systemic burden typical of the disease [22]. Moreover, emerging data also support B-cell involvement in disease pathogenesis. Patients with newly diagnosed PMR display a reduced number of circulating B cells compared to healthy subjects or patients with other chronic inflammatory diseases like rheumatoid arthritis (RA) [23]. In particular, circulating effector B cells, a specific B-cell subset known to induce T-cell activation via IL-6 secretion, have shown to be decreased in PMR at disease onset and to normalize following GC-induced remission, suggesting a contribution of B cells to the enhanced IL-6 response characterizing PMR [23].

Finally, it has been hypothesized that patients with PMR may have a relative adrenal insufficiency characterized by inadequate cortisol secretion in response to the inflammatory status [24]. In particular, several studies demonstrated reduced cortisol, adrenocorticotrophic hormone, androstenedione, dehydroepiandrosterone and 17-hydroxyprogesterone in PMR. However, it is unknown whether this hormonal deficiency is implicated in pathogenesis of the disease or whether it represents an endocrinological response to chronic inflammatory disease [25–27].

2. CLINICAL PRESENTATION AND DIAGNOSTIC CRITERIA

2.1 Clinical Presentation

Polymyalgia rheumatic remains a clinical enigma and diagnosis is mainly based on clinical judgment [28]. The hallmark clinical picture of PMR is characterized by

pain and stiffness lasting more than 30 min and worsening after rest at the shoulder girdle, hip girdle, and neck muscles. Shoulder pain is usually reported by nearly all patients, and neck and pelvic girdle are involved in approximately 70% and 50% of patients, respectively. The pain may be initially unilateral but subsequently becomes typically bilateral and usually has a sudden onset. Constitutional symptoms, including fatigue, malaise, anorexia, depression, weight loss, and low-grade fever can occur in 40–50% of patients. Moreover, about a fourth of patients may develop distal musculoskeletal manifestations including peripheral symmetrical arthritis mainly involving knees, wrists, and small joints of the hands. In this setting, a symmetrical, nonerosive metacarpophalangeal and proximal interphalangeal arthritis warrants consideration of a diagnosis of elderly onset, usually seronegative, RA [29–31]. Finally, a subset of patients may display swelling and pitting oedema of the hands and feet due to extensor tendon tenosynovitis, a peculiar picture referred to as RS3PE (remitting seronegative symmetrical synovitis with pitting oedema) syndrome [32].

About 20% of patients with PMR have concomitant GCA, a large- and medium-sized vessel vasculitis characterized by inflammatory involvement of the external carotid branches, including temporal and occipital arteries, the ophthalmic, vertebral, distal subclavian, and axillary arteries. Patients with PMR and symptoms suggestive of vasculitis such as new onset headache, jaw claudication, scalp tenderness, visual disorders, carotidynia, and limb claudication should quickly undergo diagnostic testing to evaluate for concomitant GCA [29–31].

Increased risk of malignant disease has been depicted in PMR patients in comparison to control subjects. The risk appears to be higher in the first 6–12 months after diagnosis and in patients with atypical symptoms and lack of response to low-dose GC therapy [33,34]. However, mortality due to neoplasm does not appear to be increased in patients with PMR in comparison to matched healthy subjects [35,36].

Several conditions may mimic PMR and should be considered in the diagnostic approach to a subject displaying proximal inflammatory muscular pain and stiffness. Careful clinical history and physical examination are important in distinguishing PMR from other mimicking diseases, including inflammatory and noninflammatory musculoskeletal disorders, infectious diseases, endocrinopathies, malignancies, and other disorders like hypovitaminosis D, depression, and drug-induced myopathy [29,31]. In this setting, slow gradual onset of pain, no involvement of the shoulders, absence of stiffness, predominant systemic manifestations, normal or very markedly elevated inflammatory laboratory markers, and peripheral arthritis are considered warning signs to be evaluated in the differential diagnosis of PMR [37].

The long-term prognosis of PMR is uncertain. A few studies have analyzed mortality in patients with PMR and conflicting results were reported due to different study design [4,36]. Prospective studies, however, have demonstrated that mortality from all causes is not significantly increased in patients with PMR compared to matched controls [35,38].

2.2 Diagnostic Criteria

The diagnosis of PMR is clinical and relies on a combination of typical clinical symptoms, increased inflammatory markers, favorable response to GC therapy, and exclusion of PMR mimics. The lack of diagnostic tests specific to the disease represents an undeniable challenge. Over the years, several sets of diagnostic criteria have been proposed (Table 9.1) [39–42].

Most of criteria include an age cutoff greater than 50–65 years [39,40,42]. The presence of bilateral shoulder and hip girdle pain, morning stiffness, and raised erythrocyte sedimentation rate (ESR) represent other common criteria. Bird's criteria have been demonstrated to have good sensitivity but the lowest specificity with respect to other sets in differentiating PMR from total controls [43]. In this setting, Healey and Chuang criteria appear to perform better as diagnostic criteria and to equally discriminate well between PMR and both total controls and patients with other inflammatory rheumatic disorders including RA [39,42]. Moreover, Healey criteria included the rapid response to low-dose GC therapy as a confirmation of diagnosis [42]. However, recent studies have raised concerns about the validity of rapid responsiveness to GC therapy as a diagnostic tool, highlighting that about one-third of patients receiving standard treatment still report proximal muscle pain and morning stiffness at the 3 week assessment and that the majority of patients display a complete response to GC therapy only at 4 weeks [44].

Challenges in PMR diagnosis also relate to the performance of laboratory tests. Indeed, laboratory findings in PMR are nonspecific and reflect the systemic inflammatory burden of the disease. Such abnormalities can include mild anemia related to chronic inflammation, leucocytosis, and raised markers of inflammation (ESR and C-reactive protein) [29,30,45]. However, a normal ESR rate has been reported in 6–20% of patients with the disease [45]. C-reactive protein is considered to be a more sensitive marker of inflammation than ESR and should be used to monitor the response to therapy [30]. Autoantibodies, including rheumatoid factor, and antibody to cyclic citrullinated peptide, are usually negative, and if positive, should raise the suspicion for late-onset RA [29,30,45]. In addition, other laboratory tests should be performed in the assessment of patients with suspected PMR in order to exclude other disorders that

TABLE 9.1 Sensitivity and Specificity of All Classification Criteria for PMR

Criteria	Sensitivity (%)	Specificity (%)
BIRD [40]		
Any three or more: <ul style="list-style-type: none"> • age ≥ 65 years • onset ≤ 2 weeks • bilateral shoulder pain and/or stiffness • bilateral upper arm tenderness • ESR ≥ 40 mm/h • morning stiffness ≥ 1 h • depression and/or weight loss 	89	40
JONES [41]		
All of the following: <ul style="list-style-type: none"> • shoulder and pelvic girdle pain without muscle weakness • symptom duration > 2 months • morning stiffness • ESR > 30 mm/h or C-reactive protein > 6 mg/L • negative rheumatoid factor • absence of muscle disease, inflammatory arthritis, malignant disease • prompt response to glucocorticoids 	63	97
CHUANG [39]		
All of the following: <ul style="list-style-type: none"> • age ≥ 50 years • bilateral aching and stiffness ≥ 1 month affecting two of the following areas: neck or torso, shoulder or arm proximal region, hips or proximal thighs • ESR > 40 mm/h • exclusion of other diagnosis except for giant cell arteritis 	77	81
HEALEY [42]		
Age > 50 years + any three: <ul style="list-style-type: none"> • pain in the neck, shoulder or pelvic girdle • morning stiffness > 1 h • raised ESR • rapid response to prednisone ≤ 20 mg/day 	80	81

often mimic the disease, including thyroid stimulating hormone, calcium, serum protein electrophoresis, creatine phosphokinase, transaminases, and urinalysis. In particular, raised creatine phosphokinase or persistent increased liver function tests in the setting of muscle weakness should prompt investigation for myopathies or an underlying thyroid disorder.

In recent years imaging tools have been employed to assess patients with suspected PMR in order to increase specificity of the diagnosis. Increased use of imaging modalities such as ultrasonography and magnetic resonance imaging (MRI) have allowed the detection of periarticular structural abnormalities such as bilateral subacromial-subdeltoid and trochanteric bursitis,



FIGURE 9.1 Ultrasonography. Transverse ultrasound scan of shoulder of a patient with active PMR showing mild long head biceps tenosynovitis (asterisk) and distension of subachromial bursa (arrows). Adapted from the personal collection of the authors.

bicipital tenosynovitis, and glenohumeral or hip joint effusions (Fig. 9.1) [46]. However, it is unclear how these imaging signs are able to discriminate patients with PMR from those with RA and from unaffected controls. Moreover, there is limited evidence on the use of ultrasonography to assess disease activity or to monitor response to therapy in the individual patient. Indeed, more than half of patients judged to be in clinical remission or to have low disease activity had persistent inflammatory findings on ultrasonography at follow-up [29,30]. Positron emission tomography (PET) has been used as an imaging modality for patients with active disease. Enhanced fluorodeoxyglucose (FDG) uptake has been detected in the shoulders, hips, and the cervical and lumbar interspinous processes of patients with PMR. Moreover, about one-third of patients with apparently isolated PMR display subclinical vascular inflammation, especially in the subclavian arteries, suggesting that PET or magnetic resonance angiography might be useful in the assessment of patients with suspected large-vessel vasculitis [46]. FDG-PET is not routinely recommended in the evaluation of patients in routine clinical practice. However, it has been found to be useful to disclose the presence of large-vessel vasculitis in some patients with PMR who do not achieve remission or with persistently elevated inflammatory markers and patients with PMR and severe constitutional symptoms. Finally, temporal artery biopsy is not routinely indicated in the diagnostic assessment of patients in the absence of clinical symptoms or findings suggestive of cranial GCA [29,30].

To overcome all these diagnostic challenges and in response to the lack of standardized criteria for PMR, an international collaborative initiative between the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) developed a

TABLE 9.2 European League Against Rheumatism and American College of Rheumatology Provisional Classification Criteria for PMR [47]

Required criteria: age ≥ 50 years, bilateral shoulder pain, and abnormal ESR and/or C-reactive protein	
Clinical Criteria	Points
• morning stiffness >45 min	2
• hip pain or restricted range of motion	1
• negative rheumatoid factor and antibody to cyclic citrullinated peptide	2
• absence of other joint involvement	1
Ultrasound Criteria	Points
• ≥ 1 shoulder with subdeltoid bursitis, biceps tenosynovitis, or glenohumeral synovitis + ≥ 1 hip with synovitis or trochanteric bursitis	1
• both shoulders with subdeltoid bursitis, biceps tenosynovitis, or glenohumeral synovitis	1
• with only clinical criteria: score ≥ 4 sensitivity = 68% and specificity = 78% for discriminating PMR from comparison patients	
• combination of clinical + ultrasound criteria: score ≥ 5 sensitivity = 66% and specificity = 81% from discriminating PMR from comparison patients	

ESR, erythrocyte sedimentation rate.

set of classification criteria based on prospective evaluation of a cohort of patients with new onset PMR and a cohort of patients with other disorders mimicking PMR (Table 9.2) [47]. Application of these criteria in a cohort of patients aged 50 years or older presenting with new onset bilateral shoulder pain (not explained by alternative diagnosis) and raised inflammatory markers yielded a sensitivity of 68% and a specificity of 78% for the diagnosis of PMR. The addition of ultrasonography to the classification criteria improved the specificity to 81% in the discrimination of PMR from non-PMR patients and to 89% in discrimination of PMR from other shoulder disorders at the expense of a reduction in sensitivity to 66% [47]. The ACR/EULAR taskforce suggested the concept of polymyalgic syndrome, which can indicate PMR or other diseases. According to this concept, the diagnostic strategy must be expanded considerably and exclusion criteria must be ruled out. Finally, standardized treatment with 15 mg/day of prednisone is given. Thus, the definite diagnosis of PMR can be established only at the end of follow-up, usually after 12–18 months of treatment, when the patient is fully recovered with no relapse following GC discontinuation. The target population is patients aged 50 years or older presenting with less than 12 weeks onset of bilateral shoulder pain with abnormal acute-phase response. The criteria may only be applied to those patients in whom the symptoms are not better explained by an alternative diagnosis. Mimicking

conditions, including inflammatory and noninflammatory ones, should be ruled out. EULAR/ACR criteria have been demonstrated to perform better than other criteria in discriminating between patients with PMR and those with RA or other inflammatory diseases [48]. However, prospective validation studies in other cohorts are needed to better evaluate their sensitivity and specificity. Moreover, they are classification rather than diagnostic criteria primarily intended to select patients with definite disease for inclusion in clinical trials and to study disease outcomes in homogeneous patient cohorts.

3. PATHOPHYSIOLOGY OF POLYMYALGIA RHEUMATICA ASSOCIATED CARDIOVASCULAR INVOLVEMENT

Inflammatory and autoimmune systemic diseases have been associated with an increased risk of cardiovascular (CV) involvement, and several pathophysiologic mechanisms have been demonstrated to contribute to such increased risk. In particular, a close interplay between traditional CV risk factors, chronic inflammation, and autoimmune system dysregulation may be advocated as mechanisms involved in the induction and progression of atherosclerotic vascular damage [49]. Recently, an altered balance between endothelial microparticle (EMP) release and endothelial progenitor cell (EPC) generation was demonstrated in patients

with systemic autoimmune diseases and recognized as a potential surrogate marker of endothelial injury, CV risk, and metabolic risk factors in the general population [50–52].

Physiopathogenetic factors contributing to CV involvement in PMR have not been extensively investigated. However, some plausible mechanisms underlying vascular damage in PMR can be hypothesized and may include endothelial dysfunction, damage due to the systemic inflammatory burden of the disease and the association of PMR with overt inflammatory arteritis (GCA). Moreover, long-term cumulative exposure to GC treatment should be considered a relevant contributor to the CV damage in patients with PMR. Indeed, GC therapy, especially at high doses, may exert harmful CV effects by increasing the risk due to deleterious effects on lipids, glucose tolerance, weight gain, and hypertension.

3.1 Biochemical Markers for Endothelial Injury

Under physiological conditions, vascular endothelium exerts a pivotal role in arterial wall integrity maintenance by regulation of inflammatory cell infiltration in the vessel wall, thrombus formation, and smooth muscle cell proliferation. Endothelial layer reparative potentiality is maintained by EPC ability to form new blood vessels through a process of vasculogenesis by homing on sites of vascular injury (Fig. 9.2A) [53]. Of note, a decrease in absolute number of EPC, altered functional phenotype, or reduced migratory ability have been associated with

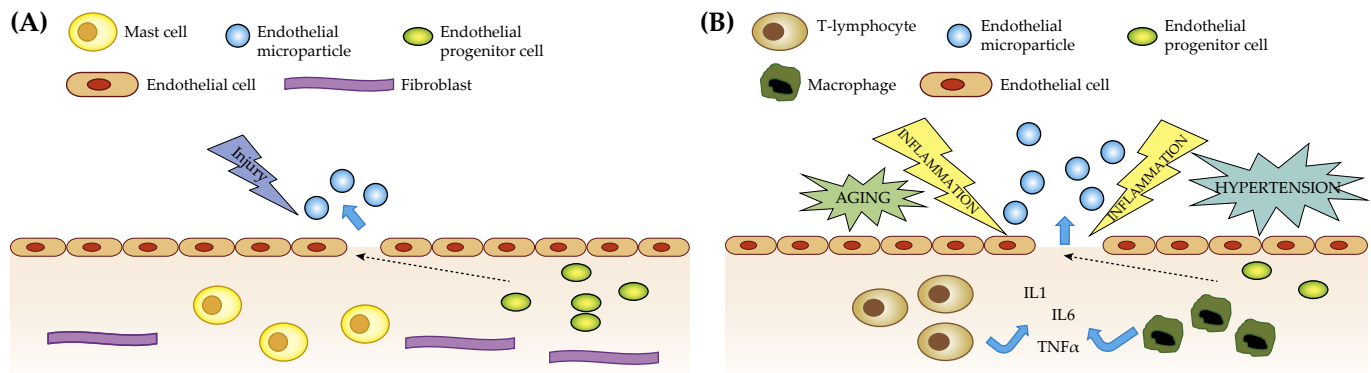


FIGURE 9.2 Schematic representation of hypothesized mechanisms underlying subclinical atherosclerosis in PMR. (A) Under physiologic condition, normal endothelial layer structure and function may be impaired under the action exerted by potentially damaging pathogenic triggers, with release of EMPs into bloodstream. Endothelial microparticles are shed into the peripheral circulation upon endothelial cell activation, apoptosis, and injury. The release of EMPs is a highly controlled process that is driven by different stimuli such as shear stress, proapoptotic stimulation, and damage. Under basal conditions, endothelial cells may also release EMPs spontaneously. Moreover, they may have important functional properties, regulating inflammation, vascular function, and coagulation. In consequence of endothelium damage, endothelial progenitor cells migrate to the site of vascular injury promoting arterial wall repair. Arterial layer resident mast cells allow immunologic tolerance against external pathogenic factors. (B) In PMR, disease-related systemic inflammatory burden, in association with aging and high arterial pressure, is associated with increase of aortic stiffness. The on-going systemic inflammation may induce endothelial damage and fragmentation, with high release of endothelial microparticles, associated with repair impairment due to reduced availability of endothelial progenitor cells. Following endothelial damage, inflammatory cells may migrate from the blood across the activated endothelium. The inflammatory infiltrate within the arterial wall is mainly characterized by macrophage and T-cell producing pro-inflammatory cytokines and contribute to impaired endothelial function in PMR. Adapted from the personal collection of the authors.

increased CV risk both in the general population and in patients with systemic autoimmune diseases [54,55]. Moreover, endothelial wall pathologic activation can lead to cell membrane disruption by apoptosis and EMP release. It is well established that abnormal EMP release is associated with alterations in endothelial homeostasis (Fig. 9.2B). In this setting, increased levels of circulating EMPs have been observed in patients with stable or unstable coronary artery disease, stroke, and peripheral artery disease, and changes in plasma EMP levels have been associated with higher risk of CV diseases [56]. The altered ratio between increased EMP release and defective EPC repair ability support the use of these factors as potential biomarkers of CV disease and as potential predictors of major CV events.

A significantly higher EMP/EPC ratio has been demonstrated in patients with recent onset PMR, in the absence of traditional CV risk factors including type 2 diabetes mellitus, uncontrolled arterial hypertension, and history of CV disease, in comparison to age and sex-matched healthy subjects [57]. The increased ratio was the result of both an increased number of circulating EMPs and a reduced number of EPCs. Moreover, the EMP/EPC rate directly correlated with parameters of systemic inflammation, suggesting that the inflammatory burden characterizing the disease at onset may induce endothelial damage and fragmentation and impaired repair due to reduced EPC availability. Of interest, a significant reduction of EMP/EPC ratio resulting from both a reduction of EMP and an increase of EPC number was observed following 1 month of GC treatment [57]. In this setting, given the pivotal role of inflammation in atherogenesis, effective control of systemic inflammation may be regarded as a plausible tool to reduce atherosclerotic risk in PMR.

Other markers of endothelial damage have been investigated in PMR patients. Plasma concentration of plasminogen activator inhibitor (PAI), an inhibitor of fibrinolysis, and of von Willebrand factor (vWF), a recognized marker of vascular damage, have been shown to be increased in untreated new-onset PMR patients [58,59]. Of interest, vWF-antigen has been identified in the luminal endothelium of temporal arteries of seven PMR patients and in the lamina elastica of the arterial wall in GCA patients [60]. Both vWF and PAI have been demonstrated to act as acute phase proteins. However, unlike acute phase proteins, levels of vWF and PAI do not decrease with GC therapy and remain high for years after disease diagnosis, suggesting that persistently high levels of both factors might reflect a subclinical disease with persistent endothelial activation [58,61]. Moreover, significantly higher levels of homocysteine, not related to the inflammatory process, were demonstrated in 39 active PMR patients in comparison to healthy controls [62]. Indeed, hyperhomocysteinemia may induce

vascular disease by two main mechanisms. First, it has adverse effects on endothelial cells, causing cell damage, smooth muscle cell proliferation, and increased oxidative stress. Second, it induces a procoagulant condition, which interferes with the coagulation mechanism [63]. In this setting, hyperhomocysteinemia may be considered an adjunctive risk factor for atherosclerosis in PMR patients.

Intriguingly, in spite of a well-known association between antiphospholipid (aPL) antibodies and thrombotic events in patients with systemic connective diseases, including systemic lupus erythematosus and primary aPL syndrome, literature data do not allow establishing a definitive role of aPL antibodies in ischemic event induction in GCA/PMR patients. Indeed, higher prevalence of aPL, in particular anticardiolipin antibodies, has been demonstrated in GCA and PMR patients with respect to healthy subjects [59,64,65]. However, a clear relationship between aPL antibodies and thrombosis in GCA and PMR has not been depicted in such studies, suggesting that aPL appearance in these patients, rather than an etiologic agent of ischemic manifestations, may be the expression of endothelial phospholipid exposure due to vascular inflammation.

Finally, plasma levels of adrenomedullin, a 52-aminoacid peptide produced by vascular endothelium in normal subjects and an indirect marker of systemic inflammation and endothelial injury in several rheumatic diseases, have been demonstrated to be significantly elevated in a small cohort of GCA patients in comparison to PMR ones and healthy subjects and to correlate with parameters of inflammation. However, no difference in adrenomedullin concentration was depicted between PMR patients and healthy subjects, probably reflecting the different severity of vascular endothelial damage in these conditions [66].

3.2 Systemic Inflammation and Atherosclerotic Risk

A growing body of evidence supports the role of inflammation in the induction and progression of atherosclerosis. Increased CV risk has been observed in several conditions associated with systemic inflammation, including autoimmune diseases and chronic infection, while levels of systemic inflammatory biomarkers, including CRP, predict CV disease [67]. Indeed, in RA, the prototype of chronic inflammatory diseases, disease activity, and severity are positively related to higher CV disease risk [68]. Moreover, in patients with chronic rheumatic disorders systemic inflammation exhibits pro-atherogenic effects by induction of dyslipidemia, insulin resistance, endothelial dysfunction, and oxidative stress and may exert an important role in the each stage of atherosclerotic damage development [69]. Furthermore,

systemic inflammation may also be associated with hypercoagulability, owing to several factors such as thrombocytosis and elevation of fibrinogen and vWF. Indeed, it is plausible that, although the cause–effect relationship between systemic inflammation and atherosclerosis might differ between low-grade and high-grade and between acute and chronic inflammation, the high systemic inflammatory burden characterizing PMR at onset may be considered as an adjunctive mechanism underlying endothelial dysfunction and vascular damage. In this setting, interleukin (IL)-6, a proinflammatory cytokine produced by activated macrophages, endothelial cells, and vascular smooth muscle cells, has been recognized to exert a relevant role in promoting all stages of atherosclerosis [70]. Moreover, IL-6 has been demonstrated to be a valuable predictor of subclinical atherosclerosis and of overt CV disease in different pathologic conditions [71]. Significantly increased levels of serum IL-6 have been demonstrated in newly diagnosed PMR patients with respect to controls and cytokine concentration correlated with disease activity [72]. In this setting, it may be hypothesized that IL-6 plays a role in atherosclerotic vascular damage in PMR patients. Inflammation may also increase arterial stiffness and damage through structural changes of the arterial wall. Indeed, inflammatory cell, in particular macrophage and T lymphocyte, infiltration within the vessel wall may induce arterial stiffening, damage of elastic fibers, and changes in smooth muscle tone and extracellular matrix. Of interest, macrophage-derived and T lymphocyte-derived cytokines have been depicted in temporal artery biopsy specimens of PMR patients without arteritis [73].

3.3 Inflammatory Arteritis

It is well known that approximately 15–30% of patients with PMR develop GCA and that PMR occurs in about 50% of patients with GCA, a disease characterized by inflammatory involvement of large- and medium-sized arteries [31]. Subclinical vasculitis has been documented in patients with PMR in the absence of overt clinical features suggestive for coexisting GCA [74]. Elevated FDG accumulation in large vessels, in particular the subclavian arteries, is a frequent finding on 18-FDG PET/CET in patients with apparently isolated PMR and correlates with laboratory inflammatory parameters [75,76]. The finding of large-vessel vasculitis is specific to PMR and is not seen in other inflammatory conditions [76]. Of interest, a greater proportion of patients with GCA and involvement of upper extremity large arteries had a previous diagnosis of PMR compared to patients with cranial GCA [77]. These data suggest that, in a proportion of patients, PMR is not isolated and may be the expression of underlying large-vessel vasculitis, as demonstrated in nearly 40% of patients with GC-resistant PMR [78].

The pathogenesis of vascular inflammatory involvement in PMR is unclear. Small-vessel vasculitis, identified as aggregates of mononuclear cells surrounding a small capillary distant from an uninflamed temporal artery, was a microscopic finding frequently detected in patients with PMR and in those with GCA presenting as PMR symptoms [79]. Moreover, *in situ* production of messenger RNA for macrophage- and T cell-derived inflammatory cytokines has been demonstrated in the temporal artery biopsy of patients with PMR despite the lack of microscopic evidence of arteritis, suggesting low-grade vascular inflammation [73]. However, a vascular FDG uptake consistent with vasculitis was depicted in only 6–14% of these patients [75,76,80]. To further support the vasculitic nature of PMR, a significantly higher uptake of gallium-67 by single photon emission tomography scintigraphy in the temporal arteries has been depicted in a small population of isolated PMR patients in comparison to controls [81].

Overall, the exact frequency and clinical significance of subtle vascular inflammation in patients with PMR remains unclear.

4. CARDIAC INVOLVEMENT

Clinical manifestations of CV involvement in patients with PMR have largely been unexplored, with limited data available to date. Subjects with PMR do not appear to have an increased risk of CV mortality since long-term survival in PMR has been demonstrated to be similar to that reported in the general population [38,82,83]. A previous report suggesting increased mortality from vascular diseases in men with PMR during the first 2 years after diagnosis has not been confirmed in subsequent studies [36]. In particular, a clear difference between the rates of CV mortality, related to stroke and coronary heart disease, in patients with PMR as compared to the general population rates has not been demonstrated [35,84]. On the other hand, it is worth noting that CV comorbidity accounts for incremental direct medical costs related to PMR, suggesting that long-term consequences related to CV events contribute substantially to the costs of care for patients with PMR [85]. In this context, there is some evidence that patients with PMR may be characterized by a significantly higher risk of incident major vascular events with respect to the general population, independent of traditional CV risk factors [84]. However, factors contributing to the increased risk have been poorly investigated and many confounders, including age, comorbidity, dosage of GC therapy, and concomitant drugs, hamper data analysis and interpretation.

4.1 Prevalence of Traditional Risk Factors for Accelerated Atherosclerosis

Traditional CV risk factors are well-recognized relevant players in the induction and progression of atherosclerosis and are considered significant predictors of CV events and mortality in the general population. Moreover, they have been demonstrated to be more prevalent in patients with systemic autoimmune diseases, to contribute to subclinical atherosclerosis, and to be useful tools for prediction of CV events [86,87]. Studies specifically aimed at analyzing the contribution of traditional CV risk factors in the risk of vascular events in PMR are lacking. Some studies have reported a variable prevalence of CV risk factors in patients with PMR (shown in Table 9.3) [85,88–90]. However, the prevalence of traditional CV risk factors was similar in patients with PMR compared with comparator populations. A statistically significant increased prevalence of all traditional CV risk factors was reported only in a Japanese PMR cohort in comparison to the general population [89]. With respect to the other studies, this is a retrospective evaluation of patients with PMR treated with GC therapy. Although data on mean dose and duration of GC therapy were not provided, it is plausible that prolonged GC therapy could have increased the prevalence of diabetes mellitus, hypertension, and dyslipidemia in patients with respect to the control population. Moreover, lack of data regarding smoking status and concomitant lipid-lowering and antihypertensive therapy in most of the studies hamper data interpretation. Similarly, the role of these traditional CV risk factors in the induction of atherosclerotic damage and in the risk of overt CV events has not been clarified.

4.2 Markers for Atherosclerosis and Endothelial Dysfunction

Endothelial dysfunction represents the very early stage of atherosclerosis, is associated with aging and CV risk factors, and predicts CV morbidity and mortality. Of consequence, evaluation of endothelium function may represent a useful tool for investigating the etiopathogenesis of CV disease by employment of either invasive, including flow-dependent or independent vasodilation studies, or noninvasive tools, such as biomarkers of endothelial damage. Similarly, arterial stiffness, evaluated by carotid-to-femoral pulse-wave velocity (PWV), reflects early changes in mechanical wall properties that predispose to major CV disease. Indeed, arterial stiffness has been identified as an independent predictor of CV morbidity and mortality in the general population [91]. Recently, arterial stiffness was also identified as a reliable surrogate marker

of CV risk in patients with systemic autoimmune diseases. Of note, both traditional CV risk and disease-related inflammatory and autoimmune factors seem to contribute to the increased arterial stiffness in these patients [92].

Aortic stiffness has been evaluated in a cohort of new onset PMR patients free from prevalent CV disease and traditional CV risk factors, including tobacco smoke, diabetes mellitus, and treated hypertension. Patients were characterized by significantly increased carotid-femoral PWV with respect to age, sex, and blood pressure matched controls (Fig. 9.3). On the other hand, no significant difference was observed between patients and controls in carotid-radial PWV and augmentation index, the latter defined as the difference between the second and first peaks of the central arterial waveform expressed as a percentage of the pulse pressure. This index is considered a composite measure reflecting intensity and timing of pulse-wave reflection from peripheral sites and is influenced by endothelial dysfunction, arterial compliance and heart rate [93]. Of interest, the disease itself resulted a major determinant of aortic stiffness, in particular age and mean arterial pressure. In patients, aortic PWV correlated directly with laboratory markers of systemic inflammation and decreased significantly following 1 month of GC therapy (Fig. 9.4). Furthermore, the change in aortic PWV showed a direct correlation with percentage change in C-reactive protein (Fig. 9.5). Such findings suggest that PMR patients may have an increased risk of accelerated atherosclerosis and that the inflammatory burden characterizing disease onset may play a relevant role in increasing aortic stiffness. Of importance, the disease itself may be considered an adjunctive causative factor, in association with age and mean arterial pressure, in determining the increased value of PWV in this population. Short-term GC therapy may have a beneficial effect on vascular function, as further supported by the demonstration of a significant reduction of augmentation index in a small PMR cohort following 1 month of steroid treatment [94].

4.3 Echocardiographic and Cardiac MRI Findings

A case report of myocardial inflammation detected by cardiac MRI was demonstrated in a patient with PMR complaining of persistent tachycardic episodes. Echocardiogram revealed diffuse left ventricular hypokinesia with a reduced left ventricular ejection fraction. A diffuse increased T2-weighted ratio of myocardial to skeletal muscle signal suggested myocardial edema. Interestingly, myocardial inflammatory

TABLE 9.3 Risk of Cardiovascular Events in PMR

Study	Group	n	Gender (F/M)	Age (years) mean ± sd	CV comorbidities	FU	Previous CV events	Testing method	Risk	Comments	References
STUDIES REPORTING CHD RISK											
Prosp	PMR	193	125/68	74 ± 9	DM (14%)	5 year Median	Yes	Electronic medical records	OR = 1.78 ^a (1.13,2.82)	>Costs related to CV morbidity in PMR	[85]
	Control	695	445/250	72 ± 10	DM (11%)		Yes				
Prosp	PMR	21.351	14188/7163	NR	DM, HTN, obesity	>10 years	No	CHD (ICD codes 7–10)	SIR = 1.57 ^a (1.53,1.62)	Persistent risk at >10 years	[105]
	Control	Tot pop	NA	NA			NA				
Prosp	PMR	3.249	2356/893	72 ± 9	Smoke (43%) HTN (51%) DM (13%) DysLip (16%)	7.8 years Mean	No	MI, angina (ICD- 9-CM codes)	HR = 2.7 ^a (2.4,3.0)	>Risk in first 6 months /<60 years	[90]
	Control	12.735	9245/3490	72 ± 9	Smoke (41%) HTN (41%) DM (10%) DysLip (11%)		No				
STUDIES REPORTING CEREBROVASCULAR EVENT RISK											
Prosp	PMR	193	125/68	74 ± 9	DM (14%)	5 years Median	Yes	Electronic medical records	OR = 1.6 ^a (1.08,2.39)	>Costs related to CV morbidity in PMR	[85]
	Control	695	445/250	72 ± 10	DM (11%)		Yes				
Retro	PMR	781	302/479	69 ± 11	DM (21%) ^a HTN (46%) ^a DysLip (26%) ^a	3 years	No	Stroke (ICD-9-CM 430–438)	HR = 2.3 ^a (1.8,2.9)	>Risk in PMR with DM, HTN, DysLip	[89]
	Control	3.905	1510/2395	69 ± 11	DM (16%) HTN (38%) DysLip (20%)		NR				
Prosp	PMR	16.496	11.183/5.313	NR	DM, HTN, obesity, CHD	>10 years	No	Stroke (ICD-9-CM 430–438)	SIR = 1.54 ^a (1.47,1.6)	>Risk first year after hospitalization	[107]
	Control	Tot pop	NA	NA			NA				
Prosp	PMR	3.249	2356/893	72 ± 9	Smoke (43%) HTN (51%) DM (13%) DysLip (16%)	7.8 years Mean	No	Stroke, TIA (ICD- 9-CM codes)	HR = 2.3 ^a (2.0,2.6)	>Risk in first 6 months /<60 years	[90]
	Control	12.735	9245/3490	72 ± 9	Smoke (41%) HTN (41%) DM (10%) DysLip (11%)		No				
STUDIES REPORTING PAD RISK											
Prosp	PMR	193	125/68	74 ± 9	DM (14%)	5 years Median	Yes	Electronic medical records	OR = 2.21 ^a (1.37,3.6)	>Costs related to CV morbidity in PMR	[85]
	Control	695	445/250	72 ± 10	DM (11%)		Yes				
Retro	PMR	364	237/127	73 ± 10	DM (26%) HTN (70%) DysLip (36%)	11 years Median	No	PAD§	HR = 2.5 ^a (1.5,4.1)	Similar survival PMR- PAD /non PAD	[88]
	Control	728	476/252	73 ± 9	DM (24%) HTN (67%) DysLip (32%)		No				
Prosp	PMR	3.249	2356/893	72 ± 9	Smoke (43%) HTN (51%) DM (13%) DysLip (16%)	7.8 years Mean	No	Intermittent claudicatio, ischemia	HR = 2.8 ^a (2.2,3.5)	>Risk in first 6 months /<60 years	[90]
	Control	12.735	9245/3490	72 ± 9	Smoke (41%) HTN (41%) DM (10%) DysLip (11%)		No				

FU, follow-up; CHD, coronary heart disease; NR, not reported; NA, not applicable; DM, diabetes mellitus; HTN, hypertension; DysLip, dyslipidemia; PAD, peripheral artery disease; *prosp*, prospective; *retro*, retrospective. § ankle-brachial index \leq 0.90 in either leg OR intermittent claudication/or ischemic pain at rest with pulse absence OR peripheral artery surgery or lower extremity amputation OR aortoiliac stenosis.

^asignificant in PMR versus controls.

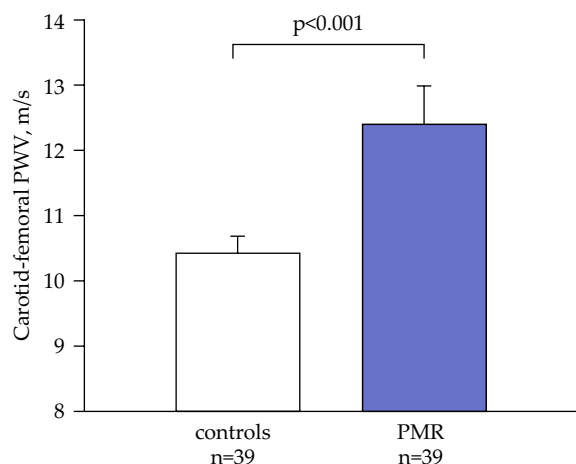


FIGURE 9.3 Carotid-to-femoral PWV in patients with untreated PMR and in age-, sex-, and blood pressure-matched healthy control subjects. Adapted from the personal collection of the authors.

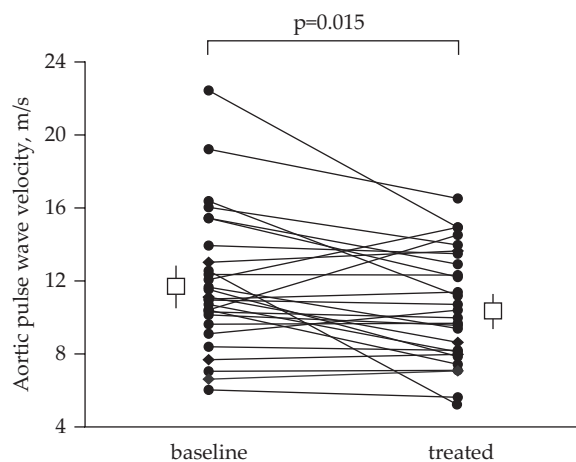


FIGURE 9.4 Carotid-to-femoral PWV in patients with PMR before and after 4 weeks of GC treatment. Adapted from the personal collection of the authors.

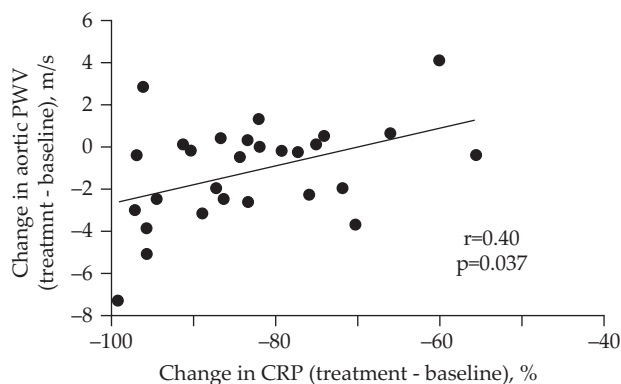


FIGURE 9.5 Bivariate correlation between treatment-induced change in carotid-to-femoral PWV and percentage change in plasma CRP concentration in patients with PMR. Adapted from the personal collection of the authors.

involvement persisted despite the complete resolution of PMR-related symptoms and normalization of laboratory inflammatory markers. This single report suggests that myocardial involvement should be evaluated in patients with PMR and relevant cardiac symptoms [95].

Pathogenesis of myocardial involvement in PMR has not been evaluated. However, increased levels of interstitial muscle proinflammatory cytokines have been demonstrated in untreated PMR patients, suggesting that local release of inflammatory cytokines within cardiac muscle may be a possible mechanism underlying myocardial involvement in these patients [16]. Of interest, a modest lymphocyte infiltration has been depicted in the deltoid muscle of a patient with PMR [96]. Such findings suggest that an inflammatory infiltrate may characterize cardiac and striated muscular tissue in patients with PMR. Finally, a coronary vasculitis impairing cardiac muscle contraction may not been ruled out [97]. In this setting, a case of fatal myocardial infarction as a consequence of a granulomatous coronary arteritis in patients with previously diagnosed PMR and biopsy-proved GCA has been described [97].

4.4 Pericardial Involvement

Few reports of PMR patients presenting with pericardial effusion have been described [98–104]. In three of them a concomitant pleural effusion was documented [101,103,104]. Two cases manifested with pericardial tamponade requiring pericardiocentesis [100,102]. Pericardiectomy was performed in one case and histologic examination revealed inflammation of the pericardium with fibrosis and interstitial hemorrhage areas without fibrin deposits. Immunohistochemical analysis reported perivascular lymphocytic infiltrate consisting of a mixed lymphocyte population, mostly T cells (CD45/CD3/CD5 positive) with a minor B-cell population (CD79a/CD20 positive) [102]. All cases were characterized by good response to GC treatment.

4.5 Prevalence of Major Cardiovascular Events

Current evidence supports that, similar to other inflammatory rheumatic disorders, isolated PMR may be associated with a significant increased risk of major CV events, including myocardial infarction, peripheral artery disease (PAD), and cerebrovascular events, in comparison to the control population, although caution is needed in data analysis due to the degree of heterogeneity of studies (Table 9.3) [84]. Moreover, potential confounding modifiers, such as cigarette smoking, alcohol consumption, dietary habits,

physical activity, and family history for CV disease and use of statins or aspirin, have not been uniformly evaluated in all studies, hampering data comparison and interpretation.

Nevertheless, prospective studies involving large cohorts demonstrated that patients with PMR, free from previous CV events, are characterized by a two- to threefold increased risk of ischemic heart disease with respect to control subjects [85,90,105]. The relative risk of ischemic heart disease was strongest for patients aged less than 60 years, was higher during the first 6 months–1 year after the diagnosis, and remained significant during a follow-up period of 8 up to more than 10 years [90,105]. This early excess risk further supports the hypothesis that the high inflammatory burden characterizing the disease at onset may have a role in increasing CV risk in these patients. The evidence of an increased risk of ischemic heart disease in patients aged less than 60 years deserves further consideration. Indeed, PMR is a disease typically affecting subjects aged more than 65–70 years. The appearance of a polymyalgic picture in a younger subject generally represents a paraneoplastic manifestation or, alternatively, the onset of a chronic inflammatory disorder, in particular RA. Moreover, the old classification criteria employed in all studies are not able to discriminate well between patients with real PMR and early inflammatory diseases. Indeed, it has been widely demonstrated that patients with RA are characterized, also in young age, by an increased risk of myocardial infarction [106]. In this setting, it is plausible to hypothesize that the younger patients with PMR enrolled in the studies may have developed a chronic systemic inflammatory disease during follow-up.

Similarly, retrospective and prospective population-based studies with long follow-up demonstrated a significant twofold higher risk of cerebrovascular events, including ischemic and hemorrhagic stroke, in PMR patients with respect to control population [85,89,107]. This risk of stroke was higher in patients with comorbidities such as hyperlipidemia, hypertension, diabetes mellitus, and coronary heart disease [89].

Finally, a more than twofold significant increased risk of clinically relevant peripheral artery disease was demonstrated to characterize PMR patients in comparison to control subjects both in retrospective and prospective studies with a median follow-up of up to 11 years [85,88,90]. The risk remained significant after adjusting for traditional CV risk factors, including hypertension, diabetes mellitus, and dyslipidemia [88,90]. All patients with PMR and PAD were symptomatic with lower limb claudication and all had abnormal dorsal pedis pulse [88]. On the other hand, no statistically significant increased risk of heart failure was demonstrated in a PMR cohort [85].

5. TREATMENT OPTIONS AND CARDIAC IMPLICATIONS OF THERAPY

5.1 Glucocorticoid Therapy

To date, low-to-medium dose GC therapy remains the cornerstone of treatment for PMR (Fig. 9.6). There are no randomized controlled trials (RCTs) assessing the efficacy of steroid therapy compared to placebo, and a universally accepted steroid regimen has not been established [108,109]. Current evidence comes mainly from retrospective case series and prospective cohort studies. In practice, the initial suggested dose is 12.5–15 mg daily of oral prednisone or equivalent. Indeed, a low dose (12.5 mg daily) is associated with a complete clinical and serologic response in the majority of patients, although a few patients may require up to 20 mg daily [110,111]. Lower doses have been associated with a higher incidence of relapses and higher doses with increased risk of GC-related adverse events [112]. Recently, the British Society for Rheumatology recommended a consensus-based regimen for GC treatment, starting with prednisone at 15 mg daily for 3 weeks. Then, the dose is tapered to 12.5 mg daily for an additional 3 weeks, 10 mg for 4–6 weeks, and finally reduced by 1 mg every 4–8 weeks thereafter until discontinuation or flare [109]. Dose tapering should also be guided according to symptom resolution and inflammatory laboratory marker normalization. In a recent trial, prednisone 25 mg/day was compared to methylprednisolone 20 mg/day in a cohort of patients with PMR [113]. The changes in inflammatory markers and clinical remission in the two groups were similar. However, prednisone was associated with a significantly longer mean time to achieve full remission, suggesting that a delayed response to prednisone may occur in PMR patients. Similarly, a prospective observational study evaluated changes in inflammatory parameters, such as ESR, CRP, and fibrinogen, circulating cytokines, including IL-6, and morning cortisol level in a cohort of patients with PMR diagnosed on the basis of the 2012 ACR/EULAR criteria after treatment with 6-methylprednisolone or modified-release prednisone [114]. Changes in inflammatory parameters were similar in the two groups, whereas morning cortisol levels remained unchanged only in the modified-release prednisone group. Of interest, during the first month of treatment, modified-release prednisone given at bedtime significantly decreased IL-6 levels, and a significantly higher percentage of patients treated with modified-release prednisone stopped GC therapy in comparison to 6-methylprednisolone treated patients [114].

The majority of patients are able to discontinue GC therapy within 8 months to 2 years after disease onset; however, some patients may require prolonged treatment to manage disease relapses. Some patients may need a more gradual GC tapering schedule or a longer period of treatment with

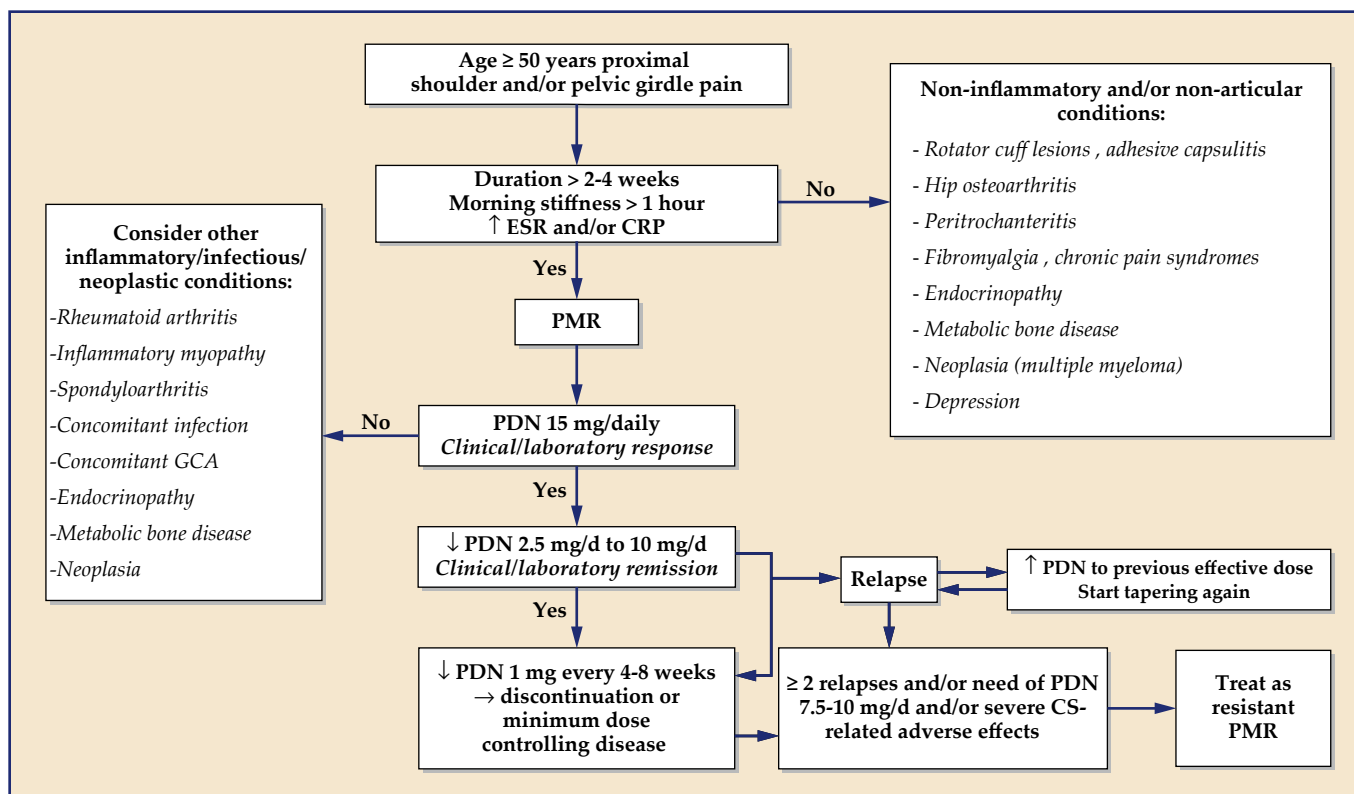


FIGURE 9.6 Diagnostic and therapeutic approach to a patient with PMR. Adapted from the personal collection of the authors.

a sustained low dose. It is important to adjust steroid dose according to comorbidity, including diabetes mellitus and CV diseases, fracture risk, and adverse events [109]. The management aim is to achieve the optimal GC dose with appropriate balance between efficacy and minimal adverse events [115]. In this setting, a PMR activity score has been proposed based on the evaluation of morning stiffness duration (minutes \times 0.1), patient and physician assessment of global pain by VAS scale, ability to elevate the upper limbs, and CRP [116]. However, prospective studies are needed to assess score usefulness in detecting disease flares and optimizing GC exposure therapy.

A randomized placebo controlled trial demonstrated that a course of intramuscular methylprednisolone was associated with a similar remission rate but with lower cumulative mean steroid dose and fewer GC-related complications than standard oral steroid therapy [117]. However, these limited data suggest that intramuscular steroid injection should be reserved for patients at high risk of GC-related adverse events.

About one-half of patients with PMR experience a disease flare upon GC tapering or discontinuation. Disease relapses, defined as recurrence of PMR symptoms with an increase in inflammatory makers during GC taper, are treated with an increase in GC doses [109]. Patients who experience disease recurrence following GC discontinuation should be restarted on GC therapy.

Long-term GC therapy may indirectly increase the risk of CV disease through its detrimental effects on traditional CV risk factors, including hypertension, diabetes mellitus, dyslipidemia, and obesity. Moreover, subjects chronically exposed to GC therapy are at higher risk of subclinical endothelial damage and CV events, including myocardial infarction, stroke, and heart failure [118]. In particular, daily GC dose, duration of treatment exposure, and higher cumulative dose have been associated with higher risk of CV events, in particular in patients with chronic inflammatory disorders [119]. On the other hand, GCs may have antiatherogenic effects mediated by anti-inflammatory and antiproliferative mechanisms on the arterial wall [120]. Of interest, use of low-dose prednisone in association with antirheumatic therapy in RA patients during the first 2 years of the disease was not associated with an increased risk of ischemic heart disease in comparison to patients not assuming GCs [121]. However, a fourfold increased risk of cerebrovascular events was observed in GC-treated patients. Moreover, there was a trend toward reduced survival in the GC-treated group. Such results suggest that it may be possible to avoid the potential negative effects of GC on future coronary ischemic events if GCs are used at low dose and in association with drugs able to control disease activity.

The effect of GC therapy on CV outcomes in patients with PMR is less clear. Duration or cumulative dose of

long-term low-dose GC therapy has been associated with a higher risk of arterial hypertension [122]. On the other hand, cumulative GC dose and long-term therapy do not appear to be associated with increased risk of overt CV events in retrospective analysis of PMR cohorts [122,123]. Of interest, significant risk reduction of CV events has been shown in patients exposed to GCs for at least one year prior to the event compared to those never exposed to GC [123]. In this setting, the high inflammatory burden that characterizes PMR at the onset compared to other chronic inflammatory diseases may induce functional and structural endothelial damage [57,93,124]. Rapid inhibition of systemic inflammation induced by short treatment with low-dose GC may have a beneficial effect on vascular endothelial homeostasis in patients with PMR [57,93]. Of consequence, even if the possibility of an excess of CV risk with GC exposure cannot be ruled out, the negative effect of GC may be counteracted by suppression of the inflammatory response. Finally, GC therapy has been demonstrated to induce a significant increase of homocysteine levels, especially in GCA patients, suggesting an atherogenic mechanism of corticosteroids [62].

5.2 Glucocorticoid-Sparing Agents

In patients experiencing more than two disease relapses or for those at high risk of GC-related adverse events, the introduction of immunosuppressant (IS) drugs should be considered (Fig. 9.7) [109].

The efficacy of Methotrexate (MTX) 7.5–10 mg weekly in newly diagnosed PMR cases has been investigated in RCTs with conflicting results [108,125]. In an RCT, MTX 7.5 mg/week proved no better than placebo in reducing time to remission, number of relapses, and cumulative steroid dose in a small PMR cohort [108,125]. On the other hand, oral or intramuscular MTX 10 mg/week was superior to placebo in reducing flares and cumulative GC dose, while allowing GC discontinuation in other trials [108,125]. However, rates and severity of GC-related adverse events were similar between the two groups, and therefore the utility of MTX is modest. The evidence of elevated blood levels of proinflammatory cytokines, including IL-6 and TNF α , in PMR prompted the investigation of TNF α inhibitors as GC-sparing agents for patients with newly diagnosed PMR. Adding infliximab to prednisone was not superior to placebo in affecting the proportion of patients without relapses, number of relapses, duration, and cumulative prednisone dose or steroid discontinuation rate in an RCT [126]. Similarly, etanercept monotherapy demonstrated a modest effect in reducing disease activity and patient morning stiffness and pain with respect to placebo in a cohort of GC-naïve PMR patients [127]. These findings suggest

very limited efficacy of TNF α inhibitors in patients with new onset PMR.

Several IS drugs have been employed as maintenance therapy in patients with relapsing disease or in patients requiring ongoing use of GC. Adding oral MTX 7.5 mg up to 12.5 mg weekly in patients previously treated with GCs showed no or modest effect in reducing the number of flares in comparison to prednisone alone [108,125]. Moreover, MTX-treated patients had the same frequency of GC-related adverse events as patients treated with steroid therapy alone at 5-year follow-up. One year of azathioprine treatment added to GCs as maintenance therapy allowed a significant reduction in cumulative prednisone doses with respect to placebo in a very small PMR cohort [108]. However, use of azathioprine was associated with an increased frequency of drug-related adverse events. Leflunomide was shown to be potentially effective as a GC-sparing agent in a very small cohort of patients with refractory PMR. However, further studies are needed to confirm the usefulness of leflunomide in reducing GC use dose in PMR [128]. On the other hand, TNF α blocking agents might be considered for the treatment of patients with relapsing disease despite previous GC or other IS therapy. Infliximab and etanercept have been shown to be effective in allowing GC dose tapering, improving patient symptoms, and reducing inflammatory markers in small case series and one open label study [129]. Finally, 13 patients with isolated or GCA-associated PMR resistant to GCs or other IS were treated with the anti-IL-6 receptor antibody tocilizumab in monotherapy or in association with GC therapy. Indeed, IL-6 levels have been demonstrated to be significantly higher in active PMR patients and to directly correlate with the risk of relapse [130]. Complete remission of clinical and laboratory parameters of disease activity has been achieved in all but two PMR patients, which reported a partial remission within 2 up to 6 months following monthly tocilizumab administration [131]. Moreover, tocilizumab has been demonstrated to be effective both as GC-sparing agent, allowing tapering or discontinuation of steroid therapy in all patients, and as monotherapy. However, the need of RCTs with a long follow-up and the high cost of tocilizumab limit its employment as first-line agent for the treatment of PMR.

In conclusion, there is lack of adequate evidence regarding the efficacy of IS drugs as GC-sparing agents or in inducing disease remission in patients with refractory PMR. This can be accounted, at least in part, by the heterogeneity of studies in terms of study designs, types, and number of patients included, different classification criteria employed, definition of outcomes, IS dose, GC tapering regimens, and length of follow-up. Moreover, the high drop-out rates reported in most of the studies and the lack of sufficient statistical power to assess

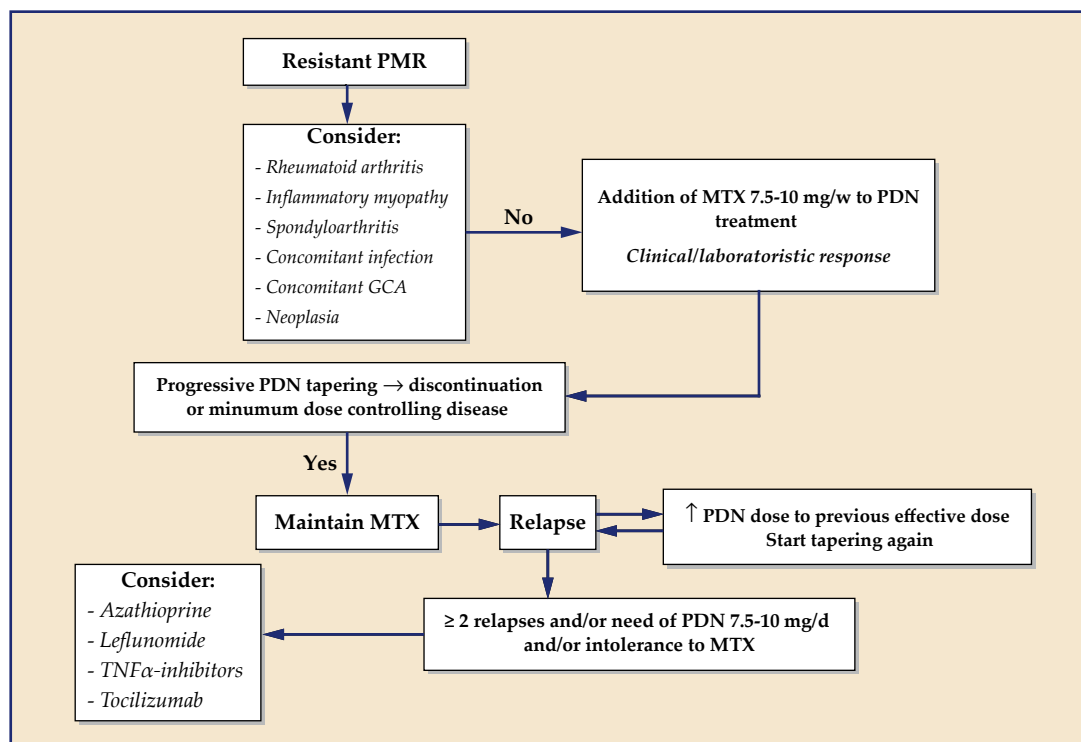


FIGURE 9.7 Therapeutic approach to a patient with PMR resistant to standard corticosteroid therapy. Adapted from the personal collection of the authors.

efficacy and measure clinically important outcomes hamper data interpretation. A summary of indication and dose of drugs employed in PMR with definition of level of evidence and grade of recommendation is illustrated in Table 9.4.

The effect of GC-sparing agents on the heart in PMR patients has not been addressed. However, nonbiological and biological drugs commonly employed in the treatment of chronic inflammatory rheumatic disorders, including RA and psoriatic arthritis, have been demonstrated to exert a positive CV effect in treated patients. Many anti-inflammatory strategies have emerged as potential therapeutic approaches to atherosclerosis, and treatment of the underlying inflammatory process may, of consequence, be associated with CV improvement in patients with systemic inflammatory diseases [132]. In this setting, a systematic review demonstrated that MTX is associated with a reduction in CV mortality and myocardial infarction, whereas associations with stroke were less clear [133]. In a subsequent meta-analysis of observational studies, MTX treatment was associated with a 21% reduced risk of total CV disease incidence and a 18% reduced risk of myocardial infarction [134]. Similarly, a systematic review and meta-analysis demonstrated that, in RA, use of TNFα blocking agents is associated with a significant reduction of 30% in the risk of CV events risk, suggesting that reducing and controlling the systemic inflammation in patients with chronic inflammatory diseases may be associated

with a better CV outcome [135]. Interestingly, IL-6 has been demonstrated to play an important role in different inflammatory effector pathways in many systemic autoimmune diseases. Moreover, elevated IL-6 levels have been independently associated with increased CV risk, including fatal myocardial infarction and cerebrovascular accident, in the general population [136]. Intriguingly, IL-6 blockade with tocilizumab in patients with active RA has been demonstrated to affect quantitative and qualitative changes in lipids and lipoproteins. In particular, tocilizumab administration was associated with an increase in low-density lipoprotein cholesterol but induced a change of high-density lipoprotein particles toward an anti-inflammatory phenotype. Moreover, significant decline of fibrinogen and D-dimer was observed in tocilizumab-treated patients, suggesting a reduction in thrombotic risk in these patients [137]. Moreover, changes in lipid parameters during tocilizumab treatment do not appear to be associated with increased risk of major CV events [138].

6. CONCLUSIONS

Due to the lack of a gold standard investigation, diagnosis of PMR represents a challenge and is based on clinical construct and laboratory evidence of systemic inflammation. However, controversy exists as to whether PMR represents a disease in its own right or is

TABLE 9.4 Glucocorticoid and Immunosuppressive Therapy in PMR

	Studies	Evidence	Recommendation
GLUCOCORTICOID THERAPY			
Dose <ul style="list-style-type: none"> • PDN 15 mg/day for 3–4 weeks • PDN 12.5 mg/day for 3–4 weeks • PDN 10 mg/day for 4–6 weeks • reduction by 1 mg every 4–8 weeks or alternate day reductions until discontinuation 	Retrospective case-series [108,109]. Prospective cohorts [108,109]. 1 prospective randomized [108,109].	B	IIa
Relapse <ul style="list-style-type: none"> • increase PDN to previous higher dose 		B	IIa
Milder cases, high risk of GC-adverse effects <ul style="list-style-type: none"> • methylprednisolone i.m. 120 mg every 2 weeks for 12 weeks then reduced by 20 mg every 2–3 months 	1 RCT [117].	B	IIa
IS INITIAL GC-SPARING AGENTS			
Drug, dose <ul style="list-style-type: none"> • MTX 7.5–10 mg/week 	2 RCTs [139,140]. 1 prospective randomized [141].	A	I Some benefit in: <ul style="list-style-type: none"> • reducing relapses • reducing GC dose • reducing GC adverse effects
<ul style="list-style-type: none"> • infliximab 3 mg/kg weeks 0,2,6,14,22 • etanercept 50 mg/week 	1 RCT [126]. 1 RCT [127].	B	IIb No efficacy
IS COTREATMENT FOR REMISSION MAINTENANCE			
Drug, dose <ul style="list-style-type: none"> • MTX 7.5 mg/week up to 12.5 mg/week if no response 	1 observational prospective [142]. 1 retrospective case-control [143].	B	IIa Some benefit in: <ul style="list-style-type: none"> • reducing relapse risk • pts at high risk of long-term GC adverse effects
<ul style="list-style-type: none"> • azathioprine 150 mg/day 	1 RCT [144].	B	IIa Some benefit in <ul style="list-style-type: none"> • reducing GC dose at long follow-up
<ul style="list-style-type: none"> • leflunomide 10 mg/day up to 20 mg/day 	1 case-series [128].	C	IIb Some benefit as GC-sparing agent
<ul style="list-style-type: none"> • infliximab 	Case series [129].	C	IIa Efficacy in: <ul style="list-style-type: none"> • reducing GC dose • clinical/laboratoristic improvement
<ul style="list-style-type: none"> • etanercept 25 mg X 2/week 	1 open label [129]. Case-series [129].	C	IIa Good efficacy in: <ul style="list-style-type: none"> • reducing GC dose • clinical/laboratoristic improvement
<ul style="list-style-type: none"> • tocilizumab 8 mg/kg monthly 	Case-series (13 pts) [131].	C	IIa Good efficacy in: <ul style="list-style-type: none"> • complete clinical/laboratoristic parameter remission • GC-sparing agent also in monotherapy

GC, glucocorticoid; MTX, methotrexate; Pts, patients.

a term used to define a clinical presentation common to a range of related conditions. Indeed, for decades, the lack of standardized classification criteria represented a major factor hampering the evaluation of patients in clinical studies, the validity of disease diagnosis, and the development of evidence-based therapeutic approaches. Of consequence, literature data should be interpreted with caution and, in the future, work must focus on further defining PMR as a distinct disease entity. Moreover, it is relevant to consider that, at the moment, there are no randomized prospective studies on patients fulfilling the EULAR/ACR classification criteria and that current therapeutic guidelines have been derived from studies employing different classification criteria.

Nevertheless, in recent years major advances have been made in the global approach to PMR, an intriguing model to be employed in the investigation of the effects of high systemic inflammatory burden on vascular outcome. First of all, the introduction of a core set of classification criteria allows to better standardize diagnostic approaches and patient management and follow-up in order to define uniform patient populations for specific studies designed to identify specific disease markers. Secondly, available data suggest that the inflammatory response characterizing the disease at onset is associated with an increased risk of endothelial damage and subclinical atherosclerosis, irrespective of patient age and concomitant CV morbidity. The clinical relevance of this association is further strengthened by the evidence of a substantial increased risk of major vascular events in these patients. This excess risk is detectable soon after the diagnosis, is higher in younger patients, and remains significant during a long follow-up period. The fact that increased risk of vascular events does not seem to exert a significant effect on mortality in PMR is also noteworthy. However, although several studies have evaluated mortality and survival in PMR, very few separately evaluated PMR and GCA and did not have uniform results. There is also lack of appropriate prospective studies with matched controls specifically addressing mortality and morbidity in PMR. Nevertheless, occurrence of CV events in these patients after disease diagnosis accounts for long-term CV morbidity and incremental disease-related costs, suggesting that adequate control of the disease may potentially reduce the CV risk and, of consequence, the economic burden of CV morbidity. The demonstration at FDG-PET of persistence of some signs of arterial vessel inflammation despite sufficiently long GC therapy suggests that PMR is not a completely benign disease and that low-grade inflammation may persist despite clinical remission. Of consequence, it is plausible that noncompliant or GC-resistant patients may be characterized by a worse long-term CV outcome with respect

to compliant ones. To further support this hypothesis, evidence showing that appropriate introduction of low-dose GC therapy as soon as the diagnosis of PMR is established may exert a favorable effect on endothelium homeostasis suggests that rapid reduction of inflammatory burden may reduce the long-term risk of CV events. Of course, further prospective studies with longer follow-up are needed to test this hypothesis.

Despite the lack of formal RCTs, empirical evidence suggests that a starting prednisone dose of 12.5–15 mg/day followed by a slow tapering regimen is an appropriate treatment for most PMR cases. In resistant or relapsing cases and in order to avoid adverse effects related to chronic GC therapy, the introduction of an IS treatment may be considered. Among traditional and biologic drugs, methotrexate may be considered the first choice as GC-sparing agent. However, larger RCTs are needed to evaluate the efficacy of all biological and traditional IS drugs as GC-sparing agents in PMR.

Further research is needed to investigate the mechanisms underlying endothelial damage and vascular disease in PMR and, more specifically, to assess the effect of traditional CV risk factors, chronic inflammation, and of therapies on long-term CV risk in these patients.

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Systemic Lupus Erythematosus

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1. INTRODUCTION

Systemic lupus erythematosus (SLE) is the prototype of systemic autoimmune diseases, mainly affecting women of reproductive age and characterized by various clinical manifestations, as well as by multiple laboratory/immunologic abnormalities.

1.1 Epidemiology

Disease incidence ranges from 1 to 10 patients/100,000 population/year, whereas prevalence is approximately 50–70 patients/100,000 population [1]. Incidence and prevalence in populations of Asian and African origin are 2–3 times higher in comparison to Caucasians [2]. Recent studies demonstrated a significant increase in disease incidence between 1950–1979 and 1980–1992 (1.5 vs 5.6 patients/100,000 population) [3, 4].

SLE is significantly more frequent in women of reproductive age and, in most studies, approximately 90% of the patients are women [1]. The most common age at disease onset is during the third and fourth decade of life; however, symptom initiation has been described at any age.

In childhood, incidence and prevalence are reportedly lower in comparison to adults as annual incidence in children under 16 is less than 1/100,000 population in Europe and North America [5].

The natural course of the disease is characterized by periods of remission and exacerbations with intervals between flares varying considerably. Nevertheless, cumulative damage worsens with time and impacts patient prognosis. Mortality is reportedly bimodal with an early peak in the first years after diagnosis due to disease activity and/or infections and a later peak due to atherosclerotic cardiovascular events and organ failure [6–8].

1.2 Pathogenesis

The exact etiology of SLE remains unknown; current pathogenetic theories involve a complex interplay

between certain genetic and environmental factors, which may lead to immune system dysfunction, auto-antibody production, immune complexes formation, and, eventually, tissue damage.

1.2.1 Genetic Factors

The genetic contribution to SLE has been revealed through linkage, gene-association, and genome-wide association studies and includes more than 30 distinct genetic loci [9]. As a result, SLE is characterized by a high inheritance risk ($8 < \lambda_s < 29$) and a higher frequency in monozygotic twins (20–40%) as compared to dizygotic ones and other siblings (2–5%) [10].

Recognized genes that confer a considerable relative risk can be divided into three categories. The first includes those genes that control the clearance of apoptotic debris through macrophages and the subsequent antigen processing and presentation process (FcγRIIA, FcγRIIIA, HLA-DR2, -DR3, -DR4). In addition, SLE has been linked to genetic deficiencies of certain complement fragments (C2, C4, C1q) or their receptors [integrin α M (ITGAM) or complement receptor 3] [11]. The second group includes those genes that control the biological action of interferon-α (IFN-α) (IRAK1, interleukin-1 receptor associated kinase 1, TREX1 three prime repair exonuclease 1, IRF5 interferon regulatory factor 5, and TNFAIP3) [12]. The third group includes genes that control the intracellular signaling in T and B lymphocytes, such as PTPN22 (protein tyrosine phosphatase nonreceptor type 22) [13] and BLK and BANK1 [11]. Interestingly, there are other genes that cannot be classified in these groups, such as PXX, XKR6, and KIAA1542 [10].

1.2.2 Environmental Factors

Ultraviolet radiation A and B (UVA, UVB) are strongly associated with SLE, since almost 70% of patients may develop a disease flare after considerable sun exposure [14]. UVB and, to a lesser extent, UVA may alter DNA structure (through increase of thymine dimers) and lead to keratinocyte apoptosis [15], release of phospholipids, and heat shock proteins (HSP) [16].

Viruses and bacteria have been implicated in clinical deterioration, in particular, Epstein-Barr virus (EBV) infection in newly diagnosed patients [17]. Several studies have reported increased frequency of antibodies against Human Immunodeficiency Virus (HIV, anti-gp24), BK virus, human retrovirus 5, cytomegalovirus (CMV), PARVO B19, and oncornavirus C virus [18]. These observations have not been confirmed in large series; therefore, the role of infectious agents in SLE pathogenesis remains controversial.

Certain drugs, such as hydralazine, procainamide, isoniazide, minocycline, methyldopa, interferon- α , and tumor necrosis factor (TNF) inhibitors have been linked to the disease called drug-induced lupus. This variant has distinct clinical features and laboratory findings and usually subsides after drug discontinuation. Common symptoms include arthritis, serositis, and constitutional symptoms (fatigue, low-grade fever), while kidney and central nervous system (CNS) involvement are rare. With regard to immunologic findings, there is a higher frequency of antihistone antibodies [19].

1.2.3 Estrogen

The role of estrogen is supported by several studies, given that SLE may relapse during pregnancy and disease severity is decreased after menopause. Furthermore, disease frequency is reportedly increased in men with Klinefelter's syndrome (47, XXY) [20]. Interestingly, hormone replacement therapy did not seem to increase the flare rate in postmenopausal women [21, 22]. In addition, oral contraceptives did not increase the flare rate in women with stable disease [23].

1.2.4 Immune System Dysfunction

The complex interplay between these genetic and environmental factors leads eventually to the breakdown of immune tolerance and the generation of immune response against self-antigens (autoimmune response). Practically, all immune cells are implicated in SLE pathogenesis.

1.2.5 Innate Immunity Abnormalities

Innate immunity dysfunction is primarily expressed through the abnormal differentiation of monocytes/macrophages and dendritic cells (DCs) that are responsible for the initiation of the autoimmune response. Nevertheless, other cellular subpopulations, such as the NK cells, $\gamma\delta$ T cells, T follicular cells, and neutrophils are increasingly recognized as important mediators.

In regards to macrophages, a disequilibrium between M1 (promoting Th1 differentiation) and M2 (mainly anti-inflammatory) macrophages has been described [24]. M1 macrophages are more activated, as suggested by increased CD86 [25] and CCL2 [26], which are implicated in lupus nephritis as well as IFN- γ , IL-6, and

CXCL10 that are implicated in central nervous system involvement [27].

Dendritic cells (DCs), considered as the professional antigen presenting cells, originate and differentiate from pluripotent stem cells to myeloid (seeding peripheral tissues and secondary lymphoid organs) and plasmacytoid (circulating in the bloodstream and migrating to secondary lymphoid organs) cells. The cytokine microenvironment in SLE, particularly high IFN- α levels, promotes the differentiation of monocytes to myeloid DCs [28]. Plasmacytoid DCs are the main cellular source of IFN- α , following their activation through nucleic acid-TLR interactions [29]; non-TLR dependent mechanisms have also been observed [30]. After maturation, DCs may promote the expansion of autoreactive CD8⁺ T cells [31], autoreactive B cells [32], and plasmablasts [33].

NK cells have been described in decreased numbers, probably because IFN- α drives their apoptosis [34]; furthermore, their cytotoxic properties are impaired [35]. Neutrophils were suggested to undergo intense apoptosis [36]; as a result, increased numbers of immature, low and dense neutrophils are observed in the periphery [37]. These cells are involved in sites of active inflammation, and their death through netosis may lead to complement activation and augment autoimmune response [38]. Concerning $\gamma\delta$ T cells, their numbers have been found decreased in active SLE, whereas they were locally increased in skin lesions [39]; their precise role in disease pathogenesis remains to be elucidated.

1.2.6 Adaptive Immunity Abnormalities

T lymphocytes are considered the key players in SLE pathophysiology and are roughly divided into CD4⁺ T helpers (Th) and CD8⁺ cytotoxic T cells (Tc). Other subpopulations include $\gamma\delta$ T cells (TCR with $\gamma\delta$ chains) and NKT cells [40]. T helper cells are further divided according to cytokine secretory profile into Th1 (mainly secreting IFN- γ , IL-2, and TNF- α), Th2 (mainly secreting IL-4, IL-5, IL-10, and IL-13), Th17 (IL-17 and IL-23), and T regulatory cells [41]. Th1 cells predominantly infiltrate the kidneys in diffuse proliferative glomerulonephritis [42], whereas Th2 response drives the deposition of immune complexes in the glomeruli [43]. In human SLE, serum levels of IL-17 and Th17 cells have been found increased [44] and correlated to disease activity [45]. Double-negative (CD4⁻CD8⁻) T cells are expanded in SLE and dominate the infiltrations in kidney biopsies, secreting considerable amounts of IL-17 [46]. Th17 response is maintained from other immune cells and soluble mediators, such as IFN- α , which may induce IL-6 and IL-23 secretion [47]. Finally, IL-17 alone or synergistically with B-cell activating factor (BAFF) is able to induce B-cell survival [48]. This generates a vicious cycle through which plasma cells secrete autoantibodies that create immune complexes that stimulate DCs to secrete IFN- α that induces IL-17.

CD8⁺ cytotoxic T cells are found in increased numbers and functionally activated in SLE; their numbers correlate to disease activity [49]. Due to their cytotoxicity, they are considered the main mediators of apoptosis induction and neoepitope formation, which will subsequently lead to autoantibody production.

B cells have been proven to exert multiple functions, such as autoantibody formation, antigen processing and presentation, and cytokine secretion. In SLE, they are polyclonally activated and produce antibodies against nuclear elements and other self-molecules. This phenomenon may not solely rely on T-cell help since data from genome-wide association studies imply that certain genes (BLK, BANK1, PTPN22, CD40 TNFAIP3, and Fcγ receptors) may affect B-cell hyper-responsiveness [50]. Immature B cells (expressing CD38, CD5, CD9, CD10, and CD24) are found in increased numbers in the periphery of lupus patients, regardless of disease activity [51]. Even from the early stages of B-cell maturation (CD10⁺CD27-IgM⁺), autoreactive cells have been isolated, underlining a defect in B-cell tolerance [52]. Furthermore, memory CD27⁺IgD⁻ B cells are increased in SLE and are resistant to immunosuppressive therapy [53]. Regarding mature B cells (plasmablasts/plasma cells), these are found in increased numbers in SLE and possess the CD19^{low}CD20⁻CD38^{high} phenotype [54]. Increased numbers reflect the ongoing maturation within germinal centers of the secondary lymphoid organs [55]. Germinal center activation relies on the function of several factors such as T follicular helper cells (Tfh), IL-21, IL-6, TNF-β, and ICOS. These molecules facilitate the interaction between T and B cells through CD40/CD40L (CD154) and ICOS/ICOSL, eventually resulting in the polyclonal B-cell activation [56]. Other molecules implicated in B-cell maturation in ectopic germinal centers are BAFF, APRIL, and TACI [57]. Furthermore, BAFF/BlyS and APRIL correlate strongly with disease activity and represent the most current biologic targets in SLE therapeutics [58].

1.2.7 Immune Regulation Abnormalities

Dysfunction of the mechanisms of immune tolerance has a central role in the escape of autoreactive lymphocytes in the periphery. Central tolerance impairment has been demonstrated only in murine models of SLE and not in humans [59]. On the other hand, dysfunction of the peripheral tolerance, mainly represented by T regulatory cells (Tregs, CD4⁺CD25⁺CD127^{low}FOXP3⁺), has been well described in human SLE [60]. These cells are able to suppress/regulate virtually every immune cell by means of IL-2 deprivation of effector cells, cell-to-cell contact, regulatory cytokine production (IL-10, TGF-β), metabolic disruption, and other indirect mechanisms [61]. Quantitative and qualitative defects of these cells have been described in SLE patients; furthermore, they

are inversely related to disease activity and restored after remission induction.

1.2.8 Apoptosis and Complement Abnormalities

Apoptosis (programmed cell death) is crucial for the homeostasis of the immune system; cellular debris is digested by cells of the reticuloendothelial system, mainly macrophages, through recognition via scavenger receptors. Further degradation or modification follows in a way that permits reuse of peptides for new protein synthesis. The ultimate goal of apoptosis is not only the renewal of cellular populations but also the degradation of potentially antigenic cellular elements [62]. Apoptosis affects virtually all cells; under normal circumstances, it does not mobilize an immune response. In SLE, apoptosis impairment, either as excessive apoptotic load or as a mechanistic disorder in the process, plays an important role [63]. However, no specific macrophage disorder has been confirmed; it is possible that this impairment may be influenced by the low levels of complement fragments C1q, C3, and C4 [64].

The impact of complement abnormalities in disease pathogenesis is decisive since homozygous genetic deficiencies (for C1q) possess a 90% risk of developing SLE and represent the strongest predisposing factor [65]. The pathophysiologic importance of this phenomenon is not completely known; however, C1q is involved in the clearance of the apoptotic debris and the activation of T and B lymphocytes [66]. C1q may be bound on the surface of the apoptotic blebs and enhance their phagocytosis by macrophages, whereas it induces the down-regulation of costimulatory molecules on the surface of DCs [67]. Other genetic deficiencies of complement fragments (C2, C3, and C4) also predispose to SLE [62]. However, most SLE patients do not have genetic deficiencies of these proteins. Low C3 and C4d are usually attributed to consumption in immune complex formation and/or the presence of specific antibodies. Immune complex phagocytosis will eventually lead to the activation of the complement cascade and tissue damage (complement-dependent cytotoxicity, CDCC).

Alternatively, immune system activation may be provoked through endogenous impairment of the apoptotic process, which may lead to the formation of neoepitopes or reveal cryptic epitopes to antigen-presenting cells. In the first instance, DCs could be stimulated through Toll-like receptors (mainly TLR7 and TLR9) that can recognize endogenous and/or exogenous nucleic acids; they will eventually differentiate into mature DCs and process the antigen, present this to the helper T cells while, in parallel, secreting proinflammatory cytokines (IFN-α, IL-6), which, in turn, are able to promote the functional differentiation of Th17 cells. In the case of apoptotic overload, monocytes/macrophages are the instigators of the autoimmune process. Antigen presentation to CD4⁺ T

TABLE 10.1 Main Immune System Abnormalities in SLE

Innate Immunity	
Monocytes/ macrophages	Disequilibrium between M1 (proinflammatory) and M2 (anti-inflammatory) macrophages, active involvement in impaired apoptosis
Dendritic cells	Derived from monocytes (myeloid), secrete IFN- α (plasmacytoid), activate CD8 $^{+}$ T cells, B cells and plasmablasts
NK cells	Decreased numbers, impaired cytotoxicity
$\gamma\delta$ T cells	Decreased numbers, involvement in skin inflammation
Neutrophils	Increased numbers, netosis leads to complement activation
Adaptive Immunity	
CD4 $^{+}$ T cells	All subtypes (Th1, Th2, Th17, Tfh9) show impaired function, visceral infiltrations, immune complex deposition, cytokine secretion
CD8 $^{+}$ T cells	Increased numbers and function (cytotoxicity)
B Cells	Polyclonally activated, multiple autoantibody production, cytokine secretion
Immune Regulation	
T Regulatory cells	Decreased numbers, impaired function

cells may lead to proliferation and functional differentiation of these cells toward the Th17 and, to a lesser extent, the Th1 and Th2 functional phenotypes (depending on the cytokine microenvironment). Th17 cells represent the main mediators causing tissue damage; simultaneously, CD4 $^{+}$ T cells drive the functional differentiation of B cells that mature into plasma cells. Autoantibody production will subsequently lead to immune complexes formation that may cause tissue damage with the activation of a complement cascade. In parallel, they have the capacity to stimulate DCs, thus creating a vicious cycle to restimulate the immune system. Tissue damage will reveal cryptic epitopes or generate neoepitopes that will augment and substantiate the autoimmune process. The aforementioned disorders depend on the defective suppression/regulation of the effector cells from the regulatory cells (mainly T regulatory cells) (Table 10.1).

2. CLINICAL FEATURES

2.1 Constitutional Symptoms

Constitutional symptoms, such as fever, weight loss, fatigue, and lymphadenopathy are not disease-specific but have to be considered in the differential diagnosis of a disease flare or infections. Fever is usually low grade and appears cumulatively in 86% of the patients; rarely may be the only initiating symptom, as in patients with fever of unknown origin, up to 5% were finally diagnosed with SLE [68]. Fatigue may be related to increased disease activity or an upcoming flare. Patients with SLE suffer from fibromyalgia in up to 47%, which may enhance fatigue and chronic pain [69]. Clinically important weight loss is rare and should be differentiated from concurrent malignancy.

2.2 Musculoskeletal Features

Arthritis is the most common manifestation, cumulatively affecting >90% of patients. Arthritis is nonerosive and rarely causes deformities. Nonerosive, deforming arthritis is called Jaccoud arthropathy [70]. Other usual musculoskeletal findings are tenosynovitis and fasciitis; myositis is seen less often.

2.3 Skin Involvement

Cutaneous involvement in SLE is classified as acute, subacute, and chronic cutaneous lupus erythematosus. Acute forms include the butterfly rash and generalized exanthema and usually heal without scarring, while subacute disease is characterized by annular lesions or erythematosquamous papules or plaques and healing is accompanied by partial loss of pigmentation. Discoid lupus is classified as chronic cutaneous lupus erythematosus, along with lupus tumidus and chilblain lupus, and represents the first disease manifestation in 10% of patients. Discoid lupus often results in pitting or scarring and hyper- and hypopigmentation. Subcutaneous lupus consists of lupus profundus and lupus panniculitis and is characterized by superficial nodules mainly in the gluteal area, thighs, and upper arms [71]. Alopecia, particularly when accompanied with inflammatory lesions of the scalp, may be related to SLE. Usually, a diffuse hair thinning and/or loss is observed, which is reversible with disease control. Photosensitivity is induced from ultraviolet radiation and usually appears as diffuse redness in the exposed parts of the skin; UVB, in a wavelength of 300–320 nm, induces the strongest immune response in the skin [14]. Livedo reticularis is associated with the presence of antiphospholipid antibodies [72]. In cases of

severe disease, vasculitic lesions may be observed in the fingers and toes of patients and are related to immune complexes deposition in the cutaneous capillaries.

2.4 Respiratory System Involvement

Respiratory involvement in SLE affects all anatomic structures of the lungs and pleura. Pleuritis is the most common feature, affecting up to 35% of patients during disease course [73]. It is usually bilateral and mild to moderate in severity; it is seldom dry. Pleuritic fluid is clear, exudative, and may contain antinuclear antibodies and inflammatory cells. Interstitial lung disease is rare (<3%) and clinically manifested with exertional dyspnea, dry cough, end-inspiratory crackles, and gas-exchange disorders. Initially, there is lymphocytic infiltration of the alveolar wall with honeycombing formation in high-resolution computed tomography (HRCT) imaging [73]. Histopathologically, nonspecific interstitial pneumonia is the most common type [74]. Diffuse alveolar hemorrhage is the most severe respiratory complication of SLE and affects <1% of patients. The pathologic background is a diffuse, immune complex-mediated pulmonary capillaritis. Prognosis is poor, with frequent development of adult respiratory distress syndrome (ARDS) and respiratory failure [75]. Acute lupus pneumonitis is a diffuse alveolar inflammation without evidence of vasculitis or hemorrhage; overwhelming infections and uremia should be differentiated [73]. Bronchiectasis, bronchiolitis obliterans, and upper respiratory tract involvement are rarely described. Involvement of pulmonary vasculature, as pulmonary arterial hypertension, can be detected with noninvasive measures in 6–14% of lupus patients; half of them do not have a clear risk factor besides SLE [76]. The pathologic basis is immune complex-mediated vasculitis with thickening of the intima and media layers of pulmonary artery branches and subsequent lumen narrowing [77]. Pulmonary embolism and chronic thromboembolic pulmonary disease are associated with antiphospholipid antibodies. Respiratory muscle involvement may lead to hemidiaphragm dysfunction and shrinking lung syndrome [78].

2.5 Nervous System Involvement

SLE may affect the central, peripheral, and autonomic nervous systems; the variety of the clinical manifestations is reflected in the ACR nomenclature for the classification of neuropsychiatric SLE (NPSLE) [79]. Epileptic seizures may resemble all known forms of epilepsy and should be differentiated from infectious, metabolic, or toxic etiology [80]. Cerebrovascular disease is clinically manifested with ischemic infarcts, solitary or multiple, which may lead over time to multi-infarct dementia [81]. They are associated with the presence of antiphospholipid antibodies or CNS

vasculitis. Lupus headache is defined as a severe migrainous-like headache nonresponsive to opioid analgesics; however, ordinary migraine may have these characteristics [82]. Lupoid sclerosis was used to describe complex neurologic lesions similar to those of multiple sclerosis [83]. Since there is no wide acceptance of its existence, ACR recommends the term demyelinating syndromes. Other features include chorea, transverse myelitis, and aseptic meningitis. Acute confusional state, defined as decreased level of consciousness or alertness with accompanying cognitive impairment of varying severity and behavioral disorder [84], is currently replacing the older term organic brain syndrome. Rarely, posterior reversible encephalopathy syndrome (PRES) is described in patients with severe disease and manifests with fever, seizures, arterial hypertension, and visual disorders [85].

Peripheral nervous system involvement includes simple or multiple cranial or peripheral neuropathies, polyneuropathies, plexopathies, and dysautonomia. SLE is rarely complicated with myasthenia gravis and acute inflammatory demyelinating polyneuropathy (Guillain-Barre syndrome) [86].

Psychiatric involvement may develop as psychosis, major depression, and anxiety disorders; the latter are not clearly associated with the disease per se. More common features are mild cognitive impairment, such as attention, focus, and memory deficits for which the use of standardized structured questionnaires is recommended [87].

2.6 Lupus Nephritis

Lupus nephritis (LN) is a major determinant of morbidity and mortality in SLE since almost 60% of patients will develop some form of kidney involvement. Identified risk factors are male gender, young age, non-Caucasian origin, and anti-C1q and anti-dsDNA antibodies [88]. LN diagnosis usually relies on the recognition of proteinuria; in 45–65% this reaches nephrotic range. Microscopic hematuria is detected in 80% of patients during disease course while macroscopic hematuria is rare [89]. Active urinary sediment with casts as well as arterial hypertension accompanies proliferative forms. Proteinuria is best assessed with a 24 h urine collection; a protein/creatinine ratio in a random sample can be used as a screening test [90]. Renal biopsy is the gold standard for LN diagnosis, classification, and management plan. In 15–20% of cases, LN is the initial manifestation and biopsy is diagnostic for SLE [88]. The most widely accepted biopsy classification system was proposed by the International Society of Nephrology and the Renal Pathology Society in 2003 [91]. This classification divides LN into six distinct forms and defines their activity and chronicity [92]. Roughly, LN class I represents the minimal change glomerulonephritis (GN), class II the

mesangial GN, class III the focal and/or segmental proliferative GN, class IV the diffuse proliferative GN, class V the membranous GN, and class VI the advanced sclerosing GN.

2.7 Blood Cell Abnormalities

All blood cell lineages can be affected and are clinically expressed as cytopenias of varying severity. Anemia is usually attributed to chronic disease with mildly reduced hemoglobin and normal or low mean corpuscular volume (MCV); it is related to disease activity and high IL-6 levels [93]. Autoimmune hemolytic anemia develops cumulatively in approximately 10% of patients and might be severe in one-third of them [94]. The Coombs' test may be positive or negative in the hemolytic anemia. Other rare causes include pure red cell aplasia and aplastic crisis. The possibility of occult gastrointestinal bleeding due to chronic nonsteroidal anti-inflammatory (NSAID) use, the coexistence of pernicious anemia, and anemia in chronic kidney disease due to erythropoietin deficiency should be considered. Lastly, various medications such as azathioprine, cyclophosphamide, and mycophenolate mofetil may suppress bone marrow [95].

Leukopenia and lymphopenia may be related to disease activity and are usually mild. Occasionally they can be caused by azathioprine and cyclophosphamide [96].

Thrombocytopenia is frequent and related to antiplatelet and antiphospholipid antibodies. Usually it is mild and does not demand specific treatment. On rare occasions it can be the initiating manifestation as autoimmune thrombocytopenic purpura. Thrombotic

thrombocytopenic purpura may accompany SLE due to antibodies against metalloprotease ADAMTS-13, which lead to the aggregation of large von Willebrand factor multimers [97]. The disease is associated with SLE activity and cumulative damage [98].

2.8 Other Clinical Manifestations

The gastrointestinal system is involved with oral ulcerations that are usually shallow and painful; they can be detected throughout the digestive tract and might also be related to chronic NSAID use. Peritonitis is considered rare (<3%), while liver involvement with elevated transaminases is reported in 10–31% of patients. Hepatic inflammation may be drug-induced, whereas less often it is related to concurrent autoimmune hepatitis or primary biliary cirrhosis [99]. Mesenteric vasculitis may develop with features resembling acute abdomen [100]. Pancreas is involved with autoimmune inflammation or vasculitis.

The endocrine system is frequently affected during the disease course with autoimmune thyroidopathies being the most prominent [94]. Furthermore, increased incidence of autoimmune diabetes mellitus, adrenal insufficiency, and hypopituitarism has been reported [101].

Frequent manifestations involving the eyes are conjunctivitis, episcleritis, uveitis, and retinal vasculitis, which may be severe and lead to blindness [102]. Xerophthalmia is associated with secondary Sjögren's syndrome and may be accompanied by xerostomia (sicca syndrome). Ear involvement in the form of immune-mediated inner ear disease (IMIED) is rare in SLE [103] (Table 10.2).

TABLE 10.2 Main Clinical Manifestations of SLE

Constitutional symptoms	Fever, weight loss, fasciitis, lymphadenopathy
Musculoskeletal features	Arthritis, arthralgias, Jaccoud arthropathy, myositis, fascitis, tendinitis, tenosynovitis
Skin disease	Acute cutaneous LE (butterfly rash, generalized exanthema), subacute cutaneous LE, chronic cutaneous LE (discoid lupus, chilblain lupus, lupus tumidus), subcutaneous LE (lupus profundus, panniculitis), livedo reticularis, vasculitis, alopecia
Respiratory system	Pleuritis, interstitial lung disease, diffuse alveolar hemorrhage, acute lupus pneumonitis, pulmonary arterial hypertension, pulmonary embolism, chronic thromboembolic lung disease, shrinking lung syndrome
Cardiovascular system	Pericarditis, myocarditis, cardiomyopathy, valvular disease, Libman–Sacks endocarditis, premature atherosclerosis
Nervous system	Seizures, cerebral infarcts, multi-infarct dementia, acute confusional state, posterior reversible encephalopathy, chorea, demyelinating syndromes, transverse myelitis, peripheral neuropathies, Guillain–Barre syndrome, psychosis
Kidney involvement	Lupus nephritis (class I–VI)
Blood cell abnormalities	Autoimmune hemolytic anemia, leukopenia, lymphopenia, thrombocytopenia
Gastrointestinal manifestations	Oral ulcerations, peritonitis, hepatitis, pancreatitis, mesenteric artery vasculitis
Endocrine involvement	Hypothyroidism, diabetes mellitus, adrenal insufficiency, hypopituitarism
Sensory organs	Uveitis, retinal vasculitis, sicca syndrome, immune-mediated inner ear disease

3. ANTIPHOSPHOLIPID SYNDROME

Antiphospholipid syndrome (APS) is characterized by the presence of antiphospholipid antibodies (aPL) in patients with recurrent vascular thrombosis and/or obstetric complications. Antiphospholipid antibodies include, but are not limited to, lupus anticoagulant (LA), anticardiolipin antibodies (aCL), antibodies against b2 glycoprotein I (anti-b2GPI), as well as antibodies against rare phospholipid epitopes (prothrombin, phosphatidylserine, phosphatidylcholine, etc.). These antibodies are detected in 30–50% of lupus patients and are clinically expressed in half of them.

Diagnosis relies on the revised Sapporo criteria that require the presence of a vascular thrombosis (arterial or venous) or recurrent fetal loss in the background of positive aPL; these should be detected on two separate occasions 12 weeks apart [104].

Antiphospholipid antibodies, detected in 2% of the general population and 12% of the healthy elderly, are directed against proteins that bind negatively charged phospholipids and are called cofactors, among which b2GPI and prothrombin are well-characterized [105]. The initial stimuli leading to aPL production is unknown, but certain infectious agents, drugs, and neoplastic diseases have been implicated with mechanisms of molecular mimicry [106]. Another hypothesis is that aPL exist as natural autoantibodies and become pathogenic under conditions of increased oxidative stress [107].

The exact pathogenesis of thrombosis in APS is largely unknown. Current hypotheses include the disruption of the normally anticoagulative properties of b2GPI [108], the inhibition of activated protein C, protein S, and antithrombin III [109], inhibition of fibrinolysis [110], and endothelial and platelet activation, resulting in a prothrombotic (overexpression of tissue factor) and proinflammatory state (adhesion molecules synthesis and secretion of proinflammatory cytokines) [111]. The role of inflammation in APS has been investigated recently [112]; aPL-induced inflammatory response from the endothelial cells is characterized by overexpression of adhesion molecules (VCAM-1 and E-selectin) and increased production of proinflammatory cytokines and chemokines.

Venous thrombosis is the most frequent manifestation of APS. Usually lower limb deep veins are affected. Rarely, thrombosis may be detected in intra-abdominal, intrathoracic, and upper limb veins. Intracranial thrombosis may present with clinical features of space-occupying lesion [109]. Arterial thromboses may be seen in all arteries; cerebral circulation is most commonly affected. Clinical presentation varies from the typical stroke to the chronic development of multi-infarct dementia. Other affected arteries are the coronary

circulation, pulmonary artery and its branches, and renal and mesenteric arteries [113].

Obstetric complications are classified among the thrombotic manifestations since their pathophysiologic background is thrombosis of trophoblast vessels and placental insufficiency. Early spontaneous abortions (first trimester) are common, and intrauterine growth retardation, premature delivery, pre-eclampsia, eclampsia, and late intrauterine death have also been described [114].

Other manifestations include cognitive impairment and demyelinating lesions, possibly due to direct toxic action of aPL on neuronal cells and the breakdown of the blood–brain barrier [109]. The etiologic relationship of aPL with migraine and transverse myelitis has not been proved, although they are more frequent in aPL positive patients [115]. Libman–Sacks endocarditis is related to high levels of aPL but without a clearly thrombotic background in the valvular area [116]. Lastly, thrombocytopenia and livedo reticularis have been reported with increased frequency in APS.

Catastrophic APS is a rare (<1% of APS patients) variant, characterized by multiple, simultaneous small-vessel thromboses in >3 target organs, and resulting in multiorgan failure in nearly 50% of cases [117].

4. IMMUNOLOGIC AND OTHER LABORATORY FINDINGS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Autoantibodies are the main characteristic of SLE and reflect the polyclonal activation of B cells. Antinuclear antibodies (ANA) are widely accepted as the cornerstone for disease diagnosis [118]. Each antibody that is directed against autologous elements of the cellular nucleus can be classified as ANA and their frequency in SLE exceeds 98% [119]. However, certain ANA can be directed against extranuclear epitopes, such as the anti-Jo-1 antibodies in polymyositis [120]. Indirect immunofluorescence assay (IFA) in a Hep-2 substrate (human epithelioid cells) is the most reliable method for their detection. Other techniques are a specific enzyme-linked immunosorbent assay (ELISA), but it does not have valid standardization. ANA are characterized by high sensitivity but low specificity since they can be detected in 5–10% of healthy individuals and up to 25% of the elderly. For this reason, their positive predictive value is limited in low titers (1/40 to 1/160 dilution) [121]. Current classification criteria do not define a threshold for ANA positivity; furthermore, their evaluation is semiquantitative and depends on precise compliance with the laboratory protocol and the experience of the interpreter. Fluorescence patterns are mainly homogeneous, speckled, peripheral, and nucleolar; a homogeneous pattern is the most common and a speckled pattern the most specific since it is related to the extractable nuclear

antigens (ENA), such as anti-RNP, anti-Sm, anti-SSA/Ro, anti-SSB/La, and anticentromere antibodies [119].

Antibodies against DNA are divided into those directed against single-stranded (anti-ssDNA) and double-stranded DNA (anti-dsDNA), which are considered highly specific for SLE [122]; the latter are detected in 50–65% of patients. The most reliable methods for their detection and quantification are IFA in *Crithidia luciliae* substrate, the Farr assay, and ELISA. Due to the lack of standardization, it is recommended that IFA should be performed for screening, and, in case of positivity, quantification with the Farr assay should follow. Anti-dsDNA antibodies are an acceptable biomarker of disease activity and have been found in the sera of patients even 10 years preceding disease diagnosis [123]. In patients with lupus nephritis, it was demonstrated that an increase in their titers is strongly related to disease flare [124].

Antibodies against extractable nuclear antigens (anti-ENA) are detected with ELISA or immunofixation in 30–50% of patients and relate to certain disease phenotypes. Anti-SSA/Ro and anti-SSB/La antibodies have been associated with secondary Sjögren's syndrome, subacute lupus, and neonatal lupus with congenital heart block [125, 126]. Anti-Smith antibodies are detected in 30% of patients and considered specific for SLE. They are usually detected in combination with the anti-RNP (ribonuclear protein) antibodies due to their relation with common small nuclear RNAs. Anti-RNP antibodies are detected in 50–60% of patients and are related to overlap syndromes with mixed connective tissue disease (MCTD). Both epitopes are not related to disease activity, but their titers may fluctuate during disease course.

Antiphospholipid antibodies are detected by ELISA in 16–60% of lupus patients and related to recurrent arterial and/or venous thrombosis and obstetric complications. Most important epitopes are LA, aCL, and anti-b2GPI antibodies; recently, other epitopes (phosphatidylserine, phosphatidylcholine, phosphatidylinositol, prothrombin, etc.) have been recognized. LA measurement should be based on at least two phospholipid-dependent assays, according to the International Society for Thrombosis and Hemostasis [127].

Antiribosomal P antibodies are related to neuropsychiatric involvement, but there is no direct proof of their pathogenetic role [128]. Antihistones are detected in 50–80% of lupus patients with immunofixation and are associated with drug-induced lupus [19]. Anti-C1q and antinucleosome antibodies are implicated in LN pathogenesis since their titers reflect disease activity and are decreased with a favorable response to therapy [124, 129].

In general, SLE-related autoantibodies exceed 170 different specificities; of clinical significance are the antierythrocyte antibodies, antiplatelet antibodies, anti-endothelial cell antibodies, and, also, antibodies that characterize other autoimmune diseases more frequently seen in SLE patients (Table 10.3).

Lupus erythematosus (LE) cells are macrophages or neutrophils that have phagocytosed apoptotic nuclear material bounded to ANA. They were included in the 1982 classification criteria but, due to low sensitivity, they were excluded in the 1997 revision [118]. C-reactive protein (CRP) and C3 and C4d complement fragments are useful in the monitoring of disease activity [130, 131]. Immune complex levels are increased in patients'

TABLE 10.3 Main Laboratory Findings in SLE

Antinuclear antibodies (ANA)	Prevalence 98%, detected with indirect immunofluorescence
Anti-dsDNA	Prevalence 50–65%, correlated to disease activity and lupus nephritis
Anti-SSA/Ro, anti-SSB/La	Prevalence 30–50%, associated with Sjögren's syndrome, neonatal lupus
Anti-Sm	Highly specific, prevalence 20–30%
Anti-RNP	Associated with mixed connective tissue disease, overlap syndromes
Antiphospholipid antibodies	Prevalence 16–60%, usually lupus anticoagulant, anticardiolipin and anti-b2GPI antibodies, more uncommon epitopes may exist
Antiribosomal P	Associated with neuropsychiatric involvement
Antihistones	Associated with drug-induced disease
Anti-C1q	Associated with nephritis
Antinucleosome	Associated with nephritis
Antierthrocyte, antileukocyte, antiplatelet	Associated with respective cytopenias
C3, C4 complement	Associated with disease activity
Immunocomplexes	
ESR, CRP, IgG	

serum as well as hyper- γ globulinemia, particularly of the IgG type [132]. Other immunologic findings include increased serum levels of proinflammatory cytokines (IL-1, IL-2, IL-6, IL-8, TNF- α , IFN- α), soluble mediators (sCD40L, sVCAM-1), etc.

Erythrocyte sedimentation rate (ESR) is a reliable biomarker of disease activity, but its specificity is low [133]. Other laboratory findings include anemia, leucopenia, lymphopenia, thrombocytopenia from the hematopoietic system, proteinuria, hematuria, and aseptic pyuria from the kidneys, elevated transaminases in cases of liver involvement, and other nonspecific findings.

5. DIAGNOSIS

The first set of classification criteria was adopted in 1971 [134]. In 1982, those criteria were revised from the American College of Rheumatology [135]. The need for revision derived from the wide use of ANA, as a decisive element of SLE diagnosis, as well as from the low specificity of the, initially included, alopecia and Raynaud's phenomenon. The second revision in 1997 incorporated antiphospholipid, anti-dsDNA, and anti-Sm autoantibodies in the 10th criterion. In parallel, LE cell detection was abandoned [118] due to the time-consuming assay. Its sensitivity was estimated to be 85% and specificity 95%.

False-positive assays for syphilis should be persistent for 6 months or more and confirmed with negative immobilization assays for *Treponema pallidum* or absorption of fluorescent treponemal antibodies (FTA-ABS).

Definite diagnosis demands the presence of four or more criteria regardless of the timeframe of the appearance of clinical or laboratory findings.

Recently, novel criteria were proposed by the SLICC study group (Systemic Lupus International Collaborating Clinics) [136]. These criteria focus on the distinction of skin involvement and provide details on the acute, subacute, and chronic skin lesions; in parallel, patchy alopecia is incorporated again. Concerning renal involvement, the protein/creatinine ratio in random urine sample has been suggested. In addition, histopathologic diagnosis of lupus nephritis with positive ANA and/or anti-dsDNA autoantibodies would be adequate for SLE diagnosis. Furthermore, in neurologic disorders, other manifestations may occur such as mononeuritis multiplex, transverse myelitis, peripheral or cranial neuropathy, and acute confusional state. Finally, in the immunologic findings, the inclusion of low complement levels (C3, C4, CH50), IgA anticardiolipin, and anti-b2GPI antibodies and positive Coombs' test have been included. These criteria have increased sensitivity as compared to the 1997 ACR criteria (94% vs. 86%) and decreased specificity (92% vs. 93%) but have more face validity in this era (Table 10.4).

TABLE 10.4 ACR 1997 Revised Classification Criteria for Systemic Lupus Erythematosus

Criterion	Definition
1. Malar rash	Malar erythema, fixed or raised, sparing nasolabial sulcus
2. Discoid rash	Erythematous plaques with hyperkeratosis and folliculitis, atrophic scars in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, from history or physician's observation
4. Oral ulcers	Oral or pharyngeal ulcerations, usually painless observed in physical exam
5. Arthritis	Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	A) Pleuritis, from history of pleuritic pain or pleural rub or evidence of pleuritic effusion B) Pericarditis, confirmed by ECG, pericardial rub, or evidence of pericardial effusion
7. Renal disease	A) Persistent proteinuria >0.5 g/24 h or >3+ in semi-quantitative evaluation B) Cellular casts from erythrocytes, hemoglobin, granular, tubular, or mixed
8. Neurologic disease	A) Seizures in the absence of drug-induced or metabolic disorders (uremia, ketoacidosis, electrolyte disorders) B) Psychosis in the absence of drug-induced or metabolic disorders (uremia, ketoacidosis, electrolyte disorders)
9. Blood disorders	A) Hemolytic anemia with reticulocytosis B) Leukopenia <4000/mm ³ on two or more occasions C) Lymphopenia <1500/mm ³ on two or more occasions D) Thrombocytopenia <100,000/mm ³ in the absence of drug causes
10. Immunologic disorders	A) Anti-DNA: Autoantibodies against DNA in abnormal titers B) Anti-Sm: Autoantibodies against nuclear antigen Sm C) Positive antiphospholipid antibodies (IgM and/or IgG ACA, lupus anticoagulant, IgM, and/or IgG anti-b2GPI, false-positive serological reactions for syphilis)
11. Antinuclear antibodies	Abnormal titers of ANA with immunofluorescence or equivalent assay in the absence of drugs involved in drug-induced lupus

The presence of ≥ 4 criteria is required for the classification of SLE.

6. HEART DISEASE IN SYSTEMIC LUPUS ERYTHEMATOSUS

Heart involvement, in the course of SLE, most commonly presents with pericarditis. However, myocarditis and valvular disease are often detected. Accelerated atherosclerosis is also increasingly recognized as one of the most important comorbidities of SLE.

6.1 Pericarditis

Pericardial effusion is detected in 11–54% of patients, depending on the diagnostic method used [137, 138], whereas acute cardiac tamponade is considered rare and described mainly in case reports [139]. Usually, pericarditis is recurrent and associated with positive ANA and fever [140]. In many cases, pleural effusion may be concurrently found. Rarely, pericarditis may have other causes (viral, tuberculosis, uremic, etc.) in the context of SLE, which should not be overlooked [141].

Diagnosis of pericarditis in SLE patients does not differ from that in nonautoimmune patients. Chest pain (retrosternal or precordial) or discomfort, improved when leaning forward and accompanied by dyspnea, palpitations, or fatigue is the cardinal symptom. Physical examination may reveal the characteristic pericardial rub, whereas ECG may show widespread ST elevation and PR depression in most of the limb and precordial limbs (sinus tachycardia usually coexists). Secure diagnosis is achieved with heart ultrasound (echocardiogram), which can roughly estimate the volume of fluid and assess the hemodynamic impact of the effusion. In rare cases of dry or chronic constrictive pericarditis, cardiac CT and/or MRI may provide further diagnostic information [142].

Autopsy studies demonstrated the presence of granular depositions of immunoglobulin and C3 on the pericardium, suggesting the role of immune complexes in promoting pericardial inflammation [143]. Pericardial fluid is typically an exudate with neutrophil predominance; autoantibodies, mainly ANA, might be detected, although this is not pathognomonic.

6.2 Myocarditis

Primary myocardial involvement is uncommon and affects approximately 3–9% of lupus patients; however, the frequency is greater in autopsy studies performed in the 1950s and 1960s, reaching 15% [143]. In more recent postmortem studies, after the wide use of corticosteroids in lupus therapeutics, its prevalence was 0–8% [144]. African American patients are at increased risk for development of myocarditis [145].

Typically, myocarditis causes chest pain with palpitations and/or clinical features of heart failure. Cardiac

troponins and pro-B type natriuretic peptide are usually elevated [138]. History of recent viral infection will usually be absent. ECG commonly shows sinus tachycardia with widespread nonspecific ST alterations and T-wave changes. Lupus myocarditis may present with arrhythmias, conduction abnormalities, and ventricular dilatation. Echocardiogram can assess the extent of ventricular dysfunction (systolic and diastolic) and cardiac function compromise as low ejection fraction is detected in the majority of patients [146]; rough assessment of the texture of ventricular wall may be feasible. Global hypokinesis is detected in 5–20% of cases, but segmental motion abnormalities may also be indicative of the disease. Cardiac MRI is the imaging modality of choice in nonischemic inflammation of the myocardium; inflammatory hyperemia and edema, necrosis and/or scar, contractile dysfunction, and pericardium involvement may be addressed thoroughly (Fig. 10.1) [147, 148]. Endomyocardial biopsy may further support the diagnosis in complicated cases; however, its diagnostic yield ranges from 10% to 30%. Typical findings include mononuclear cell infiltration, perivascular inflammation, and cardiomyocyte necrosis (Fig. 10.2) [149]. Immunofluorescence studies have demonstrated granular immune complexes and complement deposition in the wall and perivascular tissue of myocardial blood vessels, supporting the hypothesis that lupus myocarditis is an immune complex-mediated disease.

6.3 Cardiomyopathy

Cardiomyopathy, mainly manifested as global hypokinesis and impaired contractility, is multifactorial in SLE since myocardial dysfunction may be caused by disease activity, concurrent infection, uremia, hypertension, thrombosis, and other factors. In later stages, accelerated atherosclerosis leading to ischemic sequelae plays a key role. The most common initial echocardiographic finding is that of mild diastolic dysfunction and decreased exercise tolerance [140]. This dysfunction may remain undetected and deteriorate, even in adolescents, since it is largely asymptomatic and is associated with disease duration, renal impairment, and abnormal nailfold microvasculature [150]. More recently, it was shown that left atrial mechanical function and volume are impaired in SLE, particularly in asymptomatic patients with significant cumulative damage [151].

Chronic antimalarial use (chloroquine or hydroxychloroquine) has been linked to a specific form of cardiomyopathy that resembles that of lysosomal storage diseases [152]. Secure diagnosis requires endomyocardial biopsy. Characteristic findings include vacuolated cytoplasm with inclusion bodies (lipid accumulation) on electron microscopy (Fig. 10.3). This cardiomyopathy is characterized by concentric hypertrophy of all chambers

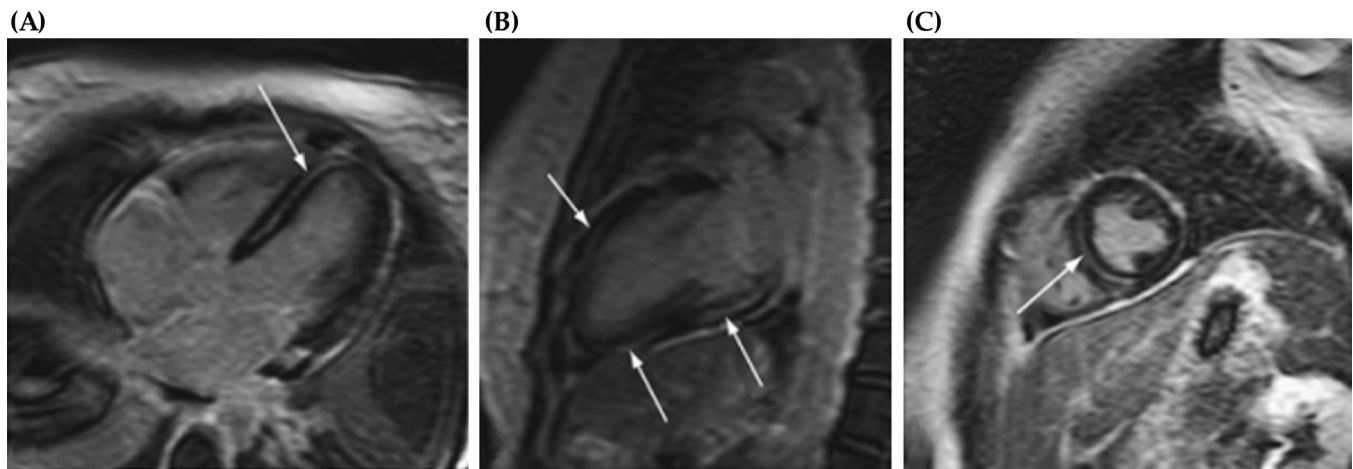


FIGURE 10.1 Lupus myocarditis. MRI shows enhancement of the myocardium, which spares the endocardium. (A–C): Contrast-enhanced inversion recovery technique with late imaging after contrast administration in horizontal long-axis plane (A), vertical long-axis plane (B), and short-axis plane (C). Strong enhancement is found in the midwall of the ventricular septum (A and C), in the apex (A), and in the midanterior and posterior wall (B). Reprinted from Elsevier Lupus Image Bank [148].

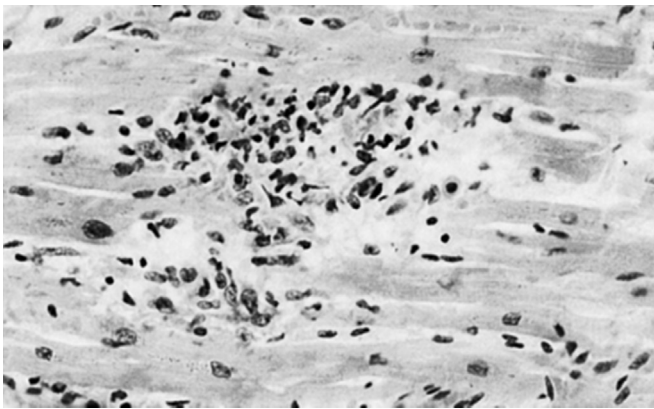


FIGURE 10.2 Endomyocardial biopsy specimen from a patient with systemic lupus erythematosus, demonstrating the typical histologic features of focal myocarditis. Reprinted from Elsevier Lupus Image Bank [149].

and restrictive functional features. Cardiac MRI is considered to reliably assess the extent and distribution of hypertrophy and myocardial fibrosis (Fig. 10.4). Differential diagnosis from Fabry's disease (with genetic testing) is warranted. From a clinical perspective, heart failure is the dominant syndrome; drug withdrawal is recommended while prognosis is poor.

More recently, Takotsubo cardiomyopathy was described in lupus patients [153]. This particular form of cardiomyopathy is associated with physical and emotional stress and most commonly affects postmenopausal women. The typical echocardiographic presentation is that of apical ballooning of the left ventricle usually accompanied by mural thrombus. Disease pathogenesis is largely unknown; microcirculation disturbances, coronary vasospasm, ischemia-reperfusion injury, and catecholamine overload may be implicated. Management is usually supportive with normalization of wall

abnormalities within weeks. Its association with SLE is currently unclear.

6.4 Valvular Disease

Valvular involvement in SLE is detected echocardiographically in almost 50% of patients and usually presents as valve thickening, sterile vegetations, and valve distortion and dysfunction. Nevertheless, its direct link to disease pathophysiology is uncertain. Many investigators have reported an association between valvular abnormalities and the presence of antiphospholipid antibodies [143]. Hohnik et al. reviewed four large studies with transthoracic echocardiograms (TTE) in patients with primary APS and concluded that 32–38% of patients had mitral and aortic valve involvement [116]. In studies using transesophageal echocardiography (TEE), the prevalence of valvular disease reached 82% (valve thickening in 63%), while this was correlated to the titer of IgG anticardiolipin antibodies [154]. Of note, valvular involvement was present in all patients who eventually developed cerebrovascular disease (stroke), suggesting that the presence of valve abnormalities may be a significant risk factor for stroke, seizures, and other CNS manifestations [155].

From a functional standpoint, mild regurgitation of the mitral and aortic valves is usually detected, while tricuspid and pulmonary valves may be secondarily affected in cases of pulmonary arterial hypertension. Rarely, clinically significant valvular insufficiency and/or stenosis develop, and in 4–6% of cases surgical treatment may be required. The mortality risk of surgical replacement is reportedly higher in patients taking immunosuppressives or having APS [156]. There is no direct evidence that treatment with corticosteroids and/

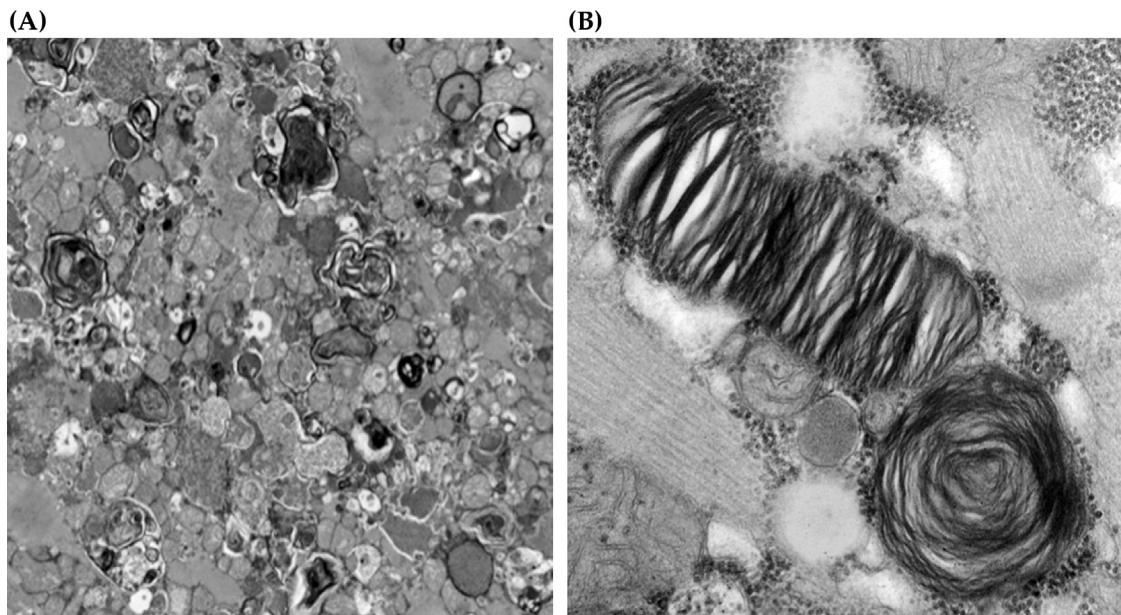


FIGURE 10.3 (A) Lamellar phospholipid membranes in stacks and whorls. (B) Lamellated myelinoid inclusions in myocytes. *Adapted from the personal collection of the authors.*

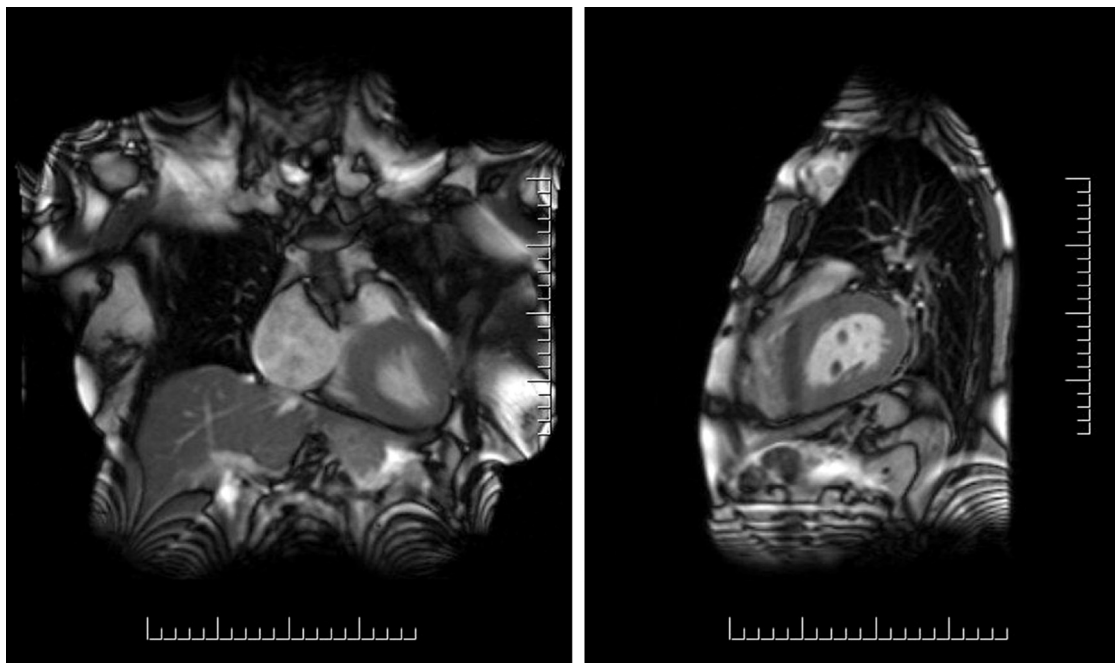


FIGURE 10.4 Dramatic LVH (LV mass index 122 g/m^2 compared with 75 g/m^2). No evidence of late gadolinium enhancement indicating myocardial fibrosis. *Adapted from the personal collection of the authors.*

or immunosuppressives may prevent or improve valvular disease. However, autopsy studies showed a decline in the prevalence of Libman–Sacks endocarditis after the introduction of corticosteroids, supporting an indirect role [143].

The most characteristic valvulopathy of SLE is Libman–Sacks endocarditis (LSE), which mainly affects the left cardiac valves [157]. In an echocardiographic

study of 342 lupus patients, LSE was diagnosed in 38 cases (24 mitral, 13 aortic, and 1 tricuspid valve) [158]. LSE may rarely affect more than one valve [159]. Its frequency is considered lower than previously reported, possibly due to the more effective disease management and estimated to vary widely between 11% and 74%. LSE is a noninfectious, verrucous, vegetative endocarditis (Fig. 10.5) [160]. The histopathological

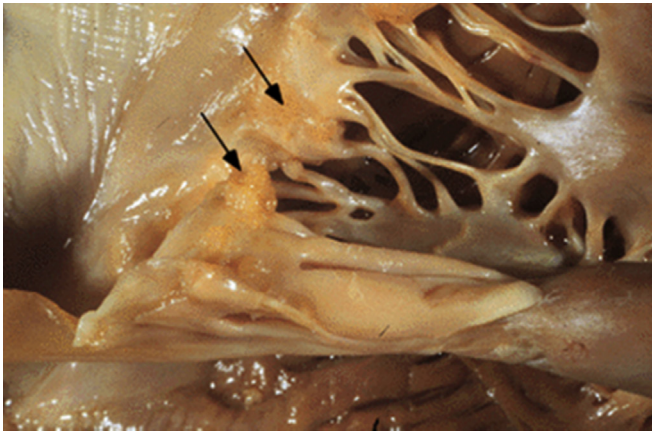


FIGURE 10.5 Libman-Sacks verrucous endocarditis with valvular vegetations (arrows) in a 52-year-old woman with systemic lupus erythematosus who died of pneumonia and chronic interstitial pneumonitis. Reprinted from *Bernas et al.* [332].

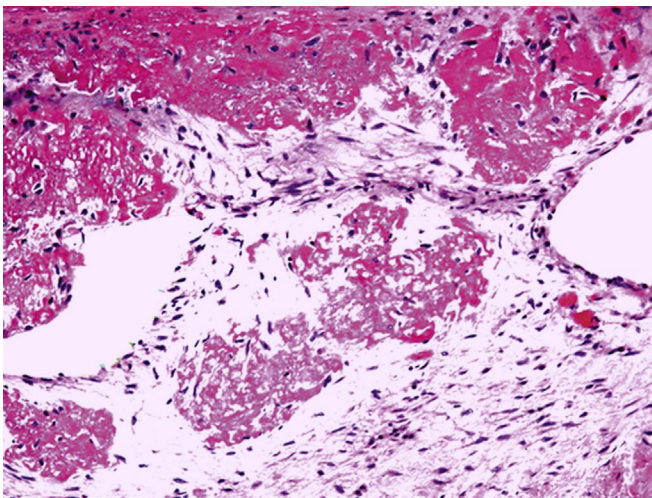


FIGURE 10.6 Libman-Sacks endocarditis. A 27-year-old woman died during an acute exacerbation of systemic lupus erythematosus. At autopsy, the mitral valve and surrounding endocardium showed small vegetations. Histologic examination showed the fibrinoid necrosis of the valve cusp that is characteristic of Libman-Sacks endocarditis ($\times 250$). Reprinted from *Elsevier Lupus Image Bank* [161].

background involves fibrin deposits, neovascularization, immune complex deposition, and mononuclear cells infiltration (Fig. 10.6) [161]. Diagnosis is based on transthoracic and transesophageal echocardiogram for the visualization of leaflet vegetations, as well as in real-time 3D ultrasound (Fig. 10.7) [162]. LSE was shown to be related to antiphospholipid antibodies and clinically present with acute embolic infarcts (mainly cerebral) [143]. Conservative management with corticosteroids and anticoagulation may be beneficial in selected patients with no hemodynamic instability. Clinically significant valvular dysfunction, requiring surgical management, develops in 1–18% of patients.

6.5 Electrocardiographic Abnormalities and Arrhythmias

Rhythm disturbances are considered rare in SLE; however, in a recent study it was demonstrated that nonspecific ST-T changes exist in 31% of newly diagnosed patients [163]. Repolarization abnormalities, mainly expressed as prolonged QT, were present in 15%, although in <1% that was severe ($QTc > 550$ msec). Other findings included ECG evidence of left ventricular hypertrophy in 5.4%, supraventricular arrhythmias in 1.3% (atrial fibrillation in 0.13%), atrioventricular heart block in 0.6%, incomplete bundle branch block (BBB) in 2.7%, right BBB in 0.8%, and left BBB in 0.3% of patients. Only the cumulative damage (as assessed by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index, SLICC/ACR DI) was associated with these repolarization abnormalities. Antibodies against SSA/Ro antigen (the main autoantibodies implicated in the atrioventricular block of neonatal lupus) were not associated with any ECG findings. Recently, QRS fragmentation was described in higher frequency in SLE patients and was associated with higher C-reactive protein levels, older age, and longer disease duration [164].

From a pathogenetic standpoint, autopsy studies revealed the presence of periarteritis of sinus nodal arteries and fibrosis of the atrioventricular node and conduction tissue [165].

Moreover, the medications used in lupus therapeutics may have a role in the prevalence of ECG abnormalities. In this context, a cumulative dose of hydroxychloroquine greater than 365 g was associated with decreased resting heart rate [166]. In line with this observation, chloroquine (and duration of use) was demonstrated to be protective against arrhythmias [167]. On the contrary, high disease activity was related to increased rates of chronic tachycardia [168].

7. ACCELERATED ATHEROSCLEROSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

The inflammatory nature of atherosclerosis has been well established during the last 15 years [169]. In this context, an initial insult causing endothelial dysfunction will drive the accumulation of macrophages and T lymphocytes into the arterial wall, which in turn will proliferate and activate other cellular subpopulations through the secretion of proinflammatory cytokines. This mechanism will facilitate the formation of atherosclerotic plaques whose rupture will give rise to the clinical equivalent, i.e., cardiovascular events.

The first studies on the impact of atherosclerotic cardiovascular events (CVEs) on SLE mortality date back

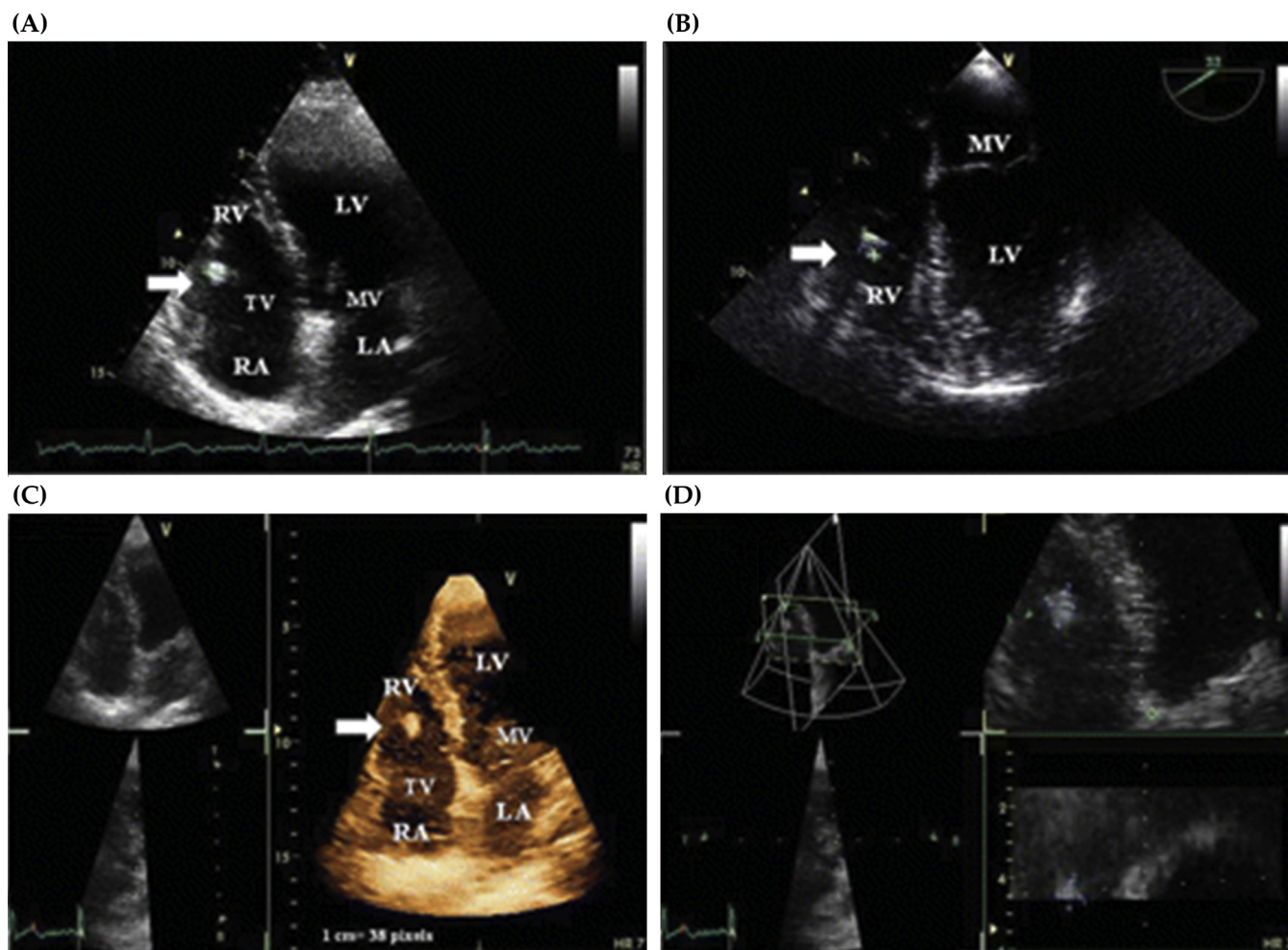


FIGURE 10.7 (A) and (B) Transthoracic two-dimensional echocardiogram and transoesophageal echocardiography images, respectively, showing a subtricuspid vegetation of $\approx 0.65 \times 0.65$ cm. (C) Real-time three-dimensional image from the apical four-chamber view, showing the Libman–Sacks vegetation. With this mode of acquisition, which is predominantly used to visualize cardiac valve morphology, a three-dimensional pyramidal dataset of $\approx 50^\circ \times 30^\circ$ is displayed in a volume-rendered manner in real-time without the need for respiratory gating. (D) Vegetation size was measured by three-dimensional echocardiography using a novel cropping tool, the 9-slice mode. A wide-angle $90^\circ \times 90^\circ$ pyramidal data set obtained from a typical apical four-chamber view is sliced into nine equidistant, parallel short-axis cut planes from base to apex. The position of the nine slices can be adapted to the real anatomy in angle and distance. Thus it is possible to pinpoint the cut plane across the vegetation so that its dimensions can be measured. A more accurate vegetation-size measurement could identify a group of systemic lupus erythematosus patients at higher risk for embolic events. Reprinted from Plastiras et al. [162].

to 1976 [6] when it was demonstrated that atherosclerotic CVEs are the major causes of death late in disease course. Subsequently, it was demonstrated that 30% of patients' deaths were attributed to coronary artery disease (CAD) [170]. At that time, it was shown that premenopausal women, aged 35–44, with SLE had at least 50-fold increased risk for myocardial infarction (MI) when compared to healthy women of the same age [171]. Following that initial observations, several studies have provided solid evidence that SLE is associated with an excessive cardiovascular risk. Recently, in a population-wide study from Sweden, it was shown that SLE patients who were hospitalized for their disease had a five-fold increased risk for readmission for a major cardiovascular event in the first year after initial admission [172]. Furthermore, SLE patients have a five-fold increased risk

for myocardial infarction in the first year after diagnosis [173] or even in the 2 years preceding diagnosis [174].

In the Toronto Lupus Clinic, 11% of all patients (and 10% of 561 inception patients) did develop a CVE in an average time of 8–9 years since diagnosis [175]. The average age of a first CAD-related event in a female lupus patient is between 48 and 50 [176], rather earlier than her healthy counterpart. Moreover, the age difference at the time of cardiovascular death was approximately 15 years for females and 11 for male patients [177].

The precise pathophysiologic mechanisms underlying accelerated atherosclerosis in SLE have not been fully elucidated. It is likely that they represent the net result of a complex interplay between traditional, disease-related risk factors, and the medications used for the long-term management of lupus.

7.1 Predictive Factors of Premature Atherosclerosis in Systemic Lupus Erythematosus

7.1.1 Traditional Risk Factors

Traditional risk factors are involved in the atherosclerotic process in SLE in a proportion that was estimated to be about 60% [178].

Age, particularly over 48 or in postmenopausal state, was shown to be a significant independent predictor for atherosclerotic CVEs with a relative risk ranging from 1.04 to 5.1 for all age groups [179]. In addition, increasing age was related to each stage of subclinical atherosclerosis, such as endothelial dysfunction, arterial stiffness, arterial wall thickening and/or plaque formation, coronary artery calcification, and angiographically defined atherosclerotic plaques [180].

Positive family history, defined by the presence of a CVE in a first-degree relative under the age of 55 for males or 65 for females, was an independent risk factor for CVEs (relative risk=3.6) [181].

Furthermore, both CVEs (HR=1.6–6.2) and subclinical atherosclerosis (as assessed by arterial stiffness, atherosclerotic plaques in the carotid and femoral arteries, coronary calcification, and angiographically proven CAD) were more frequent in male patients [181].

Obesity is a well-recognized risk factor both for CVEs and subclinical atherosclerosis in SLE patients [182]. In particular, lupus patients with BMI>30 demonstrated endothelial dysfunction, assessed by brachial artery flow-mediated dilation, increased carotid intima-media thickness (IMT) and plaque formation (HR=1.06–6.16), and coronary artery calcification [180]. Interestingly, obesity was among the major predictors of IMT progression over 3 years in pediatric SLE patients in a prospective study [183].

Arterial hypertension is detected in increased frequency in SLE patients, ranging from 25% to 74% in various large cohorts; common causes include kidney involvement and/or increased use of corticosteroids and nonsteroidal anti-inflammatory medications [184, 185]. Moreover, the activation of the renin-angiotensin system, increased levels of endothelin-1, and oxidative stress along with certain cytokines (IL-6, IL-17, TNF α) play a significant role [185]. Hypertension was found to be independently associated with increased rates of CVEs (relative risk=1.05–3.5). Furthermore, it was related to endothelial dysfunction and arterial stiffness, increased carotid IMT and plaque formation, coronary artery calcification, and angiographic CAD [180]. In addition, HTN was an independent risk factor for myocardial perfusion defects (HR=2.11–2.53) [186]. Further research from the Toronto Lupus Clinic showed that in approximately half of SLE patients, blood pressure fluctuates significantly and was associated with increasing

age and disease activity [184]. It was also demonstrated that the time-adjusted mean values of systolic and diastolic blood pressure could capture the respective CV risk more precisely than the traditional Framingham definition.

Impairment of glucose metabolism has been demonstrated by significantly decreased sensitivity to insulin in nondiabetic lupus patients. Euglycemic state is achieved by a compensatory increase in insulin secretion [187]. Insulin resistance was related to disease activity, inflammatory markers, and increased levels of oxidized LDL (oxLDL) and, less frequently, with corticosteroid therapy [188]. Diabetes mellitus was associated with an increased risk for CVEs [189, 190] and conferred a 60-fold increased risk for carotid IMT progression and a four-fold for myocardial perfusion defects [180].

Dyslipidemia, defined as any atherogenic abnormality in the basic lipid profile (increased total cholesterol, and/or LDL and/or triglycerides and/or decreased HDL), affects about one-third of SLE patients at the time of diagnosis and almost 60% after 3 years [191]. Two distinct patterns have been described; the first is characterized by increased levels of triglycerides and very low-density lipoprotein (VLDL), as well as by decreased high-density lipoprotein (HDL). This pattern reflects very active or untreated disease, as a consequence of systemic inflammation, and is attributed to disorders of chylomicron metabolism or to the presence of autoantibodies against lipoprotein lipase (LpL) and apolipoprotein A1 [192, 193]. The second and more common pattern is characterized by increased total cholesterol (TC) and triglycerides (TG) as well as increased low-density lipoproteins (LDL). This is related to kidney involvement (nephrotic syndrome), hypothyroidism, and corticosteroid therapy [194]. Multiple factors contribute to the high frequency of dyslipidemia in SLE. The most important are autoantibodies against LpL, oxidized LDL, HDL, and apolipoprotein A1. Moreover, certain cytokines such as TNF α , IL-6, and monocyte chemoattractant protein 1 (MCP-1) affect lipid metabolism by increasing the hepatic synthesis of VLDL [195]. Nevertheless, increased TC is an independent predictor for CVEs (relative risk=3.9–6.9) as well as subclinical atherosclerosis. Time-adjusted TC values may capture more precisely the increased CV risk of lupus patients, since lipid values may fluctuate over time, reflecting changes in disease activity and therapy with antimalarials that exhibit a favorable effect on lipid profile [184]. Of note, patients with sustained hypercholesterolemia were more prone to develop CVEs as compared to patients with intermittently elevated total cholesterol [196]. In addition, elevated TG were an independent predictor for CVEs [197]. Recently, the role of proinflammatory HDL in accelerated atherosclerosis in

SLE was described [198]. It is believed that chemically modified HDL molecules lose their antiatherogenic properties and induce vascular inflammation through immune-mediated mechanisms. Proinflammatory HDL was strongly associated with increased carotid IMT and plaque formation [199].

Metabolic syndrome represents a combination of traditional atherosclerotic risk factors with a common pathophysiologic basis on insulin resistance. In its most acceptable definition it comprises an “abnormal” waist circumference with elevated triglycerides, arterial hypertension, impaired glucose metabolism, and decreased HDL levels. The metabolic syndrome is reported three times more frequently in SLE as compared to the general population [200]. The main risk factors identified were central obesity, arterial hypertension, and insulin resistance. It was demonstrated that the metabolic syndrome in lupus patients was associated with higher disease activity, corticosteroid use, and renal disease, whereas the use of antimalarials was associated with lower prevalence [201]. Furthermore, it was shown that free fatty acids are significantly elevated in SLE patients with metabolic syndrome and were related to insulin resistance (as assessed with the homeostasis model, HOMA) and endothelial activation [202]. From a clinical standpoint, metabolic syndrome was associated with increased carotid IMT, coronary artery calcification, and arterial stiffness [180].

High levels of homocysteine have a significant impact on the accelerated atherosclerosis of lupus patients by causing endothelial dysfunction through increased oxidative stress and inhibition of the endothelial derived NO synthetase (eNOS). Furthermore, homocysteine may cause functional disorders of the platelets and soluble coagulation factors, thus inducing a prothrombotic state in SLE [203]. Elevated levels of homocysteine were related to coronary artery calcification and increased carotid IMT or plaque [180].

Finally, smoking was associated with a 2.2–3.7 increased risk for CVEs as well as increased rates of carotid plaque and coronary artery calcification [180].

In addition to the aforementioned traditional atherosclerotic risk factors, it was recently demonstrated that African American lupus patients are on average 10 years younger than their Caucasian peers at the time of first hospital admission for cardiovascular disease [177]. The same conclusions were also made for Hispanic lupus populations (as compared to Caucasians). It should be mentioned that socioeconomic variables were not taken into account in that study. In another relevant study (the LUMINA group) such a difference could not be reproduced [204].

Traditional risk factors, which may independently predict clinical or subclinical CAD, are presented in Tables 10.5 A and B.

7.1.2 Disease-Related Risk Factors

SLE has been demonstrated to be an independent predictor for CVEs, increasing the risk of clinical cardiovascular disease about 5–8 times. In this context, multiple disease-related factors have been associated with that increased risk.

Overall disease activity, assessed by composite indices such as SLE Disease Activity Index (SLEDAI) [205], was significantly associated with CVEs (relative risk = 1.05–1.2) and subclinical atherosclerosis. In particular, high disease activity was related to increased arterial stiffness, increased prevalence of carotid plaque formation, and a 12-fold increase in coronary artery calcification score [180]. Recently, it was shown that clinical activity is more crucial in increasing CV risk since patients with serological activity but clinically quiescent were less likely to develop CAD [206]. Cumulative damage, assessed by the Systemic Lupus International Collaborating Clinics (SLICC) Damage Index [207], was strongly related to CVEs (HR = 1.3–4.1), endothelial dysfunction, carotid IMT and plaque (HR = 1.7), and coronary artery calcification (HR = 1.2) [180]. Likewise, disease duration was an independent predictor of CVEs (HR = 1.1–1.45), arterial stiffness and decreased small artery elasticity, increased carotid IMT and plaque (HR = 1.7–3.2), and coronary artery calcification (HR = 1.2–15.1) [180].

Certain disease phenotypes are linked to an excess atherosclerotic risk. Lupus nephritis and renal impairment were independent predictors of CVEs (relative risk = 1.2–6.8), increased aortic stiffness, and carotid IMT and plaque, even in pediatric lupus patients [180]. Creatinine levels >110 mmol/L were associated with a 16.4-fold increase of coronary calcification. Proteinuria was related to CVEs (relative risk = 2.4) and increased carotid IMT and plaque. Neuropsychiatric involvement was associated with CVEs (relative risk = 2.2–5.2), probably reflecting increased disease activity.

Anticardiolipin antibodies (aCL) were implicated as independent predictors of CVEs in several studies (HR = 3.1–5.8). They were also associated with myocardial perfusion defects (HR = 4.1), carotid plaques (HR = 5.2), and coronary calcifications. Anti-b2GPI antibodies were associated with CVEs (HR = 3.4) and coronary calcifications, but not with carotid plaques or endothelial dysfunction. Lupus anticoagulant (LA) was associated with CVEs (HR = 1.74), carotid plaque (HR = 5.2), and coronary calcifications (HR = 4.4). Other antiphospholipid epitopes, such as anti-oxPAPC (oxidized palmitoyl arachinodoyl phosphocholine), were identified risk factors for increased carotid IMT and plaque formation in solitary studies (HR = 1.06). In addition, low levels of natural IgM antiphosphorylcholine antibodies were related to increased carotid IMT and plaque formation [180].

TABLE 10.5A Traditional (Modifiable and Nonmodifiable) Risk Factors With an Independent Predictive Ability for CVEs (Cardiovascular Events) or Surrogate Atherosclerosis Measures in Lupus Patients

Parameter	PWV	CP/IMT	CAC	SPECT	CA	CVEs
	(Pooled HR)	(Pooled HR)	(Pooled HR)	(Pooled HR)	(Pooled HR)	(Pooled HR)
Age	1.13	1.11–4.1	1.08–8.5		2.22	1.04–5.1
Positive family history						3.6
Male sex		8.78			2.38	1.56–6.2
BMI > 30		1.06–6.16				
Arterial hypertension		1.04–3		2.11–2.53		1.05–3.5
Diabetes mellitus	1.54	60		4		1.5
TC		1.2–3		2.51	1.89	3.9–6.9
LDL		7.6				
HDL		4.8		3.86		
piHDL		9.1–12.8				
TG						1.15–8
oxLDL						
Metabolic syndrome		3.11				
Homocysteine		1.24				
Smoking		7.7	3.8			2.2–3.7

CA, coronary angiography; CP, carotid plaque; IMT, intima-media thickness; PWV, pulse-wave velocity; SPECT, single photon emission computed tomography. For each endpoint, the respective pooled hazard ratio range (HR) is shown.

TABLE 10.5B SLE-Related Risk Factors With an Independent Predictive Ability for CVEs (Cardiovascular Events) or Surrogate Atherosclerosis Measures

Parameter	PWV	CP/IMT	CAC	SPECT	CVEs
	(HR)	(Pooled HR)	(Pooled HR)	(HR)	(Pooled HR)
Disease activity			12.3		1.05–1.2
Cumulative damage		1.7	1.2		1.3–4.1
Disease duration		1.7–3.2	1.2–15.1		1.1–1.45
aCL		5.2		4.1	3.1–5.8
Anti-b2GPI					3.4
LA		5.2	4.4		1.74
Anti-oxPAPC		1.06			
Anti-dsDNA					1.56
hsCRP		3	1.65–4.15		1.6–3.4
TWEAK		29			
IL-6			1.07		
Renal disease	7.5		16.4		1.2–6.8
Proteinuria					2.4
Neuropsychiatric SLE					2.2–5.2

CP, carotid plaque; IMT, intima-media thickness; PWV, pulse-wave velocity; SPECT, single photon emission computed tomography. For each endpoint, the respective pooled hazard ratio range (HR) is shown.

Anti-Sm antibodies were protective against carotid plaque [208]. Anti-dsDNA autoantibodies, a widely acceptable activity biomarker, were found to be associated with CVEs (HR=1.56) and noncalcified coronary plaques. Other antibodies that have been implicated in lupus vasculopathy are the antiendothelial cell antibodies (AECAs). Despite their ability to stimulate endothelial activation through upregulation of the adhesion molecules ICAM-1, VCAM-1, and E-selectin [209], their precise role in promoting atherogenesis is unknown. Other autoantibodies with potential atherogenic properties are those against HDL and its protein part apoA1, which were shown to correlate to disease activity [210].

After the recognition of the importance of immune-mediated mechanisms in the atherosclerotic process in 1999, an increasing number of soluble inflammatory mediators were evaluated in patients with SLE and premature atherosclerosis [211]. C-reactive protein (CRP) is a potent atherogenic molecule, able to induce the expression of adhesion molecules from endothelial cells, and the secretion of proinflammatory cytokines such as IL-1, IL-6, IL-8, and TNF- α , which activate macrophages for oxLDL ingestion. Furthermore, it is involved in the mechanisms of coagulation and fibrinolysis through the activation of Plasminogen Activator Inhibitor 1 (PAI-1). Newer high sensitivity assays are considered more specific and better reflect systemic inflammation and atherosclerotic risk. High-sensitivity CRP confers an increased risk for CVEs (relative risk=1.6–3.4). In addition, it was an independent predictor of endothelial dysfunction, increased arterial stiffness, carotid IMT and plaque, and coronary calcification presence and severity [180].

Elevated levels of complement C3 were implicated in the accelerated atherosclerotic process as it was shown to predict carotid IMT progression over 2 years [212]. C3 above the upper limit of normal (>120 mg/dL) was associated with carotid plaque (HR=1.9) and coronary artery calcification and increased arterial stiffness [213].

TNF- α may induce dyslipidemic profile with increased TG and decreased HDL, through interactions with its soluble receptors (sTNFR). In addition, it inhibits lipoprotein lipase and induces VLDL synthesis [214]. Serum levels of TNF- α are strongly related to disease activity and drive the activation of endothelial cells, smooth muscle cells, and macrophages, thus augmenting the atherogenic process. Recently, it was demonstrated that TNF- α is implicated in endothelial cell apoptosis (through p55 receptor) and vulnerability of the atherosclerotic plaque [215].

Low levels of the transforming growth factor β (TGF- β) in SLE are associated with the breakdown of immune tolerance that characterizes the disease. Studies in lupus-prone mice as well as in human patients demonstrated a strong correlation of decreased TGF- β with premature atherosclerosis [216]. Newer studies showed that

decreased numbers of T regulatory cells are implicated in the decreased levels of TGF- β in the periphery and within the atherosclerotic plaque [217]. Other cytokines that were shown to increase CV risk in lupus patients are tumor necrosis factor-like weak inducer of apoptosis (TWEAK), IL-6, vascular endothelial growth factor (VEGF), and type I interferons. Furthermore, certain adhesion molecules were associated with increased burden of subclinical atherosclerosis (VCAM, ICAM-1, E-selectin). Adipocytokines were recently introduced as potential atherosclerosis risk factors; leptin (particularly >34 ng/dL) conferred an increased risk for carotid IMT and plaque [180].

Soluble CD40 ligand (sCD40L) is overexpressed in SLE and associated with increased activation of the endothelial cells through CD40 binding on their surface. This molecule induces the coagulation cascade through the increase of tissue factor (TF) expression and is also related to plaque vulnerability. Increased sCD40L is associated with increased risk for recurrence after an acute coronary syndrome [218].

Other inflammatory factors implicated in accelerated atherosclerosis in SLE are fibrinogen [219], asymmetric dimethyl-arginine, as well as disorders of the clearance of immune complexes containing oxLDL, anti-b2GPI, and other phospholipid epitopes. These immune complexes bind to the C1q receptor of the endothelial cells and induce the expression of vascular cell-adhesion molecule 1 (VCAM-1), while, in parallel, are implicated in cholesterol metabolism.

Apoptosis impairment of the endothelial cells was initially considered as SLE-specific; however, newer studies demonstrated similar results in other systemic autoimmune diseases [220]. Apoptosis induction is mediated through Fas/FasL and TNF/TNFRII interactions, while its potency leads to insufficient apoptotic debris clearance from monocytes/macrophages [221].

T regulatory cells may also be implicated in premature atherosclerosis in SLE [222]. These cells are qualitatively and/or quantitatively affected in the disease and fail to suppress the immune effector cells in the periphery as well as within the atherosclerotic plaque. Novel studies demonstrated that the in vivo expansion of T regulatory cells leads to a significant repression of the atherosclerotic process [217]. Statin administration resulted in the recruitment of these cells inside the atherosclerotic plaque and the suppression of the atherosclerosis-inducing Th1 cells [223].

Finally, the role of the pharmaceutical agents used in disease therapeutics should not be overlooked. Corticosteroids may be directly atherogenic (through the modification of the composition of plasma lipoproteins) and/or augment pre-existing risk factors such as arterial hypertension, insulin resistance, and increased body weight. However, patients with higher cumulative doses are usually those with increased disease severity, making

the latter an important risk factor. High doses of steroids, either as cumulative (reflecting active disease over time), or current dose (reflecting an acute alteration in the vascular microenvironment), were independent predictors for CVEs (relative risk=2.5), increased arterial stiffness, carotid IMT, and plaque formation, as well as coronary calcifications [180].

On the contrary, antimalarials were protective against CVEs (relative risk=0.77) and associated with a favorable metabolic profile concerning lipid and glucose metabolism [180]. Moreover, they were associated with a 68% reduction of thrombotic events (HR=0.32), further underlying their value in the chronic management of SLE [224]. The precise mechanism of antimalarial-related atheroprotection has not been elucidated. It seems that the favorable effects on disease activity, steroid sparing effect, antithrombotic potency, and metabolic profile may play a role.

Disease-related risk factors with independent predictive ability for clinical and subclinical atherosclerotic vascular disease are shown in [Tables 10.5 A and B](#).

7.1.3 Atherosclerosis Imaging in Systemic Lupus Erythematosus

After successful application in the general population, several imaging techniques for the precise characterization of the atherosclerotic burden have been implemented in lupus patients; however, only carotid ultrasound with IMT and plaque assessment has been evaluated with regard to its predictive ability for CVEs.

7.1.4 Flow-Mediated Dilatation of the Brachial Artery

Endothelial dysfunction is believed to represent the initial phase of the atherosclerotic process and can be assessed through the flow-mediated dilatation (FMD).

Impaired FMD was shown to be an independent predictor for future CVEs in the general population.

Most studies in SLE have assessed FMD using widely accepted guidelines, although measurement reproducibility has been questioned [225–233]. Their main findings are summarized in [Table 10.6](#).

A recent *meta-analysis* of most relevant studies demonstrated that FMD is significantly reduced in SLE compared to healthy controls, although publication bias was significant [234]. Furthermore, FMD was inversely correlated to carotid IMT and associated with traditional and disease-related cardiovascular risk factors. SLE was a significant risk factor for impaired FMD [233].

7.1.5 Pulse-Wave Velocity

Pulse-wave analysis and the derivative parameter Augmentation Index quantify arterial stiffness and independently predict future CVEs in the general population. Most commonly, the technique assesses carotid-femoral artery or carotid-radial artery pulse-wave velocity (PWV). Several cross-sectional studies in SLE patients confirmed the association of increased PWV with various traditional and disease-related risk factors [228, 235–242]. [Table 10.7](#) summarizes the findings of the main clinical studies.

Despite the accumulating evidence of PWV impairment in lupus patients, its predictive value for future CVEs has not been tested in SLE.

7.1.6 Carotid Intima-Media Thickness and Carotid Plaque

Increased carotid IMT and plaque formation occur at a later stage of the atherosclerotic process and are characterized by restricted reversibility potential. Ultrasonographic assessment (Doppler) improves the predictive

TABLE 10.6 Main Findings of Studies Assessing Flow-Mediated Dilatation of the Brachial Artery in SLE Patients

	Authors	Publication Year	No of Patients	Main Findings
1	Ahmadi et al. [225]	2011	84	No significant associations between FMD and disease activity, disease duration, and antiphospholipid antibodies.
2	Attia et al. [226]	2011	30	Increased number of circulating endothelial cells in patients were associated with impaired FMD
3	Castejon et al. [227]	2014	46	No association between endothelial progenitor cells and FMD
4	Cypiene et al. [228]	2009	30	Impaired FMD associated with increased BMI and low HDL
5	El-Magadmi et al. [229]	2004	62	FMD was associated with systolic BP
6	Karadag et al. [230]	2007	25	Impaired FMD was related to high CRP
7	Kiss et al. [231]	2006	61	Significant correlations of FMD with age and BP
8	Lee et al. [232]	2006	35	Decreased small artery elasticity was related to age, disease duration and oxLDL
9	Zhang et al. [233]	2009	111	SLE was significantly associated with impaired FMD

TABLE 10.7 Main Findings of Studies Assessing Pulse Wave Velocity in SLE Patients

	Author	Year	n	Main Findings
1	Amissah–Arthur et al. [235]	2012	67	Increased PWV in active lupus patients was restored after treatment with intravenous corticosteroids
2	Cypiene et al. [228]	2009	30	Increased PWV was associated with age and BP
3	Karp et al. [236]	2012	26	Lupus activity related to increased PWV
4	Norby et al. [237]	2011	39	Increased PWV was associated to increased coronary calcification in kidney-transplanted lupus patients
5	Parra et al. [333]	2011	64	Augmentation Index was associated with age and triglycerides
6	Roldan et al. [239]	2014	76	Increased aortic stiffness by transesophageal echocardiography is associated with age, cumulative damage, and LV dysfunction
7	Sabio et al. [240]	2009	128	Increased PWV was associated with age, male gender, metabolic syndrome, disease duration, and CRP
8	Sacre et al. [241]	2014	41	Systolic BP and cumulative dose of steroids were associated with increased PWV
9	Selzer et al. [242]	2004	214	Increased aortic stiffness was associated with age, hypertension, C3, insulin, renal disease, and leukopenia

ability for CVD risk; however, precise standardization is still lacking, leading to significant discrepancies.

Carotid IMT was strongly associated with traditional as well as disease-related risk factors in SLE [180]. The precise mean IMT in asymptomatic lupus patients ranged from 0.37 to 0.89 mm. In longitudinal studies, IMT progressed in 28–40% of patients in 20–34 months. Furthermore, increased carotid IMT was an independent predictor of future CVEs conferring a relative risk of 1.35 after 8 years of follow-up. The main findings of related studies [183,212,233, 242–267] are summarized in Table 10.8.

The assessment of carotid plaque was shown to be a more accurate predictor of CVEs in the general population. In SLE patients, plaque detection rate ranged from 7% to 50% (Table 10.8). In one longitudinal study, carotid plaque frequency was increased from 20% to 24% of patients in 2 years [212]. Regarding its predictive ability, it was shown that total plaque area was more strongly associated with clinical CAD than carotid IMT (HR 9.55 vs. 2.02, respectively) [251]. Other investigators demonstrated a 4.26-fold increased risk for CVEs in lupus patients with carotid plaque [258]. In addition, the concurrent presence of carotid and femoral plaques was a better predictor for CVEs than carotid plaque alone [252].

7.1.7 Coronary Artery Calcification

Coronary artery calcification (CAC) evaluation, quantified with the Agatston score, achieves further CV risk stratification. Potential pitfalls, besides radiation, include the technique's inability to evaluate noncalcified plaques or plaque stability.

Several reports evaluating CAC in lupus patients [203, 268–281] showed significant correlations with traditional and disease-related risk factors (Table 10.9). The prevalence of CAC ranged from 7% to 48%. The disease itself conferred a significant relative risk of 7.7–9.8 for CAC presence [269, 281]. Most of those studies were cross-sectional and not designed to assess the method's predictive ability; nevertheless, in one prospective study, 20% of patients demonstrated an increase of CAC scores after 2 years [274]. In addition, it was recently shown that noncalcified coronary plaques, considered to be more prone to rupture, could be detected in nearly all patients with CAC and half patients without CAC; they were related to age and anti-dsDNA antibodies [275].

7.1.8 Myocardial Perfusion Evaluation With Single Photon Emission Computed Tomography

Myocardial single photon emission computed tomography (SPECT) is a reliable method for assessing myocardial perfusion in the general population. When appropriately selected, perfusion defects confer a 3.7-fold increased risk for myocardial infarction and cardiac death. In SLE, limited studies [186, 278, 282–285] have revealed significant associations between perfusion defects and traditional and disease-related risk factors (Table 10.10). The prevalence of perfusion defects ranged from 28% to 58%, while the pattern of abnormalities included reversible, permanent, and combined defects. Perfusion abnormalities confer a 13-fold increased risk for CVEs after a follow-up of 8.7 years [186]. However, later studies showed that there is a poor agreement between SPECT and coronary angiography, since

TABLE 10.8 Main Findings of Studies Assessing Carotid Intima-Media Thickness and Plaque in SLE Patients

SN	Author	Year	n	Main Findings
1	Ahmad et al. [243]	2007	200	Mean IMT = 0.48 mm, carotid plaque (CP) prevalence 29%, association with age, smoking, antiphospholipid antibodies, and azathioprine use
2	Anania et al. [244]	2010	114	Strong correlation with age and LDL levels
3	Bhatt et al. [245]	2006	50	Mean IMT = 0.417 ± 0.07 mm, CP prevalence 14%, associations with age, BMI, systolic blood pressure, total cholesterol, and cumulative damage
4	Boucelma et al. [246]	2011	153	Increased carotid IMT was associated with age, TC, LDL, and homocysteine
5	Colombo et al. [247]	2009	80	Mean IMT = 0.74 mm, CP prevalence 31.9%
6	De Leeuw et al. [248]	2006	72	Mean IMT = 0.65 ± 0.12 mm, CP prevalence 6%, age, and modified SCORE risk were predictive of increased carotid IMT
7	De Leeuw et al. [249]	2009	74	Carotid IMT progression in 40% of patients after 20 months, age, and disease duration were associated with deterioration
8	Doria et al. [250]	2003	78	Carotid plaque prevalence 17%, IMT associated with age and cumulative steroid dose, absence of hypertension was protective against progression
9	Eder et al. [251]	2014	103	Carotid plaque is a stronger predictor for CVEs than carotid IMT, significant associations with elevated LDL and low HDL
10	Frerix et al. [252]	2014	100	Atherosclerotic plaques in femoral arteries occur in the absence of increased carotid IMT, associated with age, male gender, and smoking
11	Ghosh et al. [253]	2009	60	Increased carotid IMT was associated with cumulative damage
12	Gustafsson et al. [254]	2013	281	Increased carotid IMT was associated with age, hypertension, impaired renal function, and proteinuria
13	Huang et al. [255]	2009	76	Mean carotid IMT = 0.63 ± 0.08 mm in juvenile SLE, CRP, and baseline lymphopenia predict IMT progression
14	Jimenez et al. [256]	2005	70	Carotid IMT was associated with age, disease activity, and cumulative damage
15	Kalim et al. [257]	2013	40	Carotid IMT was associated with age, BMI, disease duration, CRP, and duration of corticosteroid therapy
16	Kao et al. [258]	2013	392	Carotid plaque is a stronger predictor for CVEs than increased IMT
17	McMahon et al. [259]	2014	210	PREDICTS score (age > 48, piHDL > 0.94 FU, leptin > 34 ng/mL, TWEAK > 373 pg/mL, homocysteine > 12 μ mol/L) is associated with 28-fold increase in IMT progression
18	McMahon et al. [260]	2011	250	Age, hypertension, smoking, proinflammatory HDL, and leptin predict carotid IMT progression
19	Mok et al. [261]	2010	123	Metabolic syndrome is associated with increased carotid IMT
20	Roman et al. [262]	2007	158	28.3% of patients had carotid IMT progression after 34 months, CP prevalence 28.5%, associated with age, disease duration, and homocysteine levels
21	Rua-Figueroa et al. [212]	2010	101	Carotid plaque prevalence 20% (baseline) and 24% (after 2 years), associated with age, disease duration, homocysteine, and complement levels
22	Schanberg et al. [183]	2009	221	Increased carotid IMT in pediatric patients was associated with BMI, impaired renal function, high corticosteroid dose, and azathioprine use
23	Selzer et al. [241]	2004	214	Mean carotid IMT = 0.71 ± 0.1 mm, carotid plaque prevalence 31%, associated with age, hypertension, hyperglycemia, total cholesterol, and CRP
24	Shaharir et al. [263]	2012	39	Age and proteinuria were associated with increased carotid IMT in LN patients in remission
25	Smrzova et al. [264]	2010	63	Increased carotid IMT was associated with age, waist-to-hip ratio, disease duration, and proteinuria
26	Souza et al. [265]	2005	82	Carotid plaque prevalence 50%, associated with age, BMI, disease duration, and cumulative damage
27	Thompson et al. [266]	2008	217	Carotid plaque prevalence 31% (baseline), 40% (after 4 years), associated with age, triglycerides, C3 levels, and immunosuppressives at baseline
28	Zhang et al. [233]	2009	111	Premenopausal females had increased carotid IMT, associated with age, BMI, hypertension, disease duration, cumulative damage, and CRP
29	Zhang et al. [267]	2014	210	Mean carotid IMT = 0.74 ± 0.25 mm, carotid plaque prevalence 28.4%, associated with age, metabolic syndrome, and disease duration

TABLE 10.9 Main Findings of Studies Assessing Coronary Artery Calcification in SLE Patients

SN	Author	Year	n	Main Findings
1	Asanuma et al. [268]	2006	74	IL-6 was an independent predictor of increased CAC
2	Asanuma et al. [269]	2003	65	SLE confers a 9.8-fold greater risk for CAC compared with healthy individuals, CAC prevalence 31%, associated with age, male gender, triglycerides, and homocysteine
3	Enama et al. [270]	2010	140	CAC associated with age, uric acid, and smoking
4	Somers et al. [271]	2012	95	Type I interferons were associated with increased CAC and carotid IMT
5	Kao et al. [272]	2008	105	CAC prevalence 48%, associated with CRP and sICAM-1
6	Kiani et al. [273]	2008	200	CAC severity associated with age, BMI, and diabetes
7	Kiani et al. [274]	2011	187	20% of patients increased their CAC scores after 2 years, age, hypertension, smoking, total cholesterol, and CRP predict CAC progression
8	Kiani et al. [275]	2012	147	Age and anti-dsDNA titers predict the presence of noncalcified coronary plaques
9	Manger et al. [276]	2003	75	CAC prevalence 28%, smoking, C3>90 mg/dL, and serum creatinine>110 mmol/L were associated with CAC
10	Mok et al. [277]	2010	123	Metabolic syndrome was associated with CAC
11	Plazak et al. [278]	2011	60	Antiphospholipid antibodies are strong predictors of CAC presence
12	Rho et al. [279]	2008	109	TNFA, ICAM, VCAM, E-selectin, and VEGF were associated with CAC
13	Ribeiro et al. [280]	2010	94	CAC prevalence 12.7%, age, disease duration, and cumulative damage were associated with its presence, bone mineral density was inversely correlated to CAC
14	Romero-Díaz et al. [281]	2012	139	SLE confers a 7.7-fold higher risk for CAC, age, disease duration, and activity are independent predictors
15	Von Feldt et al. [203]	2006	152	CAC prevalence 30%, age, homocysteine, disease duration, and impaired renal function were associated with its presence

TABLE 10.10 Main Findings of Studies Assessing Myocardial Perfusion With SPECT in SLE Patients

SN	Author	Year	n	Main Findings	Perfusion Defects
1	Nikpour et al. [186]	2009	122	Fixed perfusion defects confer a 13-fold increased risk for CVEs	37.7%
2	Bruce et al. [282]	2003	129	Hypertension, total cholesterol and TC/HDL ratio were independent predictors for perfusion defects	38%
3	Nikpour et al. [283]	2011	24	Poor agreement between SPECT and coronary angiography findings	58.3%
4	Plazak et al. [278]	2011	60	Perfusion defects were associated with the presence of antiphospholipid antibodies	36.7%
5	Sella et al. [284]	2003b	82	56% of perfusion defects were reversible, hypertension, low HDL, triglycerides, and diabetes mellitus were strong predictors	28%
6	Zakavi et al. [285]	2009	20	No minor or major CVE was recorded after 40 months of follow-up	45%

approximately two-thirds of patients with perfusion defects had normal angiograms [283].

7.1.9 Magnetic Resonance Imaging

Cardiac MRI primarily aims to visualize microvascular disease. The method's predictive ability has been confirmed in the general population with an increased incidence of myocardial infarction and cardiovascular death. Limited data in SLE suggest that there may be considerable frequency of perfusion defects in the absence of obstructive

CAD [286]. Furthermore, cardiac MRI could detect more ventricular wall abnormalities than conventional transthoracic echocardiogram [287]. SLE patients demonstrated a diffuse pattern of coronary artery wall contrast enhancement (reflecting generalized vascular inflammation), while conventional CAD patients had patchy distribution of the lesions [288]. A recent study in pediatric SLE patients showed that cardiac MRI identifies heart involvement in a significant proportion of children with cardiac symptoms and normal routine evaluation [289].

7.1.10 Coronary Angiography

Coronary angiography is considered the “gold standard” for diagnosis of flow-restricting CAD. It has been demonstrated that lupus patients had comparable coronary atherosclerotic burden as non-SLE controls, although they were 20 years younger and had half the incidence of diabetes mellitus and dyslipidemia [290]. Even though 10% of patients were on hemodialysis at the time of catheterization, SLE was an independent predictor of symptomatic CAD. Additional predictive factors were age, male sex, and dyslipidemia. Previously, it was shown that postmenopausal status, hypertension, and mean number of traditional CAD risk factors were associated with more severe angiographic findings [291].

7.1.11 Composite Cardiovascular Risk Scores

Composite scores, such as the Framingham risk score (FRS) and the Systematic Coronary Risk Evaluation (SCORE), have been used to predict long-term cardiovascular risk in the general population; however, their value in SLE is questionable. In a study using CAC as an endpoint, FRS and PDAY (Pathobiological Determinants for Atherosclerosis in the Youth, a modified score for younger patients) did not differ between lupus patients and controls [292]. In a prospective study of 250 female patients, the FRS at baseline was higher in patients who developed a CVE (5.8 ± 4.2 vs. 2.9 ± 4), although this was clinically insignificant [293]. In fact, it has been demonstrated that the FRS underestimates the risk of CAD in SLE by a factor of 7.5, underlining the importance of lupus-specific risk factors [178]. In the SLICC cohort of inception patients, the mean FRS was lower than the original Framingham cohort (2.55 ± 3.38 vs. 3.29 ± 4.5) with the same age and gender distribution [294]. More recent research showed that a multiplication of FRS by two more accurately predicts CV risk in lupus patients [295].

Risk factor models composed of lupus-specific variables only rather than traditional risk factors were shown to predict subclinical atherosclerosis more accurately [243]. Using carotid plaque as a surrogate, investigators showed that older age at diagnosis, disease duration, azathioprine use, and the presence of antiphospholipid antibodies were strong predictors of plaque presence.

8. THERAPEUTIC APPROACH TO SYSTEMIC LUPUS ERYTHEMATOSUS

Therapeutic management of SLE is multifaceted and demands close collaboration of the physician and the patient. Changes in the pharmaceutical regimen and/or the doses of different agents are common. Nonpharmaceutical measures and lifestyle modifications are equally important in long-term prognosis. The main medications used in SLE therapeutics are the following.

8.1 Antimalarials

Antimalarials (chloroquine and hydroxychloroquine) are the drugs of choice in mild to moderate disease severity and rather effective in musculoskeletal and skin involvement [296]. Additionally, they prevent mild disease flares and decrease thrombosis rate but they are of limited value in active visceral involvement. They inhibit the function of lysosomes and TLR activation leading to a decrease of DC-derived IFN- α [297]. Furthermore, they are related to improved lipid profile, as they increase HDL levels. The usual dose for hydroxychloroquine is 200–400 mg/day. Side effects may include eye toxicity with corneal and retinal deposits (a dose of 6 mg/kgBW for hydroxychloroquine is considered safer) for which annual ophthalmology evaluation with visual field assessment is recommended [298]. Chronic use has been associated with elevated muscle enzymes [299] and rarely with hypertrophic cardiomyopathy [152]. It may cause hemolysis in patients with G-6PD deficiency.

8.2 Corticosteroids

Corticosteroids represent the cornerstone of lupus therapeutics and the first choice in cases of significant visceral involvement such as nephritis and neuropsychiatric involvement [300]. They are potent anti-inflammatory agents and suppress the mechanisms of natural as well as adaptive immunity. They bind to the intracellular glucocorticoid receptor 1 (GR 1) and the complex translocates to the nucleus where it inhibits the function of the transcriptional factor NFkB. With this mechanism, they inhibit the function of B and T cells, as well as monocytes and DCs [301].

Usual doses range from a few mg/day to 0.5–1 mg/kgBW/day whereas, in organ threatening cases, intravenous pulses (1 g/day for 3 to 5 consecutive days) are used. Common side effects include osteoporosis (calcium and vitamin D supplementation is recommended), metabolic disorders (impaired insulin tolerance, dyslipidemia, arterial hypertension), increase of body weight (salt restriction is recommended), predisposition to infections, etc.

8.3 Methotrexate

Methotrexate is believed to act through the inhibition of enzymes involved in purine metabolism (mainly dihydrofolate reductase). Furthermore, it inhibits T- and B-cell activation and induces T-cell apoptosis. It is mainly used for the treatment of musculoskeletal involvement in doses 15–25 mg/week. Coadministration of folic acid (5 mg/day) is recommended. Common side effects are liver toxicity, leukopenia, and pulmonary fibrosis.

8.4 Azathioprine

Azathioprine is a purine analog that is transformed in the liver and erythrocytes to its active metabolite, 6-mercaptopurine. It is a nonspecific immunosuppressive that inhibits DNA synthesis and immune cell proliferation. In SLE, it is commonly used as a steroid-sparing agent, whereas it demonstrated satisfactory results in remission maintenance after induction therapy in LN [302, 303]. The usual dose is 2–3 mg/kgBW/day and the maximum dose should not exceed 200 mg/day. Common side effects include gastrointestinal disorders (gastric discomfort, nausea, vomiting, diarrhea, elevated levels of transaminases, etc.), and dose-dependent bone marrow suppression.

8.5 Mycophenolate Mofetil

This agent is the prodrug of mycophenolic acid, a potent inhibitor of the inosine dehydrogenase monophosphate, an enzyme that controls the de novo guanine synthesis. In addition, mycophenolate may inhibit purine synthesis and B- and T-cell proliferation [304]. Mycophenolate mofetil demonstrated similar efficacy to cyclophosphamide in LN (for remission induction) with a superior safety profile [305]. Observational studies showed that the drug is also effective in nonrenal manifestations with an overall response of approximately 50% and a significant steroid-sparing effect [306]. The maximum dose is 3 g/day in divided doses; common side effects are gastrointestinal disorders, bone marrow suppression, and predisposition to infections.

8.6 Cyclophosphamide

Cyclophosphamide is a nonspecific alkylating agent that inhibits DNA doubling and, subsequently, cellular division. Its effect is more prominent in rapidly dividing cells such as primed T cells. Multiple studies in LN demonstrated that cyclophosphamide is a standard of care for patients with progressive disease [307]. Pulse therapy (usually combined with corticosteroids) is considered effective in remission induction with a response rate of approximately 80–85%. Lower doses (500 mg every 2 weeks for 6 pulses, Euro-Lupus protocol) are considered equally effective as higher doses [750–1000 mg/m² every 21 days for 6 pulses, National Institutes of Health (NIH) protocol, USA] [300]. Common side effects are gastrointestinal disorders (nausea, vomiting), bone marrow suppression, ovarian failure, hemorrhagic cystitis, infection predisposition, and neoplasias.

8.7 Cyclosporine

Cyclosporine binds to cyclophilin in the cytoplasm of T cells and the complex inhibits calcineurin that cannot

activate the nuclear factor of activated T cells (NFAT). As a consequence, cytokine secretion (particularly IL-2) is inhibited. Cyclosporine (usual dose 2.5–4.5 mg/kgBW) is recommended in refractory cases of membranous LN (class V). Common side effects include hypertension and kidney failure.

8.8 Belimumab

Belimumab is a fully human monoclonal antibody that binds and inhibits the function of soluble B-cell activating factor (BAFF or BlyS). BAFF cannot then bind to its receptors (TACI, BCMA, and BAFFR), resulting in decreased B-cell activation and autoantibody production [308]. In clinical trials, belimumab demonstrated significant improvement in disease activity with a satisfactory safety profile; the recommended dose is 10 mg/kgBW per month [309]. In 2010, belimumab became the first drug to be approved for SLE from the Food and Drug Administration (FDA) since 1960 [310].

8.9 Rituximab

Rituximab is a chimeric monoclonal antibody against the CD20 molecule on B-cell surface that was initially approved for the treatment of B-cell lymphoma and rheumatoid arthritis. CD20 is detected in various stages of B-cell maturation with the exception of plasmablasts and plasma cells. Despite initial encouraging results in small case series of patients with active lupus nephritis [311], rituximab was not shown to be superior to placebo in two randomized clinical trials (EXPLORER, LUNAR); because of this, its use is only justified in refractory cases [312, 313]. Furthermore, rituximab demonstrated considerable efficacy in specific clinical phenotypes such as autoimmune thrombocytopenia, hemolytic anemia, and lupus-related thrombotic thrombocytopenic purpura [314].

8.10 Other Modalities

Intravenous immunoglobulins (IVIGs) are used in cases of refractory thrombocytopenia and hemolytic anemia, as well as in resistant myositis and in cases of peripheral nervous system involvement (mainly in Guillain-Barre syndrome).

Plasma exchange may be useful in rare cases of concurrent thrombotic thrombocytopenic purpura.

8.11 Novel Biologic Agents in Systemic Lupus Erythematosus Therapeutics

Other biologic agents that are currently being tested in clinical trials include epratuzumab (monoclonal antibody against the CD22 molecule on B-cell surface, which

failed in two randomized controlled trials), atacicept (chimeric molecule with the extracellular part of TACI and the Fc part of IgG1), abatacept (chimeric molecule with the extracellular part of CTLA-4 and the Fc part of IgG), sifalimumab (human anti-IFN α monoclonal antibody), tocilizumab (monoclonal antibody against the IL-6 receptor), fostamatinib (Syk kinase inhibitor), and R348 and CP-690/550 (Jak3 kinase inhibitor) [315].

8.12 Treatment of Antiphospholipid Syndrome

Therapeutic management of APS is based on anticoagulants and/or antiplatelet drugs and depends on the initial clinical manifestations and concurrent comorbidities. In the case of nonsymptomatic presence of antiphospholipid antibodies (in particular, high titers of IgG aCL, IgG anti-B2GPI, or persistently positive LA), the use of low-dose aspirin (75–100 mg/day) is recommended [316], although not based on solid evidence.

In the case of deep venous thrombosis, initial management does not differ from patients without APS, which is heparin (unfractionated or low molecular weight fondaparinux) for 4–5 days followed by warfarin administration. Warfarin (or other vitamin K inhibitors) is recommended indefinitely, aiming at an INR (international normalized ratio) between 2.0 and 3.0. In the case of an arterial thrombotic event (stroke, myocardial infarction, etc.), warfarin aiming at an INR between 3.0 and 4.0 is recommended; in patients with high risk for recurrence aspirin should be coadministered.

Obstetric complications are managed with low-dose aspirin (81 mg/day) and low-molecular weight heparin from conception until the 34th week of gestation. In complicated cases, intravenous immunoglobulins may be administered [114].

Immunosuppressive medications are usually recommended in secondary APS aiming at suppressing aPL titers and the vascular inflammation from the underlying disease. High doses (or intravenous pulses) of corticosteroids, cyclophosphamide, rituximab, and/or plasma exchange are used in cases of refractory disease (in the context of severe SLE) or catastrophic APS.

8.13 Drugs For the Reduction of CV Risk in Systemic Lupus Erythematosus

Several randomized controlled trials with statins in SLE patients failed to prove clear benefit in terms of halting atherosclerosis progression. Atorvastatin (10–20 mg/day) did not prevent the progression of subclinical atherosclerosis (assessed by carotid IMT) in children and adolescents with SLE, over a 3-year period, in the Atherosclerosis Prevention in Pediatric Lupus Erythematosus Study [317]. Use of higher doses (40 mg/day) in adults stabilized the coronary artery calcium score but

had no effect on perfusion defects, assessed by myocardium SPECT [318]. Similarly, Lupus Atherosclerosis Prevention Study (LAPS) reported no benefit, in terms of coronary artery calcium and carotid IMT, using atorvastatin 40 mg/day for 2 years. Moreover, no changes were observed concerning disease activity and inflammation or endothelial cell activation markers [319].

Rosuvastatin (10 mg/day) decreased carotid IMT in lupus patients following 24-month administration [320], whereas fluvastatin significantly reduced CVEs in a subgroup of kidney-transplanted SLE patients [321]. In adolescent lupus patients, atorvastatin failed to reduce carotid IMT, although subgroup analysis showed that it might reduce the progression rate of the atherosclerotic plaque in patients with high CRP [322].

Angiotensin-converting enzyme inhibitors (ACEIs) and/or angiotensin receptor blockers (ARBs) are currently recommended for patients with lupus nephritis (LN), as an adjuvant therapy for proteinuria. Furthermore, they were shown to delay the occurrence of renal involvement and stabilize disease activity [323]. Concerning premature atherosclerosis, ACEIs nonuse was associated with increased carotid atherosclerosis, as assessed by total plaque area, in African American lupus patients [324]. However, it is not known if these agents have an atheroprotective potential with regard to clinical CVEs.

Aspirin in low doses is recommended for primary prevention of thrombotic events in patients with positive aPL [325], although there is no strong evidence for this.

9. PROGNOSIS

The survival of SLE patients has been significantly improved in the last 50 years since 5-year survival was approximately 50% in the 1950s, while in 2000 exceeded 95% [94]. In parallel, 10-year survival is estimated to be 92%. This improvement has resulted from comprehensive patient care with regard to severe visceral disease (nephritis, CNS involvement) as well as the successful management of comorbidities, such as accelerated atherosclerosis, infections, and end-stage renal disease.

The most important clinical and epidemiological factors that affect prognosis in SLE are race, gender, age at onset, visceral involvement, the development of comorbidities, and low socioeconomic status. Increased disease severity and predisposition to refractory nephritis has been documented in African American patients as well as in Hispanic and Native Americans [326]. Male patients more frequently develop refractory nephritis and serositis [327]. Younger age at disease onset is associated with higher mortality, while pediatric onset was related to more frequent visceral involvement [5]. Active nephritis and CNS involvement significantly affect prognosis [94]. Coexistence of APS is related to atherothrombotic cardiovascular events in 26.7%

of patients [328]. Low socioeconomic status was associated with higher cumulative damage and poor prognosis, mainly due to low patient compliance and lack of access to specialized centers [329].

Regarding the immunological parameters that affect prognosis in SLE, the anti-dsDNA, anti-C1q, and antinucleosome antibodies are associated with nephritis [330]. Antiphospholipid antibodies are related to arterial or venous thrombotic events as well as obstetric complications. Positive anti-SSA/Ro and anti-SSB/La antibodies in pregnant women increase the risk of neonatal lupus and congenital heart block; the same autoantibodies are associated with the development of secondary Sjögren's syndrome [125].

Increased disease activity is characterized by poor prognosis and increases the risk for major cardiovascular events. In addition, increased cumulative damage significantly affects patient outcomes [331].

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Neonatal Lupus

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1. INTRODUCTION

Neonatal lupus is an autoimmune disease that is acquired in utero after exposure to antibodies produced by the maternal immune system against Ro and La antigens. It is characterized by varying degrees of heart block and can be associated with an erythematous rash. It was first termed neonatal lupus by G.R. Hogg in 1957 who reported a full-term 2 kg baby boy with complete atrioventricular block and subendocardial fibrosis who was born to a mother with lupus. She named the condition congenital acute lupus erythematosis, thus implicating maternal disease in the pathophysiology [1]. Prior to that, in 1945, the first case of congenital heart block was published in the American Heart Journal by R.K. Plant and R.A. Steven, but they did not cite the mother's diagnosis as the causative agent [2]. By the 1970s multiple reports had been published proclaiming the association between maternal autoimmune disease and neonatal heart block and identifying maternal antibodies as the causative agent. Lev et al. noted fibrosis in the conduction system on pathology specimens of infants stricken with complete heart block [3]. In 1977, two separate groups published papers postulating that the atrioventricular (AV) node is fibrosed and essentially destroyed secondary to inflammation from maternal transfer of antibodies across the placenta [4,5]. Since then, neonatal lupus has served as a paradigm for the intersection of several fields of study in the world of medicine and acted as a springboard for the advancement of research into such areas as embryology, immunology, rheumatology, and cardiology. The study of this disease has led to questions that have furthered scientific inquiry into a myriad of other disease processes. These fascinating insights will be reviewed in this chapter.

2. EPIDEMIOLOGY

Neonatal lupus occurs in 1–2% of babies born to mothers with anti-Ro (also known as anti-SSA) and anti-La (also known as anti-SSB) antibodies [6]. It is not necessary for these women to have clinical manifestations of an autoimmune disease [7]. In fact, a frequency of 0.44% of antibodies to Ro and La was noted in 2500 healthy female blood donors between 20 and 50 years of age [8]. However, approximately one half of the mothers who test positive for these antibodies and do not carry a diagnosis of Sjögren's syndrome (SS) or systemic lupus erythematosis (SLE) at the time of pregnancy will go on to develop an autoimmune disorder. This was demonstrated in a 2009 study of 321 mothers enrolled in the Research Registry for Neonatal Lupus (RRNL). Of the 51 mothers who were asymptomatic at the time of the birth of their baby with neonatal lupus, 26 progressed to manifest autoimmune disease [7].

The RRNL, which was established in the United States in 1994, collects data from mothers and their children who are affected by Neonatal Lupus as an investigative resource for researchers [9]. The RRNL includes only mothers who give birth to babies with congenital complete heart block (CCHB) and have anti-Ro/SSA and/or anti-La/SSB (or in rare cases anti-RNP) antibodies.

Prospective studies have demonstrated an incidence of complete heart block in 1–3% of pregnancies in mothers with anti-Ro/SSA antibodies and an autoimmune disease [10–14]. In 2001, Brucato et al. noted that the risk of complete heart block (CHB) was 2% in 100 women with anti-Ro/SSA antibodies who also had clinical manifestations of a connective tissue disorder [11]. Cimaz et al. conducted a prospective study published in 2003 that noted an occurrence of CHB in 1.6% of 128 infants born to mothers with anti-Ro antibodies with or without also

being seropositive for anti-La antibodies [12]. The following year, Costedoat-Chalumeau et al. noted the incidence of CHB in neonates whose mothers were positive for anti-Ro/SSA antibodies to be 1% and refuted the notion that there was an increased finding of sinus bradycardia and prolongation of the QTc in these infants when compared to a control group [13]. In 2008, Friedman et al. published the results of the PR Interval and Dexamethasone Evaluation (PRIDE) study and noted that heart block occurred in 4% (2% complete block + 2% first-degree block) in mothers without a previous child with congenital heart block or rash and in 19% of infants born to mothers who had a previous child with congenital heart block [14]. Thus the risk of giving birth to a child with cardiac manifestations of neonatal lupus increases from approximately 2% (if no prior affected child) to 19% in women who have had a previous baby with neonatal lupus. These cardiac features include some degree of heart block as well as endocardial fibroelastosis (EFE) [15,16].

There also appears to be a relationship between the specific maternal titers to the incidence of CHB [17,18]. This concept was initially presented in 1993 by Buyon et al. who noted that high titers of anti-SS-A/Ro antibodies were present more often in mothers of children with cardiac neonatal lupus [17]. In 2010, Jaeggi et al. sought to determine if cardiac complications of neonatal lupus were related to maternal anti-Ro and anti-La autoantibody levels by performing a prospective study on 186 antibody-exposed fetuses. All fetuses or infants who developed cardiac complications had at least moderate anti-Ro/SSA antibodies as defined by >50 u/mL, with the vast majority having high levels (>100 u/mL). None of the fetuses with low-level antibody exposure developed cardiac complications of neonatal lupus [18]. Thus there may be a threshold anti-SSA/Ro antibody level associated with the development of fetal disease, but there is no evidence to suggest a dose-response relationship; greater amounts of antibodies do not correlate with severity of cardiac-neonatal lupus. Finally, a 2006 paper suggested that women with hypothyroidism and anti-Ro antibodies were at an increased risk of delivering a baby with complete congenital heart block when compared to women with antibodies alone [19]. However, it should be noted that a review of the RRNL subsequent to this paper revealed that one-third of mothers of children with neonatal lupus had antithyroglobulin antibodies without an increased prevalence of hypothyroidism found in the same cohort of women [20].

3. PATHOGENESIS

Ro and La are subtypes of extractable nuclear antigens (ENAs). They are intracellular proteins that were initially described in the 1970s in association with SS. Thus they

are also known as SSA and SSB, respectively. They are expressed on the surface of cells undergoing apoptosis. Antibodies that recognize the Ro antigen may be directed toward either the cellular protein Ro60 or Ro52. The Ro protein functions in a quality-control pathway for ribosome biogenesis and as such plays a role in the recognition or repair of intracellular damage. A knockout mouse model of TROVE-2, the gene that encodes Ro60, was initially created at Yale University in 2003. TROVE-2-null mice develop autoimmune disease, including membranoproliferative glomerulonephritis [21]. Ro52 functions as an E3 ubiquitin ligase and is capable of autoubiquitination [22]. By interacting with multiple target proteins and signaling cascades, it can regulate inflammation. Mice that lack Ro52 also develop multiorgan autoimmune disease. Espinosa et al. published these findings in 2009, revealing the Ro52 protein to be a negative regulator of proinflammatory cytokine production [23].

It is hypothesized that anti-Ro/SSA and anti-La/SSB IgG antibodies (the only isotype that can be transferred through the placenta via FcRn receptors) bind to fetal cardiac tissue. It is important to note that Ro and La antigens are intracellular; it is likely that during the physiologic process of apoptosis during cardiac embryogenesis, the cardiomyocyte translocates these proteins to the cell surface. It is speculated that when maternal antibodies bind these proteins, a marked inflammatory response ensues, injuring the AV node and causing fibrotic changes that render the conduction system incompetent. It has been demonstrated that neighboring cardiomyocytes are capable of engaging in efferocytosis in the fetal heart [24]. However, when anti-SSA/Ro and anti-SSB/La antibodies are expressed on the surface of cells undergoing apoptosis, it has been reported that efferocytosis by the healthy cardiocytes is impaired. Based on autopsy studies it has been hypothesized that macrophages are recruited. As a result of this deviation from the physiologic pathway of cardiomyocyte phagocytosis, cytokine release occurs, including the involvement of TNF- α and TGF- β [25–27]. Thus it is possible that this inflammatory cascade results in fibrotic destruction of the conduction system in the fetal heart (Fig. 11.1).

It is likely that this inflammatory response is not limited to the conduction system. Litsey et al. noted in the *New England Journal of Medicine* in 1985 that the myocardium demonstrated IgG deposition [29]. In a 2002 study by Nield et al., the documentation of EFE in two infants and one fetus with normally functioning conduction systems and diffuse deposition of IgG and T cells throughout the myocardia of the postmortem specimens lends further support to the belief that the inflammatory response includes pancarditis [30].

Furthermore, it has been suggested that maternal antibodies may directly affect the function of cardiomyocytes. Sera from mothers who had pregnancies complicated by

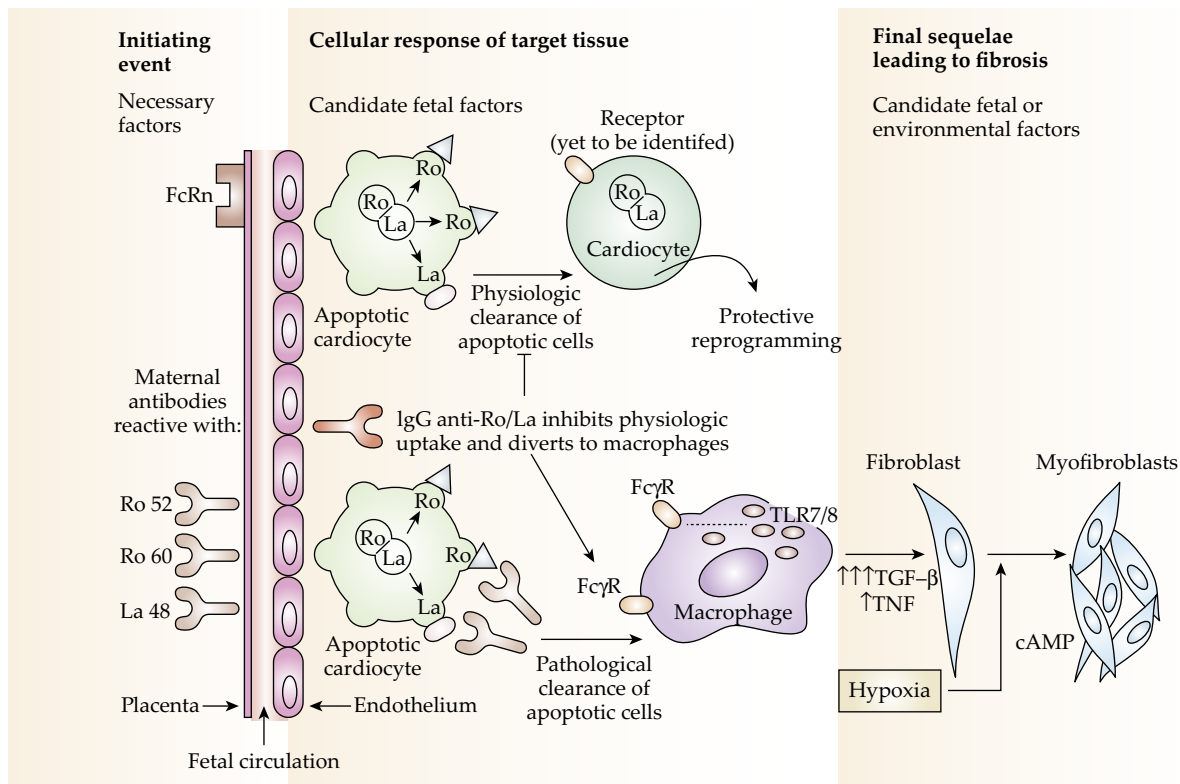


FIGURE 11.1 The pathogenesis of congenital heart block. This overview demonstrates the mechanisms of autoantibody-induced injury leading to congenital heart block. Cardiocytes participate in apoptosis. Maternal anti-SSA/Ro and anti-SSB/La in the fetal circulation divert physiologic clearance of apoptotic cardiocytes by healthy cardiocytes toward clearance by macrophages via FcγR with release of inflammatory and fibrosing cytokines. Adapted from Buyon et al. [28].

congenital heart block inhibited both L-type and T-type calcium channels but did not affect sodium and potassium channels [31]. It remains to be seen how this plays into the pathogenesis of cardiac neonatal lupus. It can be argued that inhibition of L-type channels, necessary for conduction in both the SA and AV nodes, occurs prior to the inflammatory response. The calcium channel blockade hypothesis has also been used to attempt to explain why heart failure occurs in these infants as inhibition of Ca-channels in ventricular myocytes decreases contractility [32].

In addition to maternal antibodies, maternal DNA has been found to be significantly increased in the hearts of infants with cardiac neonatal lupus as compared to controls, thus implicating microchimerism in the pathogenesis of neonatal lupus [33]. Interestingly, these maternal cells were shown to express sarcomeric α -actin, a specific marker for cardiac myocytes. Some demonstrated CD45, a hematopoietic cell marker [34]. Alternatively, it is possible that these cells were not involved in the pathogenesis of neonatal lupus but instead involved in repair.

Several studies have illustrated that although maternal antibodies are a necessary component in the pathogenesis of neonatal lupus, they are not in and of themselves the whole story. Fetal susceptibility appears to be a key factor as evidenced by the fact that heart block only develops

in a small minority of pregnancies despite the presence of maternal antibodies. HLA markers, specifically DQ alleles, may serve as antigen-presenting cells [35,36]. However, studies on monozygotic twins have revealed that additional factors must play into the pathogenesis of neonatal lupus in addition to the genetic makeup of a fetus. This was first documented in 1997 by Cooley et al. who noted that only one baby of identical twins demonstrated CCHB [37]. This curious observation prompted investigators to search for an environmental factor that might potentiate the disease process thereby explaining the discordance of disease expression. Clancy et al. suggested that this key factor could be hypoxia [38]. Through the upregulation of cAMP, hypoxia induced the differentiation of cardiac fibroblasts to myofibroblasts. These cells, which are typically recruited to perform repair, produce cytokines, thus exacerbating the inflammatory response [39].

Mysteries continue to exist around the pathophysiology of neonatal lupus, allowing for new discoveries and further advancement in multiple fields of medicine. It is apparent that anti-Ro/La antibodies are necessary to cause disease in the fetus but there does not appear to be a linear dose-response relationship with antibody titers. What other factors may be necessary to impact disease severity? Why does the risk of giving birth to a

baby with cardiac neonatal lupus increase almost 10-fold when the mother has had a previous pregnancy complicated by neonatal lupus? Fetal factors are critical and yet not sufficient to explain disease presence. Do biomarkers exist to predict disease presence in at-risk pregnancies prior to irreversible damage? Recent work by Saxena et al. suggests that inflammatory markers such as C-reactive protein (CRP) and brain natriuretic protein (BNP) are correlated with disease severity. Perhaps more importantly they noted that MMP2, a profibrotic endopeptidase expressed by macrophages and trans-differentiating fibroblasts could serve as a target for therapeutic interventions [39]. Translational research in multiple areas will be needed to dissect out both maternal and fetal modifying factors that lead to neonatal lupus.

4. CLINICAL MANIFESTATIONS

4.1 Cardiac

The most notorious complications of neonatal lupus involve the heart. These include first-, second-, and third-degree heart block as well as EFE. Neonatal lupus is the etiology most commonly associated with isolated congenital heart block, accounting for up to 95% of all cases [28,40,41]. Clinically, the fetus will likely present with bradycardia as the first indication of a cardiac complication of exposure to maternal antibodies noted on routine prenatal testing.

Less commonly, heart block secondary to neonatal lupus can present after the newborn period, but as maternal-fetal care continues to become increasingly prevalent, the more likely clinical scenario is presentation during fetal life, typically in the second trimester. In

addition to bradycardia, the fetus may also demonstrate ventricular dilation, pericardial effusion, cardiomyopathy, AV valve insufficiency, and hydrops. One study correlated poor fetal outcome with low ventricular escape rates, decreased contractility, and high umbilical arterial resistance [42].

Fetuses of mothers with anti-Ro/anti-La antibodies can also present with first- or second-degree heart block, attesting that there is a continuum of damage that may eventually culminate in third-degree heart block. However, some authors have suggested that there is not an evolution of damage moving systematically from first- to second to third-degree block but that there is a rapid onset of third-degree atrioventricular block (AVB) [43].

In those fetuses with first-degree heart block, the PR interval will be prolonged to greater than 150ms, although some authors have suggested a cutoff of 135 ms [44] (Fig. 11.2).

First-degree heart block may or may not progress to more advanced degrees of heart block. In one prospective study of 165 fetuses exposed to maternal anti-Ro antibodies, 15 were found to have first-degree AVB or Mobitz Type 1. None of these fetuses were treated with dexamethasone and none demonstrated advancement [46]. However, four out of nine babies enrolled in the RRNL who demonstrated first-degree block at birth progressed to advanced forms of heart block [47]. Persistent sinus bradycardia has also been reported, possibly due to damage to the sinus node [48,49].

Endocardial fibroelastosis can accompany conduction defects but can also occur independent of heart block. The term EFE was first introduced into the medical literature by A.J. Himmelfarb and T. Weinberg at Johns Hopkins in 1943 [50]. They described a pathologic specimen with a thickening of the left ventricle that was pearly white

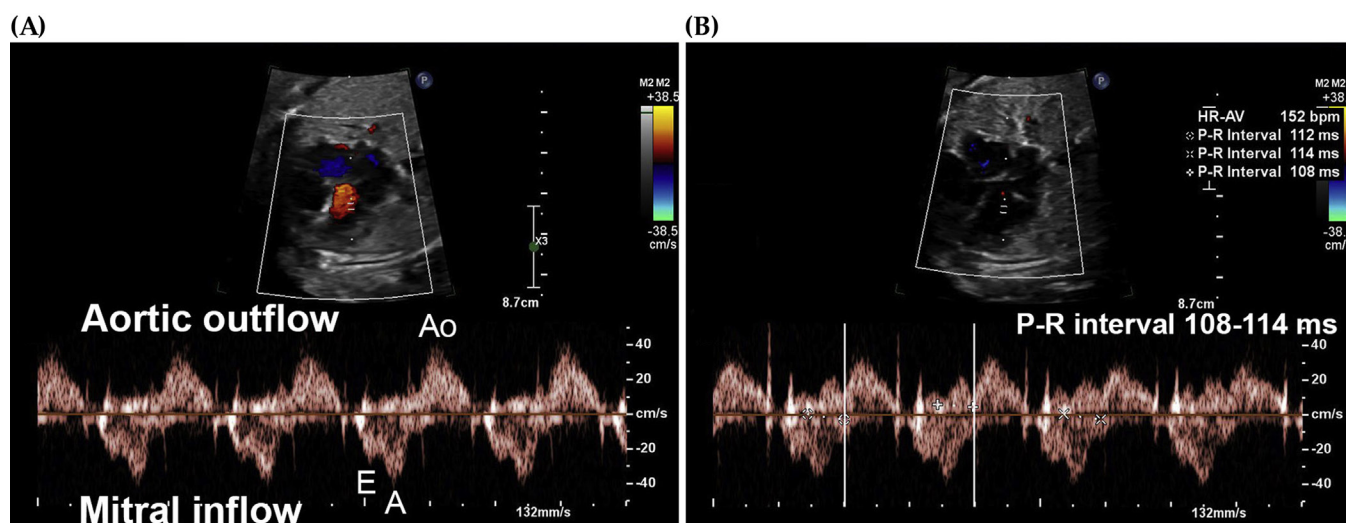


FIGURE 11.2 Determination of PR interval. Measurement begins at the onset of atrial systole until ventricular systole, representing the mechanical PR interval. See also the original paper by Glickstein et al. describing the method to determine the fetal PR interval. Adapted from Glickstein et al. [45].

in appearance. Histologic studies demonstrated hyperplasia of the fibroelastic endocardium [51]. Today the term has become less common, with the more general term dilated cardiomyopathy being used. Nonetheless, EFE is a distinct entity recognizable to pediatric cardiologists that is a negative predictor of outcome in maternal anti-SSA antibody-associated CHB [52] and accounts for 25% of all cases of pediatric cardiac transplantation [53]. When EFE was diagnosed in association with anti-Ro or anti-La antibodies, it was initially thought to be a compensatory mechanism allowing the fetus to augment stroke volume in the face of chronotropic impairment. However, it has since been shown to be a direct result of myocardial damage secondary to maternal antibodies. Support for this concept was demonstrated in a retrospective study of 13 cases of EFE. Nield et al. published historical, histologic, echocardiographic, and outcome data on 13 patients with EFE. Immunohistochemical studies demonstrated IgG throughout the myocardium [54]. In a separate study during the same time period, the authors noted that EFE was associated with maternal anti-Ro and anti-La antibodies in the absence of conduction defects in three cases [30].

Infants with EFE present with the typical symptoms of congestive heart failure, including failure to thrive, difficulty feeding, tachypnea, and respiratory distress. EFE may be clinically indistinguishable from dilated cardiomyopathy; however, as stated above, pathology specimens of the left ventricle are coated in an unmistakably white fibrous tissue with contracted appearing papillary muscles that sit in a significantly more superior position in the left ventricle than normal. When compared with pathologic specimens of dilated cardiomyopathy, the endocardium is significantly thicker [53]. Conventional ultrasound dogma implies that the endocardium will appear particularly echo bright, but definitive diagnosis by echocardiogram has not been established. Cardiac MRI is emerging as an increasingly useful imaging modality. Fibrosis is revealed through the use of gadolinium enhancement, which is demonstrated as a characteristically high-intensity signal located on the endocardial surface of the ventricles as well as atria [55,56].

Other anatomic issues have also been reported including 14 children with CHB in the RRNL that had structural lesions not causally related to conduction defects. Six had atrial septal defects, four demonstrated right-sided valvar lesions (including one with a hypoplastic RV), three demonstrated hemodynamically significant patent ductus arteriosus, and one had a hemodynamically insignificant ventricular septal defect [9]. Likely mediated directly by autoantibody injury to the valve-supporting apparatus, AV valve rupture secondary to papillary muscle atrophy [57], and multivalvar stenosis and insufficiency [58] have also been reported.

5. DERMATOLOGIC

The clinical manifestations of neonatal lupus can be confined to the skin, heart, or both. The rash can be noted at delivery but more often it becomes apparent after exposure to ultraviolet light [59]. It has been reported to occur in 15–25% of children with neonatal lupus [60]. It is self-limiting and typically resolves by 6–8 months of age. This is coincident with the disappearance of maternal antibodies from the child's circulation. The histopathology of the skin lesions resembles subacute cutaneous lupus erythematosus found in adults [61]. Immunofluorescent staining will identify IgG in the epidermis [62]. The rash appears as raised erythematous annular lesions and typically is found on the head with the most notable location occurring in the periorbital region. It is possible, however, for the rash to cover other parts of the body (Fig. 11.3).

Of the 57 infants noted in the RRNL with cutaneous manifestations, all had a rash that involved the face with the next most common locations occurring on the scalp, then trunk, arms and legs, neck, and other intertriginous areas. The least likely locations are the palms and soles [60]. The vast majority of mothers of infants with cutaneous manifestations of neonatal lupus are seropositive for anti-Ro antibodies; however, there have been reports of mothers who are seronegative for anti-Ro and anti-La antibodies but positive for anti-U1RNP [63,64]. It is important to note that none of the reported infants who were also seropositive for U1RNP but negative for anti-Ro antibodies had congenital heart block.



FIGURE 11.3 Typical rash in neonatal lupus; note the predilection for the periorbital area. Adapted from the personal collection of the authors.

6. GASTROINTESTINAL

Elevated transaminases, cholestasis, and hepatitis have all been reported. In the RRNL, 19 out of 219 patients were reported to have hepatobiliary involvement. Disease severity ranged from mild elevation in liver enzymes to severe liver failure [65]. It is possible that liver involvement may be the only presenting sign in the infant that the mother has anti-Ro and anti-La antibodies [59]. The prognosis of liver involvement is typically excellent but cases of death secondary to liver failure have been reported [66,67].

7. NEUROLOGIC

It has been postulated that there are neurological conditions associated with neonatal lupus. These include hydrocephalus, macrocephaly, hypocalcemic seizures, vasculopathy, spastic diplegia, vasculitis resulting in a cerebrovascular accident as well as neuropsychiatric disorders [68–72]. The first report of a possible association between hydrocephalus and neonatal lupus was by Nakayama-Furukawa in 1994 in a case of two female siblings [73]. This was further advanced by a prospective study documenting an occurrence rate of 8% in 87 infants born to mothers with anti-Ro antibodies, 47 of whom had neonatal lupus [74]. Nonetheless, in a recent review of the literature, Chen et al. noted that the majority of CNS findings are asymptomatic but suggest that a head ultrasound be used as a screening for potential neurologic issues in infants born to mothers with anti-Ro and anti-La antibodies [75].

8. HEMATOLOGIC AND SKELETAL

Hematologic abnormalities that have been reported include anemia, neutropenia, and thrombocytopenia, as well as a case report of an infant born to a mother with SS who had pancytopenia [76]. However, these infants were not reported to have bleeding disorders or immunologic issues such as sepsis [59]. Stippling of the epiphyses, sometimes called chondrodysplasia punctata [59], has also been associated with neonatal lupus.

9. DIAGNOSIS

At this time, universal screening for anti-Ro and anti-La antibodies in all pregnant women has not been recommended. Thus the diagnosis of neonatal lupus

is often made during pregnancy or postnatally when antibodies to Ro or La are identified in the serum of a mother who gives birth to a child with heart block and/or demonstrates the typical rash described previously. Prenatally, routine examination of the fetus can reveal bradycardia on Doppler ultrasonography or heart block on fetal echocardiography. Additionally, fetal echocardiography can demonstrate cardiomyopathy. Thus in women who have anti-Ro antibody autoimmune disease or have previously had a pregnancy complicated by neonatal lupus, anticipatory fetal echocardiography should be performed. There is a question as to the frequency and breadth of echocardiographic screening in antibody-exposed fetuses. A recent paper by Krishnan et al. looked at 636 echoes performed on 140 antibody-exposed fetuses and did not detect any cases of second- or third-degree heart block or cardiomyopathy [77], but this appears to be perhaps a statistical anomaly. A consensus of the best screening protocol has yet to be established. Nonetheless, it is important to recognize that heart block most often develops from 18 to 24 weeks gestation and frequent ultrasounds with Doppler to measure the PR interval and look for indications of cardiomyopathy are warranted. Pulsed Doppler during fetal echocardiography will demonstrate the mechanical PR interval as defined by the onset of atrial contraction to the beginning of ventricular systole [45]. Placement of the Doppler beam can be directed through the mitral and aortic valve to obtain both inflow and outflow; alternatively, the Doppler tracings from the superior vena cava and aorta, or tissue Doppler imaging, may be used to discern the atrial and ventricular contractions (Fig. 11.4).

Additionally both fetal electrocardiography (EKG) and fetal MRI are showing promise as diagnostic tools. Fetal EKG has been demonstrated in one study to have better sensitivity and specificity in differentiating normal PR intervals from first-degree AVB [79]. Fetal magnetocardiography is increasingly being used as a research tool to better aid in the understanding of fetal arrhythmia. By acquiring information from the magnetic field produced by the fetal heart, reference values for the PR, QRS, and QT intervals have been documented [80]. Fetal magnetocardiography's application in CHB is becoming established but has not made its way into most clinical practices. Nonetheless, fetal magnetocardiographic work by Zhao et al. on 28 fetuses with second- and third-degree AV block, 18 of whom were due to maternal antibodies to SSA and/or SSB, demonstrated the association of fetal heart rate reactivity on prognosis and outcome. They also proposed that the onset of third-degree block was sudden and associated with junctional ectopic tachycardia and ventricular ectopy [43].

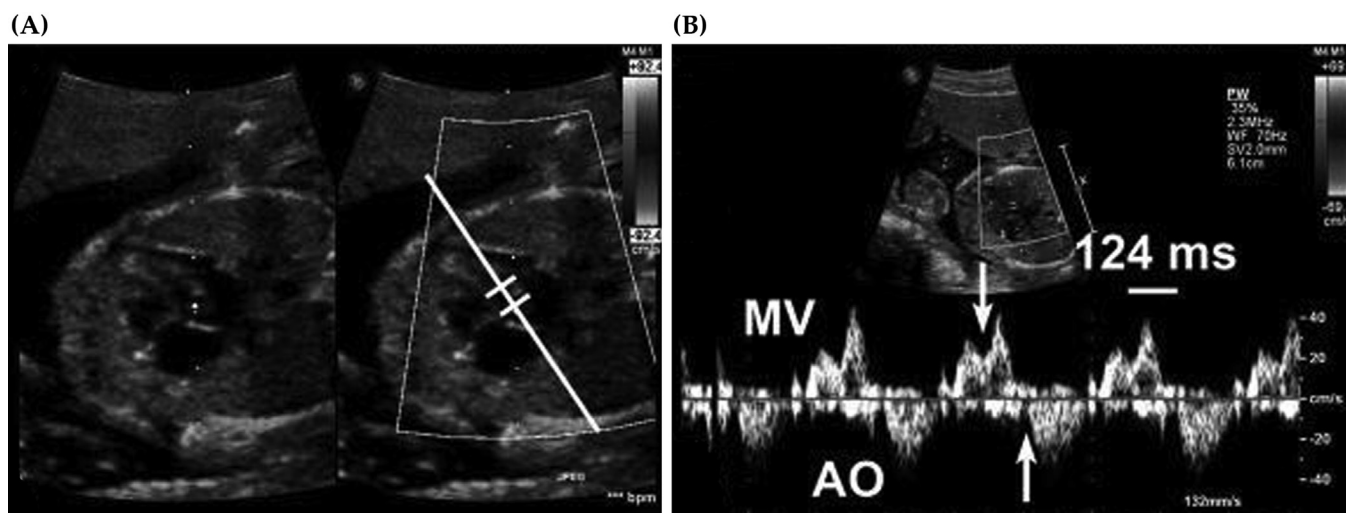


FIGURE 11.4 Obtaining mechanical PR interval on fetal echo. Mitral-aorta pulsed-wave Doppler method. The Mitral (MV)-aorta (AO) method relies on identification of the trans-mitral atrial inflow (A wave) and ejection out the left ventricular outflow tract. The Doppler pulsed-wave sample volume is placed near the mitral valve leaflet in the left ventricle (LV) outflow tract (panel A), and measurement is made from the onset of the trans-mitral A wave to the onset of LV outflow tract ejection (panel B, arrows). In this case, the MV-AO PR interval is 124 ms. Note: The mechanical PR interval may not reflect only the true electrical PR interval, as mechanical and flow events may also influence this measurement; a discussion is beyond the scope of this chapter but the reader is referred to the review by Phoon et al. for further discussion on the strengths and weaknesses of various methods. Adapted from Phoon et al. [78].

10. TREATMENT

Complete heart block has been shown to be irreversible [28]. Thus it is essential to identify earlier lesions of heart block in utero, to perhaps take advantage of a window of opportunity to treat and possibly prevent higher forms of heart block. Multiple studies have documented the utility of fluorinated glucocorticoids in the treatment of first- and/or second-degree heart block identified in utero, but data are conflicting. Fluorinated steroids such as dexamethasone are not subject to inactivation by the placenta. A retrospective study based on data from the RRNL from 1994 to 1999 of 47 mothers with positive anti-SSA/Ro or anti-SSB/La antibodies demonstrated the utility of steroids in prevention of progression of second-degree heart block [81]. However, first-degree heart block has been documented to revert to normal sinus rhythm without treatment in a neonate [5]. This was corroborated in a later study by Sonesson et al., in 2004, which also documented recovery from second-degree to first-degree block and then further reduction of the PR interval to normal sinus rhythm [82].

Since first-degree block does not invariably progress to more advanced degrees of heart block, justification of the use of steroids in a fetus with first-degree block has not yet been fully demonstrated. A French study suggested that fluorinated steroids were associated with fetal demise and intrauterine growth restriction, which is why the decision to initiate dexamethasone is not simple [83]. A subsequent prospective study known as the PRIDE study evaluated the efficacy of dexamethasone in 30

anti-SSA/Ro-exposed fetuses who demonstrated varying degrees of heart block in comparison to 10 untreated controls. This control group consisted of nine fetuses with third-degree block and one with first-degree block. Confirming previous studies, no reversal of third-degree block was seen in either group. Only one of six fetuses with second-degree heart block who was treated with dexamethasone advanced to third-degree block. There were six reports of intrauterine demise. Additionally, dexamethasone was associated with prematurity and low fetal birth weight for age [84]. Fluorinated steroids have also been associated with improvement in fetal hydrops [85,86] and possibly EFE [87]. The combination of dexamethasone and β -agonists has been advocated in cases of fetal CAVB with heart rates <55 bpm. In a 2004 paper by Jaeggi et al., 21 patients treated with dexamethasone had a 1-year survival rate of 90% as compared to a 46% survival rate in those patients not treated with steroids [88]. Plasmapheresis has also been used in combination with fluorinated steroids with divergent outcomes in case reports [89–92], but to date there have not been any controlled trials. Reviews of this topic at least suggest the use of fluorinated steroids in the treatment of second-degree AV block [93–95]. Izmirly et al. reviewed the cases of 156 infants from the RRNL and noted that in 71 children who received fluorinated steroids within 1 week of a diagnosis of advanced heart block (second or third degree), there was no benefit in terms of prevention of extranodal disease or improvement of survival compared to 85 children who were not exposed to fluorinated steroids for cardiac

neonatal lupus treatment [96]. These data seem to support a previous study of all isolated AV block (80% of whom were associated with anti-Ro/SSA autoantibodies) in which no significant effect of treatment with fluorinated corticosteroids was seen [97].

Intravenous immunoglobulin (IVIG) has been linked to improved outcome in patients with EFE in association with neonatal lupus when combined with steroid therapy. A retrospective study of 20 infants born to mothers with anti-SSA/Ro or anti-SSB/La who received IVIG during fetal or postnatal life demonstrated an improved survival rate of 80% at 3-year follow-up with none of the infants requiring heart transplant [87]. However, two prospective studies published the year before did not demonstrate the ability of IVIG to prevent recurrent heart block in pregnancies complicated by maternal antibodies [98,99]. It has been postulated that the dose used in these may have been too low. Higher doses of IVIG at 1 g/kg have been proposed [100].

Current studies aimed at prevention are also underway. One such study, the preventative approach to congenital heart block with hydroxychloroquine (PATCH), is evaluating hydroxychloroquine (HCQ) as a potential protector against heart block ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01379573) # NCT01379573). Hydroxychloroquine has been used in pregnant mothers without adverse effect to prevent flares of SLE [101,102]. It has been postulated that through its inhibition of toll-like receptors, HCQ can prevent macrophage production of inflammatory and profibrosing factors such as endothelin-1 (ET-1) [103]. Numerous studies have demonstrated ET-1's involvement in the pathophysiology of fibrosis and scarring in multiple organ systems, including the heart [104]. An elegant study by Alvarez et al. linked the production of ET-1 by macrophages activated by toll-like receptor 7 to fibroblast differentiation [103]. A 2010 case-control study by Izmirly et al. suggested that the use of HCQ in anti-SSA/Ro/anti-SSB/La antibody-exposed fetuses decreased the incidence of cardiac neonatal lupus [105]. A subsequent study looking at 257 pregnancies in mothers with a previous pregnancy complicated by cardiac neonatal lupus, 40 of whom were exposed to HCQ, demonstrated a decrease in the incidence of cardiac neonatal lupus from 21.7% to 7.5%. Furthermore, there were no deaths in the HCQ-exposed group as compared to a 21.7% fetal mortality rate in those fetuses not exposed to HCQ [106].

11. RECOMMENDATIONS

At this time no proven treatment protocols have been established for this disease other than supportive care. Based on current expert opinion we suggest the following guidelines:

- Serial fetal echocardiographic evaluation of at-risk fetuses secondary to maternal antibodies is indicated, with the most important time interval being 18–26 weeks gestation. In our labs we recommend weekly fetal echocardiograms during this period of highest risk.
- Women who have previously had a pregnancy complicated by cardiac manifestations of neonatal lupus should be considered for treatment with hydroxychloroquine 400 mg po daily (once daily, or 200 mg BID), starting at 6–10 weeks gestation.
- While the use of fluorinated corticosteroids (ie, dexamethasone, betamethasone) is not robustly supported by clinical data, most groups will treat AV block (first or second degree), abnormal echogenicity of valve annuli or myocardium, and/or cardiomyopathy. Local practice is highly variable as to the thresholds at which to initiate treatment; for example, some groups will not initiate therapy for first-degree AV block, while others will be more aggressive in attempts to halt progression.
- For infants born to mothers with anti-Ro/SSA and/or anti-La/SSB antibodies, the pediatrician caring for the newborn might consider blood tests including a CBC and a full metabolic panel that includes liver function tests, particularly if there are clinical signs of anemia and/or hepatic dysfunction. (This applies to infants with normal sinus rhythm.)
- It may be prudent to avoid, or minimize, sun exposure for the first 6–8 months.
- An EKG and an echocardiogram are recommended after birth, but the timing may vary depending on local clinical practice. While some prefer early initial evaluation in the neonatal period, others will see the infant once at a few months of age [78]. For those babies seen early, the EKG is repeated at 1 year of age to rule out late progression as progression from first-degree block to second- and third-degree block has been reported [9,45]. As the data continue to support a benign postnatal course if the prenatal course is entirely unremarkable, consideration even for only one to two EKG's postnatally may also be considered, since the risk of cardiomyopathy would be extremely low. A consultation with a pediatric cardiologist is warranted to aid in further management.
- Pacemaker implantation is indicated in those infants with complete AVB and a heart rate of <55 bpm, or for advanced second- or third-degree AVB associated with symptomatic bradycardia, ventricular dysfunction, or low cardiac output (Strength of recommendation: Class I; Level of evidence: C). For infants older than 1 year with either an average heart rate <50 bpm, significant ventricular pauses, or symptoms consistent with chronotropic impairment, a pacemaker is indicated (Strength of recommendation: Class IIa; Level of Evidence: B) [107].

- Those infants who were exposed to steroids in utero are at risk for adrenal insufficiency. Pediatricians and neonatologists caring for these newborns need to monitor and treat accordingly with steroids [83].
- There are no data confirming benefit of specific prenatal or postnatal disease-modifying therapy. Individual decision-making must be invoked, necessitating thorough parental counseling.

12. PROGNOSIS

As previously noted, morbidity and mortality is significant. In 2002, Jaeggi et al. published a retrospective review of their institution's experience with isolated complete AV block. They identified the following factors as associated with greater perinatal mortality: fetal hydrops, EFE, and delivery <32 weeks gestation [108]. In a later study by Izmirly et al., 325 infants with cardiac neonatal lupus had a mortality rate of 17.5%, with a third of those deaths occurring during fetal life. Pacing was required postnatally in 70% by 10 years of age and four infants went on to require cardiac transplantation [109]. It has also been noted that patients with congenital heart block secondary to cardiac neonatal lupus who require pacemaker implantation have a worse prognosis than those with complete AV block not due to antibody exposure. This was manifested as congestive heart failure and mitral insufficiency post-implantation [110]. Additionally there is a subset of children born with complete congenital atrioventricular block secondary to anti-Ro/anti-La antibodies who go on to develop delayed dilated cardiomyopathy despite pacemaker implantation [111–113]. A recent paper that included 127 patients status postpacemaker placement for isolated CHB suggested that those patients who had CCHB secondary to anti-SSA/Ro/SSB-La were not more likely to demonstrate abnormal left ventricular function after pacemaker placement than those patients who were not exposed to maternal antibodies [114]. Nonetheless, all patients, despite adequate ventricular pacing, should be monitored for this possibility.

13. FUTURE DIRECTIONS

The future of neonatal lupus research will prove fascinating to watch unfold. Further bench and translational research will allow for advancements in treatment when the exact pathophysiologic mechanism is made manifest. Neonatal lupus/autoantibody-mediated heart block is an antenatally acquired disease in presumably otherwise healthy fetuses; therefore, the goal ultimately is prevention, similar to Rh isoimmunization. We will await the results of the PATCH trial. If successful, universal

screening will be warranted from a cost-effective perspective but more importantly, from of a life-saving one.

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Sjögren's Syndrome

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1. INTRODUCTION

1.1 Epidemiology

Sjögren's syndrome (SS) is a chronic, systemic autoimmune exocrinopathy, affecting principally the salivary and lacrimal glands. As a result, dysfunction of the secretory processes occurs, leading to dryness of the mucous membranes [1]. The syndrome has a diverse clinical spectrum, which expands from organ-specific complaints, mainly due to lymphocytic infiltration of exocrine glands, to systemic disease, with a considerable number of individuals evolving to malignant B-cell lymphoma [2]. Sjögren's syndrome can occur either alone, or in the context of another connective tissue disorder (e.g., systemic lupus erythematosus (SLE), scleroderma, and rheumatoid arthritis (RA)) [3]. According to epidemiologic surveys, SS displays high prevalence in the female menopausal population. Studies have shown that 0.5–1% of the adult population can be affected [1].

1.2 Genetics

Several clinical studies have shown increased frequency of both organ-specific and systemic autoimmune diseases in SS relatives, implying that genetic contributors are implicated in disease pathogenesis. Gene candidate studies together with genome-wide association studies (GWAS) have revealed genetic variants predisposing to disease development. Over the last several decades, initially reported associations between human leukocyte antigen (HLA) genes and SS susceptibility (such as HLA-B8, HLA-DR15, and HLA-DR3 alloantigens) [4–9] have also been confirmed in studies conducted in large series of patients [10], and associations of these genes with certain autoantibodies, such as anti-Ro/SSA and anti-La/SSB, have also been detected [11–15].

Apart from HLA alleles, other genetic loci, affecting distinct pathways of the immune system, have been implicated in the pathogenesis of SS. Single-nucleotide polymorphisms (SNPs) in early B-cell factor 1 (EBF1), tumor necrosis factor superfamily (TNFSF4), and B lymphoid tyrosine kinase (BLK) genes, which are essential for the differentiation of cells mediating adaptive immunity, have been linked to SS development [16]. Other polymorphisms of two transcription factors with regulatory role in the interferon pathway, the interferon regulatory factor 5 (IRF-5) [10,17], and signal transduction and transcription-4 (STAT-4) [10,18] also confer increased risk for SS development. Furthermore, novel associations of cytokine-related genes with SS onset include interleukin-12 subunit α (IL-12A), TNFAIP3-interacting protein 1 (TNIP1), and C-X-C chemokine receptor type 5 (CXCR5) [10].

2. CLINICAL PRESENTATION, LABORATORY FEATURES, AND DIAGNOSTIC CRITERIA

2.1 Clinical Presentation

The wide clinical spectrum of SS prompted classification of clinical features into two major types: glandular, involving exocrine glands, and extraglandular (systemic manifestations). The latter group comprises nonspecific systemic manifestations, symptoms due to periepithelial lymphocytic infiltration of parenchymal organs, disorders related to immune complexes deposition, indicative of B-cell hyperactivity, and finally non-Hodgkin lymphoma (NHL) development [19]. In a recent study conducted in a large cohort of SS individuals of Italian origin, mild extraglandular manifestations were observed in 46.6% of patients, while 15% of SS group exhibited severe systemic manifestations, including

synovitis, peripheral neuropathy, cutaneous vasculitis, and NHL development [20].

2.1.1 Glandular Manifestations

Glandular manifestations are mainly related to lymphocytic infiltration and impairment of exocrine glands, though other inflammation independent mechanisms have also been proposed [19].

2.1.1.1 Xerostomia (Dry Mouth)

Xerostomia manifested as subjective feeling of dry mouth, together with a sensation of burning mouth and difficulty in swallowing solid foods, are the most common complaints of SS patients, related to salivary glands involvement. The tongue surface is often red and depapillated. The decreased secretion of saliva—having both lubricating and antimicrobial properties—results in increased rates of dental carries and oral infections, mainly fungal infections, causing mucosal erythema with pseudomembranous lesions and angular cheilitis. The saliva appears thick like a rope, a condition known as “ropy saliva.” A frequent complication is the enlargement—usually painless and bilateral—of major salivary glands [1,19].

2.1.1.2 Ocular Dryness (Dry Eyes)

The impairment of lacrimal glands leads to reduced tear production, promoting epithelial damage of the cornea and the conjunctiva (keratoconjunctivitis sicca). Patients often mention the sensation of sand or foreign body in their eyes, which are often irritated and itch [1,19].

2.1.1.3 Skin and Other Mucosal Dryness

Dry and scratchy skin, nasal mucosal, and tracheobronchial desiccation of the upper respiratory system, mostly manifested as dry chronic cough, as well as vaginal dryness leading often to dyspareunia are among the most common, glandular-related sicca features, beyond the salivary and lacrimal glands [3].

2.1.2 Extraglandular Manifestations

2.1.2.1 Nonspecific

Nonspecific musculoskeletal manifestations like myalgias, arthralgias, and less often nonerosive arthritis are commonly encountered in SS [19]. About two thirds of SS patients suffer from chronic fatigue, a prominent symptom adversely affecting quality of life [21]. Raynaud's phenomenon affects approximately one third of patients and can occur prior or after disease onset [22].

2.1.2.2 Periepithelial

Other systemic manifestations in SS are related to periepithelial lymphocytic infiltration of parenchymal

organs such as lungs, liver, kidney, and endocrine glands.

In the majority of cases, lung involvement results from peribronchial inflammation leading to obstruction of middle and inferior airways [23], whereas interstitial inflammation (mainly in form of lymphocytic interstitial pneumonitis) is relatively rare, although the available data regarding its prevalence is rather conflicting. A proportion of patients can present hepatomegaly and elevated liver enzymes, indicating either autoimmune hepatitis or SS-related primary biliary cirrhosis, often in association with antimitochondrial antibodies (AMA) [24]. Peritubular lymphocytic infiltration at the kidney level can lead to interstitial nephritis, a common SS-related renal complication [25–27], which can be manifested with renal tubular acidosis, leading to hypokalemia, urinary alkalization, and nephrocalcinosis [28]. In a similar pattern, lymphocytic accumulation around thyroid and adrenal epithelia can also occur as indicated by the presence of autoantibodies against, thyroid [29] and adrenal [30] antigens, respectively.

2.1.2.3 Immunocomplexes Associated

Deposition of immunocomplexes in the skin, kidney, peripheral, and very rarely the central nervous system (CNS), as a result of B-cell hyperactivity, can lead to a spectrum of clinical manifestations, including palpable purpura [31], glomerulonephritis [26], sensory and sensorimotor neuropathy [32], and occasionally to CNS lesions manifested as multiple sclerosis like symptoms or stroke [33].

2.1.2.4 Lymphoma Development

Among other systemic autoimmune diseases, SS confers the highest risk for NHL development, affecting approximately 5–10% of the SS population during the lifetime [34,35]. Non-Hodgkin lymphoma in the context of SS is mainly of B-cell origin, with mucosa-associated lymphoid tissue (MALT) being the most common type, while other histological subtypes, like diffuse large B-cell (DLBC) and follicular lymphomas, can also occur [36]. Although certain disease-related clinical manifestations, such as palpable purpura and parotid gland enlargement, laboratory findings like hypocomplementemia and cryoglobulinemia [2,37,38], histopathological features [39], and genetic aberrations including t(14:18) translocation, p53 mutations, and SNPs of the B-cell activator factor (BAFF) gene [40–42] have been previously associated with increased risk for lymphoma development, the pathogenesis of NHL in the setting of SS has not yet been elucidated.

2.2 Laboratory Features

Elevated levels of γ globulins, which constitute autoantibodies against organ-specific (antithyroid and antimitochondrial antibodies) and nonorgan-specific (antinuclear antibodies) antigens is a common serological feature. The most important autoantigens are ribonucleoproteins, consisting of RNA clustered with the proteins Ro52, Ro60, or La48KD. Anti-Ro/SSA antibodies are found in 50–90% of patients, while anti-La/SSB antibodies have high specificity, encountered in 30–60% of patients. Approximately half of patients have positive titers of rheumatoid factor, an antibody recognizing the Fc part of IgG or IgM immunoglobulin [43]. Hyper- γ -globulinemia reflects the polyclonal activation of B lymphocytes in the majority of cases, although monoclonal immunoglobulin, including monoclonal light chains [44], is detectable in a proportion of SS patients. Cryoglobulinemia and decreased C4 complement protein levels—indicative of immunocomplexes-related disorders—are common laboratory findings, while increased levels of inflammatory markers, mainly erythrocyte sedimentation rate (ESR) and more rarely C-reactive protein (CRP), are encountered in the context of SS [45].

2.2.1 Diagnostic Tests

1. Schirmer's Test

Schirmer's test is a method used for the assessment of ocular dryness, after placing a graduated strip of filter paper in the lower eyelid sac. The test is considered

abnormal, if after 5 min, 5 mm or less of the paper is wet [46].

2. Break-up Time Test

The break-up time (BUT) test evaluates the stability of the tear film, measuring the time tears need to break up after placing fluorescein in the eye. Under normal circumstances, the lacrimal film breaks after 10 s. Break-up test ≤ 10 s is considered abnormal [46].

3. Lissamine Green Staining

Lissamine green is a dye that stains the impaired epithelium of the cornea or conjunctiva. The eye is divided in three zones (nasal, central, and temporal), each of which is scored from 0 to 3. The final staining score (Bijsterveld score), ranging between 0 and 9, is indicative of keratoconjunctivitis sicca, if it is ≥ 4 [46].

4. Sialometry

Whole unstimulated salivary flow, collected in a tube, less than 1.5 mL during 15 min, indicates reduced saliva secretion, while stimulated sialometry, using lemon juice or specific gums, less than 3.5 mL in 5 min is considered abnormal [46].

5. Minor Salivary Gland Biopsy

Minor salivary gland (MSG) biopsy constitutes the hallmark for SS diagnosis. Biopsy is performed on the inner part of the lower lip and requires local anesthesia. The characteristic finding is periductal lymphocytic infiltration of salivary gland (Fig. 12.1). A cluster of 50 or more lymphocytes is called focus. The number of foci per 4 mm² of labial tissue is the average focus score. A biopsy with focus score ≥ 1 is considered compatible with the diagnosis of SS [46].

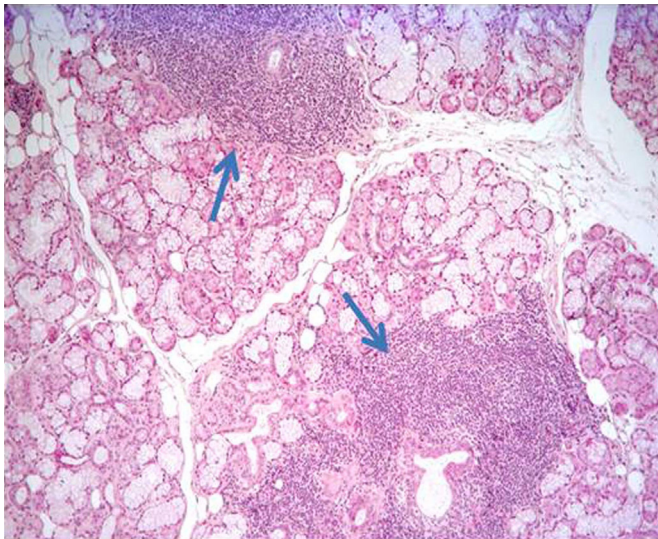


FIGURE 12.1 Minor salivary gland biopsy from a patient with primary Sjögren's syndrome. The arrows indicate the presence of periductal lymphocytic infiltrates. Adapted from the personal collection of the authors.

2.3 Classification Criteria

Various sets of SS classification criteria have been so far proposed, with the European/American being the most commonly accepted, taking into account both objective and subjective manifestations, the presence of certain autoantibodies against ribonucleoproteins, and the histopathological characteristics in the MSG biopsy (Table 12.1) [46]. Diagnosis of the syndrome requires the presence of four out of six criteria, including definitely either salivary gland biopsy compatible with SS (focus score ≥ 1) or positive antibody titers (anti-Ro/SSA or/and anti-La/SSB antibodies), or the fulfillment of three out of four objective criteria (3–6).

A new set of classification criteria for SS has recently been proposed by the American College of Rheumatology (ACR) [47]. Thus classification of a patient having SS

TABLE 12.1 Revised European/American Classification Criteria for Sjögren's Syndrome [46]

1. Ocular symptoms (at least one present)	
Persistent, daily ocular dryness for longer than 3 months	
Recurrent sensation of sand or gravel in the eyes	
Use of tear substitute more than 3 times a day	
2. Oral symptoms (at least one present)	
Daily feeling of dry mouth for more than 3 months	
Persistent or recurrent swollen salivary glands as an adult	
Consumption of liquids to aid in swallowing dry food	
3. Objective evidence of ocular dryness (at least one present)	
Schirmer's test ≤ 5 mm/5 min	
Ocular dye score ≥ 4	
4. Objective evidence of salivary glands involvement (at least one present)	
Unstimulated salivary flow ≤ 1.5 mL/15 min	
Parotid sialography (showing the presence of diffuse sialectasias (punctate, cavitory, or destructive pattern), without evidence of obstruction in the major ducts)	
Salivary gland scintigraphy (showing delayed uptake, reduced concentration, and/or delayed excretion of tracer)	
5. Histopathological features	
Positive minor salivary gland biopsy with focus score ≥ 1 (a cluster of 50 or more lymphocytes per 4 mm ² of labial tissue)	
6. Autoantibodies	
Presence of anti-SSA/Ro or/and anti-SSB/La antibodies	

Exclusion criteria

Infection with hepatitis C virus or HIV, sarcoidosis, lymphoma, graft vs. host disease, use of anticholinergic drugs, previous radiotherapy to the head and neck

requires the presence of two out of the three following: (1) either positive titers of anti-Ro/SSA or/and anti-La/SSB antibodies, or positive rheumatoid factor along with titers of antinuclear antibodies (ANA) higher than 1:320; (2) ocular dye staining score ≥ 3 ; and (3) lymphocytic infiltration of MSG, containing more than 50 lymphocytes per 4 mm² of labial tissue.

3. PATHOPHYSIOLOGY OF NON-AMYLOIDOSIS ASSOCIATED CARDIAC INVOLVEMENT

3.1 Biochemical Markers for Endothelial Injury

Similar to other chronic systemic autoimmune diseases, such as RA and SLE [48,49], SS has also been

proposed as an independent predictor of subclinical atherosclerosis, possibly through endothelial injury [50]. Endothelial dysfunction plays a pivotal role in the initiation of atherosclerosis, and several genetic and traditional CV risk factors, as well as chronic inflammation, have been implicated as contributors to endothelial damage [51,52].

Under physiologic circumstances, endothelial cells produce regulatory vasodilators, such as NO and prostacyclin-2, anticoagulant mediators like thrombomodulin—a cofactor in protein C activation—as well as fibrinolytic molecules, such as tissue plasminogen activator. In the presence of detrimental stimuli, endothelium is activated acquiring an inflammatory phenotype—known as endothelial dysfunction—characterized by reduced release of vasodilating factors, overproduction of prothrombotic molecules, and overexpression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin, promoting recruitment of leukocytes [53–55]. The latter subsequently promote cytokine production, including tumor necrosis factor (TNF- α), interleukin (IL)-1 and -6, inducing migration of smooth muscle cells in the subendothelium space, as well as differentiation and proliferation of the adherent inflammatory cells [51,55,56].

Among well-recognized culprits of endothelial dysfunction, systemic inflammatory burden plays a central role, as previously shown in chronic inflammatory disorders, such as rheumatoid arthritis [53]. Tumor necrosis factor- α (TNF- α), oxidative stress, and CRP, all features of chronic inflammation, lead to endothelial dysfunction, mainly through downregulating nitric oxide (NO) bioavailability, and CRP has also been shown to enhance the expression of vasoconstrictors and adhesion molecules on the surface of endothelial cells [51,52].

Although systemic inflammation is not implicated to a great extent in SS pathogenesis, increased levels of protein oxidation markers were detected in 26 SS patients compared to 15 healthy individuals, and were not associated with clinical and laboratory features [57]. In addition, while SS is generally considered to exhibit normal CRP levels, increased CRP response has been reported in a subset of SS patients [58].

A growing body of data suggests the presence of endothelial dysfunction in the setting of SS [50,59], as evidenced by the upregulation of several biochemical markers of endothelial injury in SS patients compared to controls. Thus soluble VCAM-1, ICAM-1, and E-selectin—surrogate markers of endothelial dysfunction [51]—and endothelial microparticles—associated with endothelial injury and proposed as markers of endothelial dysfunction [60]—have been identified in

high concentrations in the circulation of SS patients [61,62]. Another cause of endothelial dysfunction is the impaired capacity of endothelial repair, observed in SS patients with longer disease duration [62]. Furthermore, anti-endothelial cell antibodies, previously suggested to have an antibody-dependent cytotoxic role [63], were found in elevated levels among SS patients, associated with Raynaud's phenomenon [64], while Von Willebrand factor levels, another indicator of endothelial damage, produced by endothelial cells, have also been found to be increased in the SS population [65,66], as shown in a small study including 23 SS patients compared to 229 controls. No associations with antinuclear or anti-Ro/SSA and anti-La/SSB autoantibodies were detected [65]. Finally, elevated levels of a novel endogenous antagonist of NO production, the asymmetric dimethylarginine (ADMA), previously associated with atherosclerosis and endothelial dysfunction [67], have also been observed in RA and primary SS patients [59,68].

Recent evidence supports the contribution of decreased oral hygiene to the development of CV disease. Reduced oral health, defined by the frequency of tooth brushing, has been associated with endothelial dysfunction, determined by flow-mediated and nitroglycerine-mediated dilatation (FMD and NMD) [69]. Moreover, periodontal disease, defined by increased dental plaque formation [70,71], gingival inflammation [70], as well as poor oral hygiene [72], have been all related to subclinical atherosclerosis assessed by carotid intima media thickness (IMT) score and/or atherosclerosis progression [73]. Putative underlying mechanisms for this association include the activation of the host immune response against bacteria derived from periodontal space, as well as the increased prevalence of traditional CV risk factors, such as smoking and diabetes, which are encountered more frequently in patients with periodontal disease [73,74]. To this end, reduced salivary flow was observed in SS patients with evidence of carotid and/or femoral plaque formation, implying that reduced saliva production in the setting of SS can lead to increased risk of dental caries and poor oral hygiene [75]. Further studies are required in order to explore the role of dental disease in atherosclerosis development among SS individuals.

3.2 Markers for Increased Thrombogenicity

While the association of SLE with heightened risk of thromboembolic events is well defined [76], there is limited evidence regarding the prevalence of thrombosis in the setting of SS. According to a recent study including 90 primary SS patients [77], the incidence of both arterial and venous thromboembolic manifestations has been found to be 1.4% per 100 patients-years. Higher

risk of deep venous thromboembolism and/or pulmonary embolism have also been reported in a large Taiwan study of 8920 SS patients (both primary and secondary), as well as in a cohort of hospitalized SS patients [78,79], particularly during the first year after hospital admission [80]. A subsequent recent meta-analysis reported a cumulative incidence for venous thromboembolism of 2.18% in SS [81].

The etiology of increased thromboembolic risk in the setting of SS is not well defined; however, a remarkable proportion of SS patients, ranging from 15% to 30% in various reports [82–85], are carriers of antiphospholipid antibodies (lupus anticoagulant, anticardiolipin, and anti- β 2-glycoprotein-I), usually in low titers. This percentage can be even higher, approximately 46%, in patients with SS secondary to SLE [86]. Although in the majority of studies these autoantibodies have no clinical significance in the setting of SS, a recent study revealed lupus anticoagulant as a putative biomarker for stroke and venous thrombosis [87]. Moreover, in a previous small study on SS patients, low levels of the anticoagulant protein C together with its cofactor protein S were detected; however, no clear associations with thromboembolic events were reported [77]. Finally, increased serum levels of the endothelial derived molecule von Willebrand factor—previously shown to confer increased risk for thrombosis—were also found in the setting of SS [65,66,88].

3.3 Innate Immune Abnormalities

Unlike SLE and RA, in which activation of the type I interferon pathway has been proposed as a significant contributor of accelerated atherosclerosis, possibly through reduction of endothelial progenitor cells—a cardinal repair mechanism for endothelial injury—no data so far is available in the setting of SS [89–91].

3.4 Adaptive Immune Abnormalities

Autoantibodies, mainly anti-Ro/SSA, anti-La/SSB, and rheumatoid factor, indicative of adaptive immunity activation, have been related with both impaired endothelial dysfunction and increased arterial wall thickening in SS patients [50,92]. Association of these serologic features with atherosclerosis implies that immune dysregulation accounts for the accelerated atherosclerotic damage observed in SS. More specifically, taking into account that the presence of autoantibodies has been associated with the degree of salivary gland lymphocytic infiltration [93], it could be hypothesized that a similar infiltrative procedure occurs in subendothelial space [50]. Beyond their contribution to atherosclerotic process, several autoantibodies (anti-Ro/

SSA, antinuclear, anti-RNP, and rheumatoid factor) have been proposed as indicators of clinically overt cardiac abnormalities, such as pulmonary hypertension [94] and pericardial involvement [95,96], among SS individuals.

4. CARDIAC INVOLVEMENT

4.1 Cardiac Amyloidosis

Although the exact prevalence of amyloidosis in the setting of SS has not been elucidated, several cases of patients with cutaneous [97–100] or pulmonary [101] amyloidosis (mainly of AL type), usually subsequent to SS diagnosis, have been reported. Furthermore, according to data from an Italian study of 141 sicca individuals, serum amyloid A was detected in salivary gland biopsies of one third of patients with definite SS, associated with increased β two microglobulin serum levels [102].

Despite the fact that no studies on SS regarding the amyloidosis-related cardiac involvement are available, a case of a patient with concomitant diagnosis of SS and AL amyloidosis, also presenting echocardiographically confirmed diastolic cardiac dysfunction (possibly attributed to amyloidosis), was reported [103]. Similarly, another case of SS patient, displaying heart failure, with reduced ejection fraction, cardiac hypokinesia, and pericardial effusion in echocardiographic evaluation, was diagnosed simultaneously with AL amyloidosis, confirmed by myocardial biopsy [104].

4.2 Prevalence of Traditional Risk Factors for Accelerated Atherosclerosis

Increased atherosclerotic burden characterizes autoimmune diseases, including SLE and RA. Traditional CV risk factors do not entirely account for this heightened risk, implying that additional, disease-related features are implicated in this process. There is increasing evidence over the last few years that SS also constitutes an independent risk factor for subclinical atherosclerosis. The prevalence of traditional risk factors for CV disease among SS patients varies between studies, as depicted in Table 12.2.

In a large series of 788 SS patients and 4774 healthy individuals, hypertension was encountered more often in the SS population with a prevalence of 32% and 28%, respectively ($p=0.02$) [105], and similar observations derive from a study conducted in 200 patients and 200 controls (28% vs. 15.5%, respectively, $p=0.003$) [106]. However, different reports indicate either underrepresentation of hypertension in 312 SS individuals

compared to 312 healthy controls (30% vs. 46%, respectively, $p<0.001$) [107] or no significant differences in the prevalence of hypertension among groups [75,108,109]. Hypercholesterolemia has also been identified more frequently in the SS group according to two recent studies. The first study was conducted in 788 patients and 4774 controls with a prevalence of 30.0% and 23.0%, respectively ($p<0.001$) [105] and the second was carried out in 1974 patients and 9870 patients, with hypercholesterolemia frequency being 15.0% and 11.3%, respectively ($p<0.001$) [108]. Other previously published reports demonstrated no difference in cholesterol levels [107] or even reduced levels in the patient group [75,110]. Decreased high-density lipoprotein (HDL) levels also seem to characterize SS population as reported in studies with small numbers of patients [92,110]. Additionally, elevated triglyceride levels are encountered in higher rates among SS individuals (approximately 20%) compared to healthy controls [106,107].

On the other hand, smoking habits were underrepresented in 312 SS patients compared to an equal number of healthy controls (19% vs. 31%, respectively, $p<0.001$) [107], as well as in 788 SS patients in comparison with 4774 healthy volunteers (13% vs. 23%, respectively, $p<0.001$) [105]. In the latter study, similar observations were observed in regard to obesity in the SS population (11% vs. 21%, $p<0.001$) [105]. Finally, apparently conflicting are the data on diabetes mellitus, depicting either lower prevalence [105], or no difference between SS and healthy individuals [106,108]. Of interest, a study including 624 patients and controls demonstrated higher prevalence of diabetes in the SS group (27% vs. 13%, respectively, $p<0.001$) [107], while according to another recent study conducted in a smaller series of 64 patients and similar number of controls, 6.3% of the SS population and none of controls suffered from diabetes mellitus [75], although this difference was not statistically significant.

4.3 Markers for Atherosclerosis and Endothelial Dysfunction

Although previously unrecognized, in recent years, a growing body of evidence convincingly demonstrates increased prevalence of endothelial dysfunction, subclinical atherosclerosis, and cardiovascular morbidity among SS patients (summarized in Table 12.3) [50,59,75,92,109].

SS-related endothelial dysfunction was demonstrated by impaired brachial endothelium-dependent flow-mediated (FMD) and nitrate-mediated dilatation (NMD), assessed in 45 women diagnosed with SS and 59 age-matched female controls, with similar distribution of traditional CV factors, apart from HDL levels

TABLE 12.2 Prevalence of Traditional CV Risk Factors in SS Patients and Healthy Controls

Study	Group	N	Hypertension %	High cholesterol levels %	Low HDL %	High LDL %	High triglyceride levels %	Smoking %	Obesity %	Diabetes mellitus %	References
1	SS patients	788	32.0 ^a	30.0 ^a	Na	Na	Na	13.0 ^a	11.0 ^a	4.0 ^a	[105]
	Controls	4774	28.0	23.0	Na	Na	Na	23.0	21.0	7.0	
2	SS patients	200	28.0 ^a	19.0	16.5	16.5	21.0 ^a	3.8 ^a	19.5	3.0	[106]
	Controls	200	15.5	17.5	13.0	18.5	9.5	10.1	18.6	2.0	
3	SS patients	1974	24.2	15.0 ^a	Na	Na	Na	Na	Na	10.1	[108]
	Controls	9870	23.6	11.3	Na	Na	Na	Na	Na	9.5	
4	SS patients	312	30.0 ^a	30.0	10.0	21.0	22.0 ^a	19.0 ^a	18.0	27.0 ^a	[107]
	Controls	312	46.0	35.0	9.0	16.0	15.0	31.0	26.0	13.0	
5	SS patients	25	52.0	8.0	Na	Na	Na	Na	Na	Na	[109]
	Controls	25	60.0	8.0	Na	Na	Na	Na	Na	Na	
6	SS patients	64	36.5	Na	Na	Na	Na	Na	Na	6.3	[75]
	Controls	60	25.8	Na	Na	Na	Na	Na	Na	0.0	

CV, cardiovascular; SS, Sjögren's syndrome; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HC, healthy controls; Na, not applicable.

^ap < 0.05.

TABLE 12.3 Markers of Endothelial Dysfunction and Subclinical Atherosclerosis in SS Patients and Healthy Individuals

Study	Group	n	Assessment of endothelial dysfunction						References
1			Brachial FMD % ± SD	Associations	Brachial NMD % ± SD	Associations			[50]
	SS patients	45	7.4 ± 3.6	Articular involvement PGE RF	8.1 ± 3.5 ^a	Leukopenia RF anti-La/SSB articular involvement			
	Controls	59	7.7 ± 1.9		10.3 ± 2.1				
2			CFR median	Associations	PWV right/left (m/s)	Associations			[59]
	SS patients	22	2.70 ^a	Na	8.80/8.90 ^a	Na			
	Controls	22	3.20		6.86/6.89				
3			Assessment of subclinical atherosclerosis						
			Carotid IMT(mm ± SD)	Associations	Femoral IMT (mm ± SD)	Associations	Plaque formation n (%)	Associations	[92]
	SS patients	37	0.82 ± 0.24 ^a	Anti-Ro/SSA leukopenia	0.81 ± 0.26 ^a	Leukopenia	9 (24.0)	Na	
	Controls	35	0.63 ± 0.20		0.67 ± 0.23		7 (20.0)		
4			Abnormal ABI n (%)	Associations					[109]
	SS patients	25	5 (20.0)	Disease duration					
	Controls	25	1 (4.0)						
5			IMT right catotid (mm)	Associations	IMT left carotid (mm)	Associations			[59]
	SS patients	22	0.60	Na	0.60	Na			
	Controls	22	0.53		0.60				
6			IMT (mm ± SD)	Associations	Plaque formation n (%)	Associations			[75]
	SS patients	64	1.0 ± 0.3 ^a	Age	44 (68.8)	Age lymphocytes number			
	Controls	60	0.9 ± 0.2		34 (56.9)				
7			PWV (m/s)	Associations					[111]
	SS patients	44	8.2 ^a	Age at SS diagnosis SSDI blood pressure low 25[OH] D levels Framingham risk score					
	Controls	78	7.7						

FMV, flow-mediated dilatation; NMV, nitrate-mediated dilatation; SD, standard deviation; SS, Sjögren's syndrome; HC, healthy controls; PGE, parotid glands enlargement; RF, rheumatoid factor; CFR, coronary flow reserve; PWV, pulse wave velocity; IMT, intima media thickness; ABPI, ankle brachial pressure index; Na, not applicable.

^a*p* < 0.05.

(lower in the patient group). While the FMD values were similar between the two groups, after stratification according to clinical manifestations, these values were reduced in patients displaying parotid gland enlargement (mean \pm SD: 5.7 ± 3.1) and articular involvement (mean \pm SD: 5.4 ± 2.8), compared to controls (mean \pm SD: 7.6 ± 1.9). On the other hand, NMD levels were found to be significantly lower in the whole SS patient group than in the control group (mean \pm SD: 8.1 ± 3.5 vs. 10.3 ± 2.1 , $p \leq 0.001$). This reduction was more evident in the SS subgroup characterized by the presence of leukopenia (mean \pm SD: 6.4 ± 3.8), RF (mean \pm SD: 7.5 ± 4.2), and anti-SSB/La autoantibodies (mean \pm SD: 7.4 ± 4.4), as well as articular manifestations (mean \pm SD: 7.4 ± 3.9). Nitroglycerine-mediated dilatation was also directly and inversely correlated with the number of leukocytes and circulating VCAM-1 (a marker of endothelial dysfunction) levels, respectively [50]. In line with these results, pulse-wave velocity (PWV)—measuring the stiffness of arterial wall—and coronary flow reserve (CFR)—the ultrasonographic evaluation of the coronary flow after administration of vasodilators and in rest conditions—values were also found to be impaired in SS individuals [59] in a study including 22 patients and 22 age- and sex-matched healthy volunteers. More specifically, CFR values were decreased in SS individuals compared to healthy controls (median: 2.70 vs. 3.20, $p < 0.0001$), while arterial stiffness of both right and left carotid, evaluated by PWV levels, was found to be increased in the patient group (8.80 m/s and 8.90 m/s, respectively) in comparison with the control group (6.86 m/s and 6.89 m/s, respectively). Elevated levels of PWV were also detected among 44 primary SS patients compared to 78 age- and sex-matched healthy volunteers, in association with age, blood pressure, and reduced levels of 25-hydroxyvitamin D. [111].

Subclinical atherosclerosis was also demonstrated in SS patients both by carotid and/or femoral ultrasound and ankle brachial pressure index (ABPI) measurements. Thus arterial wall thickening—defined as intima media thickness (IMT) score by ultrasound (Fig. 12.2)—of both carotid and femoral artery was found to be increased in 37 SS patients (0.82 ± 0.24 mm and 0.81 ± 0.26 mm, respectively) compared to 35 healthy controls (0.63 ± 0.20 mm and 0.67 ± 0.23 mm, respectively) of Italian origin, to a statistically significant extent ($p \leq 0.001$ and $p \leq 0.02$). Arterial wall thickening was associated with leukopenia and the presence of ant-Ro/SSA autoantibodies, implying that disease activity and impaired humoral immunity are implicated in the atherosclerotic process [92]. In a similar fashion, a study conducted in a Greek population (64 SS patients and 60 controls) showed significantly higher IMT levels in patients than in controls (1.0 ± 0.3 mm vs. 0.9 ± 0.2 mm, $p = .03$) in association with traditional

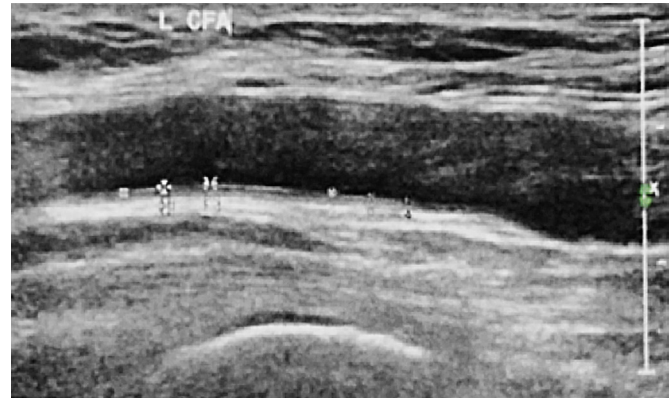


FIGURE 12.2 Ultrasonographic evaluation of a patient with Sjögren's syndrome for the identification of intima media thickness (IMT) (arrows), which represents an example of abnormal IMT. Adapted from the personal collection of the authors.

CV risk factors, including age, BMI, hypertension, and LDL, as well as manifestations related to lymphocytic periepipithelial infiltration. However, after multivariate analysis only age remained an independent contributor for arterial wall thickening [75]. The prevalence of traditional risk factors did not differ to a significant extent between patients and controls both in the Italian and Greek study, except for total cholesterol, LDL [75], and HDL [92] levels, which were lower in patients compared to controls. However, and in contrast to RA [75,112], plaque formation on the arterial wall, another marker of subclinical atherosclerosis, was similar between SS and healthy individuals, in both the aforementioned studies [75,92]. Furthermore, increased prevalence of abnormal ABPI—defined as the ratio of systolic blood pressure in the lower leg and arm, with levels lower than one indicating peripheral artery disease—was also detected in the SS group, associated with disease duration [109]. In this study, including 25 SS patients and 25 healthy individuals with similar age, sex, and rates of CV risk factors, 20% of patients and 4% of control subjects presented abnormal ABPI, although this difference did not reach significance. Contrary to the previous reports, when 18 elderly primary SS patients were evaluated with Doppler sonography of carotids for the identification of subclinical atherosclerosis—defined by IMT score, stiffness, and other hemodynamic parameters—and compared with 18 osteoarthritic patients with similar age, sex, and CV risk factors distribution, no significant differences were observed [113].

4.4 Evaluation of Coronary Flow and Markers for Cardiac Ischemia

Coronary flow in rest and after administration of vasodilator agents (CFR), a marker of endothelial

dysfunction and atherosclerosis, has been assessed in SS patients and found to be impaired compared to healthy individuals [59]. All subjects underwent transthoracic Doppler evaluation of the left anterior descending coronary artery, in the left lateral position. Coronary flow reserve values less than two are considered predictive for severe, clinically overt coronary stenosis [114]. Although CFR levels were within normal limits in SS patients (median: 2.70), they were still decreased compared to the control group (median: 3.20), implying coronary flow impairment, to some extent, in SS.

4.5 Prevalence of Ischemic Heart Disease and Heart Failure

Recent data, derived from studies conducted in a large series of SS patients, suggest increased rates of heart failure and myocardial infarction in SS compared to healthy individuals, at a rate of 1.8–3.4% [78,105] and 1% [105], respectively. Contrary to these observations, the prevalence of ischemic heart disease and heart failure, found to be 0.8% and 2.5%, respectively, was similar between 1974 SS patients and 9870 controls [108]. While CV-related deaths have been previously reported in SS patients [115], a Swedish report revealed similar risk of deaths attributed to CV disease between SS patients and the general population [116].

4.6 Echocardiographic Findings

Findings derived from echocardiographic studies have shown that cardiac complications are frequent in SS. Pericardial involvement has been observed, in forms of either echogenic pericardium [117] and clinically silent pericardial effusion [118], or as overt pericarditis accompanied by hypokinesia and abnormal systolic dimension of the left ventricle [96]. Of interest, a diagnosis of diffuse large B-cell lymphoma involving the pericardium was revealed in an SS female patient, presenting with pericardial effusion along with pericardial lesions [119]. Other commonly reported echocardiographic abnormalities include thickening of mainly mitral, aortic, and tricuspid valves [120] as well as aortic (23.4%), mitral (30%), and tricuspid (10.3%) regurgitation [118], while disturbed systolic [121] and diastolic functions [117,118,121] of the left ventricle have also been reported (Fig. 12.3). Although pulmonary hypertension was earlier considered a rare complication in the context of SS [122–125] later reports revealed high rates, ranging from 22% to 37% [117,118] in association with hypocomplementemia and cryoglobulinemia [118]. Moreover, SS patients with pulmonary hypertension displayed more frequently clinical manifestations, like Raynaud's phenomenon and interstitial lung

disease, and markers of B-cell activation, such as several autoantibodies (antinuclear, anti-Ro/SSA, rheumatoid factor, and anti-RNP), compared to those patients with no evidence of such complication [94]. Of interest, in a case of a patient with SS and echocardiographically confirmed pulmonary hypertension, atrial septal defect was concomitantly diagnosed [126].

4.7 Coronary Vasculitis

Vasculitic events involving the coronary artery are uncommon in the context of SS. However some cases of SS patients who presented symptoms of angina and underwent artery bypass, due to coronary arteritis, have been reported [127,128].

4.8 Conduction Disorders

It is well established that the transplacental transport of maternal anti-Ro/SSA antibodies account for the congenital heart block development in approximately 2% of neonates [129]. Despite the fact that the atrioventricular node in adults is considered to be resistant to the arrhythmogenic effect of these antibodies, individuals with SS carrying anti-Ro/SSA antibodies have been reported to present with complete heart block [130,131]. Furthermore, atrial electromechanical delay has been observed in SS adults [132], while prolonged PR interval, indicative of first-degree atrioventricular block, has been reported in 5 out of 51 SS individuals, with no evidence of second-degree or complete heart block. The presence of heart block was related with disease activity features, such as the extent of lymphocytic infiltrates of salivary gland biopsy (focus score) and the presence of autoantibodies (anti-La/SSB and anti-cardiolipin antibodies). However, no association between first-degree heart block and anti-Ro/SSA autoantibodies was demonstrated, implying that other antibodies may be implicated in the pathogenesis of atrioventricular block among SS adult individuals [133].

4.9 Prevalence of Ventricular Arrhythmias

A frequent clinical feature of cardiac involvement in the setting of connective tissue diseases is disturbance of the heart rhythm, including mainly conduction disorders and tachyarrhythmias [134]. Although limited evidence regarding the prevalence of rhythm disorders among SS individuals is available, higher prevalence (about 4%) of cardiac arrhythmias has been reported in a large series of SS patients [108]. Moreover, when 57 patients with rheumatic diseases (31 anti-Ro/SSA positive, including 9 SS patients and 26 anti-Ro/SSA negative, including 3 SS patients) underwent electrocardiographic evaluation, patients carrying anti-Ro/

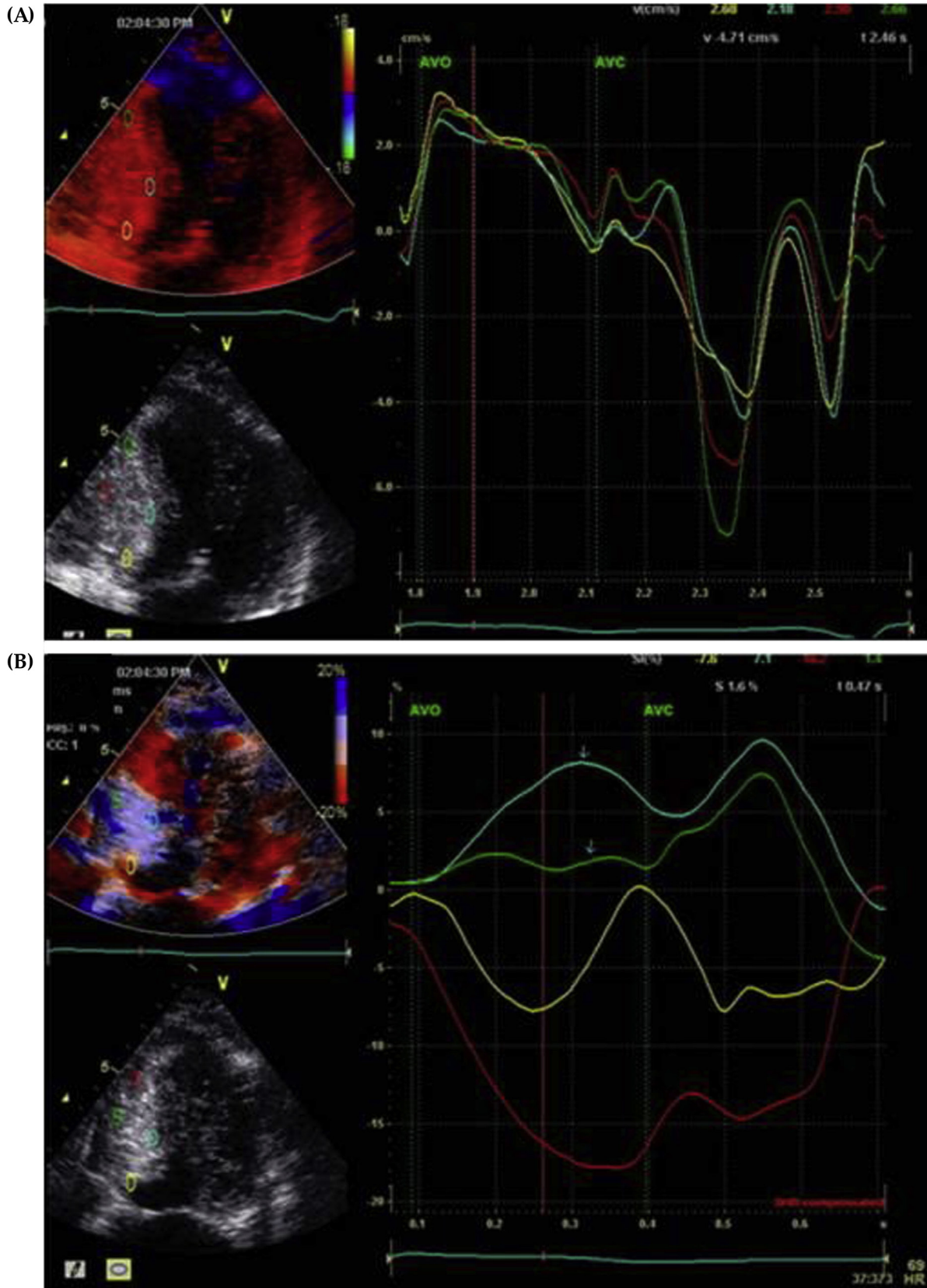


FIGURE 12.3 Assessment of left ventricular (LV) dysfunction by means of tissue Doppler (A) derived strain and 2D strain (B) imaging techniques from four representative regions of interest in a patient with Sjögren's syndrome. Note the significantly attenuated systolic and early diastolic velocities from these disparate regions in the septum (A). Note the positive longitudinal strain (systolic lengthening) or "paradoxical strain" (blue and green tracings) in two of the four depicted regions (basal septum) and attenuated longitudinal strain elsewhere (yellow tracing). Also note the striking heterogeneity of the strain tracings in contrast to tissue Doppler data. AVO, aortic valve opening; AVC, aortic valve closure. *Adapted from the personal collection of the authors.*

SSA antibodies displayed prolonged QT interval, which is associated with high risk of ventricular arrhythmias [135]. This finding indicates an arrhythmogenic role of anti-Ro/SSA antibodies, affecting the ventricular repolarization period. Taking into account the previously reported inhibition of calcium channels by anti-Ro/SSA antibodies [136], a possible additional effect of these antibodies on potassium channels, responsible for the repolarization phase, could provide a putative explanation. Longer QT dispersion, a marker of repolarization inhomogeneity, which predisposes to ventricular arrhythmias, was also observed in 18 RA patients with secondary SS (mean \pm SD: 41.3 ± 10.5 ms) compared to 40 RA patients without SS (mean \pm SD: 28.2 ± 6.7 ms) and 29 healthy individuals (mean \pm SD: 20.0 ± 3.9 ms), implying that SS is related to cardiac rhythm impairment [137]. Of interest, anti-Ro/SSA antibodies were not associated with the observed longer QT dispersion, confirming previous reports among SLE patients [138,139]. Considering that the cardinal histopathological lesion in the setting of SS is the lymphocytic infiltration of exocrine glands, as well as of parenchymal organs, the prolongation of QT dispersion, demonstrated in secondary SS patients, may be attributed to heart tissue injury due to local lymphocytic infiltration [137].

4.10 Pericardial Involvement

An increasing body of evidence supports pericardial involvement in the setting of SS. A patient diagnosed with SS and cryoglobulinemic glomerulonephritis also exhibited pleural and pericardial effusion of inflammatory origin, probably due to immunocomplexes deposition in the pericardium [140]. Another uncommon case of an SS patient complicated by hemolytic uremic syndrome and pericarditis was reported, with

immunocomplexes deposition being proposed as the main pathogenetic mechanism for both complications [141]. In a previous study, only 1 out of 64 SS patients developed acute pericarditis, while an echogenic pericardium, with no clinical signs of overt pericarditis, was observed in 21 individuals (33%) [117]. Subclinical pericarditis manifested as pericardial effusion (Fig. 12.4) has also been found to occur at increased rates (8%) in SS population compared to healthy controls (0.8%), according to a recent study including 107 patients [118]. The pericardial effusion was further associated with disease-related features, such as cryoglobulins and primary biliary cirrhosis [118]. Another study reported pericardial effusion in 20.2% of 124 SS patients, related with hypocomplementemia, elevated CRP levels, and anti-Ro/SSA positivity [95]. However, clinically overt pericarditis has been demonstrated among SS individuals at a rate ranging from 10 to 33% [96,142]. In an echocardiographic study 9 out of 27 evaluated SS patients displayed signs of pericarditis, in association with higher age, shorter disease duration, the presence of antinuclear antibodies, as well as other cardiac abnormalities, including small systolic dimension and hypokinesia of the ventricle [96]. Contrary to these observations, in another study of 18 SS patients, no pericardial involvement was demonstrated after echocardiographic evaluation [143].

4.11 Aortic Abnormalities

A limited number of SS individuals with aortic disorders, including aortitis, periaortitis, and aortic rupture, have been reported [145,146]. In a recent study carried out in a small series of 50 patients, lower aortic distensibility in the SS group compared to healthy volunteers (2.7 ± 1.1 vs. 7.1 ± 3.2 , $p < 0.001$), associated with left ventricular diastolic dysfunction, was observed [147].

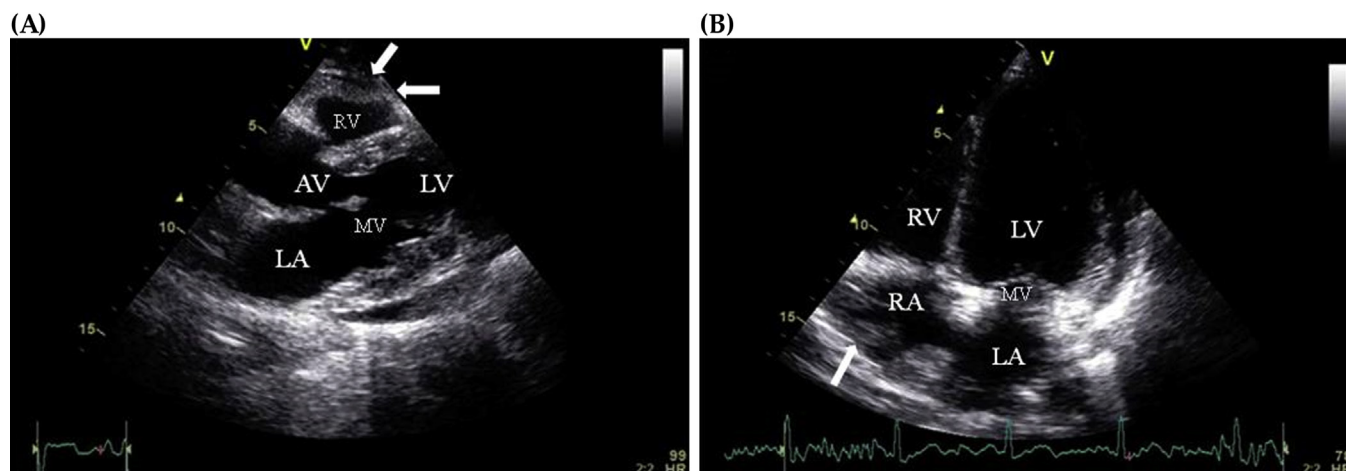


FIGURE 12.4 Echocardiography (parasternal view and 4-chamber view) showing mild to moderate pericardial effusion (arrowhead). Note the diastolic collapse of the free wall of the right ventricle (parasternal view, arrow) and the paradoxical movement of the right atrial free wall (4-chamber view, arrow). RV, right ventricle; LV, left ventricle; LA, left atrium; RA, right atrium; MV, mitral valve; AV, aortic valve. Adapted from Plastiras and Toumanidis [144].

4.12 Valvular Abnormalities

A few echocardiographic studies of SS patients have demonstrated valvular defects—predominantly insufficiency—in mitral, aortic, and tricuspid valves. More specifically, according to an echocardiographic study conducted in 107 SS patients and 112 healthy individuals with similar age and sex distribution, mitral, aortic, and tricuspid regurgitation were encountered more frequently in the SS group compared to healthy controls (29.9% vs. 10.7%, $p < 0.001$, 23.4% vs. 9.8%, $p = 0.007$ and 10.3 vs. 2.7, $p = 0.02$, respectively). Valvular abnormalities were associated with C4 hypocomplementemia—indicative of classical complement pathway activation—for mitral valve), age (for mitral and aortic valves), and pulmonary hypertension (for tricuspid valve) [118]. Furthermore, valvular thickening [96,120] without clinical significance has been observed in SS patients, possibly attributed to a combination of degenerative and disease-related factors [120]. Only one case report of a patient diagnosed with SS, who underwent double valvular replacement, has been reported [148].

4.13 Maternal Sjögren's Syndrome

Pregnancy in the setting of SS can be successful in the vast majority of cases. The pregnancy outcomes were similar among 58 women diagnosed with primary SS and 157 healthy volunteers, with an exception of neonatal congenital heart block in 3.4% of SS mothers [149]. Another study, including a small number of pregnancies, demonstrated successful outcomes, although SS women tended to deliver preterm neonates with lower birth weight [150]. Low-birth-weight neonates delivered by mothers with SS were also observed in a study of 16 SS women and 80 controls (3.010 ± 787.1 vs. 3.458 ± 606.8 , $p = 0.025$), along with higher age at delivery (33.6 ± 4.2 vs. 28.8 ± 5.5 , $p = 0.004$) and lower incidence of normal partus (56% vs. 83%, $p = 0.02$) in the SS group [151]. A recent study comparing the pregnancy outcomes between 34 SS patients and 136 controls confirmed these observations, demonstrating higher rates of preterm delivery (39% vs. 5%, $p < 0.0001$), of neonates with low birth weight (35% vs. 1%, $p < 0.0001$), of Cesarean section (57% vs. 31%, $p = 0.03$), and of spontaneous abortion (30% vs. 3%, $p < 0.0001$) in the SS population [152]. The latter complication has been demonstrated in previous studies as well [153,154], without association with autoantibodies, such as anticardiolipin, anti-Ro/SSA and anti-La/SSB antibodies [154]. On the other hand, a previous study aimed at investigating the possible association of anti-Ro/SSA autoantibodies with pregnancy outcomes found a higher frequency of pregnancy loss among anti-Ro/SSA positive patients. More specifically,

obstetric records of 154 anti-Ro/SSA positive and 142 anti-Ro/SSA negative patients and 154 healthy women were retrospectively evaluated. Anti-Ro/SSA positive women were further separated into SLE ($n = 78$) and non-SLE ($n = 76$) patients. The same was done for anti-Ro/SSA negative women (SLE = 71, non-SLE = 71). Of this group, 59.2% of anti-Ro/SSA positive non-SLE and 62.0% of anti-Ro/SSA negative non-SLE patients were diagnosed with SS. In the non-SLE group, anti-Ro/SSA positive patients exhibited more frequently recurrent pregnancy loss compared to both non-SLE anti-Ro/SSA negative patients and healthy controls (23.7% vs. 7.0%, $p = 0.006$ and 23.7% vs. 6.4%, $p = 0.0004$, respectively) [155]. Furthermore, higher incidence of spontaneous abortions has been reported in patients with SS secondary to SLE [156]. With the exception of spontaneous, induced abortions are also encountered more frequently in SS patients [157].

However, the occurrence of congenital heart block in the offspring remains the most significant complication of SS-related fetal outcomes [149,150], arising from transplacental passage of maternal anti-Ro/SSA or/and anti-La/SSB antibodies [158–160]. Several cases of SS women carrying anti-Ro/SSA or and anti-La/SSB autoantibodies and delivering neonates with heart block have been reported [161–163]. Indeed, a patient with subclinical SS, as well as anti-Ro/SSA and anti-La/SSB positivity, displayed adverse outcomes in two consecutive pregnancies, which is quite uncommon. The first fetus was diagnosed with heart block of third degree at the 23rd gestational week, while the second child developed neonatal lupus erythematosus at 20 weeks after partus [164]. Other cases of SS women, carrying anti-Ro/SSA antibodies, presented with fetal atrioventricular heart block of second degree [165] and increased myocardial echogenicity [165,166], indicative of fibrosis. Of interest, these complications regressed after administration of dexamethasone [165], while in another case the fetal third-degree atrioventricular heart block persisted despite treatment with dexamethasone [167]. Moreover, after retrospective evaluation of 33 women who gave birth to a child with congenital heart block, the laboratory features were related with SS, implying that primary SS is the main underlying autoimmune disorder of women delivering neonates with this complication [168].

The percentage of neonates afflicted by congenital heart block, born from anti-Ro/SSA positive mothers independently of disease status, has been estimated to be around 2% [129], although this frequency may increase to 5% with the additional presence of anti-La/SSB antibodies [169]. The cross-reactivity of anti-Ro/SSA and anti-La/SSB antibodies with antigens present in cardiomyocytes provides a plausible pathogenetic mechanism [170]. More specifically, La/SSB autoantigens share common sequences with laminin, an important component of cardiac sarcolemmal membrane [171,172]. Thus maternal

autoantibodies, recognizing epitopes in fetal cardiomyocytes, induce local inflammatory response at the level of atrioventricular node and myocardium, resulting in cardiac tissue injury in a crucial stage of development. Moreover, a homology between Ro52 ribonucleoprotein and the cardiac serotonergic 5-HT₄ receptor peptide could potentially explain a blocking effect of anti-Ro52 antibodies on serotonin-induced L-type Ca channel activation on human atrial cells [173]. While evidence-based treatment strategies for the prevention of congenital heart block are inconclusive, administration of intravenous immunoglobulin at an early stage of pregnancy, hydroxychloroquine [174], and to a lesser extent oral fluorinated steroids are the most commonly used treatment options in clinical practice [174,175]. Of note, recent evidence suggests intravenous immunoglobulin treatment [176] as an inducer of antiidiotypic antibodies (against anti-La/SSB idotype). The latter can serve as inhibitors of maternal anti-La/SSB antibodies resulting in decreased risk for congenital heart block onset [177].

5. TREATMENT OPTIONS AND CARDIAC IMPLICATIONS OF THERAPY

Treatment of SS is aimed at ameliorating primarily sicca complaints or extraglandular, systemic manifestations (Table 12.4). The reduced tear and/or saliva secretion can be managed with tear/saliva substitutes, ocular cyclosporine drops [178–180], and oral administration of pilocarpine [181–183] or cevimeline [184–187]. Pilocarpine, as a parasympathomimetic agent, may induce disturbances in the autonomic nervous system and can affect the heart rate, although bradycardia is a

relatively rare complication [188,189]. Among immunomodulatory drugs, hydroxychloroquine (HCQ) is the most frequently administered agent [190–193], mainly in those patients presenting systemic manifestations, such as arthralgias, myalgias, and fatigue [194]. Hydroxychloroquine use has also been associated with improvement of laboratory abnormalities [191,194–196]. Although it is considered a safe drug, several cases of HCQ-induced cardiotoxicity, manifested mostly as cardiomyopathy, have been reported [197–201]. Of interest, a recent study reported a beneficial effect of HCQ in the lipid profile of 71 SS females, leading to decreased levels of total cholesterol and increased HDL levels, resulting in the improvement of atherogenic index [202]. In line with these results, RA patients treated with HCQ also displayed reduction in LDL and triglyceride levels [203,204]. Improved lipid profile has also been observed in RA HCQ users, regardless of statin intake [205].

More severe systemic disorders can also be managed with corticosteroids, which can improve oral symptoms and serological abnormalities [206,207] and B-cell depletion therapy against CD20. Rituximab, the monoclonal antibody against CD20 protein on B-cell surface, was previously reported to improve both sicca symptoms and extraglandular manifestations [208,209], such as fatigue [209–211] and peripheral nervous system involvement [212], as well as disease activity index [213,214] and is considered a safe treatment option. Treatment with high-dose corticosteroids conferred increased risk for the occurrence of CV disease, including myocardial infarction and cerebrovascular events, while intermediate and high doses of glucocorticoids increased mortality [215]. Furthermore, low-dose corticosteroid therapy was protective for lipid profile, had an adverse effect on

TABLE 12.4 Treatment Options for Sjögren's Syndrome

Treatment	Manifestations	Studies	Level of evidence	Strength of recommendation	References
Pilocarpine	Sicca symptoms	3 RCT	A	Class I	[181–183]
Cevimeline	Sicca symptoms	4 RCT	A	Class I	[184–187]
Cyclosporine drops	Ocular symptoms	2 RCT 1 prospective	A	Class I	[178–180]
Hydroxychloroquine	Sicca symptoms	1 prospective 2 retrospective	B	Class IIa	[190, 193, 194]
	Arthralgias/myalgias/fatigue	1 retrospective	C		[194]
	Laboratory abnormalities	1 RCT 2 prospective 1 retrospective	B		[191, 194–196]
Corticosteroids	Sicca symptoms	1 RCT 1 prospective	B	Class IIa	[206, 207]
	Laboratory abnormalities	1 RCT 1 prospective	B		[206,207]
Rituximab	Sicca symptoms	1 RCT 1 prospective	B	Class I	[208,209]
	Extraglandular manifestations	3 RCT 4 prospective	A		[208–214]

RCT, randomized controlled trial.

insulin resistance, and did not influence blood pressure levels or atherosclerotic process, but was associated with increased CV events among RA patients [216]. Although there is no evidence reporting a direct cardiotoxic effect of anti-CD20 therapy, rare cardiovascular complications after therapy in isolated cases have been reported, including atrioventricular block, atrial fibrillation and chest pain [217,218].

However, the management of possible cardiovascular complications in the setting of SS remains a challenge due to the adverse effects of commonly used medications. More specifically, the administration of drugs, such as calcium-channel blockers [219], beta-blockers, diuretics [220,221] and antiarrhythmic agents [222–225], with a strong or moderate effect on lacrimal and salivary gland secretory function, may deteriorate symptoms. In a previous study conducted in an elderly population oral dryness was associated with the use of diuretics and antihypertensive agents [226], while in another recent study of 668 elderly people oral and ocular dryness were related to cardiac agents, such as thiazide diuretics, calcium-channel blockers, beta-blockers, angiotensin II antagonists and statins [227].

6. CONCLUSIONS

Sjögren's syndrome is a chronic systemic autoimmune disease, displaying mainly local manifestations, attributed to the impairment of exocrine glands, although systemic disorders can also occur. Recent data suggest cardiac involvement as a frequent complication of SS patients. Cardiovascular complications range from signs of subclinical atherosclerosis to overt clinical symptoms of pericarditis, pulmonary hypertension, valvular defects, and heart failure.

Heightened CV burden has been reported in SS, attributed to some extent to the increased prevalence of traditional CV risk factors among SS patients, such as hypertension, hypercholesterolemia and elevated triglyceride levels. Several studies have also focused on the identification of markers related to endothelial dysfunction and subclinical atherosclerosis. Arterial wall thickening (defined by IMT score), plaque formation on arterial wall, as well as abnormal endothelial vasodilation have also been described in SS individuals at increased rates compared to the healthy population. The accelerated atherosclerotic process observed in the setting of SS has been associated with disease-related features, either clinical or serological, including the presence of extraglandular manifestations or autoantibodies (RF, anti-Ro/SSA, or/and anti-La/SSB).

Moreover echocardiographic evaluation of SS patients has demonstrated the occurrence of cardiac abnormalities, such as pericardial involvement, valvular disturbances,

pulmonary hypertension, and systolic or diastolic dysfunction. Pericardial involvement can be manifested as subclinical pericardial effusion or clinically overt pericarditis. The association of pericardial involvement with SS clinical and laboratory characteristics imply that inflammation is implicated in the pathogenesis of this complication. Among valves, mitral, aortic, and tricuspid are most frequently affected, leading mainly to insufficiency, in association with age- or disease-related factors. High rates of pulmonary hypertension have also been observed in SS patients, often in those displaying manifestations, such as Raynaud's phenomenon and interstitial lung disease. Other—previously considered uncommon—cardiac complications are ventricular arrhythmias and conduction disorders, which are encountered in SS individuals, mainly in those with anti-Ro/SSA positivity. Despite the growing evidence on cardiovascular involvement in the context of SS, few studies aimed to identify the prevalence of ischemic heart disease and heart failure in large series of SS patients. In these studies such complications were found increased in SS individuals compared to the healthy population. Finally, although SS females can have in general successful pregnancies, several adverse outcomes, including preterm delivery, low birth weight of neonates, and fetal loss, are related to maternal SS. The most severe complication for neonates, attributed to the transplacental passage of anti-Ro/SSA or/and anti-La/SSB maternal autoantibodies, remains congenital heart block development.

Taking into account the increased body of evidence on the cardiovascular manifestations in the setting of SS, the physician should be aware of these complications for the better management of SS patients, considering that commonly used cardiac therapies may adversely affect the existing dryness discomfort. However, more studies are warranted to elucidate the prevalence and severity of SS-related cardiovascular abnormalities.

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Systemic Sclerosis

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1. INTRODUCTION

Systemic sclerosis (SSc) is a rare, heterogeneous connective tissue disease characterized by small vessel vasculopathy, production of autoantibodies, and fibroblast dysfunction leading to increased deposition of extracellular matrix [1]. The clinical manifestations and the prognosis of SSc are variable, depending on skin thickening and different internal organs involved. Subsets of SSc can be distinguished with regard to cutaneous involvement: limited cutaneous SSc (lcSSc), diffuse cutaneous SSc (dcSSc), and SSc without skin involvement [1–3].

1.1 Epidemiology of Systemic Sclerosis

Prevalence and incidence of SSc appears to be greater in populations of European ancestry and lower in Asian groups. As reported recently, it is estimated that the annual incidence of SSc varies from 10.9 cases/million up to 43 cases/million [4–6]. The incidence is higher in females than in males in all studies, and the cause of this discrepancy remains speculative [4–6]. African Americans and older-age-onset patients have more severe disease. The average age of onset of disease is about 50 years [7]. The prevalence varies among racial and ethnic groups from 56 to 341 per million people [4]. Survival in SSc has improved significantly compared with earlier published reports. Steen and Medsger reported an improvement in survival from 54% in the 1970s to 66% in the 1990s, in their longitudinal Pittsburgh cohort [8].

1.2 Classification Criteria for Systemic Sclerosis

The 1980's classification criteria for SSc lack sensitivity in early SSc and limited cutaneous SSc, thus the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) established a committee to provide a joint proposal for new

classification criteria for SSc. Their objective was to develop a set of criteria that would enable identification of individuals with SSc for inclusion in clinical studies, being more sensitive and specific than previous criteria.

The new classification criteria are shown in Table 13.1, showing one sufficient criterion, two exclusionary criteria, and seven items with a threshold above which cases are classified as SSc. The classification criteria may be applied to patients who may have SSc being considered for inclusion in an SSc study [9].

2. PREVALENCE OF CARDIAC INVOLVEMENT IN SYSTEMIC SCLEROSIS

Cardiac involvement in SSc may be either primary or secondary to the impairment of other organs such as the lungs and kidneys. Primary cardiac involvement, which develops as a direct consequence of the disease, may affect all cardiac tunic, endocardium, myocardium, and pericardium. This may conduct, from a clinical point of view, to pericardial effusion, arrhythmias, conduction system defects, myocardial ischemia, myocardial hypertrophy, and heart failure [10].

In the past years, cardiac involvement in SSc was considered an infrequent event, and it mainly resulted from autopsy studies. In particular, overt manifestations of ischemic heart disease were considered rare, cardiac failure was observed in about 10% of cases, and pericarditis in 15% [11–14]. By contrast, postmortem investigations demonstrated myocardial lesions secondary to SSc in more than 50% of cases [12].

Prevalence and prognostic factors for cardiac involvement in SSc differed depending on different stages of the disease and different cardiac manifestations. Most of the available data considered clinical features, routine investigations such as electrocardiogram (ECG), chest X-ray, and echocardiography, which are known to have low sensitivity [10].

TABLE 13.1 The American College of Rheumatology/European League Against Rheumatism Criteria for the Classification of Systemic Sclerosis (SSc)^a

Items	Subitem(s)	Weight/Score ^b
Skin thickening of the fingers of both hands extending proximal to the metacarpophalangeal joints (sufficient criterion)	–	9
Skin thickening of the fingers (only count the higher score)	Puffy fingers	2
	Sclerodactyly of the fingers (distal to the metacarpophalangeal joints but proximal to the proximal interphalangeal joints)	4
Fingertip lesions (only count the higher score)	Digital tip ulcers	2
	Fingertip pitting scars	3
Telangiectasia	–	2
Abnormal nailfold capillaries	–	2
Pulmonary arterial hypertension and/or interstitial lung disease (maximum score is 2)	Pulmonary arterial hypertension	2
	Interstitial lung disease	2
Raynaud's phenomenon	–	3
SSc-related autoantibodies (anticentromere, antitopoisomerase I [anti-Scl-70], anti-RNA polymerase III) (maximum score is 3)	Anticentromere	3
	Antitopoisomerase I	
	Anti-RNA polymerase III	

^aThese criteria are applicable to any patient considered for inclusion in an SSc study. The criteria are not applicable to patients with skin thickening sparing the fingers or to patients who have a scleroderma-like disorder that better explains their manifestations (eg, nephrogenic sclerosing fibrosis, generalized morphea, eosinophilic fasciitis, scleredema diabeticorum, scleromyxedema, erythromelalgia, porphyria, lichen sclerosis, graft-versus-host disease, diabetic cheiroarthropathy).

^bThe total score is determined by adding the maximum weight (score) in each category. Patients with a total score of ≥ 9 are classified as having definite SSc. Adapted from van den Hoogen et al. [9].

With the improvement of the prognosis of scleroderma renal crisis, pulmonary and cardiac involvement became the main causes for disease-related mortality in SSc.

Cardiac involvement have been observed in 15% of 953 patients with the dcSSc, considering clinical findings, ECG, 24-Holter ECG, echocardiography. Asymptomatic decrease of ejection fraction, arrhythmias, and pericardial effusion were not considered in this study [15]. In a 10-year follow-up, cardiac causes explained 20% of deaths associated to the disease [8,16], with the greatest impact in the first 5 years (14% mortality rate).

In a large Italian cohort of 1012 SSc patients, 35% had symptomatic arrhythmia, cardiopulmonary deaths represented 70% of the mortality, of whom 36% of deaths were attributed to cardiac involvement alone. Hazard ratio for cardiac death was 1.46 (95% CI 1.09–1.96) with multivariate analysis [17].

Another retrospective study observed the mortality rate in a Greek SSc population of 254 patients over 4 years. The authors found a mortality rate of 2% per year, and the incidence of cardiac involvement ranged from 7% in limited cutaneous (lc) SSc to 21% in dcSSc [18]. Although cardiac involvement could be more prevalent and more severe in the diffuse cutaneous subtype of the disease, the limited cutaneous form is not considered free of cardiac involvement [16]. In a large epidemiological Italian study, although heart symptoms were found more

frequently in the diffuse subtype (32%) as compared with the limited form (23%), the difference was not statistically significant. Another important study by Perera et al. [19] has shown that antitopoisomerase I antibody-positive patients with SSc, either of limited or diffuse cutaneous subtype, with a rapid skin thickness progression rate, have reduced survival rates, primarily due to early renal and cardiac involvement. The rapid skin thickness progression rate subgroup had the highest frequency of cardiac involvement (41%). Thirty of the 40 patients (75%) with documented cardiac involvement developed scleroderma cardiac disease within 3 years of the onset of skin thickening. Thus antitopoisomerase I antibody-positive patients with rapid or intermediate skin thickness progression rate are at considerable early risk for the occurrence of SSc-associated cardiac problems [19].

Some data have even suggested a more prevalent involvement in the limited subtype of the disease. Echocardiography showed 18 of 57 (31%) SSc patients having left ventricular abnormalities in the limited cutaneous form compared with 5 of 23 (21%) patients in the diffuse form [20].

In the large European League Against Rheumatism Scleroderma Trials (EUSTAR) database including more than 7000 SSc patients, 264 deaths were registered; out of 128 SSc-related deaths, 33 (26%) were of cardiac origin [21].

Conventional echocardiography assessed a depressed left ventricular (LV) contractility only in a minority of patients, as confirmed in the same EUSTAR cohort [reduced LV ejection fraction (EF) in 383 (5.4%) patients]. Moreover, men presenting with dcSSc and active or past digital ulcerations ($n=203$ patients; 2.8%) were at particularly high risk of LV dysfunction (OR 3.2; 95% CI 2.1–4.9) [22]. Instead, diastolic dysfunction was the most frequent feature (17.5%) [23].

Considering more sensitive diagnostic investigations such as cardiac magnetic resonance imaging (cMRI), cardiac involvement can be revealed in up to 75% of patients compared to 48% revealed at Doppler echocardiography [24].

Tissue Doppler imaging (TDI) also demonstrated a higher proportion of LV abnormalities in SSc patients. In 101 SSc patients, TDI demonstrated a reduced LVEF in 14% compared to 7% at standard echo [25].

A very recent histological study of 25 SSc patients with secondary cardiomyopathy (excluded patients with right heart failure caused by pulmonary arterial hypertension, PAH, or hypertensive heart disease) undergoing endomyocardial biopsy demonstrated cardiac fibrosis in 100% of patients, which might be considered as a consequence of inflammation in the myocardium [26]. During 22.5 months follow-up period, 28% of patients reached the primary endpoint, defined as the combination of cardiovascular death, arrhythmic endpoints (defined as appropriate discharge of ICD), or rehospitalization due to heart inflammation in the myocardium by trend. It is worth noting that patients with an event in the further course of the disease showed a higher degree of fibrosis and inflammation in their endomyocardial biopsies [26]. Therefore, the histopathological findings together with more sophisticated cardiac imaging procedures (tissue Doppler and MRI) may also reveal a prognostic impact in future studies.

These findings are largely confirmed by a recent EUSTAR autopsy study. In 10 out of 11 autopsy reports, more than one abnormal cardiac finding was described. Myocardial fibrosis and coronary arteriosclerosis (both in 5/11 SSc patients) were the most frequent abnormalities; 3/5 patients with myocardial fibrosis have concomitant coronary arteriosclerosis and 4/5 have generalized atherosclerosis at autopsy. Clinically diastolic dysfunction (6/11), conduction block (4/11), and dyspnea (8/11) were the most frequent cardiopulmonary findings [27].

When primary myocardial involvement becomes clinically evident, it assumes a deeply negative prognostic significance, with a mortality rate above 70% at 5 years [28].

A recent report evaluating a series of patients who underwent stem cell transplantation highlighted the high impact of cardiac involvement on mortality: five (6%) of 90 patients died from treatment-related causes, and four treatment-related deaths occurred because of cardiovascular complications [29].

However, data on the prognostic impact of subclinical myocardial involvement as detected by more sensitive tests in SSc patients are presently lacking.

3. PATHOPHYSIOLOGY OF PRIMARY CARDIAC INVOLVEMENT IN SYSTEMIC SCLEROSIS

3.1 Pathogenetic Mechanism

Myocardial involvement shares the general pathogenetic mechanism of SSc: microvascular alterations, collagen accumulation by altered fibroblasts, and complex immune system dysregulation. These mechanisms, variously combined, lead to ischemic, fibrotic, and inflammatory lesions, which can affect the pericardium, myocardium, and conduction system. The pathogenesis of primary myocardial involvement is still debated; the most frequent pathological features of SSc in the myocardium are focal fibrosis (in more than 50% of cases) and contraction band necrosis (CBN) (in 77% of patients) [12]. Follansbee et al. [30] found a high prevalence of CBN in SSc patients with scleroderma heart involvement, probably related to an intermittent vascular spasm of the coronary arteries with episodes of ischemia reperfusion (the so-called intramyocardial Raynaud phenomenon) [12,31].

3.2 Microvascular Involvement

Coronary microcirculation impairment is relevant in SSc, while the prevalence of atherosclerotic coronary artery disease (CAD) seems to be comparable, or slightly increased, to that observed in the general population [32–34].

The small coronary vessels show a reduced patency or obliteration due to intimal proliferation, fibrinoid necrosis, fibrosis, and intravascular coagulation [35] (Fig. 13.1B). A putative mechanism whereby microvascular injury drives tissue remodeling has been proposed, which suggests that the microvascular structural and functional abnormalities lead to the increased fibroblast activity and disseminated tissue fibrosis [36] (Fig. 13.1A), which may progress to a clinical pattern of restrictive cardiomyopathy [37].

The consequences of such anatomical damage and functional disorder were reported in subsequent studies. Kahan et al. first demonstrated the impairment of coronary vasodilator reserve using coronary catheterism [38], later confirmed by noninvasive adenosine trans-thoracic echocardiography (A-TTE) by other groups [39,40]. In addition, myocardial scintigraphy enabled several authors to observe reversible myocardial perfusion defects, induced either by exposure to the cold or by physical exercise [41–43], as expression of the functional microvascular disorder in SSc.

It is worth mentioning here, although extensively treated in other chapters, the secondary cardiac

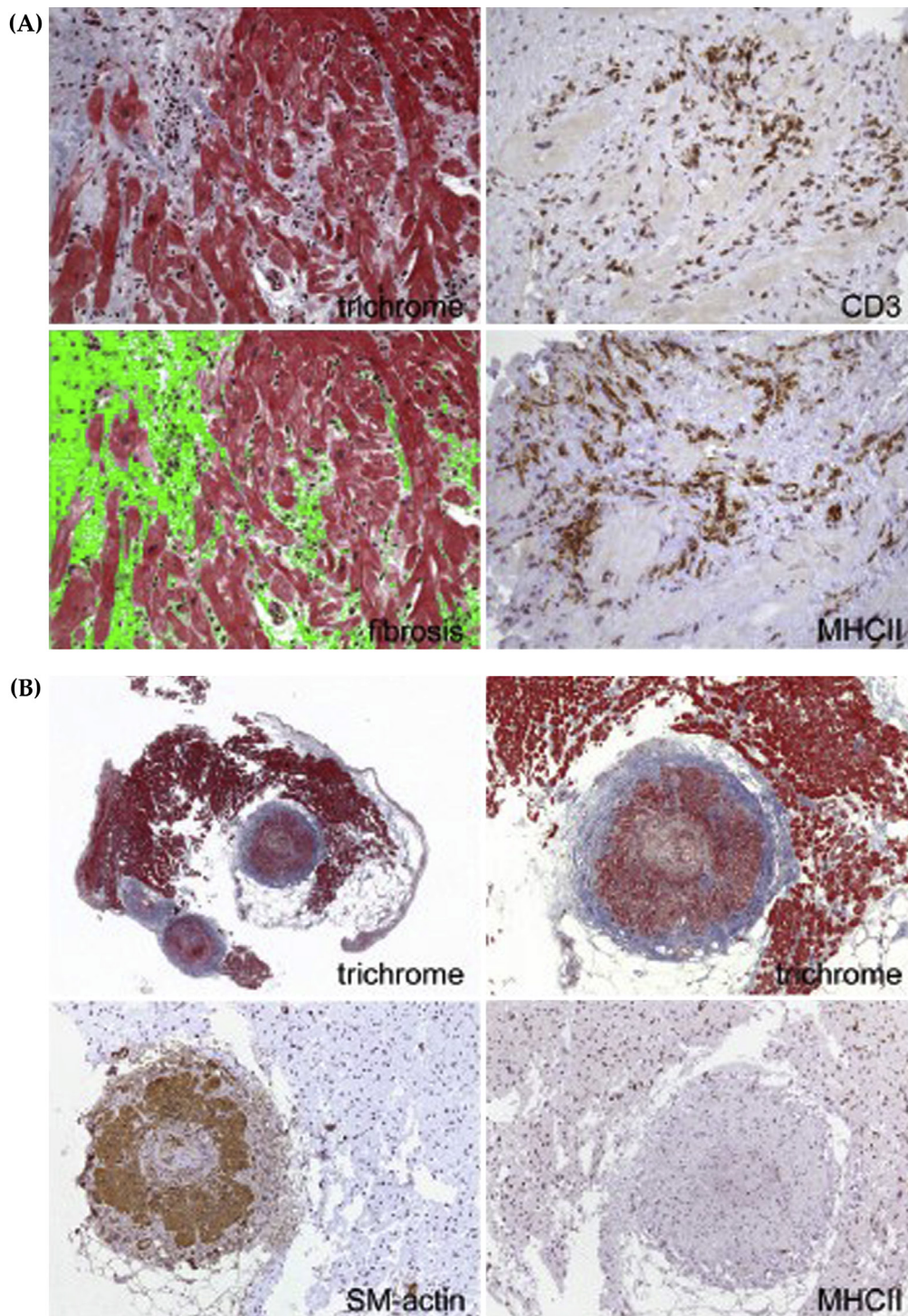


FIGURE 13.1 Histopathological and immunohistological findings in the myocardium of patients with systemic sclerosis and cardiac involvement. (A) This is a representative image of a biopsy with severe inflammation, which is characterized by the presence of numerous CD3⁺ T lymphocytes and MHC II⁺ macrophages. A severe cardiac remodeling is also present. (B) In the left picture, an overview of an endomyocardial biopsy is presented revealing two arterioles with pronounced changes of the architecture of the vessel wall including fibrosis. Immunohistological staining with SM-actin confirms a considerable hyperplasia of smooth muscle in the vessels. *Adapted from Mueller et al. [26].*

involvement that develops in response to pulmonary vascular and/or interstitial disease. From a physiologic point of view, there are compensatory mechanisms that allow the right ventricle to deal with the elevated afterload of

chronic pulmonary hypertension. Molecular mechanisms that facilitate myocardial cell hypertrophy are activated in response to elevated right ventricular (RV) wall stress and an increase in end-diastolic volume occurs. This increase

can initially improve cardiac output. However, subsequently, as the RV thickens, diastolic filling becomes more dependent on the performance of the right atrium, which, if impaired, will lead to right heart failure [44]. In patients with PAH-SSc, the ability of the RV to adapt to the pressure overload may be reduced by myocardial inflammation and scarring, as suggested by endomyocardial biopsy samples from patients with SSc, a finding not reported in idiopathic PAH [34]. In pulmonary hypertension, RV adaptation to elevated pulmonary artery pressures seems not uniform among patients. The disparity in the degree and duration of preservation of RV function may explain the variability in survival.

3.3 Myocardial Fibrosis

A “patchy” distribution of myocardial fibrosis is a pathognomonic feature of primary myocardial involvement in SSC [12,13,45]. Myocardial fibrosis in SSc can be histologically differentiated from that seen in CAD: in scleroderma heart involvement fibrotic areas are not related to the pathologic changes of a single coronary artery, hemosiderin myocardial deposits are absent, and fibrosis may be also found in the subendocardial region, usually spared in CAD. Cardiac fibrosis in SSc progresses to replace damaged muscle cells as documented by left ventricular (LV) hypertrophy at autopsy and echocardiographic studies [13,45]. A slightly increased thickness of the septum and posterior wall or asymmetric septal hypertrophy, even in absence of systemic arterial hypertension, have been demonstrated in a significant percentage of SSc patients compared to controls [13,17]. Septal hypertrophy also has been observed secondary to PAH, in a subclinical stage [13].

The exact pathogenesis of fibrosis remains uncertain [46]. Early microvascular injury as vasospasm of small coronary vessels may be the first abnormality leading to fibroblast activation and differentiation to myofibroblast. They overproduce extracellular matrix proteins that conduct to myocardial fibrosis [47]. But these alterations cannot completely explain the cardiac remodeling due to collagen deposition.

The clinical expression of fibrosis is more often diastolic LV dysfunction, less frequently, systolic dysfunction, mainly associated to severe coronary or hypertensive heart disease, with the rare evolution toward overt heart failure. Usually it can be triggered by severe systemic hypertension, as happens in scleroderma renal crisis [11]. Systolic dysfunction is less frequent in SSc [22]. Right heart overload and failure may be related to pulmonary arterial hypertension. A high prevalence of RV diastolic abnormalities has also been documented [17,48], although its occurrence can be influenced by other factors associated with impaired ventricular filling, such as arterial hypertension, CAD, pericardial disease, and pulmonary hypertension.

3.4 Myocardial Inflammation

Myocardial inflammation has been occasionally described in early stages of the disease [49–52]. Recently, in a cohort of 181 SSc, seven patients who newly developed clinical symptoms and signs of heart failure and cardiac involvement showed a biopsy-proven myocarditis. Immunosuppressive therapy improved the symptoms, with normalization of cardiac enzymes and improvement of MRI findings in the majority of cases [52].

4. CARDIAC INVOLVEMENT

4.1 Accelerated Atherosclerosis

Microvascular damage is considered to be a hallmark of SSc. Nevertheless, there have been conflicting reports regarding the presence and extent of macrovascular disease that might evolve due to ATS in SSc patients [53]. Comparable to other autoimmune diseases such as rheumatoid arthritis [54] (RA) and systemic lupus erythematosus [55] (SLE), there is emerging data that early accelerated atherosclerosis occurs in SSc. A meta-analysis by Au et al. reported on increased prevalence of atherosclerosis involving coronary arteries, carotid and cerebrovascular arteries, and peripheral arteries in SSc. The authors focused on carotid intima-media thickness (CINT) and flow-mediated dilation (FMD) as surrogate markers for atherosclerosis [56]. However, none of the studies addressed the unresolved question of whether subclinical atherosclerosis led to an increased risk of cardiovascular disease. A population-based cohort provided evidence that SSc patients had increased incidence rates of myocardial infarction and stroke. The study involved 865 patients compared with 8643 age-matched, sex-matched, and study entry-matched controls. The incidence rates of myocardial infarction (MI) and stroke were 4.4 and 4.8 per 1000 person-years versus 2.5 and 2.5 per 1000 person-years in the comparison cohort [57]. The impact of microvascular disease on the development of atherosclerotic macrovascular disease and cardiovascular events still remains unclear, due to the relative paucity of data in the literature.

4.2 Prevalence of Traditional Cardiovascular Risk Factors

Limited information regarding the prevalence of traditional cardiovascular risk factors in SSc is available. Overall, it has been found to be similar [57,58] or reduced [59–61] compared to controls, and SSc appeared to be an independent risk factor for CAD, in addition to age, hypercholesterolemia, male gender, hypertension, and diabetes. Besides, SSc-related factors such as PAH, renal involvement, and disease duration should be considered [59–61].

4.3 Markers for Atherosclerosis and Endothelial Dysfunction

Other risk factors may include systemic inflammation, endothelial dysfunction, altered lipid profile, and disease-related factors.

Inflammation is a pivotal component of atherosclerosis, and it is well documented that elevation of inflammatory markers (eg, C-reactive protein) is predictive of cardiovascular events in general population [62]. There have been reports of increased inflammatory markers, such as tumor necrosis factor- α , interleukin 6, and high-sensitivity C-reactive protein in SSc patients compared to controls [63]. However, the relationship between these mediators and cardiovascular disease in SSc remains unclear; it is possible that chronic systemic inflammation could promote accelerated atherosclerosis in patients with SSc.

Endothelial dysfunction is a key point in the development of both SSc and atherosclerosis, and represents a loss in vasodilatory function, together with increased platelet aggregation and leukocyte adhesion due to decreased nitric oxide, a well-recognized vasodilator [64]. In SSc, endothelial cell damage leads to enhanced expression of adhesion molecules and elevated levels of circulating soluble adhesion molecules. Soluble E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 (VCAM-1) levels are all significantly elevated in SSc, reflecting endothelial activation [60,63,65]. Enhanced endothelial cell expression of adhesion molecules results in adhesion of inflammatory cells, transmigration across the vessel wall, and infiltration of the extracellular matrix [63].

An altered lipid profile may play a more pertinent role than hypercholesterolemia alone. Elevated circulating oxidized-LDL/ β 2-glycoprotein I complexes and antioxidant-LDL antibodies have been found in SSc patients [64–67]. The consumption of oxidized LDL by monocytes contributes to the formation of atherogenic foam cells. LDL is oxidized by reactive oxygen species, as a result of enhanced oxidative stress that is associated with endothelial dysfunction. SSc patients have also been reported to have higher levels of proinflammatory HDL [58] and lipoprotein-a [68]. A similar association between proinflammatory HDL and atherosclerosis has been described in the general population, as well other autoimmune diseases such as SLE and RA, but its role as the primary mediator of endothelial dysfunction remains unclear.

Antiphospholipid antibodies have been associated with cardiovascular disease, although their role in subclinical atherosclerosis remains to be defined. An increased prevalence of anticardiolipin (aCL) and anti- β 2-glycoprotein I (β 2GPI) antibodies in the absence of antiphospholipid syndrome has been demonstrated in patients with SSc compared with controls [69]. Boin and colleagues showed that anti- β 2GPI antibodies are associated with both higher mortality and vascular disease, including digital ischemia and PAH in SSc. However,

the authors did not report the cause of mortality or the prevalence of cardiovascular disease in their cohort [70].

Asymmetric dimethylarginine (ADMA) is the major endogenous inhibitor of nitric oxide (NO) synthase and may be involved in endothelial dysfunction [71]. It is recognized as an important parameter in determining cardiovascular mortality and morbidity [72]. Recently, increased ADMA levels have been found in a series of SSc patients, without clinical signs of cardiovascular disease, who also presented impaired coronary flow reserve (CFR), thus postulating a role of ADMA in subclinical cardiovascular involvement [73]. Other studies demonstrated elevated ADMA levels in SSc patients with active disease [74] or with pulmonary hypertension [75].

4.4 Surrogate Markers of Atherosclerosis

Different surrogate markers of atherosclerosis have been investigated in SSc but with conflicting results [53,56,75,76]. Intima media thickness (IMT) and FMD are the most studied, as they represent a marker of subclinical atherosclerosis and of endothelium-dependent vasodilation, respectively [61].

The systematic review and meta-analysis by Au et al. [56], showed an increased ATS rate in SSc patients compared to healthy controls. Sixteen studies assessed the carotid IMT [76–91], and in 7 (44%) of these studies a significantly greater carotid IMT value [summary mean difference 0.11 mm, 95% confidence interval (95% CI) 0.05 mm, 0.17 mm; $P=0.0006$] were observed in SSc patients compared to controls [76,79,80,84–86,90]. Three studies evaluated carotid plaque and in two of these (67%) the rate of carotid stenosis and plaque was higher in SSc patients than in controls [92,93].

The same meta-analysis found a significantly higher frequency of peripheral vascular disease in SSc. Brachial artery FMD% was significantly lower (summary mean difference -3.07% , 95% CI -5.44% , -0.69% ; $P=0.01$) in SSc patients compared to controls in four of seven studies [80,85,87,88,94–96]. Four studies evaluated the ankle brachial pressure index (ABPI) [84,86,92,97] + and in three of these [84,86,97] there were no significant differences in the ABPI between SSc patients and controls.

Nordin et al. in their population-based case-control study found ischemic arterial events being more common in patients than in controls due to more prevalent ischemic heart disease and ischemic peripheral vascular disease. There was no difference regarding the occurrence of ischemic cerebrovascular disease, the frequency of plaques, and IMT between SSc patients and controls. Subgroup analyses revealed that patients with anticentromere antibodies (ACA+) had more plaques and more ischemic arterial events compared to other SSc patients (67% vs 39% and 32% vs 11%; $P=0.006$ and $P=0.01$, respectively) and compared to controls (67% vs 41% and 32% vs 7%, $P=0.02$ and $P=0.0003$, respectively).

TABLE 13.2 Major Findings and Technical Aspects of Diagnostics in Studies Investigating IMT, FMD, and ABPI in SSc

Study	Group	<i>n</i>	Female (%)	Age (years)	lcSSc (%)	Testing Method	IMT (mm)	FMD (%)	ABPI	References		
1	SSc	12	100	49 ± 14	NA	Carotid/Brachial artery DUS	0.83 ± 0.3*	3.6 ± 7.0*		[80]		
	Control	12	100	49 ± 14			0.46 ± 0.2	11.9 ± 4.6				
2	SSc	53	81	55.43 ± 11.35	NA	Carotid DUS	0.65 ± 0.24			[81]		
	Control	20	80	42.75 ± 15.58			0.63 ± 0.19					
3	SSc	40	NA	NA	NA	Carotid DUS	0.69 ± 0.25			[82]		
	Control	45	NA	NA			0.59 ± 0.1					
4	SSc	10	100	Range 45–71	NA	Carotid DUS	0.66 ± 0.88*			[83]		
	Control	52	52	Range 14–87			0.80 ± 0.19					
5	SSc	91	83	46 ± 9.1	NA	Carotid DUS	0.75(0.69–0.81)			[78]		
	Control	90	90	45 ± 7.7		Median (range)	0.76(0.67–0.79)					
6	SSc	35	83	61 ± 6	68	Carotid DUS/Brachial artery DUS	0.93 ± 0.29*	3.41 ± 4.56*		[84]		
	Control	20					0.77 ± 0.13	7.66 ± 4.24				
7	SSc	53	89	60.4 ± 10.68	85	Carotid DUS	0.85 ± 0.03		1.018 ± 0.10	[85]		
	Control	43	93	56.3 ± 10.23			0.68 ± 0.01		1.091 ± 0.11			
8	SSc	66	89	60.5 ± 12.2	83	Carotid DUS	0.90 ± 0.036 (lcSSc)		1.040 ± 0.097	[86]		
							0.87 ± 0.04 (dcSSc)					
	Control	20	79	58 ± 12.3			0.69 ± 0.013		1.097 ± 1.106			
9	SSc	44	NA	62 ± 11	86	Carotid DUS	0.9 ± 0.36			[79]		
	Control	32	NA	60 ± 11.8			NA					
10	SSc	29	86	51.8 ± 10	66	Carotid DUS	0.67 ± 0.26	4.82 ± 3.76*		[87]		
	Control	29	79	49.3 ± 9.6			8.86	4.82 ± 3.56				
11	SSc	49	84	55.4 ± 11.6	92	Carotid DUS	0.69(0.260.62–0.79)			[77]		
	Control	32	91	50.9 ± 10.1		Median (IQR)	0.68(0.56–0.75)					
12	SSc	42	90	51 ± 13	79	Carotid DUS/Brachial artery DUS	0.53 ± 23	11(10.9)		[88]		
	Control	33	91	52 ± 14			0.51 ± 0.11	11.4(8.1)				
								Median (IQR)				
13	SSc	81	NA	62.4 ± 12.6	NA		0.69 ± 0.11			[89]		
	Control	80	NA	62.2 ± 12.8			0.69 ± 0.14					

Continued

TABLE 13.2 Major Findings and Technical Aspects of Diagnostics in Studies Investigating IMT, FMD, and ABPI in SSc—cont'd

Study	Group	<i>n</i>	Female (%)	Age (years)	lcSSc (%)	Testing Method	IMT (mm)	FMD (%)	ABPI	References
14	SSc	50	NA	40.82±9.35	NA	Carotid DUS	0.63±0.08*			[90]
	Control	50	NA	NA			0.40±0.06			
15	SSc	60	92	56±13.9	33	Carotid DUS	0.77±0.2*			[76]
	Control	51	88	51±15.5			0.59±0.14			
16	SSc	50	92	Median 52 (range 22–63)	70	Carotid DUS	0.613±0.240			[91]
	Control	41	90	Median 52 (range 27–64)			0.654±0.173			
17	SSc	111	81	61±8	78	Carotid DUS	0.68±0.13		1.13	[60]
	Control	105	86	61.5±12.3			0.68±0.13		1.12	
18	SSc	24	83	57.9±15.1	83	Brachial artery US		4.63(0.71–7.58)		[94]
								Median (IQR)		
	Control	24	NA	57.7±14.6				4.55(2.23–6.82)		
								Median (IQR)		[95]
	SSc	98	NA	NA	NA	Brachial artery US		11.8±4.9		
	Control	40	NA	NA				14.6±1.4		
20	SSc	43	86	51±10.8	70	Brachial artery US		8(3–9)*		[96]
								Mean(range)		
	Control	27	74	45±13				15 (12–16)		

ABPI, ankle brachial pressure index; DUS, Doppler ultrasound; FMD, flow-mediated dilation; IMT, intima-media thickness; IQR, interquartile range; lcSSc, limited cutaneous SSc; NA, not available; SSc, systemic sclerosis; **p*-value <0.05.

[60]). Table 13.2 summarizes major findings and technical aspects of diagnostics in studies investigating IMT, FMD, and ABPI in SSc.

Older autopsy studies [12,45] and more recent angiographic ones [33] described that myocardial infarctions were common in SSc patients, despite normal coronary arteries. These studies suggest that vasospasm rather than atherosclerosis is a major pathogenic mechanism behind SSc-related heart disease. Therefore, all of these studies suggested an increased prevalence of ATS in SSc compared to age- and sex-matched controls, but we are far from identifying its mechanism and its role in determining CAD. Some conjectures might be drawn. Atherosclerosis and SSc seem to share some pathophysiological mechanism. Proposed mediators of the vasculopathy of

SSc, which have also been implicated in atherosclerosis, include endothelial dysfunction, a reduced number of circulating endothelial progenitor cells, and an increased number of microparticles [32].

Thus, a diagnostic method that might differentiate between the two processes is of primary importance in SSc. We previously described the absence of epicardial stenosis by coronary contrast angiography with myocardial multidetector computed tomography (MDCT) in a series of seven SSc patients asymptomatic for CAD but with severe impairment of CFR, expression of early microcirculation involvement: no defects in coronary size and lumen were detected in SSc patients studied, parietal spots of calcium deposition were found just in one female who showed high cholesterol serum levels [98] (Fig. 13.2).

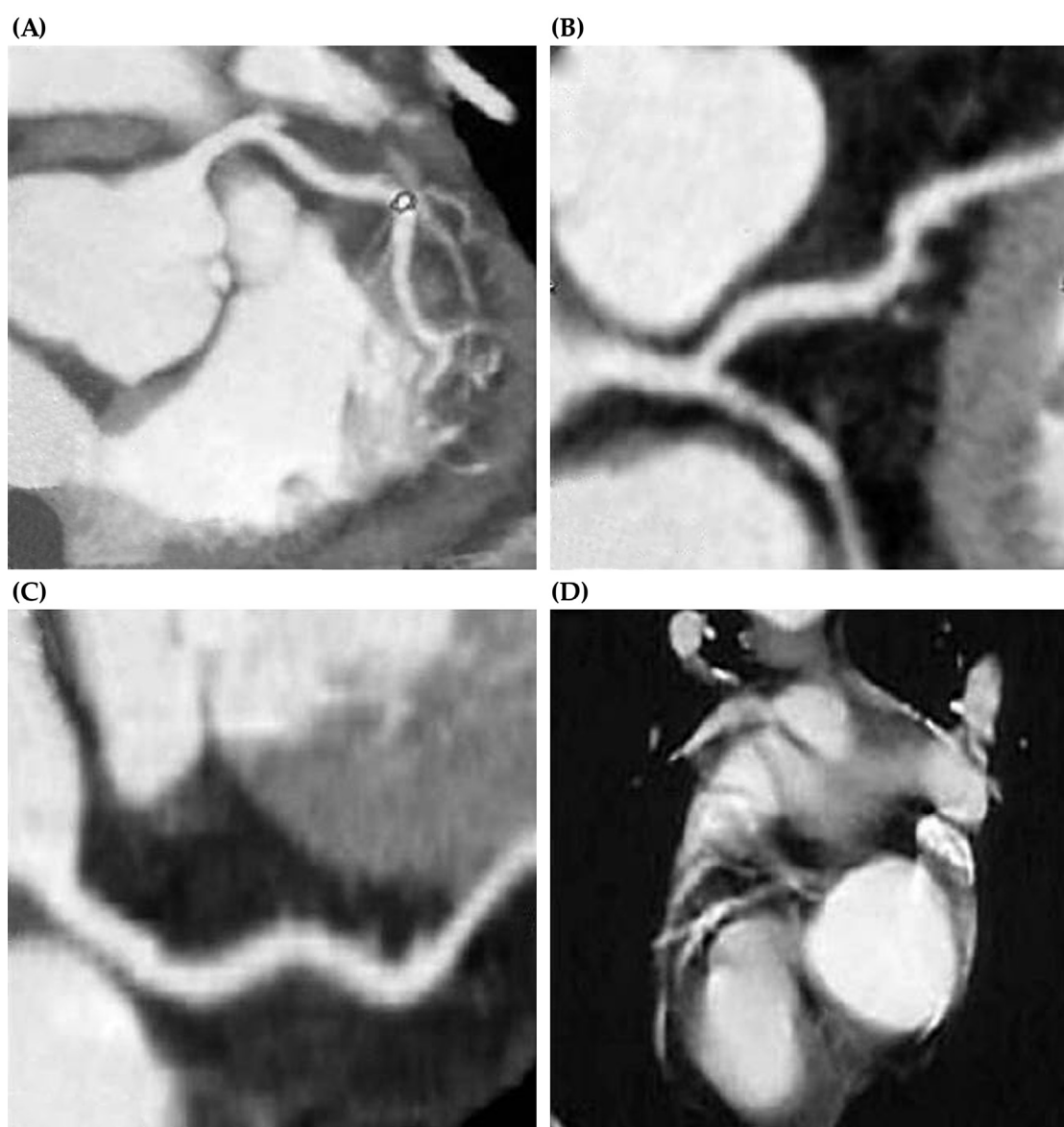


FIGURE 13.2 (A) and (B) Normal left coronary artery. (C) Normal wall and diameter in the left coronary artery. (D) Isolated parietal spotty calcifications in the left anterior descending coronary artery, in SSc patients with hypercholesterolemia. Adapted from Vacca et al. [98].

A qualitative systematic review of the prevalence of CAD in SSc has been recently published [99]. In this systematic review, the authors specifically address CAD outcomes in SSc, including coronary artery calcium scores (CACS), autopsy findings, coronary angiographic findings, and physician- or patient-reported diagnosis of CAD, MI, or ischemia. CACS measures coronary artery calcification that occurs in atherosclerotic plaques and has a good negative predictive value for CAD in the general population [100]. The authors concluded that SSc is associated with an increased prevalence or incidence of CAD, and they also highlighted that the contribution of traditional cardiovascular risk factors to CAD in SSc was small, and other disease-related factors, such as longer disease duration, renal involvement, and PAH, could be involved. The limits of these studies are the heterogeneous outcomes and inclusion criteria in a small number of studies, which did not allow to pool the data on prevalence/incidence to conduct a meaningful meta-analysis, and the fact that different methods of measuring the outcome were used [99].

5. EVALUATION OF CARDIAC FUNCTION

5.1 Doppler Echocardiography

Doppler echocardiography is the recommended screening modality for routine cardiac assessment in patients with SSc, although it may lack sensitivity and does not allow a prompt diagnosis in a preclinical stage of heart involvement. It represents a reliable noninvasive evaluation of valvular abnormalities, pericardial disease, and ventricular wall motion defects. Doppler analysis can give more information on left ventricular diastolic filling, valve function, and pulmonary pressures.

Echocardiographic techniques used in SSc patients include: standard transthoracic Doppler echocardiography, tissue Doppler echocardiography (TDE), speckle tracking echocardiography (STE), stress echocardiography with coronary flow reserve evaluation, or dobutamine/dipyridamole stress echocardiography.

An analysis with conventional echocardiography in more than 7000 SSc patients included in the EUSTAR database reported only 5.4% of patients with depressed LV contractility, moreover age, male gender, digital ulcerations, myositis, and lung involvement were independently associated with an increased prevalence of LV dysfunction (OR 3.2; 95% CI 2.1–4.9) [22].

These data are confirmed in other studies using conventional echocardiography; besides the rare depressed LV contractility, up to 40% of patients had relaxation abnormalities, valvular regurgitation, and possible RV involvement [101].

The recent development of TDE allows direct measurement of myocardial velocities and strain rate (SR), which means to assess myocardial contractility, systolic and diastolic dysfunction. Strain rate has demonstrated to be a powerful indicator of myocardial contraction, independent of myocardial translational motion, becoming a more sensitive and accurate echocardiographic tool for evaluating subclinical myocardial dysfunction [102,103].

The most important TDE indexes are systolic mitral annular velocity (S_M) for evaluating LV contractility; mitral lateral annulus early (E') diastolic velocity for LV diastolic dysfunction; systolic tricuspid annular velocity (S_T) for RV contractility; and the ratio between early diastolic flow velocity and E' (E'/E' ratio) for LV filling pressure abnormalities.

Diastolic dysfunction arises when an impaired myocardial relaxation, preventing early filling, or increased myocardial stiffness occurs. LV diastolic dysfunction is defined by $E' < 10 \text{ cm/s}$; LV filling pressure abnormalities are detected when the E'/E' ratio is >15 . As well as LV, TDE allows to assess RV diastolic dysfunction, evaluating tricuspid E' and isovolumic relaxation time. A mitral E'/A' (A' : atrial contraction) and a tricuspid E'/A' are considered indicative of LV and RV diastolic dysfunction, respectively.

In a controlled study of 100 SSc patients without PAH or overt heart failure, TDE demonstrated a wider mean left atrial diameter, impaired LV relaxation, a trend toward a reduced LV ejection fraction, and a higher pulmonary arterial pressure. Moreover, 15% of SSc patients showed reduced LV contractility compared to none of the controls ($p = 0.004$), 30% presented impaired relaxation versus 8% of controls ($p = 0.002$), and 15% had reduced RV contractility compared to none among controls ($p = 0.04$). TDE measures of LV and RV contractility and LV relaxation correlated with each other but not with lung abnormalities or other disease characteristics [25].

In a recent 3-year longitudinal study, a total of 74 consecutive SSc patients and 71 controls underwent cardiac assessment at baseline and at three-year follow-up. At baseline, patients showed impaired LV and RV diastolic function, subtle LV and RV systolic dysfunction, and higher pulmonary artery systolic pressure, compared to controls. At three-year follow-up, SSc patients showed a further deterioration of biventricular diastolic and systolic function and a further sPAP increase. TDE evidence of new abnormalities in RV and/or LV diastolic function was associated with a baseline cardiac Medsger severity score ≥ 1 ($p = 0.01$). Neither diastolic or systolic abnormalities nor sPAP changes correlated with treatment [104].

These findings allow to confirm early occurrence of myocardial involvement in SSc; abnormal diastolic dysfunction and impaired LV contractility precede the

development of LV systolic dysfunction, which is considered rare [34].

A novel observation comes from the study of Hinchcliff et al., which suggested a possible association between LV diastolic dysfunction and increased risk of death (they confirmed previous results on prevalence of LV diastolic dysfunction among 153 consecutive SSc patients (23%) and LV systolic dysfunction (5.2%), moreover they found disease duration, age, coronary artery disease, and systemic hypertension independently associated with LV diastolic dysfunction at multivariate analysis. During a mean follow-up of 1.9 ± 1.3 years, LV diastolic dysfunction was independently associated with increased risk of death (hazard ratio 3.2, 95% confidence interval 1.1–9.5, $p=0.034$ per each standard deviation decrease in TDE E' velocity [105]. Obviously, these data need to be confirmed before concluding that LV diastolic dysfunction may predispose to adverse events or to a worse disease, or if it simply represents an epiphenomenon.

A cross-sectional controlled study of 104 SSc patients evaluated with cardiopulmonary exercise testing, 24-h Holter ECG monitoring, and Doppler echocardiography, when STE examination was performed, showed subtle LV systolic dysfunction despite having normal LV ejection fraction and LV dimension. Besides, STE findings (LV global longitudinal and circumferential strains) but not conventional echocardiographic parameters were independently associated with functional capacity and ventricular arrhythmias [106].

The RV might be affected very early in the course of the disease as it might be involved in both primary myocardial involvement and/or lung vascular or lung interstitial disease, which are common in SSc [107–109].

While several studies reported possible RV alterations in SSc patients with PAH [110–113], only a few, small series investigated RV function in unselected SSc patients [25,104,114–117]. The limitations of the sample size and of the various tools used for the assessments in those studies did not permit a clear picture of the prevalence, impairment of diastolic function, and risk factors.

The largest study comes from Meune et al., which evaluated LV and RV, systolic and diastolic functions, using echocardiography and TDE indexes, in a cohort of 212 consecutive SSc patients seen during a nine-month period at two tertiary rheumatology centers, compared to 50 healthy controls. They observed that right heart dysfunction is more common in SSc patients than LV alterations, mainly diastolic impairment (25% of the SSc patients), both in SSc patients with PAH and those without PAH. Moreover, they reported an association between RV systolic dysfunction, LV systolic and diastolic function parameters, as well as diffusing lung capacity for carbon monoxide (DLCO) and creatinine concentration, strengthening the existence of overall myocardial involvement [118].

In this view, RV should be evidenced by sensitive methods (possibly TDE/strain echocardiography and cardiac MRI) and considered to further optimize specific treatment.

5.2 Cardiovascular Magnetic Resonance

Cardiovascular magnetic resonance (CMR) is a noninvasive, nonradiating imaging technique, which provides novel information for the evaluation of cardiovascular diseases. Specifically, CMR allows the identification of small subendocardial perfusion defects, coronary flow reserve determination, myocarditis evaluation as well as morphological evaluation of fibrotic myocardium compared to viable tissue, subclinical right myocardial involvement determination, and assessment of the right ventricle in secondary heart involvement related to PAH [119].

Previous studies have shown the useful role of CMR in identifying the different patterns of myocardial involvement in SSc, focusing on delayed contrast enhancement abnormalities, on ventricular volumes and ejection fraction, or on perfusion index [12,24,120–123].

The high prevalence of cardiac abnormalities observed with CMR is consistent with necropsy studies that approximately 80% of patients had histological lesions of heart involvement, suggesting that CMR is highly sensitive [24].

Delayed enhancement MRI (DE-MRI) has been used to detect myocardial fibrosis in a series of SSc patients. Among the 36 patients, myocardial fibrosis was detected in 24 (66%). Late enhancement was characteristically midwall, with a linear pattern in all patients, sparing the subendocardium and epicardium. Myocardial fibrosis had a noncoronary distribution, and it was preferentially located in the basal and midcavity segments of the left ventricle (Fig. 13.3). Myocardial fibrosis did not differ between dcSSc and lcSSc and appeared to be more severe in patients with abnormal Holter study results and in patients with a relatively long duration of Raynaud phenomenon [121].

In a very recent cMRI study on 62 SSc patients with no traditional cardiovascular risk factors and without heart disease prior to the onset of SSc, the prevalence of myocardial fibrosis was 45%, higher and more severe in dcSSc (59%). Moreover, it was associated with lower LV ejection fraction and affected mainly basal walls. Microvascular damage was shown to be common and associated with elevated ultrasensitive CRP levels. The myocardial perfusion evaluation showed that 79% of the patients had subendocardial perfusion defects, reflecting microvascular perfusion impairment (Fig. 13.4). As in previous studies, myocardial damage was not associated with CAD, as confirmed by the noncoronary distribution of myocardial fibrosis and normal coronary CT scans [124].



FIGURE 13.3 Representative delayed enhanced magnetic resonance images in a patient with systemic sclerosis. (A) and (B) Four-chamber views demonstrating globular focal enhancement (arrows) at the basal segment of the left ventricular free wall as well as a small focus of enhancement at the basal segment of interventricular septum (arrowhead). (C) Short-axis view at the midventricular level showing linear midwall enhancement at the free wall (arrow) as well as at the interventricular septum (arrowhead). Adapted from Tzelepis et al. [121].

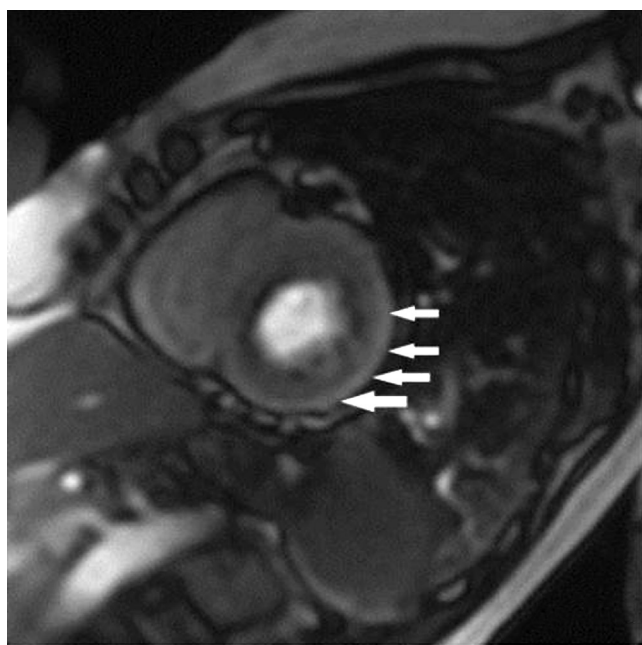


FIGURE 13.4 Cardiac MRI in TI gradient echo/echo planar imaging pulse sequence showing a diffuse subendocardial perfusion defect in the left ventricle wall of an SSc patient. Adapted from Rodrigues-Reyna et al. [124].

An integrated cardiac CMR evaluation, using edema and stress perfusion-fibrosis CMR, can identify the various patterns of cardiac pathophysiology. Mavrogeni et al. demonstrated severe cardiac involvement in early, asymptomatic dcSSc, presenting either as myocardial inflammation or severe reduction in myocardial perfusion reserve index (MPRI) and diffuse fibrosis. Moreover, CMR follow-up evaluation after 2 years showed further asymptomatic MPRI deterioration and presence of diffuse subendocardial fibrosis, without any change in LV, RV volumes and ejection fractions [125].

Cardiac MRI appears accurate also in determining subclinical right myocardial involvement and assessing the

right ventricle in secondary heart involvement related to PAH. Although CMR does not yet seem ready to replace RHC to confirm PAH diagnosis, it provides a noninvasive assessment of cardiovascular structure and function, including hemodynamic parameters in pulmonary circulation, and seems to be more efficient in assessing the changes in RV structure and function [126].

In the near future, as a result of continuous improvements in image acquisition, spatial and temporal resolution, CMR will be more efficient than invasive and radiation-based techniques that are currently routinely used in PAH, and it will be preferable for ongoing serial monitoring of treatment response (Fig. 13.5) and for correlating clinical improvement with hemodynamic parameters [127].

5.3 Evaluation of Coronary Flow Reserve

CFR is an indicator of coronary microvasculature [38–40]. Kahan et al. investigated coronary vasodilator reserve at catheterization, in patients with clinically symptomatic dcSSc. At rest, the mean coronary sinus blood flow did not differ from that of controls, but after maximal coronary artery vasodilation with intravenous dipyridamole, CFR was significantly reduced; coronary arteriograms were normal and endomyocardial biopsies showed fibrosis and vascular lesions with concentric intimal hypertrophy [38].

A new completely noninvasive method that allows serial evaluation of CFR has been developed and validated [128]. This method involves advanced ultrasound technology (second harmonic) and intravenous infusion of an ultrasound contrast agent. We have applied this technique to SSc patients, and we detected a frequent CFR impairment among individuals without clinical evidence of coronary heart disease and absence of epicardial stenosis. Fourteen out of 27 (52%) patients with SSc had severe reduction of the CFR (≤ 2.5) compared with controls (1/23, 4%, $p < 0.002$). A nonsignificant

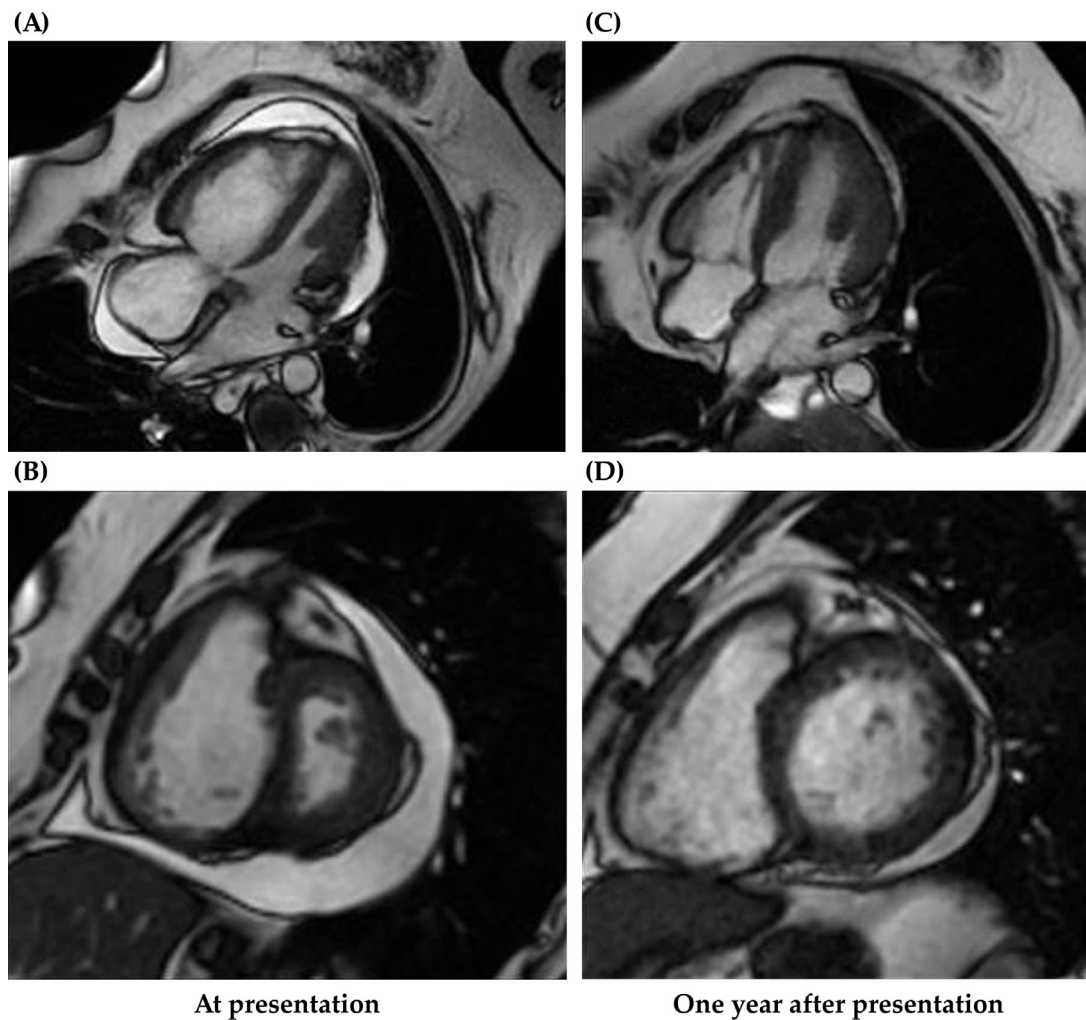


FIGURE 13.5 An example of cardiac MRI in a SSc patients for ongoing serial monitoring of treatment response. Upper side: end-systolic images showing right ventricular enlargement and pericardial effusion before (A) and after starting combination therapy for PAH with bosentan, sildenafil, and iloprost inhalation (C). Lower side: short axis of the right and left ventricle before (B) and after starting combination therapy (D). Adapted from Garau et al. [127].

trend between mean CFR and the severity and duration of the disease was also seen. CFR impairment in patients with SSc is likely to indicate an anatomical and/or functional impairment of coronary microvasculature because the presence of structural impairment of small coronary arteries has been frequently demonstrated in these patients, in absence of abnormalities of epicardial coronary arteries [39].

Follansbee et al. studied 26 patients with SSc, without cardiac symptoms and with normal coronary angiograms, and found that 79% of their patients had perfusion defects at thallium-201 scan [129].

In addition, myocardial scintigraphy enabled several authors to observe reversible myocardial perfusion defects, induced either by exposure to the cold or by physical exercise [41–43].

Dobutamine stress echocardiography (DSE) enables evaluation of the dynamics of left LV wall motion, which correlates to perfusion and oxygen supply, during

chronotropic and inotropic pharmacological stress. This test is a well-established diagnostic and prognostic tool that has widespread applicability because of its clinical accuracy and cost effectiveness [130].

Some authors demonstrated that the simultaneous evaluation of CFR and left ventricular wall motion (LVWM) by dipyridamole or dobutamine stress echocardiography increases the diagnostic power of each test to detect coronary macro- and micro-vascular involvement [131,132].

Nineteen out of 41 (46%) patients with SSc showed reduced CFR (≤ 2.5) and in 16/41 (39%) SSc patients wall motion abnormalities (WMA) (hypokinesia), which was absent on baseline rest examination, was observed during dobutamine infusion (Fig. 13.6).

Thirteen out of 41 patients (32%) showed both CFR and DSE test impaired; these combined abnormal findings were more frequent in the subgroup with dcSSc (8/15: 53%) than in the lcSSc subset (5/26: 19%) ($p < 0.04$), while

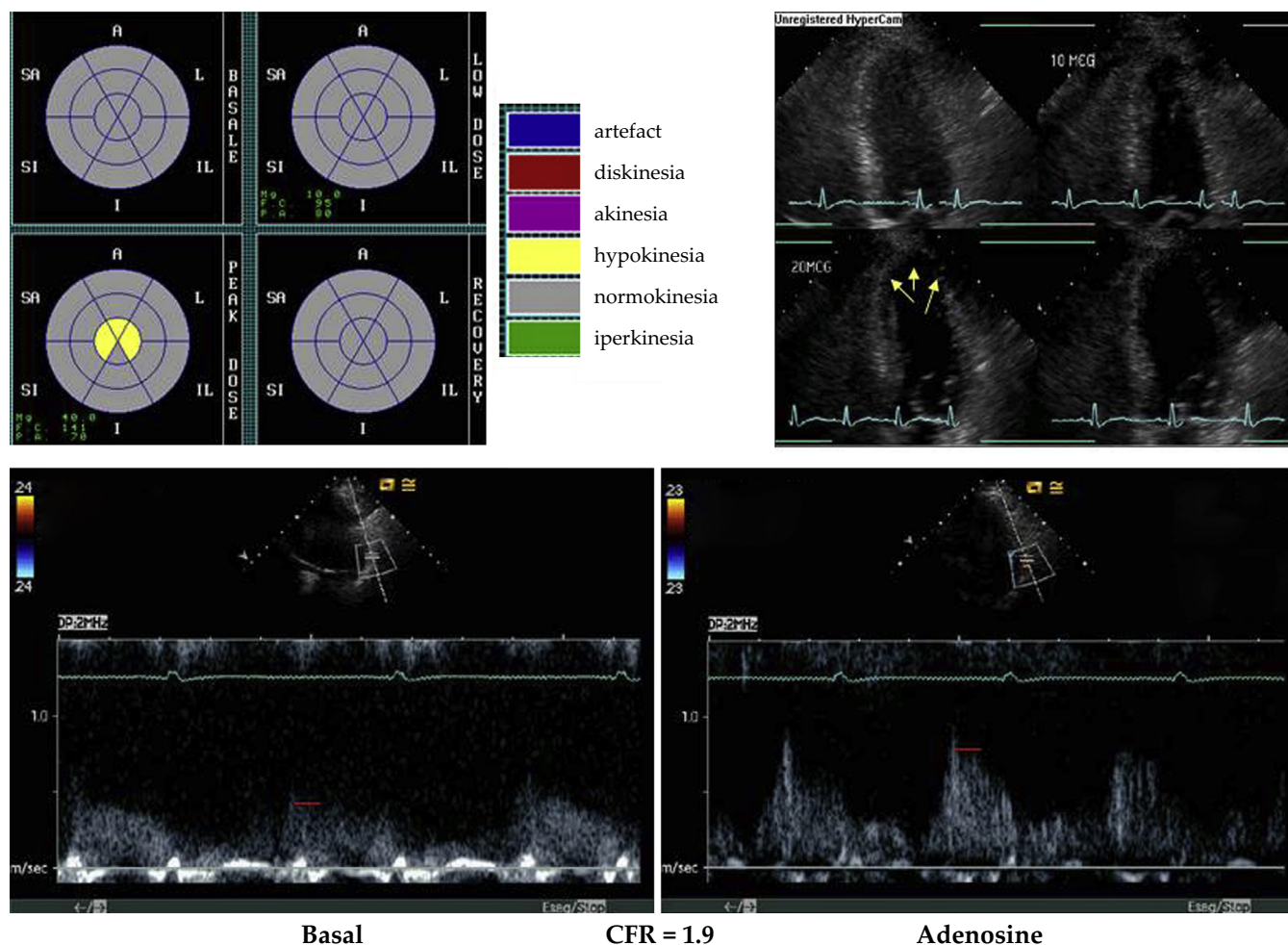


FIGURE 13.6 Doppler echocardiography image of a SSc patient with wall motion abnormalities (hypokinesia of apical segments) during Dobutamine Stress Echocardiography (upper panel) and abnormal Coronary Flow Reserve (lower panel). Adapted from Vacca et al. [133].

they were irrespective of age, disease duration, presence of anti Scl-70 or anticentromere antibody, esophageal and lung involvement, digital ulcers, high-cholesterol serum levels, and blood pressure. During a 10-year follow-up, seven patients with both abnormal coronary functional tests died of disease-related causes, compared to only one patient with normal tests, suggesting a prognostic value of these tests, similar to other myocardial diseases [133] (Fig. 13.7).

5.4 Electrocardiographic Findings, Cardiac Conduction, and Rhythm Abnormalities

Arrhythmias and conduction defects are important and frequent manifestations of cardiac involvement in patients with SSc. These abnormalities range from mild to severe and can also lead to a fatal outcome. The underlying arrhythmogenic mechanisms are not well understood, but it seems to be the result of myocardial damage and fibrosis [14,31]. Arrhythmias may be associated with

a worse prognosis and represent 6% of overall causes of death in the large EUSTAR database [21]. Out of 128 SSc-related deaths, 33 (26%) were of cardiac origin with about half of them due to malignant arrhythmias [21]. Another report from the GENISOS cohort, which included 250 patients with early onset (3 years of disease duration), showed a total of 52 deaths with almost 56% related to SSc. Seven variables were found to be independent predictors of mortality in the final multivariable Cox model: body mass index (BMI), age, forced vital capacity, blood pressure, pulmonary fibrosis, anticentromere antibody, and cardiac arrhythmias (HR of 2.18 (1.05–4.50); $p=0.035$ for cardiac arrhythmias) [134]. In the same cohort, right bundle branch block has been found as an independent predictor of mortality in patients with early SSc [135].

In a study by Ferri et al., 24-h Holter monitoring revealed many more rhythm disturbances than resting ECG (30%), ranging from 66% of SSc patients with supraventricular arrhythmias, 90% with ventricular arrhythmias, 40% with multifocal ventricular

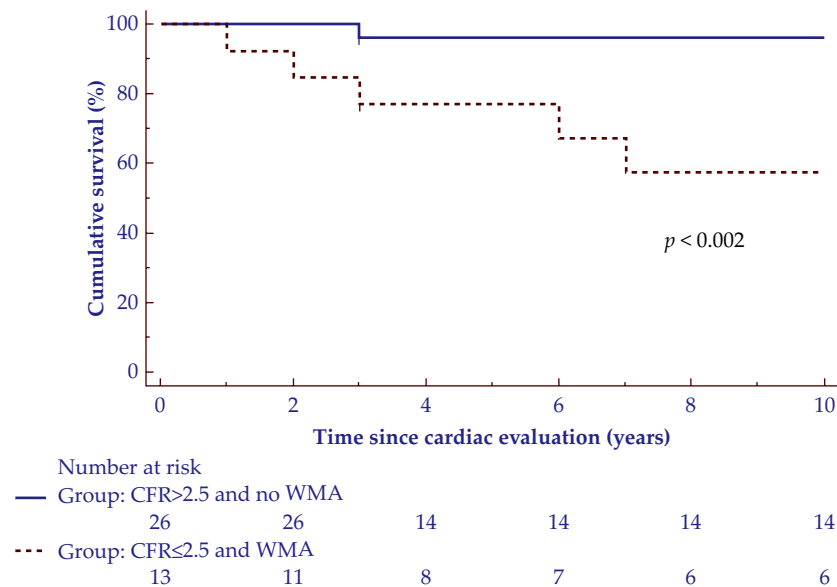


FIGURE 13.7 Kaplan–Meier survival curves in patients stratified according to normal coronary flow reserve (CFR) and no wall motion abnormalities (WMA) versus abnormal CFR and WMA. The worst survival is observed in patients with abnormal CFR and WMA. Adapted from Vacca et al. [133].

premature beats, 28% with pairs of runs of ventricular tachycardia, and 13% with one or more run of ventricular tachycardia. The prevalence and severity of ventricular arrhythmias did not correlate with clinical features. Abnormal ventricular arrhythmias were more likely in patients with echocardiographic abnormalities, although ECG findings were normal in about half of the patients who had ventricular arrhythmias. The high incidence of arrhythmias in this study may be explained by both the high sensitivity and lower specificity of Holter ECG [136].

A multicenter ambulatory ECG study in 183 SSc patients showed ventricular ectopy in 67% of patients, which correlated with total mortality and sudden death. Episodes of ventricular tachycardia and supraventricular tachycardia were also observed in 7% and 21% of patients, respectively [137]. Despite the very frequent occurrence of ventricular arrhythmias, sudden cardiac death is not very common in SSc. A large observational study reported sudden cardiac death in 18 (5%) of 391 deaths occurring in 1258 SSc patients, and concomitant skeletal and cardiac muscle involvement worsened the prognosis of SSc patients with severe cardiac arrhythmias [138].

In a meta-analysis evaluating 436 consecutive cases, Follansbee et al. reported the presence of an abnormal ECG in 46% of patients. Focusing the analysis on 100 selected SSc subjects presenting with evident cardiac abnormalities or who died of more advanced disease, an abnormal ECG was found in 95% of cases [139].

Many electrophysiological abnormalities can be revealed only by conducting invasive studies. Rokas et al. assessed SSc patients without evidence of myocardial

involvement and arrhythmias, by rest and 24-h Holter ECG, echocardiography, and radionuclide ventriculography. They found significant supraventricular or ventricular tachyarrhythmia, sinus node dysfunction, and atrioventricular conduction delay in 57% of the patients [140].

Another parameter that can be evaluated in SSc patients during 24-h Holter ECG monitoring is heart rate turbulence (HRT), which is not only a measure of cardiovascular autonomic activity but also of baroreflex sensitivity [141,142].

Several studies demonstrated impairment of HRT, which seems to be a potentially useful tool for the identification of patients at risk for ventricular arrhythmia, and might be considered an independent risk factor for mortality in SSc [142].

An interesting autopsy study in 35 SSc patients showed focal fibrotic changes in the specialized conduction tissue of the heart, but they were not unequivocally related to the presence of SSc type myocardial fibrosis. Although conduction abnormalities were more frequent in SSc patients with myocardial disease, specific conduction system disease was not the cause of death in most patients. In fact, the conduction system appeared to be relatively spared from the myocardial changes of SSc, and the high incidence of conduction disturbances may be a consequence of, rather than be caused by, specific damage to the proximal portion of the specialized conduction tissue. Moreover, it seemed to be less sensitive to ischemic injury, arguing against the so-called “intramyocardial Raynaud phenomenon,” which suggested that focal contraction band necrosis

was caused by intermittent spasm before evolving to focal replacement fibrosis [31].

In a prospective study, 16 (32%) of 50 SSc patients had resting ECG conduction abnormalities. The most common alteration was left bundle branch block (16%), followed by first-degree atrioventricular block (8%), whilst second- and third-degree atrioventricular block were infrequent (<2%) [57]. In the study by Ferri et al., resting ECG demonstrated conduction defects in 19% and ST-T changes in 5%. Moreover, this prevalence increased to 34% for ST-T changes and to 33% for AV block when 24-h Holter monitoring was performed [136]. Other authors showed a similar prevalence of conduction disturbances. In addition, QTc prolongation, which can lead to life-threatening tachyarrhythmias, has also been reported [143–145]. An electrocardiographic study performed in 102 SSc patients investigated the functional correlates of the abnormalities found in 48 patients with cardiac physiologic data available. Almost 50% presented normal findings on ECG; the most common ECG abnormality was isolated nonspecific ST-T waves changes (14%), and conduction abnormalities were common (17%). Functional correlations showed that 5/48 (10%) had septal infarction pattern, and 10/48 (21%) had ventricular conduction defects. Four of the five (80%) patients with septal infarction pattern had septal or antero-septal thallium perfusion defects, and the fifth had an inferoapical perfusion defect. In four of the five cases, the perfusion defects were fixed, suggesting underlying myocardial fibrosis. Six of the 10 (60%) patients with ventricular conduction abnormalities also had septal or antero-septal thallium perfusion abnormalities. All six had fixed perfusion defects, and four of the six also had redistribution defects. Therefore, 10 of the 15 (67%) SSc patients with one of these ECG abnormalities had a septal or antero-septal thallium perfusion defect compared with six of 33 (18%) of the remainder ($p < 0.005$). It is worth noting that three patients with septal infarction pattern and three with ventricular conduction defects showed normal coronary angiography results, even if in the presence of septal thallium perfusion abnormalities. These fixed defects suggested underlying myocardial fibrosis, consistent with the hypothesis that septal infarction pattern or ventricular conduction abnormalities identify more advanced myocardial involvement in patients with SSc [139].

Of 265 SSc patients with average disease duration of 2.5 years, 140 (53%) showed abnormal ECG findings. The most common ECG abnormality was ST-T wave abnormalities, and these were not associated with cutaneous subtype or autoantibody profile; by contrast they were associated with more severe heart and lung involvement.

Right bundle branch block (RBBB) was associated with an increased risk of mortality in this early cohort of SSc patients. Seventy-five patients (28%) died over a follow-up time of 10 years. Complete RBBB, present in 7 (2.6%) patients, predicted a higher risk of mortality (HR:

5.3; CI 2.1–13.4; $p < 0.001$). The predictive significance of RBBB for mortality was independent of age at enrollment, gender, ethnicity, and risk for CAD [135].

In a large general population study, presence of RBBB was not associated with higher overall or arrhythmic mortality [146], supporting the fact that the association between RBBB and mortality in the GENISOS group could be secondary to SSc-related lung or cardiac involvement. Thus, RBBB should be considered a marker of early disease severity.

In conclusion, it is unclear whether patchy myocardial fibrosis represents the only underlying issue responsible for these conduction defects [14,31].

In contrast to the previous data, other authors found an association between atrioventricular node fibrosis and first-degree atrioventricular block [14]. Interestingly, two cardiac MRI study on SSc patients reported a correlation between the presence of cardiac arrhythmias, conduction disturbances and myocardial fibrosis [120,121], while a more recent study, which excluded those with heart disease prior to SSc onset, did not find any association of abnormal ECG with cardiac fibrosis or abnormal perfusion [124]. Analysis of Holter monitoring data showed that 19 out of 36 patients (53%) had abnormal study results. Among the 19 patients, 12 (63%) had premature ventricular contractions, 2 (10.5%) had supraventricular tachycardias, 3 (16%) had premature atrial contractions, 2 (10.5%) had atrial fibrillation, and 2 (10.5%) had nonsustained ventricular tachycardias. Two additional patients (10.5%) had right bundle branch block, and one patient (5%) had left bundle branch block. Compared to the 17 patients with normal Holter study results, patients with abnormal results were more likely to have pulmonary hypertension by Doppler echocardiography, a decreased left ventricular ejection fraction, increased right ventricular diameter, and a greater number of enhancing myocardial segments at delayed enhanced cardiac MRI study [121].

Studies that correlated autoptic findings with cardiac MRI abnormalities are not presently available, but we can make some indirect correlations. Cardiac MRI enabled to analyze the different patterns of heart involvement in SSc, by differentiating morphological, functional, perfusion, and delayed contrast enhancement abnormalities. The high frequency of heart abnormalities observed on cardiac MRI is consistent with necropsy studies that showed that about 80% of patients with SSc had histological lesions of heart involvement [24].

In the EUSTAR database conduction abnormalities on ECG have been observed in 33% of patients with depressed LV ejection fraction [22].

The frequent ECG abnormalities observed in these studies indicate the presence of alterations in the cardiac centers of impulse formation and conduction, and suggest

that electrical instability of the heart is an integral clinical feature of primary cardiac involvement in SSc [14].

Figs. 13.8 and 13.9 show ECG tracking of two SSc patients with representative arrhythmias and conduction abnormalities.

5.5 Pericardial Involvement

Similar to other cardiac manifestations, the prevalence of pericardial abnormalities varies according to the examination methods used. Only in 7–20% of patients it is clinically manifested [147].

At echocardiography, pericardial effusion is a common finding, although not often hemodynamically significant, as shown in a tissue Doppler study that demonstrated pericardial effusion in 15% of SSc patients compared with 4% of controls [25].

An Italian study provided a higher proportion of SSc patients with pericardial effusion (43% vs 4% of controls), but only 14% presented a significant effusion [148].

Necropsy studies reported pericardial effusion in 33–72% of patients with SSc. The pathological findings were represented by fibrinous pericarditis, chronic fibrinous pericarditis, pericardial adhesion, and

pericardial effusion [147]. At histological examination of 44 autopsies, chronic pericarditis was found in 77% of SSc patients compared to one of matched age and sex controls. The degree of fibrosis was higher in SSc, myocardial fibrosis was present in 37.5% of SSc patients but in none of controls, suggesting that pericarditis may be primary rather than secondary to uremia. Constrictive pericarditis has rarely been reported as a cause of death in SSc [21,149]. Pericarditis can be rarely secondary to uremia, renal crisis, and pulmonary hypertension. In this context, pericarditis may be a sign of overall disease activity and progression, and a poor prognosis after the diagnosis of large pericardial effusion has been found in these patients [150]. Among patients with PAH, investigating for pericardial effusion is mandatory due to recognizing the role of bad prognostic factors [151].

5.6 Valvular Involvement

A higher prevalence of aortic regurgitation (18%) and a trend toward more prevalent mitral regurgitation have been detected in a series of 100 SSc patients; however, valvular regurgitations were associated with age, and most patients had grade 1 aortic or mitral regurgitation,

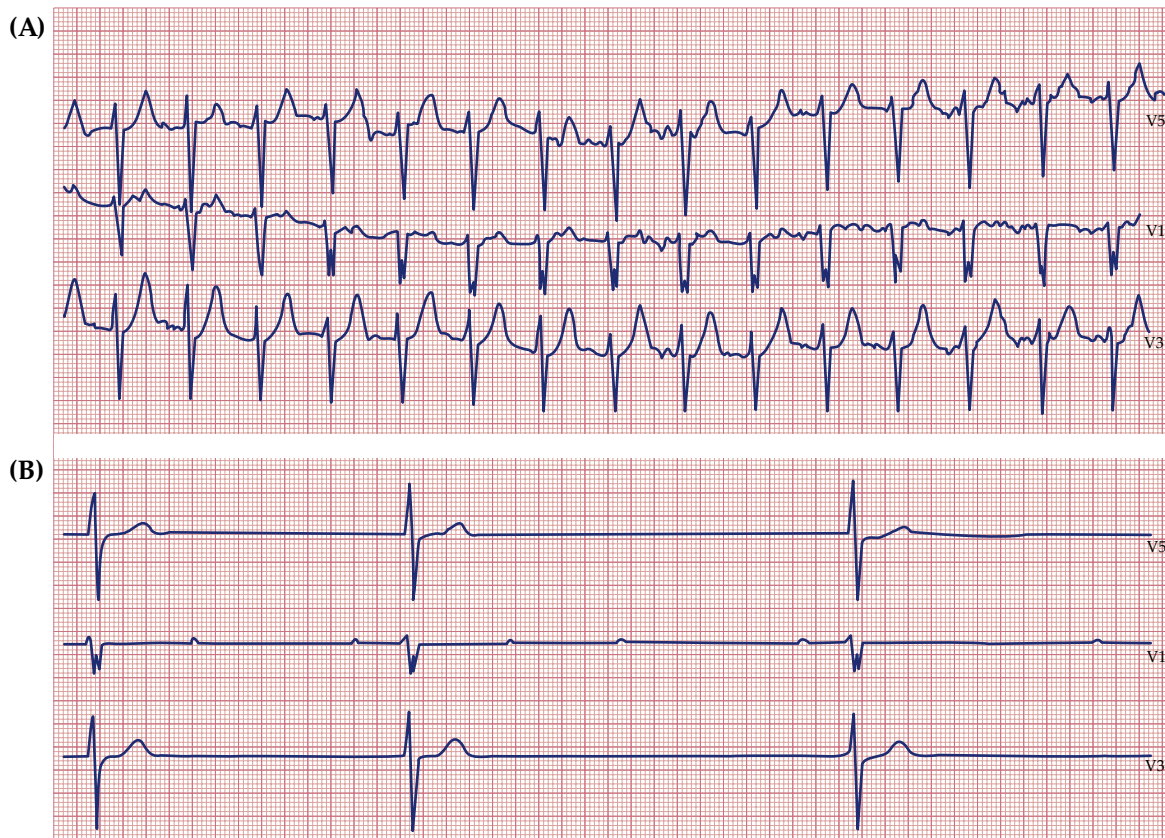


FIGURE 13.8 24-Holter ECG tape of a 68-year-old SSc male patient: first-degree atrioventricular block, second-degree atrioventricular block, and 2:1 complete block. He underwent pacemaker implantation. Adapted from the personal collection of Dr. A. Vacca, University Hospital of Cagliari, Monserrato, Italy.

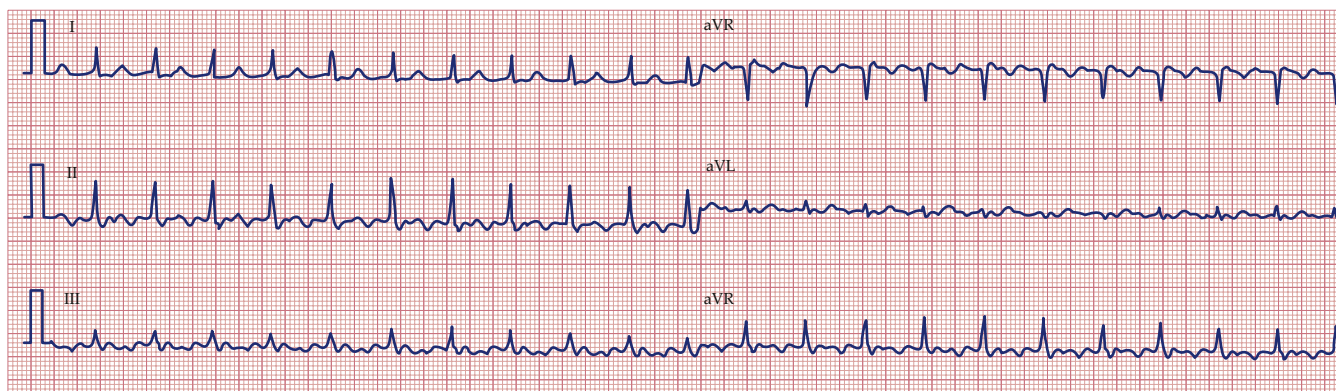


FIGURE 13.9 ECG of a 52-year-old SSc female patient: atrial flutter (heart rate 150/min). Although asymptomatic, she started verapamil 80 mg three times a day and anticoagulated. Adapted from the personal collection of Dr. A. Vacca, University Hospital of Cagliari, Monserrato, Italy.

or both, considered anyway as a benign finding [25]. Valvular vegetations are rarely documented in patients with SSc. At autopsy studies, they were documented in 5 out of 28 SSc cases, including lesions of the mitral, tricuspid, and aortic valves [152,153]. However, they seem to not have any clinical significance. Endocarditis may occur together with severe myocardial involvement [154].

6. PATIENT EVALUATION, CLINICAL DIAGNOSIS, AND CARDIOVASCULAR ASSESSMENT

6.1 Clinical Manifestation

Symptoms of cardiac involvement can be nonspecific, often difficult to distinguish from clinical presentation of other complications, such as vascular or interstitial lung involvement, esophageal dysfunction, myositis, and chest wall disorders (Table 13.3).

Fatigue and dyspnea on exertion or at rest usually are the most common symptoms of cardiac involvement, although they can also be related to other concomitant SSc manifestations like lung fibrosis and pulmonary hypertension, anemia, and musculoskeletal involvement. The severity of dyspnea should be classified according to the New York Heart Association classification, which suggests obtaining an objective evaluation of functional exercise capacity, addressing further diagnostic examinations.

Chest pain may be a symptom of pericarditis, less frequently of underlying CAD, while angina pectoris and typical precordial pain are uncommon [13]. The presence of atypical chest pain, in addition to pericarditis, also can be associated with esophageal reflux and musculoskeletal involvement.

Palpitations, tachycardia, dizziness, syncope, or sudden death can be correlated to either rhythm and

conduction abnormalities or autonomic cardiac neuropathy [155].

On physical examination further clinical information on heart rate, rhythm disorders at auscultation, murmurs suggestive for valve incompetence, signs of RV and pulmonary vasculature overload such as jugular congestion, hepatomegaly, and peripheral edemas can be collected.

However, cardiac involvement can be silent, and when symptoms and signs are present they can be misinterpreted.

6.2 Diagnosis and Assessment

The cardiac workup in patients with SSc includes careful interpretation of symptoms and signs, routine and second-level investigations, both for electrical assessment and heart evaluation, as summarized in Table 13.3.

Standard 12-lead ECG as well as Doppler echocardiography should be performed routinely in all SSc patients, even if the patient is asymptomatic. If the patient complains of palpitations, syncope, or dizziness, the next steps must include exercise testing, upright tilt-table testing, and 24-h Holter monitoring.

When significant changes are detected in the conduction and rhythm systems, careful investigations for heart disease must be performed. Firstly, classic cardiovascular risk factors should be assessed together with any potential signs or symptoms of ischemic heart disease. If primary cardiac involvement is suspected, echocardiography should be performed, when possible, by pulsed Doppler. In addition, cardiac magnetic resonance imaging (MRI) to evaluate tissue damage may be considered, particularly if there are any signs of myositis, in order to rule out myocarditis, which demands specific management [156].

TABLE 13.3 Cardiac Diagnostic Workup in Patients With Systemic Sclerosis

Clinical Manifestations	Routine Assessment	Second Level or for Research Purpose Assessment
Asymptomatic	CV risk factors assessment	Pulsed Doppler echocardiography
Fatigue	Standard 12-lead ECG	Stress echocardiography
Dyspnea	Exercise testing	Invasive electrophysiological studies
Chest pain	Upright tilt-table testing	Measurement of HRT and HRV
Angina	24-h Holter monitoring	Coronary angiography
Palpitations	Color Doppler echocardiography	Cardiac MRI
Syncope	NT-proBNP plasma levels	Endomyocardial biopsy
Dizziness		

CV, cardiovascular; ECG, electrocardiogram; HRT, heart rate variability; HRV, heart rate turbulence; MRI, magnetic resonance imaging.

A 24-h Holter monitoring might be considered as part of routine evaluation in SSc patients, even if they are asymptomatic. The patient should be also questioned about the presence of systemic illnesses that can be associated with arrhythmias such as chronic obstructive pulmonary disease, hyperthyroidism, pericarditis, and congestive heart failure. Moreover in SSc, several complications might favor arrhythmia such as life-threatening infections related to severe motility disorders of the intestine or electrolyte imbalance because of gut or kidney involvement [157]. Measurement of HRT should be considered in selected patients after detailed clinical, echocardiographic and standard ECG, and Holter monitoring evaluations. Invasive electrophysiological studies are indicated in patients who have atrioventricular block, intraventricular conduction disturbance, sinus node dysfunction, tachycardia, and unexplained syncope or palpitations [158].

6.3 Cardiac Biomarkers in Systemic Sclerosis

Recently, some biomarkers emerged as strong predictors of cardiac disease, particularly cardiac troponin T (cTnT) and brain natriuretic peptide (BNP). The recently available high-sensitivity (HS)-cTnT has prognostic value in patients with various cardiac diseases, such as acute MI, chest pain, heart failure, acute and chronic myocardial ischemia, and in low-risk populations [159,160]. Moreover, HS-cTnT has been identified as a novel prognostic biomarker in idiopathic and chronic thromboembolic pulmonary arterial hypertension [161].

The N-terminal pro-brain natriuretic peptide (NT-proBNP) is released concomitantly with BNP by ventricular myocytes, as a consequence of increased strain. The circulating half-life of BNP is 23 min, whereas NT-proBNP has a longer half-life of 60–120 min, explaining

why the latter is preferable as biomarker in clinical practice.

NT-proBNP predicts cardiac mortality and morbidity in the general population as well as in cohorts of patients with heart failure and stable coronary heart disease [162–164]. NT-proBNP plasma levels can be raised also in noncardiologic condition such as anemia, hyperbilirubinemia [165,166] and can be influenced by age >65 years, BMI, gender and ethnicity, although its diagnostic utility remains unchanged [167]. NT-proBNP showed an established role in diagnosis and follow-up of SSc-related PAH as well as in reduced left/right ventricular contractility [168–170].

Several studies evaluated NT-proBNP as an independent marker for SSc-related PAH. SSc patients with PAH have shown higher NT-proBNP plasma levels compared to those without. Besides, several studies reported strong correlation between NT-proBNP levels and other hemodynamic parameters [171–174]. NT-proBNP showed a sensitivity of 56% and a specificity of 95% in predicting PAH in SSc patients at a cutoff value of 395 pg/mL [173]. Similar results were reported by other authors: Oravec et al. [175] found a sensitivity of 100% and a specificity of 72.3% at a cutoff value of 157.8 pg/mL, with a positive predictive value of 39.1% and a negative predictive value of 100%. Besides as a screening tool in PAH, NT-proBNP has a prognostic value for PAH development [168,176,177] and is related to PAH severity and survival [173,178]. Moreover, NT-proBNP may be used for monitoring response to treatment, as suggested by its decrease after bosentan and sildenafil therapy for PAH in SSc patients [179–181].

The European Society of Cardiology guidelines suggest NT-proBNP sampling at baseline and every six months in the follow-up of PAH patients or every three months after starting, changing therapy or clinical deterioration [182].

Higher NT-proBNP levels have also been found in SSc patient with cardiac involvement compared to SS controls without heart disease [176,183]. Allanore et al. demonstrated a correlation between NT-proBNP and depressed myocardial contractility measured at tissue Doppler echocardiography. Moreover, they identified a cutoff of 125 pg/mL offering a sensitivity of 92% and a specificity of 71% in revealing a depressed myocardial contractility, while a sensitivity of 94% and a specificity of 78% was evidenced for detection of overall cardiac involvement with a negative predictive value of 97.6% [176]. Another interesting finding is the identification of BNP as the only independent predictor of incident atrial fibrillation in a prospective observational study of 49 SSc patients followed for 72 ± 24 months [184].

The only published study suggesting the clinical utility as a biomarker of HS-cTnT plasma levels comes from Avouac et al. The study group included 161 patients with SSc and 213 matched control subjects. HS-cTnT and NT-proBNP plasma levels were significantly increased in SSc patients compared with controls (both $P < 0.001$). Similar results were confirmed in the subgroup of patients with SSc who had no cardiovascular risk factors ($n = 72$). Multivariate logistic regression analysis evidenced diabetes mellitus, high blood pressure, precapillary pulmonary hypertension, and the diffuse cutaneous SSc as factors independently associated with an HS-cTnT level of >14 ng/L. The association with diabetes and hypertension suggests that subclinical atherosclerosis promoted by cardiovascular classic risk factors may influence elevation of cardiac biomarkers in SSc. Moreover, the elevated levels of HS-cTnT and NT-proBNP measured also in SSc patients without traditional cardiovascular risk may reflect subclinical myocardial damage and dysfunction. Increased NT-proBNP concentrations were associated only with the presence of precapillary pulmonary hypertension. Normal concentrations of both HS-cTnT and NT-proBNP had a high negative predictive value for precapillary pulmonary hypertension (92%), and notably, the combination of increased values of these two markers had the highest strength of association with precapillary pulmonary hypertension in logistic regression analysis [185].

Thus, these two biomarkers could identify, after validation, a category of SSc patients at risk of developing precapillary pulmonary hypertension to be more closely followed and specifically treated [185].

In this setting, NT-proBNP should be used routinely in patients with SSc, particularly those with dyspnea, either to indicate that accurate cardiac is required or, conversely, to show the presence of pulmonary involvement when a normal value is obtained, while more evidence is needed for HS-cTnT before accepting it as a validated cardiac biomarker in SSc.

7. TREATMENT

7.1 Vasodilators: Calcium Channel Blockers and ACE Inhibitors

Vasodilators such as calcium channel blockers and ACE inhibitors (level C, class IIa) are drugs of proven efficacy in SSc patients with myocardial involvement. This indication comes from the observation of beneficial effect on Raynaud phenomenon, which has been hypothesized to be present also in myocardium.

In patients with SSc, some vasodilating approaches (prostacyclin or NO/endothelin, calcium channel blockers) may counteract the microvascular dysfunction at peripheral and pulmonary level; similarly, few vasodilators such as nifedipine, nicardipine, and captopril, and more recently bosentan (level C, class IIb) have been reported to acutely improve both myocardial perfusion and function as assessed by myocardial scintigraphy, TDI, and cardiac MRI [123,186,187]. Kahan et al. studied 20 patients with SSc without coronary stenoses and found perfusion defects at myocardial scintigraphy in all of them. After nifedipine, myocardial perfusion improved in 43% of segments [188]. Another small study evaluated the potential of acute administration of L-propionylcarnitine (L-Pc) in this clinical context since it is a metabolic substance that exerts beneficial effects on both microcirculation and myocyte function during ischemia reperfusion. Acute administration of L-Pc has been demonstrated to exert a short-term beneficial effect on CFR in the SSc patients studied [189].

Improved myocardial perfusion was also seen after treatment with ACE inhibitors [190].

These beneficial effects of vasodilators can be partially explained by the concomitant presence of ischemic lesions accessible to reperfusion after vasospasm of coronary microvasculature and irreversible lesions, like morphological vessel pathology or myocardial fibrosis [16]. In this setting, vasodilators may influence the reversible component of myocardial ischemia.

Patients with reduced LV ejection fraction should be treated with ACE inhibitors or angiotensin II blockers, but a significant number of patients are treated also with dihydropyridine calcium channel blockers.

7.2 Antiarrhythmic Drugs

Some therapeutic options in SSc patients with cardiac arrhythmias and conduction defects may differ from those recommended in other populations, keeping in mind that possible multiple organs can be involved with concomitant use of different drugs.

Some drugs commonly used in SSc may trigger a rhythm or conduction abnormality: corticosteroids (tachyarrhythmias), methotrexate (RBBB and ventricular

arrhythmias), and prokinetics (serious ventricular arrhythmia or sudden cardiac death).

As no randomized controlled trials have been performed specifically in SSc patients, the choice of therapy should be similar to that of patients without SSc. Class Ia (quinidine, procainamide) and class Ib (flecainide) antiarrhythmic drugs are contraindicated in patients with cardiac dysfunction or myocardial ischemia. Calcium channel blockers have a potential tachycardiac effect, but this seems to be of limited impact in SSc, in which they can improve LV ejection fraction, LV contractility, LV filling, and RV contractility. Verapamil-like calcium channel blockers might be recommended in the treatment of atrial or intranodal tachycardia, although their efficacy still remains limited. β -blockers are effective but can deteriorate Raynaud phenomenon. Class III antiarrhythmic drugs (amiodarone) are probably the most effective drugs (level C, class IIa). There is no evidence that preexisting pulmonary fibrosis increases the risk of immunological pneumonia, but it will worsen its consequences. Implantable cardioverter defibrillator (ICD), which monitors the cardiac rhythm and can deliver competing pacing stimuli and low- and high-energy shocks, has been used effectively in selected patients to prevent malignant ventricular arrhythmias. There is no specific recommendation in SSc patients; ICD should be considered in patients at high risk of sudden death in secondary prevention and in primary prevention for patients with LVEF <30% (35% if ischemic), and with proven symptomatic ventricular tachyarrhythmia (level A, class I). Catheter ablation therapy allows the destruction of a delimited atrial/ventricular zone, and it should be considered as first-line therapy in patients with recurrent reentrant tachycardia, or atrial flutter, in patients with symptomatic, sustained, monomorphic ventricular tachycardia, resistant or intolerant to pharmacological treatment, in patients with recurrent symptomatic atrial fibrillation despite antiarrhythmic drugs (level B, class I). Pacemaker implantation is the treatment of choice for complete heart block and other serious bradyarrhythmias [55] (level A, class I).

Heart transplantation might be an option for end-stage primary SSc heart disease, although it is rarely described in the literature [109,191].

7.3 Treatment of Pericarditis

Pericarditis does not need any specific treatment in the majority of patients. If symptomatic, it can be treated with nonsteroidal anti-inflammatory drugs and most rarely with corticosteroids, especially if myocarditis is associated (level C, class IIa). In this case the starting dose of 60–80 mg of equivalent prednisone should be tapered to zero in a week. Colchicine (1 mg twice a day or 1 mg daily after loading dose of 2–3) may be

added to other treatments or can be used as a first-line therapy (level C, class IIa). Pericardiocentesis is indicated in case of tamponade, which is associated with a bad prognosis [192]. Renal and cardiac monitoring are mandatory during the therapy due to the risk of scleroderma renal crisis, PAH, and cardiac arrhythmias in these patients [150].

7.4 Treatment of Myocarditis

Prompt intervention with corticosteroids and immunosuppressors such as cyclophosphamide and azathioprine (level C, class IIa) must be reserved for SSc patients who develop myocarditis [52].

Table 13.4 summarizes diagnostic tools and therapeutic approaches according to cardiac manifestations in SSc.

8. CONCLUSIONS

Primary myocardial involvement has a documented high prevalence in patients with SSc, both in diffuse and limited cutaneous form, and may present with various manifestations, both clinical and subclinical. The presence of cardiac involvement in SSc is often underestimated due to the occult nature of the signs and symptoms, and reports of the prevalence of cardiac disease vary depending on the methods used. Moreover, symptoms of cardiac manifestations are often attributed to noncardiac causes such as pulmonary, musculoskeletal, or esophageal involvement. But whenever present, it is an indicator of poor prognosis and early diagnosis and staging of the disease have been sought.

The best diagnostic approach for cardiac evaluation has not been defined yet, although ECG and echocardiography would be the first-step imaging method easily available in any hospital. While several possible diagnostic approaches have been proposed, some of them might have practical value for diagnosis, and others may provide pathophysiological insight or prognostic information. The determination of the appropriate role of imaging is of great concern in the era of multimodality cardiac imaging, as well as the exact time of monitoring for repeating such examinations must be individualized.

Although no specific therapy exists for primary heart involvement, early recognition of the presence and characteristics of the involvement may lead to more effective management of patients with SSc.

In summary, while the influence of scleroderma on cardiac function has been known for nearly a century, only recently have we begun to gain a new understanding of the prevalence and prognosis in this patient population. Through new and more refined imaging modalities as well as more frequent use of

TABLE 13.4 Diagnosis and Treatment of Cardiac Manifestations in Systemic Sclerosis

Cardiac Manifestations	Diagnostic Tools	Treatment
LV systolic dysfunction	Color Doppler echocardiography, cardiac CMR, NT-proBNP plasma levels	Beta-blockers, ACE-inhibitor/ARB; calcium channel blocker if microvascular coronary disease is present
LV diastolic dysfunction	Color Doppler echocardiography, TDE, NT-proBNP plasma levels	Treatment of heart failure if present; calcium channel blockers if microvascular ischemia present
Primary RV dysfunction	Color Doppler echocardiography, TDE, CMR, NT-proBNP plasma levels, invasive hemodynamic testing to exclude PH	Digoxin, diuretics if right heart failure
Microvascular coronary artery disease	Evaluation of CFR, stress echocardiography, nuclear imaging, CMR	Calcium channel blockers, ACE-inhibitors/ARB; statins
Macrovascular coronary artery disease	Coronary angiography; calcium score and coronary CT for screening	Coronary stenting or standard medical management of coronary artery disease
Pericardial effusion	Color Doppler echocardiography	Diuretics in patients with dyspnea or right heart failure; pericardiocentesis for severe symptoms/cardiac tamponade
Constrictive pericarditis	Color Doppler echocardiography, TDE, CMR, cardiac CT, echo, right and left heart catheterization	Diuretics, sodium and fluid restriction; pericardial stripping surgery contraindicated in most cases
Myocarditis	Color Doppler echocardiography, cardiac troponin T, CMR, endomyocardial biopsy	Immunosuppressors such as cyclophosphamide, intravenous pulse steroids, azathioprine; if symptomatic systolic heart failure is present, treat according to guidelines; comanagement with heart failure specialist
Tachyarrhythmias	Standard 12-lead ECG, 24-h Holter monitoring, invasive electrophysiological studies, color Doppler echocardiography	Verapamil calcium channel blockers, beta-blockers such as carvedilol, amiodarone, ICD to prevent malignant ventricular arrhythmias, catheter ablation
Bradyarrhythmias and cardiac block	See above	Pacemaker

CFR, coronary flow reserve; CMR, cardiovascular magnetic resonance; CT, computed tomography; ECG, electrocardiogram; ICD, implantable cardioverter device; LV, left ventricular; NT-proBNP, N-terminal pro-B natriuretic peptide; PH, pulmonary hypertension; RV, right ventricular; TDE, tissue Doppler echocardiography.

invasive hemodynamics, we will be able to better assess patients for subclinical disease and gain new insight as to the long-term prognosis in patients with SSc. Moreover, early detection will permit improving quality of life and survival in SSc patients with cardiac involvement.

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Dermatomyositis and Polymyositis

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1. INTRODUCTION

Idiopathic inflammatory myopathies (IIMs), collectively called myositis, are a heterogeneous group of diseases affecting adults and children (Fig. 14.1). The IIMs are characterized clinically by symmetrical, proximal muscle weakness and by inflammatory infiltrates in skeletal muscle. Based on differences in clinical and histopathological features, they can be subgrouped into dermatomyositis (DM), polymyositis (PM), cancer-associated myositis (CAM), sporadic inclusion body myositis (sIBM), and a more recent subset named immune-mediated necrotizing myopathy (IMNM). In children, juvenile dermatomyositis (JDM) is the far most common subgroup, whereas juvenile polymyositis is a very rare entity. Histopathologically, the IIMs are characterized by inflammation in skeletal muscle tissue with degeneration and regeneration of muscle fibers. The inflammatory infiltrates are typically composed of T lymphocytes and B lymphocytes in PM, DM, and IBM and by macrophages in IMNM. These observations from muscle biopsies together with the frequent presence of autoantibodies support that the IIMs are immune-mediated disorders. During recent years, a large number of myositis specific autoantibodies (MSAs) have been detected. Importantly, they are associated with distinct clinical phenotypes, and another way to subgroup patients with IIM is according to autoantibodies, as will be discussed in the following. Other organs are frequently involved in patients with IIM such as skin in dermatomyositis, lungs, joints, gastrointestinal tract, and the heart, particularly in PM, DM, and JDM, emphasizing that these are systemic inflammatory diseases. Myositis may also present in patients with other defined rheumatic diseases, most often systemic sclerosis and mixed connective tissue disease (MCTD). These are called overlap syndromes. In

this chapter, we will focus on adult PM and DM and JDM as little is known about cardiac involvement in the other subsets.

1.1 Epidemiology

The IIMs are rare disorders. The prevalence of adult IIM is approximately 10 per 100,000 and the annual incidence is approximately 1 per 100,000 inhabitants. The annual incidence for JDM is 2 to 4 per 100,000 children, whereas prevalence figures are lacking. The IIMs may occur at any onset of life with a peak incidence of adults at approximately 50–60 years of age and in children 7–8 years of age [1,2].

The IIMs are present all over the world and interestingly with a variation of the frequencies of myositis subgroups in that DM is more frequent in countries closer to the equator and PM more frequent in countries of northern latitudes. This distribution between subgroups is related to the ultraviolet (UV) light exposure, suggesting that UV light is an environmental risk factor for DM.

1.2 Genetics

The IIMs belong to complex genetic diseases. As these are rare disorders, large international networks are needed for genetic studies. Recently, such an international collaboration, the Myositis Genetics Consortium (MYOGEN) has resulted in the first publication on genome wide association studies (GWAS) in Caucasian patients with DM and JDM. A more recent study used ImmunoChip analyses and also included PM and IBM patients. The strongest genetic association is to the major histocompatibility complex (MHC) on chromosome 6 including alleles *HLA-DRB1*03:01* and *HLA-B*08:01* of the 8.1 ancestral haplotype (8.1AH). This supports the

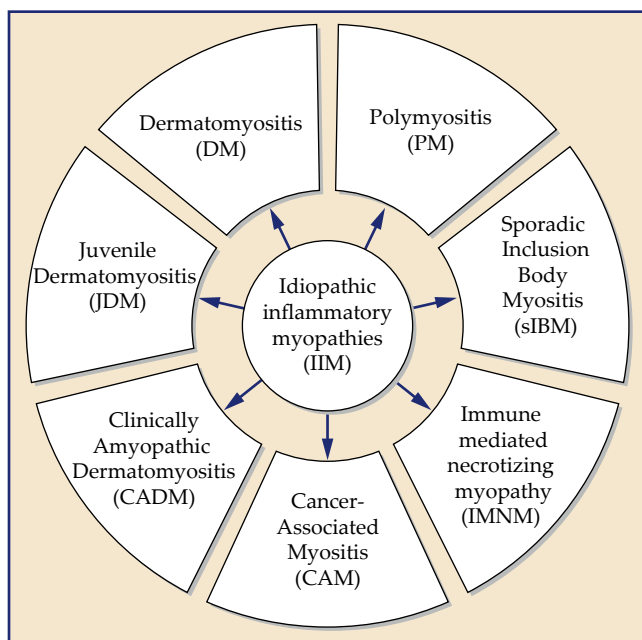


FIGURE 14.1 The subgroups of idiopathic inflammatory myopathies. Adapted from the personal collection of the authors.

notion of an immune component of myositis, since a major role for the MHC molecule is to present antigens to the T lymphocytes. Interestingly, different HLA-DR genotypes are associated with different autoantibodies, again emphasizing the role of the adaptive immune system in the pathogenesis of these disorders. Other genetic associations are to immune related genes like PTPN22 and STAT4, which are also associated with other autoimmune diseases.

2. CLINICAL PRESENTATION AND DIAGNOSTIC CRITERIA

2.1 Clinical Presentation

2.1.1 Clinical Features

Dermatomyositis (DM) and polymyositis (PM) are both characterized by symmetrical, proximal muscle weakness, whereas characteristic skin manifestations are only seen in DM [3]. Clinically amyopathic DM (CADM) is a clinical phenotype where patients have typical dermatomyositis rash, but lack evidence of the myositis component [4]. Being systemic diseases, PM, DM, and CADM are all associated with extra muscular manifestations, like lung, heart, and gastrointestinal involvement. The antisynthetase syndrome (ASS) is a subgroup of patients with DM/PM characterized by interstitial lung disease (ILD), Raynaud phenomenon, polyarthritides, mechanic's hands, fever, and production of antisynthetase antibodies (ASA) [5,6]. Juvenile DM, or JDM (which starts before the age of 18 years), is the far most common phenotype of IIMs in children [7]. JDM shares many

common features with DM, but also has some distinct characteristics, like more often vasculopathic changes, calcinosis, and less commonly lung involvement [8]. Malignancy is linked to DM (cancer-associated myositis: CAM), but to a lesser extent PM or JDM. The onset in DM/PM and JDM may be acute (days), or insidious (days to months). The features can be divided as constitutional and organ specific.

2.1.1.1 Constitutional Features

Fever, malaise, weight loss, and fatigue may be seen, especially at symptom onset, and more often in JDM.

2.1.1.2 Musculoskeletal Features

The cardinal symptom in both DM and PM is proximal and symmetric *muscle weakness*, which is seen as the presenting symptom in more than 90% of PM patients, and in 50% of DM cases [9]. The muscle weakness leads to difficulties in eg, climbing stairs, getting up from a chair, and lifting objects, and may lead to difficulties getting up from the floor and frequent falls in JDM. Distal muscle weakness may be seen, especially in JDM and in advanced cases of DM, but is usually mild and does seldom cause significant functional problems. Neck muscles, especially flexor muscles, may be involved in all types, and abdominal muscles typically in JDM. Although rarely seen, a severe weakness of neck extensor muscles as in dropped head syndrome may be observed. Muscle inflammation can also involve the pharynx, larynx, or the proximal esophageal tract, which can lead to progressive dysphonia, dysphagia, and even tracheal aspiration [10]. In serious and in some acute cases, respiratory muscles can be involved as well. *Myalgia* and *muscle tenderness* can be seen in JDM [7] and also in PM and DM [3], but if myalgia is the prominent symptom, other diseases should be excluded. *Muscle atrophy* is typically a late symptom in patients with advanced disease. Arthritis is seen in more than half of patients during disease course, both in adult onset [11] and juvenile onset disease [12] and arthralgia is even more common.

2.1.1.3 Cutaneous Features

A rash is present in DM, but not in PM, and may precede or accompany muscle weakness at the time of DM presentation. The skin manifestations may be pathognomonic of DM [13], can be modest or quite prominent, and are often similar in juvenile and adult onset disease. The most common types of rashes are:

- *Gottron's papules or sign* on the knuckles, or at the knee or elbows, typically violaceous and symmetrical, and may evolve into a scaling discoloration.
- Heliotrope (meaning blue-purple) rash mainly with a periorbital localization, which may be accompanied by periorbital edema.
- Erythematous rash at the upper chest (V-sign) and upper back (shawl-sign), which is rarely seen in JDM.

- Facial erythema or malar rash is most common in JDM, and as opposed to the similar rash seen in systemic lupus erythematosus (SLE), the nasolabial fold is often spared.
- Periungueal hyperkeratosis and telangiectasias, which represent dilated capillary loops at the base of the fingernail, are most often seen in JDM and in cancer-associated dermatomyositis.
- Skin ulcers, due to vasculopathic changes in skin, are most often seen in JDM.
- Calcinosis in subcutaneous tissue, but also in fascia or muscles, is most commonly seen in JDM (up to 40% of cases), and less commonly described in DM.
- Lipodystrophy, characterized by loss of subcutaneous fatty tissue, is most common in JDM.
- Hyperkeratosis and fissured skin on the palmar and lateral aspects of the fingers, so-called mechanic's hands, are most often seen in patients with ASS.
- Various other rashes are also seen, as previously reviewed [14,15].

2.1.1.4 Pulmonary Features

The prevalence of interstitial lung disease (ILD) in adult onset DM and PM myositis ranges from 20% to 78% (depending on which diagnostic method is used), and is associated with increased morbidity and mortality [16]. ILD is associated with certain autoantibodies, especially with anti-aminoacyl-tRNA synthetase antibodies. Patients with CADM may also develop ILD, and particularly in association with anti-MDA5 antibodies this can rapidly progress with poor prognosis requiring intense immunosuppressive therapy. Clinically significant ILD in JDM is rare. Respiratory function may also be impaired (hypoventilation) due to weakness of respiratory muscles, especially in patients with generalized muscle weakness [3]. Of notice, tracheal aspiration due to pharyngeal or esophageal dysmotility may lead to aspiration pneumonia [17].

2.1.1.5 Vascular Features

Vascular involvement can be prominent in DM, especially in JDM, and histologically, endothelial swelling, inflammation, and obliteration may be seen [7]. This can affect blood vessels in several organs. Ischemic vasculopathy of the bowel can result in gastrointestinal bleeding or even perforation, which is rarely seen in adults [18]. Transient digital ischemia can lead to Raynaud's phenomenon, and ischemia in the skin to ulcerative lesions.

2.1.2 Disease Activity and Damage

In IIMs, like in other rheumatic diseases, it is important to distinguish between features caused by disease activity (potentially reversible with treatment) and disease damage (irreversible changes in anatomy, physiology, or function). To assess these important aspects, tools have

been developed by the International Myositis Assessment and Clinical Studies Group (IMACS). The myositis disease activity assessment tool (MDAAT) assesses disease activity in muscle and six extra muscular organ systems (domains) during the previous 4 weeks, including the cardiac domain [19,20]. The cardiac items that are included in the MDAAT are pericarditis, myocarditis, severe and other arrhythmias, and sinus tachycardia. The myositis damage index (MDI) measures cumulative organ damage in 11 organ systems/domains [19], including cardiovascular damage. The cardiovascular items included in the MDI are hypertension requiring treatment, ventricular dysfunction/cardiomyopathy, angina or coronary artery bypass, and myocardial infarction. These tools are used for both juvenile and adult onset IIMs.

2.1.3 Autoantibody Phenotypes

Over the last years, several new myositis-specific autoantibodies (MSA) have been described and linked to specific clinical phenotypes in IIMs (Table 14.1) [6,8,21–26]. For instance, autoantibodies against aminoacyl-tRNA synthetases are seen in 15–25% of PM and DM patients, and share a common constellation of clinical features, referred to as antisynthetase syndrome. The antihistidyl antibodies (anti-Jo-1) are the most frequent ASA. Interestingly, clinical phenotype in patients with the same antibody may differ between subgroups, best demonstrated by anti-NXP2 (anti-MJ), which has been linked to malignancy in adult PM/DM [27], but is a marker of calcinosis in JDM [24,28]. Likewise, malignancy is linked to the presence of anti-TIF1 γ antibodies in adults but not in children [24,27]. The anti-MDA5 antibody is associated with rapidly progressive ILD, particularly in CADM patients. Anti-SRP was initially reported to be associated with heart involvement, but this has not been confirmed in more recent studies [29].

Other autoantibodies have also been found, so-called myositis-associated autoantibodies (MAAs). When MAAs are detected, an overlap syndrome to other autoimmune rheumatic diseases—like SLE or systemic sclerosis—may be present. The most frequently present MAAs in PM and DM is anti-SSAs, more specifically anti-Ro-52 and anti-PMScl [30].

2.2 Diagnostic Criteria

2.2.1 Diagnostic and Classification Criteria for IIMs

In 1975, Bohan and Peter developed a classification scheme and diagnostic criteria for myositis, which have, until now, been most widely used both for clinical workup and clinical trials in juvenile and adult onset IIMs (Table 14.2) [31,32]. Importantly, there are several exclusion criteria to rule out mimicking conditions.

TABLE 14.1 Autoantibody Phenotypes in Dermatomyositis, Polymyositis, and Juvenile Dermatomyositis

MSA	Frequency adult IIM (% of total)	Adult disease feature	Frequency juvenile IIM (% of total)	Juvenile disease features
Anti-Jo1, -PL-7, -PL-12, -EJ, -OJ, -KS, -Ha, -Zo	40	Antisynthetase syndrome ^a	1–5	Muscle atrophy, ILD
Mi2	10–30	Classic DM rash, good prognosis	4–5	Classic DM rash, good prognosis
SRP	4–8	Necrotizing myopathy, PM, dysphagia	1–2	Necrotizing myopathy
TIF1-γ	20	Severe DM rash associated with malignancy	23–32	Severe DM rash, ulceration, and lipodystrophy described
NXP2	25	Associated with malignancy	20–25	DM, rash, calcinosis, worse functional status
MDA5	20–30% in Asian population, rare in Caucasians	Amyopathic or mild myositis, DM rash, arthritis, ILD, RP-ILD (especially in Japan)	7	ILD rare
SAE	5	Initially amyopathic, DM rash, dysphagia	<1	

ILD, interstitial lung disease; RP-ILD, rapidly progressive ILD.

^aMyositis can be absent, mechanic's hands, Gottron's sign common, nonerosive polyarthritis, interstitial lung disease, Raynaud phenomena.

Adapted from Tansley et al. [8], Lazarou and Guerne [23], Rider et al. [24], Tansley and Gunawardena [25], and Zong and Lundberg [26].

TABLE 14.2 Bohan and Peter Criteria

1. Characteristic cutaneous changes (eg, heliotrope, Gottron's sign)
2. Symmetric, often progressive weakness of proximal musculature
3. Elevation of the serum level of one or more of the muscle enzymes, creatine kinase (CK), lactate dehydrogenase (LD), aspartate aminotransferase (ASAT), or aldolase
4. Electromyographic (EMG) changes characteristics of myopathy and denervation
5. Muscle biopsy documenting histological evidence of perifascicular atrophy, perivascular inflammatory infiltrates, and necrosis of muscle fibers

Definite DM requires the skin criterion (criterion 1) and 3 of the muscle criteria (criteria 2–5), whereas *probable DM* requires the skin criterion and 2 muscle criteria.

Exclusion criteria: The diagnosis of PM/DM requires that all other forms of myopathy (eg, infectious, metabolic, endocrine disorders, and dystrophic myopathies) are excluded by appropriate clinical, laboratory, genetic, or pathologic techniques.

Adapted from Bohan and Peter [31,32].

Definite DM requires the skin criterion (criterion 1) and 3 of the muscle criteria (criteria 2–5), whereas *probable DM* requires the skin criterion and 2 muscle criteria. *Definite PM* requires all four criteria including muscle variables and probable 3 of these, [Table 14.2](#).

Exclusion criteria: The diagnosis of PM/DM requires that all other forms of myopathy (eg, infectious, metabolic, endocrine disorders, and dystrophic myopathies) be excluded by appropriate clinical, laboratory, genetic, or pathologic techniques.

There have been several attempts to modify and improve these criteria. Some of them have acknowledged

immunopathological features [33,34]. Given advances in radiological technology, and the discovery of new MSAs, Targoff et al. suggested in 1997 criteria that included MSA (at that time antisynthetases, anti-Mi-2, or anti-SRP). He also proposed that the presence of muscle inflammation on MRI could substitute muscle biopsy findings [35]. An international collaboration in the International Myositis Classification Criteria Project and in the IMACS network has resulted in the development of new EULAR/ACR classification criteria for IIM [36]. They are not finally published, but integrate muscle weakness, skin manifestations, laboratory measurement included muscle enzymes and anti-Jo-1, to decide whether a patient can be classified as having IIM or not. If a patient is defined as IIM, a classification tree is applied to decide the IIM subgroup, eg, PM or DM. If age of onset is ≤18 years, it is defined as juvenile form.

It should be emphasized that most of these criteria are developed as *classification criteria*, and thus have limitations for use in diagnostic workup. Importantly, classification criteria will almost always mirror diagnostic criteria, but are not synonymous. Rather, a diagnosis results from a clinical evaluation for features that suggest the presence of disease [37].

2.2.2 Clinical Features and Relevant Examinations for Diagnostic Workup

The diagnosis of DM/PM and JDM is based on a constellation of clinical and laboratory tests as described in the Bohan criteria, but it has been recognized that, at least in children, few physicians do all the necessary tests to

TABLE 14.3 Criteria Supporting the Diagnosis of DM, PM, and JDM

	PM	DM	JDM
Muscle weakness	Subacute onset of proximal symmetric weakness in adults	Subacute onset of proximal symmetric weakness	Subacute onset of proximal symmetric weakness
Characteristic skin rash	Not present	Present	Present
CK level	High, up to 50 times upper limit	High, up to 50 times upper limit	Moderately elevated, may be normal, LD, AST, and ALT often elevated [7]
EMG	Myopathic units (active and chronic)	Myopathic units (active and chronic)	Myopathic units (active and chronic)
Muscle biopsy	Endomysial infiltrates, CD8+ cells invading healthy fibers; wide-spread expression of MHC class I antigen; no vacuoles	Perivascular, perimysial, and perifascicular inflammation; necrotic fibers in “wedge-like” infarcts; perifascicular atrophy; reduced capillaries	Swelling of the capillary endothelium with obliteration of the lumen, perivascular inflammation, perifascicular atrophy, reduced capillaries [7]
Autoantibodies	Antisynthetase antibodies (often seen in overlap myositis) associated with interstitial lung disease, arthritis, fever, and “mechanic’s hands”	Anti-MDA-5, anti-Mi-2; anti-TIF-1 and anti-NXP-2 (implicated in cancer-associated dermatomyositis)	Anti-TIF-1 and anti-NXP common; antisynthetase rare [29]
Magnetic resonance imaging of thigh muscles with STIR or T2 weighted images	Often show active inflammation	Often show active inflammation	Often show active inflammation
Nailfold capillaroscopy [38,39]	Microvascular alterations unusual	Often microhemorrhages, capillary enlargement, and capillary loss	Often microhemorrhages capillary enlargement, and capillary loss

Data on PM and DM adapted from Dalakas [3].

ensure that the patients meet these criteria; in particular, muscle biopsies and EMG are avoided (Table 14.3) [3,22,38,39].

Also, individual supplementary diagnostic tests should be considered in every patient to reveal extramuscular manifestations according to the symptoms (eg, functional lung tests, high-resolution computed tomography (HRCT), echocardiography, electrocardiogram, swallowing assessments, etc.).

3. PATHOPHYSIOLOGY OF CARDIAC INVOLVEMENT

Polymyositis, dermatomyositis, and juvenile dermatomyositis are considered autoimmune disorders due to the typical finding of infiltrates of T lymphocytes in muscle biopsies and due to the high frequency of autoantibodies. The adaptive immune system may have different effects in the pathophysiology of myositis as discussed below. There is also evidence to support a role of the innate immune system in the pathophysiology of these diseases as will also be discussed. Inflammatory molecules may be markers of systemic inflammation but some may also have a role in the pathogenesis by direct effect on muscle fibers leading to impaired

skeletal muscle fiber contractility. Whether similar mechanisms could affect myocardial function is not known. Here we present some information on general molecular mechanisms involved in the pathophysiology of myositis as well as some mechanisms that are in particular relevant for the heart involvement in these diseases.

3.1 Inflammatory Markers

Even though lower inflammatory markers are often seen in IIMs compared to other rheumatic diseases [40], some studies addressing cardiac involvement in IIMs have shown elevated ESR and CRP compared to healthy controls, whereas others have not [41]. Elevated CRP is a known marker of cardiovascular disease, as discussed below.

Several cytokines and chemokines have been found upregulated in blood and muscle in IIMs [42] and include proinflammatory cytokines such as interleukin IL-1 α , IL-1 β , TNF- α , type I interferons (includes interferon- α and - β), and chemokines including MCP-1 (CCL2) and IP-10 (CXCL10). It has been shown that serum levels of IL-6, IL-8, and TNF- α correlate with disease activity in DM and JDM [43], but the relative importance of the various cytokines and chemokines in

relation to disease progress in patients with myositis is still not clarified. Cytokines and chemokines may play several roles in the pathophysiology of myositis. They may contribute to recruitment of inflammatory cells into the affected tissues. Some cytokines such as type I interferons may have a direct effect on muscle fibers by upregulation of MHC class I as well as of MHC class II expression. MHC class I expression can affect muscle fiber contractility and lead to muscle weakness, as has been demonstrated in a mouse model for myositis (the MHC class I transgene mouse as discussed below under innate immunity). Whether this is the case for heart muscle is less studied.

Interestingly, many of the same mediators have shown to be important for development of heart disease, eg, in atherosclerosis [44], and heart failure in the general population [45], and plasma levels of inflammatory cytokines and chemokines appear to be elevated in direct proportion to deterioration of functional class (NYHA class) and cardiac performance [46]. However, to our knowledge, only one study has addressed the association between cytokines and cardiac affection in IIMs [47]. In this controlled study, the chemokines MCP-1, eotaxin, and IP-10 were found to be elevated in serum in JDM patients after median 16.8 years' disease duration. MCP-1 and eotaxin were also associated with diastolic and systolic dysfunction. MCP-1 is one of the key mediators associated with myocardial inflammation, atherosclerosis development, and cardiac fibrosis in the general population, and may likely cause diastolic dysfunction, and maybe systolic dysfunction [47–49]. As opposed to MCP-1, there is not much evidence for eotaxin upregulation in myositis. A small study has shown elevated eotaxin in DM patients [50], and, as mentioned above, Schwartz et al. found eotaxin upregulated in JDM compared to controls [47]. Eotaxin has been associated with fibrosis in different tissues [51,52]. It is possible that eotaxin induces similar tissue fibrosis in the heart, either by recruiting granulocytes that release profibrotic substances, or by itself. It should be noted that there is 49% homology and 64% shared protein structure between MCP-1 and eotaxin [53].

TNF- α together with IL-6 and IL-1 have been shown to be elevated in plasma in patients with heart failure in the general population, TNF- α is involved in heart failure with negative inotropic effect and promotion of left ventricular remodeling leading to hypertrophy or dilatation [54]. The inflammatory cytokine IL-17 has also been shown to regulate myocardial fibrosis in experimental models of heart failure through a RANKL-dependent mechanism. However, limited information is available on the role of these cytokines in relation to heart involvement in patients with IIM [55].

3.2 Markers for Endothelial Injury and Dysfunction

There is evidence for premature, subclinical atherosclerosis in IIMs compared to controls, shown by decreased flow-mediated dilatation of the brachial artery (FMD) in DM/PM [56], increased carotid intima media thickness in adult JDM patients [57] and increased coronary calcification in PM/DM measured by CT scanner [58]. The risk for manifest coronary artery disease in IIMs is also increased by at least three-fold, as shown in large population-based studies [59,60]. The mechanism for this increase in atherosclerotic burden in IIM is not fully understood, but one explanation might be the higher prevalence of traditional CVD risk factors (eg, diabetes, hypertension, obesity) in PM, DM, and JDM populations, compared to healthy controls [47,58,61]. There is also evidence for dyslipidemia (mainly hypertriglyceridemia and low HDL) in untreated patients both with DM and PM [62,63], and in JDM, including long-term follow up studies [47,57]; an increased prevalence of metabolic syndrome is also seen in DM and PM patients [64,65]. Cholesterol levels do not seem to be elevated in patients with IIM, but may still be associated with cardiac dysfunction. It might be that the threshold for unfavorable cholesterol levels is lower in IIMs, as described in RA [66]; further studies are needed to address this in myositis.

Numerous epidemiological, clinical, and laboratory investigations suggest that chronic inflammation and immune dysregulation exert a key role in accelerating atherosclerosis in autoimmune diseases [67], thus it seems like the inflammatory process in atherosclerosis and rheumatologic conditions is linked. Increased CRP is a well-established risk factor for atherosclerosis [44], and it is known that several proinflammatory cytokines are involved in this process as well. Whether this is applicable for patients with IIM is not known. Interestingly, the risk for being hospitalized for coronary heart disease is already high during the first year after DM/PM diagnosis [60], which might indicate that active inflammatory disease is a strong driving force that may lead to local and systemic imbalances in the coagulation system [68].

3.3 Markers for Increased Thrombogenicity

It is known that children with JDM demonstrate a prothrombotic milieu mediated by thrombospondin-1, which is associated with the TNF- α -308 polymorphism [69], present in twice as many JDM as ethnicity-matched controls [70]. Whether this is applicable to adult patients with PM/DM is not known.

3.4 Myocardial Inflammation and Fibrosis

IIMs are characterized by chronic inflammation of the skeletal muscles. It is assumed that the muscle inflammation might involve the cardiac muscle as well, causing myocarditis. Support for this comes from autopsy studies [71,72], ^{99m}technetium pyrophosphate scintigraphy [73–75], and cardiac MRI [75,76]. However, histological proof for myocarditis is in most instances lacking, and most studies do not include healthy controls. The findings of left ventricular diastolic dysfunction in DM/PM [74,77,78] and JDM [79] could also be secondary to increased ventricular stiffness due to myocarditis. Myocarditis can also lead to fibrosis, another cause of ventricular stiffness. However, it is not only cardiac filling that can be impaired; diffuse or focal fibrosis can also lead to either block or arrhythmias, as shown in the general population [80].

3.5 Innate Immune Abnormalities

There is a lack of correlation between degree of inflammatory cell infiltrates in skeletal muscle and muscle weakness, thus, some patients may have profound inflammation in skeletal muscle but limited muscle weakness, whereas others may have no detectable inflammatory infiltrates and still have weakness. These observations have contributed to a hypothesis that there is a role for a so-called nonimmune-mediated immune mechanism or nonadaptive immune or innate immune mechanisms in the pathophysiology leading to muscle weakness in myositis. In muscle biopsies of patients with myositis with muscle weakness in the absence of inflammatory infiltrates a phenotypical change in muscle fibers has been observed, called major histocompatibility complex (MHC) class I expression. This is found in most patients with myositis. Importantly, most nucleated cells in our body express MHC class I molecules with a few exceptions, differentiated muscle fibers being one. Support for a role of MHC class I expression in the pathophysiology of muscle weakness comes from genetically modified mice with MHC class I upregulation in muscle fibers (MHC class I transgene mice). These mice develop weakness before immune cells can be detected in muscle tissue. The molecular mechanisms that explain this have partly been delineated, one mechanism being endoplasmic reticulum (ER) stress as has been suggested from the MHC class I transgenic mice as well as from molecular expression in skeletal muscle tissue from patients with PM/DM [81].

Interferons are strong inducers of MHC class I expression and are also likely to be involved in the pathogenesis of PM/DM and JDM. However, they are released from inflammatory cells, mainly the plasmacytoid dendritic cells. An endogenous proinflammatory molecule

that also has the capacity to upregulate MHC class I molecules in muscle fibers is the alarmingly high mobility group box (HMGB)1, which is bound to DNA in all nucleated cells and can be actively released from necrotic cells eg, from muscle fibers after some kind of trauma or hypoxia. HMGB1 can act through TLR4 on muscle fibers and induce muscle weakness and muscle fatigue as has been demonstrated in ex vivo models [82]. HMGB1 can thus be a molecule involved in the early event of muscle inflammation. HMGB1 also has well-known negative effects on cardiomyocytes, but whether this is applicable for patients with PM/DM or JDM is not known [83].

3.6 Adaptive Immune Abnormalities

There are multiple indications to support involvement of the adaptive immune system in the pathophysiology leading to PM/DM and JDM. The infiltrates in skeletal muscle tissue are composed of both CD4⁺ and CD8⁺ T lymphocyte phenotype as well as by dendritic cells and macrophages. In some patients, large infiltrates of B lymphocytes or plasma cells may be seen. Another support for the adaptive immune system in the pathogenesis of these disorders is the frequent presence of autoantibodies. Interestingly, the myositis specific autoantibodies (MSAs) are associated with distinct clinical phenotypes as well as with distinct HLA-DR genotype, further supporting a role of the adaptive immune system in IIM (Table 14.1) [6]. Thus several new autoantigens have been identified (Table 14.1). These are ubiquitous autoantigens present in all cells but interestingly with different levels of expression in different tissues and with particularly high levels in regenerating muscle fibers. Where the first immune reaction takes place, in the muscle or in another organ, eg, the lung, has not been clarified.

Two major patterns of inflammatory infiltrates have been identified in muscle tissues from patients with myositis [84]. One is predominated by endomysial infiltrates mainly composed of CD8⁺ and CD4⁺ T cells together with macrophages and dendritic cells, surrounding the muscle fibers. This pattern indicates that the muscle fiber is the target of the immune reaction. The other pattern has a predominance of inflammatory cell infiltrates with a perivascular localization often in the perimysium. This latter pattern indicates that the blood vessels are targets of the immune response. The latter pattern is preferentially observed in adult patients with DM and in JDM whereas the first pattern with endomysial infiltrates is mainly reported in patients with PM, although in some patients these patterns overlap and in other only diffusely spread inflammatory cells can be detected. These different patterns of inflammatory cell infiltrates indicate that different molecular pathways may predominate in different subsets of myositis patients.

One prevailing hypothesis as a cause of muscle weakness is that cytotoxic CD8 T cells may attack muscle fibers leading to muscle fiber necrosis. Newer data also indicate a possible cytotoxic effect of a specific subset of T cells, so-called CD28^{null} T cells. These T cells predominate in infiltrates in skeletal muscle and have a limited TCR repertoire [85]. Moreover, both CD4 CD28^{null} and CD8 CD28^{null} T are easily activated producers of IFN- γ , TNF, and of perforin and are apoptosis resistant [85]. Thus they may have a role in perpetuating the inflammatory response in skeletal muscle and may contribute to muscle fiber necrosis and weakness. Whether these T-cell subsets are of relevance for cardiac involvement is not known.

4. CARDIAC INVOLVEMENT

IIMs are associated with increased mortality, and cardiovascular involvement has been recognized as the main prognostic factor for death. Processes affecting the heart in IIM include coronary artery disease and cardiac affection caused by inflammation (Fig. 14.2).

4.1 Prevalence of Traditional Risk Factors for Accelerated Atherosclerosis

It has been known for the last few decades that the prevalence of traditional cardiovascular (CV) risk factors is higher in patients with inflammatory rheumatic diseases than in the general population, especially well-documented in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [86,87]. Traditional CV risk factors include diabetes, obesity, hypertension, dyslipidemia, older age, smoking, and male gender.

In adult IIM, increasing evidence suggests a similarly higher burden of traditional risk factors like in other rheumatic diseases [57,58,61–65,88]. In a large cohort of patients with biopsy-proven IIM retrospectively collected, the prevalence of hypertension (62%) and diabetes (29%) was higher than the background prevalence of 9% and 4% [61]. Similar results have been noted in later case-control studies on patients with DM and PM [58,64,65]. Increased prevalence of dyslipidemia (68–88%) and obesity (33%) was found in these patient cohorts compared to healthy controls (50–73% and 10%, respectively) and abdominal obesity was prominent [58,64,65]. At the time of assessment, approximately half of the patients were receiving prednisolone in all three studies.

In JDM increased prevalence of CV risk factors has also been suggested [57,88–90]. A minor, noncontrolled study of children (4.6–16 years) with refractory myositis revealed that 23.5% of the patients had hypertension, 41.2% elevated fasting insulin level, 17.1% increased cholesterol levels, and 47.1% hypertriglyceridemia [88]. All patients received prednisolone (in combination with hydroxychloroquine

and/or methotrexate), and an effect of chronic steroid treatment cannot be ruled out. Correspondingly, adults (24–44 years) with a history of JDM had higher systolic and diastolic blood pressure but no significant increase in fasting insulin or triglycerides levels compared to healthy controls [57]. These patients had not taken any immunosuppressive medication for a median of 20 years, but all had a medication history of prednisolone and other immunosuppressives. Higher frequency of hypertension (20%) was confirmed in a cohort of patients with JDM (6.7–55.4 years) compared to none in a healthy matched control group [79]. By contrast, the same patient group had lower levels of cholesterol than controls [91]. However, even though cholesterol level was lower in patients than in controls, total cholesterol level correlated with parameters of both systolic and diastolic dysfunction, albeit only in patients with sustained active disease [47].

The basic mechanism behind the higher burden of CV risk factors in IIM patients cannot be elucidated in the setting of these recent studies. Immunosuppressive treatment-related complications have been suggested [61] as long-term glucocorticoid treatment has been linked to CV risk factors, including obesity, dyslipidemia, hypertension, and glucose intolerance [92]. However, patients with IIM were more likely to be diagnosed with hypertension prior to the myositis diagnosis rather than as a complication of treatment [61]. In addition, dyslipidemia has been recognized not only in patients with long-term disease [47,64,65] but also in patients with early and untreated DM (mean age 44.6 ± 13.6) and PM (mean age 42.9 ± 12.5) compared to an age- and gender-matched control group [62,63].

4.2 Markers for Atherosclerosis and Endothelial Dysfunction

Cardiovascular disease has been recognized as a leading cause of morbidity and mortality in RA and SLE, and increased prevalence of subclinical atherosclerotic disease has been detected [93,94]. Patients with IIM also have increased mortality compared with the general population, predominantly due to cardiovascular disease (CVD) [95–98]. Large epidemiological studies demonstrate a pooled overall 2.24-fold increased risk of coronary artery disease (CAD) in patients with IIM compared with non-IIM subjects [59,60,68,99–101]. However, very few studies have examined the atherosclerotic status of myositis patients.

Within the last few years, there has been growing evidence suggesting accelerated atherosclerosis in patients with IIM. Several noninvasive methods—biomarkers—exist to measure subclinical atherosclerosis [102]. However, a limited number of these modalities have been applied to patients with IIM [56–58,103]. Arterial stiffness, which is known to predict CV events and mortality, was assessed in 27 patients with DM or PM by ultrasonography with

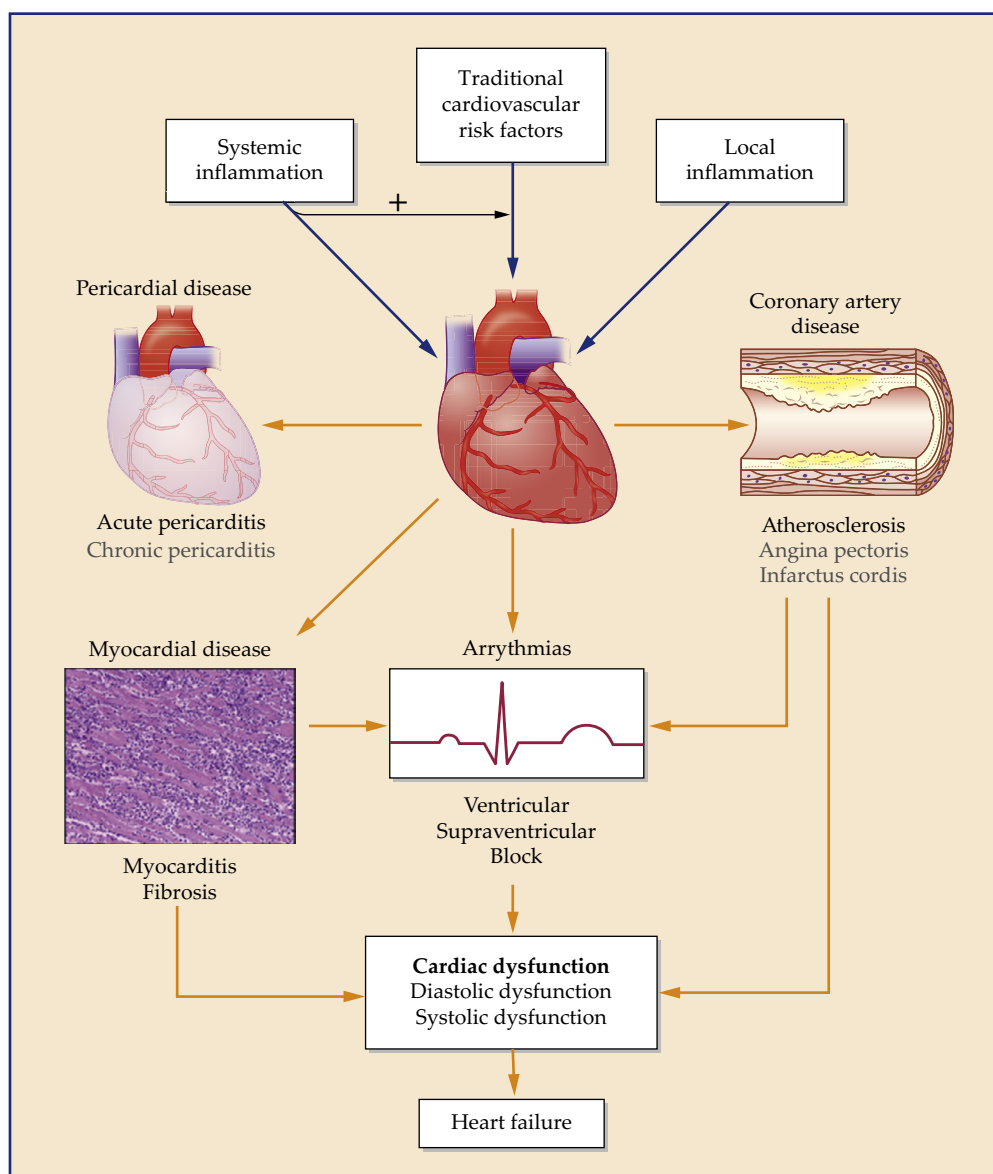


FIGURE 14.2 Traditional cardiovascular risk factors can give cardiac disease in IIM patients. Systemic and local inflammation may either have a direct effect on the myocardium or make the heart more susceptible to traditional risk factors. In the heart, disease can occur in the pericardium, coronary arteries, or the myocardium, and arrhythmias can appear. Myocardial disease can result in diastolic or systolic dysfunction, and when clinical symptoms arise, the patient has developed heart failure. Myocardial disease may result in arrhythmias, or they can occur as a result of inflammation directly influencing cardiomyocyte function. Both arrhythmias and coronary artery disease, such as myocardial infarction, can directly result in heart failure. Adapted from Schwartz et al. [178].

flow-mediated dilation of the brachial artery (FMD) [56]. Compared with healthy controls, patients with DM had decreased FMD consistent with increased arterial stiffness. Decreased FMD correlated with hypertriglyceridemia. Similarly, eight adults with a history of JDM showed lower FMD values as well as greater carotid intima media thickness than healthy controls [57].

Coronary artery calcification (CAC), a measure of sub-clinical atherosclerosis by computed tomography (CT), is associated with the degree of atherosclerotic plaque, and is predictive of CV events [104,105]. The technique is well-established in patients with suspected coronary artery

disease and has been used repeatedly in RA and SLE [102]. To date only one study has investigated CAC in IIM patients [58]. In a Danish cohort of patients with DM or PM, 20% had severe atherosclerosis compared with 4% of the healthy controls, ie, five times more frequent than in the controls (Fig. 14.3). Four patients (5%) complained of chest pain, and subsequent coronary angiography revealed significant stenosis of a coronary artery in one patient.

Inflammation has been implicated in the pathogenesis of atherosclerosis and subsequent CVD [106], and chronic inflammation associated with inflammatory rheumatic diseases is thought to contribute to the increased risk of

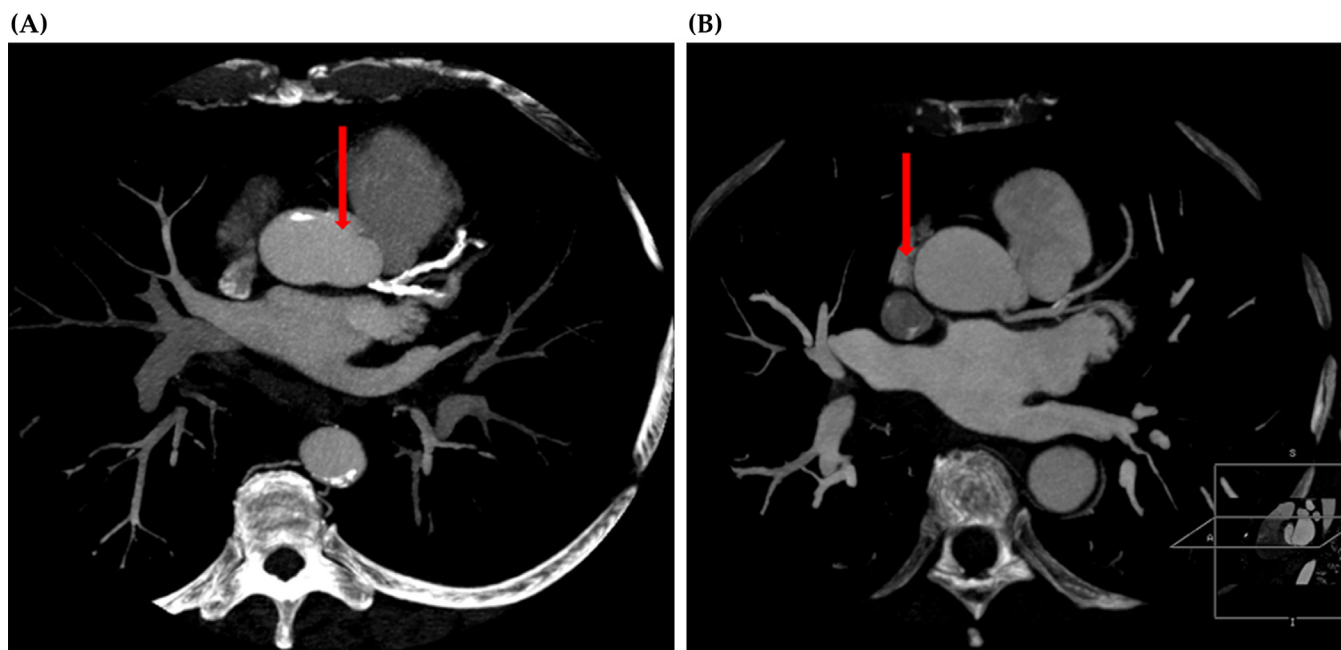


FIGURE 14.3 (A) Strongly increased coronary artery calcification in a patient with myositis (red arrow). (B) Normal coronary arteries in a healthy subject (red arrow). Adapted from the personal collection of Associate Professor Axel CP Diederichsen, Odense University Hospital, Denmark.

ischemic heart disease [107]. Although an association between high CAC score and inflammatory mediators and disease-specific factors has been reported in RA and SLE, severe CAC was not linked to disease activity in patients with PM or DM, but rather with traditional CV risk factors [58]. These findings suggest that inflammation in myositis-related atherogenesis may be less important than in other chronic inflammatory diseases. In light of the sparse current available evidence, CV risk stratification in myositis should so far be based on the same tools as for the general population [108].

4.3 Cardiac Dysfunction and Underlying Pathology

The incidence and prevalence of heart failure (HF) in myositis is not known. Although clinically evident heart failure may be rare, the presence of severe heart failure is well-documented in case reports [109–122]. Systolic and diastolic function of the heart, heart structure and valves, pericardium, and pulmonary artery pressure can be evaluated by various imaging modalities, some of which have been applied to patients with myositis. Echocardiography is the most widely used technique to assess cardiac function. With the development of more sensitive echocardiographic (ECHO) techniques within the last decade, it is now possible to assess even subclinical systolic and diastolic dysfunction. Left ventricular strain is the most sensitive method to evaluate systolic function [123] while tissue Doppler imaging (TDI) is the echocardiographic

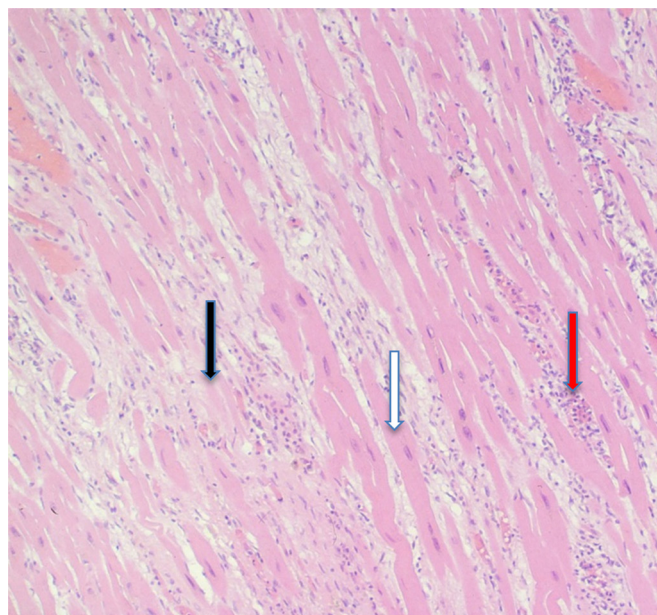


FIGURE 14.4 Cardiomyocytes (white arrow) surrounded by fibrosis (black arrow) and inflammatory infiltrates (red arrow) (Hematoxylin and eosin stain). Adapted from the personal collection of Professor Ulrik Baandrup, Vendsyssel Hospital, Aalborg University, Denmark.

technique of choice to estimate left ventricular diastolic dysfunction (LVDD) [124].

Primary heart affection due to inflammation of the myocardium has been suggested as the leading cause of HF in myositis, and inflammatory characteristics similar to those found in skeletal muscle biopsies of myositis patients have been verified in some cases by endomyocardial

biopsy (Fig. 14.4) [111,113,117–120,125,126]. In two minor autopsy studies, myocarditis was documented in 25–30% of the patients with IIM, all of which had nonspecified clinical symptoms of congestive heart failure or ECG abnormalities [71,72]. Although endomyocardial biopsy is considered to be the gold standard for confirmation of myocarditis, it is an invasive method that is not routinely performed. Furthermore, the possibility of sample error must be considered. It is possible to detect subclinical inflammation within the myocardium with the advent of noninvasive, highly sensitive imaging techniques such as magnetic resonance imaging (MRI) and ^{99m}Tc -pyrophosphate (^{99m}Tc -PYP) scintigraphy (the latter is mostly used in scientific settings).

4.3.1 Systolic and Diastolic Dysfunction— Echocardiographic Findings

Left ventricular systolic dysfunction as evaluated by left ventricular ejection fraction (LVEF) is uncommon in patients with adult onset myositis. In minor IIM cohorts, none of the patients had decreased LVEF [127–130]. More recently, cross-sectional studies from different centers confirmed preserved systolic function (LVEF) in PM/DM [41,74,75,78]. However, LVEF is not the most sensitive method to measure reduced systolic function. In a recent study, systolic function was preserved in terms of LVEF in 59 patients with JDM examined at long-term follow-up, median 17 years after disease onset [91]. On the other hand, left ventricular long axis strain, the suggested superior parameter for evaluation of LV systolic function, was reduced in JDM compared with healthy controls. The observed reduction in LV function was subclinical, but correlated to important disease characteristics. Similarly, LVEF was within the normal range in a cohort of 30 adult patients with recent onset PM/DM [131]. However, measurements of systolic function by a more sensitive and detailed TDI method showed subclinical systolic dysfunction restored after 3-month of immunosuppressive treatment including steroids [131].

There is growing evidence of increased risk of LVDD in patients with myositis, not only in patients with long-term disease, but even as an early feature of cardiac involvement. In 26 adults with long-term PM/DM (mean disease duration 6 years), 11 patients (42%) had LVDD [128]. In this minor series, LVDD was not associated with clinical symptoms, comorbidities, or disease activity. Two studies on Asian populations reported significantly higher frequency of LVDD in patients with PM/DM compared to healthy controls [41,78]. Forty-six patients with no clinical signs or history of cardiovascular disease and with newly diagnosed PM/DM (mean disease duration 4.8 months) and 21 matched controls underwent conventional ECHO and TDI [41]. Patients had indices of higher left ventricular filling pressure, a marker for LVDD, which correlated to female sex, late disease onset, and longer

disease course. Similar findings have been noted in 51 patients with DM independently associated with longer disease duration (mean disease duration 8 months) [78]. Notably, patients had no evidence of clinical cardiovascular disease, hypertension, or diabetes. In a recent multicenter study, LVDD was not linked to traditional CV risk factors but associated with longer disease duration, presence of autoantibodies, and high myocardial ^{99m}Tc -pyrophosphate (^{99m}Tc -PYP) uptake, the latter a marker of inflammation [74]. The possible link between myocardial inflammation and LVDD is further supported by the findings of development of LVDD in patients with newly diagnosed PM/DM during a 3-month follow-up period [131].

In JDM, LVDD was found in a Norwegian JDM cohort and the echocardiographic parameters correlated with early disease activity 1 year after diagnosis [79]. In a subgroup of these patients ($n=54$), high levels of proinflammatory cytokines (eotaxin, MCP-1) were correlated with both systolic and diastolic dysfunction in patients with active disease [47].

In the general population, LVDD is related to traditional CV risk factors as hypertension, diabetes mellitus, obesity, female sex, and older age, and is widely recognized as an independent predictor of mortality [132]. In the context of myositis, the current literature points to the myositis disease as the primary cause of decreased diastolic function. However, the prognostic implication of subclinical diastolic dysfunction in myositis patients with preserved systolic function cannot be predicted based on existing literature, but will have to be addressed in future, prospective studies.

4.3.2 Underlying Myocardial Pathology

4.3.2.1 MRI Findings

Cardiac MRI plays a central role in the diagnosis of acute myocarditis [133], which is an important cause of cardiac dysfunction. In the setting of IIM, cardiac MRI has been used to identify myocarditis using two types of techniques: delayed enhanced images after injection of a contrast agent and T2-weighted images [134]. In recent years, T1 mapping has been used to investigate cardiac fibrosis [135]. T1 mapping really measures extracellular volume, and this will reflect edema in acute disease. However, in chronic disease, T1 mapping signals are correlated to degree of diffuse fibrosis. The technique has so far not been used in IIM.

Case reports have described subclinical myocarditis by MRI in patients with recent onset and untreated disease and also later during disease course [136,137]. In minor IIM cohorts, 62–75% of patients had signs of myocarditis assessed by gadolinium enhancement MRI [76,138,139]. EF was normal evaluated by functional cardiac MRI in two of the studies [138,139] but abnormal MRI correlated with lower EF in another [76]. However,

those findings were not validated against histopathology or compared with age matched healthy controls. Notably, no generally accepted standardized cut-off value between normal and abnormal myocardium for a given cardiac MRI technique exists, and the reported high rate of subclinical myocarditis in IIM should be interpreted with some caution, which stresses the importance of control subjects.

In a recent controlled study, no significant differences of the cardiac MR findings, including T2-weighted and EF values, were detected between 14 patients with newly diagnosed myositis and 14 healthy controls [75]. However, the distribution pattern of T2-weighted values differed among the groups, ie, more patients had high T2-weighted values, which is indicative of inflammation (Fig. 14.5). Due to the small sample size, no correlation to clinical parameters was made.

4.3.2.2 Scintigraphic Findings

Like MRI, ^{99m}Tc pyrophosphate (^{99m}Tc -PYP) scintigraphy has been reported to be useful in the diagnosis and monitoring of myositis by demonstrating sites indicative of skeletal muscle inflammation [73,125,140,141]. The method is also capable of detecting cardiac muscle involvement [73–75,125].

Abnormal myocardium PYP-uptake was observed in 57% of patients with PM/DM in different stages of the disease, with a close association between the magnitudes

of PYP-uptake and the frequency or severity of ECG changes [73]. Two patients with high PYP-uptake, severe ECG abnormalities, and left ventricular dysfunction died. Autopsy showed inflammation, degeneration, and fibrosis of the myocardium in accordance with PYP scintigraphy results.

A controlled study recently established a correlation between the magnitudes of myocardium PYP-uptake and diastolic dysfunction of the heart, which strongly suggests that inflammation is a significant contributor to heart affection in IIM [74]. Although these findings were subclinical and need further prospective studies, they imply awareness of tight disease control in terms of inflammation.

4.4 Electrocardiographic Findings

The most frequently reported cardiac abnormalities in patients with IIM are rhythm and conduction abnormalities including nonspecific S-T deviations, axis rotation, signs of chamber hypertrophy and enlargement, various degrees of bundle branch blocks, and atrial and ventricular arrhythmias, sometimes even fatal [9,79,128–130,142–145].

Rhythm and conduction disturbances can be caused by structural abnormalities in the conduction system or the myocardium that result from necrosis, fibrosis, calcification, infiltrative lesions, or impaired vascular supply. In myositis, disturbances of conduction and rhythm have been attributed to pathological changes in the conduction system with microscopic findings in autopsies consistent with direct involvement by the disease process [71,72,117,143,146–148]. Different degrees of inflammation and fibrosis have been detected in all parts of the conduction system, sinoatrial and atrioventricular nodes, A-V bundle (bundle of His), right and left bundle branches, and Purkinje fibers, changes that corresponded with the respective ECG findings of the patients. The role of inflammation and/or fibrosis is not obvious in individual cases.

4.4.1 Conduction Disorders

Conduction disorders are common in myositis, and a variety of conduction disorders have been described casuistically, including first, second, and third degree atrioventricular blocks, incomplete and complete right and left bundle branch blocks, and left anterior fascicular block [109,111–118,146,147,149–152]. Isolated ECG changes can be found, or in combination with heart failure.

In previous studies, different blocks have been described in 10–38% of IIM patients [126,128–130,142]. A retrospective study of 77 patients with myositis (13 patients <16 years old) showed 9% with right bundle branch block, 3% with left bundle branch block, and 13% with left anterior fascicular block [142]. In a recent

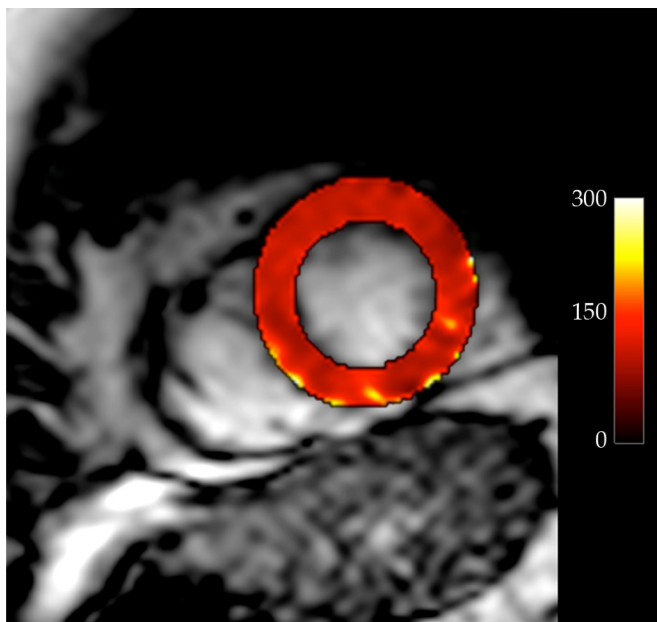


FIGURE 14.5 Cardiac magnetic resonance image in the left ventricular short-axis view with overlying color-coded T2 map showing increased T2 values, indicating diffuse myocardial edema as a sign of myocarditis. The color bar shows T2 values. Adapted from the personal collection of Esben SS Hansen, MSc., Aarhus University Hospital, Denmark.

controlled study, which used strictly quantitative criteria for pathological ECG defined by international guidelines [153–155], ECG abnormalities were observed in 18% of patients versus 10% of healthy controls [74]. Patients had significantly longer QRS and QTc intervals compared with healthy controls. Similarly, 10 of 58 patients with a history of JDM (17%) had pathological ECG compared with 4 of 57 healthy controls (7%), but without prolongation of QRS or QTc intervals compared with controls [79]. ECG changes may even be more pronounced in newly onset myositis. ECG was pathological in 6 of 14 adults with newly diagnosed and untreated IIM (43%) compared to 2 of 14 healthy controls (14%) with prolonged PQ, QRS, and QTc intervals [75].

Measurements of the QT interval are a matter of great importance because of the relationship between prolongation of the QT interval and potentially lethal ventricular arrhythmias. The presence of the QT interval in an ECG report should call for careful clinical evaluation [154]. As conduction disorders—including prolonged QT interval—seem to occur frequently, ECG may be recommended on a regular basis during disease course. However, this needs clarification in future, prospective studies.

4.4.2 Supraventricular and Ventricular Arrhythmias

For detection of arrhythmias, ECG recording is the first performed. For example, in atrial fibrillation, this

often will suffice. However, paroxysmal arrhythmias usually require continuous electrocardiographic monitoring, such as Holter monitoring (24 h ECG). Abnormal Holter monitoring has been found in 52–88% of patients with myositis, showing supraventricular and ventricular extrasystoles, supraventricular and ventricular tachyarrhythmia, and atrial fibrillation [128,130,143].

Supraventricular arrhythmias including atrial fibrillation have been reported in 12–50% of patients with myositis [128,130,143]. In addition, the frequency of supraventricular tachyarrhythmia was significantly higher in myositis (47%) compared with healthy controls (26%) [74]. The same study did find left atrial enlargement in myositis patients, a known marker of increased risk for supraventricular arrhythmias.

Episodes of nonsustained ventricular tachycardia (VT) have been documented in patients during 24-h Holter (Fig. 14.6) [74,130,156,157], which might ultimately lead to cardiac arrest by ventricular fibrillation [115]. Children with JDM have also been reported with nonsustained VT, a finding that was associated with disease activity [158,159]. However, after long disease duration (median 13.5 years), neither nonsustained VT or other significant arrhythmias were observed by 24-h Holter, although only 50% of the patients had inactive disease according to the Paediatric Rheumatology International Trials Organisation (PRINTO) criteria [145].

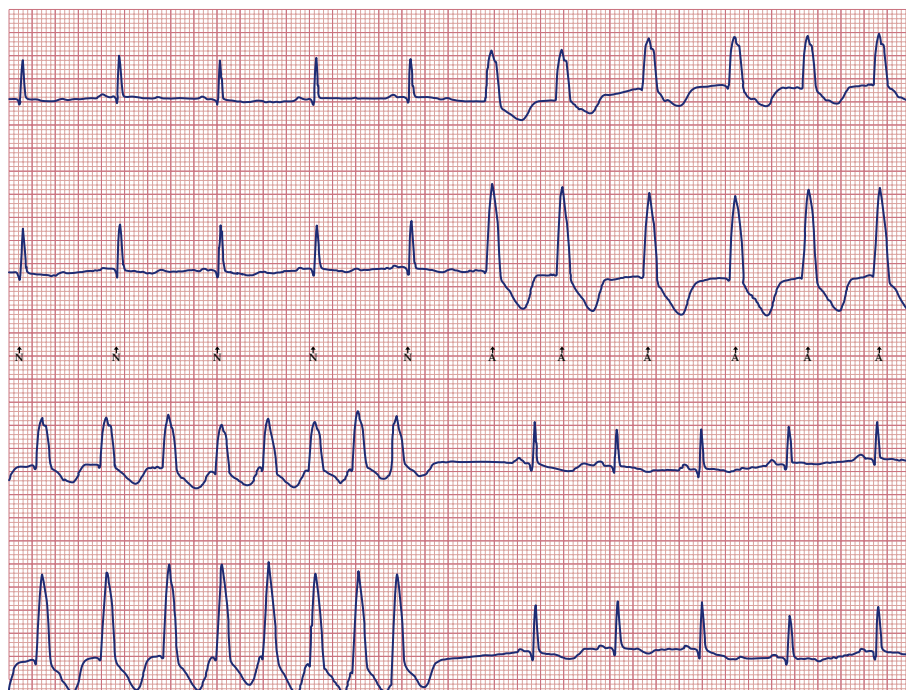


FIGURE 14.6 Nonsustained ventricular tachycardia (VT) in a patient with Antisynthetase Syndrome. Adapted from the personal collection of Associate Professor Louise Pyndt Diederichsen.

4.5 Other Cardiac Diseases

4.5.1 Heart Structure and Valves

The prevalence of structural changes of the heart in IIM is uncertain. Various valvular abnormalities (mitral valve prolapse, insufficiency, or stenosis) have been described in patients with myositis [127–130,143] but without significant difference compared to controls in recent controlled studies [41,75].

4.5.2 Pericardial Disease

Pericardial involvement might occur, and hemodynamic insignificant pericardial effusion (Fig. 14.7) has been observed in 4–25% of adults with IIM [128–130,143]. Likewise, pericarditis was observed in 12–15% of patients with JDM, during disease course [79,160]. Pericarditis may even be more common in subgroups of patients with antisynthetase syndrome (ASS). In a series of patients from European multicenter study, 9 of 18 anti-PL-7- positive patients (50%) had pericardial effusion during disease course [161]. Some ASS patients might even have hemodynamic significant pericardial effusion requiring pericardiocentesis [162,163].

4.5.3 Pulmonary Hypertension

ECHO is currently the most effective screening tool for pulmonary hypertension (PH). PH is a potentially life-threatening disorder that can lead to death through right ventricular failure. PH is common in connective tissue disorders and consists of pulmonary artery hypertension (PAH), PH caused by myocardial involvement, pulmonary veno-occlusive disorders, and interstitial lung disease. PAH has been studied particularly

in systemic sclerosis, with an estimated prevalence of 7–12% [164]. In antisynthetase syndrome (ASS), occurrence of severe PAH is evident in case reports [165–168]. Based on right heart catheterization, PH has been confirmed in 8% of the ASS patients in two major retrospective cohort studies [169,170] and dramatically worsened the prognosis with a 3-year survival rate of 58% [170]. PH was systematically associated with interstitial lung disease (ILD) in ASS, although other mechanisms than lung disease could not be excluded in the setting of this study [170]. These findings support the routine of screening for PH in ASS by ECHO at baseline and annually, as recommended by ESC guidelines for systemic sclerosis [164].

5. CONSIDERATIONS FOR TREATMENT OF CARDIAC DISEASE IN IIM

5.1 Traditional Cardiovascular Risk Factors

Increasing evidence suggests a higher burden of traditional cardiovascular (CV) risk factors in myositis like in other inflammatory rheumatic diseases [57,58,61–65,88]. Furthermore, patients with IIM have an increased risk of coronary artery disease (CAD) compared to non-IIM subjects [59,60,68,99–101]. However, knowledge regarding the potential influence of and associations between disease specific parameters, traditional CV risk factors, and CAD is so far lacking [47,56,58].

For the time being, until clear evidence suggesting otherwise has emerged, the recommendations for CV risk stratification and CV prevention should be the same for patients with myositis as for the corresponding general population (I-C) [108,171]. However, special attention must be drawn to the use of lipid-lowering agents, which may be associated with myopathic side effects and may in some individuals be the main cause of disease, ie, statin-associated autoimmune necrotizing myopathy [172]. A recent study on the use of lipid-lowering therapy in patients with IIM treated by IIM specialists showed that statins are commonly used. In some of the patients, myositis also became more severe during statin treatment [173]. This study points to the fact that treatment of dyslipidemia in patients with IIM is complex and requires careful attention until further prospective studies have been carried out.

5.2 Cardiac Involvement

Currently, there are no special guidelines for the treatment of heart involvement in myositis. Development of cardiac manifestations in myositis may occur early, at disease onset, as well as during disease course. Clinical heart disease can occur under immunosuppressive

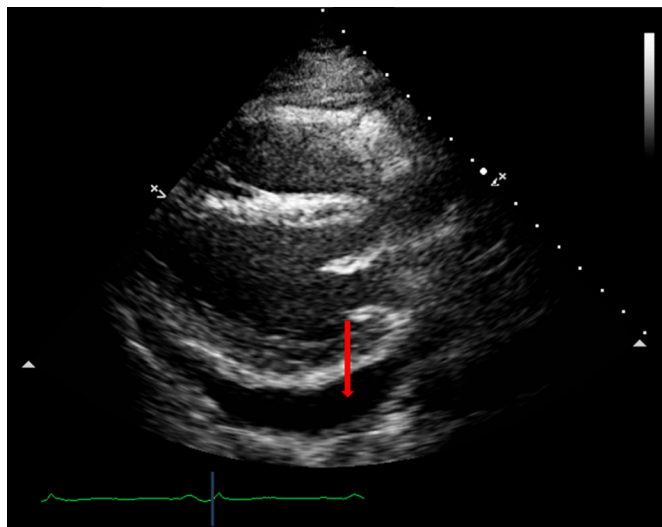


FIGURE 14.7 Echocardiographic image of the heart in the longitudinal plane showing pericarditis (red arrow) in a patient with myositis. Adapted from the personal collection of Mikkel Hougaard, MD, PhD, Odense University Hospital, Denmark.

treatment, in the course of disease flare, or even in disease remission [109,111–114,116,118,119,137,142,143,146,148–152,157,159,174,175]. Conflicting results exist regarding the associations between disease activity and cardiac involvement and no firm conclusions can be drawn.

Although inflammatory changes within the myocardium form the basis for the cardiac dysfunction seen in myositis, the impact of immunosuppressive therapy on the presence and severity of cardiac involvement is unknown. Casuistically, there are case reports on successful pharmacological treatment of arrhythmia, conduction disorders, and heart failure in both children and adults with myositis [111,113,120,142,143,151,159,174,176]. However, in addition to anti-inflammatory therapy these patients received antiarrhythmic drugs, heart failure management, or pacemakers according to their cardiac manifestations. It is, therefore, difficult to estimate the beneficial effect of immunosuppressive treatment alone on the improvement of cardiac dysfunction. However, it is reasonable to assume that an early and sustained reduction in inflammation may reduce the risk of developing diffuse interstitial myocardial fibrosis. This is an important aspect, since inflammation is treatable, while fibrosis to a large extent is irreversible.

Few studies have investigated the effect of treatment for concomitant cardiac disease in patients with newly onset myositis. A study of eight newly diagnosed patients with PM/DM showed subclinical or clinical heart affection by ECG, Holter, ECHO, or cardiac scintigraphy [143]. All patients improved during prednisolone treatment, except two patients, who had persistent elevation of CK and prolonged cardiac manifestations. Six of the patients required antiarrhythmic drugs, diuretics, and/or digitalis, in addition to corticosteroids. The effects of anti-inflammatory drugs have been evaluated in four patients with recent onset and untreated IIM and MRI verified myocarditis [136]. All four patients complained of dyspnea, but only one patient had abnormal cardiac screening tests, which included TnI/NT-pro-BNP, ECG, and ECHO, and none had heart failure. The patients were treated with methylprednisolone (1000 mg for 3 days) followed by per oral prednisolone and (1) monthly infusion of cyclophosphamide for 6 months followed by azathioprine in three patients, or (2) hydroxychloroquine and azathioprine in one patient. After 2 months, dyspnea had improved in all patients. Cardiac MRI after 6 months of treatment showed normalized contractile function and reduced inflammation. Similarly, in another study, subclinical myocarditis was verified with MR at disease onset in 15 of 20 patients with PM/DM [139]. At follow-up, inflammation was reduced in 10 of 12 patients after 3 months of treatment with glucocorticoids; the dosage was not stated. In a recent study of 30 patients with newly onset PM/DM, subclinical systolic dysfunction measured by ECHO returned to normal after a 3-month

period of immunosuppressive therapy including high-dose glucocorticoid (initially 0.5–3 mg/kg/day) [131].

Cardiac dysfunction may also develop during disease course. A patient with DM treated with prednisolone and methotrexate for 8 months developed recurrent palpitations, dizziness, and dyspnea in addition to persistent disease activity [151]. ECHO showed normal EF but Holter demonstrated multifocal atrial tachycardia, ventricular premature beats, and a few second-degree atrioventricular blocks. Rituximab (1.000 mg every 2 weeks for 2 doses) and methylprednisolone (1.000 mg for 3 days) was initiated, followed by oral prednisolone. The clinical condition improved and the cardiac symptoms disappeared. At the 8-month follow-up, Holter demonstrated normal heart rhythm.

If cardiac involvement—even subclinical—is rendered probable by cardiac symptoms and/or basic cardiac measurements (physical examination, cardiac troponins (TnT/TnI) [177], ECG, etc.), we recommend an evaluation by a cardiologist, if not already involved. If heart abnormalities due to myocarditis are confirmed, a multidisciplinary approach between the rheumatologist and the cardiologist should be undertaken. Based on case reports, glucocorticoids are a cornerstone in the treatment of myocarditis (I-C), as monotherapy or in combination with other immunosuppressive drugs (IIa-C). In addition to immunosuppressive therapy, management of cardiac involvement may require individual treatments, anti-arrhythmic drugs, clinical or subclinical heart failure management, or pacemaker according to the cardiac manifestations (I-C). Hemodynamic insignificant pericarditis seems to respond readily to corticosteroid alone (IIa-C) [143,161].

6. CONCLUSIONS

IIMs are associated with increased mortality, and cardiovascular involvement has been recognized as the main prognostic factor for death. The main processes affecting the heart in IIM include coronary artery disease and cardiac affection caused by inflammation. Clinically manifest heart involvement with heart failure and/or severe rhythm and conduction disturbances may be rare but subclinical heart affection with systolic/diastolic dysfunction, minor rhythm, and conduction disturbances and pericarditis is well-recognized, even though the prevalence is unknown.

Increased coronary calcification in PM/DM has been identified by CT scan, and the risk for manifest coronary artery disease in IIM is increased by at least three-fold, as shown in large population-based studies. The mechanism for this increase in atherosclerotic burden in myositis is not fully understood, but one explanation might be the higher prevalence of traditional CVD risk factors

(eg, diabetes, hypertension, obesity) in IIM, compared to healthy controls. Also, there is evidence for dyslipidemia (mainly hypertriglyceridemia and low HDL) in patients with DM, PM, and JDM. Cholesterol levels do not seem to be elevated in patients with IIM, but may still be associated with cardiac dysfunction. It might be that the threshold for unfavorable cholesterol levels is lower in IIM, as described in RA; further studies are needed to address this in myositis.

Inflammation has been implicated in the pathogenesis of atherosclerosis, and subsequent cardiovascular disease and chronic inflammation associated with inflammatory rheumatic diseases is thought to contribute to the increased risk of ischemic heart disease, as is evident in RA and SLE. Whether this is applicable for patients with IIM is not known. Interestingly, the risk for being hospitalized for coronary heart disease is high already during the first year after DM/PM diagnosis, which might indicate that active inflammatory disease is a strong driving force that may lead to local and systemic imbalances in the coagulation system.

IIMs are characterized by chronic inflammation of the skeletal muscles. It is assumed that the muscle inflammation might involve the cardiac muscle as well, causing myocarditis. Support for this assumption comes from case reports and autopsy studies of myositis patients with findings in endomyocardial biopsies resembling the inflammation in the skeletal muscle biopsies. In addition, highly sensitive imaging techniques as cardiac 99m technetium pyrophosphate scintigraphy and MRI have shown subclinical myocarditis, in some cases confirmed by biopsy.

In general, systolic function is preserved in myositis. However, using sensitive TDI techniques subclinical systolic dysfunction has been identified, which might be related to myocarditis. In favor of this potential association, subclinical systolic dysfunction disappeared during 3-months of immunosuppressive treatment in newly diagnosed patients with PM/DM. The evident findings of left ventricular diastolic dysfunction in DM/PM and JDM could also be secondary to myocarditis. Myocarditis can lead to myocardial fibrosis as revealed by endomyocardial biopsy in myositis, another cause of ventricular stiffness. The possible link between myocardial inflammation/fibrosis and diastolic dysfunction is further supported by the findings of development of diastolic dysfunction in patients with newly diagnosed PM/DM during a 3-month follow-up period. Furthermore, diastolic dysfunction has been associated with disease duration in both PM/DM and JDM.

However, it is not only cardiac function that can be impaired in myositis; diffuse or focal fibrosis can also lead to either block or arrhythmias, as shown in the general population. In case reports, conduction and rhythm disturbances have been attributed to pathological changes in the conduction system with microscopic

findings in autopsies consistent with direct involvement by the disease process. Different degrees of inflammation and fibrosis have been detected in all parts of the conduction system, changes that corresponded with the respective ECG findings of the patients.

Pericardial involvement might occur and hemodynamic insignificant pericardial effusion has been observed in 4–25% of adults with IIM. Pericarditis may even be more common in subgroups of patients with antisynthetase syndrome, some of which even might have hemodynamic significant pericardial effusion. In the same subgroup of patients, occurrence of severe pulmonary hypertension is evident in case reports and has been confirmed in 8% of these patients in two major retrospective cohort studies. These findings support the routine of screening for pulmonary hypertension in antisynthetase syndrome by ECHO at baseline and annually.

Currently, there are no special guidelines for diagnostic algorithm or treatment of heart involvement in myositis. Development of cardiac manifestations in myositis may occur early, at disease onset, as well as during disease course. For the time being, until clear evidence suggesting otherwise has emerged, the recommendations for CV risk stratification and CV prevention should be the same for patients with myositis as for the corresponding general population. Although inflammatory changes within the myocardium form the basis for the cardiac dysfunction seen in myositis, the impact of immunosuppressive therapy on the course of cardiac involvement is largely unknown. If cardiac involvement—even subclinical—is rendered probable by cardiac symptoms and/or basic cardiac measurements, a multidisciplinary approach between the rheumatologist and the cardiologist should be undertaken. In addition to immunosuppressive therapy, management of cardiac involvement may require antiarrhythmic drugs, clinical or subclinical heart failure management, or pacemaker according to the cardiac manifestations.

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Gout

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1. INTRODUCTION

1.1 Epidemiology of Gout

Gout is among the most common forms of inflammatory arthritis. The most recent data from the National Health and Nutrition Examination Survey (NHANES) 2007–2008 estimate the prevalence of gout to be 3.9% in the United States [1]. Several reports suggest the prevalence of gout is increasing [1–6] while others do not [7]. This rise in gout prevalence coincides with a rise in the prevalence of obesity and metabolic syndrome. Fructose, abundantly present in sugar-sweetened beverages, has been incriminated as a possible explanation of this rise in the prevalence of gout, obesity, and metabolic syndrome [8–11]: Fructose may cause a rise in serum uric acid by increasing the degradation of ATP to AMP, which is further metabolized to uric acid [12]. Cardiovascular diseases are the main cause of death worldwide, with coronary heart diseases being the most prevalent. For the past years, evidence has shown that there is an association between gout and cardiovascular diseases and that they share possible common pathophysiological pathways.

As in cardiovascular diseases, men are more frequently affected by gout than women. The NHANES 2007–2008 estimated the prevalence of gout in men at 5.9% and in women at 2.0% [1]. This difference between men and women has been attributed, at least partially, to the uricosuric effects of estrogen. In the postmenopausal period, the prevalence of gout in women tends to rise, but never attains the same levels as in men [3,13–15]. In men, gout prevalence rises from the age of 35 onward [3,13,15].

The prevalence of gout is also dependent on ethnicity. In the NHANES 2007–2008 study, the prevalence of gout was reportedly higher in black people (5.0%, 95% CI 3.3–6.6%) compared to white people (4.0%, 95% CI 3.3–4.8%) [1].

In a recent study from Atherosclerosis Risk in Communities (ARIC), blacks had a 1.5 times higher risk of gout, even after adjustment for well-known confounders [16]. In the ARIC study, higher uric acid levels may partially explain the higher risk of gout, especially in men. In another study comparing the risk of black and white people, the higher risk of gout in black men was suggested to be related to hypertension [17]. But this study did not adjust for differences in uric acid levels and renal function disturbances. Further, gout prevalence seems to be higher in particular populations such as New-Zealand Maori and Hmong Chinese [15].

1.2 Genetics of Gout

1.2.1 Genetics

Until the last decade, knowledge about the contribution of specific genes in the pathogenesis of gout was limited to those that were associated with rare metabolic and renal disorders, such as hypoxanthine guanine phosphoryl transferase-related disease (Lesch–Nyhan Syndrome), phosphoribosyl pyrophosphatase synthetase-related disease, glycogen-storage diseases, or medullary cystic kidney diseases [18]. In the last decade, the Human Genome Project and several genome-wide associations scans (GWAS) have contributed to the identification of genes involved in the renal urate-transport [18,19]. Because of the associations between uric acid, gout, and cardiovascular diseases, a few studies have evaluated the causal character of this association, applying the principle of Mendelian randomization. Mendelian randomization is based on the principle that alleles are randomly allocated during gamete formation. A Mendelian randomization allows estimating the unbiased effects of a putative risk factor, in this case uric acid, on a disease

outcome, eg, cardiovascular risk factors and/or cardiovascular diseases. Only one of four studies has shown an association between serum uric acid and cardiovascular risk factors or coronary heart disease [20–24]. The only study demonstrating an association between uric acid levels and systolic blood pressure was done in a more controlled setting and in a more homogeneous population, which could explain the different results [18,23].

2. CLINICAL PRESENTATION AND DIAGNOSTIC CRITERIA

2.1 Clinical Presentation

The phenotype of gout distinguishes three clinical stages: a first stage defined as “asymptomatic hyperuricemia,” a second stage known as “acute gout,” and a third, more chronic stage called “tophaceous gout [25].”

2.1.1 Asymptomatic Hyperuricemia

This stage describes patients with *hyperuricemia* who are at risk of developing gout but who do not yet have any clinical symptoms. Eventually, 22% of patients with chronic hyperuricemia will develop gout [26] but 78% will not. While hyperuricemia predisposes to clinical gout, the association is not strong enough to have imminent clinical repercussions [27]. Duskin-Bitan et al. followed 5234 individuals for 7 years and identified the level of uric acid as being the only statistically significant risk factor for eventually developing gout in men (OR 11.2 95% CI 3.6–35.2 for uric acid levels higher than 7 mg/dL (0.42 mmol/L), increasing further to OR 624.8 95% CI 134.0–2913.1 for uric acid levels higher than 10 mg/dL (0.60 mmol/L)) [28]. Other risk factors, such as the use of thiazides, alcohol consumption, and renal function did not remain statistically significant in the multivariate analysis. However, in another study in which 2479 women and 1951 men were followed up for 52 years, increasing age, obesity, alcohol consumption, diuretic use, and hypertension were all associated with a higher risk of clinical gout [29]. In women, higher levels of uric acid increased the risk of gout but at a lower rate than in men [29]. There was also a stronger age effect in women than in men. This could be explained by a higher clearance of uric acid, possibly caused by estrogen. It could be argued that the definitions for hyperuricemia should differ between men and women.

There is no international consensus about how hyperuricemia should be defined. Physiologically, monosodium urate (MSU) crystal deposition may occur at 6.8 mg/dL (0.41 mmol/L) and above. One could also define hyperuricemia according to the risk of gout and to the risk of developing cardiovascular disease [30]. Hyperuricemia could then be defined as a serum uric

acid level above 6 mg/dL (0.36 mmol/L), or—more strictly—above 5.7 mg/dL (0.34 mmol/L) in men and above 4.5 mg/dL (0.27 mmol/L) in women—when taking differences in cardiovascular risk into account [30].

Another important recent observation is that MSU crystal deposition can even be found in asymptomatic hyperuricemic patients, raising the hypothesis that asymptomatic hyperuricemia may not be as asymptomatic as previously thought but should be seen as an early stage of chronic gout [31].

2.1.2 Acute Gout

The typical clinical presentation of acute gout is also known as “podagra,” which means “foot trap” in Greek. It presents with a suddenly occurring extremely painful, red, and swollen “big toe” that usually spontaneously resolves within a few days. The arthritis typically starts by the end of the night, in the early morning [32].

Usually, the presentation is mono- or oligoarticular, but 3–14% of patients present with polyarticular arthritis [25]. This is especially the case in older patients [33]. Any joint, even those of the spine, can be affected by gout but the disease more often affects the lower limbs; shoulder and hip joints are rarely affected [25].

The period between the attacks is called “intercurrent” gout: During this period, the patient is asymptomatic, meaning that he does not have signs and symptoms of arthritis. However, when left untreated, most patients will develop a second attack within 6 months to 2 years [25]. This can be explained by the fact that MSU crystal depositions remain present, which implies a chronic rather than an acute disease, and may evoke subsequent acute attacks [31]. In addition, it has been demonstrated that inflammation, albeit subclinical, remains present during the “intercurrent period [34].”

2.1.3 Chronic Gout

Chronic gout is longstanding gout associated with complications, such as tophus depositions and/or bone/joint damage [31]. Tophi are accumulations of MSU crystals in soft tissues that are surrounded by inflammatory cells [35]. When clinically apparent, tophi are subcutaneous nodules that often appear white (due to the MSU crystals) (Fig. 15.1). They are mostly located in the fingers (pulp or joints), elbows, hallux, Achilles’ tendon, and in the ears, but may appear everywhere [25,35]. A few case reports have demonstrated the presence of tophi in the heart, usually on the mitral valve in patients with longstanding, untreated, tophaceous disease [36–38]. The transthoracic echocardiogram reveals a hyperechoic mass that usually does not interfere with the function of the valve [37,39]. Tophi usually appear after longstanding disease, but it has recently been demonstrated that 16% of patients with gout for less than 10 years already have tophi at presentation of

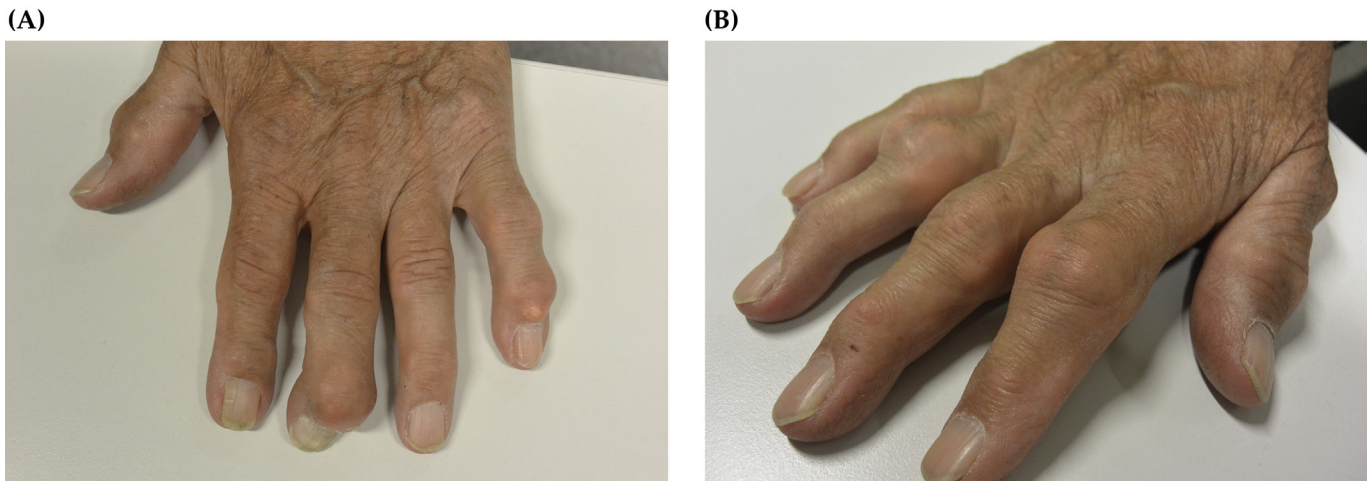


FIGURE 15.1 Tophi present on the hand of a patient. *Adapted from the personal collection of the authors.*

arthritis [156]. Impaired renal function, the presence of genetic variants, and increased serum urate levels have been identified as risk factors for tophaceous gout [35]. Tophi cause structural damage to the joints. In a recent MRI study, the presence of tophi was associated with bone erosion, but not with edema and synovitis, suggesting they are key factors in the occurrence of joint destruction in gout [40]. Tophaceous gout is also associated with higher economic burden [41] and poor functional outcome and quality of life, [42–44] and with increased mortality due to cardiovascular diseases [45].

2.2 Diagnostic Criteria

The detection of MSU crystals in the synovial fluid of an affected joint is considered to be the gold standard for the diagnosis of gout (Fig. 15.2). Different classification criteria have also been developed (Table 15.1). It should be stressed that the criteria from Mexico and the Netherlands were developed to be diagnostic criteria, whereas the Rome and the American Rheumatism Association (ARA) are considered classification criteria. Comparison of those criteria with the gold standard and identification of MSU crystals showed that the sensitivity of all the existing criteria sets was satisfying (over 80%) in early and established disease but that they lack specificity especially later in the disease [46]. This led to the development of new classification criteria by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) [47].

When considering each item of the classification criteria, it seems that only the presence of tophi and a previous response of an acute arthritis episode to colchicine are associated with a higher likelihood of having gout, whereas features systematically used as a diagnostic aids, such as podagra/1st MTP involvement, have shown poor

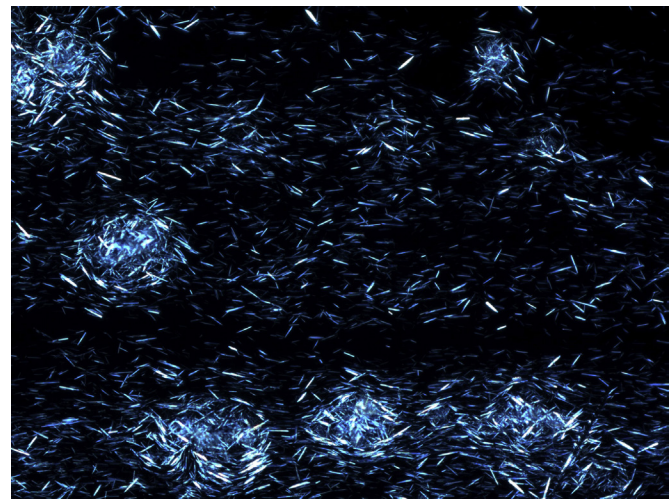


FIGURE 15.2 Birefringent needle-shaped monosodium urate crystals in synovial fluid under a polarized microscope (magnification: 200×). *Adapted from the personal collection of the authors.*

diagnostic accuracy [48]. This implies that diagnosing gout is rather challenging and that at this stage the identification of monosodium urate crystal in synovial fluid is still the preferred procedure. Emerging imaging techniques, such as ultrasound and Dual-Energy Computed Tomography, are promising and may assist the clinician in making an accurate diagnosis of gout [48].

3. PATHOPHYSIOLOGY OF HYPERURICEMIA, GOUT, AND CARDIOVASCULAR DISEASES

3.1 Pathophysiology of Hyperuricemia and Gout

Uric acid is the end product of the purine metabolism (Fig. 15.3). The origin of purines is as follows: 20% of the purines present in the body stems from the diet and 80%

TABLE 15.1 Sensitivity and Specificity of All Classification Criteria for Gout

Criteria	Sensitivity	Specificity
ROME 1963 [49]		
Two or more of any criteria or presence of MSU crystals in SF or on deposition:	0.64–0.82 ^a	0.99 ^a
<ul style="list-style-type: none"> • serum uric acid >7 mg/dL in men and >6 mg/dL in women • presence of tophi • MSU crystals in SF or tissue • history of attacks of painful joint swelling with abrupt onset and resolution within 2 weeks • at least two attacks of painful joint swelling with complete resolution with 2 weeks • history or observation of podagra • presence of tophi • rapid response to colchicine treatment, defined as a major reduction in the objective signs of inflammation within 48 h 		
AMERICAN RHEUMATISM ASSOCIATION 1977^c [50]		
6 of 12 clinical criteria required or presence of MSU crystals in SF of in tophus:	0.70–0.85 ^a	0.64–0.97 ^a
<ul style="list-style-type: none"> • more than one attack of acute arthritis • maximum inflammation developed within 1 day • monoarthritis attack • redness observed over joints • first metatarsophalangeal joint attack • unilateral tarsal joint attack • tophus (proven or suspected) • hyperuricemia • symmetric swelling within a joint on X-ray^a • subcortical cysts without erosions on X-ray • monosodium urate monohydrate microcrystals in joint fluid during attack • joint fluid culture negative for organisms during attack 		
MEXICO 2010 [51]		
MSU crystal identification or four of eight criteria required:	0.88–0.97 ^a	0.96 ^a
<ul style="list-style-type: none"> • current of past history of more than one attack of arthritis • rapid onset of pain and swelling (less than 24 h) • mono- and/or oligoarticular attacks • podagra • joint erythema • unilateral tarsal joint attack • tophus (suspected or proven) • hyperuricemia (more than 2 s.d. Greater than the normal population average) 		

Criteria	Sensitivity	Specificity
NETHERLANDS 2010 [52]		
Each item contributes its weighted score as shown. A summed score of 4 or less excludes gout; 8 or more suggests gout; between 4 and 8 suggests the need for SF analysis:	0.95 ^b	0.59 ^b
<ul style="list-style-type: none"> • male sex: 2 • previous patient-reported arthritis attack: 2 • onset within 1 day: 0.5 • joint redness: 1 • MTP1 involvement: 2.5 • hypertension or more than one cardiovascular disease: 1.5 • serum uric acid level >5.88 mg/dL: 3.5 • presence of a tophus: 13 		
2015 EULAR–ACR CLASSIFICATION CRITERIA [53]		
Each item contributes its weighted score as shown. A score of ≥8 classifies a subject as having gout.	0.92 ^b	0.89 ^b
<ul style="list-style-type: none"> • Step 1: <i>entry criterion</i>: at least 1 episode of swelling, pain, or tenderness in a peripheral joint or bursa • Step 2: <i>sufficient criterion</i> (if met can classify for gout without applying criteria below): presence of MSU crystals in a symptomatic joint or bursa (ie, in synovial fluid), or tophus • Step 3: <i>criteria</i> (to be used if sufficient criterion not met) <ul style="list-style-type: none"> • <i>pattern of joint/bursa involvement during symptomatic episode(s) ever</i>: <ul style="list-style-type: none"> - ankle or mid-foot: 1 - first metatarsophalangeal joint: 2 • <i>characteristics of symptomatic episode(s) ever</i>: <ul style="list-style-type: none"> - erythema overlying affected joint - can not bear touch or pressure to the affected joint - great difficulty with walking or inability to use affected joint 		
One characteristic: 1		
Two characteristic: 2		
Three characteristics: 3		
<ul style="list-style-type: none"> • Presence of typical episode (ever), defined as the presence of ≥2 of the following characteristics: <ul style="list-style-type: none"> - time to maximal pain <24 h - resolution of symptoms ≤14 days - complete resolution (to baseline level) between symptomatic episodes 		

TABLE 15.1 Sensitivity and Specificity of All Classification Criteria for Gout—cont'd

Criteria	Sensitivity	Specificity
One typical episode: 1 Recurrent typical episodes: 2		
• <i>clinical evidence of tophus</i> Present: 4		
• <i>Laboratory</i> - <i>serum urate</i> : <4 mg/dL (<0.24 mmol/L): -4 6–<8 mg/dL (0.36–<0.48 mmol/L): 2 8–<10 mg/dL (0.48–<0.60 mmol/L): 3 >10 mg/dL (>0.60 mmol/L): 4		
- <i>Synovial fluid analysis</i> : MSU negative -2		
• <i>imaging</i> : - <i>imaging evidence of urate deposition in symptomatic (ever) joint or bursa</i> : Ultrasound evidence of double-contour sign or DECT demonstrating urate deposition → presence: 4		
- <i>imaging evidence of gout-related joint damage</i> : conventional radiography of the hands and/or feet demonstrates at least 1 erosion → presence: 4		
A Web-based calculator can be accessed at: http://goutclassificationcalculator.auckland.ac.nz/		

MSU, monosodium urate.

^asensitivity and specificity adapted from Dalbeth, N, et al. *New classification criteria for gout: a framework for progress*. *Rheumatology (Oxford)* 2013;52:1748–53.

^bsensitivity and specificity adapted from Neogi, T, et al. 2015 Gout classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2015;74:1789–98.

^cNow American College of Rheumatology.

stems from endogenous sources (cell turnover and de novo synthesis of nucleic acids). Purines are degraded into hypoxanthine and xanthine, which in turn are converted into uric acid by the enzyme xanthine oxidase (XO). Xanthine oxidase is one of the two interchangeable forms of the enzyme xanthineoxidoreductase (XOR): xanthine oxidase (XO) and xanthine dehydrogenase (XD) [55]. XOR is present in the liver, gut, intestinal epithelium, kidney, heart, blood vessels, and brain [34]. During the oxidation of hypoxanthine to uric acid, superoxide radicals (O_2^-) are produced. These superoxide radicals can then react with hydrogen ions and form hydrogen peroxide (H_2O_2) and hydroxyl radicals ($-OH$) [55]. Furthermore, the generated reactive oxygen species (O_2^-) can react with Nitric Oxide (NO) to form peroxynitrite, which in turn leads to more free radicals.

All species, except humans and primates, further break uric acid down to allantoin by uricase. Allantoin is water-soluble and is then excreted in the urine. In humans and primates who lack this uricase, uric acid is mostly filtered through the kidneys, and a small part is excreted through the intestine. In the kidneys, 90% of the filtered uric acid is reabsorbed, mostly through URAT-1 and GLUT9 [56]. It is still unclear how uric acid is excreted through the intestine; the recently discovered urate-transporter ABCG2 may play a role in this process [57].

Under physiological condition, uric acid exists in his ionized form urate. As said previously, MSU crystals may form if the urate concentration of 6.8 mg/dL (0.41 mmol/L) is surpassed. The solubility of urate decreases by increasing sodium concentration, by decreasing temperature and by decreasing pH [56]. In an acute gout attack, the uric acid precipitates in crystals, which in turn will activate the inflammasome and cause inflammation primarily by the production of interleukin-1 [58].

3.2 Uric Acid, Gout, and the Heart

Most pathophysiological mechanisms linking gout to the heart involve uric acid.

3.2.1 Uric Acid and Endothelial Function/Atherosclerosis

During the production of uric acid, ROS are produced that can react with NO and thus reduce the production of NO (Fig. 15.3). This imbalance of ROS and NO is called oxidative stress and may cause endothelial dysfunction. Endothelial dysfunction is considered the initial step in the process of atherosclerosis. It may lead to remodeling, platelet aggregation, loss of vasodilatation, inflammation, and smooth muscle cell growth [59]. It has been demonstrated that uric acid, when entering a cell, can react with oxidants to form radicals and reduce the availability of NO, inducing endothelial dysfunction [60]. Uric acid has also been associated with markers of early atherosclerosis, confirming this possible pathophysiological link with induction of oxidative stress [55]. It has also been demonstrated in animal studies that soluble uric acid itself may directly activate inflammatory pathways in vascular smooth muscle cells and produce inflammatory cytokines, such as monocyte chemoattractant protein-1 (MCP-1) that are also present in early arteriosclerotic plaques [61]. This inflammatory response eventually induces oxidative stress and thus endothelial dysfunction [60].

Another mechanism by which arteriosclerosis can be promoted in gout is via low-density lipoprotein (LDL) oxidation under oxidative stress. Oxidized LDL is an important contributor to the development of arteriosclerosis

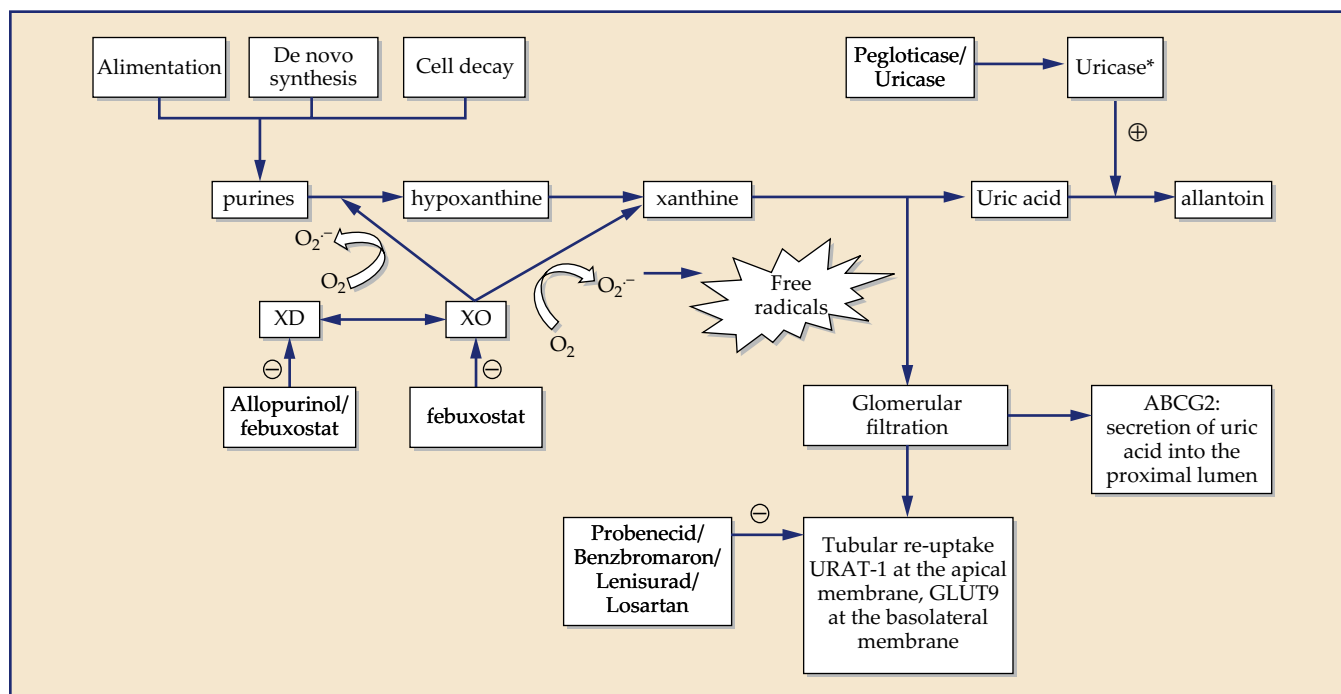


FIGURE 15.3 Formation, elimination of uric acid, and impact of drugs. ABCG-2, ATP-binding cassette sub-family G member 2; GLUT9, glucose transporter 9; URAT1, urate transporter-1; XD, xanthine dehydrogenase; XO, xanthine oxidase. Adapted from Puddu et al. [55] and Richette and Bardin [25].

[62]. Uric acid levels correlate with the level of oxidized LDL [63]. It has been demonstrated that oxidative stress is indeed present in untreated gout patients as measured by increased levels of malondialdehyde (MDA), which is an end product of the lipid peroxidation and is a reliable and widely used index of oxidative stress [64]. It was also demonstrated in the same study that the antioxidant defense system was depleted as indicated by a low level of superoxide dismutase activity and of paraoxonase-1. Such a mechanism may contribute to the higher prevalence of atherosclerosis in gout patients.

However, one of the theories to explain the evolutionary loss of uricase by primates and humans is that uric acid acts as a potent antioxidant in plasma. Indeed, uric acid may act as an NO-transporter and high urate levels in gout patients may be interpreted as a favorable response to oxidative stress in cardiovascular diseases [55]. The explanation for these different roles (uric acid as oxidant vs. uric acid as antioxidant) could lie in the localization of uric acid: intra- or extracellularly. Gout and the antioxidant effects of uric acid being mediated by extracellular uric acid and the effect of uric acid on the cardiovascular system being mediated by intracellular uric acid.

3.2.2 Uric Acid and Hypertension

The effect uric acid has on cardiovascular outcomes could, at least partially, be mediated by hypertension. The endothelial dysfunction induced by hyperuricemia and the associated decrease in NO cause renal vasoconstriction,

which results in activation of the renin-angiotensin system. This may lead to a uric acid-induced vaso-reactive hypertension, which has shown to be reversed by allopurinol in animal studies [34,65]. It has been demonstrated in vitro that the proliferative effect of uric acid on vascular smooth muscle cells is partly mediated through the activation of the RAS system and that inhibition of the RAS system could inhibit the uric acid-induced proliferation, further linking uric acid to atherosclerosis [66]. Another mechanism by which uric acid could cause hypertension is the induction of microvascular kidney damage. This would eventually lead to a salt-sensitive hypertension, which cannot be reversed by xanthine oxidase inhibition [34,65].

3.2.3 Uric Acid and Diabetes

The relationship between uric acid and diabetes is rather intriguing. Hyperinsulinemia is inversely correlated to the excretion of uric acid and may thus play a role in the development of hyperuricemia [67,68]. This may be mediated through GLUT9, one of the uric acid transporters in the kidney [68]. On the other hand, it has also been demonstrated that there is a bell-shaped relationship between uric acid and HbA1c: Uric acid levels increase following increasing levels of HbA1c up to 6.9%, upon which there is a decrease in uric acid levels with increasing HbA1c levels [69]. This phenomenon is thought to be explained by the uricosuric effect of glycosuria [70]. However, hyperuricemia often precedes the development of hyperinsulinemia

and diabetes. One study has demonstrated that lowering uric acid concentration may improve insulin resistance [71], raising the hypothesis that uric acid plays a causative role in the development of diabetes. Uric acid is also known to cause endothelial dysfunction (see above). Glucose uptake in the muscles relies in part on increased blood flow, which is mediated by the insulin-stimulated release of nitric oxide from the endothelial cells. Since uric acid can alter the endothelial function, it could be stipulated that it reduces the glucose-uptake in the muscles [65]. This illustrates the complexity of the interactions of uric acid with multiple processes in the body.

3.2.4 Uric Acid and Heart Failure

Uric acid could be linked to heart failure by its effects on hypertension and atherosclerosis as described above. But uric acid could also be a biomarker of heart failure, an epiphenomenon, as there is evidence of increased XOR activity in the myocardium of the failing heart [56]. This increased activity could be due to hypoxia and apoptosis, resulting in increased production of uric acid and thus of ROS (Fig. 15.1). Attesting a role for XOR in heart failure, several studies have demonstrated an advantageous effect of allopurinol in the treatment of patients with heart failure [125,136,151,152]. This effect could not be demonstrated with other uric acid-lowering drugs [71]. Studies also demonstrated that hyperuricemia was associated with a poor outcome in heart failure, raising the suggestion that increased levels of uric acid in heart failure may be caused by increased XOR activity [157,158]. An important limitation of studies like this is that confounding cannot be ruled out.

3.2.5 The Role of Inflammation

Gout is characterized not only by hyperuricemia and the deposition of urate crystals in joints and soft tissue but also by inflammation. How MSU crystals trigger inflammation is an important area of research. The role of the inflammasome in the inflammation process of MSU crystals is particularly interesting, since it has been recently demonstrated that cholesterol crystals, which are present in the atherosclerotic plaque, may also trigger the inflammasome (Fig. 15.4) [72]. Until now, three different mechanisms have been identified through which the inflammasome can be activated: (1) by phagocytosis of MSU crystals, which activate the inflammasome via cathepsin B; (2) by the rupture of crystal-containing lysosomes inducing the production of ROS (by the lysosome itself or by mitochondria) and a subsequent efflux of potassium (K^+) [58,73,74]; and (3) MSU crystals can also bind to Toll-Like Receptors (TLRs), which in turn prime the nucleus to the production of proinflammatory cytokines, such as prointerleukin-1 (pro-IL-1 β). The inflammasome activation results in the activation of caspase-1,

which in turn activates interleukin-1 β (IL-1 β). Activated IL-1 β results in the influx of neutrophils to the site of MSU depositions and subsequent inflammation. The fact that cholesterol crystals could also trigger this inflammation has led to the hypothesis that targeting inflammation in gout may also ameliorate the cardiovascular risk [72]. Furthermore the production of inflammatory cytokines such as IL-1 β promotes the expression of adhesion molecules on endothelial cells such as E-selectin, which in turn promotes neutrophil influx at the site of inflammation [75]. Neutrophils have also been demonstrated to play an important role in the pathogenesis of atherosclerosis [75]. In analogy with other chronic inflammatory diseases such as rheumatoid arthritis [76], chronic inflammation may thus, at least partially, explain the increased cardiovascular risk in gout patients. The fact that it has been demonstrated by MRI that inflammation in gout persists in the intercurrent periods, independently of uric acid level, supports this hypothesis [77].

4. CARDIAC INVOLVEMENT

4.1 Gout and Cardiovascular Risk Factors

The NHANES 2007–2008 study showed that patients with gout have a 2–3 times higher prevalence of cardiovascular risk factors, such as hypertension, diabetes, and obesity [78]. The prevalence of cardiovascular risk factors is also increased in hyperuricemia, with a clear association between the level of hyperuricemia and the presence of the risk factors (the higher the uric acid concentration, the higher the prevalence of cardiovascular risk factors). Multiple studies have investigated the risk of developing those cardiovascular risk factors in patients with gout and/or hyperuricemia. It has also been demonstrated that patients with cardiovascular risk factors present have a higher risk of developing gout. This suggests that uric acid, gout, and cardiovascular diseases and cardiovascular risk of factors are strongly linked, but the debate on whether or not the associations are causal is still ongoing (Fig. 15.5).

4.1.1 Hypertension

While the link between hyperuricemia and hypertension has been thoroughly investigated, the link between gout and hypertension has not been studied. A recent systematic literature review confirmed that patients with hyperuricemia have a moderately increased risk of developing hypertension, especially women (HR for women 1.9 vs. 1.4 for men) [79]. This elevated risk was also confirmed in two meta-analyses [80,81]. An important observation is that, after adjustment for the other cardiovascular risk factors, there was an attenuation of the risk for hypertension. The risk of hypertension in hyperuricemic patients also decreases with age and with

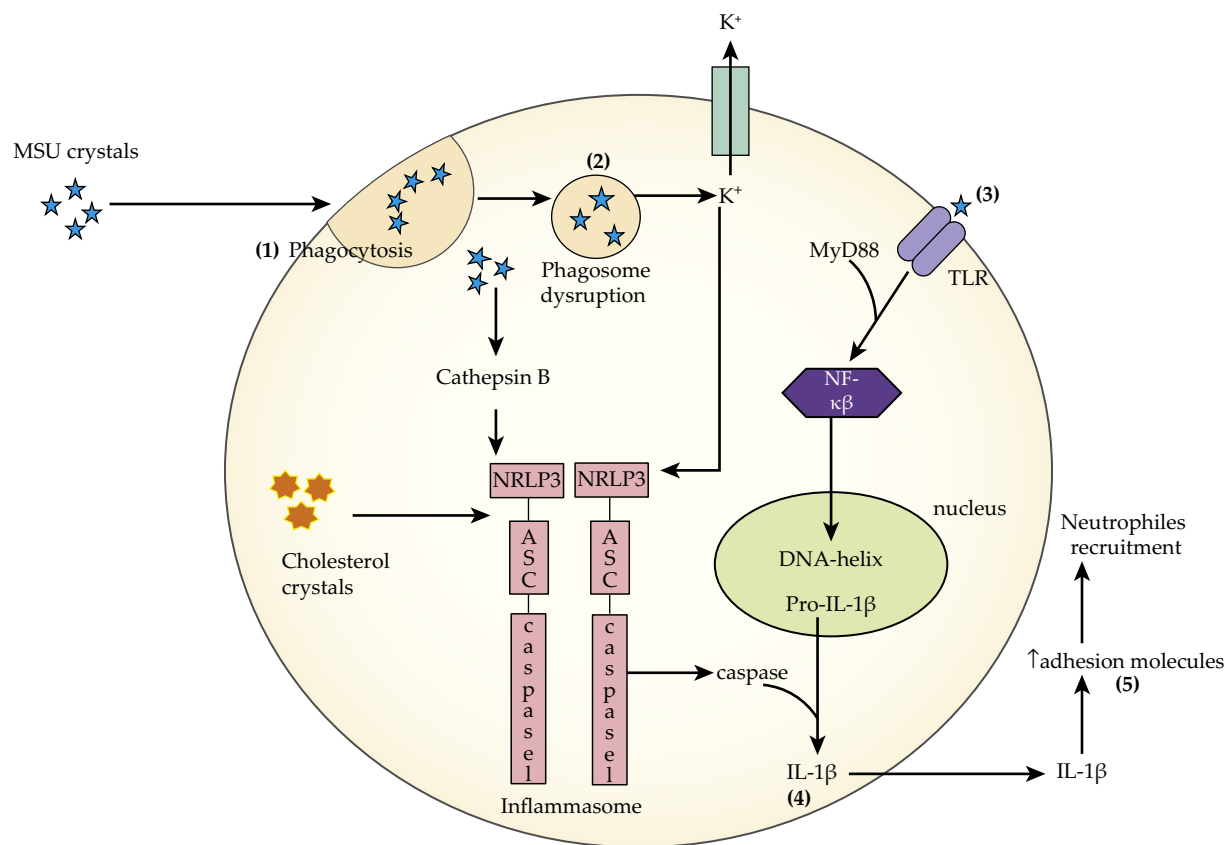


FIGURE 15.4 Inflammation pathway in inflammatory cells.

1. Phagocytosis of the MSU (Monosodium Urate) crystals and activation of the inflammasome through cathepsin B.
2. Disruption of the lysosomes and production of ROS (Reactive Oxygen Species) which lead to the efflux of potassium (K^+) and subsequent activation of the inflammasome and production of caspase.
3. Binding of the MSU crystals to Toll-Like Receptors (TLRs) and Priming of the nucleus to production of pro-Interleukin-1 β (pro-IL-1 β) through the activation of NF- κ B in the presence of Myeloid Differentiation factor 88 (MyD88).
4. Production of active IL-1 β through caspase.
5. Promotion of expression of adhesion molecules and influx of neutrophils to the site of inflammation.

Abbreviations:

MSU, Monosodium Urate; ROS, Reactive Oxygen Species; K^+ , potassium; TLRs, Toll-Like Receptors; IL-1 β , Interleukin-1 β ; NF- κ B, Nuclear Factor- κ B; MyD88, Myeloid Differentiation factor 88.

Adapted from the personal collection of the authors.

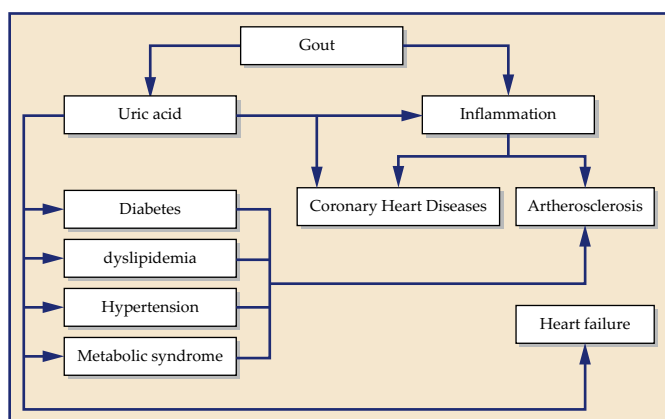


FIGURE 15.5 Relationships between gout, uric acid, and the heart.

Adapted from the personal collection of the authors.

duration of hypertension, suggesting a more important role of uric acid in younger subjects and in early-onset hypertension [65].

Vice versa, it has been demonstrated that patients with hypertension have a two-fold increased risk of gout [16]. The risk remained increased, but at a slightly attenuated level (HR 1.6) when limiting the analysis to patients who did not use diuretics.

The treatment of hypertension may also influence the risk of gout. In a large population-based nested-case control study, Choi et al. demonstrated that patients with hypertension using calcium antagonists and losartan had a lower risk of incident gout than patients with hypertension who did not use these drugs [82]. Diuretics, β -blocking drugs, and angiotensin-converting-enzyme inhibitors as well as nonlosartan angiotensin II receptor blockers conveyed an increased risk of incident gout.

With regard to diuretics, Bruderer et al. demonstrated that lis-diuretics, thiazide, and thiazide-like diuretics were associated with a higher risk of gout, while potassium-sparing diuretics were not [83].

4.1.2 Diabetes

The relationship between diabetes and gout is complex. When looking at the risk of gout patients to develop diabetes type 2, an increased risk, ranging from HR 1.15 to 1.41, was found in three studies. This relative risk was even higher in women, ranging from 1.48 to 1.78 [84–86]. However, when looking at the risk of patients with manifest diabetes developing gout, one case–control study demonstrated that individuals with type 2 diabetes are at lower risk of developing gout [87] and that this risk is even lower when diabetes is poorly controlled [88]. A potential explanation is that glycosuria coincides with urine loss of uric acid, thus preventing gout.

4.1.3 Dyslipidemia

Patients with gout have elevated concentrations of triglycerides, cholesterol, VLDL, and a low level of HDL [89,90]. The presence of hypertriglyceridemia is independent of alcohol use or body weight [90]. Recently Chen et al. demonstrated in a prospective cohort study that hypertriglyceridemia is an independent risk factor for gout in men, even when hyperuricemia is not present at baseline (HR 1.39) [91]. This could be partially explained by an enhanced production of fatty acids by triglycerides breakdown in adipose tissue, which may cotrigger an acute gouty arthritis through TLRs (inflammasome) [92]. Another explanation is that VLDL plays a role in the renal excretion of uric acid: there is an inverse relationship between the serum VLDL-concentration and the renal urate excretion as demonstrated by Tinahones et al. [93]. Furthermore, a reduction in VLDL by a hypocaloric diet may cause an increase in the renal excretion of urate [94]. As VLDL and triglycerides are known to increase the cardiovascular risk [95,96], these findings suggest at least common pathophysiological pathways, possibly through the excretion of uric acid.

4.1.4 Obesity

The prevalence of obesity is high in gout patients [78]. Studies have demonstrated that BMI is a risk factor for gout in men and women, although it is uncertain if this risk is independent of other known risk factors [91,97,98]. The risk of gout further increases by increasing BMI [91,97,98]. One study has shown that uric acid was a predictor of “unhealthy obesity” (obesity associated with other components of the metabolic syndrome) in adolescents and adults [99]. Furthermore, it has been demonstrated that obesity or excessive weight gain at a younger age was associated with a higher risk of gout

[98,100] and that weight loss may reduce that risk, at least in men [101]. These data stress the importance of weight counseling, especially in young people.

4.1.5 Smoking

The expectation that smoking could influence the risk of developing gout is based on the following pathophysiological mechanisms: (1) the antioxidant capacity of uric acid that can buffer the oxidative stress caused by smoking or other inhaled agents, and (2) the inactivation of xanthine oxidase by cyanides in cigarette smoke. It has indeed been demonstrated that smokers have a lower uric acid level [102,103]. Wang et al. even demonstrated that cigarette smoking was associated with a lower risk of gout [104]. This does not mean that smokers who are at risk of developing gout should be encouraged to continue smoking but rather stresses the potential role of uric acid as a physiological counter-regulator in the human organism. Whether or not this effect of smoking on uric acid and gout is important in the context of the cardiovascular risk of gout patients is not known to date.

4.1.6 Metabolic Syndrome

Longitudinal cohort studies assessing the risk of developing the metabolic syndrome have only been performed in patients with hyperuricemia. They found an independently increased risk of 65% [105,106]. There is also growing evidence that uric acid may act as a predictor of metabolic syndrome [107]. With regard to gout, the prevalence of the metabolic syndrome seems to be high (82% in a study by Vazquez–Mellado et al. [108]). A first acute gout attack often precedes the development of other features of the metabolic syndrome [109]. The observation that the presence of hypertension, diabetes, dyslipidemia, and obesity on the one hand and the risk of gout on the other are associated may indicate that they are all part of the same syndrome. The presence of gout should always trigger clinicians to evaluate the presence of the metabolic syndrome.

4.2 Gout and Cardiac Function

Ultrasound is a widely used diagnostic instrument in cardiology. Recently, a few researchers have looked at the possible effects of gout on the cardiac function as can be measured by ultrasound, in an attempt to better understand a link between cardiovascular diseases and gout. When comparing the different stages of gout (asymptomatic hyperuricemia, gouty arthritis, and tophaceous arthritis), it was shown that the severity of gout was associated with the degree of left ventricle dysfunction and with subclinical systolic dysfunction as measured by left ventricular (LV)-mass and LV-mass index and Em and Em/Am ratio [110]. It was also demonstrated in that study that the left atrial function (measured by

the left atrial ventricular index (LAVi)—and more specifically the left atrial booster pump function (as measured with ALS (Atrial Longitudinal Strain)_{late})—were independently associated with the severity of gout and thus tophi [110,111]. Another study demonstrated that the mitral annular peak velocity (Em) was significantly lower in gout patients with tophi compared to those without tophi and to asymptomatic hyperuricemic individuals. However, in a multivariate regression analysis the presence of renal insufficiency appeared to be more important in explaining this association than the stage of gout [112]. Considered together these three studies suggest that gout is associated with poorer cardiac function and that possibly inflammation, apart from uric acid, contributes to this deterioration of function.

4.3 Gout and Markers of Arterial Stiffness

Arterial stiffness is a clinical hallmark of atherosclerosis. As such, multiple studies have demonstrated a relationship between uric acid levels and markers of atherosclerosis [106,113–115]. In addition, it has recently been demonstrated that the level of uric acid may predict mortality in patients with asymptomatic carotid atherosclerosis [116]. The data for gout are scarcer. One study has demonstrated that carotid intima thickness (IMT) was significantly increased in gout patients compared to individuals with asymptomatic hyperuricemia, and were even higher than in patients with rheumatoid arthritis, another well-known chronic inflammatory disease with an elevated cardiovascular risk [76,117]. Another study measuring vascular stiffness using the common carotid artery resistive index (CCARI) demonstrated that tophi were independently associated, just as hypertension, with an abnormal CCARI [112].

4.4 Gout and Coronary Heart Disease

A recent systematic review of the literature showed that the risk of incident coronary heart disease (CHD) and of mortality due to CHD is, when adjusted for additional cardiovascular risk factors, slightly increases in gout patients (HR 1.3–1.6 for incident CHD, 1.4–1.8 for mortality), especially in females [79]. A recent retrospective cohort study confirmed that the relative risk for coronary heart disease seems to be more pronounced in females than in males [118]. A recent meta-analysis showed that when taking only myocardial infarction into account, the risk was not elevated in patients with gout [119]. These discrepancies in risks between the broader diagnosis of CHD and the more specific diagnosis of myocardial infarction could be explained by a common problem of epidemiological studies: Data collection and case ascertainment. At this time it is still unclear whether the risk of CHD in gout patients is related to the higher prevalence of cardiovascular risk

factors in those patients, to uric acid itself, or to ongoing inflammation due to the deposition of crystals in tissues. The fact that these risks decrease by adjustment for traditional risk factors confirm that traditional risk factors play a role in the development of coronary heart disease but cannot fully explain the relationship. It is well known that other inflammatory rheumatic diseases, such as rheumatoid arthritis and systemic lupus erythematosus, which are not related to uric acid, also have a higher incidence and mortality due to cardiovascular diseases [120,121]. This increased risk is explained by ongoing inflammation in those diseases. In gout, there is growing evidence that the inflammation is not only present during the attacks but also in the intercurrent phase [77,122].

4.5 Gout and Heart Failure

Heart failure is more prevalent in gout patients when compared to healthy controls [1]. A systematic review of the literature showed that higher uric acid levels are associated with an increased risk of heart failure and that this higher risk seems to be “dose-dependent [123]”. With regard to gout, only one study assessed the risk of developing heart failure and showed that this risk is increased in gout patients [124]. Gout even seemed to be a poor prognostic factor in patients who have developed heart failure. Furthermore, the ejection fraction of the left ventricle as well as the global left ventricle function assessed in patients who had not yet developed heart failure were worse in gout patients compared with healthy controls, which may suggest a direct role of uric acid on cardiac function [124]. Another mechanism through which uric acid could play a role in heart failure is the xanthine oxidase system [125]. Hyperuricemia upregulates xanthine-oxidase, which in turns produces free radicals. These free radicals may then affect the cardiac function by inhibiting nitric oxide. This is why, in the last decade, studies have looked at the effect of allopurinol (a xanthine-oxidase inhibitor) in patients with heart failure, yielding different results.

When assessing the risk of developing gout in patients with heart failure and hypertension as a cause of heart failure, NYHA (New York Heart Association) class III/IV, Fractional Excretion of Uric Acid (FEUA) <4%, lower Glomerular Filtration Rate (GFR), and male gender below ≤64 years are the strongest risk factors [126].

Diuretics are a corner stone of the treatment of patients with heart failure and are known to increase the risk of gout [82]. An interesting study by Janssens et al., however, demonstrated that when correcting for the indication for the use of the diuretics (hypertension, heart failure, or myocard infarction), there did not seem to be an increased risk of incident gout. With regard to the diuretics class, spironolactone even seems to reduce the risk of gout in patients with heart failure [126].

4.6 Gout and Arrhythmia

Multiple studies have looked at the association between uric acid and tachyarrhythmia and atrial fibrillation in particular [127–131]. They have all found that an elevated uric acid level was a predictor of future atrial fibrillation. One study even demonstrated that in patients with atrial fibrillation, hyperuricemia was a significant risk factor for stroke, which could refine the clinical risk stratification [132]. With regard to gout, only one study has suggested a slightly increased adjusted hazard ratio (HR) on atrial fibrillation when compared to nongout patients (HR 1.21, 95% CI 1.11, 1.33) [133]. An important limitation of this study is the potential effect of other comorbidities, especially heart failure and chronic obstructive pulmonary disease. At this stage, the pathophysiological link between those two conditions still remains to be uncovered.

4.7 Gout as a Prognostic Factor for Cardiovascular Disease

Uric acid has antioxidant properties but the production of uric acid and uric acid itself can induce oxidative stress and endothelial dysfunction. Therefore one could easily suggest that uric acid is a biomarker for outcome in cardiovascular disease. As gout is the last step of chronic hyperuricemia, it could also serve as a marker for CVD.

High uric acid concentrations at admission after an acute myocardial infarction seem to be associated with higher rates of adverse events and with mortality [134,135]. It has also been demonstrated that higher uric acid levels were associated with a poor outcome in patients with heart failure [123,136]. With regard to patients with gout, data are scarce. One observational study including 444 patients (of whom 48 had an acute gout attack) showed that an acute gout attack during a hospitalization for acute myocardial infarction is associated with an 88% higher rate of short-term nonfatal adverse events, mainly due to a higher rate of revascularization procedures compared to patients who do not experience a gout attack during hospitalization [137]. On the long-term, patients with gout had an 82% higher risk of late adverse events (more than 30 days after admission), mainly because of readmission due to heart failure. The short- and long-term mortality rate did not differ between the two groups. A post hoc analysis from the Aspirin Myocardial Infarction Study showed that only untreated gout was associated with a higher mortality rate when compared to controls without gout [138]. A study by Choi showed in a subanalysis of 235 patients with gout and previous coronary heart disease a 26% increased risk of cardiovascular mortality [139].

In heart failure, patients with heart failure and a history of gout (before the first admission for heart failure)

have a 63% increased risk of being readmitted for heart failure or to die from heart failure [125].

Although the data are limited and the underlying mechanisms are not clear, the presence of gout could be seen as a poor prognostic factor in patients with cardiovascular disease.

5. TREATMENT OPTIONS AND CARDIAC IMPLICATIONS OF THERAPY

5.1 Treatment Guidelines for Gout

The treatment of gout has two goals: one is to treat the inflammation during an acute gout attack and the second one is to lower the uric acid concentration to prevent future attacks of gout and deposition of urate crystals in soft tissues, which may lead to the formation of tophi and destruction of joints.

Treatment of acute gout includes: colchicine, nonsteroidal-anti-inflammatory drugs (NSAIDs), oral glucocorticoids, intra-articular glucocorticoids, and the more recently developed Interleukin 1 inhibitors (anti-IL-1) [140]. The choice of the drugs should be based on the individual patient characteristics, especially the comorbidities and the risk of adverse events [27].

The second phase of treatment consists of lowering the uric acid levels. The recommended concentrations are 0.36 mmol/L for nontophaceous gout and 0.30 mmol/L for tophaceous gout [27,141]. This can be achieved by xanthine-oxidase inhibitors (allopurinol and febuxostat) or by uricosurics (Fig. 15.3). Uricase is only recommended in severe refractory tophaceous gout (Fig. 15.3) [27]. Because starting urate-lowering-therapy (ULT) can provoke acute gout it is recommended to use a prophylaxis during the first 3–6 months. Low-dose colchicine (0.5 mg daily) is generally used for this indication [140]. There has been a lot of debate regarding the recommended doses of allopurinol in patients with impaired kidney function. It is now recommended to start at a low dose (50–100 mg of allopurinol) and slowly uptitrate in patients with mild to moderate renal impairment [27]. With febuxostat no drug adjustment is needed in patients with mild to moderate renal impairment [27]. The use of uricosurics is not recommended in patients with severe renal impairment since they will not be able to increase their uric acid excretion.

5.2 Effect of Allopurinol and/or Febuxostat and/or Colchicine on Cardiovascular Risk Factors

In patients with hypertension one small, randomized crossover trial has demonstrated in 30 adolescents with newly onset hypertension that allopurinol 200 mg once a day resulted in a reduction of blood pressure

[142]. Furthermore, 67% of the patients achieved normal blood pressure after treatment with allopurinol. In older patients, one cohort study investigated the effect of allopurinol on blood pressure in adults that either did not receive treatment for hypertension or continued the same treatment. There was a rather small decrease in blood pressure (3 mm Hg) that was independent of uric acid level before initiation and tended to be higher with higher allopurinol dose [143].

With regards to diabetes, one study of patients with congestive heart failure demonstrated that lowering the concentration of uric acid did improve insulin resistance [71]. Benzbromarone was used in this study, suggesting that this effect is independent of xanthine-oxidase inhibition.

Concerning atherosclerosis, one study evaluated the effect of febuxostat and allopurinol in patients with untreated tophaceous gout on pulse-wave-velocity (PWV), which is a reliable indicator of arterial stiffness and of markers of oxidative stress [144]. They showed that after 1 year of therapy the PWV increased in the allopurinol group but not in the febuxostat group. An other interesting finding was that febuxostat significantly decreased both TNF- α and NADPH-oxidase activity, suggesting that febuxostat can influence both the inflammatory and the uric acid-induced oxidative stress pathways, explaining why the PWV did not increase in the febuxostat group.

5.3 Effect of Allopurinol, Colchicine, and/or Febuxostat on the Outcome and/or Prognosis of Heart Diseases

Because of the potential effect of free radicals (produced by the xanthine oxidase system) on cardiac function, several studies have addressed the role of xanthine-oxidase inhibitors, allopurinol, and febuxostat on the outcome of cardiovascular diseases. A few studies have demonstrated that the use of allopurinol may indeed improve the endothelial function [56]. But Kim et al. did not demonstrate any influence of allopurinol or febuxostat on cardiovascular mortality in a study with poor treatment compliance [145].

Allopurinol seems to be associated with a lower risk of acute myocardial infarction and a reduced risk of recurrence [146,147]. This effect seems to be dose-dependent (at least 300 mg a day) and occurs only at longer treatment duration (more than 6 months) [146].

Targeting atherosclerosis with colchicine has recently gained interest, especially since the elucidation of the role of neutrophils in the pathophysiology of atherosclerosis. In the LoDoco (low dose colchicine) trial, it was demonstrated that in patients with stable coronary heart disease adding colchicine to the secondary treatment of stable coronary heart disease was associated with a better outcome [148]. A retrospective study of 1-year

follow-up in 1288 gout patients using colchicine as gout prophylaxis showed a decreased prevalence of myocardial infarction (RR=0.46, P value=0.03 for the colchicine vs. the noncolchicine group) [149]. In a trial of 151 patients with ST-segment elevation myocardial infarction treated with percutaneous coronary intervention who were randomly assigned to colchicine for 5 days or placebo, colchicine reduced the infarct size [150]. Interestingly, this decrease in infarct size was also associated with a decreased inflammatory response, as measured by neutrophils count and CRP-level, confirming the role of inflammation in myocardial infarction and the role that colchicine may play in it.

In chronic heart failure, several studies investigated the effects of allopurinol in patients with heart failure and found improved survival and heart function [125,136,151,152]. One study showed a small but statistically significant risk reduction on heart failure readmissions or on death in patients with heart failure when using at least 100 mg of allopurinol, suggesting that, as demonstrated for myocardial infarction, the effect of allopurinol might be dose-dependent [125]. Malek et al. found that allopurinol therapy, together with an elevated uric acid level, was a poor prognostic factor in acute heart failure admission. This may be explained by the fact that such patients have a lower ejection fraction and more severe symptoms. Unfortunately the allopurinol dosage was not registered so that a possible dose-related effect could not be measured [136]. Interestingly, it was shown in an RCT in patients with chronic heart failure in which lowering of uric acid levels was achieved by benzbromarone that heart function was not improved. This finding suggests the hypothesis that it is the XO inhibition rather than the inhibition of uric acid itself that may play a role in heart failure [71].

In arrhythmia, two studies have looked at the effect of colchicine on preventing atrial fibrillation. One trial in patients who underwent cardiac surgery found no effect of colchicine in preventing postoperative atrial fibrillation although a first trial was promising. It did find a beneficial effect of colchicine for preventing postpericardiotomy syndrome [153,154]. However, adverse events were a major concern. Another trial in patients with paroxysmal atrial fibrillation who underwent a pulmonary vein ablation were randomized to a 3-month course of colchicine or placebo and showed a reduced risk of recurrence of atrial fibrillation in favor of colchicine [155]. This was accompanied by a better quality of life.

6. CONCLUSIONS

The association between uric acid and cardiovascular diseases is obvious. Clinical gout is the ultimate consequence of hyperuricemia and is therefore also involved.

What is still not clear is whether the relationship between these three conditions (hyperuricemia, gout, and cardiovascular disease) is a causative relationship (ie, hyperuricemia causes CVD) or that uric acid and gout should rather be considered as metabolic markers of CVD. At this point, a firm genetic basis for a causal relationship between uric acid and cardiovascular diseases is also lacking. However, there could be different phenotypes of hyperuricemia and gout, since not all hyperuricemic patients develop gout and as not all patients with gout develop cardiovascular disease. This area of research is very relevant because of its potential therapeutic consequences.

There is also growing evidence that gout should be seen as a poor prognostic factor in cardiovascular disease. Whether or not this can be influenced by treatment still remains to be elucidated. Another issue of interest is whether it is the reduction of uric acid itself (therapeutic target below 6 mg/dL (0.36 mmol/L)) that improves the cardiovascular prognosis or rather the inhibition of the xanthine-oxidase pathway by xanthine oxidase inhibitors (allopurinol, febuxostat).

At this point, current evidence indicates that physicians treating gout patients should carefully check for the presence of cardiovascular risk factors and CVD in their patients with gout. In return, cardiologists should be aware of the potential interaction between UA and CVD and the observations suggesting that hyperuricemia/gout is a poor prognostic marker for survival: They may ask the rheumatologist for help in timely treating this condition.

In conclusion, treating gout is more than lowering uric acid levels in order to prevent gout attacks. Uric acid lowering therapy may have favorable effects on the cardiovascular system and may influence the prognosis of cardiovascular diseases.

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Giant Cell Arteritis

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1. INTRODUCTION

1.1 History and Nomenclature

While giant cell arteritis (GCA) is now well-recognized as a systemic inflammatory vasculopathy of large- and medium sized-vessels, early observations of this disease focused on its classic cranial manifestations. Hutchinson is credited with the first account of GCA in 1890, described at that time as *thrombotic arteritis of the aged*, in which an 80-year-old male displayed inflamed, tender, and swollen temporal arteries bilaterally, which eventually became pulseless [1]. This report went largely unnoticed until 40 years later when similar symptoms were observed in two patients evaluated at the Mayo Clinic and described by Horton and colleagues in 1932 [2]. Temporal artery biopsies from these patients demonstrated the first evidence of histopathologic lesions described as transmural “chronic periarteritis and arteritis.”

Over the subsequent three decades greater recognition of patients with similar presentations resulted in reports of the condition defined as *Horton's disease*, [3] *giant cell arteritis*, [4] *cranial arteritis* [5], *granulomatous arteritis* [6], and *senile arteritis* [7]. With the development of the 1990 American College of Rheumatology (ACR) classification criteria for vasculitis, the term *giant cell (temporal) arteritis* was selected as the preferred description due to the frequent histopathologic presence of multinucleated giant cells among the temporal artery specimens [8]. However, not all patients with GCA have temporal artery involvement and other forms of vasculitis may involve the temporal arteries. Therefore the 2012 Revised International Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitides recommended the use of the term *giant cell arteritis* and determined *temporal arteritis* to no longer be a suitable alternative term [9].

1.2 Epidemiology

GCA is the most common form of idiopathic systemic vasculitis in Europe and North America, affecting almost exclusively patients ≥ 50 years of age. The frequency of diagnosis increases with age and a peak incidence has been observed in the seventh decade [10–12]. The incidence of this disease varies by population, with the highest rates reported among white individuals in Northern European (Scandinavian) countries and in Minnesota, USA where the annual incidence rates are 18–29 cases per 100,000 population aged ≥ 50 years [13–16]. Epidemiologic studies have identified a north-south gradient, with lower annual incidence rates among people observed in Southern Europe [11,17] and Israel [18] (7–11 cases per 100,000 population aged ≥ 50 years) as well as fewer than 4 cases per 100,000 population among Hispanic, Asian, African American, and Australian populations [12,19–22]. Women, among all populations studied, are 1.5–3.0 times more commonly affected than men. The lifetime risk for GCA is approximately 1.0% for women and 0.5% for men [23,24].

1.3 Genetics, Etiology, and Pathogenesis

GCA exhibits a complex pathogenesis in which both genetic and environmental factors likely influence the development and progression of this disease. Genetic polymorphisms of both HLA class I and II alleles have been implicated. Genetic variation among HLA class II genes has been consistently associated with GCA susceptibility, particularly carriage of HLA-DRB1*0401 and DRB1*0404 alleles [25–30]. Overrepresentation of these alleles in patients with GCA suggests a contribution of T-cell selection and antigen recognition in GCA susceptibility. Carmona et al., in a recent large-scale

genetic analysis of 1651 patients with GCA compared to 15,306 unaffected controls, provided further confirmation of HLA-DR β 1 involvement and additionally identified novel genetic susceptibility factors for GCA including HLA-DQ α 1 and HLA-B alleles [31]. This international study also observed significant risk association loci outside of the HLA region in genes encoding key proteins involved in immunoregulation and T-cell function including: protein tyrosine nonreceptor type 22 (PTPN22), leucine-rich repeat-containing protein 32 (LRRC32), and v-rel avian reticuloendotheliosis oncogene homolog (REL) [31]. Multiple other genetic polymorphisms in genes encoding endothelial cell molecules, cytokines, and their receptors, and the innate immune system underscore the polygenic nature of this condition.

GCA has been depicted as an antigen-driven disease, and isolation of identical T-cell clones from different vasculitic sites suggests a response to a specific antigenic stimulus. Studies have proposed that arterial wall dendritic cells may be activated by environmental infectious agents or autoantigens [32]. Several microorganisms have been suggested as an infectious trigger including *Mycoplasma pneumonia*, *Chlamydia pneumonia*, parvovirus B19, varicella zoster virus, herpes simplex virus, human herpes virus 6, and Epstein-Barr virus. Attempts to identify pathologic organisms in temporal artery biopsy specimens have yielded inconsistent results for a causal infectious agent. Other environmental factors have not been identified with certainty; an interesting association with solar cycle has been reported, but is of uncertain significance [33].

While the specific immunostimulatory triggers are unknown, the immunopathology of GCA is better understood and originates from a dysregulated interaction between the vessel wall and both the innate and adaptive immune system [34,35]. Unlike small vessels that rely on oxygen through luminal diffusion, large vessels require a microvascular network (vasa vasorum) to supply oxygen to the media-adventitia vascular cell layers. Arteries with vasa vasorum contain vascular dendritic cells (vasDCs) at the media-adventitial border, which in vasculitic lesions become activated via toll-like receptors (TLRs) and redistribute throughout the vessel wall [36]. Stimulated vasDCs are able to attract and activate T lymphocytes (predominantly CD4⁺), which recruit macrophages through secretion of interferon- γ (IFN- γ). Macrophages participating in the vasculitic process infiltrate the arterial wall through the vasa vasorum and further specialize into M1 and M2 phenotypes according to the arterial microenvironment. In the adventitia, activated M1 macrophages primarily secrete proinflammatory cytokines (eg, IL-1 and IL-6) [37], while M1 macrophages in the medial layer injure vascular smooth muscle cells and endothelial cells through local oxidative

stress mechanisms [38] and degrade the arterial matrix through secretion of matrix metalloproteinases. M2 macrophages, on the other hand, reside closer to the intima-media border and synthesize proangiogenic molecules and growth factors (vascular endothelial growth factor, fibroblast growth factor, and platelet-derived growth factor), which result in myofibroblast relocation, proliferation, and the characteristic marked thickening of the arterial intima.

Recent studies have further elucidated the role of CD4⁺ T cells in vasculitic lesions of GCA and two distinct lineages have been identified as key regulators; type 17 helper T cells (Th17) and type 1 helper T cells (Th1) [39]. These T-cell lineages appear to be stimulated by independent signals from different antigen presenting cells (APC), with Th17 cells requiring IL-1 β /IL-6/IL-21/IL-23, whereas the Th1 pathway is dependent on APCs secreting IL-12/IL-18 [35,39]. In early untreated GCA, both T-cell lineages are increased in the peripheral circulation as well as within vascular inflammatory infiltrates. The Th17 pathway appears to be very sensitive to treatment and glucocorticoids (GC) rapidly control the Th17 effector cytokine production of IL-1, IL-6, IL-17 and IL-23 [35,40] with concomitant depletion of both circulating and infiltrating Th17 cells.

Rapid reduction in these cytokines contributes to the prompt resolution of systemic features of GCA upon glucocorticoid initiation. Despite the effective reduction of the Th17 pathway, a Th1-cell response persists in both biopsy and blood samples of treated patients with GCA [40]. The Th1 cytokine signature associated with chronic vasculitis in GCA is identified by production of IL-12 and IFN- γ and is poorly susceptible to GC-mediated suppression. Indeed, persistence of vascular Th1 cellular infiltrates despite immunosuppression is likely responsible for the chronic relapsing nature of GCA [35].

2. CLINICAL PRESENTATION AND DIAGNOSIS

2.1 Clinical Presentation

The clinical presentation of patients with GCA is highly variable and partly depends on the distribution of vascular inflammation. The main clinical phenotypes, which are not mutually exclusive, include cranial vascular manifestations, arteritis of large vessels, systemic inflammatory syndrome, and polymyalgia rheumatica.

2.1.1 Cranial Arteritis

Predominant cranial symptoms of GCA are due to arteritis of the external carotid artery branches and include headache, scalp tenderness, and jaw claudication. Headaches, which occur in 50–75% of patients with

GCA, are most often persistent, bitemporal, and refractory to simple analgesics [41]. Approximately half of patients report associated scalp tenderness, commonly located over the temporal regions [41]. Examination of the superficial temporal arteries may reveal abnormalities such as tenderness, thickening, nodularity, erythema, or diminished pulse. Jaw claudication, a result of vascular insufficiency to the mastication muscles during chewing, occurs in one-third of patients, can be unilateral or bilateral, and is highly specific for GCA. Scalp, tongue, or lip necrosis can occur but are considered rare findings. Although uncommon, patients may report upper respiratory symptoms such as nonproductive cough, sore throat, or hoarseness. Cerebral ischemia due to inflammation of the carotid, vertebral, or basilar arteries affects 3–6% of patients. Involvement of the intracranial vessels is considered to be extremely rare.

Ocular manifestations including visual impairment, amaurosis fugax, and diplopia are common in GCA. Visual loss has been reported in up to 15–20% of patients and is more prevalent at time of diagnosis than in later stages of the disease [42,43]. The frequency of visual symptoms and vision loss due to GCA has decreased in recent decades, likely due to earlier disease recognition and treatment. A population-based cohort study from Olmsted County, MN, USA reported lower incidence of ischemic optic neuropathy in the 1980–2004 cohort versus 1950–1979 (6% vs 15%, $p = .03$) [44]. The most frequent ocular manifestation in GCA is anterior ischemic optic neuropathy (AION) resulting from occlusion of the ophthalmic or posterior ciliary arteries. Precursors of impending ocular ischemic occlusion include transient blindness (amaurosis fugax), local perfusion deficits in the retina (cotton-wool spots), and diplopia.

Unfortunately, a limited subset of patients will present with visual loss as the first symptom of GCA. Partial or total monocular visual loss is considered an ophthalmologic emergency because if left untreated, the second eye will often become involved within 1–14 days [45]. In a patient with established AION, glucocorticoid therapy is unlikely to improve vision in the affected eye. Therefore in a patient with unilateral AION, the main goal of treatment is protection of the contralateral eye to minimize the risk of blindness [44]. Impaired vision from AION, however, can be difficult to distinguish clinically from nonarteritic ischemic optic neuropathy (NAION). The latter is characterized by a hyperemic optic disk and is not associated with increased inflammatory markers or clinical features of GCA [46].

2.1.2 Large-Vessel Arteritis

Although there is a noted tropism of GCA for the extracranial branches of the carotid artery, many patients also demonstrate involvement of the aorta and its primary branches (large-vessel involvement, LV-GCA (Table 16.1)).

TABLE 16.1 Topography of Inflammatory Arterial Changes Observed on Vascular Imaging at Time of Diagnosis in Patients With Giant Cell Arteritis [49,51,55]

Vascular territory involved	Percentage
Aorta	
Thoracic aorta	45–68
Abdominal aorta	27–54
Panaortitis ^a	23
Primary and Secondary Aortic Branches	
Brachiocephalic trunk	48
Carotid	35
Subclavian	43–74
Axillary	18–47
Brachial	22
Splanchnic	20–22
Renal	7
Iliac	11–37
Common femoral	14
Superficial femoral	30–37
Deep femoral	6–8
Popliteal	5–6

^aPanaortitis = all aortic segments affected.

Aortitis is generally clinically silent; however, vasculitis of the upper extremity arteries often produces symptoms and signs of vascular insufficiency. The exact prevalence of LV-GCA in newly diagnosed patients is uncertain. Retrospective studies focusing on the presence of symptomatic vascular insufficiency estimate that only 10–26% of patients with GCA have arteritic involvement of the aorta and its major branches [47–49]. Studies employing vascular imaging, on the other hand, demonstrate that up to 83% of patients may have radiographic evidence of LV-GCA depending on the modality and extent of vascular imaging that is utilized [50–58]. The latter estimates are more analogous to autopsy studies identifying histopathologic evidence of arteritis in the aorta and subclavian/axillary vessels in 90–100% of patients with GCA [59,60].

The diagnosis of LV-GCA requires comprehensive vascular imaging studies as temporal artery biopsy is negative in about 50% of this patient subset, and diagnosis is often delayed [49,58,61]. Compared to patients with classic GCA, patients with LV-GCA tend to be younger at diagnosis and have less frequent cranial symptoms [58,62,63]. In GCA, upper extremity critical limb ischemia from stenotic lesions is rare. Indeed, among patients with upper extremity claudication symptoms, 89–100%

remain stable or improve with glucocorticoid therapy [64]. Deterioration with conservative medical therapy is unusual and reconstructive surgical procedures are uncommon. However, patients with LV-GCA affecting the lower extremities have a higher risk of progression requiring revascularization, which may be necessary in 11–26% of cases [49,65].

2.1.3 Systemic Manifestations

Nearly half of patients with GCA have symptoms of a systemic inflammatory process including low-grade fever, fatigue, night sweats, and weight loss. If cranial features or ischemic complications are absent, these non-specific constitutional symptoms with accompanying elevated inflammatory markers may be the only presenting manifestations. In such cases, clinicians typically pursue a thorough evaluation to initially exclude occult infection or malignancy before considering GCA. Given that approximately 15% of patients with GCA present as fever of unknown origin [66], clinicians should consider GCA in the early differential diagnosis of unexplained fever among elderly patients.

2.1.4 Polymyalgia Rheumatica

Polymyalgia rheumatica (PMR) is a clinical syndrome characterized by aching and stiffness in the neck as well as the shoulder and pelvic girdles [67]. It is characterized by articular and periarticular inflammation of shoulders, hips, and axial structures. Symptoms are commonly bilateral and frequently worse in the morning or after prolonged inactivity. Approximately 40–50% of patients with GCA experience symptoms of PMR, whereas 16–21% of patients with PMR develop GCA [68]. However, the exact frequency of GCA among patients with PMR is difficult to ascertain, as some patients have subclinical vasculitis that is only detectable by sensitive imaging studies. For example, almost one-third of patients with isolated PMR were found to have FDG uptake of large arteries by PET imaging [69]. Since GCA and PMR also share overlapping epidemiologic characteristics they are often thought to represent different phenotypes along a spectrum of the same disease.

2.2 Classification Criteria and Diagnosis

The American College of Rheumatology (ACR) 1990 criteria for the classification of GCA (Table 16.2) were formulated as a way to distinguish GCA from other types of vasculitis. As such, these criteria are useful to classify rather than diagnose this condition. However, providers often use the 1990 criteria in clinical practice, particularly for the diagnosis of patients with cranial symptoms and negative temporal artery biopsy. Another caveat of the 1990 criteria is that patients with

LV-GCA confirmed by imaging will often not meet sufficient criteria for diagnosis [58]. Future iterations of the ACR criteria will likely include reference to vascular imaging as this has become an integral component of the diagnostic evaluation of patients with suspected GCA. Although algorithms for the diagnosis of GCA have been proposed, no current schemas have been validated. The diagnosis of GCA is therefore based on the combination of symptoms, clinical examination findings, laboratory results, histopathology, and diagnostic imaging.

2.2.1 Laboratory Investigations

Specific diagnostic biomarkers for GCA are not currently available. General laboratory evaluation typically discloses features of systemic inflammation. As such, leukocytosis, thrombocytosis, and normochromic/normocytic anemia are commonly observed. Elevation of the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are considered hallmarks for this condition. Both parameters are sensitive for clinical use in diagnosis (ESR 77–96%; CRP 95–98%) but lack specificity because increases can be seen in several other conditions including trauma, infections, malignancy, and other autoimmune disorders [70]. Although elevated serologic inflammatory markers raise suspicion for GCA, normal levels do not exclude the possibility of this diagnosis. Indeed, up to 4% of patients may fail to demonstrate elevated ESR and CRP levels at time of presentation [71]. Following an established diagnosis, monitoring

TABLE 16.2 1990 American College of Rheumatology Criteria for the Classification of Giant Cell Arteritis^a

1. Age at disease onset ≥ 50 years
Development of symptoms or findings beginning at age 50 years or older
2. New headache
New onset of or new type of localized pain in the head
3. Temporal artery abnormality
Temporal artery tenderness to palpation or decreased pulsation, unrelated to arteriosclerosis of cervical arteries
4. Elevated erythrocyte sedimentation rate
Erythrocyte sedimentation rate ≥ 50 mm/h by the Westergren method
5. Abnormal artery biopsy
Biopsy specimen with artery showing vasculitis characterized by a predominance of mononuclear cell infiltration or granulomatous inflammation, usually with multinucleated giant cells

^aFor purposes of classification, at least three criteria must be fulfilled. Sensitivity: 93.5%; specificity: 91.2%.

Adapted from Hunder et al. [8].

of inflammatory markers is recommended to assess response to therapy and disease activity.

2.2.2 Temporal Artery Biopsy

Temporal artery biopsy is still considered the diagnostic gold standard for GCA. Inflammatory vascular lesions (“skip lesions”) can be scattered irregularly along the course of the vessel and therefore an artery length of at least 1 cm should be obtained to decrease the likelihood of false-negative results [72,73]. The use of simultaneous bilateral biopsies remains debatable. Although bilateral biopsies may increase the diagnostic yield by about 10%, most patients undergo unilateral biopsy in routine clinical practice. In select cases where the diagnosis remains unclear, a subsequent contralateral biopsy can be considered. In a recent study, the use of color duplex sonography to guide temporal artery biopsy did not increase the sensitivity of biopsy for diagnosing GCA [74]. Indeed, while a positive temporal artery biopsy confirms the diagnosis, negative results among patients with GCA are common. This is particularly observed in patients with extracranial LV-GCA in which histologic findings compatible with GCA are absent in 42–48% of cases [58,61]. Although treatment with glucocorticoids may decrease the likelihood of a positive temporal artery biopsy, studies have shown that evidence of inflammation persists even 2–4 weeks after glucocorticoid initiation [75–77]. Therefore temporal artery biopsy should still be considered in patients already on GC.

Characteristic histologic findings of affected temporal artery segments include a predominant mononuclear cell infiltrate seen throughout the intima, media, and

adventitia (Fig. 16.1A). Within the media, infiltrating macrophages and activated T cells frequently form granulomas. Fragmentation of the internal elastic lamina is often seen and commonly associated with nearby multinucleated giant cells (Fig. 16.1B). Giant cells, though considered pathognomonic, are not required for diagnosis of GCA and may be absent in up to 50% of biopsies demonstrating arteritis [78]. In addition, the arterial intima may undergo concentric proliferation leading to luminal stenosis or, in some cases, occlusion.

Histopathologic findings compatible with GCA in the aorta differ slightly from temporal artery lesions. Inflammatory cell infiltrates in the aorta tend to localize predominantly in the adventitial and medial vessel wall layers with relative sparing of the intima. Furthermore intimal proliferation and luminal stenosis do not occur. Another distinguishing feature is “laminar medial necrosis” in which the medial elastic layers of the aorta collapse as a result of intramural infarction and loss of vascular smooth muscle cells (Fig. 16.2).

2.2.3 Diagnostic Imaging

2.2.3.1 Ultrasonography

Color-coded duplex ultrasonography allows for non-invasive examination of the superficial temporal arteries, extracranial vessels, and branches of the aortic arch. The most sensitive and specific ultrasonographic finding is the presence of hypoechogenic wall thickening (“halo sign”), which is due to vascular wall edema and myointimal hyperplasia [79]. In the temporal arteries, a unilateral halo sign has a sensitivity of 68–75% and a specificity of 83–91% for the diagnosis of GCA, using the ACR 1990 classification criteria as reference standard.

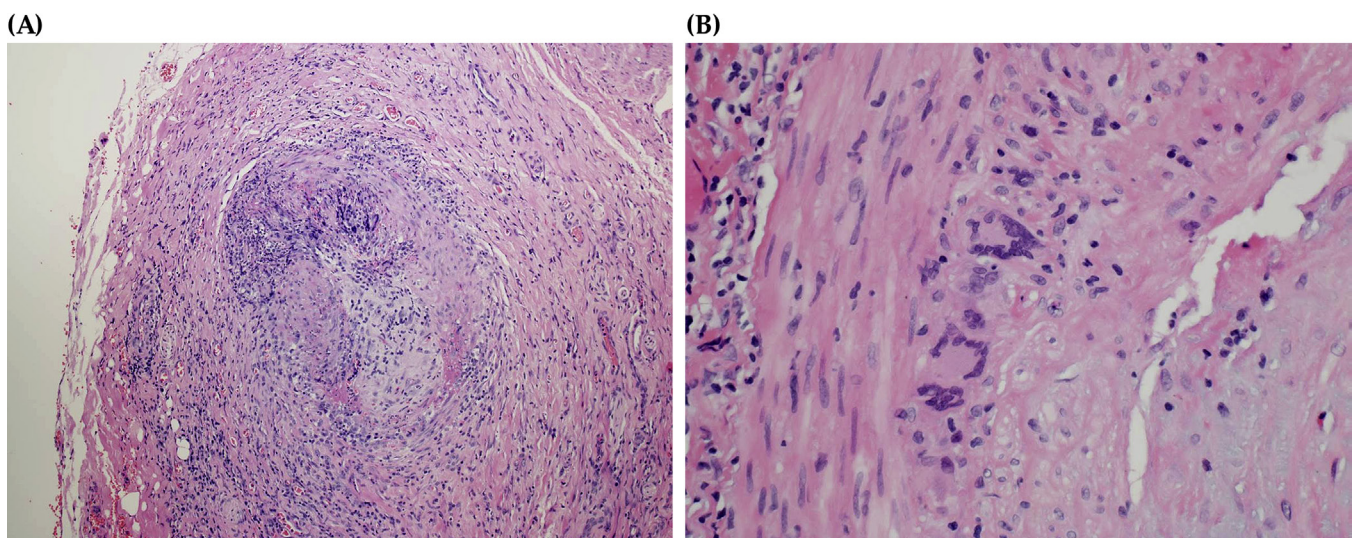


FIGURE 16.1 (A) Active giant cell arteritis in the temporal artery with transmurial lymphohistiocytic inflammation and luminal occlusion (10× magnification, hematoxylin & eosin). (B) High-power view of multinucleated giant cells present in the temporal artery medial layer (40× magnification, hematoxylin & eosin). Adapted from the personal collection of Dr. Peter Lin, Department of Pathology, Mayo Clinic, Rochester, MN, USA.

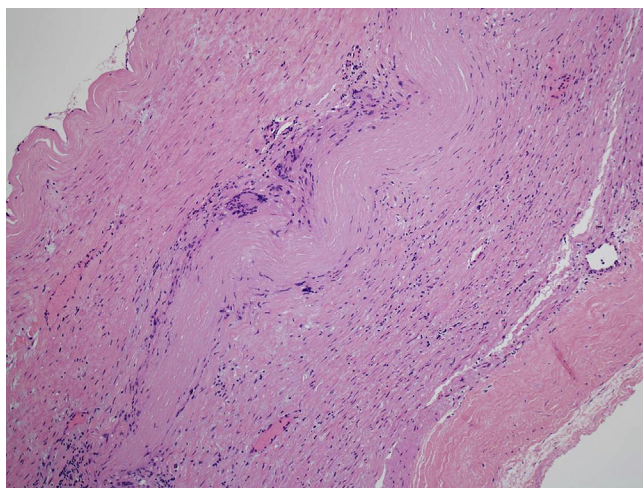


FIGURE 16.2 Active giant cell aortitis with mild lymphohistiocytic medial inflammation and giant cells adjacent to region of laminar medial necrosis (10× magnification, hematoxylin & eosin). Adapted from the personal collection of Dr. Peter Lin, Department of Pathology, Mayo Clinic, Rochester, MN, USA.

Specificity is further increased to nearly 100% if the halo sign is demonstrated bilaterally [80]. Additional imaging findings may include stenoses and occlusions of affected vessels, most commonly seen in the subclavian, axillary, and proximal brachial arteries. In a prospective study of patients with GCA, comprehensive vascular ultrasound revealed that 30% had involvement of the upper extremities arteries [81]. Advantages favoring the use of ultrasonography as a diagnostic modality include a higher resolution compared to magnetic resonance imaging, real-time image acquisition, absence of exposure to ionizing radiation, and limited cost. However, the requirement for an experienced sonographer and the inability to assess the thoracic aorta provide limitations to current widespread use.

2.2.3.2 Computed Tomography

A systematic evaluation of patients with vascular imaging techniques such as contrast-enhanced computerized tomography angiography (CTA) and high-resolution magnetic resonance imaging angiography (MRA) reveals the extent of extracranial involvement in GCA. The advantage of these imaging techniques is that they allow evaluation of vessel-wall inflammation as well as assessment of the vascular lumen for stenoses or dilatation. Radiographic evidence of large-vessel inflammation can be seen in about 68% of patients at the time of GCA diagnosis by CTA [51]. Characteristic findings observed on CTA include thickening of the arterial wall and mural enhancement involving the aorta and arch branches. In general, concentric aortic wall thickness of ≥ 2 mm (in the absence of atherosclerosis) is considered to be consistent with vasculitis [51,57]. In the

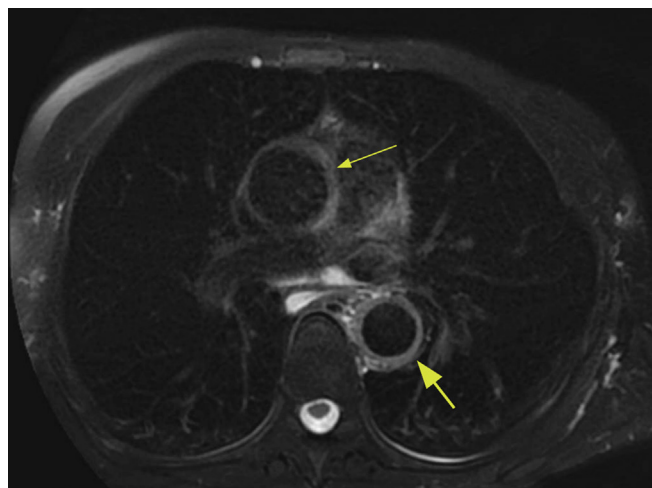


FIGURE 16.3 Axial T2-weighted fat-suppressed magnetic resonance angiography demonstrating abnormal wall thickening, edema, and delayed enhancement in the ascending (thin arrow) and descending thoracic aorta (thick arrow) in a patient with giant cell arteritis. Adapted from the personal collection of Dr. Kenneth Warrington, Division of Rheumatology, Mayo Clinic, Rochester, MN, USA.

subset of patients with arterial occlusive disease of the upper extremities, CTA typically reveals long segments of tapered stenoses. The risk of radiation exposure, especially with serial CT studies, needs to be carefully considered when using this imaging modality.

2.2.3.3 Magnetic Resonance Imaging

Similar to CTA, MRA is able to detect increased arterial wall thickness and mural enhancement (T1-weighted sequences) (Fig. 16.3), but can additionally identify vessel wall edema (in T2- and fat-suppressed sequences). Mural enhancement and wall edema are notably affected by glucocorticoid therapy and such changes can decrease within days of treatment initiation with near resolution at 2–3 months [82]. While the number of affected aortic segments and the degree of arterial thickening typically decreases with treatment, persistence of residual vessel wall thickening can be seen in up to two-thirds of patients; the clinical significance of which is currently unknown [54]. Imaging of the superficial cranial arteries using 3T high-resolution MRI is a promising noninvasive diagnostic modality in patients with GCA. Protocols employing contrast-enhanced T1-weighted images and T2-weighted inversion recovery fast-spin echo protocols have provided the ability to detect inflammatory contrast enhancement, luminal stenosis, as well as mural thickening and edema [83,84]. Compared to temporal artery biopsy, the sensitivity and specificity of MRI are 81–89% and 75–100%, respectively [85,86]. However, similar to evaluation of larger vessels by MRI, signs of vasculitis in the temporal artery tend to decrease with glucocorticoid treatment of 5 days or longer. Therefore if incorporated in

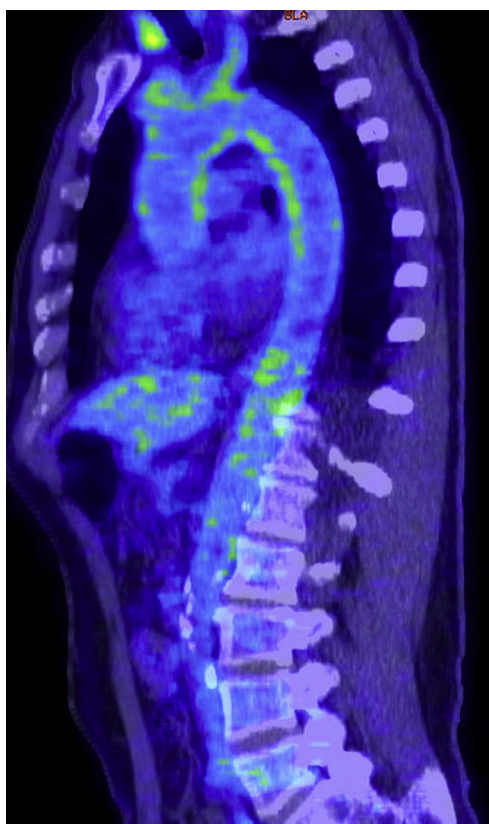


FIGURE 16.4 Positron emission tomography (sagittal view) with grade 2–3 ^{18}F -fluorodeoxyglucose uptake demonstrated in the proximal aortic braches and aortic segments (ascending/descending thoracic and abdominal). Adapted from the personal collection of Dr. Kenneth Warrington, Division of Rheumatology, Mayo Clinic, Rochester, MN, USA.



FIGURE 16.5 Attenuation-corrected positron emission tomography (coronal view) with vascular-FDG uptake outlining increased metabolic activity in the bilateral internal carotid (red arrows) and subclavian arteries (black arrows). Adapted from the personal collection of Dr. Kenneth Warrington, Division of Rheumatology, Mayo Clinic, Rochester, MN, USA.

the diagnostic evaluation of patients with suspected GCA, MR imaging should not be delayed [86]. The cost and limited timely availability of MRI may constrain its widespread use as a diagnostic test for GCA. On the other hand, color Doppler sonography of the temporal arteries is often more readily available and less expensive than MR scanning. In a study comparing the diagnostic performance of ultrasound with that of MRI, the sensitivity and specificity of these two imaging modalities for detecting GCA was similar [87].

2.2.3.4 ^{18}F -fluorodeoxyglucose Positron Emission Tomography

Positron emission tomography (PET) is a nuclear medicine technique that uses a radiolabeled glucose analogue (^{18}F -fluorodeoxyglucose [FDG]) to evaluate the degree of uptake in metabolically active cells. The intensity of FDG uptake correlates with the level of inflammation and is semiquantitatively classified on a four-point scale: none (grade 0), less than liver uptake (grade 1), similar to liver (grade 2), and higher than liver (grade 3) [88]. In GCA, inflammatory cells in the wall of the affected arterial segments are highly metabolic and FDG-avid. Vessel uptake,

with grades 2 or 3, is reasonably specific for vasculitis (Fig. 16.4). FDG uptake on PET can also be analyzed semiquantitatively using the maximum standardized uptake value [SUV_{max}] or SUV_{max} normalized by standard uptake value of the liver or inferior vena cava [89]. According to a recent meta-analysis PET has a sensitivity of 90% and specificity of 98% for the diagnosis of GCA [89]. A unique advantage of PET is the capability, through whole-body scanning, to demonstrate concurrent visualization of all large vessels except the temporal arteries and renal arteries, thus providing information on both the extent and severity of large-vessel vasculitis (Fig. 16.5). However, PET alone is limited by the inability to assess structural abnormalities in the vasculature. When PET is combined with low-dose CT (PET-CT) vascular anatomic detail (eg, aneurysm or stenosis) can be correlated with metabolic activity. The diagnostic accuracy of PET markedly decreases following the initiation of glucocorticoids and therefore limits use beyond early diagnosis. In addition, the utility of PET during follow-up is poorly defined and cost may limit its routine use.

TABLE 16.3 Systematic Review of Giant Cell Arteritis Cases With Coronary Arteritis

Author(s)	Year	Age (yr)/Sex	ESR (mm/h)	Presentation	Arteritic involvement	Main cause of death
Cooke et al. [90]	1946	73/F	–	PMR, HA, vision loss, fatigue, wt loss	T, A, Co, O, R, Ret, S mes	Stroke
Ritama [91]	1951	63/F	100	PMR, fatigue, malaise	T, A, Ca, Co	Stroke
Morrison & Abitol [92]	1955	67/M	61	HA, vision loss	T, Bas, Co, O	MI
Bonnin & Lander [93]	1956	75/F	–	HA, vision loss	T, A, Ca, Co, O, Lingual	MI
Crompton [94]	1959	71/M	45	Vision loss	T, Ca, Cil, Co, V	MI
Spencer & Hoyt [95]	1960	77/M	–	Vision loss, HA,	T, A, Ca, Co, I, O	MI, embolic stroke
Ainsworth et al. [96]	1961	85/–	–	No data	T, Co	MI
		86/–	–	No data	T, Co	Aortic aneurysm rupture
Harrison & Bevan [97]	1967	68/M	123	PMR, HA, vision loss	T, Co	MI
Harris [98]	1968	70/F	55	Aortic aneurysm rupture	*A, Ca, Co, Lingual	Aortic aneurysm rupture
Hamrin et al. [99]	1968	74/M	133	PMR, vision loss	T, A, Ca, Co, Sc, S mes	Malignancy, pulmonary edema
Östberg [59]	1973	3 cases of Co among 6 patients with GCA – presentation and cause of death not detailed				
Klein [47]	1975	73/F	98	PMR	*A, Co, Ren, Sc	MI
Martin [100]	1980	77/F	85	HA, wt loss, jaw claudication	T, Co, V	MI
Bengtsson & Malmvall [118]	1981	82/F	50	PMR	Bas, Co	MI
Lie et al. [102]	1986	84/M	–	PMR, HA	T, A, Co	MI
Säve-Söderbergh et al. [103]	1986	73/F	106	HA, PMR, jaw claudication, wt loss	T, A, Co	MI, ventricular wall rupture
		85/F	50	PMR	A, Bas, Co	MI, ventricular wall rupture
		75/F	62	HA, scalp tenderness, wt loss	T, A, Bas, Co, V	Stroke
		79/F	78	HA, fever	T, A, Bas, Co	Aortic dissection
Hupp et al. [104]	1990	82/F	88	PMR, HA, jaw claudication, fatigue, vision loss	T, Bas, Ca, Cil, Co, O, Sc	MI
Morris & Scheib [105]	1994	68/M	114	HA, PMR, jaw claudication, fatigue	T, Co	MI
Freddo et al. [106]	1999	75/M	121	PMR, wt loss, vision loss	T, Co	Stroke
Kumar et al. [107]	2002	74/F	–	No data	*Co	MI
Karger & Fechner [108]	2006	84/F	93	No data	*Co	MI
Godoy et al. [109]	2007	83/F	110	HA, jaw and arm claudication, wt loss, vision loss	T, Ca, Cil, Co, O, V	MI, ventricular wall rupture
Lin et al. [110]	2007	74/F	–	No data	*Co	Alive after heart transplant

A, aorta; Bas, basilar; Br, brachial; Ca, carotid; Cil, ciliary; Co, coronary; F, female; HA, headache; I, iliacs; M, male; MI, myocardial infarction; O, ophthalmic; PMR, polymyalgia rheumatica; R, radial; Ren, renal; Ret, retinal; Sc, subclavian; S mes, superior mesenteric; T, temporal; V, vertebral.

*Temporal artery biopsy not performed or not reported.

3. GIANT CELL ARTERITIS-ASSOCIATED CARDIOVASCULAR INVOLVEMENT

3.1 Coronary Arteritis

The exact frequency of vasculitis involving the coronary arteries in GCA is unknown, but is considered to be rare. Less than 30 autopsy-confirmed cases of coronary artery involvement have been published to date [47,59,90–110] (Table 16.3). While additional cases have been suspected based on the temporal association of GCA diagnosis and myocardial infarction, pathologic confirmation of coronary arteritis in these reports is absent [111,112].

Nevertheless, coronary arteritis may represent an underrecognized manifestation of GCA for several reasons. Coronary events due to arteritis among elderly patients may be incorrectly attributed to the more frequently occurring cause of atherosclerotic disease. As such, elderly patients suffering myocardial infarction are unlikely to undergo postmortem examination to determine if coronary arteritis was present [104]. In addition, asymptomatic coronary arteritis in patients treated with glucocorticoids for GCA may not be detected clinically [105].

Early reports of coronary arteritis frequently described myocardial infarction occurring within days or weeks following GCA diagnosis [94,95,97,104] or glucocorticoid initiation [92,93,95,100,103]. The majority of these cases report delayed diagnosis of GCA, late presentation with advanced vasculitis burden, and failure to initiate or adequately treat patients with appropriate doses of glucocorticoid therapy. In recent years, reports of coronary arteritis in GCA have become less frequent. Case reports now often highlight atypical presentations in which GCA symptoms were absent prior to myocardial infarction and arteritis tends to be confined to the cardiac vessels [107,108,110]. Increased physician awareness of GCA and earlier diagnosis and treatment likely contributed to this shift in disease expression.

Though coronary arteritis has often been observed in association with proximal aortitis, involvement of the ascending aorta is not requisite [100,105]. Furthermore, similar to the “skip lesions” observed in temporal arteries, coronary arteritis may be uni- or multifocal and is infrequently contiguous along the full length of a single vessel [59,90,113]. Branches of both the left and right coronaries are affected without apparent anatomical predilection. Inflammation in the coronary ostia and epicardial branches is more regularly observed. Although giant cell arteritis in the intramural and septal branches has been reported, such involvement is considered less common [107,110].

The histopathologic abnormalities observed in coronary vessels affected by GCA are identical to those seen in the superficial temporal arteries, and intimal hyperplasia results in luminal narrowing. Given the age group of patients affected, concomitant atherosclerosis is often

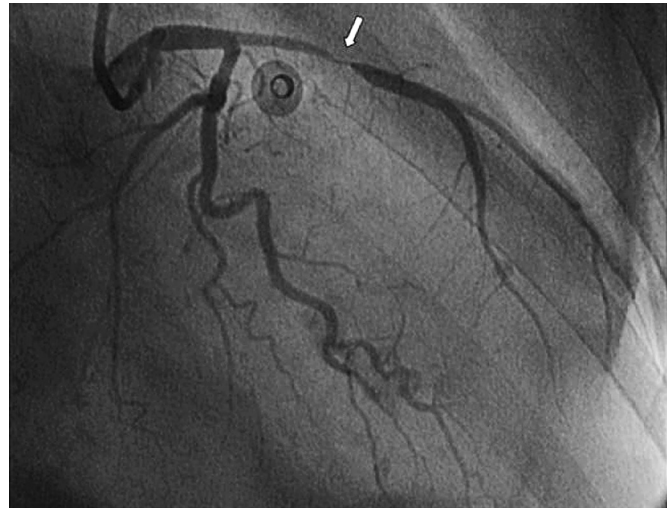


FIGURE 16.6 Coronary angiography of the left anterior descending artery revealing tapered smooth narrowing (arrow) in patient with giant cell arteritis. Adapted from the personal collection of Dr. James J. Jang, Mount Sinai School of Medicine, New York, NY, USA.

present in the majority of vessels involved by vasculitis. However, evidence of myocardial infarction due to coronary arteritis in the absence of associated atheromatous disease has also been detected [94,100,102].

Patients with either established or suspected GCA presenting with new onset chest pain and evidence of myocardial ischemia should be evaluated and managed according to current acute coronary syndrome guidelines with interventions aimed at myocardial reperfusion [114,115]. Neither electrocardiogram nor cardiac biomarker elevation can immediately differentiate patients with symptoms due to coronary arteritis versus unstable plaque rupture. In addition, inflammatory markers are nonspecific and can be elevated in myocardial infarction of any etiology [116,117] preventing their use as reliable screening measures in this population. Coronary angiography features in GCA are indistinguishable from atherosclerotic lesions; however, evidence of a *tapered smooth narrowing* has been described as suggestive for coronary arteritis (Fig. 16.6) [113]. If coronary arteritis is suspected, patients should undergo thorough clinical evaluation for systemic vasculitis. Moreover, the clinician may consider initiation of glucocorticoids while awaiting diagnostic confirmation and rheumatology evaluation.

3.2 Atherosclerosis and Coronary Artery Disease

Atherosclerosis is now well-recognized as a local, chronic inflammatory disease of the arterial wall mediated by proinflammatory cytokine release, enhanced expression of cellular adhesion molecules, and endothelial recruitment of activated inflammatory cells [119,120]. Premature atherosclerosis is common in patients with

rheumatic conditions due to the presence of ongoing or uncontrolled inflammatory mechanisms and immune dysregulation, which can promote accelerated vascular plaque formation [121]. While systemic inflammation may act synergistically with traditional cardiovascular risk factors, the direct pathophysiologic link between inflammation, endothelial dysregulation, and atherosclerosis remains elusive [122].

The vasculitides are distinct from other rheumatic diseases due to the direct vascular injury related to the disease process itself. Therefore, complex interaction of overt vascular inflammation as well as the effects of systemic inflammation and traditional cardiovascular risk factors

likely contributes to cardiovascular disease in vasculitis. Although evidence of accelerated atherosclerosis has been seen in the antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides and Takayasu arteritis, this association has not been observed in patients with GCA [122]. Compared with other vasculitides and autoimmune conditions, the advanced age at onset of GCA provides notable limitations in differentiating the pathophysiologic impact of the disease itself from an already high prevalence of atherosclerotic disease in this elderly population.

Whether the risk of coronary artery disease is increased in patients with GCA is uncertain and epidemiologic studies have provided conflicting results

TABLE 16.4 Characteristics of Studies Evaluating Coronary Artery Disease in Giant Cell Arteritis

	Ray et al.	Le Page et al.	Molloy et al.	Amiri et al.	Tomasson et al.	Udayakumar et al.
Country	Canada	France	USA	Canada	United Kingdom	USA
Study design	Retrospective	Prospective	Retrospective	Retrospective	Retrospective	Retrospective
year of the study	2002	2006	2007	2013	2014	2015
Diagnosis of GCA	All patients diagnosed with GCA in Ontario Health Insurance Plan database from January 1, 1995 to March 31, 2002	Patients with new diagnosis of GCA recruited from participating hospitals (January 1991 to April 2004)	All patients hospitalized with diagnosis of GCA in year 2004 identified through the National Inpatient sample database	All patients in province of British Columbia with GCA diagnosed between 1990 and 2010	All patients diagnosed with GCA in the Health Improvement database from January 1990 to June 2010	All patients in Olmsted county, Minnesota, USA diagnosed with GCA between 1950 and 2009 by 1990 ACR classification criteria
Controls	Age > 65 years, subjects randomly selected from the same database	Age-, sex-matched subjects randomly selected from general population	Age > 50 years randomly selected from same database	Age-, sex-matched subjects randomly selected from same database	Age-, sex-, and time of entry-matched subjects randomly selected from same database	Age-, sex-matched subjects from the same database
Coronary artery disease definition	MI, angina, or coronary artery revascularization	MI or angina	Not available	MI	MI	Unstable angina, MI (STEMI or NSTEMI)
Mean age of cases (yrs)	75.2	75.1	Not available	75.0	73.1	76.2
Number of cases	1142	432	4807	834	3408	245
Number of controls	200,000	483	19,228	8340	17,027	245
Average follow-up (yr)	2.7	2.0	Not available	Not available	3.9	9.7
Confounder adjustments	Age, sex, HTN, DM, dyslipidemia, medications	Age, sex, HTN, DM, cancer, dyslipidemia, smoking, PVD	Age, sex, race, income, HTN, DM, obesity, dyslipidemia, PVD	Age, sex, angina history, hormone replacement, COPD, obesity, DM, dyslipidemia, NSAIDs, number of hospitalizations	Age, sex	Age, sex, calendar year of index date
Risk ratio (95% CI)	1.90 (1.27, 2.85)	1.67 (0.72, 3.88)	0.83 (0.77, 0.90)	3.00 (2.25, 4.01)	2.03 (1.70, 2.43)	0.74 (0.44, 1.25)

ACR, American College of Rheumatology; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; GCA, giant cell arteritis; HTN, hypertension; MI, myocardial infarction; NSAIDs, nonsteroidal antiinflammatory drugs; NSTEMI, non-ST elevation myocardial infarction; PVD, peripheral vascular disease; STEMI, ST-elevation myocardial infarction; USA, United States of America.

Adapted from Ungprasert et al. [131].

[101,123–130] (Table 16.4). However, a recently conducted systematic review and *meta*-analysis evaluated all cohort and case–control studies reporting coronary artery disease incidence in GCA. The study concluded that when comparing the combined 10,868 patients with GCA and 245,323 controls, the incidence of coronary artery disease in GCA was not significantly increased (risk ratio [95% CI] 1.51 [0.88, 2.61]) [131]. Nevertheless, considering the age of the patients affected and the frequent accumulation of traditional cardiovascular risk factors (induced or exacerbated by glucocorticoid treatment), attention to the prevention or adequate control of diabetes, obesity, dyslipidemia, and hypertension is recommended. Obtaining an accurate blood pressure reading can be challenging in patients with bilateral upper extremity arterial occlusive disease due to GCA. In these patients, arm (brachial) blood pressure measurements underestimate the actual arterial blood pressure. Therefore, lower extremity noninvasive vascular studies and blood pressure measurements at the ankle can be helpful in such cases (only if there is no significant lower extremity arterial disease or femoropopliteal vasculitis).

3.3 Coronary Artery Dissection

The occurrence of coronary artery dissection (CD) in the general population is considered rare. An observational

study using the Nationwide Inpatient Sample (NIS) database evaluated 8 million hospital stays from 1000 US hospitals during the period 2009–2010 and found the prevalence of coronary artery dissection to be 0.02% [132]. Of the 11,255 patients with CD, only 0.05% had International Classification of Disease, version 9 (ICD-9) codes for GCA listed at time of admission or discharge [132]. In comparison, coronary artery disease (92%), hypertension (70%), hyperlipidemia (64%), diabetes (30%), and smoking (22%) were much more frequently associated comorbid conditions. Given the retrospective design of this database study, information was not available regarding confirmation of GCA or its activity at the time of CD diagnosis. Therefore the contribution of GCA to CD is unknown.

3.4 Involvement of Heart-Wall Layers

3.4.1 Pericardium/Epicardium

The occurrence of pericardial disease in GCA is unusual [133]. Indeed, a large survey of 95 patients with active GCA found that only 2% developed pericardial inflammation [118] and an extensive literature review yielded only 21 reports of GCA-related pericarditis [134–152] (Table 16.5). Chest pain and dyspnea were presenting features in 80% of cases, while a few asymptomatic patients were identified incidentally. Cardiac auscultation alone is not reliable for diagnosis, as detectable friction rubs and murmurs are present in as few as 50% of cases. Constitutional symptoms are frequent but less than one-half demonstrate cranial manifestations and merely one-third note polymyalgia rheumatica symptoms at presentation. An exudative pericardial effusion is universally present but tamponade is notably rare [141].

Unless presenting with features that raise suspicion for GCA, exclusion of more common etiologies in this age group (infection, malignancy, collagen vascular disease, hypothyroidism, or metabolic irregularities) should be pursued. In atypical cases, vascular imaging may disclose large-vessel inflammation [134,135,138] but temporal artery biopsy should still be obtained for confirmation. Among elderly patients with “idiopathic” pericarditis, additional features that may raise suspicion for GCA include failure to respond to nonsteroidal antiinflammatories [135] and rapid reaccumulation of pericardial fluid following pericardiocentesis [138].

3.4.2 Myocardium

Myocarditis is an extremely rare feature of GCA and has been detailed in five clinical cases [153–156] and four autopsies [47,99,157,158]. The pathophysiology of GCA-associated myocarditis is unknown. Diffuse vasculitis of the small myocardial vessels has been proposed [159]; however, histopathologic findings of intramyocardial

TABLE 16.5 Clinical Data of Patients With Pericarditis Due to Giant Cell Arteritis

Clinical features	Number	Percent
Mean age, years (range)	68.4 (55–86)	–
Sex (female/total)	18/21	86
Pericardial manifestations	21/21	100
Murmur/rub/gallop	9/18	50
Effusion	21/21	100
Pain	17/21	81
Shortness of breath	15/19	80
Classical GCA manifestations	13/21	62
Cranial symptoms	9/19	47
Polymyalgia rheumatica	6/18	33
Fever	8/16	50
Weight loss	12/16	75
ESR, mm/h (range)	31–140	–
>75	14/19	74
>100	10/19	53
Temporal artery positive	20/20	100

ESR, erythrocyte sedimentation rate.

vasculitis on autopsy have not been reported in all cases [47,157].

The most frequent presenting symptoms are precordial chest pain and progressive exertional dyspnea. Cardiac biomarker elevation is variable but dynamic electrocardiogram changes are frequent, often mimicking acute coronary syndrome with T-wave inversions, ST-segment elevations, or the presence of Q waves [153,154,156]. Coronary angiography is often performed during evaluation but is invariably negative for focal coronary occlusion. Echocardiography frequently discloses small-to-moderate pericardial effusions without hemodynamic compromise [153,154]. Mild reductions in left ventricular ejection fractions can be seen as well as apical or global hypokinesis [155,156]. Cardiac-gated magnetic resonance imaging typically demonstrates contrast enhancement consistent with myocarditis [153] but is not specific for GCA.

All reported patients presented with malaise, anorexia, weight loss, and low-grade fevers in the context of markedly elevated inflammatory markers raising the suspicion for an infectious cause, which must be considered and ruled out. Only one patient presented with characteristic cranial symptoms of GCA [154], while in other cases the diagnosis was delayed due to lack of typical presenting features. Despite normal temporal artery examinations and absence of cranial features, temporal artery biopsies were positive in all cases when performed [153–155].

Following the initiation of high-dose glucocorticoids (40–60 mg/day of prednisone equivalent) prompt resolution of chest pain is seen, often within 24–72 h. Disappearance of pericardial effusion and dynamic electrocardiogram changes can occur as soon as 2–8 weeks after treatment is started. Daumas and colleagues [153] have suggested that electrocardiogram and echocardiography should be systematically performed in patients presenting with GCA to determine if myopericarditis is present. However, GCA-associated myopericarditis generally responds to glucocorticoid doses recommended for initial treatment of GCA. Therefore if no cardiac symptoms are present and examination does not disclose signs of cardiac insufficiency or heart murmur (ie, friction rub, pericardial knock, summation gallop) then such investigations are considered to be of low utility and should not be routinely obtained.

3.4.3 Endocardium

Involvement of the endocardium in the context of GCA has not been reported clinically or in autopsy studies. Dilation of the ascending aorta and aortic annulus (annuloaortic ectasia) can occur in the context of proximal aortitis, resulting in aortic valve insufficiency [160,161]. Significant regurgitation through the aortic valve is secondary to the dilated aortic root and not due

to inflammatory destruction of the valve leaflets. Aortic valve insufficiency in isolation of annuloaortic ectasia has not been described. Similarly, mitral, tricuspid, and pulmonary valve insufficiency do not occur unless in conjunction with advanced heart failure from severe aortic insufficiency [162].

3.5 Aortic Aneurysm/Dissection

Patients with GCA are at an elevated risk of developing aortic complications that are associated with significant morbidity and mortality. The ascending aorta is preferentially involved with thoracic aortic aneurysms and/or dissections occurring three times more frequently than abdominal aortic complications [163,164] (Fig. 16.7A–C). In a population-based study, patients with GCA were 17.3 times more likely to develop thoracic aortic aneurysms and 2.4 times more likely to develop abdominal aortic aneurysms than the general population [165].

Earlier population-based retrospective studies in the United States [163,165] and Europe [164] have demonstrated an incidence of aortic aneurysm and/or dissection in patients with GCA to be 19 per 1000 person-years at risk. These studies also reported a prevalence of aortic aneurysm of 9.5–18% [163,164]. However, the incidence and prevalence of aortic structural damage in patients with GCA is likely to be underestimated, particularly in retrospective cohort studies in which only clinically detected events are recorded. In a cross-sectional study of patients undergoing systematic screening for aortic dilatation, aortic structural damage was detected in 22% of patients at a median of 5 years after GCA diagnosis [166]. The same study cohort was followed for a median of 10 years from GCA diagnosis, at which time the prevalence of aortic damage had risen to about 33% [53].

Vascular damage to the aorta characterized by histopathologic patterns of intramural infarction, depletion of smooth muscle cells, and breakdown of the medial elastic layer by upregulated gelatinases and matrix metalloproteinases leads to aortic-wall weakening and aneurysmal development [167,168].

It is unknown, however, whether patients with ongoing but subclinical aortic inflammation are at a higher risk of aneurysmal formation. Indeed, histopathologic evaluation of aortic specimens obtained at surgical removal or autopsy in a prospective series found extensive destruction of medial elastic fibers in all patients with GCA-related aneurysms but only two of six samples displayed minimal residual inflammatory infiltrates [51]. In contrast, in another case series, five of seven patients with thoracic aortic dissection were found to have active aortitis at autopsy [163]. The utility of chronic low-dose glucocorticoids to prevent late-stage aortic complications in asymptomatic patients with GCA who have achieved clinical remission is uncertain.

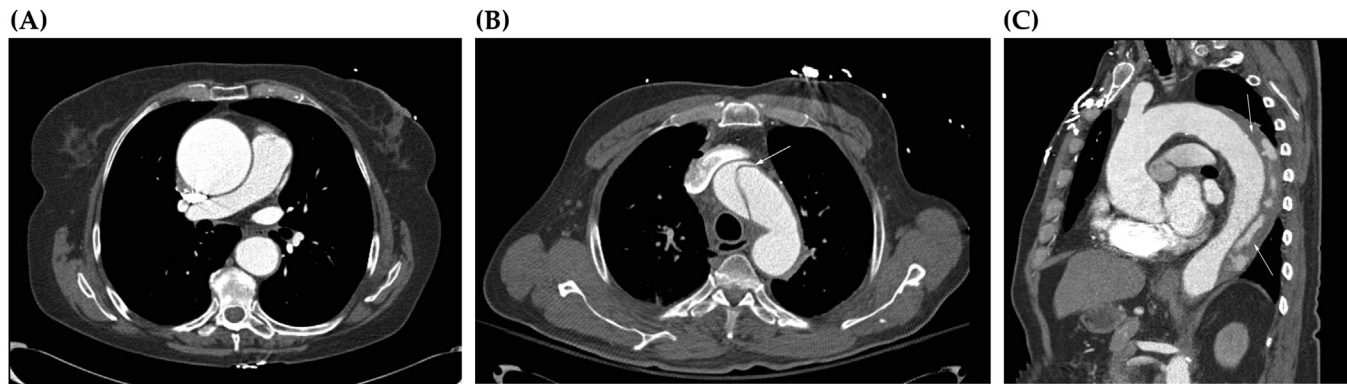


FIGURE 16.7 Computed tomography angiography of patients with GCA demonstrating 6.4 cm ascending aortic aneurysm (panel A – axial view), proximal thoracic aortic dissection (panel B – axial view) and type B dissection of thoracic aorta with false lumen (arrows) posteriorly (panel C – sagittal view). Adapted from the personal collection of Dr. Kenneth Warrington, Division of Rheumatology, Mayo Clinic, Rochester, MN, USA.

The disease-specific risk factors identified for the development of aneurysm/dissection include increased aortic FDG uptake by PET at GCA diagnosis [56], younger age at GCA diagnosis [164], and cumulative time since GCA diagnosis [63]. Garcia-Martinez et al. reported that a weak inflammatory response at diagnosis of GCA, lower frequency of disease relapses, and shorter duration of glucocorticoid therapy were associated with the development of aortic damage [166]. Additional influences of smoking [169], hyperlipidemia [163,166], and hypertension [164,169] are analogous to modifiable risk factors that contribute to increased threat of aortic structural damage in the general population and underscore the requirement for aggressive management of such comorbidities in patients with GCA.

Although several studies have shown that the overall mortality in patients with GCA is the same as the general population, Kermani and coworkers observed that the subset of patients with GCA who develop aortic aneurysm and/or dissection have a 2.6-fold increased mortality compared to non-GCA patients [63]. In view of the adverse outcomes associated with aortic aneurysm, a screening strategy for early detection may be of benefit.

There are only limited data regarding optimal screening methods and frequency for aneurysms in patients with GCA due to the absence of controlled trials. Current practice patterns for screening are variable and based on consensus opinion. The United Kingdom guidelines for management of GCA recommend a chest radiograph every 2 years following diagnosis to screen for thoracic aortic aneurysm [170]. Nevertheless, in a cohort of non-GCA patients with acute aortic complications, chest radiography had a sensitivity of only 60% for the detection of thoracic aortic aneurysm [171]. In addition, among patients with GCA, there is a low correlation between conventional chest radiography and computed tomography findings [166]. The American cardiology and vascular medicine societies advocate that all patients with

GCA undergo computed tomographic or magnetic resonance angiography at the time of diagnosis to assess for aortic dilatation; however, recommendations for longitudinal screening are absent [172].

While a recent systematic review determined that, on average, seven patients with GCA would need to be screened by an imaging modality to detect one thoracic aortic aneurysm/dissection, the heterogeneity of the evaluated studies prevented development of specific recommendations on the type of imaging modality or appropriate screening intervals [173]. Furthermore, the lone prospective study evaluating systematic screening for aortic aneurysm/dissection with long-term follow-up suffered from a high dropout rate due to the advanced age of the GCA population with only 14 of the initial 54 patients completing a third screening stage [53].

In addition to resource allocation and cost, patient suitability for aneurysm repair must be considered prior to screening. Indeed, of the 16 aneurysms detected among the 54 patients screened by Garcia-Martinez and colleagues, 8 patients were eligible to undergo corrective surgery but only 3 did so; the remainder were not operated on due to patient preference or due to high surgical risk related to comorbidities [53].

While the ascending aorta is preferentially involved in GCA compared to the descending aorta, the opposite is true for degenerative atherosclerosis. Therefore it remains uncertain if the natural history of established aortic dilatation in patients with GCA differs from that of patients with noninflammatory aortic aneurysms. In the absence of conclusive prospective clinical trial data, current surveillance and treatment strategies have been based on the recommended management of patients with degenerative or atherosclerotic aneurysms [172,174].

In general, surgical management of aortic aneurysm due to GCA requires a tailored approach and should be done at experienced institutions. In cases where the

ascending aorta is involved, it is unclear whether valve-sparing techniques should be performed. Gelsomino et al. [161] recommended that all patients undergo replacement of the aortic root and aortic valve with a Bentall procedure since those undergoing valve-sparing repair subsequently developed dilation of the native sinuses and required reoperation. However, Zehr and colleagues [162] observed that since the aortic valve tissue was uninvolved with the inflammatory process, a valve-sparing technique could be performed provided that a functional aortic valve was present.

Whether aneurysmal dilation of the remaining aorta and great vessels can be abrogated by an aggressive regimen of postoperative glucocorticoids is unknown. In cases where complete arch reconstruction is performed, the use of a free-floating graft (elephant trunk) extending from the aortic arch into the descending aorta is suggested to allow for subsequent repair of the descending aorta [162]. The comparative efficacy of endovascular repair of the descending aorta has not been studied; however, favorable outcomes have been reported in high-risk patients unable to undergo open surgical replacement [175–177].

4. TREATMENT OPTIONS AND CARDIAC IMPLICATIONS OF THERAPY

4.1 Glucocorticoid Therapy

Prompt initiation of high-dose glucocorticoids (GCs) remains the cornerstone for remission induction treatment of GCA. Based on expert opinion and decades of experience with the use of glucocorticoids for GCA, the European League Against Rheumatism (EULAR) recommends an initial prednisolone dose of 1 mg/kg/day (maximum 60 mg/day) to be initiated and maintained for 1 month [178]. Patients presenting with severe ischemic manifestations, particularly visual loss, may benefit from pulsed intravenous methylprednisolone (1 g daily for 3 consecutive days) (Table 16.6); however, prospective, randomized-controlled trials demonstrating superiority of intravenous therapy over high-dose oral treatment are lacking and these treatment strategies require further investigation. Delay in treatment initiation can increase the risk of severe ischemic complications, particularly in patients presenting with recent visual symptoms [179]. Therefore glucocorticoid therapy should be started promptly in patients with a strong suspicion of GCA and should not be deferred pending confirmation by imaging or biopsy.

After attaining remission, the GC dose should be gradually decreased. Although treatment response to glucocorticoid tapering is highly variable, the British

Society for Rheumatology (BSR) provides general recommendations. After 3–4 weeks of high-dose GCs, prednisolone can be reduced by 10 mg every 2 weeks to a dose of 20 mg, then down by 2.5 mg every 2–4 weeks to 10 mg, then by 1 mg every 1–2 months, provided no flares occur [73]. Within 2 years 30–50% of patients will experience a clinical relapse. If a relapse occurs, it can be managed by increasing the GCs temporarily with subsequent tapering. The duration of treatment in most cases is approximately 2–3 years; however, some patients may have a chronic-relapsing course and require long-term GCs [180].

GC treatment in the affected age group is associated with a high number of adverse effects. Prophylaxis for osteoporosis should be provided. Physicians should additionally monitor for the development or worsening of arterial hypertension and steroid-induced diabetes and institute treatments when clinically indicated. Patients with GCA may be at increased risk of infection, particularly early in the disease course [181]. Age-appropriate immunizations should be provided, although live vaccines (eg, zoster vaccine) are contraindicated in the context of high-dose glucocorticoid therapy. Pneumocystis jiroveci pneumonia (PJP) occurs rarely in patients with GCA. However, this preventable infection is associated with significant morbidity and mortality. Therefore unless contraindicated, PJP prophylaxis is recommended until the prednisone dose reaches 15 mg daily [182].

4.2 Glucocorticoid-Sparing Agents

Adjunct immunosuppressive agents should be considered for patients with significant GC-related adverse effects and/or relapsing disease (Table 16.6) [178]. Methotrexate has been best studied in prospective clinical trials, although its efficacy in GCA is modest. A meta-analysis of three randomized-controlled trials evaluating the efficacy of methotrexate (dose range 10–15 mg/week) added to GC therapy found that the combination therapy led to lower cumulative GC doses and reduced the risk of first relapse by 35% and second relapse by 51% [188]. Cyclophosphamide is only used in exceptional circumstances for patients with life- or organ-threatening disease that is refractory to conventional therapy, but the benefit of this therapy is uncertain at best [192].

Anti-TNF- α therapies added to GCs have shown no clear benefit in the treatment of newly diagnosed [196] or refractory patients with GCA [195]. The humanized monoclonal interleukin-6 receptor antagonist, tocilizumab, has shown promising results in a number of case series and is currently being tested in a multicenter randomized-controlled trial. Tocilizumab often causes an increase in the serum lipid levels; however, the potential cardiovascular consequences are as yet unclear.

TABLE 16.6 Treatment of Giant Cell Arteritis

Glucocorticoid therapy			
Dose	Studies	Evidence	Recommendation
GCA with recent/impending vision loss			
<ul style="list-style-type: none"> Methylprednisolone i.v. 1 g daily for 3 days, followed by standard oral prednisone (as below) 	Retrospective cohort [183]	C	I Pulse glucocorticoids may increase likelihood of vision improvement
GCA (no vision symptoms)			
<ul style="list-style-type: none"> Prednisone 40–60 mg daily for 4 weeks Reduction by 10 mg every 2 weeks to a dose of 20 mg daily Reduction by 2.5 mg every 2–4 weeks to a dose of 10 mg daily Reduction by 1 mg every 1–2 months 	Retrospective cohort studies [179,180,184]; Decades of clinical experience, expert consensus [178]	C	I Glucocorticoids: <ul style="list-style-type: none"> reduce risk of vision loss if started promptly rapidly improve symptoms and inflammatory markers
Relapse			
<ul style="list-style-type: none"> Dosing depends on relapse severity Severe flare with ischemic symptoms: Repeat induction regimen Nonsevere flare: Increase prednisone to previous effective dose 		C	I Glucocorticoids: <ul style="list-style-type: none"> rapidly improve symptoms and inflammatory markers
Immunosuppressive agents			
Drug, dose	Studies	Evidence	Recommendation
<ul style="list-style-type: none"> Methotrexate 7.5–20 mg/week 	Three randomized trials [185–187] Meta-analysis [188]	A	IIa Methotrexate reduced: <ul style="list-style-type: none"> risk of first and second relapse exposure to GC
<ul style="list-style-type: none"> Azathioprine 150 mg/day 	Small randomized trial [189]	B	IIb Small possible benefit as GC-sparing agent
<ul style="list-style-type: none"> Leflunomide 10–20 mg/day 	Case-series [190]	C	IIb Possible efficacy as GC-sparing agent
<ul style="list-style-type: none"> Cyclophosphamide oral or i.v. pulses 	Small open label study [191] Retrospective case series [192]	B	II Used rarely for patients with refractory and life or organ-threatening disease
Biologics			
Drug, dose	Studies	Evidence	Recommendation
<ul style="list-style-type: none"> Tocilizumab i.v. 4–8 mg/kg monthly 	Small multicenter open-label trial [193]; Cohort study [194]	B	I Favorable clinical and laboratory response in patients with relapsing/refractory GCA
<ul style="list-style-type: none"> Etanercept 25 mg s.q. twice weekly 	Small randomized trial [195]	B	III No evidence of benefit
<ul style="list-style-type: none"> Infliximab i.v. 5 mg/kg at 0, 2, and 6 weeks and every 8 weeks for 22 weeks 	Multicenter, randomized, double-blind, placebo-controlled trial [196]	B	III No efficacy of infliximab as maintenance therapy in patients with GCA treated with glucocorticoids

4.3 Antiplatelet Medications

Preclinical studies using mice engrafted with inflamed human temporal arteries have shown that acetylsalicylic acid (ASA) is a highly effective inhibitor of cytokine transcription through suppression of IFN- γ .

In these murine studies, the combination of ASA and corticosteroids had synergistic effects with further suppression of proinflammatory cytokines in the vascular lesions of GCA [197]. Neshor and colleagues [45], in a retrospective review of 175 patients with GCA, observed that patients treated with low-dose

ASA were 3.6 times less likely to present with cranial ischemic events at diagnosis and 4.3 times less likely to further develop ischemic complications during treatment with GC.

Variable results have been observed in additional retrospective studies. While some studies conclude ASA may reduce cardiovascular events [128,129] and decrease visual and cerebrovascular ischemic complications [198], other studies have not replicated these findings [199,200]. In one cohort study, patients on antiplatelet/anticoagulation therapy had a higher frequency of ischemic complications [201]. A recent *meta-analysis* concluded that treatment with antiplatelet/anticoagulation therapy prior to GCA diagnosis does not protect against the development of severe ischemic complications. However, antithrombotic therapy may provide modest protection (odd ratio 0.32; 0.10–0.99) against incident severe ischemic complications once GCs are initiated [202]. Current EULAR recommendations for the management of GCA include treatment with ASA 75–150 mg/day, unless contraindicated [178].

4.4 Lipid-Lowering Therapies

Inhibitors of 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase, or “statins,” have shown pleotropic properties including improving endothelial dysfunction, reducing thrombogenicity as well as providing antiinflammatory and immunomodulatory effects [203]. Therefore the potential benefit of these medications in patients with GCA is of clinical interest. Although one population-based study observed that patients treated with statins were less likely to develop GCA [204], these results have not been replicated by other investigators [205]. The use of statin medications among patients with GCA in additional retrospective studies has not shown a significant clinical impact in relapse prevention, glucocorticoid therapy reduction, or decrease in ischemic events [204,206,207]. Consequently, statin medications are not considered requisite adjuncts in the management of GCA and should be reserved for patients with approved clinical indications for lipid-lowering treatment.

5. CONCLUSIONS

Among the systemic vasculitides, GCA is the most frequent in patients aged 50 years or older. Clinicians should be familiar with the common manifestations and associated complications of GCA in order to promptly initiate treatment in suspected cases. The 1990 ACR classification criteria, while helpful clinically, incompletely identifies patients presenting with

predominant extracranial disease. Advances in vascular imaging have been helpful in recognizing the extent of vascular inflammation in patients with GCA. While clinically silent, radiographic evidence of aortitis is frequently present at disease onset. Aortic inflammation and damage gives rise to the most concerning cardiovascular complications of aortic aneurysm and dissection. To date, specific protocols for screening and monitoring progression of aortic dilatation are lacking and prospective trials are needed.

Coronary vasculitis is uncommon in GCA, and accelerated atherosclerosis, if present, does not appear to markedly impact the incidence of coronary artery events in this population. Nevertheless, given the advanced age of the affected patients, appropriate cardiac risk stratification and treatment with clinically indicated antihypertensive and lipid-lowering agents is prudent. Treatment with GCs remains the mainstay for remission and maintenance, but is associated with a high frequency of GC-associated adverse events. Identification of GC-sparing agents is needed and biologic medications, including tocilizumab, remain under investigation.

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Takayasu's Arteritis

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1. INTRODUCTION

1.1 History

Takayasu's arteritis (TA) is a chronic idiopathic granulomatous large-vessel vasculitis causing inflammation of the aorta, its major branches, and pulmonary arteries. Its first description dates from the 1700s and 1800s when several patients were reported with pulselessness and aortic disease. In 1761, Morgagni found large-vessel aneurysms and stenosis in the autopsy of a 40-year-old woman and in 1830, Yamamoto described a 45-year-old man who originally presented with complaints of fever and developed pulselessness in the radial arteries 1 year later [1,2]. In 1908, Mikito Takayasu of the department of Ophthalmology of Kanazawa University presented the case of a young woman with sudden vision loss in whom the fundus examination revealed wreathlike arteriovenous anastomosis around the optic disc. The name "Takayasu disease" was first used by Yasuzo Niimi in 1942 [3]. In 1951, Shimizu reported cases of similar findings and used the term "pulseless disease" [4]. In 1962, Huang first put forward the concept of "constrictive arteritis" and indicated that it is the main cause of renovascular hypertension [5]. In 1975, the research committee of the Japanese Department of Health and Welfare officially proposed adopting the term "Takayasu's arteritis" for the disease. Although TA has been the most widely used name around the world, it is known in other countries as aortic arch syndrome, pulseless disease, middle aortic syndrome, occlusive thromboarteropathy, and so on [6–9].

1.2 Epidemiology

TA has often been characterized as an illness commonly affecting young women of Eastern ethnic background. However, TA has been described in different

populations throughout the world (Fig. 17.1) [10–13]. In Japan, the number of newly diagnosed patients per 3-year period ranged between 200 and 400, and has tended to decrease over time. According to a Japanese nationwide registry, there were at least 5881 TA patients in Japan in 2011 and the prevalence is thought to be >0.004% [14].

The female-to-male ratio varies, and depends on the populations. The ratio is 9:1 in Japan, 7:1 in Italy, 9.7:1 in the United States, and 8.2:1 in Turkey [15–17]. However, the ratio in Korea, France, and India is 5.4:1, 4.9:1, and 4:1 [18–20]. The median age at TA onset was below 40 years in most studies, especially between the ages of 20 and 40 years [16–19,21,22], but it is controversial whether this applies to both female and male patients. The diagnosis of TA in patients >40 years is low, but not uncommon. The proportion with an age of onset >40 years is 17.5% in Italian and 32.0% in French patients [17,18]. According to the most widely used criteria for the diagnosis of TA, age at disease onset is usually <40 years. Furthermore, most patients >40 years do not have an acute stage due to no involvement of important blood vessels, which may lead to misdiagnosis of TA in patients >40 years.

The distribution of vascular involvement is different in each part of the world. In the United States, Arabs, Africa and some of the Asian countries, such as China and Japan, the aortic arch branches are more frequently involved [16,23–27]. In India, the aorta, especially the abdominal aorta, is more prevalent [28]. As to the type of lesions, stenosis lesions are more common in China and Japan, while India and Mexico are characterized by aneurysmal lesions.

1.3 Genetics

The etiology of TA is still unknown, but previous studies have indicated that genetic factors and cellular

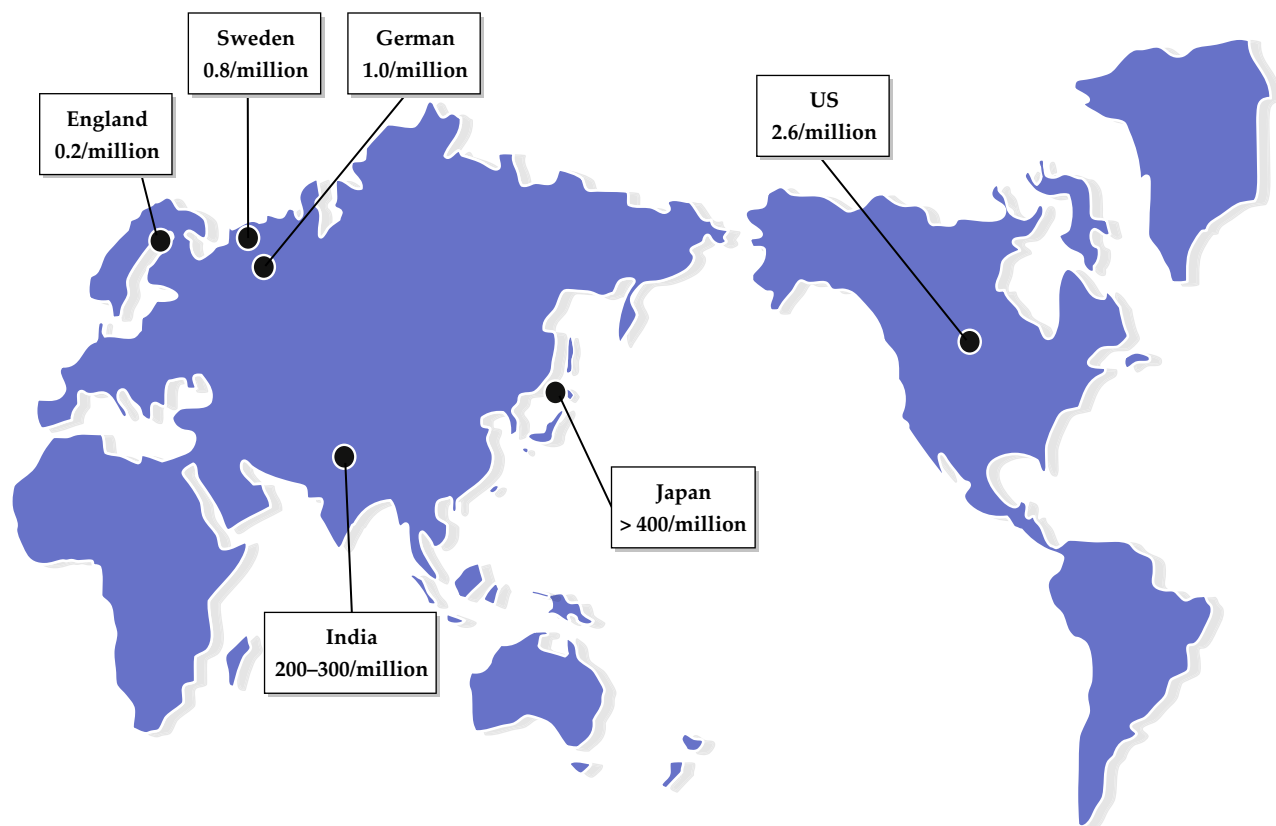


FIGURE 17.1 Incidence of Takayasu's arteritis around the world [10–14]. Adapted from the personal collection of the authors.

immunity may play a role in its pathogenesis. Human leukocyte antigen (HLA) is a risk allele for TA and HLA typing that provides important clinical information for diagnosis. The HLA-B52 allele appears in approximately 44% of Japanese patients with TA. Some patients show familial history of TA, and some studies show that HLA-B52 is found in some familial TA [29]. The HLA-B67 allele could be a new and important marker of TA because of its high odds ratio compared to HLA-B52, although its prevalence in TA is lower [29]. Terao et al. performed genome scanning of TA cases and health controls, followed by a replication study, and found that the IL-12B region on chromosome 5 (rs6871626) and the MLX region on chromosome 5 (rs6871626) as well as the HLA-B region (rs9263739) exhibited significant associations. A significant synergistic effect of rs6871626 and rs9263739 was found with a relative excess risk of 3.45, attributable proportion of 0.58, and synergy index of 3.24 ($p \leq 0.00,028$) in addition to a suggestive synergistic effect between rs665268 and rs926379 ($p \leq 0.027$). They also found that rs6871626 showed a significant association with clinical manifestations of TA, including increased risk and severity of aortic regurgitation, a representative severe complication of TA [30].

2. CLINICAL PRESENTATION AND DIAGNOSTIC CRITERIA

2.1 Clinical Presentation

TA was once thought to progress in three phases. The first phase was characterized by an inflammatory period with constitutional symptoms such as fever, headache, myalgia, and arthralgia. The second phase was characterized by vessel inflammation with symptoms of vessel pain and tenderness, or carotodynia. The final phase was characterized by vessel fibrosis or aneurysmal degeneration with signs of ischemia and aneurysm. Symptoms may occur with vascular narrowing that has occurred because of scarring after inflammation, but inflammation does not have to be present at the time of these symptoms. At the time of diagnosis, about 10–20% of patients with TA are clinically asymptomatic, and the remaining 80–90% of patients present with clinical signs and symptoms originating from the systemic inflammation or from local vascular complications. Due to recent advances in imaging techniques that allow early diagnosis and increased awareness of medical caregivers, serious complications such as visual loss, severe hypertension, and stroke are becoming less frequent. Systemic symptoms

may be absent in 60–80% of TA patients [16,31,32]. The nonspecific nature of constitutional symptoms such as fever, general fatigue, gastrointestinal discomfort, and diarrhea could be insidious and therefore missed.

The clinical presentation of patients with TA varies by geographic region, which is probably associated with ethnic factors. Though HLA is associated with pathogenesis of TA, different ethnics may have different increased frequencies of HLA subtypes. In Japan, increased frequencies of HLA-A10, B39, B52, Bw52, B5, and DR2 were confirmed, while in Korea, increased frequencies of HLA-Bw52, Cw6, DR7, and DQw2 were observed [33–35]. In Mexico, HLA-B44 is reported to be susceptibility in TA, though HLA-B39 and B52 are also reported [36]. Furthermore, focal symptoms and signs are different depending on the location of the affected arteries. Aorta involvement also showed regional difference, eg, the ascending aorta is often affected in Japan, while in other Asian countries, the thoracic and abdominal aortas are usually involved [37]. Additionally, geographic region differences are probably the reason for the extreme clinical symptom ranges.

Cerebrovascular signs and symptoms such as dizziness, headache, syncope, and visual disturbance are common due to the frequency of involvement of the aortic arch and its branches. Cerebrovascular accidents (transient ischemic attack and stroke) are observed in 8–22.2% of patients, and visual disturbance occurs in 4.6–59.3% of patients.

Hypertension occurred in 2–77% of TA patients and is one of the most common reasons TA patients seek medical attention. Some of these reasons include hypertension due to steno-occlusive lesions of renal artery (Fig. 17.2) and/or descending aorta and/or abdominal aorta (Fig. 17.3). Patients with involvement of the ascending aorta may present with hypertension due to aortic regurgitation (AR). Some patients who have been administered glucocorticoids (GC) are at high risk of retention of water and sodium, and thus could cause or increase the severity of hypertension. Premature atherosclerosis has been observed in TA, and TA patients may have some atherosclerotic risk factors, which may contribute to damaged blood vessels. A decrease in elasticity of arterial walls may also contribute to the elevation of blood pressure [38]. The carotid lesions with baroreceptor's hyposensitivity presumably contributes to the genesis of hypertension [39]. Hypertension in TA may present with headaches or with evidence of end-organ damage including congestive heart failure, hypertensive retinopathy, stroke, and renal disease [40]. However, it is worth noting that bilateral severe stenosis or occlusion of the subclavian arteries can mask hypertension. Those patients may go unrecognized and can have central aortic hypertension on catheter directed angiography. Thus patients who are suspected of TA should have blood pressure measurements of both upper and lower extremities. Patients whose blood pressure in both upper limbs is much higher than in both



FIGURE 17.2 Renal angiogram of a 22-year-old woman with 2 months of hypertension. Note the 80% stenosis of the middle segment of left renal artery. Adapted from the personal collection of the authors.

lower limbs, and imaging examination shows stenosis of the aortic isthmus, should be suspected of coarctation of aorta. Ongoing monitoring of blood pressure remains a challenging dilemma. Pressure gradient to the arm can be measured and used as a surrogate to monitor a patient's central aortic blood pressure, but it is invasive. There is no reliable method of noninvasive assessment of central aortic pressure. As persistent hypertension is a poor prognostic factor, evidence of end-organ damage should be monitored for urinalysis, retinal examination, echocardiogram, and at least in selected patients, periodic catheter directed angiography [41].

Upper extremity involvement includes diminished or absent pulse, intermittent claudication, and subclavian steal syndrome. Intermittent claudication is more common in upper extremities than in lower extremities. Subclavian steal syndrome occurs in patients with posterior cerebral circulatory insufficiency (such as stenosis of vertebral artery) aggravated by upper-limb exercise, and can cause lightheadedness, syncope, vertigo, ataxia, diplopia, motor deficits, or upper-limb claudication. Duplex ultrasonography may help to identify reversal flow in a vertebral artery. Claudication of the lower limbs is considered related to abdominal lesions since femoral, tibial, and peroneal arteries stenosis is rare in patients with TA [17].

The rate of pulmonary arterial involvement (PA) is 20–56% on autopsy [42,43], and PA is observed by pulmonary arterial angiography in 12.2–86.5% in different areas (Fig. 17.4) [18,23,44,45]. Generally, PA in TA patients is underdiagnosed. Some results show that respiratory symptoms such as shortness of breath and hemoptysis



FIGURE 17.3 Computed tomography 3D reconstruction of the aorta demonstrating the pseudoaneurysm on the superior segment of the descending aorta, diffused stenosis of the descending aorta. *Adapted from the personal collection of the authors.*

are often the initial symptoms in patients with PH because of TA [46]. Systemic signs related to TA are not specific and may be masked by symptoms originating from the involvement of other large vessels, which may lead to miss or incorrect diagnosis of TA. Thus in young women with a reduced or absent uptake on pulmonary perfusion scans, with general signs of systemic vascular damage and with specific pulmonary lesions on imaging studies, the diagnosis of PA in patients of TA should be considered. In late-phase PA, patients usually present as symptoms of pulmonary arterial hypertension (PHTN) and/or right heart failure, such as progressive dyspnea, fatigue, and/or bilateral leg edema. The rate of PHTN is 12–13% in various reports. TA can cause PHTN through pulmonary vasculopathy, left heart disease, and chronic thromboembolic pulmonary hypertension [15,42]. PHTN associated with PA has been thought to result exclusively from mechanical obliteration of the

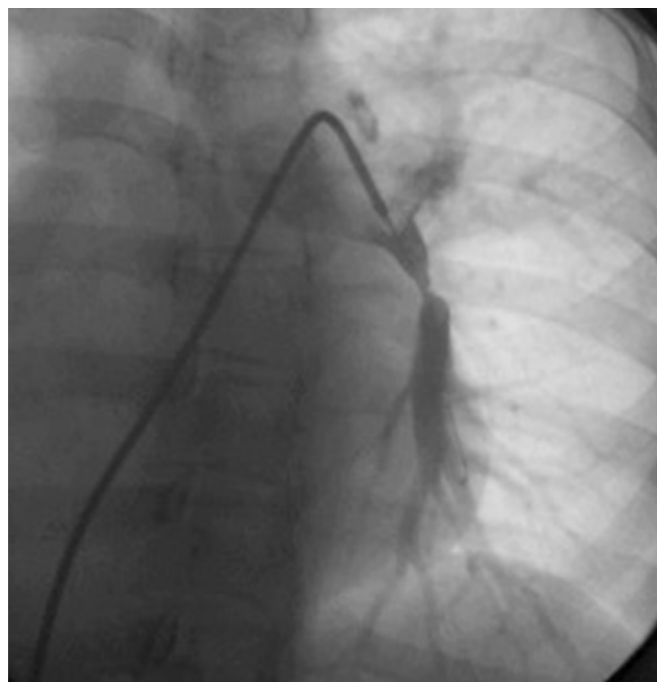


FIGURE 17.4 Pulmonary angiogram in a 19-year-old patient with Takayasu's arteritis. Note the 99% stenosis in the proximal segment of the left pulmonary artery. *Adapted from the personal collection of the authors.*

vascular bed and is a life-threatening complication itself, perhaps even more serious in patients with TA, and is a major determinant of the severity of PHTN [47]. Steno-occlusive rather than dilation or aneurysm and upper-lobe pulmonary arterial branches show abnormalities most frequently and the right pulmonary artery in the upper lobe is most often involved.

Cardiac involvement of TA includes coronary artery stenosis and/or aneurysms, heart failure, coronary vasculitis, pericardial involvement, and valvular abnormalities. However, the signs and symptoms are not specific in TA patients with cardiac involvement. Chest pain occurs with involvement of coronary arteries, aortic valve, and coronary vasculitis. In addition, as some patients are asymptomatic, or with cardiac involvement alone, TA may be misdiagnosed or missed completely.

Physical examination is crucial to the diagnosis of TA. On physical examination, peripheral pulses may decrease or be absent. Vascular bruit may be noticed in the neck, abdomen, scapular region, and chest. Visual disturbance is temporal, and hearing disturbances are especially easily diagnosed. Due to no involvement of important blood vessels, most patients don't have an acute stage. Additionally, due to the nonspecific nature of the clinical manifestations, patients with TA come to the hospital much later than at the time of disease onset. With the development of imaging technology and further understanding of this disease, the duration

from onset to diagnosis is becoming shorter. While physical examination is a simple and first step for disease assessment in TA, it has some limitations [48]. Grayson et al. conducted a study to assess the utility of the vascular physical examination (absent pulse, bruit, and blood pressure difference) to detect arteriographic lesions (defined as stenosis, occlusion, or aneurysm of the carotid, subclavian, and axillary arteries) in patients with established larger vessel vasculitis, including TA ($n=68$) and giant-cell arteritis ($n=32$). They found that individual physical examination findings had good specificity (71–98%) but poor sensitivity (14–50%) to detect arteriographic lesions, and at least 30% of arteriographic lesions were missed. Indeed, normal findings on physical examination do not exclude the possibility of arterial disease, and serial angiographic assessment is advisable to monitor arterial disease in patients with established TA.

2.2 Diagnostic Criteria

Diagnosis is sometimes difficult due to the nonspecific nature of TA. Differential diagnosis includes giant-cell arteritis and infective aortitis. There were no diagnostic criteria available for TA until Ishikawa proposed a set of diagnostic criteria in 1988, which included an obligatory criterion, two major criteria, and nine minor criteria (Table 17.1). Characteristic signs and symptoms of TA included in Ishikawa's criteria for TA contain cardinal limb signs or symptoms and minor signs or symptoms. The criteria were based on a cohort of 96 Japanese patients with TA and 12 patients with other diseases of the aorta. The sensitivity and specificity are not so high and besides, patients with single lesions of pulmonary artery or coronary artery do not fulfill Ishikawa's criteria for TA [49].

In 1990, the American College of Rheumatology published a new set of diagnostic criteria for TA. Sixty-three patients with TA were compared to 744 patients with an established diagnosis of other vasculitic syndromes [50]. Six criteria were selected for the traditional format classification (Table 17.1). The presence of three or more of these six criteria demonstrates a sensitivity of 90.5% and a specificity of 97.8% [50]. A classification tree was also constructed with five of these six criteria, omitting claudication of an extremity, which demonstrates a sensitivity of 92.1% and a specificity of 97.0%. One criticism of the 1990 ACR Classification Criteria for TA is the age restriction. Though TA frequently presents in patients below the age of 40, and over half of patients presented between the ages of 20–40, the proportion with an age of onset >40 years was low, but not uncommon. Previous studies have suggested that the proportion was 17.5–32.0% [17,18]. The other criticism is the fact that the control group used to establish these criteria comprised

mainly patients with small-vessel vasculitis but not patients with atherosclerotic disease or congenital aortic disease, thus limiting its usefulness in clinical practice [43,51]. In fact, when the 1990 ACR Classification Criteria for TA were applied to Indian patients with angiographically proven TA, the sensitivity decreased to 77.4%, though the specificity was as high as 95% [43].

In 1995, Sharma et al. modified Ishikawa's diagnostic criteria for TA, removing the obligatory criterion, adding the characteristic signs and symptoms of TA as a major criterion, removing the age in the definition of hypertension, excluding the absence of aorto-iliac lesions, and including coronary lesions in patients younger than 30 years in the absence of risk factors as minor criterion (Table 17.1) [43,52].

In 2005 the vasculitis working group of the Pediatric Rheumatology European Society (PRES) proposed new classification criteria for pediatric vasculitides, which was endorsed by the European League Against Rheumatism (EULAR) [53]. However, these proposed modifications were mainly based on a literature review and a consensus-based process and were not formally validated. Thanks to support from EULAR, the Pediatric Rheumatology International Trials Organization (PRINTO) [54] and PRES, a formal statistical validation process, with a large-scale, web-based data collection was undertaken. The project culminated finally at the 2008 Ankara Consensus Conference, which had, as its primary objective, the validation of the aforementioned EULAR-endorsed criteria for pediatric vasculitides, including childhood TA (c-TA) [55]. The final EULAR/PRINTO/PRES criteria should be used in patients younger than 18 years (Table 17.1). In c-TA, angiographic findings are crucial for diagnosis, and thus this criterion was already mandatory in the c-TA criteria. In addition to conventional angiography, more recent imaging modalities, such as computed tomography or magnetic resonance imaging had also been considered which were not considered in the 1990 ACR criteria. Other differences from the ACR were the combination of pulse deficit and claudication, which were both very frequent (75%) and highly specific, the addition of hypertension (more frequent than in the other childhood vasculitis), and the removal of the age-limit criterion. Moreover, the addition of increased acute phase reactants as an extra criterion led to properly classify all cases in which this laboratory sign, associated with angiographic abnormalities, was an important finding before the onset of complications such as hypertension or pulse deficit or claudication.

In 2015 Kong et al. from China compared 131 TA patients and 132 control patients with other types of vascular disease, and proposed new TA diagnostic criteria [56]. The new criteria are more convenient and applicable than the Ishikawa's criteria. Furthermore, the criteria do not limit the age of onset to ≤ 40 years, and

TABLE 17.1 Sensitivity and Specificity of All Diagnostic Criteria for Takayasu's Arteritis

Criteria	Sensitivity	Specificity
Ishikawa [49]		
(1) 2 major criteria; or (2) 1 major criterion plus ≥ 2 minor criteria; or (3) ≥ 4 minor criteria:	96% of young patients and 80% of older patients with active disease	67% of young patients and 64% of older patients with inactive disease
<ul style="list-style-type: none"> • Obligatory criterion: age 40 years at diagnosis or at onset of "characteristic signs and symptoms" of 1 month duration in patient history • Two major criteria: <ul style="list-style-type: none"> • left mid subclavian artery lesion • right mid subclavian artery lesion • Nine minor criteria: <ul style="list-style-type: none"> • ESR > 20 mm/h • Carotid artery tenderness • Hypertension (persistent blood pressure $> 140/90$ mmHg brachial or $160/90$ mmHg popliteal at age 40 years, or presence of the history at age 40 years) • Aortic regurgitation or annuloaortic ectasia • Pulmonary artery lesion • Left mid common carotid lesion • Distal brachiocephalic trunk lesion • Descending thoracic aorta lesion • Abdominal aorta lesion 		
The 1990 American College of Rheumatology Classification Criteria [50]		
Any 3 or more:	90.5%	97.8%
<ul style="list-style-type: none"> • Age at disease onset < 40 years • Claudication of extremities • Decreased brachial artery pulse of 1 or both brachial arteries • Systolic blood pressure difference > 10 mmHg between arms • Bruit over subclavian arteries or aorta • Arteriogram abnormality, not due to arteriosclerosis, fibromuscular dysplasia, or similar causes; changes usually focal or segmental 		
Sharma [43]		
(1) ≥ 2 major criteria; or (2) 1 major plus ≥ 2 minor criteria; or (3) ≥ 4 minor criteria:	92.5%	95.0%
<ul style="list-style-type: none"> • Three major criteria: <ul style="list-style-type: none"> • Left mid subclavian artery lesion • Right mid subclavian artery lesion • Characteristic signs and symptoms of at least one month duration (these include limb claudication, pulselessness or pulse differences in limbs, an unobtainable or significant blood pressure difference > 10 mmHg systolic blood pressure in limb, fever, neck pain, transient amaurosis, blurred vision, syncope, dyspnea, or palpitations) • Ten minor criteria: <ul style="list-style-type: none"> • ESR > 20 mm/h (Westergren) • Carotid artery tenderness • Persistent blood pressure $> 140/90$ mmHg brachial or $> 160/90$ popliteal • Aortic regurgitation or annuloaortic ectasia • Pulmonary artery lesion • Left mid common carotid lesion • Distal brachiocephalic trunk lesion • Descending thoracic aorta lesion • Abdominal aorta lesion • Coronary artery lesion documented on angiography below the age of 30 years in the absence of risk factors 		
EULAR/PRINTO/PRES criteria [55]		
Mandatory criterion plus any other criteria:	100%	99.9%
<ul style="list-style-type: none"> • Mandatory criterion: <ul style="list-style-type: none"> Angiographic abnormality of the aorta or its main branches and pulmonary arteries, not due to fibromuscular dysplasia, or similar causes • Other criteria: <ul style="list-style-type: none"> • Pulse deficit or claudication • Blood pressure discrepancy of four limb systolic blood pressure > 10 mmHg difference in any limb • Bruits • Systolic/diastolic blood pressure greater than 95th percentile for height 		
Kong [56]		
≥ 8 scores:	91.92%	93.94%
Age < 40 years (4 points), female (3), chest pain/chest distress (2), amaurosis (3), vascular bruit (2), decreased/absent pulse (5), involvement of the aortic arch or its major branches (4), and involvement of the abdominal aorta or its branches (3)		

ESR, Erythrocyte sedimentation rate.

cover more involved arteries (including the abdominal aorta or its branches). Moreover, the criteria not only contain general information, symptoms, and signs but also the characteristics of advanced imaging and systematic assessment of TA. However, the control group had only one giant-cell arteritis patient, and the study was carried out only in China and thus was limited to some extent.

Thus far, there is no global consensus on the best diagnostic criteria. The ongoing international effort of the Diagnostic and Classification Criteria in Vasculitis Study (DCVAS) aims to develop and validate diagnostic and classification criteria for systemic vasculitis that can be used in daily clinical practice and for use in clinical trials [57]. DCVAS (clinicaltrial.gov registration no. NCT01066208) aims to enroll 2000 patients over 18 years with a new or an established diagnosis of vasculitis and 1500 patients over 18 years with a presentation similar to vasculitis, but an alternative diagnosis. As of March 2015 DCVAS had recruited 4923 patients from 128 sites worldwide [58].

2.3 Classification

Classification of TA is based on the distribution of vessel involvement. The world's first classification of TA was proposed by Ueno in 1967 [59], in which TA was divided into three types. However, this classification method does not consider pulmonary and coronary involvement. In 1977, Lupi-Herrera et al. added type IV of pulmonary artery involvement [60], and then Hata et al. put forward a new classification at the Tokyo International Conference on Takayasu Arteritis, which is listed in Table 17.2 [61].

Classification of disease activity is important in determining therapy strategy and response to treatment (Table 17.3). However, the classification criteria are not uniform throughout the literature. The gold-standard histological results, is difficult to make the diagnosis of active disease without open surgical operation. The widely used classification created by the NIH observed 60 patients between 1970 and 1990 with an average age at disease onset of 25 years [31]. Researchers at the Mayo Clinic had defined active disease by two or more clinical, pathologic, laboratory, or operative criteria [62]. These criteria use ESR as a marker of disease activity, but in fact it is neither sensitive (72% of sensitivity) nor specific (56% of specificity) enough to monitor disease activity in TA [31]. Patients can have symptoms with normal ESR and CRP because the active inflammation that caused the scar may be gone at the time that the scarring lead to sufficient narrowing of a blood vessel for symptoms to appear.

TABLE 17.2 All Classification Criteria for Takayasu's Arteritis

Classification criteria	Definition
Ueno et al. [59]	
Type I	Confined to the aortic arch and its branches
Type II	Involved the descending and abdominal aortas and their branches
Type III	Combination of the two types above
Lupi-Herrera et al. [60]	
Ueno's criteria plus	
Type IV	Pulmonary artery involvement
Hata et al. [61]	
Type I	Involvement of the aortic arch and its branches
Type II a	Involvement of the ascending aorta, aortic arch, and its branches
Type II b	Involvement of the ascending aorta, aortic arch, and its branches, and thoracic descending aorta
Type III	Involvement of the thoracic descending aorta, abdominal aorta, and/or renal arteries
Type IV	Involvement of the abdominal aorta and/or renal arteries
Type V	Combination of type II b and IV

TABLE 17.3 Classification of Takayasu's Arteritis Activity

Criteria
Gold standard
Histological result
NIH [31]
New onset or worsening of ≥ 2 of the following features of TA:
1. Systemic features such as fever, musculoskeletal symptoms (no other cause identified)
2. Elevated erythrocyte sedimentation rate (ESR)
3. Features of vascular ischemia or inflammation, such as claudication, diminished or absent pulse, bruit, carotodynia, asymmetrical blood pressure
4. Typical angiographic features
Mayo Clinic [62]
≥ 2 of the following features of TA:
1. Systemic features such as fever, myalgias, or arthralgias
2. Active inflammation on pathologic specimen (eg, lymphoplasmacytic infiltrate, giant cells) taken from diseased arteries
3. Elevation of serum markers such as ESR or C-reactive protein
4. Acute inflammation of the artery and surrounding soft tissues at the time of operation

3. PATHOPHYSIOLOGY

While our knowledge of the pathogenesis of TA has considerably improved during the last decade, the exact etiology remains unclear. Many causative factors have been implicated in TA. Immune-mediated mechanisms have been suggested as a cause of TA (Fig. 17.5) [63].

Cell-mediated autoimmunity mechanisms in vessel lesions associated with TA have been illustrated. T-cell-dependent immunity, chemokine- and cytokine-dependent immunity, and B-cell-dependent vascular inflammation were thought to be the main pathological mechanisms resulting in the arterial wall injury involved in TA.

The natural history of vascular lesions is not well understood in TA, but previous studies have suggested that TA lesions mostly develop in a symmetric manner rather than in a contiguous way in paired vascular beds [63]. Pryshchep et al. compared the expression profile of Toll-like receptors (TLR) 1 to 9 and found that there was a specific pattern of vascular involvement in TA, and that TLR signatures were major determinants of this pattern [64]. It is well established that the adventitia in TA contains T cells colocalizing with dendritic cells [65,66], and immature dendritic cells have been found in temporal arteries of healthy subjects and are exclusively located near the media-adventitia border of the adventitia [67]. Additionally, natural killer cells and $\gamma\delta$ T cells can trigger apoptosis of vascular cells in

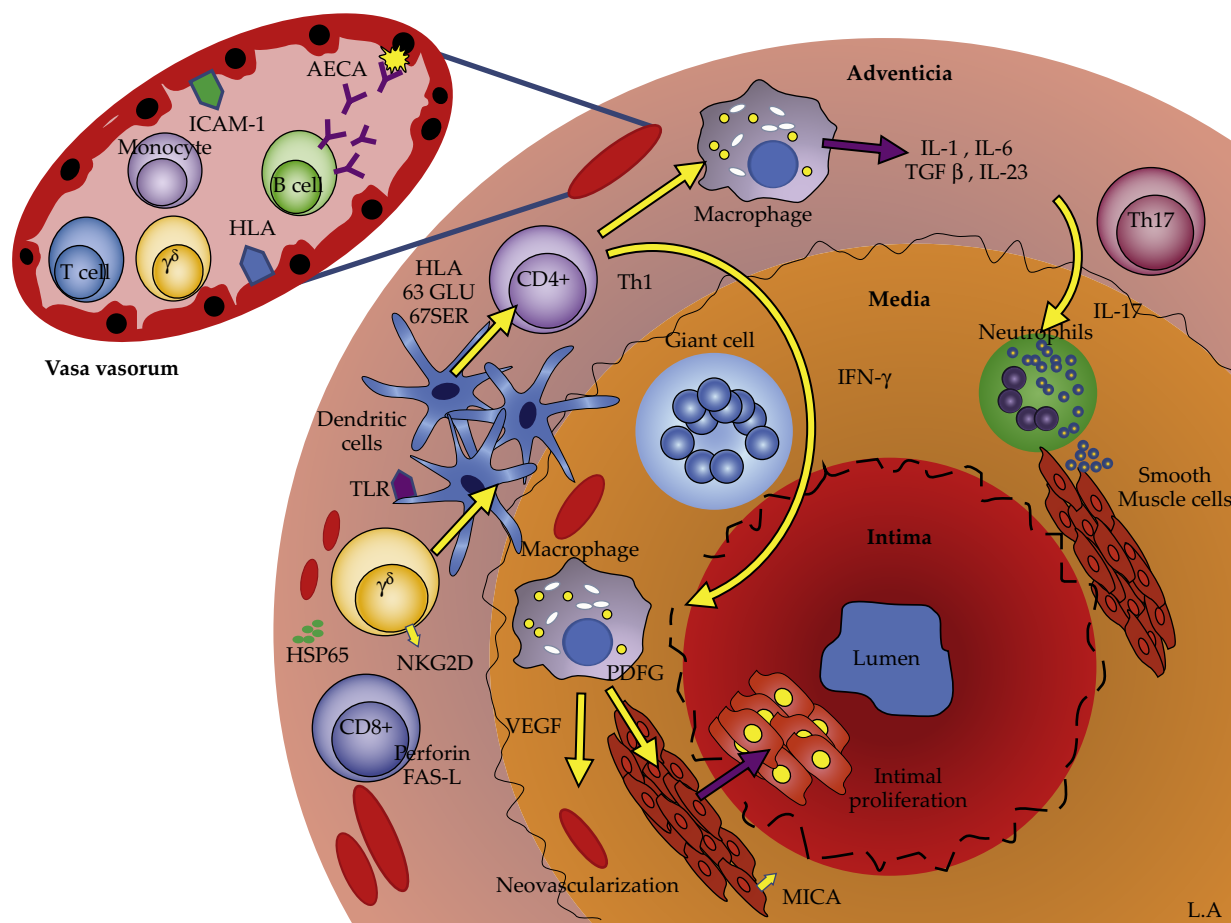


FIGURE 17.5 Pathogenesis of Takayasu's arteritis. An unknown stimulus triggers the 65 kDa heat-shock protein expression in the aortic tissue, which, in turn, induces the Major Histocompatibility Class I Chain-Related A (MICA) on vascular cells. The $\gamma\delta$ T cells and NK cells expressing NKG2D receptors recognize MICA on vascular smooth muscle cells and release perforin, resulting in acute vascular inflammation. Proinflammatory cytokines increase the recruitment of mononuclear cells within the vascular wall. T cells infiltrate and specifically recognize one or a few antigens presented by a shared epitope associated with specific major histocompatibility complex alleles on the dendritic cells, the latter being activated through Toll-like receptors. Th1 lymphocytes drive the occasional formation of giant cells through the production of interferon- γ , and activate macrophages with the release of VEGF resulting in increased neovascularization and PDGF, resulting in smooth muscle migration and intimal proliferation. Th17 cells induced by the IL-23 microenvironment also contribute to vascular lesions by activation of infiltrating neutrophils. Although still controversial, dendritic cells may also cooperate with B lymphocytes and trigger the production of antiendothelial cell autoantibodies, resulting in dependent cytotoxicity against endothelial cells. In the near future, novel drugs specifically designed to target some of the pathogenic mechanisms described above could expand the physician's therapeutic arsenal in Takayasu's arteritis. Adapted from Arnaud et al. [63].

TA. However, the stimulus that drives innate immune responses in TA remains unknown. Heat shock protein (HSP) is extremely conserved molecules and may provide valuable links between infections and autoimmune diseases. The cellular infiltrate in TA contains about 15% each of CD4⁺ and CD8⁺ T cells [68], which play a key role in the pathogenesis of large-vessel vasculitides. Furthermore, the study of peripheral blood cells from TA patients shows that T cells are in an activated state, with an elevated ratio of CD4⁺/CD8⁺ lymphocytes [69], an increased number of HLA-DR circulating T lymphocytes [70], with increased basal activity of protein kinase C and intracellular calcium levels [71], and lower levels of cyclic adenosine monophosphate, indicating the activation of protein kinase C – calcium pathway in patients with TA [71].

The Th1 and Th17 T-cell subsets are also involved in the pathogenesis of large-vessel vasculitides [72]. Th1 lymphocytes drive the formation of giant cells through the production of interferon- γ (IFN- γ), and activate macrophages with the release of VEGF, resulting in increased neovascularization and PDGF, smooth muscle migration, and intimal proliferation. Th17 cells induced by the IL-23 microenvironment also contribute to vascular lesions through activation of infiltrating neutrophils. Saadoun et al. observed an expansion of Th1 and Th17 cell pathways that correlated with disease activity in TA. They found an increased expression of Th1- and Th17-related cytokines in patients with TA. Patients with active TA had significantly increased levels of IL-2, IFN- γ , and IL-17A compared with those in patients with TA in remission, and observed increases in the percentages of Th1 cells and Th17 cells in the peripheral blood of patients with active TA compared with TA in remission and healthy donors. They provide the first evidence that Th1 and Th17 immunity appears to play an important role in driving TA-related inflammation, both systemically and in blood vessels. In addition, only one of these pathways was susceptible to glucocorticoid-mediated suppression. GC decreased Th1 cytokine levels but not Th17 cytokine levels in patients with TAK. Th17 cytokine-targeted intervention would be needed for optimal control of TA [73].

Whether antibody-mediated mechanisms take part in the pathogenesis of TA needs to be clarified. Antiaorta antibodies have long been reported in TA research, and Dhingra et al. reported that levels of antiaortic endothelial cell antibodies are 20 times greater in patients with TA compared with controls [74]. Hoya et al. found that most patients with active TA (≥ 2 NIH criteria) had higher numbers of plasmablasts than those with inactive disease, and TA activity correlated with the number of plasmablasts and with ESR. Circulating plasmablasts could be a useful biomarker of disease activity and a tool for selecting appropriate candidates for B-cell depletion therapy (BCDT). In fact, the study reported that three

patients with refractory TA and expansion of circulating plasmablasts were successfully treated with BCDT (treated with rituximab) [75]. However, Batazares et al. did not observe differences between antibodies reactive against a total human aorta extract and its main protein components between TA patients with control subjects [76]. In addition, the role of antiendothelial cell autoantibodies and antiphospholipid antibodies in TA is controversial [77,78]. Thus, whether humoral immunity is an epiphenomenon or plays a direct role in the pathogenesis of TA remains controversial.

Previous studies found that there was a link between tuberculosis (TB) and TA, thus indicating the role of mycobacterial antigens as a trigger of an abnormal immune response in TA showed promise. Tuberculin skin test (TST) and Quantiferon-TB Gold test (QFT) are the two main means of diagnosing latent TB infection. Karadag et al. conducted a study investigating latent TB infection among TA patients by the use of TST and QFT. They found that although TST positive was higher in the TA group (62.5% vs. 41.4%, $P=0.008$), QFT positivity was similar between the two groups (22.3% vs. 22.4%, $P>0.05$). QFT may be a good and favorable test compared with TST in detecting latent TB infection in TA patients [79]. A retrospective study in India found that one-fifth of TA patients had strongly positive skin tests for tuberculosis, and had been started on antitubercular therapy [80]. Seko et al. found that expression of HSP65 was increased in aortic tissue, and Aggarwal et al. found an abnormally elevated level of circulating antimycobacterial HSP65 antibodies in TA patients, indicating that HSP65 whether exogenous or endogenous may be a putative antigen-stimulating immune response in TA [81]. However, some studies found that only a minority of TA patients have a clearcut history of mycobacteria exposure [18]. The true association between TA and tuberculosis may be impossible because no improvement has been observed after giving antituberculosis therapy.

Biopsy specimens are seldom available and hence morbid anatomic features are based on autopsy findings or segments excised during surgery. Histologic examination of TA reveals a pan arteritis of all three layers of the vessel wall, with skip areas along the length of the vessel (Fig. 17.6) [82]. The course of TA includes active and quiescent phases, demonstrating that there are different inflammatory states of arterial lesions. Active inflammatory lesions initiate around the vasa vasora and at the media-adventitial junction, extend through the media and adventitia, and terminate in diffuse or nodular fibrosis. There is edema and mononuclear cell infiltration (CD4 and CD8 lymphocytes, plasma cells, and macrophages) in the outer thirds of the media and adventitia. Giant-cell granulomatous reaction and laminar necrosis can also be present. Reactive fibrosis and increased ground substance

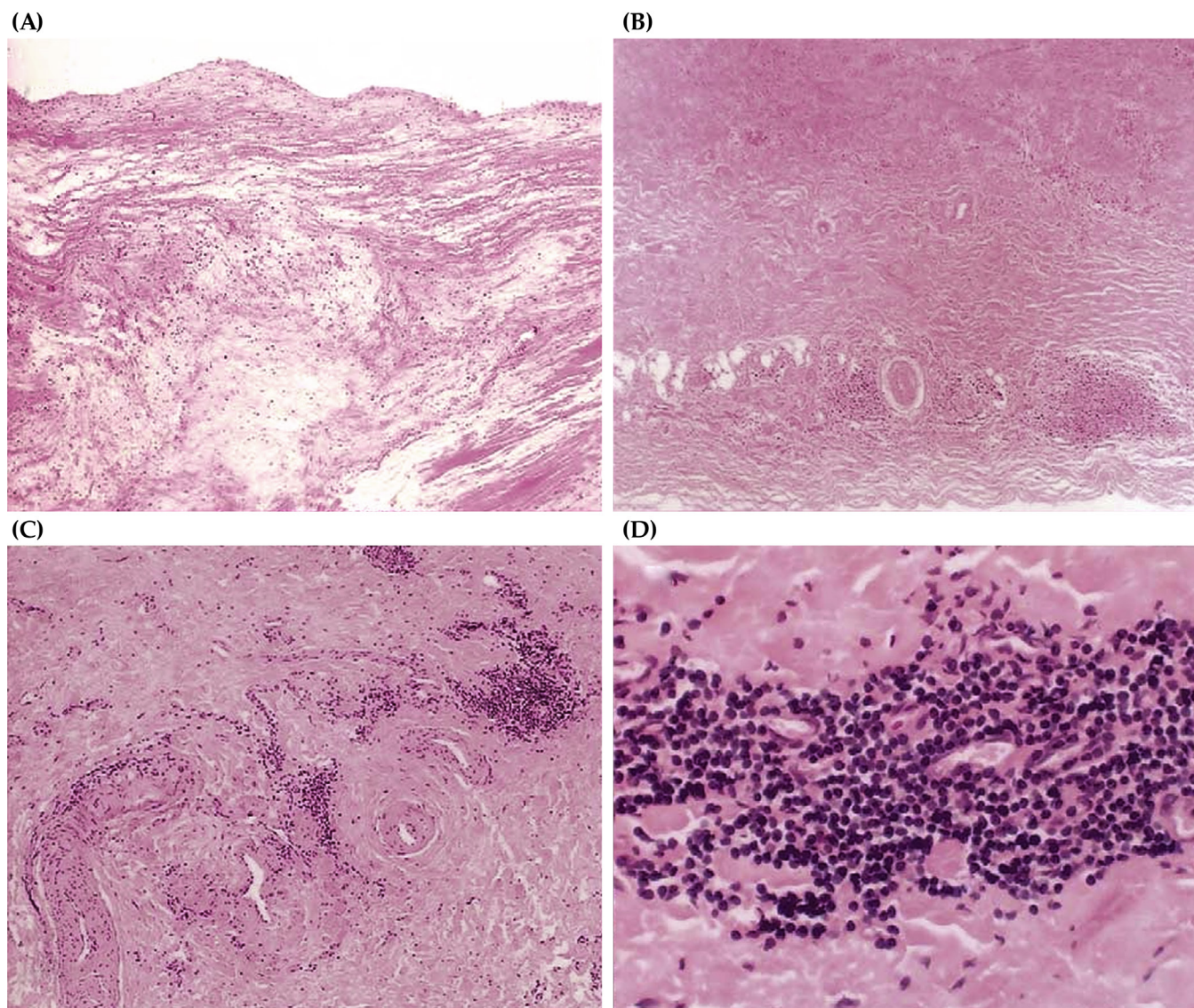


FIGURE 17.6 Histologic findings of Takayasu's arteritis. Aortic cusp (A) shows intimal fibrosis and myxoid degenerative changes of media without any significant inflammatory cells. Aortic wall shows fibrosis of media (B), and vascular proliferation and infiltration of inflammatory cells around vasa vasorum (C and D). Inflammatory cells are predominantly mononuclear lymphocytes. *Adapted from Song et al. [82].*

in the intima, myxoid degenerative changes in media and a band of neo-vascularization at the intimo-media junction are observed. Occasionally, intimal thickening of the peripheral arteries may lead to end-organ ischemia. Thickening of the entire vessel wall led by inflammation is frequently seen in the chronic phase. There is patchy mononuclear inflammatory infiltrate with medial vascularization, while the healed phase shows only fibrosis in all layers [83]. Aneurysm formation, which occurs when destruction of the elastic component of the media happens before fibrosis of the adventitia, is less frequent in TA than stenotic lesions [84].

Generally, it is hypothesized that unknown factors trigger inflammatory cell infiltration (mainly including natural killer cells, $\gamma\delta$ T cells, cytotoxic T lymphocytes, T helper cells, and macrophages), and cell-mediated immune

response affects intima, media (mainly involved), and adventitia. Although controversial, dendritic cells may cooperate with B lymphocyte and trigger the production of antiendothelial cell autoantibodies, resulting in complement-dependent cytotoxicity against endothelial cells. Eventually, the affected aorta and its main branches are thickened, stenosis, occlusion, dilation, or aortic aneurysm formation/dissection [63,86].

4. CARDIAC INVOLVEMENT

Cardiac involvement of TA includes coronary artery involvement (stenosis and/or aneurysms), heart failure, coronary vasculitis, pericardial involvement, and valvular abnormalities.

In 1951, Foving and Logan first reported narrowing of coronary arteries in TA [87]. However, coronary artery lesions were considered to be an uncommon, although potentially fatal complication in TA. Cardiac involvement in TA may manifest as dyspnea, palpitations, angina, myocardial infarction, heart failure (caused by myocardial infarction, systemic hypertension and AR), and sudden death [88,89]. Unlike coronary artery atherosclerosis, the onset age of TA with coronary artery involvement is much younger, usually ≤ 40 years. Cardiac involvement may influence prognosis of TA patients. Previous studies have found that occurrence of major complications (including Takayasu's retinopathy, hypertension, AR, and aneurysm) is one of the factors that can influence outcome [23,89].

It is reported that the incidence of coronary artery involvement is 10–30%. Studies in Japan found that about 10–24% of TA patients had coronary artery involvement, mainly with ostial left main coronary artery disease [90,91]. A study in China revealed that 45 (7.7%) of the 587 TA patients were coronary artery involved, and among them 23 (51.15%) cases had ostial left main coronary artery involvement [92]. Another study revealed that 66 (11.7%) of the 566 TA patients had coronary artery involvement, and the ostia of the left main coronary artery were observed in 25 (37.9%) cases [23]. Studies conducted in Western countries revealed a high rate of ostial left main coronary artery involvement [93–96]. It is possible that the incidence of cardiac involvement is underestimated because it is usually not evident until the occurrence of symptoms. Cardiac involvement has been noted mainly in autopsy. Nagata reviewed 82 autopsied cases of TA in Japan from 1975 to 1984, finding that congestive heart failure occurred in 49 cases (59.8%), AR in 21 (25.6%), and myocardial infarction in 10 (12.2%), which were much higher than the incidence reported previously. Some patients may go to see a dentist because of tooth pain before TA is diagnosed. Urgent assessment of coronary arteries is warranted in patients who complain of chest pain more typical of angina.

According to autopsy findings, most coronary artery lesions are thought to be due to the extension of intimal proliferation and fibrous contraction, leading by the inflammatory process that involves the ascending aorta [97,98]. In some cases, coronary artery stenosis may be caused by coronary arteritis as one of the skip lesions of TA, but even in these cases the lesions mainly affect the proximal segments of the coronary arteries [99]. Generally, on the basis of pathological features, coronary artery lesions are divided into three types: type 1, stenosis or occlusion of the coronary ostia and the proximal segment of the coronary arteries (Fig. 17.7); type 2, diffuse or focal coronary arteritis, which may extend diffusely to all epicardial branches or may involve focal segments, so-called skip lesions; and type 3, coronary aneurysm.



FIGURE 17.7 Coronary angiography of a 24-year-old female with complaint of postexercise chest pain. Note the 90% stenosis of the ostia and occlusion of the middle and distal segments of right coronary artery. Adapted from the personal collection of the authors.

Type 1 is the most frequently observed, while type 2 and type 3 are very rare [99]. TA may present as an isolated coronary lesion in fewer than 5% of cases [100]. Sun's study revealed that 3.74% (22/587) of TA patients had isolated coronary lesions [92]. It is known, however, that lesions in the aorta or its branches may develop even 5 to 20 years after the early "prepulseless" stage of the disease.

4.1 Prevalence of Traditional Risk Factors for Accelerated Atherosclerosis

In TA, premature and accelerated atherosclerosis is well recognized [101,102]. The associated atherosclerotic plaques promote the formation and occurrence of atherosclerosis in TA [84]. Seyahi et al. performed ultrasonographic longitudinal scans of the common carotid artery in 30 female patients with TA, finding that atherosclerotic plaques were much more frequently observed in TA patients than in the age- and sex-matched controls (27% vs. 2%) [101]. During active disease, TA patients may experience acceleration of the atherosclerotic process. However, when inflammation is controlled, these patients may have atherosclerotic development as in healthy subjects, as endothelial function and arterial stiffness return to normal values. Due to the persistent occurrence of damage to blood vessels, every disease reactivation may damage blood vessels further, resulting in acceleration of the atherosclerotic process compared with healthy age-matched controls [103].

The major pathophysiologic process of coronary atherosclerosis is a defect or injury of the arterial endothelial function, and determined by cardiovascular risk factors. TA patients showed increased prevalence of traditional risk factors for cardiovascular disease (CVD) compared to healthy controls, and TA patients with atherosclerotic plaques were older and had higher levels of total cholesterol compared to TA patients without atherosclerotic plaques. Diabetes, hypertension, dyslipidemia, abdominal obesity (metabolic syndrome), impaired renal function, and persistent proteinuria are more common in patients with systemic vasculitis than in healthy controls [103,104]. A study in Italy revealed that 30.6% and 11.1% of 104 patients with TA were overweight and obese, respectively [17]. A study led by Seyahi et al. revealed that 75% of patients with TA were hypertensive compared to 23% of healthy controls ($p < 0.001$). Both mean systolic and diastolic blood pressure levels were higher among patients with TA compared to healthy controls. However, other atherosclerotic risk factors including obesity, smoking, and cholesterol levels did not differ between the study participants [105]. A study in China investigated 566 patients with TA, finding that diabetes mellitus, hypertension, and dyslipidemia were observed in 11.0%, 3.0%, and 4.9% cases, respectively. Renal failure was observed in six patients [23].

4.2 Markers for Atherosclerosis and Endothelial Dysfunction

Early vascular aging has been used to describe structural and functional changes occurring in large arteries with aging, which are accelerated in individuals at increased cardiovascular risk [106]. Markers of arterial stiffness have been correlated with cardiovascular outcomes, and have been classified as an emerging risk factor that provides prognostic information beyond standard stratification strategies involving hypertension, diabetes, obesity, dyslipidemia, and smoking [107]. Multiple epidemiologic studies have correlated markers of diffuse intimal thickening, usually measured as carotid intimal media thickness (C-IMT) [108], arterial stiffness, measured as carotid-femoral pulse-wave velocity (PWV) [109], and endothelial dysfunction, measured as flow-mediated dilatation (FMD) [110,111] (Table 17.4).

Common carotid arteries are frequently involved in TA, which show prominent long-segment homogeneous circumferential C-IMT (usually 2.5–5.0 mm), calling “macaroni sign” on sonography, with relative sparing of the carotid bulb and internal carotid artery in the early or active phase [112,113]. C-IMT is significantly increased in patients with TA compared to controls [101]. However, C-IMT decreases in the chronic phase (usually 1.1–2.0 mm), and substantial arterial stenosis,

occlusion, and dissection can develop [114,115]. Sometimes, multifocal intimal denudation and an intimal flap in the common carotid artery will develop [116]. Due to the specificity of a heterogeneous increase in density covering shorter areas with an irregular appearance for atherosclerotic plaques, previous studies have reported that the plaques are reliable for the presence of atherosclerosis. In Seyahi's study, 27% of patients with TA had atherosclerotic plaques in carotid arteries, while it was 2% among age-matched and sex-matched controls. Plaques presented only among patients with TA who had carotid involvement and mainly in patients who had classic atherosclerotic risk factors. Thus local and systemic persistent inflammation from the disease itself, arterial injury due to the functional abnormalities, or turbulent blood flow and shear stress in stenotic segments all may play a part in the pathogenesis of the atherosclerosis observed in TA [101].

Arterial distensibility is a measure of the artery's ability to expand and contract with cardiac pulsation and relaxation. Decrease in arterial distensibility, which has effects on central pressure and arterial stiffness on the left ventricle, brain, and kidney, is a common pathologic mechanism among CVD [117]. The assessment of brachial-ankle pulse-wave velocity (baPWV) is considered to be the “gold standard” measurement of aortic stiffness, as it is a simple, noninvasive, and reproducible method and has the largest amount of clinical evidence, providing the predictive value of aortic stiffness for cardiovascular events [118]. Current guidelines suggest that a threshold value of carotid-femoral PWV (cfPWV) greater than 10 m/s is considered as an index of large artery stiffening and an indicator of subclinical organ damage [119]. It was previously demonstrated that the degree of arterial stiffness measured based on the cfPWV is increased in TA patients with or without traditional CVD risk factors [38,120]. Although cfPWV is currently recognized as the most prevalent index for assessing arterial stiffness, the procedure is complex and specialized techniques are required. Recent studies have demonstrated that arterial stiffness can also be measured simply using the brachial-ankle PWV (baPWV) [121]. Arterial stiffness in patients with coronary artery disease caused by TA is well documented. Cainzos-Achirica et al. conducted a study to evaluate the association between baPWV and coronary artery calcium (CAC), a reliable marker of coronary atherosclerosis, in a large sample of young and middle-aged asymptomatic adults and to assess the incremental value of baPWV for detecting prevalent CAC beyond traditional risk factors. The results revealed that baPWV was independently associated with the presence and severity of CAC and baPWV may be a valuable tool for identifying apparently low-risk individuals with increased burden of coronary atherosclerosis [122]. Wang's study investigated 48 TA

TABLE 17.4A Clinical Characteristics of Investigated Patient Groups in Studies Investigating IMT, PWV, AI and NT-proBNP

Study	Group	<i>n</i>	Gender (M/F)	Age (y)	Disease duration (y)	BMI (Kg/m ²)	SBP (mmHg)	DBP (mmHg)	Other CV comorbidities	Ref.
1	TA	30	All female	35.4±8.0	8.8±5.6	25.6±3.7	136.8±27.0*	78.2±13.1*	23.3% smoking* 53.3% HTN 10.0% DM	[101]
	Control	50	NA	38.2±5.7	NA	26.3±4.2	108.8±14.1	70.1±7.95	48.0% Smoking	
2	TA	27	All female	32.37±8.26		22.3±2.64	121±20	68±15	None	[120]
	Control	27	All female	33.89±10.12		23.7±2.99	113±13	73±10	None	
3	TA	10	All female	41.0±12.5	NA	26.3±3.1*	NA	NA	NA	[38]
	Control	11	All female	32.4±5.5		22.2±1.9				
4	TA associated CAD	48	36/12	45.0±6.7	NA	25.2±5.6	131.0±10.2 [◇]	80.2±9.2 [◇]	12.5% Smoking 50.0% HTN 16.7% dyslipidemia [◇] 10.4% DM [◇]	[123]
	Non-TA with CAD	40	29/21	44.5±5.6		24.8±5.2	124.2±9.9	73.6±10.2	20.0% Smoking 27.5% HTN 35.0% Dyslipidemia 27.5% DM	
5	Active TA	30	2/28	34.9±12.9	7.2±8.9	23.8±2.6	NA	NA	70.0% HTN 6.7% DM 30.0% Hyperlipidemia	[126]
	Inactive TA	38	4/34	37.6±12.4	11.0±9.4	24.2±2.9			57.9% HTN 2.6% DM 13.2% Hyperlipidemia	
	Control	90	10/80	37.2±5.7		23.9±1.3			NA	
6	TA with high cfPWV	24	NA	36.6±12.0	4.5 median	24.5±3.0	146.0 ±26.1 ^λ	81.4±19.2 ^λ	79.2% HTN ^λ 8.3% DM 37.5% hyperlipidemia ^λ	[130]
	TA with low cfPWV	48		33.4±11.9	5.0 median	22.7±3.5	122.6±19.6	65.8±11.8	43.8% HTN 4.2% DM 14.6% Hyperlipidemia	

BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CV, cardiovascular; HTN, hypertension; DM, diabetes mellitus; CAD, coronary artery disease; cfPWV, carotid –femoral pulse wave velocity; NA, not available; *TA vs. Control, $p < 0.05$; [◇]TA associated CAD vs. non-TA with CAD; $p < 0.05$, ^λTA with high cfPWV vs. TA with low cfPWV, $p < 0.05$.

TABLE 17.4B Laboratory Findings and Technical Aspects of Diagnostics in Studies Investigating IMT, PWV, AI, and NT-proBNP

Study	Group	ESR (mm/h)	HsCRP (mg/L)	CRP (mg/L)	Testing protocol and method	IMT (mm)	PWV-CF (m/s)	PWV-CR (m/s)	AI carotid (%)	AI radial (%)	NT-proBNP (pmol/L)	Ref.
1	TA	27.9±22.2*	11.4±19.0*	NA	Mean-IMT-mean value of 6 measurements	0.95±0.31*	NA	NA	NA	NA	NA	[101]
	Control	13.8±8.98	3.3±4.1			0.59±0.08						
2	TA	NA	NA	NA	PWV-the average value of two measurements of the right carotid-femoral PWV	NA	9.77±3.49*	NA	NA	NA	NA	[120]
	Control						7.83±1.06					
3	TA	NA	NA	NA	PWV-the average of 10 recordings of the carotid-femoral PWV and carotid-radial PWV;		12.0±4.3*	10.4±2.8	40.0±13.8*	33.1±10.3*	NA	[38]
	Control				AI-the mean of two recordings performed at each site		8.3±1.1	10.9±1.4	26.6±8.7	14.9±9.9		
4	TA associated CAD	23.3±15.4 [◇]	NA	NA	NA	NA	17.0±3.8 [◇]	NA	NA	NA	NA	[123]
	Non-TA with CAD	10.2±14.9					13.8±3.0					
5	Active TA	29.1±22.0 [#]	NA	8.5±5.0 ^{*#}	NT-proBNP-determined using specific NT-proBNP assay kits (Biomedica)	NA	NA	NA	NA	NA	915.0±328.0 ^{*#}	[126]
	Inactive TA	8.7±4.9		2.3±2.4							618.2±243.4*	
	Control	NA		1.4±1.4							427.2±81.4	
6	TA with high cfPWV	12.5 median	5.4 median ^λ	NA	NT-proBNP-determined using specific NT-proBNP assay kits (Biomedica)	NA	19.0±4.08 ^λ	NA	NA	NA	864.6±381.3 ^λ	[130]
	TA with low cfPWV	10.0 median	2.3 median				11.8±1.51				694.4±282.9	

BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CV, cardiovascular; HTN, hypertension; DM, diabetes mellitus; CAD, coronary artery disease; cfPWV, carotid –femoral pulse wave velocity; NA, not available; *TA vs. control, $p<0.05$; [◇]TA-associated CAD vs. non-TA with CAD; $p<0.05$; [#]Active TA vs. Inactive TA, $p<0.05$; ^λTA with high cfPWV vs TA with low cfPWV, $p<0.05$.

patients and 40 age-, gender, and severity-matched (assessed by SYNTAX SCORE) patients with coronary artery disease receiving drug eluting stent (DES). The results revealed that TA patients exhibited increased baPWV compared with patients with coronary artery disease, and multiple linear regression analysis revealed that baPWV was independently associated with the extent of coronary artery disease in TA patients. In addition, logistic regression analysis identified that a baPWV of 17.00 m/s or higher may be considered as an independent predictor of DES restenosis. Moreover, the multivariate Cox proportional hazards model demonstrated that a baPWV of 17.00 m/s or higher was an independent predictor of major adverse cardiac events (MACE) in TA patients who underwent DES implantation. Thus baPWV might be used as a biomarker for increased coronary artery risk in TA patients who received DES and as a target for earlier detection of a higher risk for late MACE [123].

Augmentation index (AI) is strongly correlated with direct measurement of arterial distensibility and can be considered a good surrogate for the evaluation of arterial stiffness [124]. A study led by Ng et al. found that both aortic AI derived from the radial artery ($P=0.002$) and carotid AI ($P=0.03$) were higher in TA patients than in controls [38].

N-terminal probrain natriuretic peptide (NT pro-BNP), a diuretic peptide secreted from cardiomyocytes in response to ventricular wall stretching, is considered to be a useful marker for evaluating the risk of CVD [125]. Previous studies have found that compared to the controls, NT pro-BNP levels were significantly increased in patients with active TA, as well as patients in remission. In addition, patients with severe TA showed significantly higher NT pro-BNP levels than those with mild to moderate TA [126]. These results indicate that NT pro-BNP may be a useful marker to assess the status, severity, and progression of TA. Liu et al. evaluated the relationship between the NT pro-BNP level and arterial stiffness in patients with TA, finding that the NT pro-BNP level was independently associated with the baPWV values. Several possible factors accounted for this association. First, increased arterial stiffness results in the earlier arrival of reflected pulse waves and increased left ventricular (LV) afterload, thus stimulating NT pro-BNP release [127]. Second, an increase in the PWV shifts pressure-wave reflections from diastole to systole, leading to increased systolic blood pressure and decreased diastolic blood pressure, which subsequently increases the level of myocardial oxygen and decreases coronary perfusion [128]. Consequently, subclinical myocardial ischemia may induce the production of NT pro-BNP from the intima in human coronary arteries [129]. Hence, the alterations related to increased arterial stiffness may stimulate the synthesis and release of NT pro-BNP [130].

4.3 Coronary Artery Stenosis

Clinical manifestations range from asymptomatic to acute myocardial infarction. Patients can present as acute coronary syndrome (ACS) when severe narrowing of the coronary artery exists (Figs. 17.7 and 17.8). Pectoris angina and myocardial infarction were reported to be presented in 1.2–75% and 1.0–25% of TA patients, respectively [15,18,22,131–136].

Several cases have reported that coronary ischemia is one of the main causes of death in TA, with a mortality of up to 50% at 5 years [137]. There are cases reported of sudden death in patients with ostial stenosis in the coronary arteries [138,139]. ACS must be diagnosed promptly in order to begin proper therapy. Radiologic evaluation can provide an extensive assessment of the pathologic process in TA. Digital subtraction angiography (DSA) was thought to be the only reliable radiologic method of evaluating patients with TA. However, DSA could not show blood vessel wall lesions and thus had little significance in early diagnosis of TA. Due to its invasive nature, DSA could not be performed in active TA. Coronary CTA is a noninvasive technique to assess the presence of coronary involvement in patients with TA, and it can be used in the early stages of the disease. One study suggested that coronary CTA allows the assessment of coronary artery involvement in patients with TA, finding that 8 of 18 of TA patients with symptoms of angina or dyspnea (44%) had coronary lesions,



FIGURE 17.8 Coronary angiography of a 34-year-old woman with complaint of postexercise chest pain. Note the 90% stenosis of the middle segment of left main coronary artery. Adapted from the personal collection of the authors.

and most lesions were noncalcified, ostial, or proximal in location [140]. ^{18}F -fluorodeoxyglucose PET (^{18}F -FDG PET) is useful not only in early diagnosis but also in accurately monitoring treatment efficacy in TA patients [141]. A meta-analysis revealed that ^{18}F -FDG PET had moderate diagnosis value in assessing TA activity, with pooled estimates of sensitivity and specificity of 70.1% and 77.2%, respectively. Although it was not evaluated enough to monitor TA activity, ^{18}F -FDG PET may add additional value to current diagnosis methods [142]. Thallium-201 (TI-201) myocardial scintigraphy has been employed to evaluate myocardial perfusion in patients with TA [143]. Nishimura et al. demonstrated that in comparison with coronary angiography, the sensitivity and specificity of exercise TI-201 scintigraphy for the detection of myocardial ischemia were 100% and 100%, respectively, in patients with TA presented chest pain. However, ^{18}F -FDG PET could not distinguish between atherosclerosis and TA in older patients [141].

Myocardial fibrosis could be found in TA patients with myocardial infarction, which was characterized by late gadolinium enhancement in the subendocardium in MRA. Keenan's study identified late gadolinium enhancement in 4 (27%) of 15 patients with TA, but none of these 4 patients was known to have coronary artery disease. Thus it is of great significance to exclude coronary artery involvement in TA, especially in young patients who are asymptomatic [144].

Whether there is a relationship between ACS and the levels of inflammatory markers remains unclear. Most studies show a correlation between ACS and elevated inflammatory markers such as ESR and CRP [135,136]. However, some case reports do not show this relationship [145]. O'Connor et al. reported a 24-year-old female with a 5-year history of TA who presented with stroke-like symptoms and evidence of left main coronary artery occlusion on imaging, despite a history of decreasing ESR and CRP. Computed tomography angiography revealed complete occlusion of the left common carotid artery, left subclavian, and left main coronary artery from their origins. This case suggested that inflammatory markers alone may not be a reliable method to evaluate disease progression in patients with TA, and should be taken in the context of both the patient's clinical picture and the imaging [146].

Ostial lesions in the right and left coronary arteries are the most common findings. However, due to the accelerated development of atherosclerosis in TA, lesions may also occur in distal segments. Diagnosis is based on clinical features and coronary angiography.

4.4 Coronary Artery Aneurysms

Aneurysmal coronary artery disease is seen in 0.3–5% of patients undergoing coronary angiography and

is defined as a localized luminal dilation measuring at least 1.3 to 2 times the diameter of a normal, adjacent reference segment [147]. Direct extension of the inflammatory process from the ascending aorta into the coronary ostia and the coronary artery roots as well as severe disruption of the media also seem to be causes of coronary artery aneurysms in TA [99]. Coronary artery aneurysm (CAA) formation is extremely rare in TA. The left main coronary artery is the least frequently involved artery. Several studies have shown CAA to be associated with TA. Most CAAs are asymptomatic, but some patients can present with angina pectoris, myocardial infarction, or sudden death. There is no universally accepted definition of giant CAA, and diameters of greater than 20, 40, and 50 mm and quadruple the reference vessel diameter have all been proposed [148]. Thus far, only Suzuki et al. have reported one case of giant CAA of the left main coronary artery in TA [149]. CAA often causes stasis of blood flow, resulting in mural thrombosis and myocardial infarction, and saccular aneurysms are usually thrombosed, ruptured, or enlarged, resulting in myocardial infarction, cardiac tamponade, or sudden death [150]. Thus surgical treatment of CAA should be performed in order to prevent these severe complications.

4.5 Heart Failure

Congestive heart failure (CHF) occurs in 2.44–28% of TA patients [16,18,19,23,60]. CHF is associated with moderate or severe AR, severe hypertension, and pulmonary artery involvement. Patients with heart failure that is caused by severe hypertension usually have stenosis of double or severe single renal artery and stenosis of the descending aorta and/or abdominal aorta [40,151]. Possible pathophysiological mechanisms include water and sodium retention caused by over-reactivity of renin angiotensin aldosterone system or weakness of pressure diuresis and hyperpermeability caused by damage of pulmonary capillary blood gas barrier. Patients usually present sudden or progressive dyspnea, cough, fatigue, and/or bilateral leg edema. Although rare, some patients may present isolated pulmonary artery involvement. This diagnosis is made according to the following points: (1) onset age is young, especially ≤ 40 years; (2) inflammatory markers such as ESR and CRP are abnormal, while serological tests of connective disease are all negative; (3) pulmonary angiographic findings are consistent with TA, which present with wall thickness and multiple narrowing, occlusion, or dilation of the affected vessel lumen; and (4) other etiologies that result in pulmonary stenosis have been excluded [152].

In addition to ultrasonography, cardiac function can be evaluated by MRA in TA. Cardiac MRA can identify

dynamic LV systolic function in TA. The body surface area-indexed LV end-systolic volume has shown to be significantly less in TA patients, resulting in increased ejection fraction (74% versus 67% in controls, $P < 0.001$). After indexing for BMI, the LV end-diastolic volume and LV end-systolic volume is significantly lower in TA patients [153]. However, MRA is not applicable in patients with implant of metallic materials, such as artificial heart valve, pacemaker, and some types of stent [154].

4.6 Coronary Vasculitis

The lesions caused by TA usually affect the ostial or proximal segments of the coronary arteries. Most patients of coronary vasculitis are middle-aged or elderly males, which is very different from type 1 and type 3 patients. Diffuse lesions of the coronary artery are very rare. Kreidstein et al. describe a case of TA presenting as idiopathic adult respiratory distress syndrome, with a pathologic diagnosis of acute interstitial pneumonia. Rarely occurring, diffuse coronary vasculitis also developed in this case [155]. Even in the patients with diffuse lesions of coronary artery, the lesions mainly affect the proximal segments of the arteries. Heart failure caused by acute or chronic myocardial infarction is the main cause of death. Due to the diffuse lesions, coronary artery bypass grafting (CABG) is at higher risk for patients with coronary arteritis.

4.7 Pericardial Involvement

Pericarditis in TA was rarely reported. Lupi-Herrera et al. reported a series of 107 TA patients, finding that pericardial rub was present in only 2% of patients [60]. Several case reports have described pericardial effusion caused by TA, which may present as an initial manifestation of TA [156–158]. Sometimes specific acute recurrent pericarditis may occur [159]. Patients may present with chest pain and dyspnea, with or without other typical TA manifestations. Pericarditis recurrence may be due to steroid treatment withdrawal or reduction, which may be efficiently treated with a high-dose steroid regimen.

4.8 Valvular Abnormalities

AR is the most frequently valvular dysfunction in TA patients. AR in TA was first described by Jervell in 1954 [160]. The incidence of AR in TA is about 5.4–20%. AR is caused primarily by annular dilation resulting from extensive dilatational changes of the ascending aorta and secondarily, by directed valvular lesions such as fibrous thickening, enrolling, retraction, and calcification, and by aneurysms arising from the aortic annulus [161–163]. A study in Korea found that the incidence and severity of the aortic dilation were not

significantly different between the active group and nonactive group, which suggested that direct inflammation of the aortic leaflets rather than dilation of the aortic root is the main mechanism of AR developing in active TA [135]. AR is one of the major complications that can affect outcome in TA, and can cause heart failure [31]. In addition to aortic valve, lesions of the mitral valve, tricuspid valve, and pulmonary valve can also be seen in TA, which is usually complicated with AR. Although the mechanism of other valvular abnormalities is unclear, it is generally thought that instead of direct inflammatory changes, it may be functional changes secondary to AR.

The mitral-aortic intervalvular fibrosa (MAIVF) is the fibrous junction between the noncoronary and left coronary cusps of the aortic valve and the anterior mitral leaflet [164]. In TA, the MAIVF is exposed to high LV intracavitary pressure due to elevated central aortic pressure. Davarparand et al. described a 52-year-old woman with TA diagnosed by pathological evaluation, with transthoracic and transesophageal echocardiographic examinations revealing severe central AR and increased anterior and posterior aortic root thickness (12 and 16 mm, respectively). The thickened aortic root extended to the mitral-aortic intervalvular fibrosa and two-thirds of the basal part of the anterior mitral leaflet (15 mm), which resulted in the thickening of the anterior mitral valve and aortic root and mitral-aortic intervalvular fibrosa abscess formation (Fig. 17.9). Intraoperatively, the thickened ascending aorta and aortic arch had extended to the MAIVF and anterior mitral valve leaflet, without vegetation or infectious tissue [165]. Tufekcioglu et al. described a 35-year-old with TA and moderate AR. Transthoracic echocardiography revealed a suspected aneurysm in the subaortic region adjacent to the left sinus of Valsalva, and subsequent 3D real-time transthoracic echocardiography clearly demonstrated a pseudoaneurysm in the MAIVF. Specific echocardiographic features identified the lesion as a pseudoaneurysm: expansion in systole and collapse in diastole, and communication between the sac and the LV outflow tract [166]. Though pseudoaneurysm of the MAIVF is unusual in TA, the prognosis is grave because of the high risk of rupture, peripheral embolization, and destruction of the aortic or mitral valves [164,167,168]; thus surgical correction is life-saving. Clinicians should keep in mind that such lesions can occur in TA patient groups.

Mitral and tricuspid annular calcification was less reported in TA. Ashmeik et al. identified mitral annular calcification by using transthoracic echocardiography in three patients with TA, in whom none had any of the reported causes of mitral annular calcification. Two of them also had concomitant tricuspid and aortic annular calcification [169].

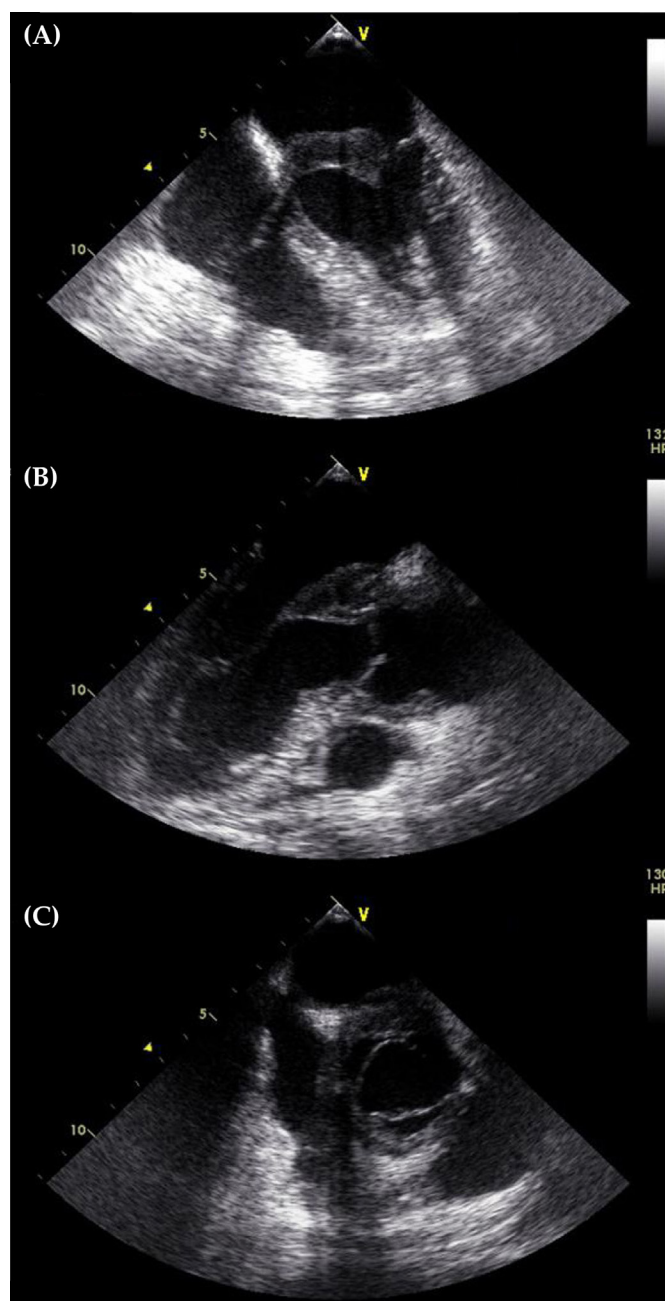


FIGURE 17.9 Anterior mitral valve thickening in transesophageal echocardiography (four-chamber view) (A), anterior mitral valve thickening and aortic root wall thickening in transesophageal echocardiography (aortic valve long-axis view) (B), and aortic root wall thickening in transesophageal echocardiography (aortic valve short-axis view) (C). Adapted from Davarband et al. [165].

Previous case reports described simultaneous occurrence of aortoarteritis and granulomatous myocarditis surrounding an invagination of endocardium that was believed to be a precursor of subaortic left ventricular aneurysm [170–172]. Kumar et al. reported a 20-year-old female aortoarteritis patient with involvement of the abdominal and descending thoracic aorta identified during cardiac catheterization. Biplane transesophageal

echocardiography found a thin-walled, loculated, aneurysmal structure arising from the posterior-medial aspect of the left ventricle just beneath the posterior mitral leaflet, and the aneurysm extended upward behind the left atrium. Though it is uncommon, the combination of TA and subvalvular aneurysm may be identified with greater frequency if aortography is routinely performed in patients with subannular left ventricular aneurysm [173].

4.9 Takayasu's Myocarditis

Inflammatory myocarditis causing left ventricular dysfunction can occur in TA patients. Previous studies have reported autopsy findings of necrosis of myofibers associated with lymphocytic infiltration consistent with Takayasu myocarditis in about 50% of TA patients in India [42,88,174,175]. In Brereinholt's study, an endomyocardial biopsy demonstrated increased HLA-DR on the endothelium and evidence of immune complex deposition in the walls of small vessels, indicating that the inflammatory, vasculitic process affecting the large vessels in TA may also involve the endomyocardium and its small vessels resulting in ventricular dysfunction [176].

Cardiovascular MRA using the paramagnetic contrast agent gadolinium can give useful information about the presence of myocardial inflammation. Mavrogeni et al. documented myocarditis with severe left ventricular dysfunction by applying a cardiovascular MRA protocol for the detection of myocardial inflammation in these patients. Evaluation of myocardial inflammation was performed using STIR T2-weighted (T2-W), T1-weighted before and after injection of 0.1 mmol/kg Gd-DTPA (T1-W) and late-enhanced images (LGE). Measurements after Gd-DTPA were started within 1 min of injection. Immediately after the second set of T1-W images, 0.1 mmol/kg Gd-DTPA was given again and LGE images were taken 15 min later. The functional evaluation to calculate the left ventricular ejection fraction (LVEF) was performed in the short-axis cine. Using this protocol they identified the presence of myocarditis in two out of six TA patients, who presented during the acute phase of the disease, and verified the findings by myocardial biopsy [176].

Myocardial injury is revisable in TA patients. Kotake et al. reported a 24-year-old female TA patient with severe left ventricular dysfunction (20% left ventricular ejection fraction). Myocardial biopsy revealed active myocarditis based on histological investigations, thus a diagnosis of myocarditis with TA was made. After 2 weeks of steroid, immunosuppressive, and conventional heart failure therapies, late gadolinium enhanced images did not show left ventricular wall enhancement, suggesting no necrosis or fibrosis of the myocardium [177].

4.10 Electrocardiographic Findings and Arrhythmias

Very few studies have examined the prevalence of arrhythmias and conduction defects in TA. Electrocardiographic findings in TA patients are associated with comorbidities such as hypertension (left ventricular hypertrophy), aortic regurgitation, and myocardial damage.

Siburian et al. studied the prevalence, severity, and clinical significance of ventricular arrhythmias in 78 female patients with TA by 24-h ambulatory electrocardiography monitoring. Fifty (64%) of 78 patients had no or less than 30 beats/h premature ventricular contractions (Group A). The remaining 28 (36%) patients exhibited frequent or complex premature ventricular contractions (Group B). Echocardiographically determined left ventricular mass (309 ± 94 vs. 166 ± 64 g; $P < 0.01$), frequency of complicated aortic regurgitation (77% vs. 24%; $P < 0.01$), and abnormal thallium-201 scintigraphic findings (76% vs. 38%; $P < 0.05$) were found higher in Group B as compared with those in Group A. These data indicate that frequent or complex ventricular arrhythmias in patients with TA were associated with the presence of left ventricular hypertrophy, aortic regurgitation, and decreased coronary reserve [178].

Kato et al. studied 21 consecutive patients with TA and no significant coronary artery disease. Twelve-lead electrocardiogram and exercise-induced thallium-201 myocardial scintigraphy were performed in all patients. Patients were divided into two groups by the presence (group *P*, $n = 10$) or absence (group *N*, $n = 11$) of exercise-induced thallium-201 myocardial scintigraphic perfusion abnormalities, including permanent defects in three, reversible defects in four, and slow washout in three. The QT dispersion at rest was significantly greater in group *P* than in group *N* (54 ± 12 vs. 40 ± 8 ms, $p < 0.005$), as well as that in group *P* than in group *N* (59 ± 15 vs. 43 ± 11 ms, $p < 0.01$). In patients with TA, myocardial involvement suggested by exercise-induced thallium-201 myocardial scintigraphic perfusion abnormalities is not rare, even when no significant coronary stenosis is present on angiography. Increased baseline QT dispersion was associated with scintigraphic abnormalities and may be a useful marker of myocardial involvement in patients with TA [179].

Only sporadic case reports describe conduction defects in TA. Naitoh et al. described a 56-year-old woman who had received an aortic valve replacement and had suffered from complete left bundle-branch block and advanced atrioventricular block. On the basis of marked systemic inflammatory findings on admission and histopathological findings on previous cardiac surgery, this patient was considered to have conduction disturbances that were a consequence of myocardial involvement of

TA. The conduction disturbances improved rapidly after treatment with steroids [180]. Yokoi et al. described an autopsied case of TA associated with complete atrioventricular (AV) block. The findings of scar formation and diffuse infiltration of lymphocytes into the cardiac conduction system, particularly the AV node, were similar to those in patients with connective tissue diseases or congenital complete heart block. The degree of AV block progressed with aggravation of the disease. These findings suggest that complete AV block may have been induced by acquired autoimmunity involving the cardiac conduction system [181].

5. TREATMENT OPTIONS AND CARDIAC IMPLICATIONS OF THERAPY

5.1 Medical Treatment

It is well acknowledged that TA patients with cardiac involvement should be treated with as much care as possible in order to improve outcomes. GC treatment is the first-line agent and gold standard in the medical treatment of TA. The clinical response to oral GC is varied and ranges from 20% to 100% [60,182]. No comparative trials have been conducted to determine the optimal dose and length of GC treatment. Initial prednisone doses range from 20 to 100 mg/day in retrospective series [60,183]. In a retrospective analysis of 150 patients who had received 30 mg/day prednisone, 51% had improved quality of life, 37% experienced no change, and 12% worsened [183]. In the largest prospective standardized experience with GC for TA, in which patients initially received prednisone at a dosage of 1 mg/kg/day (60 mg daily) for the first 3 months, which was then tapered, 60% of patients treated with prednisone alone achieved remission at least once, and first-time therapy with GC resulted in 52% of patients achieving remission [31]. When clinical and laboratory findings continue to improve for ≥ 2 weeks after the initiation of GC therapy, the dose should be tapered by about 5 mg every other week, during which time periodic evaluation of severity and disease activity should be performed. The typical maintenance dose is 5–10 mg/day prednisone, and withdrawal from GC therapy should be attempted whenever possible (Level A, Class I) [3]. More recent studies reinforce the fact that disease control with prednisone alone is difficult and most patients require other immunosuppressive agents [15,16,134]. Several issues should be considered when giving GC. First, although the initial dosage of 1 mg/kg/day is widely used, a lowering starting dosage may be considered in individuals at high risk for GC toxicity. Second, adverse effects including adrenal suppression, opportunistic infections, steroid myopathy, sleep disturbance, etc., are common, so patients often need other medications to

counteract the side effects of GC. Third, GC can cause hypertension by affecting plasma lipoproteins, promoting insulin resistance and sodium retention, thus patients who suffer from hypertension should be alerted.

Other alternative immunosuppressive agents are typically added in 40–73% of patients who relapse or never achieve remission. Some small, uncontrolled studies and case reports have reported various agents, discussed as follows.

Cyclophosphamide (CYC). Toxicity of CYC includes bone marrow suppression, infection, infertility, bladder injury, transitional cell carcinoma of the bladder, and myeloproliferative disease. Although it was the first cytotoxic agent studied for TA, CYC is rarely used for TA given the predilection for relapse and the predominantly young female population affected [184] (Level C, Class III).

Methotrexate (MTX). Hoffman et al. conducted an open-label pilot study of weekly low-dose MTX in a GC-resistant population. Weekly administration of MTX (mean stable dose of 17.1 mg) and GC resulted in remissions in 13 of 16 patients (81%). However, 44% of patients had relapses as GC was tapered to or near discontinuation, and 18.8% of patients experienced disease progression in spite of treatment. Although this study has limitations, it supports that weekly low-dose MTX is an effective means of inducing remission and minimizing GC therapy and toxicity in most TA patients (Level B, Class I). Further long-term studies are needed to assess the durability of remission and the need for maintenance MTX therapy in this subset of TA patients [185].

Azathioprine (AZA). Valsakumar et al. studied 15 patients with active TA for 1 year using AZA combined with GC. All patients had improvement in systemic symptoms and laboratory measures of disease activity within a period of 3 months of onset of treatment. Repeat angiograms revealed no significant changes compared to baseline. No new lesion appeared in any patient [186]. Toxicity of AZA includes cytopenia, infection, allergic reactions, and leukemia. AZA combined with GC can be used in patients who cannot receive MTX or who relapsed after MTX treatment (Level B, Class I).

Mycophenolate mofetil (MMF). Shinjo et al. used MMF (2 g/day) combined with GC to treat 10 active TA patients for an average of 23.3 months. Clinical activity disappeared in all patients with MMF therapy, except in one patient who abandoned the study because of a serious headache, which was attributed to the drug. Moreover, the MMF therapy allowed significant tapering of the prednisone dose in the other nine patients, and a significant reduction in inflammatory laboratory parameters was observed [187]. MMF therapy may be a promising third-line immunosuppressive drug, particularly in refractory cases and as a steroid-sparing agent (Level B, Class I).

Anti-TNF therapy may be an additional treatment option in difficult to treat TA patients who cannot achieve remission with standard drug therapies (Level C, Class II a). Hoffman et al. published the first series of anti-TNF agents for TA in 2004. They described 15 patients with active or relapsing disease despite prior treatment with prednisone and a second agent. After a median follow-up of 12 months, 93% patients responded, with 67% achieving a sustained steroid-independent remission for between 1 and 3.3 years [188]. A study led by Quartuccio et al. which is notable for providing the longest follow-up to date (mean 71 months), treated 15 patients with infliximab. Overall, 73.3% patients responded and were able to taper steroid therapy. This study also revealed that infliximab can significantly improve health-related quality of life [189]. Several studies reported anticytokine treatment (anti-IL-6 receptor antibody tocilizumab) for TA [190,191]. Tocilizumab holds promise for patients who fail to respond to conventional immunosuppressive treatment, even to TNF- α blockers [192]. Youngstein et al. reported for the first time sustained response to both anti-TNF- α and IL-6R antagonists in refractory TA [193]. Mekinian et al. conducted a retrospective multicenter study to assess the safety and efficacy of biologics (TNF- α antagonists and tocilizumab) in patients with TA. Eighty-eight percent of the 49 patients were inadequately controlled with, or intolerant to, conventional immunosuppressive therapy. The overall response to biological-targeted treatments (80% of TNF- α antagonists and 20% of tocilizumab) at 6 and 12 months was 75% and 83%, respectively. CRP levels and daily prednisone dosage significantly decreased after 12 months of biological-targeted treatments, and the 3-year relapse free survival was 90.9% (83.5–99) over the biologic treatment period compared to 58.7% (43.3–79.7) with DMARDs. After a median follow-up of 24 months, 21% of adverse effects occurred. This nationwide study shows high efficacy of biological-targeted treatments in refractory TA patients with an acceptable safety profile [194]. Although the results from these studies are encouraging, it remains difficult to determine the efficacy and relative safety of biologic agents for TA without a randomized controlled trial.

Treatment should be considered with statins and acetylsalicylic acid in order to prevent accelerated atherosclerosis in TA [103]. De Souza et al. performed a study including 48 TA patients and found that the use of antiplatelet therapy (aspirin) was associated with a lower frequency of ischemic events and had a protective effect with a low frequency of bleeding complications, suggesting that in patients without contraindications, aspirin 100–200 mg daily may reduce the risk for acute ischemic events in TA patients [195] (Level B, Class II a).

5.2 Nonmedical Treatment

Surgery should be undertaken to improve prognosis of TA patients with coronary artery involvement or AR. Surgery procedures are often unsuccessful when performed in active TA. The risk of restenosis was seven-fold higher when there was evidence of inflammatory at the time of procedure [196]. Moreover, univariate analysis revealed active inflammation to be a risk factor for detachment of the valve or graft, emphasizing the necessity of preoperative and postoperative immunosuppression when possible [197]. Adachi et al. reported on a patient who suffered from Behcet disease with active inflammatory findings who had valve detachment 5 months after aortic valve replacement (AVR). However, the absence of inflammatory signs does not necessarily preclude the risk of valve detachment. There are patients who had been well controlled with anti-inflammatory drugs before AVR and had valve detachment 7 years after AVR. The patient was well controlled with anti-inflammatory drugs after the first surgery, and the wall of aortic root obtained during reoperation showed no inflammatory changes in the histologic examination. In cases like this, long-term steroid therapy may conversely make the aortic annulus fragile, which is thought to be one of the causes of detachment of the prosthetic valve and pseudoaneurysmal formation [198]. Kaku et al. did not prescribe postoperative steroids on the basis of intraoperative findings to avoid unnecessary administration of steroids because long-term administration may cause tissue fragility, leading valve detachment [199]. Additionally, the dosage of steroids is needed to confirm. A study led by Yang et al. found that a dose of 0.5 mg/kg of body weight of prednisone could reduce the side effects of drugs and improve compliance of patients [23]. Randomized, controlled studies should be initiated to find a proper steroid dosage. When patients have unstable angina, revascularization must be performed without delay to avoid cardiac events, but GC and/or immunosuppressive therapy should be administered simultaneously [91]. Indications for revascularization are TA-associated ischemic symptoms: cardiac ischemia in the setting of proven coronary artery stenosis, progressive aneurysmal enlargement, and dissection that has become aneurysmal [84]. Only the lesions considered responsible for the ischemic symptoms or signs are determined to be revascularized at the time of the procedure [200]. Strategies for revascularization in TA include percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass graft (CABG) surgery.

The optional revascularization method for coronary artery stenosis remains undetermined. The presence of ostial lesions of the aorta and possible involvement of

the subclavian arteries as well as increased inflammatory activity make the decision more complex not to mention the feasibility of performing revascularization procedures. There is more experience with CABG in TA settings, which is generally accepted to achieve superior results [90,201]. Surgical intervention could not only reduce the complications caused by TA but also increase the long-term survival of patients. Endo et al. conducted a 10-year follow up study, finding that the actual survival rate (including in-hospital deaths) and cardiac event-free (free from cardiac death, reCABG, PTCA, and new myocardial infarction) rate at 10 years were 81.4% and 72.6%, respectively. In a study led by GY et al., 11 TA patients with a total of 19 autologous grafts used and 17 patients with PTCA performed were studied. During a 26.4 months follow-up, the patency rate for the CABG and PTCA were 69.2% and 36.6%, respectively [200]. Sun's study showed a higher mortality in patients who had PTCA compared to CABG [92]. Because subclavian arteries and internal thoracic arteries may be involved in inflammation, when selecting the type of grafts, saphenous vein grafts are a better choice as they are less likely to develop vasculitis, and graft stenosis, which is very different from atherosclerotic coronary lesions. But if the coronary artery has severe calcification, the mammary artery graft is preferred. Before using the internal mammary artery (IMA), imaging examinations of the subclavian arteries should be done to ensure their patency. In a study in which two cases used left IMA in situ grafts were used, the patients were still asymptomatic after 20 months [136]. However, surgery was also accompanied by more complications. Anastomotic aneurysm was the usual observed complication with a cumulative incidence of 13.8% at 20 years, making regular imaging modality follow-up necessary [90].

There is less about the results of coronary stenting for TA, even in a small group of patients. The most possible reason is that lesion locations are usually ostial or proximal coronary segments like the left main coronary artery, which are unsuitable for percutaneous angioplasty. Moreover, blood access sites are limited in many cases if morbid changes occur at main branches of the aorta such as the iliac, subclavian, and innominate arteries. Aortic disease itself, like aneurysm, may disturb intraaortic catheter manipulation [202]. Indications for PTCA in TA patients with coronary artery are unclear. Several cases have reported restenosis after PTCA, with or without stenting, which is very high [31,203]. Implantation of bare-metal stents in patients with TA is followed by restenosis in up to 78% of cases. A possible explanation for restenosis may be that the vessel lesions in TA are usually long, fibrotic, and almost completely occluded. In the meantime, PTCA in itself is a kind of vascular injury,

which could promote vascular endothelial cell proliferation and stenosis. PTCA with or without stenting may just treat the superficial results and not address the disease itself [85]. Drug-eluting stents were previously thought to be effective in treating TA patients with coronary artery stenosis, since they may have a potential therapeutic benefit in TA due to their local anti-inflammatory properties. Apart from decreased inflammatory-induced intimal hyperplasia, these eluted drugs may have the capacity to attenuate the arteritis [204,205]. However, with isolated cases reported reocclusion after drug-eluting stents in TA patients with coronary involvement, questions regarding the safety and efficacy of this procedure have been raised [206,207]. In fact, some authors suggest that drug-eluting stents are only useful as bridges while immunosuppression is optimized and until CABG can be performed [11,208]. Bioresorbable vascular scaffold (BVS) is an emerging technology for the management of coronary stenosis and an attractive alternative to metallic stents [209]. Camuglia et al. reported the case of a 21-year-old female who implanted a bioresorbable vascular scaffold. Repeat coronary angiography performed 8 months following the initial presentation showed no evidence of stenosis. This case outlines the potential role of BVS as an alternative method of treatment in young TA patients with ostial major vessel coronary disease. With the expected resorption of the scaffold after 2 years, the risk of very late stent thrombosis would be eliminated [210].

TA patients with moderate AR (grade two New York Heart Association) are candidates for surgery [84]. The procedures include AVR and composite graft repair (CGR). Matsuura et al. demonstrated a favorable late outcome of surgical treatment (aortic valve replacement or composite graft repair) of AR due to TA in 90 patients, with an overall 15-year survival rate of 76.1% [197]. Kaku et al. reported that the 5-year survival rate was 90.9% in 22 TA patients with AR. Although surgical outcomes have improved dramatically due to the development of not only surgical equipment and technologies but also preoperative evaluation and postoperative management [89,211], surgical treatment for this disorder is still difficult because of the need to manipulate fragile and inflamed tissue. Valve detachment after AVR or anastomotic aneurysm after CGR may still occur as a result of the fragility of the aortic wall or annular tissue caused by TA [89,211,212]. Surgical reintervention may be required because of late dilation of recurrence AR, and longstanding follow-up is needed. Active inflammation may be a predictor of pseudoaneurysm, thus aggressive postoperative control of inflammation is necessary to prevent complication [197].

6. CONCLUSIONS

TA is a chronic nonspecific inflammatory disease that frequently occurs in young women. TA mainly involves the aorta and its major branches and the coronary and pulmonary arteries. Autoimmune, inflammatory, and genetic factors are associated with the occurrence and development of TA.

Cardiac lesions are a major cause of death in cases of TA. Cardiac involvement of TA includes coronary artery involvement (stenosis and/or aneurysms), heart failure, coronary vasculitis, pericardial involvement, and valvular abnormalities.

Premature and accelerated atherosclerosis is well known, and atherosclerotic plaques are much more frequently observed in TA patients than in age- and sex-matched controls. The major pathophysiologic process of coronary atherosclerosis is a defect or injury of the arterial endothelial function. TA patients showed increased prevalence of traditional risk factors for CVD compared to healthy controls, which may increase in individuals at increased cardiovascular risk. C-IMT, PWV, AI and NT pro-BNP are markers for atherosclerosis and endothelial dysfunction in TA. It is suggested that baPWV may be used as a biomarker for increased coronary artery risk in TA patients who received DES and as a target for earlier detection of higher risk for late MACE.

About 10–30% of patients with TA have coronary artery involvement. According to the pathologic features coronary artery lesions can be divided into three types: type 1, stenosis or occlusion of the coronary ostia and the proximal segment of the coronary arteries; type 2, diffuse or focal coronary arteritis, which may extend diffusely to all epicardial branches or may involve focal segments, so-called skip lesions; and type 3, coronary aneurysm. Type 1 is the most frequently observed lesion in TA. Coronary artery lesions are caused by extension of intimal proliferation and fibrous contraction lead by the inflammatory process that involves the ascending aorta, or by coronary arteritis as one of the skip lesions of TA. Patients may present myocardial infarction, angina pectoris, and sudden death. Aneurysmal coronary artery disease is rare in TA, which is often asymptomatic or occasionally manifests as myocardial infarction or sudden death. Coronary vasculitis is very rare, often affecting middle-aged or elderly male TA patients. Unlike type 1, CABG is a higher risk for patients with coronary aneurysm or coronary vasculitis.

CHF in TA is associated with moderate or severe AR, severe hypertension, and pulmonary artery involvement. CHF is the most common reason for death in TA. AR is the most frequently seen valvular dysfunction in TA patients, which is caused primarily by annular dilation resulting from extensive dilatational changes

of the ascending aorta and secondarily, by directed valvular lesions such as fibrous thickening, enrolling, retraction, and calcification, and by aneurysms arising from the aortic annulus. AR is one of the major complications that can affect prognosis of outcome in TA, and can cause heart failure. Inflammatory myocarditis can cause left ventricular dysfunction in TA. Diagnosis of myocarditis can be determined by myocardial biopsy and myocardial injury is revisable in TA. Frequent or complex ventricular arrhythmias in patients with TA are associated with the presence of left ventricular hypertrophy, aortic regurgitation, and decreased coronary reserve. Increased baseline QT dispersion is associated with scintigraphic abnormalities and may be a useful marker of myocardial involvement in patients with TA. Complete AV block is observed in TA and may have been induced by acquired autoimmunity involving the cardiac conduction system.

TA patients with cardiac involvement should be treated with as much care as possible in order to improve outcomes. GC treatment is the first-line agent and gold standard in the medical treatment of TA. Other alternative immunosuppressive agents are typically added in patients who relapse or never achieve remission. MTX and AZA are second-line agents, and MMF is a promising third-line immunosuppressive drug. Anti-TNF therapy may be an additional treatment option in difficult to treat TA patients who cannot achieve remission with standard drug therapies. Treatment should be considered with statins and acetylsalicylic acid in order to prevent accelerated atherosclerosis in TA.

Revascularization procedures cannot be performed until inflammation is controlled. CABG, especially saphenous vein graft, is preferred due to the nature and specific lesions of TA. PTCA with drug-eluting stents may play a role as a bridge until inflammation is effectively controlled and surgical grafting can be applied. BVS may be an alternative method of treatment in young TA patients with ostial major vessel coronary disease. TA patients should be administered appropriate immunosuppressive pharmacotherapy given the systemic nature of TA.

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Polyarteritis Nodosa

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1. INTRODUCTION

Küssmaul and Maier were the first to describe polyarteritis nodosa (PAN) in 1866 [1]. This systemic necrotizing vasculitis predominantly affects medium-sized arteries in all organs, except the lungs. Infection with hepatitis B virus (HBV) [2] or sometimes other pathogens can cause PAN, but the etiology can also be noninfectious or attributable to malignancies [3–5]. The availability of anti-HBV vaccines, hygiene measures, and specific prophylaxis against infectious diseases in developed countries has made PAN, among vasculitides, even scarcer.

1.1 Definition

Vasculitides are defined by their histological features. The elementary vascular involvement present in all vasculitis types shows fibrinoid necrosis of vessel walls, perivascular inflammatory infiltrates, and their subsequent replacement by fibrotic scarring. Usually, PAN vascular lesions have a segmental distribution pattern and exhibit a predilection for arterial bifurcations. Occurring very early and predominating in the internal layer of the arterial media, fibrinoid necrosis appears simultaneously with the infiltration of inflammatory lymphocytes, plasma cells, histiocytes, and some neutrophils in or around vessel walls. Microaneurysms may result from segmental necrosis in medium-sized vessels, usually progress to fibrosis and thrombosis, and can cause tissue ischemia and damage. Because the activity pattern of some vasculitides is successive flares, different histological stages may be seen in a single tissue specimen.

PAN can be classified according to the 1990 American College of Rheumatology classification criteria [6] or the more accurate and complete Nomenclature of Systemic Vasculitides, established at the Chapel Hill Consensus

Conference [7]. The latter puts PAN in the group of medium-sized artery vasculitides and classifies it as primary or secondary vasculitis.

1.2 Epidemiology

PAN is less common than other vasculitides, and the treatment and prevention of HBV infection has made it even rarer. All racial groups can be affected. PAN prevalence in Seine-Saint-Denis County (France) in 2000 was estimated at 34/1,000,000 inhabitants [8]. Its incidence remains imprecise. The annual incidence in general populations of PAN-type systemic vasculitides ranged from 4.6 per 1,000,000 inhabitants in England [9], and 9.0 per 1,000,000 inhabitants in Olmsted County, Minnesota, to 77 per 1,000,000 inhabitants in a hepatitis B-hyperendemic Alaskan Eskimo population [10]. The PAN incidence in Germany was extremely low (0.3–0.4 per 1,000,000 inhabitants according to the year and region considered) [11]. No differences were found between the PAN incidences in Lugo, Spain, and Norwich, United Kingdom [12], respectively: 6.2 and 9.7/1,000,000. However, the development of vaccines, vaccination campaigns, treatments, and improved hygiene in developed countries mean that data on these epidemiological aspects need to be updated.

Although the exact proportion of HBV infections responsible for PAN has not been clearly established, it represented more than one-third of largest patient series [13]. However, it is probable that most PAN patients have been infected by HBV or other microorganisms, as suggested by epidemiological studies. Moreover, the progressive disappearance of PAN in developed countries is a strong argument in favor of the responsibility of an infectious cause, with the absence of clinical differences between HBV-positive and -negative PAN patients further supporting their having similar etiologies, eg, “cryptoHBV” or an unidentified close virus.

2. DIAGNOSTIC CRITERIA

Theoretically, histological proof is needed to diagnose vasculitis (Fig. 18.1). However, because only non-specific inflammation or no anomalies may be seen in tissues easily accessible for biopsy, the diagnosis is sometimes based on a combination of clinical findings, and results of biological, immunological, and radiological investigations.

The best chance of obtaining histological proof of vasculitis is by biopsying organs exhibiting clinical manifestations. For patients with general symptoms, muscles can be biopsied, with the sought after proof most likely found in a distal leg biopsy.

A skin nodule or purpura is readily accessed. For mononeuritis multiplex patients with sensory and motor deficits, a neuromuscular biopsy, usually a sensory branch of the superficial peroneal nerve, is recommended. When severe clinical manifestations, like bowel perforation, require surgical intervention, intra-operative specimens can be obtained for histological examination.

However, in the absence of histological proof, a set of clinical and biological criteria can be applied. Importantly, classification criteria cannot be used to diagnose vasculitis. They were established explicitly to classify vasculitides, once the diagnosis has been made. We devised and validated a mathematical model based on a combination of criteria (symptoms and immunological parameters) to diagnose PAN (Table 18.1) [14].

Autoantibodies, specifically antineutrophil cytoplasm antibodies (ANCA), have never been found in PAN. Indeed, ANCA-positivity should be a criterion excluding the diagnosis of PAN [14].

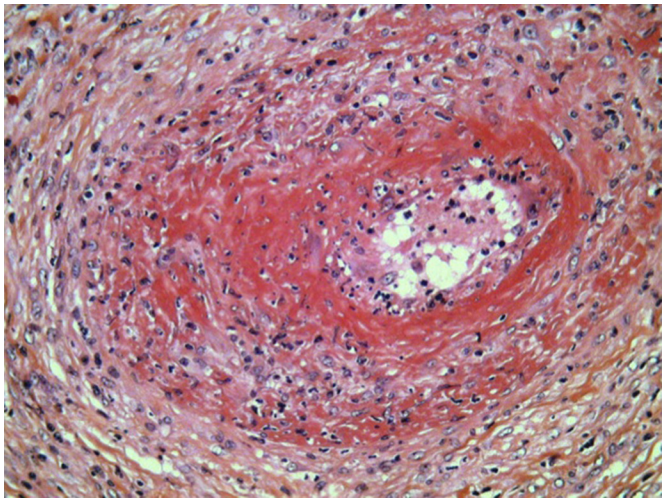


FIGURE 18.1 Biopsy showing fibrinoid necrosis of small artery, endothelium injury, and adventitial inflammation. Adapted from the personal collection of Dr. L. Guillevin, University of Paris, Paris, France.

Most PAN patients have angiographic findings, eg, multiple aneurysms 1–5 mm in diameter or irregular vessel narrowing (stenoses) (Fig. 18.2). Although

TABLE 18.1 Proposed Diagnostic Criteria for Polyarteritis Nodosa^a

Criterion	Odds Ratio	95% CI	R ²
POSITIVE FOR PAN			
HBV infection	16.85	6.30–45.08	0.320
Myalgias	1.93	1.06–3.53	0.517
Mononeuropathy or polyneuropathy	3.36	1.93–5.86	0.619
Angiographic abnormalities	20.40	7.30–56.99	0.640
Testicular pain or tenderness	5.27	1.98–28.26	0.661
NEGATIVE (EXCLUSION) FOR PAN			
ANCA-positivity	0.11	0.05–0.23	0.668
Glomerulonephritis	0.07	0.02–0.29	0.674
Recent asthma onset	0.01	0.01–0.06	0.433

^aBased on the analysis of 582 systemic vasculitis patients with all data available in the French Vasculitis Study Group’s database: 194 PAN (among whom 117 had HBV-related PAN) and 388 other systemic vasculitides (granulomatosis with polyangiitis, n=144; eosinophilic granulomatosis n=115; microscopic polyangiitis, n=101; cryoglobulinemia, n=28). Adapted from Henegar et al.[14].

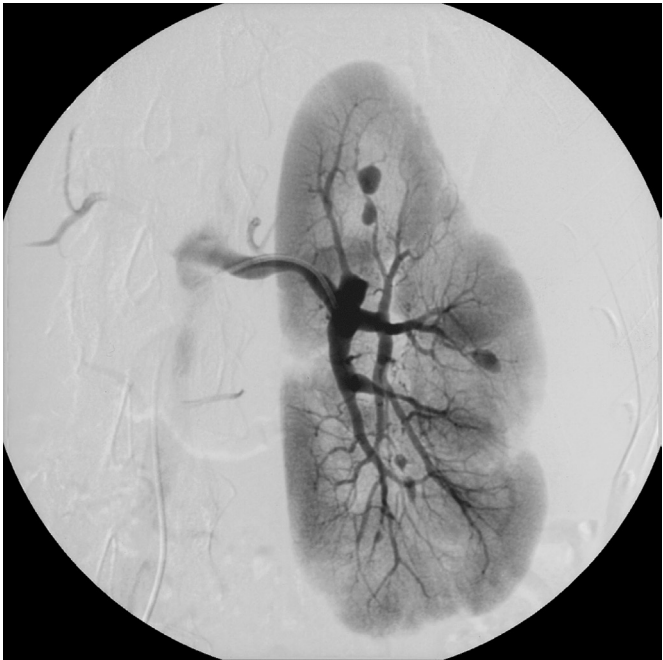


FIGURE 18.2 Microaneurysms located in kidney parenchyma. Adapted from the personal collection of Dr. L. Guillevin, University of Paris, Paris, France.

suggestive, those findings are not absolutely PAN-specific, because arterial aneurysms are also seen in thrombotic thrombocytopenic purpura, mycotic aneurysms, fibromuscular dysplasia, atrial myxoma, malignant arterial hypertension, and rare patients with small-vessel vasculitides [15]. Multiple stenoses of medium-sized arteries are more frequent in digestive and renal arteries than coronary arteries. Microaneurysms disappear when patients recover, usually within a few months [16]. This normalization of vascular imaging results from aneurysm thrombosis and a histological fibrotic process.

Noninvasive imaging, for example magnetic resonance angiography of angio computed tomography (CT) scans, can be informative. However, although the “resolution” of these new techniques is promising, they are usually not sufficient to detect the smallest aneurysms. Such investigative techniques cannot be recommended for screening PAN vascular lesions but, in patients with clinical or biological manifestations suggestive of PAN, they can help determine the diagnosis. Abdominal ultrasound and CT scanning can show visceral infarcts, which are suggestive of the diagnosis.

3. DIFFERENTIAL DIAGNOSIS

The main differential diagnosis is Kawasaki disease, which affects medium-sized vessels, with cardiac manifestations being one of its major characteristics. This vasculitis mostly affects young children but can occur in adults. Kawasaki patients have coronary arteritis, characterized by microaneurysms and stenoses of coronary arteries. Some may also suffer from cardiac insufficiency. Neither histology nor cardiac imaging can distinguish between adult PAN and Kawasaki disease. PAN is usually diagnosed when other organs, rarely affected by Kawasaki disease, are involved.

Although heart involvement can also occur in other vasculitides, like granulomatosis with polyangiitis (Wegener’s) (3.3%) [17], microscopic polyangiitis (17.9%) [18], and eosinophilic granulomatosis with polyangiitis (Churg–Straus) (27.4%) [19], PAN diagnosis is usually based on clinical, biological, and/or immunological symptoms.

It was recently shown that some vasculitides were caused by recessive mutations in the cat-eye syndrome chromosome-region candidate-1 (*CECR1*) gene encoding adenosine deaminase 2 (ADA2). The disease has been observed in Caucasians, including Jewish families from Georgia, Germany, and Turkey [20]. This vasculopathy is responsible for strokes [21] but other vascular manifestations, including cardiac manifestations, will probably be reported once larger patient series

will have been evaluated. At present, we do not know if ADA2 mutations are a cause of PAN or a new distinct entity, similar to it and sharing common clinical manifestations.

All the cardiovascular manifestations occurring in the context of vasculitis must be distinguished from those caused by other organ involvement(s) or therapeutic adverse events. Infective endocarditis, hypertensive cardiomyopathy, uremic pericarditis (which is now rarely seen), traumatic perforation and dissection after endovascular investigations or biopsies, or long-term therapy with corticosteroids (CS) and immunosuppressants may be responsible for, or detrimental to, heart involvement in vasculitides.

4. PATHOPHYSIOLOGY OF POLYARTERITIS NODOSA

PAN is an immune-complex disease, without any known relationship with autoantibodies. Some etiological agents have been identified: HBV for PAN and hepatitis C virus for cryoglobulinemic vasculitis, another immune-complex vasculitis. It is most likely that other, still unidentified pathogens, probably viruses, can cause PAN. This hypothesis comes from epidemiology: (1) patients with or without HBV infection have similar clinical manifestations and other characteristics, and (2) the hygiene measures, eg, increased blood-transfusion safety and anti-HBV vaccination, used for the general population have markedly decreased the PAN incidence. PAN, which had been one of the most frequent necrotizing vasculitides in France and other developed countries, has become rare and almost disappeared. In developed countries, most cases are now seen in non-natives, who spent most their lives in Africa or Asia, or homosexuals and/or IV drug abusers not vaccinated against HBV. In France, fewer than 10 cases of HBV-attributable PAN are seen annually. PAN has also been observed in patients with hairy-cell leukemia or other malignancies [4,5].

Because PAN is a rare disease, very little progress has been made in understanding its pathogenesis. Obviously, other mechanisms not limited to immune-complex deposition are involved. Hemodynamic factors can explain microaneurysm formation, usually at arterial branches, which suggests that turbulent blood flow facilitates immune-complex deposition at these sites and, thus, microaneurysm constitution. Cytokines, adhesion molecules, and endothelial cells also participate in the inflammatory response. Complement, too, is involved in immune-complex deposition, and immunofluorescence enables visualization of the deposits in vessel walls in small-sized artery vasculitis. But that is not usually the case for medium-sized vessel vasculitis, like

PAN. Hence, pathogenic mechanisms remain unclear and partly unelucidated.

5. CARDIAC INVOLVEMENT

Rarely, vasculitis can involve all heart tissues: from myocardium to epicardium, endocardium, conductive nodal tissue, and coronary arteries.

5.1 Cardiomyopathy

Cardiac manifestations of PAN, already noted in the first publication [1] that described a case of “nodular coronaritis,” were subsequently reported with frequencies ranging from 10% in a clinical PAN study [22] to 78% in a histopathological investigation [23].

Congestive heart failure occurs in 6–57% of PAN patients, is the predominant manifestation [22,23], and is specific and/or can be a consequence of other vasculitis-related organ involvement or disorders, usually hypertension and/or renal disease. Specific cardiomyopathy occurs during the acute phase of PAN but, in some patients, cardiac involvement can develop later as a sequela. Interstitial myocarditis was seen in 14% of autopsied patients [24].

Coronary artery or myocardial arteriolar infarcts can also cause congestive heart failure, generating disseminated necrotic foci, most often in the left ventricle. However, the right ventricle too may be affected, as for 6/8 patients with cardiac involvement [25]. Notably, clinical angina is rare, affecting 2–18% of the patients [22,25], as are myocardial infarctions that occurred in 1–12% of PAN patients, but signs of the latter were not that uncommon at autopsy [22]. Among 66 autopsied patients, 41 (62%) had features of myocardial infarction, but only three of them had clinical symptoms and three had coronary atherosclerosis [23]. Schrader et al. [24] autopsied 36 PAN patients: 50% had evidence of coronary arteritis, with small subepicardial vessels containing severe lesions just as they entered the myocardium and 8% had gross infarcts. Angiography can prove PAN coronary involvement in 85% of patients with clinical signs of infarction, whereas infarction may be due to arteritis of small coronary vessels or spasms in the remaining 15% [26] (Fig. 18.3). To date, no guidelines have been formulated regarding the indications and modes of coronary artery exploration for asymptomatic PAN patients.

Murmurs, found in one-third of PAN patients [23], are unusually innocuous, further emphasizing the rarity of valve involvement in this vasculitis [27,28]. PAN valvulopathy is unlikely and the reported cases of PAN-related mitral and tricuspid regurgitation [25,29] remain unconfirmed by histological examination or

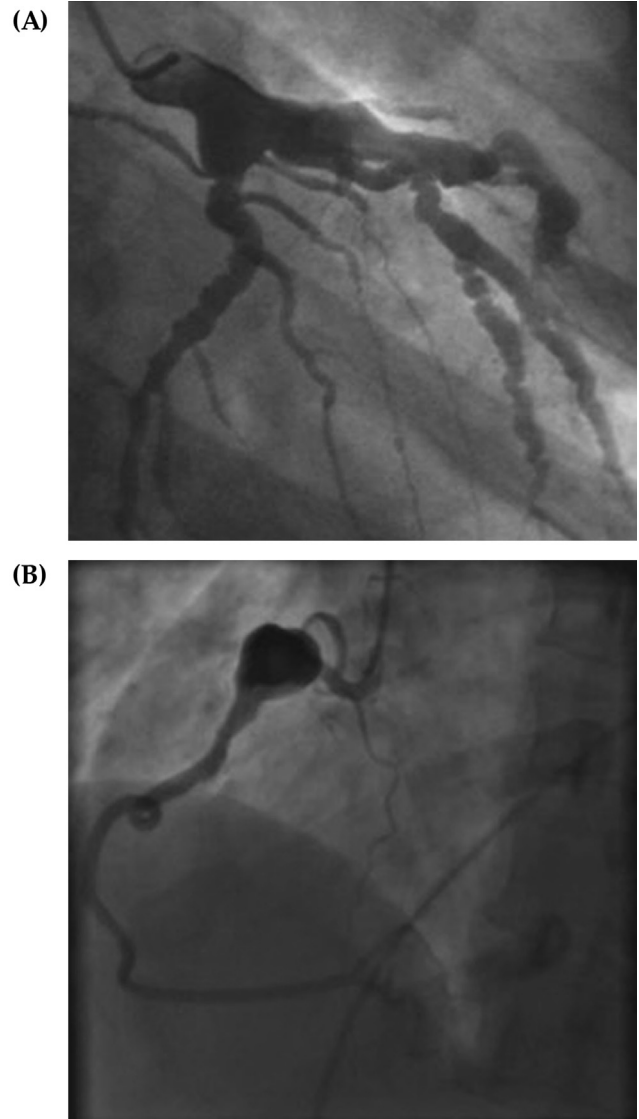


FIGURE 18.3 Coronary angiogram showing microaneurysms and stenoses in the left coronary artery, in a patient with PAN. Adapted from the personal collection Dr. L. Guillemin, University of Paris, Paris, France.

surgery. Valve involvement is rarer in PAN than ANCA-associated vasculitides. Notably, most valvular symptoms were described before the discovery of ANCA and their role, and it is possible that some valvulopathies ascribed to PAN should be reclassified as ANCA-associated vasculitides. Sinus tachycardia is common and nonspecific. Arrhythmias and conduction disorders, mainly supraventricular, occurred in 2–19% of PAN patients [23,25] because of arteritis of the sinus node or neighboring nerve fibers. Aortic dissection, albeit a rare complication, is attributed to diffuse vasculitis of the vasa vasorum, but was reported in one patient with HBV-related PAN and evolved to fatal tamponade [30]. Dissections of proximal aortic branches have also been

described, but some were attributed to other causes, eg, atherosclerotic aneurysms [31–34], syphilis, cystic media necrosis, trauma, sepsis, or hypertension.

5.2 Coronary Arteritis

Aneurysms, thromboses, dissections, and/or stenoses are signs of coronary arteritis that can lead to myocardial infarction. Although coronary angiography may visualize some abnormalities (Fig. 18.3), it is rarely done, because of the rarity of angina in this setting [22,23]. Nonetheless, a postmortem study on PAN patients found coronary involvement in half of them [24]. In clinical practice, Kawasaki disease remains the vasculitis with the most frequent coronary arteritis and up to 20% of the patients develop aneurysms [35]. Coronary angiogram should be done for patients with clinical manifestations of angina pectoris or myocardial infarction. The investigation should not be delayed and is not more risky when performed in PAN than in patients with another cause of coronary disease.

5.3 Pericarditis

Pericardial involvement affects 0–5% of PAN patients [36], rising to 19% [24] to one-third [23] of autopsy series. Notably, severe renal insufficiency was responsible for about half of the pericardial effusions initially reported but its frequency has diminished markedly now that patients undergo dialysis. Pericarditis also often accompanies cardiomyopathy.

5.4 Pulmonary Arterial Hypertension

Pulmonary arterial hypertension is rare, having been reported only anecdotally in PAN patients [37,38]. We never observed it in our series of patients [13], and the dates of publication of those case reports mean that misclassification of diseases erroneously considered to be PAN cannot be excluded.

5.5 Thromboembolic and Proximal Vascular Complications

In addition to heart and aortic involvement, thromboembolic events can complicate PAN, as in other vasculitides [39], most often when necrotizing vascular inflammation is present. More thromboses occur in granulomatosis with polyangiitis (Wegener's) [40] and eosinophilic granulomatosis with polyangiitis (Churg–Strauss) [41], and, although not directly studied, because PAN also affects the vascular endothelium (Fig. 18.4), thromboses can be suspected in it.



FIGURE 18.4 Posterior tibial artery stenoses and microaneurysms. Adapted from the personal collection of Dr. L. Guillemin, University of Paris, Paris, France.

6. TREATMENT OPTIONS AND CARDIAC IMPLICATIONS OF THERAPY

6.1 Vasculitis Severity

The Five-Factor Score (FFS), first devised in 1996 [42] and revised in 2011 [43], has significant prognostic

value and could guide physicians' therapeutic choices and avoid overtreatment. The following items, each accorded +1 point, are associated with higher mortality: proteinuria >1 g/day, renal insufficiency (creatininemia >140 μ mol/L or 1.6 mg/dL), specific cardiomyopathy, gastrointestinal (GI) manifestations, and central nervous system involvement. As mentioned above, cardiac manifestations are associated with increased mortality. According to a study on 342 patients with PAN or eosinophilic granulomatosis with polyangiitis (Churg–Strauss), for FFS=0, 1, or 2, respective 5-year mortality rates were 12%, 26%, or 46%.

6.2 Recommendations for Specific Conditions

For patients with potentially fatal diseases, supportive care contributes strongly to the therapeutic regimen. Because immunosuppression is at its maximum at treatment onset, preventing opportunistic infections, like *Pneumocystis jiroveci* pneumonia, may be necessary and prophylaxis should be prescribed on an individual basis [44,45]. Mononeuritis multiplex therapy requires pain control, prevention of pressure sores, and physical therapy. Angiotensin-converting enzyme inhibitors are effective against the severe hypertension caused by renal vasculitis and improve renal function. They and angiotensin-II inhibitors are other molecules used to treat cardiac insufficiency. GI involvement, renal failure, cardiac insufficiency, and/or cerebral involvement are usually present in fulminating PAN. Sometimes, PAN does not respond to treatment and patients die within a few months postdiagnosis.

When GI involvement with abdominal pain persists despite medical treatment, exploratory laparotomy is recommended to identify and treat possible bowel perforations. Radiological investigations usually show microaneurysms of mesenteric and hepatic arteries, and sometimes visceral infarcts or pancreatitis. However, they do not provide enough information to diagnose bowel perforations. Those patients' medications should probably be administered intravenously to avoid undertreatment due to impaired drug absorption. For patients with cardiac insufficiency, fluid intake should be limited and a low-salt diet recommended. Parenteral nutrition should be used to counter the rapid and severe weight loss caused by severe GI symptoms. Although weight loss has not been shown to be a factor of poor prognosis, good general condition should always be preferred, since it contributes to a lower infection rate under cytotoxic agents.

Echocardiography, cardiac catheterization, and coronary angiography can visualize PAN involvement in angina or cardiac insufficiency, and detect comorbid conditions, eg, treatable atherosclerosis. Clinicians should know that cardiac insufficiency can persist

beyond remission [46] and can be responsible for late death [47].

6.3 How Should PAN be Treated?

Nearly all the reports on the treatments and outcomes of PAN patients also included some with microscopic polyangiitis [22,48,49] and sometimes eosinophilic granulomatosis with polyangiitis (Churg–Strauss) [50]; thus, their results regrouped multiple distinct entities and cannot be considered the standard PAN treatment.

CS have been prescribed for 60 years to treat PAN. They increased the 5-year survival rate from 10% for untreated patients to about 55% in the mid-to-late 1970s [22,48,49]. For the most severely ill patients or those whose disease was refractory to CS, an immunosuppressant, either azathioprine or cyclophosphamide (CYC), was added to the treatment regimen [51], attaining a 5-year survival rate of 82% for CS and CYC recipients [48]. Because classification criteria have changed and the mean CS and CYC duration has been shortened over time, the therapeutic response could be different today. However, it is clear that treatment based on a strategy detailed below improved survival.

6.3.1 Corticosteroids

All PAN patients are prescribed CS (level C, class I). For HBV-related PAN, CS should be administered for only a few days (see below). For the other PAN forms, treatment lasts about a year, with initial high doses being potentially useful. Methylprednisolone pulses (usually 7.5–15 mg/kg IV over 60 min, repeated at 24-h intervals for 1–3 days) have become widely used at treatment onset for severe systemic vasculitis, especially when life-threatening organ involvement is present or during the extension phase of mononeuropathy multiplex. Pulse methylprednisolone side effects are usually mild and transient; they include bitter taste, facial flushing, headache, asthenia, sharp blood pressure rise, and temporary glucose intolerance. Oral CS (1 mg/kg/day of prednisone) is given in a single morning dose. As the patient's clinical status improves and the biological markers of inflammation (C-reactive protein, erythrocyte sedimentation rate) return to normal, usually within 3 weeks, prednisone-dose tapering can begin. We now recommend trying to reach 20 mg of prednisone at 3 months, 10 mg at 6 months, and 5 mg at 12 months.

6.3.2 Cyclophosphamide

Because of its low therapeutic/toxic index, oral CYC (2 mg/kg/day) to induce remission is recommended less frequently. Its adverse events have been thoroughly described. Major side effects associated with daily CYC intake include hemorrhagic cystitis, bladder cancer [52], bone-marrow suppression, ovarian failure, and

hematological malignancies [53]. Severe infections are a major cause of death of systemic necrotizing vasculitis patients, especially while they are on high CS doses with adjunctive immunosuppression [47,54].

Increasingly prescribed and perhaps preferable to oral CYC, IV pulse CYC now has a codified schedule: 0.6g/m² for the first three pulses infused at 2-week intervals, followed by 0.7g/m² for the following three pulses given at 3-week intervals (level C, class I). We recently demonstrated (level B) that, for patients over 65 years old, 500mg/pulses (total dose: 3g) were able to obtain full remissions while minimizing side effects [55]. Because high-dose IV CYC may be dangerous in patients with renal and cardiac insufficiency, reducing the dose according to renal function is advisable. Like for oral CYC, intense hydration is essential and combination with sodium 2-mercaptoethanesulfonate (mesna) administration may be useful. For patients with cardiac insufficiency, fluid overload is contraindicated. The response to remission-induction therapy is usually achieved in 3–4 months. Recently we showed that it was possible to effectively treat PAN in elderly patients (among others with systemic necrotizing vasculitides) with lower CS doses and a maximum dose of 500mg/pulse, with no more than six pulses. Side effects were less frequent than those observed under conventional therapy [55].

6.3.3 Other Cytotoxic Agents

Azathioprine, methotrexate, and several other cytotoxic agents have been tried in PAN patients (level C, class IIa). They are reserved for use when CYC contraindications are present or as maintenance therapy for the more severe PAN forms after CYC discontinuation; the recommended duration for their use is 12–18 months. Mycophenolate mofetil for maintenance is empirically prescribed, without the medicine-based evidence of large series or a prospective trial.

6.3.4 Biotherapies

Antitumor necrosis factor and anti-CD20 monoclonal antibodies are not effective against PAN. No clinical trial data support this statement but case reports have not indicated biotherapy efficacy. IV immunoglobulins can be effective in some patients with PAN. They have been successfully prescribed to patients with concomitant parvovirus infection [56,57] or idiopathic PAN with predominant skin disease [58]. IV immunoglobulins are more often prescribed for children, whose PAN criteria are not fully comparable to adult disease thereby explaining the different therapeutic approaches [59–61].

6.3.5 Plasma Exchanges

To date, no argument supports the systematic prescription of plasma exchanges when HBV-related PAN

is diagnosed [62]. However, they could be beneficial for sudden-onset mononeuritis multiplex with rapid progression or severe clinical manifestations. Plasma exchanges might be useful as second-line therapy of PAN refractory to conventional treatment and might be able to limit disease sequelae, as shown for severe renal disease in ANCA-associated vasculitides (level C, class IIb) [63].

6.3.6 Treatment Duration

Because PAN has a very low relapse rate [13], its treatment duration may be shorter than for other systemic necrotizing vasculitides. Indeed, remission-induction treatment often cures PAN, making maintenance therapy unnecessary. For PAN patients with FFS poor-prognosis factors, CS and CYC are given for about 4 months, followed by CS and a less toxic drug, eg, azathioprine or methotrexate.

6.3.7 Therapeutic Specificities of HBV-related PAN

For HBV-related PAN, the conventional CS-and-CYC regimen for vasculitis allows virus replication, which facilitates evolution toward chronic hepatitis and liver cirrhosis. Thus, CYC and prolonged CS are contraindicated. The preferred initial therapeutic strategy combines plasma exchanges, an antiviral drug and CS, to rapidly control the most severe life-threatening PAN manifestations common during the first weeks of the disease. CS is then abruptly withdrawn to enhance immunological clearance of HBV-infected hepatocytes and favor seroconversion from HBe-antigen-positivity to anti-HBe antibody-positivity (level B, class I/II).

Excellent overall therapeutic results were obtained by the adjunction of antiviral agents [64], and this approach should be preferred to conventional regimens that jeopardize the outcome. This strategy's efficacy was confirmed and new antiviral agents contribute markedly to treatment efficacy by increasing the seroconversion rate.

6.3.8 Cardiovascular Response to Treatment

A clinical response is obtained under treatment. Microaneurysms disappear and the healing process is responsible for aneurysm or vessel thrombosis.[16,65] Clinical manifestations regress and can disappear. However, visceral infarcts can lead to severe injury and sequelae, like renal or cardiac insufficiency. Long-term CS can also increase endothelial injury [66–68] and favor atherosclerosis that have long-term vascular consequences.

7. CONCLUSIONS

Cardiac involvement is one of PAN's most severe manifestations. However, it is a rare manifestation of PAN and is not the first cause of death of patients with

this vasculitis, unlike eosinophilic granulomatosis and polyangiitis (Churg–Strauss), an ANCA-associated vasculitis whose cardiac manifestations are the primary cause of mortality. Because PAN affects coronary arteries, myocardial ischemia can be responsible for myocardial infarction and cardiac insufficiency. The prognosis of cardiac manifestations is poor, as demonstrated by the FFS. Cardiac symptoms are one of the poor-prognosis factors accorded +1 point in the FFS. Cardiac manifestations usually occur during the first disease flare; their severity deserves induction therapy combining CS and CYC, except when concomitant HBV infection necessitates a specific therapeutic strategy. CYC is, at present, the best drug to combine with CS for induction. Other cytotoxic agents are less effective alternatives. Biotherapies do not seem to control PAN effectively and are not used as first-line agents or for flares. Treatment is effective and, if cardiac injury is not too extensive, the long-term prognosis can be favorable.

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Kawasaki Disease

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1. HISTORY

Kawasaki disease (KD) is named after Dr. Tomisaku Kawasaki who first reported the clinical entity in 1967. Beginning in 1961, Kawasaki identified Japanese infants and young children who manifested a distinctive constellation of signs that included prolonged high fever, unilateral cervical lymphadenopathy, bilateral conjunctival injection, polymorphous erythematous rash, changes of the mucosa of the mouth and lips, and edema and erythema of the extremities, with subsequent desquamation of the finger and toes. By the early 1970s, children were identified in Japan who died suddenly within 30 days of KD onset, with evidence of coronary aneurysms and acute myocardial infarction (MI) secondary to acute thrombosis. It became evident that some patients who recovered apparently uneventfully from this acute illness were at risk for sudden cardiac death. Further research showed findings of a severe coronary artery vasculitis process [10]. With the availability of echocardiography in the late 1970s, researchers determined that 20–25% of KD patients developed evidence of coronary artery abnormalities [11]. Prior to Dr. Kawasaki's definition of KD, there had been reports of fatal coronary arteritis in children (usually labeled infantile polyarteritis nodosa) published in non-Japanese pediatric and pathology literature. Patients with KD in previous decades were likely misdiagnosed as having measles, scarlet fever, rubella, or other once common conditions, with vaccine-related reductions in the numbers of cases of these illnesses helping to facilitate recognition of KD [12]. The clinical syndrome has also been previously known as mucocutaneous lymph node syndrome, lymphomucocutaneous syndrome, and infantile polyarteritis nodosa. In 2004, the American Heart Association Committee on rheumatic fever, bacterial

endocarditis, and KD published the first guidelines for the management of patients with incomplete KD [7].

2. EPIDEMIOLOGY

In the absence of a confirmatory diagnostic test, the epidemiologic case definitions of KD are relatively strict and exclude from surveillance data of other febrile exanthematous conditions that could dilute incidence reports of “true” cases. The incidence of KD varies throughout the world, with the highest being in Japan. Rates in Japan have climbed steadily, with an annual rate of 243.1 per 100,000 children younger than 5 years of age in 2011 (22nd National Survey) [13]. The highest reported rate in Korea was 118 per 100,000 children younger than 5 years old in 2007 [14]. In countries with predominantly non-Asian populations, the rate is approximately 15–20 per 100,000 children younger than 5 years of age [15–17]. Rates of KD can increase substantially during epidemics in all ethnic groups.

2.1 Race

The incidence of KD in white children is about 10-fold lower than in Asians. Children of Asian descent in predominantly non-Asian countries continue to experience high attack rates of KD. A study utilizing the Kids' Inpatient Database in 2006 reported that children in the United States of Asian and Pacific Islander descent had a rate of 30.3/100,000 compared to 12/100,000 in White children, 17.5/10,000 in Black children, and 15.7/100,000 in Hispanic children. This study identified approximately 5520 hospitalizations for treatment of KD in the year 2006 [16]. The higher rates of KD in those of

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Japanese and other Asian backgrounds suggest a genetic basis rather than environmental factors, as supported by increased rates among third- and fourth-generation immigrants from Japan to Hawaii [18].

2.2 Gender

In virtually all population-based studies in many countries, the ratio of male-to-female patients with KD is approximately 3:2 [17,19–21]. In addition, serious and fatal complications also are significantly more common findings among male patients with KD than female patients [17,22,23]. The basis for the preponderance of KD in male patients and for the even greater predominance of serious coronary artery disease in male patients with KD remains unclear.

2.3 Age

Kawasaki disease occurs almost exclusively in children. In the United States and Japan, adult cases are quite rare, although some reports of adults diagnosed by accepted diagnostic criteria have been published [24,25]. The distribution of KD by age in childhood is highly characteristic. The disease occurs most frequently in young children: about 50% are younger than 2 years of age, 80% are younger than 5 years of age, and cases are uncommon in those older than 12 years of age [20,26,27]. The features of 28 patients with KD who were aged 8 years and older at the time of diagnosis at our Chicago institution were reported [27]. Delays in establishing the diagnosis and in providing treatment were common in this series and were at least partially related to the prominence of arthritic and gastrointestinal symptoms in this population. In the United States age less than 1 and over 8 years are associated with increased risk of development of coronary abnormalities [17]. Earlier Japanese mortality data suggested that fatality rates are approximately three times higher in children younger than 1 year at the time of onset of KD, compared with older children, and that fatalities occur predominantly in the first several months after onset of KD [28].

2.4 Other Factors

Japanese investigators noted large nationwide outbreaks between 1979 and 1986 as well as more localized outbreaks of KD [22,29,30]. In the United States and elsewhere, community-wide outbreaks were documented starting in the late 1970s. No further nationwide Japanese outbreaks have been identified since 1986, suggesting that the epidemiology of KD may be evolving [31,32].

KD has been diagnosed throughout the United States and Japan and in virtually all developed and many

developing countries on all continents, including temperate and tropical zones, without a notable urban–rural or geographic pattern [33–40]. In Japan, KD occurs year around but is most prevalent in the winter, with peaks usually occurring in December or January, with a lower peak in June, and the lowest number of cases in October [23,41–43]. In the most recent national Japanese survey, the number of patients diagnosed was highest in the winter with a peak in January [13]. In the United States and other temperate areas, the number of cases peaks in the winter and early spring and is lowest in late summer; nonetheless, cases occur throughout the year [17,26,44–46].

Simultaneous or sequential cases of KD in siblings, twins, or other family contacts have also been reported, particularly during outbreaks in Japan. However, these figures are difficult to interpret due to potential recognition and reporting biases. Japanese epidemiologists have documented secondary sibling cases in approximately 1% of cases, a rate that is approximately 10 times greater than that in the general Japanese child population [47]. Secondary or coprimary cases in families occur but are not common. Little direct evidence of person-to-person spread of KD exists, although the presence of winter-to-spring predominance of cases, epidemics, age of typical onset, and possible protection by transplacental antibodies in the first 6 months of life are all consistent with a ubiquitous infectious microbial trigger.

Of note, the original epidemiologic case definitions were not intended for clinical application. Thus less strict application of clinical case criteria is appropriate for management of patients. Clinicians must be aware that children often present with clinical illnesses that do not completely fulfill the diagnostic criteria for KD but who are nonetheless at risk for developing coronary artery sequelae and therefore warrant therapy. These patients generally are considered to have incomplete KD [7,48–50] (refer to “Incomplete KD” section below).

3. ETIOLOGY

The origin of KD remains unknown, but clinical and epidemiologic features strongly suggest an infectious cause. An attractive hypothesis is that KD is caused by a ubiquitous, probably respiratory, infectious agent that produces clinically apparent disease only in selected, genetically predisposed individuals, with particular predilection for Asians. However, efforts to identify an infectious agent of KD using conventional bacterial and viral culture and serologic methods as well as animal inoculations have failed to yield a specific infectious cause [51–53]. Others have theorized that many diverse agents could trigger KD in susceptible hosts. Some researchers have proposed that KD is caused by a superantigen causing nonspecific activation and expansion of autoreactive

T cells [54,55]. While features of immune cell activation characterize the acute phase of KD, investigations indicate that the immune response in KD is oligoclonal (antigen driven, ie, a response to a conventional antigen) rather than polyclonal (as typical in superantigen-driven responses) [56,57]. The presence of IgA plasma cells, oligoclonal IgA response in arterial tissue, and cytoplasmic inclusion bodies in bronchial epithelial cells in KD patients suggests a respiratory tract viral pathogen [51,57–63]. Some researchers studying major KD epidemics have suggested that the potential environmental trigger or causative agent may be carried by large-scale wind currents [64]. Development of a useful diagnostic test likely needs to be based on the etiologic agent. The hope is that the use of modern molecular biology techniques will clarify the origin of KD and provide insights into the mechanisms of disease pathogenesis [53,59–62].

4. PATHOGENESIS

KD is a systemic vasculitis that primarily affects medium-sized arteries with a marked predilection for the coronary arteries [65–71]. Small arterioles, larger arteries, capillaries, and veins also are affected to a lesser extent [66,72]. In the acute stage of KD, systemic inflammatory

changes are also evident in many organs, including myocardium, pericardium, cardiac valves, cerebrospinal fluid, lung, lymph nodes, pancreas, spleen, joints, and liver [72,73]. The overall mortality rate in Japan has dropped from the original 2% to more recent Japanese estimates of approximately 0.01% [13]. Cardiac death in KD generally occurs in the subacute or convalescent stages of illness but also can occur earlier [22,74]. In the vast majority of cases, death is caused by acute thrombosis of inflamed coronary arteries, with resultant myocardial infarction, although inflammation of the myocardium and acute coronary rupture can also rarely occur. Deaths months to years after acute KD often are secondary to coronary stenosis and myocardial infarction.

Classic studies on the pathology of children with fatal KD led to a sequential model theory of vasculopathy with stages of inflammatory infiltrate and remodeling (Fig. 19.1). The initial neutrophilic infiltration of the coronary arteries in the first 1–2 weeks is followed by displacement of the neutrophils by mononuclear cells, and resolution of the inflammation within about 2 months of disease onset [65,67]. There was associated destruction of collagen and elastin and thus loss of the structural integrity of affected coronary arteries. This change in architecture led to aneurysm formation, disruption of blood flow,

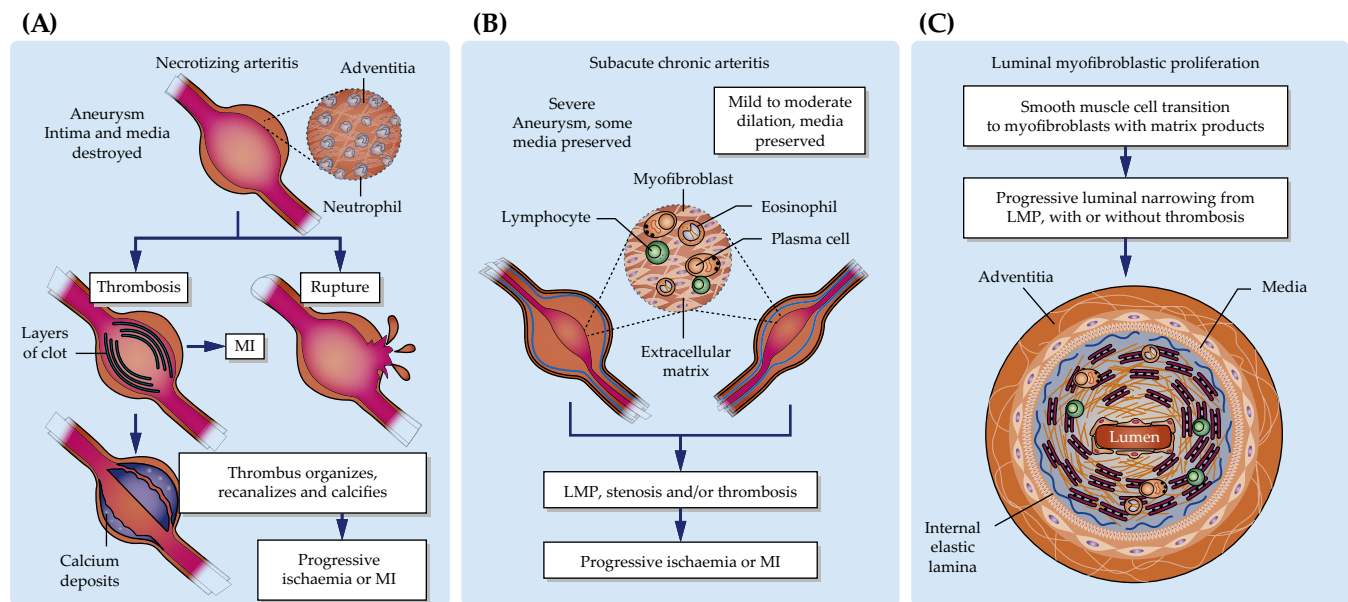


FIGURE 19.1 Three linked processes of KD arteriopathy are necrotizing arteritis, subacute chronic arteritis, and luminal myofibroblastic proliferation. Medium-sized muscular arteries, especially the coronary arteries, are predominantly affected. (A) Beginning at the luminal endothelium, neutrophil-mediated necrotizing arteritis involves necrosis of the intima, media, and part of the adventitia. Although necrotizing arteritis resolves within 2 weeks after fever onset, large saccular aneurysms may form. These can rupture, although this is rare. More commonly, these aneurysms fill with gradually formed thrombus, which can lead to myocardial infarction, while others may calcify or recanalize. Subacute chronic arteritis and luminal myofibroblastic proliferation (LMP) also begin in the first 3 weeks after fever onset, but can persist for months to years. (B) Subacute chronic arteritis begins in the adventitia and extends toward lumen but usually leaves some intact media. It involves the progressive formation of an infiltrate of lymphocytes, plasma cells, and eosinophils that can lead to fusiform arterial dilatation. (C) LMP is a unique process involving proliferation of medial smooth-muscle-cell-derived myofibroblasts and buildup of their matrix products. This may result in progressive arterial stenosis with or without thrombosis. Adapted from Shulman and Rowley [76].

and thrombus formation on the arterial luminal surfaces [65–67]. It was thought that these features resolve spontaneously within 1–2 years in most patients.

However, more recent studies of 41 KD autopsy specimens or explanted hearts by Orenstein et al. suggest that three linked pathologic processes, necrotizing arteritis, subacute chronic vasculitis, and luminal myofibroblastic proliferation, begin in the first 2 weeks after fever onset, but that only necrotizing arteritis with neutrophilic infiltration of the arterial wall leading to aneurysms is self-limited [75]. Subacute chronic vasculitis, which can affect all blood vessels, is associated with lymphocyte, plasma cell, and eosinophil infiltration. Luminal myofibroblastic proliferation is a unique but closely related process that involves smooth muscle cell-derived myofibroblasts that proliferate and form a concentric mass that can progressively obstruct the arterial lumen. The latter two processes can persist for months to years following KD onset [75].

Several autoantibodies have been suggested to be associated with KD pathogenesis. Antibodies to type III collagen have been detected, although without clear relation to coronary complications [77]. There are reports of IgM antimyosin antibodies [78], anti-endothelial cell antibodies [79–82], antibodies to anti-oxidative peroxiredoxin [83], anti- α -enolase antibody [84], IgM and IgA anticardiolipin antibodies [85], 4-trimethylaminobutyraldehyde dehydrogenase [86] and other autoantibodies in serum of KD patients that may provide insight into disease pathogenesis and/or serve as diagnostic tools in the future. Specific genetic factors likely contribute to disease pathology, as indicated by the much higher rates of KD in individuals of Japanese, Korean, and Chinese ethnicity and increased incidence of KD among siblings and parents of affected patients [47,87]. However, the genetic basis is complex and no single human leukocyte antigen (HLA) or MHC Class II gene is common to most patients with KD. Transmission disequilibrium studies have identified single nucleotide polymorphisms (SNPs) affecting function of molecules in pathways of calcineurin (ITPKC gene), TGF- β signaling, and CASP3, which are involved in immune cell apoptosis and T-cell differentiation. Genome-wide association studies have also identified polymorphisms in genes for CD40, B lymphoid tyrosine kinase, and notably FCGR2A that have been associated with increased susceptibility to KD in some populations [88–94]. Some of these SNP associations with risk of KD have been replicated in various Asian and Caucasian populations [88–91,95]. Understanding the genetic basis of KD could help identify at-risk individuals and potentially lead to further understanding of pathogenic mechanisms useful as new targets for treatment.

5. CLINICAL MANIFESTATIONS

5.1 Phases of Illness

The typical clinical presentation of KD can usually be divided into three phases: the acute, subacute, and late or convalescent phases [7,96]. KD begins with an *acute* febrile phase that lasts 7–14 days. Although this phase is self-limited, without therapy it can last from 7 or fewer days up to 30 days with a mean duration of 11 days. The clinical symptoms during this phase include high fever, polymorphous rash, nonexudative conjunctival injection, “strawberry” tongue, red swollen lips, edema, erythema of the hands and feet, and cervical lymphadenopathy (Table 19.1). This constellation of signs and symptoms forms the basis of the classic diagnostic criteria. However, it is important to note that the features may not all occur concurrently, and some may have already resolved prior to presentation [97]. Young children are often very irritable. This phase is sometimes associated with aseptic meningitis and mild hepatic dysfunction. During this phase, there may begin to be evidence of myocarditis and cardiac dysfunction that may be manifested as tachycardia even in the absence of fever. Although this phase rarely includes congestive heart failure or arrhythmias, other cardiac findings such as pericardial effusion, mitral regurgitation, or depressed myocardial function may be found on echocardiogram.

A relatively recently recognized, although rare, presentation of acute KD is hypotension or shock [98,99]. Approximately 2–3% of KD patients may require admission to an intensive care unit for suspected septic shock or toxic shock but are ultimately recognized to have KD features. Kawasaki Shock Syndrome (KDSS) is a rare form of KD presentation and has been defined as the presence of any of the following: systolic hypotension (<-2 SD blood pressure as defined for age and sex), a $>20\%$ decrease in systolic blood pressure from baseline, or clinical signs of poor perfusion with accompanying features of KD [99]. In the few small series of cases, patients presenting with shock are more likely to be female, older, and with associated consumptive coagulopathy and cardiac abnormalities. They have also

TABLE 19.1 Diagnostic Criteria for Kawasaki Disease

Fever for at least 5 days and

At least four of the following clinical findings:

- bilateral conjunctival injection
- mucous membrane changes (erythematous oral mucosa, red, chapped/fissured lips, and/or “strawberry tongue”)
- changes of the extremities (erythema and/or edema of hands and feet, periungual desquamation)
- polymorphous erythematous rash
- at least one cervical lymph node ≥ 1.5 cm in diameter

Exclusion of other possible diagnoses

been reported as more likely refractory to therapy compared to control KD patients, and thus at higher risk for developing coronary artery abnormalities [99–102]. In a case–control study by Chen et al. in 2015, KD patients presenting with shock were less likely to have a diagnosis of KD at admission (22.2% vs 66.7%) and had a higher risk of coronary artery dilatation (77.8% vs 11.1%) [103]. Lab studies in these patients reveal more dramatically elevated markers of inflammation and higher neutrophil counts as well as hyponatremia compared to more typical KD patients with normal blood pressure [103].

The *subacute phase* of KD begins after the resolution of fever. During the subacute phase, patients, although now afebrile, remain irritable and anorexic. Due to arthritis and arthralgias, primarily of the large joints, many of these patients still have decreased activity levels. Some of the physical findings of the acute phase may persist, particularly conjunctival injection. The rash that may have been present during the acute phase classically progresses to periungual desquamation on the fingers and toes. Thrombocytosis commonly manifests during this period.

The *convalescent phase* begins when all clinical signs and symptoms have disappeared and continues until all inflammatory markers are normal, which, even without treatment, usually occurs at about 6–8 weeks after the onset of fevers. Of note, the subacute and early convalescent phase are the periods during which the patient has the greatest risk of sudden death due to acute coronary artery thrombosis.

5.2 Differential Diagnosis

There are many illnesses that closely mimic the classic clinical features of KD and thus must be excluded before making a diagnosis of KD (Table 19.2). The most commonly encountered diseases to consider include viral illnesses, especially measles and adenovirus, acute

streptococcal and staphylococcal infections, and drug hypersensitivity reactions. Adenovirus in particular presents very similarly to KD with fever, rash, adenopathy, conjunctivitis, and mucous membrane changes. However, the conjunctivitis is more likely exudative with adenovirus than with KD [104]. Jaggi et al. demonstrated that molecular-based adenovirus detection is not uncommon with KD. As a result, the molecular adenovirus detection cannot be used to effectively rule out KD as this may represent concurrent infection versus reactivation or persistence of viral DNA due to a previous infection [105].

5.3 Clinical Criteria

KD has no definitive diagnostic test and thus the diagnosis of typical or classic KD is dependent on the presence of at least 5 days of fever plus four of the five clinical criteria and the exclusion of other illnesses that mimic KD. The five classic clinical features (Table 19.1 and described in detail below) include generalized polymorphous erythematous rash, nonexudative conjunctival injection, characteristic changes of the lips and tongue, characteristic changes of the hands and feet, and unilateral cervical lymph node enlargement greater than 1.5 cm [7]. Of the five classic clinical features, cervical lymphadenopathy is the least likely to be present and is only reported in approximately 50% of patients. On the other hand, conjunctival injection is fairly common and occurs in 80–90% of patients with KD [106]. All of these features are often not present at the same time, making it essential for clinicians to ask about features that may have resolved prior to presentation to medical care. The Japanese diagnostic guidelines for KD are similar to the US guidelines with the distinction that fever is considered a sixth clinical criteria and a patient must meet at least five of those six criteria for diagnosis [107]. In addition, a patient may be diagnosed with KD on or before the fifth day of fever with fewer than four of five classical clinical features if they have a coronary artery z-score (standard deviation from the mean normalized for body surface area of the internal diameters of the coronary arteries) greater than or equal to 2.5 [7].

Many children do not fulfill the classic clinical criteria for diagnosis of KD but may still have what is considered as “incomplete” KD. However, these patients are still at risk of developing the complications of KD, including coronary artery disease, and as a result, it is important that KD be considered in the differential for all children with at least 5 days of fever (refer to section on “Incomplete KD”) [7,50]. In some cases, if all of the typical clinical findings are present in a child with fever for less than 5 days, experienced clinicians can still make the diagnosis and initiate treatment [7]. Patients under 6 months of age are also more likely to present atypically [108].

One of the main clinical features of KD is *fever*. Typically in KD, a patient has high-spiking fevers with peak

TABLE 19.2 Differential Diagnosis of Kawasaki Disease

Infectious	Viral: Measles, Adenovirus, Enterovirus, Epstein–Barr virus
	Bacterial: Scarlet fever, cervical lymphadenitis, Rocky Mountain spotted Fever, leptospirosis
Toxin-mediated	Staphylococcal scalded skin syndrome
	Toxic shock syndrome (associated with <i>Staphylococcus aureus</i> or <i>Streptococcus</i>)
Hypersensitivity reactions	Drug hypersensitivity reactions
	Stevens–Johnson syndrome
Other	Systemic onset juvenile idiopathic arthritis
	Acrodynia (mercury toxicity)

temperatures generally exceeding 39°C (102°F), and frequently above 40°C (104°F). While the fever has no particular pattern, it occurs even with the use of antipyretic medications. Fevers usually persist for a mean of 11 days without therapy. However, in some patients, fevers have been reported to last up to 3–4 weeks. Once patients receive appropriate treatment with high-dose aspirin and a single 2g/kg dose of IVIG, fever generally resolves within 1–2 days of treatment [6,7,109,110]. Prolonged fever or recurrent fever after apparent resolution is associated with increased risk of coronary artery sequelae. In one study of 378 patients, those who remained febrile had an almost nine-fold increased risk of developing coronary artery (CA) abnormalities compared with those who responded to initial IVIG (12.2% vs 1.4%) [111]. Fever persists or returns within 48 h of completion of treatment with IVIG and aspirin in about 10–15% of patients with KD [19,111–113].

Although classically described as bilateral, non-exudative limbic sparing, bulbar *conjunctival injection* (Fig. 19.2), which occurs in 80–90% of patients with KD during the first week of illness, there are other key findings described [106]. Conjunctival biopsies of patients with KD showed prominent dilated vessels without evidence of edema or inflammatory cell infiltrate [114]. Due to the lack of edema of the conjunctivae, the white limbic area is easily visualized, giving a halo appearance around the cornea. The lack of exudate, conjunctival edema, or corneal ulcerations is important to distinguish the eye findings of KD from causes of purulent conjunctivitis, such as adenovirus, measles, and Stevens–Johnson syndrome [115]. If a slit-lamp examination is performed early on during the illness, many patients have mild acute iridocyclitis or anterior uveitis. If present, this usually resolves rapidly and completely and is not associated with photophobia or eye pain [114,116–119]. Other, even less common, ocular findings include superficial punctate keratitis, vitreous opacities, vitreous or chorioretinal inflammation, lateral rectus palsy, periorbital vasculitis, and papilledema [120–123].

Like the ocular findings, the *mucous membrane changes* (Fig. 19.3) seen with KD are varied and overlap with other illnesses. The typical changes of the lips seen in KD include erythema, dryness, cracking, and peeling. Sometimes fissures and bleeding occur. Patients with KD may have an erythematous tongue with prominent papilla, also referred to as a “strawberry tongue”. This occurs due to sloughing of the filiform papillae of the tongue so that the tongue appears glossy red with prominent fungiform papillae. While the “strawberry tongue” of KD is identical to the tongue findings in streptococcal scarlet fever or staphylococcal toxin-mediated diseases, this physical finding is not typically seen with viral infections. The third most frequent mucous membrane change in KD is diffuse erythema of the oropharyngeal mucosa. Oral ulcerations, pharyngeal exudates, and Koplik spots, if found on exam, likely indicate an alternate diagnosis than KD [104,115].



FIGURE 19.2 Nonexudative conjunctival injection in KD. Adapted from the personal collection of Dr. Stanford Shulman, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA.



FIGURE 19.3 Strawberry tongue and dry, chapped, fissured lips in a patient with KD. Adapted from the personal collection of Dr. Stanford Shulman, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA.

Extremity changes in KD are distinctive, common, and usually helpful features for ruling-out alternate diagnoses. During the acute phase of illness, the dorsum of the hands and feet become edematous. The palms and soles may become diffusely erythematous, often with an abrupt transition to normal skin at the wrist and ankle (Fig. 19.4) [7,106]. This blanching erythema is distinct from a patient's exanthem, if present. This sharply contrasts with scarlet fever, where the palms and soles are typically normal. Between 2 weeks and 2 months after the onset of fever during the subacute to convalescent phase, a unique pattern of periungual desquamation of fingers and toes may occur. In Dr. Kawasaki's original report of 50 Japanese patients, he noted periungual desquamation of the fingers and toes in all but one of his patients during the second week following the onset of fever [124]. The peeling in KD usually is usually a full-thickness epidermal desquamation that begins in the periungual area and may extend to involve the entire palms and soles [7].



FIGURE 19.4 Diffuse erythematous rash and plantar erythema in KD. Adapted from the personal collection of Dr. Stanford Shulman, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA.



FIGURE 19.5 Periungual desquamation in KD. Adapted from the personal collection of Dr. Stanford Shulman, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA.

This desquamation should prompt healthcare providers to consider the diagnosis of missed KD in a child with a history of a previous prolonged fever of unknown origin. Periungual desquamation (Fig. 19.5) occurs in ~50–70% of patients with KD and is distinctive from poststreptococcal diffuse desquamation of the palms and soles [125]. In one study of long term follow up of 259 cases of KD, 11% of children had episodes of recurrent skin peeling several years after their recovery from KD. These episodes were usually associated with an upper respiratory tract infection, but the etiology for these repeated episodes of peeling remains unclear. Repeeling was significantly less frequent in children who had a history of coronary artery dilatation and was more frequently seen in patients with nasal staphylococcal colonization [126]. Transverse grooves across the nails, called Beau's lines, may appear at the nail base 1–2 months after a case of acute KD, but grow out after a few months. Peripheral gangrene is a very rare complication [127].



FIGURE 19.6 Unilateral cervical lymphadenopathy in a patient with KD. Adapted from the personal collection of Dr. Stanford Shulman, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA.

The erythematous *exanthem* associated with KD is polymorphous and nonspecific. The most common rash is a diffuse, maculopapular, primarily truncal erythematous rash that may involve the face and extremities (Fig. 19.4). Occasionally the rash may present as a diffuse scarlatiniform erythroderma, urticarial or an erythema multiform-like rash with target lesions. This rash may be difficult to distinguish from a drug-reaction rash as many patients may have received antibiotics prior to presentation. Pustular rashes are much less likely to occur and vesicular or bullous rashes are not seen [128]. There is commonly an area of confluent erythema in the perineum and groin with early desquamation in the diaper area during the acute, febrile phase of KD [129–131]. Skin biopsies of the rash are not particularly useful as the histology shows nonspecific edema and dilated vessels. Of note, KD may trigger a psoriatic or eczematous rash in patients not previously diagnosed with psoriasis. These psoriatic or eczematous flares may be particularly severe and require consultation with a pediatric dermatologist [132–135].

Cervical lymphadenopathy is the least common of the five principal diagnostic criteria [7,106,136,137]. When present, lymphadenopathy is usually unilateral, transient, and found within the anterior cervical triangle (Fig. 19.6). To fulfill diagnostic criteria, the enlarged node

or mass of nodes must be nonfluctuant, nontender, or minimally tender without overlying skin erythema and at least 1.5 cm in diameter [7,115]. Due to inflammation of the sternocleidomastoid muscle, some patients may have limited rotation of their neck and present with torticollis. In some cases it may be the only dominant clinical feature besides fever, thus delaying diagnosis [138]. Thus it is important to consider KD in children first diagnosed with presumed acute bacterial lymphadenitis that are unresponsive to empiric antibiotic therapy [138,139]. The absence of suppurative changes in the lymph nodes on imaging in KD helps to differentiate these nodes from acute bacterial adenitis [140]. Similar to the other features of KD, cervical adenopathy generally resolves soon after the administration of appropriate therapy.

5.4 Additional Clinical Manifestations

In addition to the classic clinical criteria, the many other associated features of KD reflect its multisystemic nature. Many infants and young children with KD develop arthralgias or overt arthritis, often in the ankles or other lower extremity joints, which may present as refusal to bear weight or decreased activity level. Early-onset arthritis during the first week of illness can be polyarticular or oligoarticular, including both small interphalangeal joints as well as large weight-bearing joints [7,107,141]. This may mimic systemic juvenile idiopathic arthritis (JIA) [142,143]. The prevalence of arthritis varies from approximately 7.5% to 35% [141,144]. Late-onset arthritis after the 10th day of illness has a predilection for large weight-bearing joints, especially the knees and ankles, with a somewhat lower synovial fluid white blood cell count [141]. This form of arthritis may last for 6–8 weeks following the resolution of the acute phase of KD. This arthritis often responds well to treatment with a few weeks' course of a high-dose nonsteroidal anti-inflammatory medication, such as Naproxen 10–15 mg/kg/day divided into two or three doses per day [7,141,142,145]. Recently, there have been reports of arthritis developing in 2% of KD cases after defervescence following high-dose IVIG therapy [146]. MRI findings appear consistent with a nonerosive synovitis [147].

Central nervous system involvement including aseptic meningitis occurs in almost half of patients [148]. Irritability, while not a diagnostic criterion, is pronounced and very common. Because the irritability mimics that seen in bacterial or viral meningitis, some of this may be due to aseptic meningitis [7,148]. One study reported that 50% of patients were described as irritable in the 10 days prior to the diagnosis of KD, especially younger patients [149]. However, some patients with normal cerebrospinal fluid studies were markedly irritable, so this feature is unlikely solely due to aseptic meningitis [148,150]. Other neurologic findings including transient unilateral

lower motor neuron facial nerve palsy [151,152] and sensorineural hearing loss [153] occur rarely.

Gastrointestinal complaints, including nausea, abdominal pain, and diarrhea, occur in approximately one-third of patients, especially older patients [106,149]. These findings may be related to gallbladder hydrops, pancreatitis, or appendicular vasculitis [154,155]. Acalculous distention (hydrops) of the gallbladder manifests clinically as upper-right quadrant tenderness with or without obstructive jaundice, but rarely requires a cholecystectomy [7,156]. Obstructive jaundice due to cholestasis is also not uncommon [157,158], whereas mild-to-moderate elevations of serum transaminases occur in almost half of patients. Hepatic involvement appears to be entirely self-limited and has not been associated with chronic liver disease. Abdominal pain and diarrhea in the early acute stage usually respond to intravenous hydration and supportive care.

In addition to the findings mentioned thus far, there are other less common multisystem findings reported in patients with KD. Reactivation of inflammation with erythema and crusting or induration at a previous bacillus Calmette–Guérin (BCG) vaccination site is reported in children with acute KD [19,159–161]. In Taiwan, where the incidence of KD is relatively high and BCG vaccination is widely administered, erythema at the BCG vaccination site has been noted in 24–48% of children with KD [161]. Some of the variability in incidence of this inflammatory reaction may be related to the specific preparation of tuberculin skin test used [162,163]. Various pulmonary manifestations of KD have been reported, including isolated pneumonitis, pulmonary nodules [164], pleural effusions, acute respiratory distress syndrome [165], and pulmonary infiltrates [58,60]. Sterile pyuria as a manifestation of urethritis, occasionally with meatitis, is found in approximately half of patients. Patients with KD sometimes develop hemophagocytic syndrome (HPS), also known as macrophage activation syndrome (MAS), as a complication, but this is rare [166–168]. This syndrome manifests as persistent fever associated with cytopenias, hepatosplenomegaly, hepatic dysfunction, often hyperferritinemia, elevated serum lactate dehydrogenase, hypofibrinogenemia, and hypertriglyceridemia. Therapy with high-dose prednisone or other immune modifiers is indicated for this rare but serious complication. Consultation with a center that treats large numbers of patients with KD should be sought by the physician faced with rare or serious complications.

6. DIAGNOSIS

6.1 Laboratory Findings

A specific diagnostic test for KD is not currently available and its development likely depends on the ultimate discovery of the underlying causative agent of this

illness. Laboratory features of patients with KD, while very nonspecific, are nonetheless characteristic and thus may be useful in diagnosing patients with atypical clinical features. Leukocytosis, especially with neutrophilic predominance, is common in the acute stage, and the total white blood cell count exceeds $15,000/\text{mm}^3$ in approximately 50% of patients [7]. Leukopenia, on the other hand, is quite rare. Toxic granulations and Döhle bodies occasionally are seen on peripheral blood smear [169]. Normocytic anemia may develop, particularly in patients with more prolonged duration of inflammation. Severe hemolytic anemia requiring transfusions has been reported but is unusual and usually secondary to IVIG therapy [170–173]. Thrombocytosis is a very characteristic feature of the subacute phase of KD, with platelet counts ranging from 500,000 to more than $1,500,000/\text{mm}^3$, although average peak counts are approximately $700,000/\text{mm}^3$. While thrombocytosis is rarely present in the first week of illness, it begins to appear in the second week and peaks in the third week. As a result, after the seventh day of illness, many patients with KD have platelet counts higher than $450,000/\text{mm}^3$. Platelet counts gradually return to normal by 4–8 weeks after illness onset. In one study, infants younger than 1 year with fever without a source who had platelet counts greater than $800,000/\text{mm}^3$ were 17 times more likely to be diagnosed with KD than were infants with platelet counts lower than $800,000/\text{mm}^3$ [174]. There appears to be no difference in autologous platelet survival between cases and controls and minimal correlation between thrombocytosis and increased platelet aggregation [175,176]. Patients with acute KD rarely present with thrombocytopenia, which is likely due to a low-grade consumptive coagulopathy. These are often younger patients who are at increased risk for development of coronary artery disease and myocardial infarction [177,178].

As KD is a systemic inflammatory condition, it is not surprising that elevated acute-phase reactants, such as ESR and CRP, are nearly universal in KD. Characteristically, CRP values increase and fall much more quickly than ESR values. However, both should be measured during the acute phase because they may be discordant [179]. IVIG therapy leads to elevated ESR levels that last for several weeks even without ongoing inflammation. As a result, trending CRP levels rather than ESR levels is the best method for following the degree of inflammatory activity in patients with KD after they have received IVIG [7]. In the AHA guidelines for incomplete KD, a CRP $\geq 3.0\text{ mg/dL}$ and/or an ESR $\geq 40\text{ mm/h}$ is supportive of the diagnosis of KD [7]. Clinical experience suggests that KD is unlikely if all acute-phase inflammatory reactants (eg, ESR, CRP) and a platelet count are normal after the seventh day of illness.

Plasma lipids can be significantly altered in acute KD, an effect that is also seen in other inflammatory conditions. Kawasaki patients tend to exhibit decreased plasma cholesterol, high-density lipoprotein (HDL) cholesterol, and apolipoprotein A-I (apo A-I) levels [180–182]. However, these patients also have a markedly increased amount of serum amyloid A (SAA) protein in the plasma [180,181,183]. Cabana and colleagues showed that all of these lipid and plasma protein levels normalize over the course of several weeks, including the disappearance of SAA [180,181,183]. Hypoalbuminemia is another common finding and is associated with more severe disease and/or treatment failure [184]. The likely underlying mechanism for this finding is vascular leak secondary to microvascular inflammation causing increased permeability [185]. Mild-to-moderate elevations in serum transaminase levels are present in as many as 40% of patients. Mild hyperbilirubinemia occurs in approximately 10% [115]. Plasma γ -glutamyl transpeptidase levels are also elevated in most patients with KD [186]. Hyponatremia has also been very commonly reported with KD and is associated with more prolonged and more severe coronary artery disease [187–189]. While the underlying mechanism causing hyponatremia is not fully understood, current hypotheses include a syndrome of inappropriate antidiuretic hormone secretion, which may be related to underlying inflammation, hyponatremic dehydration and ingestion of hypo-osmolar fluids [188,190,191]. Urinalysis shows intermittent mild-to-moderate sterile pyuria in approximately one-third of patients, which is likely related to urethritis as suprapubic urinary specimens generally do not show pyuria [192,193]. In approximately 50% of children who undergo lumbar punctures, there is evidence of aseptic meningitis, with a predominance of mononuclear cells, normal glucose, and normal to mildly elevated protein levels [148].

The aforementioned laboratory findings, even though nonspecific, can thus provide diagnostic support in patients with clinical features that are suggestive, but not diagnostic, of classic KD [7]. In addition, some of these laboratory findings are important indicators of worse clinical outcomes or increased risk of unresponsiveness to IVIG therapy (see below) [189,194,195]. Despite the difficulties with diagnosing KD, it should be considered in the differential diagnosis for children with prolonged fever, rash, and nonpurulent conjunctivitis, especially in children <1 year old and in adolescents, in whom the diagnosis is frequently missed.

6.2 Incomplete Kawasaki Disease

Incomplete Kawasaki refers to children who present with prolonged fevers and some of the clinical features

of KD without completely fulfilling diagnostic criteria but who still may be at risk for the complications of KD, including the development of coronary artery aneurysms [7,48–50,196]. While some texts and physicians in the past have referred to these patients as having “atypical KD” the preferred terminology is incomplete KD. Unfortunately, incomplete KD occurs most frequently in young infants, who not only tend to have subtler, short-lived clinical manifestations, but are also the population at greatest risk of developing coronary artery disease with KD [50,108,197,198]. Although incomplete KD is difficult to diagnose since there is no single confirmatory diagnostic test, supportive laboratory results and/or echocardiographic findings can increase the likelihood of the presence of KD in a particular patient [7].

A committee of the American Heart Association (AHA) developed a valuable algorithm to assist in the evaluation of patients with suspected incomplete KD (Fig. 19.7). This algorithm includes a clinical assessment as well as measurement of acute-phase reactants (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]) in patients with 5 or more days of fever and 2 or 3 features of KD, or in infants 6 months old or younger with 7 days or more of fever without other explanation [7]. The laboratory findings in incomplete KD appear similar to classic KD cases and thus are useful for heightening or reducing the suspicion of KD. In addition to elevated ESR (>40 mm/h) and/or CRP (>3.0 mg/dL), a set of six supplementary laboratory criteria that can be useful in this regard is as follows: serum *albumin*, 3.0 g/dL or less; *anemia* for age; increased *alanine transaminase* (ALT); *platelets* after day 7 of more than 450,000/mm³; *white blood cell count* of 15,000/mm³ or more; and 10 or more white blood cells/high-power field in the *urine* [7,262]. While hyponatremia is not mentioned in this statement, it can also be considered a useful laboratory criterion when evaluating for incomplete KD. A retrospective study by Yellen et al. demonstrated that this AHA algorithm identified greater than 97% of patients at risk for developing coronary artery aneurysms [199].

Echocardiograms may be useful to evaluate patients with prolonged fevers and concern of KD. The presence of characteristic echocardiographic findings such as coronary artery dilation, decreased left ventricular contractility, mitral regurgitation, or pericardial effusions would support the diagnosis of KD. While echocardiographic evidence of perivascular brightness of coronary arteries was suggested as a criterion for early diagnosis of KD, a recent report did not confirm that this is a reliable finding to assist with the diagnosis of incomplete KD [200]. Although KD can be diagnosed retrospectively based on certain echocardiogram findings, ideally this algorithm can help identify patients with incomplete KD before coronary changes have occurred so they can receive appropriate therapy.

6.3 Diagnosis

The diagnosis of KD, in the absence of a diagnostic test, is thus based on the presence of characteristic clinical, laboratory, and echocardiographic findings. For the diagnosis of classic KD, a patient must have at least 5 days of fever plus 4 of the 5 classic clinical features. Patients with prolonged fever and only a few of the classic clinical features must thus be evaluated in the context of laboratory findings and additional evaluation for other illnesses that may mimic KD. Some studies have demonstrated the importance of echocardiographic screening in cases concerning for incomplete KD so as not to misdiagnose patients presenting without the classic clinical features, so the aforementioned algorithm may be used to determine which patients should have an echocardiogram [7,201–203]. Until a confirmatory diagnostic test is identified, patients with clinical features suggestive of incomplete KD should be referred to a clinical expert in KD for further evaluation and work-up.

7. CARDIOVASCULAR MANIFESTATIONS

By far, the most important associated feature of KD is cardiac involvement, which can be prominent in acute KD and is the major cause of long-term morbidity and mortality. KD is the leading cause of acquired heart disease in childhood in the United States and most other developed countries [1,2]. While the primary concern is the development of coronary artery disease, acute KD may also affect the pericardium, myocardium, endocardium, and cardiac valves. Clinical and auscultatory features may include a hyperdynamic precordium, tachycardia out of proportion for the child's age and temperature, a gallop rhythm, and a flow murmur [97]. Some infants may exhibit ECG changes, including ST-segment and T-wave changes, prolonged PR interval, and/or arrhythmias [97,204].

7.1 Coronary Artery Disease

The major complication of KD is coronary artery aneurysms (CAA), which when complicated by thrombosis or stenosis can lead to myocardial ischemia or even death. Coronary artery disease develops in 15–25% of children with untreated KD and in less than 5% of patients who receive appropriate therapy with high-dose IVIG [97,205–209]. CAA are described as small (<5 mm in diameter or z-score <5), large (5–8 mm in diameter or z-score >5 to <10) or giant (>8 mm in diameter or z-score >10) and may be classified as saccular, fusiform, or ectatic (diffusely dilated without segmental aneurysm) [7,210]. Aneurysms are often found in the more proximal segments of the coronary arteries or at

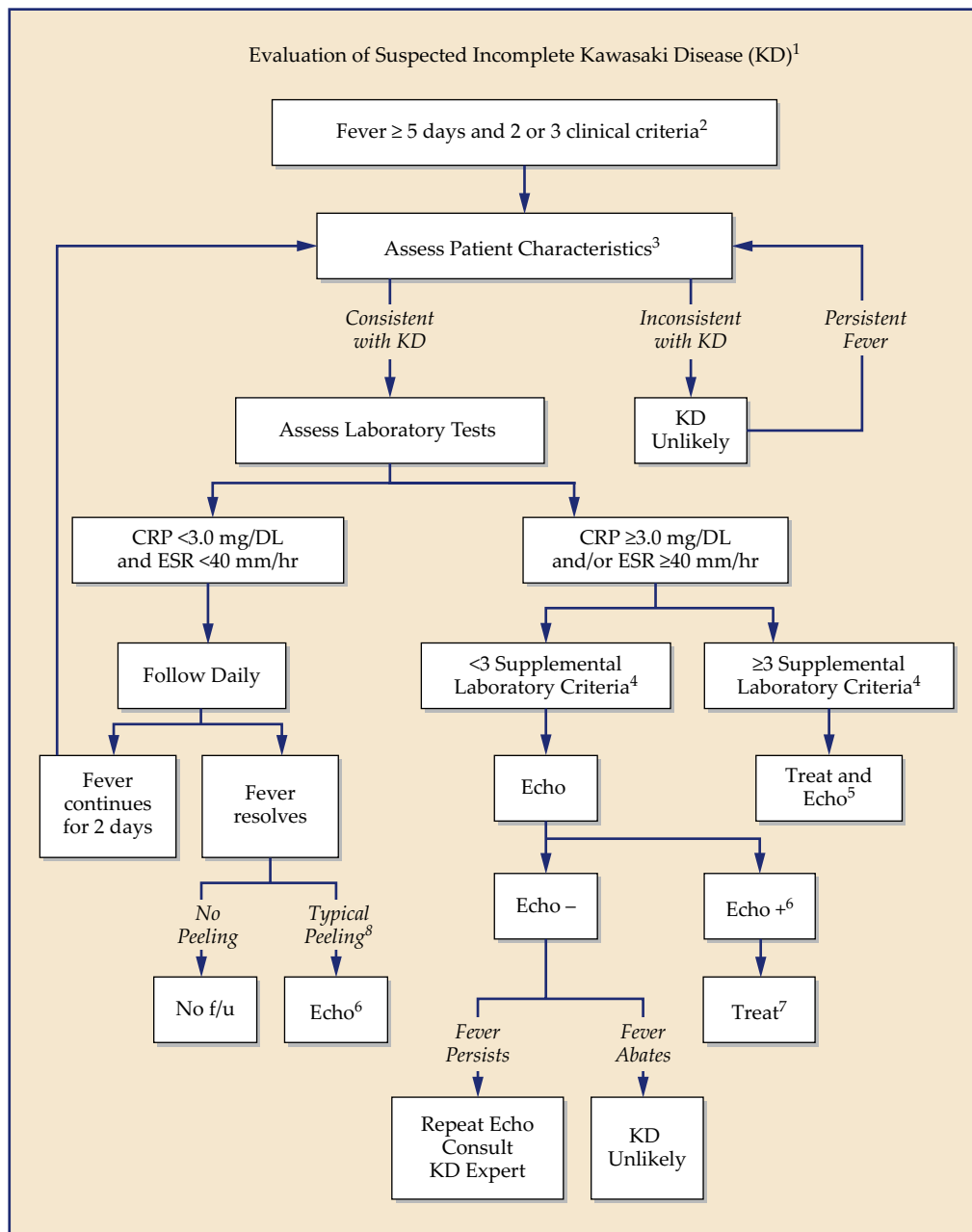


FIGURE 19.7 Evaluation of suspected incomplete KD. (1) In the absence of a gold standard for diagnosis, this algorithm cannot be evidence-based but rather represents the informed opinion of the expert committee. Consultation with an expert should be sought anytime assistance is needed. (2) Infants ≤ 6 months old on day ≥ 7 of fever without other explanation should undergo laboratory testing and, if evidence of systemic inflammation is found, an echocardiogram, even if the infants have no clinical criteria. (3) Patient characteristics suggesting KD are listed in Table 19.1. Characteristics suggesting disease other than KD include exudative conjunctivitis, exudative pharyngitis, discrete intraoral lesions, bullous or vesicular rash, or generalized adenopathy. Consider alternative diagnoses (see Table 19.2). (4) Supplemental laboratory criteria include albumin ≤ 3.0 g/dL, anemia for age, elevation of alanine aminotransferase, platelets after 7 d $\geq 450,000/\text{mm}^3$, white blood cell count $\geq 15,000/\text{mm}^3$, and urine ≥ 10 white blood cells/high-power field. (5) Can treat before performing echocardiogram. (6) Echocardiogram is considered positive for the purpose of this algorithm if any of three conditions are met: z score of LAD or RCA ≥ 2.5 , coronary arteries meet Japanese Ministry of Health criteria for aneurysms, or ≥ 3 other suggestive features exist, including perivascular brightness, lack of tapering, decreased LV function, mitral regurgitation, pericardial effusion, or z-scores in LAD or RCA of 2–2.5. (7) If the echocardiogram is positive, treatment should be given to children within 10 d of fever onset and those beyond day 10 with clinical and laboratory signs (CRP, ESR) of ongoing inflammation. (8) Typical peeling begins under nail bed of fingers and then toes in the second or third week of illness. Adapted from Newburger et al. [7].

bifurcations [211]. The proximal left anterior descending and proximal right coronary arteries are the most common location for aneurysms [212]. The echocardiographic or angiographic appearance of spontaneous resolution may occur in ~50% of CAA, especially with small aneurysms. However, multiple studies have demonstrated persistent structural damage to the vascular walls [206,213,214]. Giant aneurysms, which rarely regress, are associated with a higher risk of rupture, thrombosis, and development of stenosis over time, all of which may lead to myocardial infarction or death [205,215,216]. Patients with significant coronary artery disease during the convalescent and late phases of KD often remain asymptomatic, although it is important to be vigilant for signs of myocardial ischemia.

Several scoring systems have been developed to determine clinical and laboratory features that indicate increased risk for CAA. These include young or older age, especially <1 year of age or >8 years of age, male sex, prolonged fever, and delayed diagnosis and treatment [97,217–219]. Infants <6 months are at particularly high risk of CAA [197]. These young infants often present with fewer complete KD features and thus treatment is often delayed [220–222]. Late diagnosis and delayed treatment with IVIG appears to be the most important modifiable risk factor for CAA [223]. Laboratory findings associated with increased risk for CAA include leukocytosis, thrombocytopenia, anemia, hyponatremia (<135), and hypoalbuminemia [217,218,224]. Regardless, multiple studies have demonstrated that IVIG administration within the first 10 days of illness markedly reduces the prevalence of CAA [3,6,7].

7.2 Myocardial Infarction

Myocardial infarction is the most common cause of death in KD. In a Japanese study of 195 cases, myocardial infarction usually occurred in the first year after onset of disease, although one-fourth of patients had a myocardial infarction more than a year after resolution of the acute phase of KD. The first myocardial infarction was fatal in 22% of patients and asymptomatic in 37% [225]. Of those patients who survived a first infarct, 16% had a second myocardial infarct [225]. However, another study of 60 patients with myocardial infarction due to KD found a 30-year survival rate of 63% with worse outcomes occurring in patients with poor postinfarction left ventricular ejection fractions (LVEF) [226]. Unfortunately, chest pain complaints with myocardial infarction primarily occur only in older children. As a result, patients with a history of KD and significant coronary abnormalities should be evaluated immediately if they develop dyspnea, lethargy, inconsolable crying, significant abdominal pain, or vomiting without clear viral syndrome, diaphoresis, or syncope [225]. Patients with giant

(>8 mm or $z > 10$) coronary aneurysms are at greatest risk for having infarcts, particularly related to thrombi and/or to stenotic areas adjacent to a giant aneurysm. Most fatal infarctions are the result of obstruction of the left main coronary artery or both the right coronary and left anterior descending coronary arteries. Approximately half of the KD survivors of acute myocardial infarction had one or more complications, including ventricular dysfunction, mitral regurgitation, and arrhythmias [225]. As with adults, when diagnosed with an acute coronary thrombosis, prompt fibrinolytic therapy should be attempted at a tertiary care center [7,227], but the degree of reversibility of coronary thrombosis in children with KD may be less than that in adults with atherosclerotic disease. Late cardiac sequelae of KD sometimes do not manifest until adulthood [228].

7.3 Other Cardiovascular Complications

In addition to the coronary artery abnormalities that may develop, myocarditis is one of the most important clinical features of KD. Myocarditis is evident clinically in 50–70% of children during the acute phase of KD [7,205]. While overt evidence of heart failure is rare [229], myocardial inflammation is almost universally present on biopsy [230]. In addition to myocarditis and coronary artery abnormalities, pericardial effusions, diastolic dysfunction, or arrhythmias may occur [7,231,232]. In a study of 198 patients with KD, baseline echocardiograms at the time of diagnosis demonstrated left ventricular dysfunction in 20%, although this generally improved rapidly after IVIG administration [233,234]. Pericarditis with pericardial effusions is reported in about 25% of patients in the acute phase [235]. Patients rarely develop pericardial tamponade, though this has been a reported complication in KD patients with a ruptured coronary aneurysm [236–238]. Although death due to rupture of a coronary artery aneurysm is extremely rare, an autopsy study showed that coronary artery aneurysms rupture more commonly occurred among older children who died of KD within the first months after disease onset, whereas myocardial infarction tended to be seen more commonly in younger fatal cases and later after onset of KD [60,215].

Valvular disease, in particular mild mitral regurgitation, is also commonly reported in up to one-fourth of patients [233,239,240]. Although the exact etiology is unclear, it may result from papillary muscle dysfunction, rupture of the chordae tendinae, myocardial infarct, or valvulitis [206,215,235]. Mild aortic root dilation with associated aortic regurgitation, while less common, is also reported in as many as 1% of KD patients in the acute phase and may persist for up to a year [233,241]. In the pre-IVIG era, echocardiographic evidence of mitral regurgitation, impaired LV function, and pericardial

effusion in the acute stage was shown to be predictive of subsequent coronary abnormalities [242]. At least one patient with KD developed severe aortic and mitral regurgitation that necessitated double-valve replacement [243].

Peripheral artery aneurysms develop in up to 1% to 2% of patients with KD and occur almost always in patients who also have significant coronary abnormalities or in patients that were not treated with IVIG [205,215,244]. These abnormalities generally involve medium-sized muscular arteries, such as subclavian, brachial, axillary, iliac, or femoral arteries, but may occasionally involve the hepatic or renal arteries or the abdominal aorta [245]. These aneurysms also have a tendency to regress. Although the vasculitis of KD generally spares visceral vessels, there are rare case reports of KD presenting with acute encephalopathy [246], stroke [247], or acute surgical abdomen potentially related to vascular insufficiency [155].

7.4 Peripheral Gangrene

A rare but very serious complication in the acute febrile stage of KD is severe peripheral ischemia and dry gangrene of distal extremities, which has sometimes led to amputations [127]. Virtually all of these patients have been young infants up to approximately 7 months of age with giant coronary aneurysms, and some have developed peripheral arterial aneurysms as well. Interestingly, this complication is virtually unknown in Japan and has been reported primarily in non-Asian children in North America [127,205,215,248]. Possible pathogenic mechanisms of peripheral gangrene include the following: severe arteritis of digital or other small peripheral arteries; arteriospasm of peripheral arteries, perhaps in association with severe vasculitis; thrombosis of inflamed or spastic arteries as a result of stasis and damaged endothelium; thrombosis of a more proximal aneurysm (especially axillary) with embolism distally; rarely, cardiogenic shock; and, most likely, a combination of these factors [249]. Due to the unclear mechanism of disease, therapy is empiric and varied and has included aggressive use of anti-inflammatory agents, prostaglandin infusion, and antiplatelet, anticoagulant, and vasodilation therapies [127,248].

7.5 Cardiac Imaging

Early cardiac imaging is essential in all patients with possible KD to help confirm the diagnosis, to evaluate for potential cardiac complications, to determine baseline coronary artery measurements as well as to ensure adequate treatment and follow-up. Echocardiography remains the technique of choice for initial cardiac evaluation as it is noninvasive, widely available, and does

not require radiation. When conducted by experienced technicians and with adequate images, echocardiography has high sensitivity and specificity for detecting proximal coronary artery abnormalities as well as other cardiac complications, such as pericardial effusions and myocardial or valvular dysfunction [97,205,250,251]. Sedation is often required in younger infants to obtain optimal images. In addition to standard imaging with parasternal, apical, subcostal, and suprasternal notch windows, 2D evaluation of patients with suspected KD should also involve imaging the left anterior descending coronary, right coronary artery, and left circumflex coronary artery as well as posterior descending coronary arteries. This may require multiple imaging planes and transducer positions to achieve optimal images. In addition to measuring the size of the coronary arteries, the overall appearance of the arteries should be noted as well as the number, location, size, and type of aneurysms, if present. Echocardiograms are also useful for identifying other cardiac complications of KD such as pericardial effusions, myocarditis, impaired myocardial contractility, or valvular dysfunction and regurgitation.

Echocardiograms are the most commonly used imaging modality for collecting baseline and follow-up coronary artery measurements and assessing for aneurysm formation. Standard practice involves taking full measurements of the left main coronary and LAD and RCA internal luminal diameters. Originally coronary artery sizes were classified based on Japanese Ministry of Health criteria, which classify coronary arteries as abnormal if the internal lumen diameter is >3 mm in children <5 years old or >4 mm in children ≥ 5 years old; or if the internal diameter of a segment is ≥ 1.5 times that of an adjacent segment or if the coronary lumen is clearly irregular [7]. However, the current standard system of measurement involves using body surface area-adjusted coronary dimensions (z-scores) that appear to be more accurate [210,252]. Based on this new classification system, a z-score <2 is considered normal and any z-score greater than 2.5 is considered abnormal. Manlhoit and colleagues defined small aneurysms as z-scores of 2.5 to <5.0 , large aneurysms as z-scores of >5.0 to <10.0 , and giant aneurysms as z-scores of >10.0 [210]. Based on absolute internal dimensions, small aneurysms are defined as having an internal dimension between 2.5 and <5 mm, medium aneurysms between 5 and <8 mm, and giant aneurysms are ≥ 8 mm. The proximal left anterior descending artery (LAD) and the right coronary artery (RCA) are most commonly affected by CAA. z-scores are only available for the LMCA, proximal LAD, and proximal RCA. In most children, if the coronary artery dimensions are normal in the subacute period, it is highly unlikely that the child will subsequently develop dilation of the coronary arteries. Regardless, echocardiograms should be obtained at diagnosis, 1–2 weeks

following the first echocardiogram, and 6–8 weeks following discharge from the hospital. However, patients with abnormal baseline echocardiograms, especially with severe disease, or patients with recurrent fevers or KD symptoms may require repeat echocardiograms earlier and more frequently. However, echocardiography may have some technical limitations, such as inadequate acoustic windows, difficulty delineating distal coronary artery segments, and low-sensitivity for intraluminal thrombus or stenosis. As a result, other techniques such as angiography, computed tomography (CT), and magnetic resonance (MRI) may be required for further evaluation of coronary lesions.

Selective coronary angiography is the “gold standard” for assessing coronary abnormalities, but its clinical utility is limited by its invasiveness, high cost, and risk of complications such as hematomas, arterial dissection, vascular rupture, arrhythmias, or even death [253]. CT and MRI are useful alternatives in some patients for further evaluation of coronary lesions (Fig. 19.8). Several studies have shown that coronary CT angiography has a high negative predictive value for assessment of coronary disease, ranging from 95% to 100%. In addition, coronary CT angiography is fast, widely available, and enables improved assessment of all coronary segments, including distal regions, to more accurately exclude CAA or thrombi [254–257]. In patients with

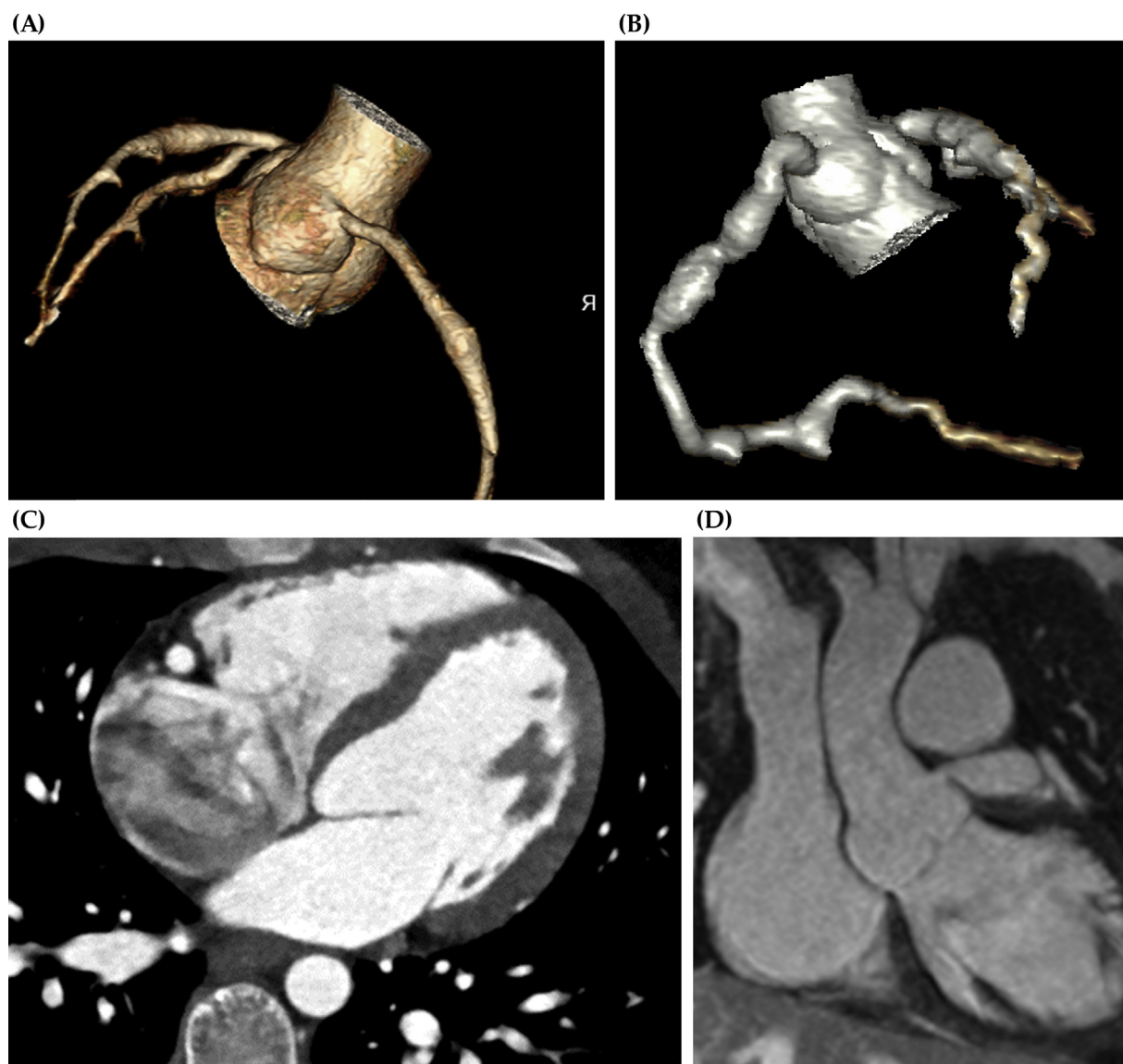


FIGURE 19.8 (A) 3D contrast-enhanced CT reconstruction showing severe dilation of the proximal to midright coronary artery and moderate dilation of the proximal to midleft anterior descending coronary artery. (B) 3D contrast-enhanced CT reconstruction showing diffusely dilated right and left coronary arterial trees containing multiple fusiform and saccular aneurysms. (C) Axial contrast-enhanced CT image showing severe dilation of the midright coronary artery in the right atrioventricular groove. (D) Coronal contrast-enhanced MR image showing a giant aneurysm involving the left main coronary artery and extending into the proximal left anterior descending coronary artery. Adapted from the personal collection of Dr. Cynthia Rigsby, Northwestern University Feinberg School of Medicine, Ann & Robert H. Lurie Children’s Hospital of Chicago, Chicago, IL, USA.

KD undergoing coronary arteriography, it is often recommended to do concurrent abdominal aortography and subclavian arteriography [7]. However, even with techniques to reduce radiation exposure to less than 1 mSv, the dose of radiation with this study remains a potential concern [258,259].

Magnetic coronary angiography is a desirable non-invasive technique due to its lack of ionizing radiation and its ability to assess for both coronary and noncoronary abnormalities. Multiple studies have shown near 100% consistency between MR coronary angiography and selective coronary angiography for diagnosing CAA in patients with KD, especially coronary artery luminal diameters and aneurysm length measurements [260–262]. While MRI is less precise in the detection of stenotic lesions compared to multidetector CT angiography or coronary catheterization [262], it is useful for detecting intraluminal thrombi [263]. MRI is also beneficial as it can assess better for LV systolic function and thus evaluate for evidence of myocarditis, myocardial infarction, or pericardial inflammation or even peripheral artery aneurysms [250,264,265].

8. TREATMENT

8.1 Acute Phase—Standard Therapy

Treatment of KD in the acute phase remains mainly empirical [4,7,266]. Patients with KD should be admitted to the hospital and receive IVIG in a single 2g/kg dose over 10–12h with high-dose aspirin at 80–100mg/kg/day in four divided doses (Table 19.3, class I recommendation) [7,96]. When administered by the 10th day of illness (defined as day of fever onset), this regimen is highly effective in reducing the development of coronary abnormalities [109]. Patients diagnosed after the 10th day of illness and who are still febrile may benefit from therapy, but the ability to prevent coronary changes is less certain. IVIG and aspirin prevent the development of giant coronary aneurysms, yet the precise mechanism of action of IVIG in KD remains unknown [267,268]. Single or multiple infusions of IVIG at

doses less than 2g/kg are not as effective as the 2g/kg dose [9,269,270] (evidence level A). A study that randomized US patients to receive salicylates at 80–100mg/kg/day or at 3–5mg/kg/day for initial therapy (each regimen with 2g/kg of IVIG) found no difference in coronary outcome, but a more prompt clinical anti-inflammatory benefit was noted in the high-dose aspirin group [271] (evidence level B). Of note, IVIG is occasionally associated with aseptic meningitis, which occurs 12–24h after infusion and is self-limited. High-dose aspirin is used for its anti-inflammatory activity, whereas the much lower dose inhibits platelet aggregation. In the absence of IVIG, aspirin therapy does not decrease the frequency of coronary abnormalities [209].

Patients should generally remain hospitalized until afebrile for at least 24h, as 10–20% may need retreatment (see below). Practices regarding the duration of high-dose aspirin vary across institutions, many reducing aspirin dose after 48–72h afebrile and others continuing until day 14 of illness and >48h after fever cessation. When high-dose aspirin is discontinued, low-dose aspirin (3–5mg/kg/day) is administered until there is no echocardiographic evidence of coronary artery changes for 2 months after the onset of illness (evidence level C, class I recommendation). Patients beyond the 10th to 12th illness day who have become afebrile and resolved their clinical features of KD without therapy (including normal CRP) and do not have coronary artery abnormalities are unlikely to benefit from IVIG (evidence level C). Such children should be treated instead with low-dose aspirin and be evaluated by serial echocardiograms. Reye syndrome has been reported rarely in children taking high-dose aspirin for a prolonged period after KD, but there are few data to suggest that low-dose aspirin poses this risk [272]. To reduce the theoretical risk of Reye syndrome in patients receiving low-dose aspirin who develop influenza or varicella illness, clopidogrel at 1 mg/kg per day can be substituted for aspirin (class IIa recommendation). Clopidogrel can also be used in the occasional patient allergic to or intolerant of aspirin.

8.2 Acute Phase—Adjunctive Therapy

The most studied choice for adjunctive initial KD therapy is a corticosteroid, which should be considered in patients at particularly high-risk for coronary complications (class IIa recommendation). The potential value of adding corticosteroid therapy to IVIG and aspirin for primary therapy has been addressed in several trials [5,273–275]. A controlled US trial studied addition of single-dose, high-dose intravenous methylprednisone to standard therapy and found little if any benefit [5]. Multiple Japanese studies, including the more recent Randomized controlled trial to Assess Immunoglobulin plus Steroid Efficacy for Kawasaki Disease (RAISE study) in 2012, found that patients predicted to have high-risk of nonresponse to standard therapy and at higher risk of developing coronary artery

TABLE 19.3 Treatment for Kawasaki Disease in Acute and Subacute Stages

IVIG 2g/kg infusion over 10–12h

Plus

Aspirin, 80–100mg/kg/day in four divided doses (until 14th illness day and patient afebrile at least 2–3 days), then 3–5mg/kg once daily for 6–8 weeks.

IVIG may be repeated if fever persists or recurs together with at least one classic sign of KD and/or elevated CRP level (see text for other alternative “rescue therapies”).

IVIG, intravenous immunoglobulin.

abnormalities based on a clinical score had improved outcomes with adjunctive corticosteroids [275]. The high-risk group in the RAISE study that received IVIG and aspirin with intravenous prednisone (5 days) and then oral prednisone followed by a 15-day taper after CRP normalization had lower coronary artery diameters, quicker resolution of fever, fewer cases necessitating retreatment, and lower nonresponse rates at 4 weeks after disease onset. Unfortunately, the Japanese scoring systems to identify high-risk patients are not useful in ethnically diverse populations as in the United States and it is thus more difficult to target adjunctive therapy to high-risk patients outside of Japan [276,277].

Infliximab, a monoclonal anti-TNF- α antibody, has been studied in a double-blinded, placebo-controlled randomized trial of adjunctive therapy. All patients with KD (not risk stratified) were randomized to receive standard IVIG and aspirin with or without a single 5 mg/kg dose of infliximab. Results showed no significant difference in response to treatment nor in the proportion of patients with coronary artery abnormalities [278].

8.3 Rescue Therapy for Refractory Kawasaki Disease

Most patients with acute KD respond promptly to treatment with IVIG and aspirin, with defervescence and subsidence of inflammatory manifestations within 48 h [6,8,180]. Approximately 10–20% of patients, however, fail to show significant clinical response to IVIG. This may be manifested as persistent or recurrent fevers at least 36 h after receiving IVIG and/or persistently elevated CRP levels. The CRP level should decrease by approximately 50% per day following successful IVIG therapy. Of note, ESR is an unreliable measure of inflammation following IVIG administration. This subpopulation of patients is at increased risk of coronary complications [7]. In these patients, administration of a second dose of 2 g/kg of IVIG generally is effective in suppressing disease activity [7,279,280] (evidence level B, class I recommendation).

The 2–4% of KD patients who do not respond to a second IVIG dose are considered to have refractory disease [281]. There are several treatment options for refractory patients, but no comparative data to determine the optimal regimen (evidence level C). Options include a third 2 g/kg IVIG dose or high-dose pulsed intravenous methylprednisolone (typically 30 mg/kg/day for 3 days) (class IIa recommendation). In some refractory patients, a slow oral steroid taper over several weeks to months once inflammatory activity appears controlled has been used. Infliximab as a single 5 mg/kg infusion was reported in an open-label experience to be effective in most refractory acute KD patients [282,283]. Additional options in the few patients who do not respond to the above include plasma exchange, single low-dose methotrexate, and cyclosporin (2–3 week

course with dose adjustments based on serum levels). Because controlled data are lacking, the relative roles of these treatment options for patients (class IIb recommendation) with refractory KD remain uncertain [284].

More recently, new scoring systems, such as the Japanese Kobayashi scoring system, were developed to identify patients at initial presentation at high-risk not only for developing coronary abnormalities but also for IVIG treatment failure who thus might benefit from more aggressive therapy [194,275]. High-risk features include age less than 12 months and greater than 7 years, male gender, prolonged or recurrent fever, anemia, hypoalbuminemia, thrombocytopenia, CRP greater than 8–10 mg/dL, hyponatremia, elevated AST, and neutrophil predominance [113,194,285]. The Kobayashi score yields 86% sensitivity and 67% to 68% specificity for IVIG unresponsiveness among Japanese patient populations [194,275]. However, the Kobayashi scoring system has not been effective in predicting treatment failure or increased risk for coronary disease in multiracial populations as demonstrated by studies in the United States [194,276]. Another study in the UK also demonstrated that the Kobayashi scoring system did not successfully predict IVIG resistance and or coronary artery abnormalities for UK patients [286].

8.4 Management After the Acute Phase

KD typically is an acute and self-limited illness. However, earlier cardiac abnormalities may be progressive, and prognosis is related to the coronary artery status.

Children with KD without initial cardiac sequelae return to their previous states of health. Patients should be re-evaluated within 2 weeks and 6–8 weeks after onset of illness because echocardiography at these time points is most likely to detect coronary aneurysms should they develop. If baseline study and these two follow-up echocardiograms fail to detect coronary abnormalities, performing further echocardiograms is likely not indicated [287], although a 6–12 month follow-up echocardiogram is performed at many centers [7,288]. Low-dose aspirin (3–5 mg/kg/day) can be discontinued after the 6–8 week follow-up echocardiogram unless evidence of coronary abnormalities is present (class I recommendation).

The management of coronary disease in KD patients depends on the severity and extent of coronary involvement. With few prospective data and randomized studies to choose optimal regimens, recommendations are based on known pathophysiology, case series, and extrapolation from management of adult coronary disease. Therapy may include antiplatelet therapy and/or anticoagulant therapy.

8.5 Long-Term Management of Cardiac Complications

Regression of small and medium aneurysms as seen on echocardiogram is common. Approximately half of

all coronary aneurysms at 2–8 weeks after onset demonstrate regression by 1–2 years, with apparently normal angiographic or echocardiographic appearance [231,289]. Aneurysm regression is more likely with smaller aneurysms, age less than 1 year at KD onset, fusiform rather than saccular morphology, and involvement of distal rather than proximal coronary segment [290,291].

The risk of coronary thrombosis or stenosis resulting in myocardial ischemia and infarction remains the most important long-term clinical problem in KD patients who develop significant coronary abnormalities. Patients who develop moderate to severe coronary abnormalities are at risk of myocardial ischemia, myocardial infarction, and even sudden death for many years after onset of illness, into adulthood [225,292]. Patients with medium or large aneurysms are at substantial risk for development of stenosis years after having the acute illness, compared with patients with small aneurysms or no aneurysmal changes [215,231,244,289,290,293,294]. Although the exact etiology is unclear, stenosis with resultant myocardial ischemia likely occurs in these patients due to calcification, myofibroblastic luminal proliferation, and/or thrombosis of the markedly abnormal vessels [215,216]. In 10- to 20-year follow-up studies the right main and left anterior descending coronary arteries are the most likely to develop stenosis [215,216,293,295,296]. Apart from myocardial infarction, patients who have severe coronary disease may have angina pectoris, mitral regurgitation, arrhythmias, and congestive heart failure.

Antiplatelet agents are important at each stage and severity of illness. Low-dose aspirin alone is sufficient for mild, stable disease. As the severity of coronary dilation increases, therapy favors a combination of low-dose aspirin with other antiplatelet agents, most commonly clopidogrel (evidence level C). When coronary disease is rapidly worsening or giant aneurysm is present, the risk of thrombosis is particularly high. Heparin with low-dose aspirin is recommended in the acute phase, and warfarin or low molecular weight heparin with low-dose aspirin for longer-term management.

In treatment of acute coronary thrombosis and occlusion in KD, the goals are to re-establish coronary patency and salvage the myocardium to improve survival. Most recommendations are derived from adults with acute coronary syndromes, although the pathophysiology of coronary disease KD is unique. Anticoagulants such as urokinase, tissue plasminogen activator (tPA), and platelet glycoprotein IIb/IIIa inhibitor (abciximab) have been used. Surgical management of KD-related disease includes coronary artery bypass grafting of obstructive lesions [297,298] particularly after recurrent myocardial infarction [124,125] (evidence level C). Catheter interventions such as angioplasty, stent placement, and rotational

ablation have been performed in relatively small numbers of children with KD complications. Recent Japanese recommendations suggest that catheter interventions should be considered in patients with ischemic symptoms, those without ischemic symptoms but with reversible ischemia on stress testing, and those with $\geq 75\%$ stenosis in the LAD [299] (evidence level C). A small number of patients with very severe KD complications have required cardiac transplantation for severe, irreversible myocardial dysfunction and coronary lesions for which bypass surgery nor catheterization procedures are feasible [300].

Patients who have had KD are divided into five risk levels for long-term management by the American Heart Association, as outlined below [7].

Patients with no evidence of coronary artery abnormalities at any time (*risk level I*)

These patients have no need for either aspirin or other antiplatelet medication beyond 6–8 weeks after onset of illness or for restriction of physical activities in the convalescent stage. Only routine pediatric follow-up beyond 1 year, with routine cardiovascular risk assessment, is indicated.

Patients with transient coronary ectasia or dilatation (*risk level II*)

Patients with transient coronary artery abnormalities that resolve by 6–8 weeks should be treated with aspirin, 3–5 mg/kg/day, until resolution of abnormalities. No restrictions are indicated after 6–8 weeks, angiography is not indicated, and risk assessment and counseling are recommended at 3–5 year intervals.

Patients with isolated (solitary) small-to-medium (3- to 6-mm or z-score = 2.5–<5) coronary aneurysm in one or more coronary arteries (*risk level III*)

Patients with solitary small-to-medium coronary artery aneurysms should be maintained on daily low-dose aspirin (3–5 mg/kg) at least until apparent regression is documented by annual echocardiographic follow-up. For patients younger than 11 years old, no restriction on physical activity is indicated, but for those 11 years and older, physical activity should be guided by a biennial stress test or myocardial perfusion study. Angiography should be performed if stenosis or ischemia is suggested.

Patients with one or more large (>6 -mm or z-score = 5–10) or giant (>8 -mm or z-score >10) coronary aneurysm, or multiple (segmented) smaller or complex aneurysms without obstruction (*risk level IV*)

Long-term antiplatelet therapy with aspirin (3–5 mg/kg once daily) or clopidogrel (1 mg/kg/day up to adult dose of 75 mg) is indicated and should be continued indefinitely.

Anticoagulant therapy with warfarin, with the international normalized ratio (INR) maintained at approximately 2.0–2.5, or daily subcutaneous low-molecular-weight heparin should be added for patients with giant aneurysms who have substantial risk for coronary thrombosis [301]. All such patients should be under the care of a pediatric cardiologist with experience in managing patients with KD. Cardiac evaluation with echocardiogram and ECG should be performed approximately every 6 months, with stress testing approximately annually. Angiography should be performed 6–12 months after recovery from the acute stage of disease to define the coronary anatomy, and repeated if symptoms or stress tests suggest myocardial ischemia. Physical activity should be regulated on the basis of annual stress test results and level of anticoagulation, and strenuous or contact athletics should be discouraged.

Patients with coronary artery obstruction (*risk level V*)

Patients with obstructive lesions or signs of myocardial ischemia should be evaluated urgently for possible intervention. Balloon angioplasty, rotablator angioplasty, coronary artery bypass grafting, stent placement, and even cardiac transplantation all have been employed for KD patients with particularly serious coronary artery disease [212,215,225,295,297–300,302]. Arterial bypass grafts are clearly superior to venous grafts in these patients. Balloon angioplasty procedures have been associated with high rates of recurrent stenosis in patients with KD and coronary stenosis [303].

9. LONG-TERM FOLLOW-UP AND PROGNOSIS

The risk of recurrence of KD is best documented in Japanese literature [7]. Yanagawa's epidemiologic studies in Japan from 1995 to 1996, reported a recurrence rate of 3.3% [28]. Other studies have reported a rate of recurrence ranging from 2.9 to 6.89 per 1000 person years [304–306]. A 1996 case-control study of patients with recurrent KD reported a higher risk of recurrence in patients who were treated with IVIG [307]. Besides a patient's ethnicity and receiving IVIG therapy, there is inconsistent evidence regarding other potential risk factors for KD recurrence reported at this time [305,307]. Based on clinical experience in large centers, the risk of a second episode of KD is approximately 1 out of 100 in Asian children. In children of other ethnic backgrounds, the risk of recurrence is approximately

1 out of 300–400. Typically, a recurrent episode of KD occurs within the first 12–24 months following initial diagnosis [304,305].

While the clinical manifestations of KD are normally acute and self-limited, cardiac abnormalities initially present during the acute phase may progress, with overall prognosis closely related to the severity of any underlying coronary artery abnormalities. Approximately 20–25% of patients not treated with IVIG develop detectable coronary abnormalities based on echocardiography or angiography. However, when IVIG is given within the first 10 days of illness, the risk of development of coronary aneurysms is reduced five-fold to approximately 2–3% overall [6,109]. Small aneurysms generally have a favorable prognosis with low risk of myocardial ischemia or death [308]. However, giant CAA (internal diameter >8 mm or with z-score ≥ 10) have a high risk of morbidity and mortality as up to one-half of these ultimately may become obstructed [215]. Among patients who develop aneurysms, mortality is highest between 15 and 45 days after onset of KD [225]. A recent analysis of 76 subjects with giant aneurysms from one Japanese center with median follow-up of 19 years showed 10-, 20-, and 30-year survival rates of 95%, 88%, and 88%, respectively. Catheter and surgical coronary interventions resulted in 28%, 43%, and 59% cumulative coronary procedure rates at 5-, 15-, and 25-year follow-up, respectively [216]. Children without apparent cardiac sequelae during the first month after onset of KD return to their previous states of health, typically without any clinical signs or symptoms, although there is concern for the future risk of cardiac complications, even in children without CAA.

Patients who develop moderate-to-severe coronary abnormalities are at risk of myocardial ischemia, myocardial infarction, and even sudden death for many years after the onset of KD [225,292]. There are reports of “missed KD” in childhood presenting with myocardial infarction as adults [309]. There are a limited number of studies of young adults with ischemic heart disease and history of diagnosed KD [228,310]. A survey of Japanese adult cardiologists identified 130 adult patients with coronary aneurysms detected by angiography to evaluate myocardial infarction or ischemia [228]. Twenty-one of these patients (mean age, 34 years; range, 20–63 years) had a history compatible with KD in childhood. These patients had severe coronary disease with myocardial infarction, angina pectoris, mitral regurgitation, arrhythmias, congestive heart failure, and need for coronary bypass grafting. Daniels et al. evaluated 261 adults <40 years who underwent angiography for suspected myocardial ischemia. Sixteen of these patients had coronary aneurysms, 13 of whom had definite or presumed history of KD as the likely etiology [310]. Both studies indicate that KD contributes to ischemic heart disease in young adults.

The natural course of CAA varies based on severity, but small and medium aneurysms more commonly appear to regress by echocardiogram and/or angiography. While aneurysmal segments can rupture, due to progressive calcification, these very rare events usually occur in the first few months after illness onset. In addition to stenosis and rupture, the primary clinical concern with aneurysmal segments over time is the potential development of thrombotic occlusions. Aneurysms may increase in size over the first 4–6 weeks after onset of illness. After reaching peak luminal diameter, approximately one-half to two-thirds of all children with aneurysms at 4–8 weeks after onset demonstrate return to normal internal lumen diameter over the next 2 years [215,231,291,311]. Further change in size is unlikely more than 2 years after the onset of illness. Giant aneurysms are the least likely to show apparent reduction in luminal diameter. Aneurysms in children younger than 1 year of age at the onset of KD, fusiform rather than saccular morphology aneurysms, smaller aneurysms, or aneurysms that are distally located are more likely to return to normal internal diameter [290,291,311,312].

Despite the return to normal coronary artery measurements, the coronary segments with apparently regressed aneurysms by echocardiogram or angiography likely still have altered physiology. Intravascular ultrasounds of such aneurysm segments demonstrate intimal thickening [313–315] and other studies demonstrate impaired coronary and peripheral vascular reactivity [315–318]. Return of the internal diameter of the vessel to normal occurs by luminal smooth muscle-derived myofibroblastic proliferation and/or by thrombus organization and recanalization as shown by Orenstein [31,67,75]. Orenstein et al. recently reported that stenotic lesions in patients who died due to complications from KD were likely due to persistent vasculitis and myofibroblastic proliferation, rather than atherosclerosis. Orenstein studied specimens from 32 autopsies, 8 cardiac transplants, and an excised aneurysm of patients with KD via light ($n=41$) and transmission electron microscopy ($n=7$) [75]. Three characteristic vasculopathic processes were identified in both coronary and noncoronary arteries: acute self-limited necrotizing arteritis (NA), subacute/chronic (SA/C) vasculitis, and luminal myofibroblastic proliferation (LMP). NA is a self-limiting process within the first 2 weeks of fever onset. NA leads to the progressive destruction of the adventitia, which leads to saccular aneurysm formation. Thrombosis or rupture of these aneurysms is the primary cause of early morbidity/mortality with KD. SA/C vasculitis is an asynchronous process that can commence within the first two weeks and continue for years. It starts in the adventitia/perivascular tissue and causes gradual inflammatory destruction of the vascular wall. SA/C vasculitis likely causes the transition of medial and adventitial smooth

muscle cells (SMC) into classic myofibroblasts. Subsequent luminal myofibroblastic proliferation then creates progressive stenosing luminal lesions. These regressed aneurysmal segments appear to have decreased vascular reactivity in response to exercise or pharmacologic agents such as isosorbide dinitrate or acetylcholine [31,315,318,319]. A few studies suggest the possibility of generalized endothelial dysfunction in patients with KD, even in some patients without documented coronary abnormalities. These abnormalities include altered lipid metabolism [183], increased brachial-radial artery mean pulse-wave velocity [320], lower myocardial flow reserve [321], and abnormal endothelium-dependent brachial artery reactivity [319,322,323]. The functional and histological abnormalities of the coronary vasculature in KD patients, even in the absence of a history of aneurysm formation, and their long-term clinical implications still warrants further investigation.

Only a limited number of aneurysms that return to normal luminal diameter progress to stenosis [215,244,294,296,318,324,325]. Patients with giant coronary aneurysms are at the greatest risk for the development of significant stenosis with resultant myocardial ischemia [215,216]. Significant stenosis usually develops at the inlet or outlet of a moderate to large coronary aneurysm [215,216,296]. In 10- to 20-year follow-up studies of patients with KD, the arteries most likely to develop stenosis are the right main and left anterior descending coronary arteries [215,216,296].

10. ATHEROSCLEROSIS AND KAWASAKI DISEASE

KD patients with regressed or persistent coronary aneurysms may be at higher risk than the general population for early atherosclerosis, although this is highly controversial. While there is some indirect supportive evidence, the recently reported coronary artery pathologic features must be considered (see the “Long-term Follow-up” section above) [75]. For instance, in Orenstein’s extensive study of the coronaries of a large group of very severely affected children after KD, no evidence of atherosclerosis was found, although myofibroblastic proliferation possibly could be misinterpreted as suggesting atherosclerosis [75]. One study compiled a review of 12 case-control studies comparing carotid artery ultrasounds of patients at least 1 year after KD diagnosis to those of controls to evaluate for differences in carotid intimal media thickness (CIMT) [326], but the results were inconclusive. When comparing a standardized version of CIMT using age-specific reference values (CIMT-SDS), the CIMT-SDS among the 12 studies was significantly different between KD patients and controls despite the absolute CIMT values

not differing significantly. However, increased CIMT is not necessarily synonymous with pathological atherosclerotic progression, especially in the absence of any plaque formation. In fact, based on previous studies in KD patients, this increase in CIMT may be related to the development of postinflammatory arteriosclerotic remodeling after KD [327], as characterized by intimal proliferation and neoangiogenesis [328]. Coronary angiograms also demonstrate increased arterial stiffness and diminished vascular reactivity in patients at 10-year follow-up [315], but this more likely represents fibroblastic proliferation rather than atherosclerosis. Such findings may also be attributable to persistent low-grade systemic inflammation or coronary vasculitis in patients with KD, even in the setting of normal inflammatory markers. Although the risk for atherosclerosis is still debatable, other known vascular abnormalities and remodeling via myointimal thickening increase the risk for stenosis, and thus ischemic heart disease, as discussed above [215,313,329–333]. Some, but not all, studies suggest the possibility of generalized endothelial dysfunction in patients following KD, even in some patients without documented coronary abnormalities. Other abnormalities, including altered lipid metabolism [183], increased brachial-radial artery mean pulse-wave velocity [320], lower myocardial flow reserve [321], and abnormal endothelium-dependent brachial artery reactivity [319,322,323] have been reported as well.

11. CONCLUSION

KD is an acute vasculitis of unknown etiology that is the most common cause of acquired heart disease in children in the developed world. Patients are diagnosed with KD based on a specific constellation of clinical symptoms and laboratory findings. While there is much that remains unknown about the etiology and pathogenesis of this illness, treatment with IVIG and high-dose aspirin has been shown to reduce the risk of long-term cardiac complications, namely coronary artery aneurysms. Further investigation is still required to understand the etiology to help target future therapies, diagnostic testing, and preventative measures.

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ANCA-Associated Vasculitis: Microscopic Polyangiitis, Eosinophilic Granulomatosis With Polyangiitis (Churg–Strauss Syndrome) and Granulomatosis With Polyangiitis (Wegener’s Granulomatosis)

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1. INTRODUCTION

Antineutrophil cytoplasmic antibodies (ANCA) associated systemic vasculitis (AAV) is a grouping of systemic small-vessel vasculitides, autoimmune diseases wherein patients have pathogenic autoantibodies reacting to myeloperoxidase (MPO) or proteinase 3 (PR3) [1,2]. AAV comprises three disease types, including microscopic polyangiitis (MPA), eosinophilic granulomatosis with polyangiitis (EGPA; formerly known as Churg–Strauss Syndrome) and granulomatosis with polyangiitis (GPA; formerly known as Wegener’s granulomatosis). The disease types differ with respect to clinical manifestations and histological features. Necrotizing granulomatous lesions are found in GPA and EGPA but not in MPA [3]. On the other hand, EGPA and MPA are mostly associated with ANCAs directed against MPO, whereas GPA is more associated with PR3-ANCA specificity [3]. AAV has an annual incidence of approximately 10–20 cases per million [4,5], with the highest overall prevalence in Caucasian people [6,7]. MPA is more frequently observed as compared to EGPA, with an annual incidence of 4.9/million and 1.4/million, respectively [5]. Geographical variation in the distribution of specific syndromes have been observed [4,8–10] and recently confirmed by a prospective study [11]. Although the latter study demonstrated no major difference in general AAV incidence between

Japan and the UK, MPA was more common in Japan [11] and previous studies showed higher incidences of MPA in Southern Europe [12]. Disease onset of AAV usually occurs at 65–74 years, although it can occur at any age [4]. Both genders are equally affected and the disease is far more common among Caucasians.

With current therapy, AAV is now characterized mostly as a remitting-relapsing condition, although outcomes are often still poor with a mortality of 25% at 5 years [13]. Both renal and cardiac involvement is independent predictors of poor outcome [14–17]. To date, the reported prevalence of cardiac involvement remains highly variable, ranging from 6 to 92% as will be discussed in the following sections. In addition, the severity of involvement may range from subclinical to life threatening. These differences might be explained by variances in disease activity and duration, selection bias, but also differences in applied diagnostic methods for the detection of cardiac involvement. Nevertheless, small-vessel vasculitides can affect the myocardium, pericardium, valves, and coronary arteries. Immunosuppressive treatment improves overall survival in these patients [13], but might also attenuate or even resolve cardiac abnormalities if treated adequately [18,19]. Therefore early detection of cardiac involvement in AAV patients is crucial to further improve prognosis.

2. CLINICAL PRESENTATION AND DIAGNOSTIC CRITERIA

2.1 Signs and Symptoms

There is substantial overlap in many of the clinical features of the ANCA-associated vasculitides. In some cases, distinguishing between the different subtypes on the basis of clinical features alone is difficult (Table 20.1). In particular, GPA and MPA are characterized by an extensive overlap in terms of clinical manifestations [20]. As both diseases have historically been considered different ends of the same disease spectrum, they are frequently included together in clinical trials [21].

Patients typically present with prodromal “flu-like” symptoms of several weeks’ or months’ duration, such as fever, polymyalgia, polyarthralgia, headache, malaise, anorexia, and unintended weight loss. Although these nonspecific symptoms overlap with many other non-vasculitis processes (ie, postviral syndrome, infections, malignancy), vasculitis should always be considered in patients with general symptoms and signs of inflammatory disease. Although most symptoms overlap between the three syndromes, some are more common in one or the other. Common symptoms include ear, nose, and throat problems such as hearing loss, otalgia, (bloody) rhinorrhea, otorrhea, sinusitis, nasal crusting, and recurrent otitis media in approximately 90%, 48%, and 35% in GPA, EGPA, and MPA, respectively [17,21–28].

In general, GPA patients typically present with symptoms of the upper respiratory tract, such as bloody nasal

discharge, nasal ulceration, chronic sinusitis, and/or otitis. The systemic symptoms, such as malaise, arthralgias, and myalgias, are frequently present. Later on, manifestations of AAV may occur in virtually every organ. In EGPA, most patients suffer from nasal obstruction due to nasal polyposis, asthma, lung infiltrates, and systemic symptoms. Multiple mononeuropathies may dominate the clinical picture of these patients. In MPA, most patients present with systemic symptoms, such as fever, malaise, arthralgia, myalgia, and skin vasculitis. Later on, a renal-pulmonary syndrome often occurs.

With current immunosuppressive treatment strategies, remission of disease is achieved in the majority of patients. However, in a subset of these patients relapse of disease, or so-called grumbling disease occurs. This may explain the increased rate of cardiovascular events. Although remission induction is currently almost always achieved, a tendency for recurrent or ongoing disease activity remains in a subset of patients. During the ongoing search for biomarkers that can predict this relapse of disease, several studies confirmed that patients with anti-MPO antibodies have lower relapse rates than patients with anti-PR3 antibodies [24,29]. Recently, it was shown that a rise in ANCA levels predicts clinical relapse only in patients with renal vasculitis [30]. Moreover, a gene expression signature seen only in CD8⁺ T cells showed a strong prediction of a tendency to remain in sustained remission, a finding confirmed in another cohort, but also in a different disease, namely systemic lupus [31]. Both these novel findings may be useful in future clinical decision-making.

TABLE 20.1 Extra-Cardiac Clinical Features of Antineutrophil Cytoplasmic Antibody-Associated Vasculitis

Feature	GPA	MPA	EGPA
ANCA-positivity ^a	80–90%	70%	30–40%
ANCA antigen specificity ^a	PR3 (70–80%) » MPO (20–30%)	MPO (80%) > PR3 (10–15%)	MPO (80–90%) > PR3 (<5%)
Fundamental histology	Leukocytoclastic vasculitis; necrotizing, granulomatous inflammation (rarely seen in renal biopsies)	Leukocytoclastic vasculitis; no granulomatous inflammation	Eosinophilic tissue infiltrates and vasculitis; granulomas have eosinophilic necrosis
Ear/nose/throat	Nasal septal perforation, saddle-nose deformity, conductive, or sensorineural hearing loss, subglottic stenosis	Absent or mild	Nasal polyps, allergic rhinitis, conductive hearing loss
Eye	Orbital pseudotumor, (epi)scleritis, uveitis	Occasional eye disease: scleritis, episcleritis, uveitis	Occasional eye disease: scleritis, episcleritis, uveitis
Lung	Nodules, infiltrates, or cavitory lesions; alveolar hemorrhage	Alveolar hemorrhage	Asthma, fleeting infiltrates, alveolar hemorrhage
Kidney	Segmental necrotizing glomerulonephritis, rare granulomatous features	Segmental necrotizing glomerulonephritis	Segmental necrotizing glomerulonephritis
Peripheral nerve	Vasculitic neuropathy (10%)	Vasculitic neuropathy (60%)	Vasculitis neuropathy (50–60%)
Eosinophilia	Mild eosinophilia occasionally	None	All

GPA, Granulomatosis with polyangiitis; MPA, microscopic polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; ANCA, antineutrophil cytoplasm antibody; MPO, myeloperoxidase; PR3, proteinase 3.

^aApproximate numbers of large cohort studies including Flossmann et al. [13] and Cormand et al. [17].

With respect to clinical features related to cardiac involvement, no specific signs or symptoms suggesting cardiac involvement exist. As a large proportion of AAV patients initially present with pulmonary disease, dyspnea due to pulmonary vasculitis or cardiac failure is difficult to discriminate. A recent prospective study including 50 EGPA and 41 GPA patients demonstrated that cardiac involvement is still present in up to 50% of these patients without typical clinical cardiac symptoms. Vice versa, cardiac symptoms were present in 50% of patients without evidence of cardiac involvement [32]. This reflects the difficulties in detecting cardiac involvement purely based on signs and symptoms.

2.2 Diagnostic Criteria

As diagnostic criteria changed over time, discriminating syndromes of vasculitis and their cardiac involvement retrospectively may be difficult due to different classifications. Of note, the first classification criteria of the American College of Rheumatology (ACR) discriminated between Wegener's granulomatosis (currently GPA), Churg–Strauss syndrome (currently EGPA), and polyarteritis nodosa (PAN) (Table 20.2) [33–36].

TABLE 20.2 ACR Criteria (1990) for the Diagnosis of ANCA Vasculitis Before ANCA Testing Was Available

	GPA	MPA	EGPA
Diagnostic criteria	<ol style="list-style-type: none"> 1. Nasal or oral inflammation (painful or painless oral ulcers or purulent or bloody nasal discharge) 2. Abnormal chest radiograph showing nodules, fixed infiltrates, or cavities 3. Abnormal urinary sediment (microscopic hematuria or red cell casts) 4. Granulomatous inflammation on biopsy of an artery or perivascular area 	Not existing	<ol style="list-style-type: none"> 1. Asthma (a history of wheezing or the finding of diffuse high pitched wheezes on expiration) 2. Eosinophilia of >10 percent on differential white blood cell count 3. Mononeuropathy (including multiplex) or polyneuropathy 4. Migratory or transient pulmonary opacities detected radiographically 5. Paranasal sinus abnormality 6. Biopsy containing a blood vessel showing the accumulation of eosinophils in extravascular areas
Diagnostic performance	≥2 out of 4 criteria positive: • sensitivity of 88% • specificity of 92%		≥4 out of 6 criteria positive: • sensitivity of 85% • specificity of 99.7%

GPA, Granulomatosis with polyangiitis; MPA, microscopic polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis.

Importantly, the distinct syndromes PAN and MPA were not differentiated and criteria were developed before the widespread availability of ANCA testing. Four years later, the Chapel Hill Consensus Conference (CHCC) defined MPA and PAN as two different vasculitides, a small-to-medium sized blood vessel vasculitis and a medium-sized blood vessel vasculitis, respectively. With the availability of ANCA detection, GPA, EGPA, and MPA were grouped together as AAV due to their association with ANCA. Therefore, cardiac involvement studies performed before the 1994 CHCC definitions included MPA and PAN as one disease, which should be taken into consideration. Finally, in 2012, 18 years after the initiation of the first CHCC nomenclature, definitions were revised due to improved insight in the spectrum of vasculitis. To overcome differentially used nomenclature in the previous years vasculitis syndromes are currently referred to as descriptive titles (Table 20.3). The definitions are based on histology and clinical manifestations, although recent insights for classification using genetic studies in large cohorts might differ from this approach [14,37]. Herein, patients are classified according to ANCAs directed against myeloperoxidase (MPO) or proteinase

TABLE 20.3 Definitions of ANCA-Associated Vasculitis According to the Chapel Hill Consensus Conference in 2012

ANCA-associated vasculitis	Necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels (ie, capillaries, venules, arterioles, and small arteries), associated with MPO-ANCA or PR3-ANCA. Not all patients have ANCA. Add a prefix indicating ANCA reactivity, eg, PR3-ANCA, MPO-ANCA, and ANCA negative.
Microscopic polyangiitis	Necrotizing vasculitis, with few or no immune deposits, predominantly affecting small vessels (ie, capillaries, venules, or arterioles). Necrotizing arteritis involving small and medium arteries may be present. Necrotizing glomerulonephritis is very common. Pulmonary capillaritis often occurs. Granulomatous inflammation is absent.
Granulomatosis with polyangiitis (Wegener's)	Necrotizing granulomatous inflammation usually involving the upper and lower respiratory tract, and necrotizing vasculitis affecting predominantly small-to-medium vessels (eg, capillaries, venules, arterioles, and veins). Necrotizing glomerulonephritis is common.
Eosinophilic granulomatosis with polyangiitis (Churg–Strauss)	Eosinophil-rich and necrotizing granulomatous inflammation often involving the respiratory tract, and necrotizing vasculitis predominantly affecting small to medium vessels, and associated with asthma and eosinophilia. ANCA is more frequent when glomerulonephritis is present.

ANCA, Antineutrophil cytoplasm antibody; MPO, myeloperoxidase; PR3, proteinase 3.

Adapted from Jennette et al. [3].

3 (PR3), revealing novel insights in epidemiological, clinical, and genetic subtypes [38]. Future knowledge will determine which (combined) approach is most useful in the classification of this elusive disease.

2.2.1 Etiopathophysiology of ANCA-Associated Vasculitis and Associated Cardiac Involvement

The etiopathophysiology of AAV is complex, and although the exact causes are unknown, the systemic vasculitis probably results from interplay between genetic and environmental factors (Fig. 20.1) [37,39,40].

2.3 Genetics and Environmental Factors

Evidence indicates that a genetic contribution is at play in the pathogenesis of AAV, with a population study showing increased familial occurrence [40] and several candidate gene approach studies over the years [41–44]. Importantly, the understanding in the field of AAV genetics has increased after the publication of two genome-wide association studies (GWASs). The first GWAS was conducted by the European Vasculitis Genetic Consortium (EVGC; 2687 cases of GPA and

MPA, as well as 6858 controls) [37]. The second by the US Vasculitis Clinical Research Consortium (VCRC; 987 GPA cases and 2731 controls) [45]. The main focus of these studies was to explore the genetic profile of PR3- and MPO-ANCA vasculitis patients. Interestingly, the latter studies confirmed the presence of a certain single-nucleotide polymorphism (SNP) in the HLA-DPB region on chromosome six in a large percentage of PR3-AAV patients as opposed to MPO-AAV patients. The association between this allele with PR3-ANCA was stronger than with the clinical diagnosis of GPA. In addition, a correlation appears to exist between GPA and *SERPINA-1* (encoding $\alpha 1$ -antitrypsin), *PRT3* (encoding PR3, the main GPA-related autoantigen) and *SEMA6A* (semaphoring 6A) genes, while MPA seems to be much more correlated with polymorphisms in *HLA-DQ* genes. Currently, no GWAS have been conducted in EGPA as shown previously.

To summarize, both GWASs demonstrate that particular genes are primarily aligned with ANCA subtypes rather than diagnostic subgroups. The importance of these MPO- and PR3-ANCA subtypes in classifying patients, rather than histology and clinical manifestations, is in line with previous results from a large cluster

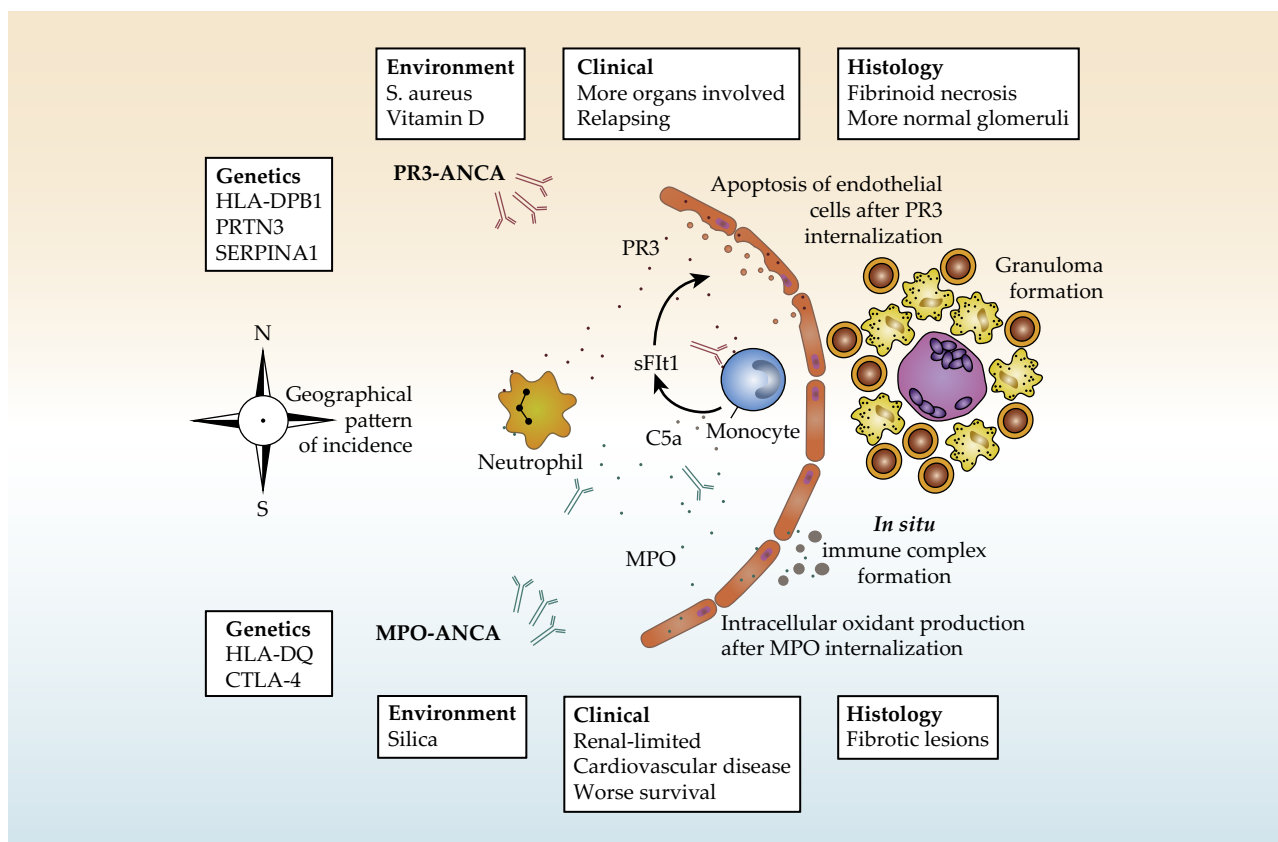


FIGURE 20.1 Pathogenic model of ANCA-associated vasculitis and the interplay between genetic and environmental factors. Pathogenic model highlighting the differences between PR3-ANCA and MPO-ANCA vasculitis as opposed to the clinical subtypes of GPA, MPA, and EGPA. PR-3-ANCA, proteinase-3 antineutrophil cytoplasmic antibody; MPO, myeloperoxidase; HLA-DPB1, human leukocyte antigen-DP β chain gene; PRTN3, proteinase-3 gene; SERPINA1, serpin peptidase inhibitor clade A member one gene ($\alpha 1$ -antitrypsin); CTLA-4, cytotoxic T-lymphocyte-associated protein 4; sFlt1, Soluble fms-like tyrosine kinase-1. Adapted from Hilhorst et al. [38].

analysis [14]. These studies suggest that MPO- and PR3-AAV might be distinct syndromes [38]. Interestingly, a difference in 5-year cardiovascular (CV) risk is observed when differentiating between PR3- and MPO-ANCA. Herein, 6.6% of PR3-ANCA vs. 19.2% of MPO-ANCA patients without premorbid CV disease demonstrated ≥ 1 cardiovascular event over a 5-year follow-up period [16].

With respect to environmental factors, several have been implicated including occupational exposure to silica (such as farming, construction work) [46,47], drugs including cocaine, antithyroid, and antihypertensive drugs (propylthiouracil and hydralazine), and several microbial agents, particularly *S. aureus* [48]. Peptides from *S. aureus* have strong homology with peptides from complementary PR3, and *S. aureus* infection has been associated with initiation and relapse of GPA [49].

2.4 ANCA Development

All these factors contribute to the development of ANCAs, which are generally considered pathogenic. ANCAs with specificity for either proteinase 3 (PR3) or myeloperoxidase (MPO) are hallmarks of AAV. ANCA is usually tested using indirect immunofluorescence, where two main patterns are observed, cytoplasmic (c-ANCA) and perinuclear (p-ANCA), then confirmed using ELISA, which in most cases shows reactivity against PR3 or MPO. GPA is usually associated with c-ANCA, directed against PR3, whereas EGPA and MPA are mostly associated with ANCAs directed against MPO showing a p-ANCA pattern [14]. However, significant overlap exists with regards to ANCA status between GPA and MPA. Therefore ANCA specificity toward PR3 or MPO cannot be used to distinguish GPA from MPA [50].

The development of ANCAs is not fully understood. However, once ANCA has developed, priming of circulating neutrophils by cytokines (eg, TNF- α), possibly during infection, causes expression of PR3 and MPO on the neutrophil cell membrane, where it becomes accessible to ANCA. ANCAs degranulate neutrophils, produce reactive oxygen species, and release proteolytic enzymes causing vasculitic and endothelial damage. Of note, the term AAV may be misleading as not all patients have positive ANCA. Besides ANCA and neutrophils, the adaptive immune system further contributes to the pathogenesis of the disease [1,2]. Other studies also indicate an important role of the alternative pathway of the complement system, danger-associated molecular patterns, and dendritic cells in the development of AAV [1]. These pathophysiologic features result in a systemic inflammatory response affecting different vessels and organs, which may include coronary arteries and the heart.

2.5 Pathophysiology of Cardiac Involvement in ANCA Vasculitis

In ANCA vasculitis, several key processes are at play that can lead to cardiovascular disease and subsequent cardiac involvement, both in the acute and chronic phase of the disease. These key processes include (low-grade) chronic inflammation, endothelial dysfunction, increased thrombogenicity, and accelerated atherosclerosis.

2.5.1 Markers of Inflammation

As known, ANCA vasculitis is characterized by ongoing systemic inflammation. Over the years, several biomarkers of inflammation have been discovered and tested in AAV patients. Moreover, the prominent role of inflammation at all stages of atherosclerotic plaque development, the main cause of cardiovascular disease, is now well recognized [51]. Importantly, increased levels of C-reactive protein (CRP), an acute phase protein, is a well-known risk factor for increased cardiovascular morbidity and mortality in the general population. Acute phase proteins, such as CRP and pentraxin 3, are modulated during inflammation. They are produced by hepatocytes stimulated by interleukin-6 (IL-6), an interleukin produced by ECs, mononuclear phagocytes, fibroblasts, and other types of cells in response to stimulation by IL-1 β and, to a lesser extent, tumor necrosis factor α [52]. Serum CRP concentration was elevated in patients with active GPA as compared to reference value for CRP in normal, healthy individuals [53]. In addition, elevated CRP levels were found in patients with asymptomatic MPA (6.39 ± 2.95 mg/dL) or GPA (8.21 ± 3.98 mg/dL) vs. healthy controls (<0.8 mg/dL) [54]. Pentraxin 3 showed several-fold higher levels in patients with MPA, GPA, and EGPA (MPA 3.25 ± 4.8 ng/mL; GPA 3.14 ± 2.58 ng/mL; and EGPA 3.28 ± 2.34 ng/mL, respectively) compared to healthy controls (1.0001 ± 0.4 ng/mL) [55]. Pentraxin three did not correlate with CRP levels in this study, but this may be due to the fact that CRP is an IL-6 dependent protein produced in the liver, whereas pentraxin three is a IL-1 β and TNF- α dependent protein [52].

With respect to cytokines and chemokines, increased serum levels of IL-6, IL-8, and IL-1ra were demonstrated in GPA and MPA as compared to control subjects [56]. Similar findings were shown a few years later, demonstrating elevated serum levels of IL-8 and monocyte chemoattractant protein-1 in GPA patients as compared to healthy controls [57].

The above-mentioned evidence clearly demonstrates a chronic state of systemic inflammation, both in the active and remission phase of the disease. This might (in part) explain the bimodal mortality pattern, with a second peak due to cardiovascular disease as

demonstrated by the four-fold increase in risk for coronary artery disease and cardiovascular disease as an independent risk factor for death in AAV patients [14–16].

2.5.2 Markers of Endothelial Activation, Damage, and Dysfunction

The endothelial cell is the primary target of PR3 and MPO in ANCA vasculitis. When these enzymes are released in the circulation, internalization by endothelial cells can occur [58]. Herein, PR3 induces apoptosis and MPO induced the production of intracellular oxidants [59]. This in turn results in endothelial damage and dysfunction. Of note, endothelial dysfunction is a key process in atherosclerosis [60–62] and independently predicts cardiovascular events [63,64]. In AAV patients, endothelium dysfunction has been shown to be present and is independent of disease activity or renal involvement [65]. A variety of mechanism may cause endothelial activation and damage, including complement-dependent cytotoxicity, antibody-dependent cytotoxicity, direct effect of adhesion molecules, and cytokines [1]. Besides the pathogenicity of ANCAs themselves, they bind to neutrophils and endothelial cells having differential but synergistic effects on both cell types. ANCAs promote degranulation of neutrophils and monocytes facilitating endothelial damage [66]. In addition, the endothelium is activated, thereby enhancing neutrophil adherence [67]. This endothelial activation and damage leads to amplification of the inflammatory cascade, resulting in leukocyte tissue infiltration, T-cell driven granuloma formation, and further damage [1,68].

Over the years, many soluble biomarkers of endothelial activation have been demonstrated (eg, CRP, sIL-6, sIL-8, TNF- α , soluble von Willebrand Factor circulating endothelial cells, endothelial microparticles). These activated endothelial cells are capable of producing endothelial adhesion molecules, pro- or anticoagulant molecules, cytokines, chemokines, and acute phase reactants [52].

Several endothelial adhesion molecules are considered biomarkers for activated endothelial cells, including soluble E-selectin, solely expressed on activated endothelial cells, and intercellular adhesion molecule –1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1). Both GPA and MPA patients showed increased levels of E-selectin (sE-selectin: 88 ± 42 ng/mL), soluble ICAM (sICAM-1: 427 ± 184 ng/mL), and soluble VCAM (sVCAM-1: 1720 ± 1174 ng/mL) as compared to healthy controls [54]. Similar increases for either sICAM or sVCAM or both but not E-selectin were found in other studies including GPA and MPA patients [69–71].

Recent studies suggest that bone-marrow-derived circulating endothelial progenitor cells (EPCs) play a

pivotal role in the balance of endothelial cell injury and repair in AAV patients [72]. Herein, mature circulating endothelial cells (CECs) and circulating endothelial microparticles (EMPs) can serve as a marker of endothelial injury, whereas immature EPCs can reflect endothelial repair [73,74]. AAV patients demonstrated significant lower levels of EPC as compared to the increased numbers of CECs and EMPs [75,76]. Importantly, it is suggested that the combination of CECs, EMPs, and EPCs may serve as markers to detect disrupted endothelial integrity at early preclinical stages [77].

2.5.3 Markers for Increased Thrombogenicity

Although the mechanisms underlying the increased occurrence of venous thromboembolic events (VTEs) in AAV patients is not fully understood, several risk factors influencing thrombogenesis are suggested including endothelial damage, coagulation factors, systemic inflammation, and associated immunosuppressive therapies [78,79,80].

Endothelial cell dysfunction, as previously mentioned due to the interaction between neutrophils and endothelial cells, with consequent massive oxidative stress finally leads to atherothrombotic complications [81]. Recently, an additional mechanism of neutrophil activation has been described, termed NETosis. Neutrophils are able to release extracellular nucleic acids associated with histones and granular proteins capable of entrapping and killing microbial invaders. These neutrophil extracellular traps (NETs) have also been implicated in thrombotic events and seem to be a potential bridge between autoimmunity and coagulation. In particular, neutrophils primed by ANCA degranulate and release NETs, which in turn contain MPO and PR3, creating a self-amplifying process [82]. In active AAV neutrophils release high levels of tissue factor expressing NETs [83]. In addition, NETs are able to promote thrombosis by inhibiting the tissue factor pathway inhibitor and recruiting platelets [84]. Finally, increased platelet activation by neutrophil-derived platelet microparticles, which include platelet activating factor, adhesion molecules, and MPO, was demonstrated in active AAV patients [83].

In EGPA, in addition to neutrophils, eosinophils may also promote vascular injury via the release of pre-formed granules during active disease. It is known that eosinophils release tissue factor as well as other cationic proteins. The former initiates coagulation while the latter inhibit natural anticoagulant activity and activate platelets, eventually leading to excessive thrombin generation [85].

With respect to the coagulation system, AAV patients can harbor antibodies with dual reactivity to plasminogen and complementary PR3, a recombinant protein translated from the antisense strand of PR3

cDNA. Antiplasminogen antibodies delay the conversion of plasminogen to plasmin and increase the dissolution time of fibrin clots and, therefore, can probably promote thrombotic complications [86,87]. Recently, patients with AAV in remission demonstrated a more procoagulant state as compared to healthy controls, indicated by increased endogenous thrombin generation potential and Factor VIII [80]. This enhanced coagulation with increased Factor VIII was also demonstrated in both the active and remission phase, whereas active AAV was associated with accumulation of prothrombotic features, in particular thrombin formation and fibrin [88]. Finally, the prevalence of prothrombotic factors and genetic mutations such as Factor V Leiden, prothrombin (G20210A), and methylenetetrahydrofolate reductase and β -2 glycoprotein-I antibodies did not differ between GPA and the general population [89].

As previously stated, thromboembolic disease is an increasingly recognized complication in AAV patients. An overview of larger and geographical different AAV studies is demonstrated in Table 20.4. In the 1990s, markers for coagulation were already reported elevated in patients with GPA and MPA during active disease as compared to healthy controls. These markers included serum thrombin-antithrombin-3-complexes (STAT), soluble TM, von Willebrand Factor, and D-dimer levels, whereas sTM and sSTAT were closely correlated with disease activity [90]. Increased risk of VTEs was first reported in pediatric AAV patients [91]. Definitive evidence was demonstrated by Wegener's granulomatosis Etanercept Trial (WGET) in 2005 [92]. In this study 180 patients with GPA followed for a mean of 27 months had an increased incidence of VTE, in particular during active disease. Moreover, GPA patients still demonstrated increased incidence of VTE when compared to patients treated with etanercept for other indications, patients with lupus, rheumatoid arthritis, or the general population [93]. Subsequent studies confirmed an increased occurrence of VTEs in AAV patients as compared to the general population [93–96], not only during early or active AAV, but also when patients are in remission [80,97]. In a large cohort of 357 AAV patients studied between 2006 and 2014, VTEs in EGPA was higher (10.1%) than in GPA (8.2%) and MPA (6.7%), probably due to the thrombogenic effects of eosinophils [98]. In a recent Australian study of 19 EGPA patients an increased incidence of VTE and pulmonary embolism was reported [99]. A very recent retrospective GPA patient study including long-term follow-up (median 7.2 years) confirmed a significant risk of VTE in both early and late during the course of follow-up with subsequent hospitalizations for deep venous thrombosis and pulmonary embolism [100].

2.5.4 Accelerated Atherosclerosis

Development of accelerated atherosclerosis and resultant ischemic heart disease is an important cause of morbidity and mortality in systemic autoimmune diseases, including rheumatoid arthritis, systemic sclerosis, systemic lupus erythematosus, and AAV [101–104]. In AAV, patients have a two- to four-fold increased relative risk of coronary artery disease (CAD) and the relapsing remitting and nature of the disease may accelerate atherosclerosis. This accelerated atherosclerosis results from systemic inflammation and immunologic abnormalities, independent of classic cardiovascular risk factors [104]. In addition, renal dysfunction—which occurs frequently in AAV patients—is an established contributor to cardiovascular disease by affecting metabolic, inflammatory, and hemodynamic pathways [105]. Furthermore, glucocorticoid therapy can cause accelerated atherosclerosis and ischemic heart disease both directly and indirectly by increasing the incidence of diabetes mellitus, dyslipidemia, and hypertension—known risk factors for CAD. In contrast, they may also have a protective role in vasculitis by reducing systemic inflammation and improving endothelial dysfunction [106].

3. VASCULITIS-RELATED CARDIAC INVOLVEMENT

Although clinically overt cardiac manifestations are infrequently observed, many studies have demonstrated (subclinical) cardiac involvement including rhythm disturbances, myocarditis, pericarditis, endocarditis, valvular disease, cardiomyopathy, myocardial infarction, and cardiac tamponade. The majority of these studies performed cardiac evaluation merely in patients presenting with clinical symptoms suggesting cardiac involvement, thus the true prevalence of involvement is likely to be underestimated. Moreover, the largest studies including AAV patients are predominantly randomized controlled trials conducted to evaluate treatment strategies for the disease itself. Only recently has cardiac involvement shown to be an independent predictor of adverse outcome by pooling data of these randomized trials [14–16]. With respect to EGPA patients, several articles have suggested that cardiac involvement is more frequent in ANCA-negative as compared to ANCA-positive patients. Herein, ANCA-positive patients have a vasculitic phenotype, with more frequent glomerulonephritis, mononeuritis multiplex, and relapse. Although ANCA-negative patients have less relapse, they have a poorer prognosis, most likely due to the more frequently observed cardiac involvement in these patients [17,107,108]. These results emphasize the need for cardiac screening

TABLE 20.4 Incidence Rates of Venous Thromboembolic Events (VTE) in AAV Patients

Study#	Study	Study type	Patient population	Gender (M/F)	Age (years)	Follow-up	Main result	Comment
1	Merkel et al. [93]	Ancillary study from RCT	180 GPA patients enrolled between 2000 and 2002	108/72	49.8	Mean follow-up 27 months	Incidence rate of 7.0 VTEs per 100-patients-years Absolute frequencies of 16.1%	Incidence rate higher than SLE, RA and normal population
2	Weidner et al. [94]	Uncontrolled retrospective cohort analysis	105 AAV (EGPA excluded) patients followed between 1986–2001	NA	VTE: 54 (16–74) No VTE: NA	NA	Incidence rate of 4.3 VTEs per 100-patients-years Absolute frequencies unavailable	VTE event occurred all during active or relapse phase
3	Stassen et al. [97]	Uncontrolled retrospective cohort analysis	198 AAV (EGPA excluded) patients followed between 1990 and 2005	118/80	VTE: 60 (22–86) No VTE: 55 (14–81)	Mean follow-up 73 months	Incidence rate of 1.8 VTEs per 100-person-years Absolute frequencies of 6.9% for GPA and 30% for MPA	Incidence rate 6.7 events per 100-person-years during active disease
4	Allenbach et al. [95]	Uncontrolled retrospective cohort analysis	377 GPA, 236 MPA, and 232 EGPA patients followed between 1985 and 2006	NA	52.5 ± 17	Mean follow-up of 58.4 months	Overall incidence rate of 1.58 VTEs per 100-person-years. Absolute frequencies of 8% for GPA, 7.6% for MPA, 8.2% for EGPA	Incidence rate 7.3 events per 100-person-years during active disease
5	Faurschou et al. [100]	Uncontrolled retrospective cohort analysis	180 GPA patients were followed between 1993 and 2011	91/89	54 (41–62)	Mean follow-up 86 months	Overall incidence rate of 1.1 per 100-person-years Absolute frequency 8.3%	Incidence rate highest in first 2 years: 2.5 events per 100-person years
7	Novikov et al. [98]	Uncontrolled retrospective cohort analysis	243 GPA, 45 MPA, 69 EGPA patients followed between 2006 and 2014	NA	NA	NA	Overall incidence rate of Absolute frequencies 8.2% for GPA, 6.7% for MPA, 10.1% for EGPA	Most VTEs occurred during 1st year

AAV, ANCA-associated vasculitis; GPA, granulomatous polyangiitis; EGPA, eosinophilic GPA; MPA, microscopic polyangiitis; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; VTE, venous thromboembolic event.

in AAV patients, as cardiac involvement was still a strong predictor even though patients did not undergo standard cardiac screening.

We will be addressing the more historical studies. Although most of them did not provide details regarding cardiac assessment, they do clearly demonstrate an overview of the general, overt cardiac involvement in currently the largest dataset of AAV populations (Tables 20.5 and 20.6). In more recent years, several groups performed (prospective) cardiac evaluation in AAV patients, predominantly using cardiac MRI (Tables 20.7 and 20.8). First, the three distinct syndromes and their level of evidence for cardiac involvement will be briefly discussed, with more detailed information regarding each diagnostic modality and their associated findings.

3.1 Level of Evidence for AAV

3.1.1 Microscopic Polyangiitis (MPA)

To date, prospective studies evaluating cardiac involvement in MPA are lacking. The prevalence of heart involvement in GPA patients ranges from 8% to 31% (Tables 20.5 and 20.6) [109]. Using the previous classification criteria for PAN, which largely included MPA patients, cardiac involvement has frequently been demonstrated in these patients. Although clinically apparent features were rare, autopsy studies demonstrated myocardial necrosis and coronary vasculitis [110,111]. The first case report in 1999, using the new classification, demonstrated a case of acute heart failure, most likely related to active MPA [112]. Several case reports followed demonstrating different MPA-related cardiac abnormalities, including myocardial infarction [113], valvular disease [114], pericardial disease [115], and cardiac tamponade [116]. Several large datasets have been published over the last few years, although none of these studies prospectively performed cardiac screening.

3.1.2 Granulomatosis With Polyangiitis (GPA)

As mentioned in the previous section, six RCTs including GPA patients have been conducted, all providing details regarding cardiac involvement. More importantly, several prospective studies have been conducted to evaluate cardiac involvement, predominantly using cardiac MRI. The frequency of cardiac involvement varies widely, ranging from 6% to 44% in earlier studies, compared to 6–86% in more recent studies. One of the largest series of 155 GPA patients demonstrated a 25% prevalence of cardiac involvement [117]. Differences in reported prevalence might be explained by differences in disease activity and extent and selection bias, but also differences in applied diagnostic methods.

3.1.3 Eosinophilic Granulomatosis With Polyangiitis (EGPA)

The evidence for cardiac involvement in EGPA patients is substantial, with early autopsy studies already demonstrating extensive cardiac abnormalities [118–120]. To date, the reported prevalence of cardiac involvement ranges from 16–92%, depending on the variables mentioned in the previous GPA section, with 27% of patients demonstrating cardiac involvement in a large study including 383 EGPA patients [17]. In general, it seems that cardiac involvement is more prevalent and severe in EGPA as compared to GPA or MPA. Of note, several studies demonstrated that cardiac involvement in EGPA patients is associated with the absence of ANCA [121–123], in contrast to our prospective cardiac screening study [32].

3.2 Electrocardiographic Changes

Although rhythm disturbances are less well described, patients with AAV may develop a variety of conduction system disorders. Most studies involve case series, in particular GPA, with only limited studies including large AAV patient populations.

Most case reports demonstrated supraventricular arrhythmias (eg, atrial tachycardia, flutter, and fibrillation) and complete AV-block in GPA patients (Fig. 20.2) [18,124–127]. Atrial arrhythmias are a frequent observation in GPA patients, which is probably due to the extent of histopathological involvement of the sinus node and the frequent observation of pericarditis (~50%), as the sinus node is close to the epicardium [124]. In most cases the conduction disorder terminated after initiating glucocorticosteroids or cyclophosphamide, with only a few cases requiring a permanent pacemaker implantation. In 1999, Guillevin was the first to publish clinical manifestations in 96 EGPA patients [128]. Herein, transient heart block was present in 3 (3%) EGPA patients. Of note, not all patients underwent ECG and/or 24-h Holter registration, therefore electrocardiographic changes are likely to be underestimated. In a smaller but more comprehensive study of 49 EGPA patients, right or left bundle-branch block was found in 5 (10%), atrial fibrillation in 1 (2%), and nonsustained ventricular tachycardia in 2 (4%) patients [123]. A very recent prospective study published by our group performed cardiac screening in 50 EGPA and 41 GPA patients using multiple diagnostic methods also including electrocardiography (ECG) and 24-Holter ECG [32]. Herein, T-wave abnormalities were most frequently observed in 16 (32%) and 6 (15%) patients with EGPA and GPA, respectively. These included predominantly prolonged QT intervals and abnormally inverted T-waves. In addition, left or right bundle-branch block was demonstrated in 5 (10%) and 4 (10%) followed by atrial fibrillation in 2 (4%) and 2 (5%) patients with EGPA and GPA, respectively.

TABLE 20.5 Clinical Characteristics of AAV Patients Investigated With Echocardiography

Study#	Study/study type	Number of patients	Gender (M/F)	Age (years)	Detection of cardiac involv.	Follow-up (years)	Main result	Other CV abnormalities
1	Morgan et al. [148] retrospective cohort	12 EGPA 20 controls	NA NA	NA NA	Echocardiography, all patients	NA	4 (40%) cardiac involvement	NA Age-sex matched controls
2	Hoffman et al. [28] retrospective cohort	158 GPA	79/79	41 (Mean)	NA	8 (Mean)	5% Cardiac involvement	NA
3	Matteson et al. retrospective cohort [143]	77 GPA	49/28	45 (Mean)	NA	7.1 (Mean)	18% Cardiac involvement	NA
4	Guillevin et al. [138] prospective cohort	52 MPA	NA	53 ± 15	NA	>6	Five factor score, CMP increased relative risk of 2.18 for mortality	NA
5	Guillevin et al. [109] retrospective cohort	82 EGPA 85 MPA	NA 47/38	48 ± 15 57 ± 15	NA	5,8 ± 5,1	15 (18%) cardiac failure, 9 (11%) pericarditis, 2 (3%) myocardial infarction	29 (34%) hypertension
6	Guillevin et al. [128] retrospective cohort	96 EGPA	44/52	48 (Mean)	NA	NA	22% Pericardial effusion, 9% Cardiac failure	3% Transient AV block, 6% stroke, 1% VTE
7	Morelli et al. [150] retrospective cohort	9 GPA	2/7	54 ± 14	Echocardiography, all patients	NA	100% Cardiac involvement	NA
8	Reinhold–Keller [117] retrospective cohort	155 GPA	76/79	48 (13–74)	Echocardiography, nr. of pts underwent echo unknown	7 (Median)	30% Cardiac involvement	NA
9	Agard et al. [139] retrospective cohort	36 MPA	NA	60 (Mean)	NA	6.7 (Mean)	6 (17%) cardiac involvement	NA
10	Oliveira et al. [144]	85 GPA	55/35	58 ± 16	Echocardiography, all patients	8 ± 6 y	86% Cardiac involvement, 36% attributed to GPA	CAD 21%, hypertension 44%, DM 11%, smoking 53%
11	Bourgarit et al. retrospective cohort [140]	148 MPA 190 EGPA 257 PAN	352/243	54 ± 17	NA	NA	MPA: 16 (11%) CMP EGPA: 39 (21%) CMP	MPA: 20 (14%) CV involvement EGPA: 44 (23%) CV involvement
12	Pela et al. [151]	16 EGPA 20 controls	9/7 10/10	49 ± 10 49 ± 10	Echocardiography, all patients			Age-sex matched controls
13	Gibson et al. [141]	73 GPA 28 MPA	40/33 17/11	66 (Mean) 71 (Mean)	NA	5	14% Cardiac involvement 25% Cardiac involvement	NA
14	Neumann et al. retrospective cohort [123]	49 EGPA	23/26	43 ± 14	Echocardiography, all patients	NA	22 (45%) cardiac involvement	8 (16%) ECG abnormalities

15	de Souza et al. retrospective cohort [145]	134 GPA	64/70	43 ± 16	NA	3	15 (11%) cardiac involvement	70 (52%) hypertension, 14 (10%) DM
16	Szczeklik et al. prospective cohort [135] (QT-disp)	20 EGPA 20 controls	8/12	44 ± 9	Echocardiography, all patients	NA	13 (65%) cardiac involvement 0 (0%) cardiac involvement	NA Age-sex matched controls
17	Szczeklik et al. prospective cohort [134] Likely overlapping with study nr 16	20 EGPA 20 controls	7/13 7/13	43 ± 9 43 ± 10	Echocardiography, all patients	NA	18 (90%) cardiac involvement 0 (0%) cardiac involvement	14 (70%) patients cardiac history (5 pericarditis, 8 myocarditis, 1 myocardial infarction)
18	Miszalski-Jamka et al. [147] prospective cohort	22 GPA 22 controls	11/11 NA	47 ± 12 NA	Echocardiography, all patients	NA NA	7 (32%) cardiac involvement 0 (0%) cardiac involvement	12 (55%) hypertension, 11 (50%) hypercholesterolemia, 4 (18%) DM, 6 (27%) obesity Age-sex matched controls
19	Miszalski-Jamka et al. [149] prospective cohort	22 EGPA 22 controls	8/14 NA	43 ± 10 NA	Echocardiography, all patients	NA NA	7 (32%) cardiac involvement 0 (0%) cardiac involvement	7 (32%) hypertension, 8 (36%) hypercholesterolemia, 0 (0%) DM Age-sex matched controls
20	Cormamond et al. [17]	383 EGPA	199/184	50 ± 16	NA	5,6 ± 5,2	16% CMP 15% Pericarditis	8% VTE
21	Mahr et al. [14] meta-analysis of 5 intervention RCTs	277 MPA 396 GPA	143/134 215/181	62 ± 13 55 ± 14	NA	4,5 ± 3,0	23 (8%) cardiac involvement 41 (10%) cardiac involvement	NA
22	Guillevin et al. Intervention RCT [27]	87 GPA 23 MPA 5 renal- Limited AAV	65/50	55 ± 13	NA	2,3	25 (22%) cardiac involvement	NA
23	McGeoch et al. prospective cohort [146]	517 GPA	248/269	46 ± 20	Echocardiography, nr. of pts underwent echo unknown	NA	3.3% Cardiac involvement	NA
24	Hazebroek et al. [32] prospective cohort	50 EGPA 41 GPA 50 controls	33/17 25/16 30/20	59 ± 11 61 ± 10 61 ± 9	Echocardiography, all patients	4,4 ± 1,5	27 (54%) 18 (44%) 10 (20%)	Controls were age, gender, cardiovascular co-morbidities matched

AAV, ANCA-associated vasculitis; GPA, granulomatous polyangiitis, EGPA, eosinophilic GPA; MPA, microscopic polyangiitis; M, male; F, female; CV, cardiovascular; NA, not available; CMP, cardiomyopathy; CAD, coronary artery disease; DM, diabetes mellitus; VTE, venous thromboembolic event.

TABLE 20.6 Echocardiographic Details of AAV Patients

Study#	Study	LVEF (%)	WMA	DCM/CMP	Valvular disease	Pericardial effusion	Mitral regurgitation	Aortic regurgitation	Tricuspid regurgitation	Diastolic dysfunction (>2)
1	12 EGPA	25–47 (%FS)	NA	NA	4 (40%)	2 (20%)	4 (40%)	0 (0%)	0 (0%)	NA
2	158 GPA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	77 GPA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	52 MPA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	82 EGPA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	85 MPA	NA	17 (20%)	NA	NA	9 (11%)	NA	NA	NA	NA
6	96 EGPA	NA	10 (9%)	NA	NA	21 (22%)	NA	NA	NA	NA
7	9 GPA	NA	2 (22%)	NA	8 (100%)	5 (55%)	3 (33%) (all grades)	8 (100%) (all grades)	3 (33%) (all grades)	NA
8	155 GPA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	36 MPA	NA	NA	NA	NA	NA	NA	NA	NA	NA
10	85 GPA	51 ± 13	31 (36%)	23 (27%)	NA	7 (8%)	38 (45%) (all grades)	11 (13%) (all grades)	23 (27%) (all grades)	NA
11	148 MPA 190 EGPA 257 PAN	NA	NA	MPA: 16 (11%) EGPA: 39 (21%)	NA	NA	NA	NA	NA	NA
12	16 EGPA	68 ± 7	3 (19%)	NA	0 (0%)	6 (36%)	0 (0%)	0 (0%)	0 (0%)	5 (31%)
	20 controls	71 ± 5	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA
13	73 GPA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	28 MPA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14	49 EGPA	NA	11 (22%)	NA	6 (12%)	9 (18%)	3 (6%)	0 (0%)	3 (6%)	NA
15	134 GPA	NA	NA	NA	NA	NA	NA	NA	NA	NA
16	20 EGPA	52 ± 14	6 (30%)	NA	NA	13 (65%)	NA	NA	NA	NA
	20 controls	NA	0 (0%)	NA	NA	0 (0%)	NA	NA	NA	NA
17	20 EGPA	56 ± 16	7 (35%)	NA	1 (5%)	1 (5%)	1 (5%)	0 (0%)	0 (0%)	4 (20%)
	20 controls	NA	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
18	22 GPA	65 ± 7.5	7 (32%)	NA	0 (0%)	0 (0%)	6 (27%)	0 (0%)	0 (0%)	
	22 controls	NA	NA	NA	NA	NA	NA	NA	NA	
19	22 EGPA	57 ± 15	7 (32%)	NA	0 (0%)	4 (18%)	0 (0%)	0 (0%)	0 (0%)	6 (27%)
	22 controls	64 ± 3	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

20	383 EGPA	NA	NA	63 (16%)	NA	58 (15%)	NA	NA	NA	NA
21	277 MPA 396 GPA	NA	20	1	NA	17	NA	NA	NA	NA
22	87 GPA 23 MPA 5 renal-limited AAV	NA	2 (2%)	NA	NA	7 (6%)	NA	NA	NA	NA
23	517 GPA	NA	NA	5 (1%)	2 (0,4%)	7 (1%)	NA	NA	NA	NA
24	50 EGPA	57 ± 11	16 (32%)	11 (22%)	3 (6%)	2 (4%)	2 (4%)	2 (4%)	0 (0%)	9 (20%)
	41 GPA	58 ± 11	6 (15%)	2 (5%)	3 (8%)	1 (2%)	1 (2%)	2 (5%)	0 (0%)	7 (19%)
	50 controls	61 ± 7	3 (6%)	0 (0%)	3 (6%)	0 (0%)	2 (4%)	1 (2%)	0 (0%)	8 (16%)

AAV, ANCA-associated vasculitis; GPA, granulomatous polyangiitis; EGPA, eosinophilic GPA; MPA, microscopic polyangiitis; LVEF, left ventricular ejection fraction; WMA, wall-motion abnormalities; DCM, dilated cardiomyopathy; CMP, cardiomyopathy; FS, fractional shortening.

TABLE 20.7 Clinical Characteristics of AAV Patients Who Underwent Cardiac Magnetic Resonance Imaging (CMR)

Study#	Study	Number of patients	Sex (M/F)	Age (years)	Detection of cardiac involv.	History of cardiac disease	Clinical remission	Follow-up (years)	Main result	Other CV abnormalities
1	Wassmuth et al. [165] retrospective cohort	11 EGPA	4/7	44 (Median)	CMR, all patients	11 (100%)	NA	4 (Median)	100% Cardiac involvement	NA
2	Mavrogeni et al. [156] retrospective cohort	16 MPA 11 GPA 9 EGPA Controls: 20 RA 13 SLE 40 healthy	11/5 8/3 2/7 11/9 2/11 22/18	66 (Median) 60 (Median) 58 (Median) 65 (Median) 42 (Median) 61 (Median)	CMR, all patients	8 (50%) 5 (45%) 3 (33%)	NA	3–6 months	2 (13%) MPA LGE 3 (38%) EGPA LGE Coronary involvement predominantly in MPA (88%)	MPA: 3 (19%) smoking
3	Neumann et al. [123] retrospective cohort	49 EGPA	23/26	43 ± 14	CMR, 12 (25%) patients	1 (2%)	0 (0%)	Yes, duration unknown	22 (45%) cardiac involvement	8 (16%) ECG abnormalities
4	Marmursztejn et al. [166] retrospective cohort	20 EGPA	14/6	50 ± 14	CMR, all patients	4 (20%)	NA, exact number unknown	2,2	13 (65%) cardiac involvement	12 (60%) CV risk factors 6 (30%) hypertension, 8 (40%) hypercholesterolemia, 3 (15%) DM, 1 (5%) smoking
5	Marmursztejn et al. [167] prospective cohort	8 EGPA	4/4	43 ± 7	CMR, all patients	2 (25%)	0 (0%)	6 months	6 (75%) cardiac involvement	NA
6	Szczeklik et al. [134] prospective cohort	20 EGPA 20 controls	7/13 7/13	43 ± 9 43 ± 10	CMR, all patients	17 (85%)	Yes, all	NA	17 (85%) cardiac involvement	EGPA: 4 (20%) hypertension, 0 (0%) DM, 0 (0%) smoking Controls: 1 (5%) hypertension, 0 (0%) DM, 0 (0%) smoking
7	Miszalski-Jamka et al. [160,161] prospective cohort	11 EGPA 10 GPA 21 controls	7/4 4/6 11/10	43 ± 10 45 ± 11 44 ± 11	CMR, all patients	5/21 (24%)	Yes, all	NA	EGPA: 9 (82%) cardiac involvement GPA: 8 (80%) cardiac involvement Controls: 0 (0%) cardiac involvement	EGPA/GPA: 9 (43%) hypertension, 9 (43%) hypercholesterolemia, 1 (5%) DM, 1 (5%) obesity
8	Marmursztejn et al. [168] prospective study	20 EGPA	12/8	49 ± 9	CMR, all patients	10/20 (50%)	Yes, all	Yes, duration unknown	14 (70%) cardiac involvement	1 (5%) hypertension, 6 (30%) hypercholesterolemia, 2 (10%) DM, 2 (10%) smoking

9	Mavrogeni et al. [108] retrospective cohort	28 EGPA Controls: 28 disease 28 healthy	9/19 4/24 8/20	42±7 45±8 40±8	CMR, all patients	3/28 (11%)	21 (75%)	2	12 (43%) cardiac involvement 12 (43%) cardiac involvement 0 (0%) cardiac involvement	EGPA: 2 (7%) hypertension, 2 (7%) hypercholesterolemia, 1 (4%) DM, 0 (0%) smoking Disease: 1 (4%) hypertension, 2 (7%) hypercholesterolemia, 2 (7%) DM, 0 (0%) smoking Healthy: no risk factors
10	Dunogue et al. [169] retrospective cohort Partly overlapping with study 4	42 EGPA	25/17	47 (Mean)	CMR, all patients	NA	NA, exact number unknown	4,6 (mean)	25 (60%) cardiac involvement	8 (19%) hypertension, 10 (24%) hypercholesterolemia, 9 (21%) smoking, 5 (12%) DM, 5 (12%) obesity
11	Hazebroek et al. [32] prospective cohort	50 EGPA 41 GPA 50 controls	33/17 25/16 30/20	59±11 61±10 61±9	41 (82%) 28 (68%) NA	4 (8%) 3 (7%) 4 (8%)	Yes, all	4,3±2 4,0±1,3 4,7±-0,8	27 (54%) 18 (44%) 10 (20%)	Controls were age, gender, cardiovascular co-morbidities matched

AAV, ANCA-associated vasculitis; GPA, granulomatous polyangiitis; EGPA, eosinophilic GPA; MPA, microscopic polyangiitis; M, male; F, female; CV, cardiovascular; NA, not available; CMP, cardiomyopathy; CAD, coronary artery disease; DM, diabetes mellitus.

TABLE 20.8 Details of Cardiac Magnetic Resonance Imaging Performed in AAV Patients

Study#	Study	LVEF (%)	LVEDV (mL/m ²)	LVEF <50%	Edema	EGE	LGE	Pericardial effusion	RV involvement	MR	AR	TR	Diastolic dysfunction (>2)
1	11 EGPA	45 ± 15	94 ± 23	6 (55%)	4 (36%)	6 (55%)	9 (82%)	7 (64%)	4 (36%)	5 (45%)	0 (0%)	0 (0%)	NA
2	16 MPA	NA	NA	NA	NA	NA	2 (13%)	NA		NA	NA	NA	NA
	11 GPA						0 (0%)						
	9 EGPA						3 (38%)						
	Controls:												
	20 RA						0 (0%)						
	13 SLE						0 (0%)						
	40 healthy						0 (0%)						
3	12 EGPA	43 ± 9	NA	10 (83%)	NA	NA	10 (83%)	9 (18%)	NA	3 (6%)	0 (0%)	3 (6%)	NA
4	20 EGPA	52 ± 13	NA	7 (35%)	6 (30%)	NA	13 (65%)	10 (50%)	NA	NA	NA	NA	NA
5	8 EGPA	45 ± 19	NA	3 (38%)				6 (75%)					
6	20 EGPA	58 ± 17	76 ± 24	6 (30%)	6 (30%)	6 (30%)	17 (85%)	1 (5%)	12 (60%)	NA	NA	NA	7 (35%)
	20 controls	61 ± 3	74 ± 11	0 (0%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)	0 (0%)	NA	NA	NA	0 (0%)
7	11 EGPA	68 ± 6	66 ± 4	0 (0%)	NA	NA	9 (82%)	2 (18%)	4 (36%)	0 (0%)	0 (0%)	0 (0%)	NA
	10 GPA	66 ± 5	66 ± 9	0 (0%)	NA	NA	8 (80%)	2 (20%)	3 (30%)	0 (0%)	0 (0%)	0 (0%)	NA
	21 controls	63 ± 3	70 ± 8	0 (0%)	NA	NA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	NA
8	20 EGPA	47	NA	6 (30%)	4 (20%)	NA	14 (70%)	NA	NA	NA	NA	NA	NA
9	28 EGPA	67 ± 4*	126 ± 20**	7 (25%)	9 (32%)	NA	12 (43%)	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	NA
	Controls:												
	28 disease	64 ± 4	114 ± 17	1 (4%)	2 (8%)	NA	12 (43%)	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	NA
	28 healthy	65 ± 3	114 ± 17	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	NA	0 (0%)	0 (0%)	0 (0%)	NA
10	42 EGPA	NA	NA	17 (41%)	6 (14%)	8 (19%)	25 (60%)	12 (29%)	NA	NA	NA	NA	NA
11	41 EGPA	53 ± 14	84 ± 28	10 (24%)	1 (2%)	NA	9 (22%)	2 (5%)	1 (2%)	1 (2%)	2 (5%)	0 (0%)	NA
	28 GPA	58 ± 11	82 ± 20	4 (14%)	0 (0%)	NA	5 (19%)	1 (4%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	NA

AAV, ANCA-associated vasculitis; GPA, granulomatous polyangiitis; EGPA, eosinophilic GPA; MPA, microscopic polyangiitis; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end diastolic volume; EGE, early gadolinium enhancement; LGE, late gadolinium enhancement; RV, right ventricle; MR, mitral regurgitation; AR, aortic regurgitation; TR, tricuspid regurgitation.

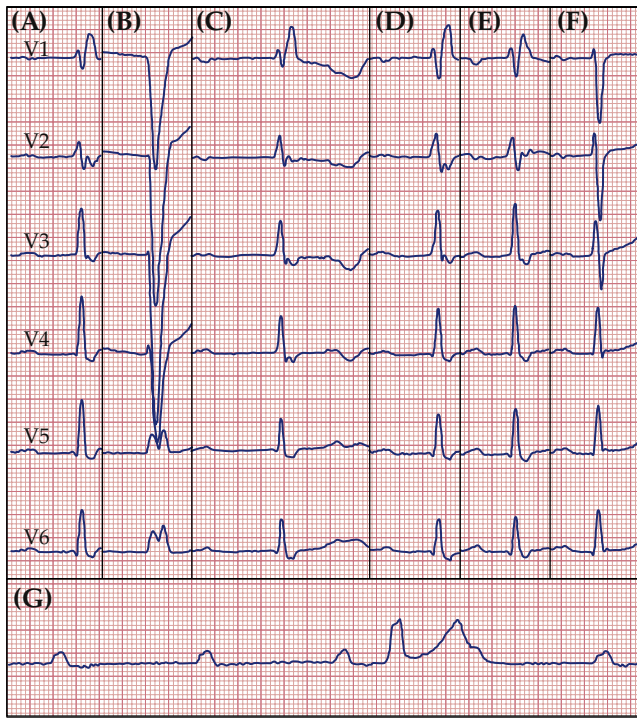


FIGURE 20.2 Time course of atrioventricular and intraventricular conduction disturbances demonstrated by the precordial leads (V1–V6) of representative electrocardiographic recordings. On admission I AV-block with complete right bundle-branch block was observed (A). Progressive worsening of conduction resulted in changes to intermittent left bundle-branch block configuration (B), II AV-block, type Mobitz (C), and complete AV-block (G). Under immunosuppressive therapy a gradual normalization of PR- and QRS-intervals was observed with recovery from all conduction defects (after 9 days (D), 12 days (E), and 42 days of therapy (F)). Adapted from Reinhard et al. [137].

Ventricular arrhythmias and sudden cardiac death are relatively rare in AAV patients, with only a few case reports published [129–133]. In 2010, a total of 20 EGPA underwent ECG and 24-Holter ECG demonstrating ventricular extrasystoles in 5 (including bigeminy, trigeminy, pairs, and ventricular tachycardia) and supraventricular extrasystoles in 4 patients [134]. Sustained ventricular tachycardia or fibrillation was not demonstrated in this study. In the same year, the latter research group studied the role of QT dispersion in 20, most likely the same, EGPA patients versus 20 control subjects [135]. As mentioned in previous chapters of this book, QT dispersion (QTd) is a simple 12-lead ECG marker reflecting the heterogeneity of ventricular repolarization. An increased QTd has been reported as an arrhythmogenic risk factor in various clinical groups [136]. QTc (heart rate corrected QT) dispersion (QTcd) was higher in EGPA patients as compared to healthy controls at both initial diagnosis (45.4 ± 14.2 vs. 26.1 ± 6.5 , $p < 0.0001$) and remission (38.6 ± 13.4 vs. 26.1 ± 6.5 , $p = 0.002$). At baseline, the 13 EGPA patients with cardiac involvement

demonstrated significantly higher QTd and QTcd as compared to EGPA patients without cardiac involvement (37.7 ± 12.7 vs. 24 ± 11.4 , $p = 0.008$ and 52.2 ± 12.1 vs. 34.7 ± 10.7 , $p = 0.007$, respectively). This difference remained significant at remission for QTcd, but not for QTd. Importantly, although significantly higher QTd and QTcd in EGPA patients were found as compared to controls, values are still within the normal range, which may explain the relatively low frequency of ventricular arrhythmias or sudden cardiac death in these patients.

3.3 Echocardiographic Changes

As mentioned before, the largest studies of AAV patients did provide prevalence of cardiac involvement based on imaging studies, although none presented actual echocardiographic data (Tables 20.5 and 20.6). As most cardiac abnormalities in these large studies are discovered using echocardiographic assessment, these studies are grouped in Table 20.6, and clearly demonstrate high prevalence of cardiac involvement. Importantly, only 12 studies reported actual echocardiographic parameters in either GPA or EGPA patients, with only seven studies providing comparison with control subjects. To date, no detailed echocardiographic data is available in MPA patients.

3.3.1 MPA

Early studies demonstrating cardiac involvement in MPA patients are scarce, most likely due to the nonexistent classification of MPA in the 1990 ACR criteria for vasculitis. In the 1990s, Guillevin and his national network of 93 centers published two large datasets including MPA and EGPA patients [109,138]. In 1996, although no actual echocardiographic data per patient group is provided, their national network was one of the first to establish the importance of cardiac involvement in 342 vasculitis patients, which included only 52 MPA and 82 EGPA patients [138]. Nevertheless, cardiomyopathy showed an increased relative risk of 2.18 for mortality and was included in the currently well-known five-factors score (FFS) together with central nervous system involvement, severe gastrointestinal involvement, renal failure (ie, serum creatinine >1.58 mg/dL, and high proteinuria (>1 g/dL)). Three years later, the same French network published a large, retrospective analysis of 85 MPA patients with associated data regarding heart involvement [109]. They observed heart failure, pericarditis, and myocardial infarction in 15 (18%), 9 (11%), and 2 (2%) patients, respectively. During a mean follow-up of 70 ± 61 months, a total of 6 (7%) patients developed cardiac events, including myocarditis ($n=3$), cardiomyopathy ($n=2$), and heart failure ($n=1$). A total of 4

(5%) patients eventually died of cardiac involvement, including heart failure ($n=2$) and myocardial infarction ($n=2$). Unfortunately, no details were given with respect to the diagnostic methods or definitions of cardiac involvement. A second larger study by the same group followed in 2003, including 36 MPA patients. At baseline, 3 (8%) MPA patients demonstrated cardiac involvement with an increase during a mean follow-up of 6.7 years to 6 (17%) patients [139]. Abnormalities included pericarditis and dilated cardiomyopathy with a higher prevalence in those patients diagnosed >90 days after the onset of symptoms. In 2005, a large retrospective study including 595 patients with polyarteritis nodosa, MPA, and EGPA was published evaluating their prognosis. Although the diagnostic methods for cardiac involvement are not provided, a total of 20 (14%) MPA patients showed cardiovascular involvement. Moreover, cardiomyopathy was present in 16 (11%) MPA patients [140]. New Zealand, a different geographical region, published data of 28 MPA patients demonstrating cardiac involvement in 7 (25%) of them [141]. In the following years, six randomized controlled trials including MPA and GPA patients were conducted, all focusing on novel treatment strategies. A comprehensive meta-analysis of five of these six RCT studies including 673 patients, 277 (41%) with MPA and 396 (59%) with GPA, was recently conducted [14]. A total of 64 (10%) patients, of which 23 (8%) were MPA patients and 41 (10%) were GPA patients demonstrated cardiovascular manifestations. Cardiovascular details were available in 40/64 patients including 17 with pericarditis, 13 with congestive heart failure, 7 with myocardial infarction or angina, 6 with bruits, 4 with pericardial pain or rub, and 1 with cardiomyopathy [14]. A very recent study including 115 AAV patients (including 87 GPA patients, 23 MPA patients, and 4 renal-limited AAV patients) demonstrated cardiac involvement in 25 (22%) patients. In line with previous studies pericarditis, cardiomyopathy and conduction abnormalities were predominantly observed in these patients [27]. Of note, cardiac examination in these MPA and GPA patients was only performed upon clinical indication and the imaging modality used is not specified in these RCTs.

3.3.2 GPA

In the 1990s, three studies including large GPA cohorts were performed involving 158, 265, and 77 patients, respectively [28,142,143]. Hoffman et al. demonstrated cardiac involvement in 8 (5%) of 158 GPA patients at disease onset. Unfortunately, the largest study performed by Anderson et al. did not provide information regarding cardiac involvement. Matteson showed 14 (18%) GPA patients with cardiac involvement at presentation, and cardiac disease was the

second cause of death, involving 5 (18%) of 28 deaths. The latter three studies did not provide information regarding the detection of cardiac involvement, as the main focus involved prognosis in GPA patients. In 2000, Reindholdt-Keller et al. evaluated prognostic factors in 155 GPA patients. Upon clinical indication, patients underwent ECG, echocardiography, thallium scintigraphy, coronary angiogram, or myocardial biopsy. Although no details were given with respect to cardiac involvement, they did demonstrate involvement in 8% and 30% at onset of disease and over the whole course of the disease, respectively [117]. Of the 22 patients who died after a median follow-up of 7 [0.3–27.3] years, 2 (9%) patients demonstrated cardiac involvement at diagnosis. Of note, 4 (18%) of the 22 deaths were related to coronary artery disease. A retrospective study from the Mayo Clinic in 2005 was the first to include a large group of 85 GPA patients who all underwent echocardiographic analysis [144]. Although biased, as only 27% of patients were asymptomatic, a total of 73 (6%) patients were found to have echocardiographic abnormalities. In 26 (36%) of these 73 patients, abnormalities could be directly related to GPA as they could not be explained by other comorbidities and cardiac risk factors. Nine patients had acute dilated cardiomyopathy during an acute flare of GPA. Two of these patients demonstrated full recovery of echocardiographic parameters after initiation of specific immunosuppressive therapy. Sporadic findings included intracavitary thrombus in 1 patient and a large granulomatous mass in the left ventricular outflow tract in another. An important finding was the presence of wall-motion abnormalities, which, in most cases, were not confined to a specific coronary artery territory and occurred predominantly in asymptomatic patients. Moreover, a significant increased mortality rate was demonstrated in patients with cardiac involvement attributed to GPA ($p=0.02$), with a high 1- and 5-year mortality rate of 29% and 43%, respectively. An Australian cohort including 28 MPA and 73 GPA patients demonstrated cardiac involvement in 15 (20%) and 7 (25%), respectively [141]. Four (40%) of the 10 deaths in MPA patients were related to cardiomyopathy and three (16%) of the 19 deaths in GPA patients were related to cardiomyopathy. In 2010, a Brazilian tertiary center published data on 134 GPA patients [145]. Cardiac involvement was present in 15 (11%) of all patients and during follow-up 8 (6%) developed myocardial infarction, 7 (5%) new strokes, and 3 (2%) VTE. Interestingly, 4 (20%) of the 20 deaths were related to myocardial infarction. A large GPA population from Northern America was very recently published, including 517 patients [146]. They specifically focused on cardiac involvement secondary to GPA, and although prospectively including patients

between 2006 and 2013, their study lacked screening of (asymptomatic) patients and cardiac involvement was solely based on patient history or medical files. This resulted in a very low detection of cardiac involvement (3.3%) in this large GPA population.

Data on actual prospective evaluation of cardiac involvement is scarce, with currently only a handful of studies published [32,147]. One study from Poland prospectively evaluated 22 GPA patients using speckle-tracking echocardiography [147]. They demonstrated regional wall-motion abnormalities in 7 (32%) patients using standard echocardiographic measurements. Of note, normal cardiac function was present in all patients indicated by LVEF > 50% in all. Using speckle-tracking echocardiography, global systolic dysfunction could be shown in 16 (73%) GPA patients. Although these abnormalities corresponded to the extent and severity of GPA,

the prognostic and therapeutic role of these speckle-tracking echocardiographic abnormalities should be further investigated. The largest prospective study to date performing in-depth cardiac screening in 41 GPA patients in clinical remission was recently published by our research group [32]. We found a two-fold higher prevalence of cardiac involvement in GPA patients as compared to age, gender, and cardiovascular comorbidities matched control subjects (44% vs. 20%, $p = 0.01$; Fig. 20.3). Importantly, echocardiographic abnormalities could still be detected in approximately one-third of these patients without clinical symptoms. On the other hand, echocardiographic abnormalities in control subjects were predominantly observed in those with symptoms. This illustrates the fact that predicting cardiac involvement in GPA patients purely based on symptoms or ECG abnormalities is unreliable, in contrast to control

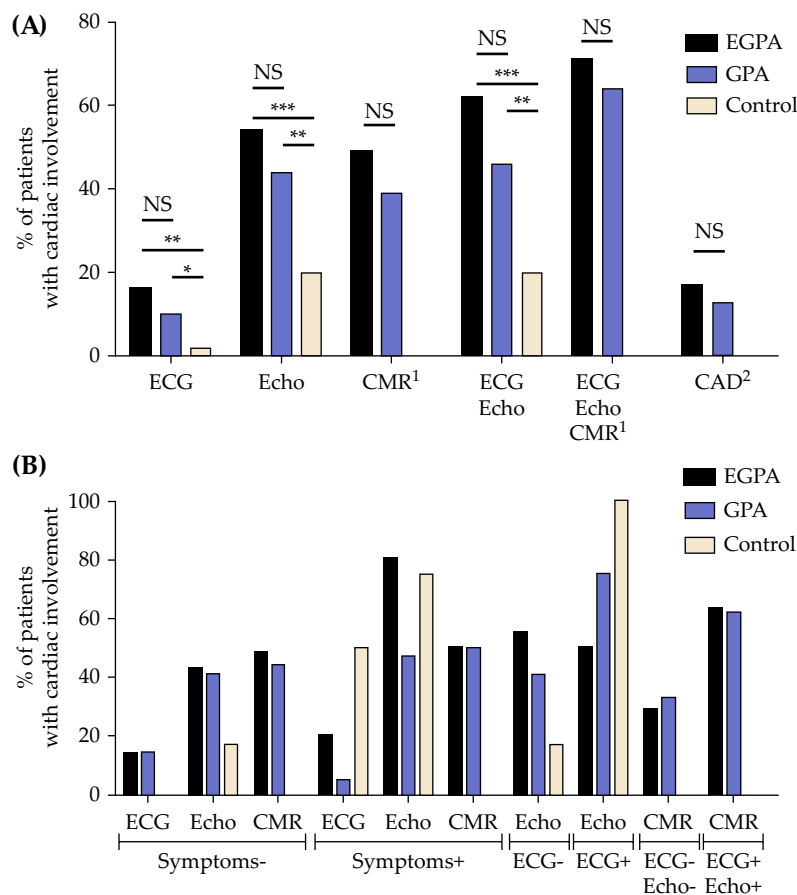


FIGURE 20.3 Cardiac evaluation in EGPA and GPA versus controls by different diagnostic modalities. (A) Cardiac involvement assessed by different diagnostic modalities in EGPA, GPA, and control patients. Percentages demonstrate the number of patients with cardiac involvement based on the respective diagnostic method used [1]. Includes the 69 patients who underwent CMR [2] and the 65 patients who underwent coronary artery disease assessment. (B) Cardiac involvement assessed by different imaging modalities including symptoms and presence of cardiac abnormalities on ECG and/or echocardiography as defined by the criteria used in the methodology. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; NS, not significant; ECG, electrocardiogram; CMR, cardiac magnetic resonance imaging; CAD, coronary artery disease; EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis. *Symptoms-*, no cardiac symptoms; *symptoms+*, cardiac symptoms present; *ECG+*, major ECG abnormalities; *ECG-*, no major ECG abnormalities; *Echo-*, no cardiac involvement on echocardiography; *Echo+*, cardiac involvement on echocardiography; *CMR-*, no cardiac involvement on CMR; *CMR+*, cardiac involvement on CMR. Adapted from Hazebroek et al. [32].

subjects. In the majority of GPA patients with cardiac involvement, regional and global wall-motion abnormalities were found (15%), followed by valvular regurgitation (8%).

3.3.3 EGPA

With respect to EGPA patients, an overwhelming amount of case reports and series showing cardiac involvement has been published in recent decades. In the 1980s, Lanham et al. drew attention to cardiac involvement as it accounted for death in 48% of patients with the condition [118]. A few years later (1989) one of the first larger series using echocardiography in 12 EGPA patients was published [148]. Cardiac involvement was seen in 4 (40%) EGPA patients, reflected by moderate to severe mitral regurgitation, followed by pericarditis in 2 (20%) EGPA patients. In the previously mentioned study of Guillevin in 1999, one of the first large case series evaluating clinical manifestations in EGPA patients, pericardial effusion was seen in 21 (22%) and cardiac failure in 9 (9%) of EGPA patients at presentation [128]. During follow-up, one additional patient presented with pericarditis and another with cardiac failure. Importantly, of the 10 EGPA patients presenting with cardiac failure (ie, all LV dysfunction) 8 patients died during the acute phase of the disease despite immunosuppressive therapy that was available in that time period. The large retrospective study from Bourgarit et al. including 595 PAN, MPA, and EGPA patients, revealed 44 (23%) EGPA patients with cardiovascular involvement and 39 (21%) with cardiomyopathy [140]. In 2009, a total of 49 EGPA patients underwent ECG, echocardiography, cardiac MRI, and endomyocardial biopsy [123]. On echocardiographic assessment, 9 (18%) showed pericardial effusion, 6 mitral valve insufficiency (3 mild, 3 moderate), 3 aortic valve insufficiency (all mild), and 7 tricuspid valve insufficiencies (4 mild, 3 moderate). Cardiac impairment, excluding those with valvular problems, was seen in 11 patients: mild (LVEF 40–55%) in 6 patients, moderate (LVEF 30–39%) in 1 patient, and severe (LVEF <30%) in 4 patients. In addition, 6 patients revealed pulmonary hypertension and thrombotic structures within the ventricular chambers were observed in 4 patients. In summary, 22 (45%) of 49 EGPA patients demonstrated cardiac involvement using echocardiography. In 2013, the French Vasculitis Study Group published a large dataset of 383 EGPA patients diagnosed between 1957 and 2009, including 6 prospective trials not previously mentioned here [17]. They demonstrated cardiomyopathy and pericarditis in 63 (16%) and 58 (15%), respectively.

Similar to GPA, only a handful of studies prospectively evaluated cardiac involvement in EGPA patients. A recent study from 2011, although focusing on QT dispersion, also assessed all 20 EGPA patients using echocardiography demonstrated cardiac involvement in 13

(65%) patients [135]. The same group published several articles, most likely with overlapping patient populations. In the same year, 7 (35%) of 20 EGPA patients demonstrated wall-motion abnormalities [134]. In 2012, 22 EGPA patients underwent speckle-tracking echocardiography, demonstrating abnormalities in 7 (32%) patients using standard echocardiographic abnormalities [149]. Applying speckle-tracking echocardiography, they suggested that impaired systolic dysfunction may result from impaired contraction of inner and middle but not outer myocardial fiber layers. To date the largest prospective study was performed by us, evaluating 50 EGPA patients (Fig. 20.3). Similarly to GPA patients, we found a two-fold higher prevalence of cardiac involvement in EGPA patients as compared to matched control subjects (54% vs. 20%, $p < 0.001$; Fig. 20.3). Moreover, also in these patients echocardiographic abnormalities could still be detected in approximately one-third without clinical symptoms. This again emphasizes the fact that predicting cardiac involvement in EGPA (and GPA) patients purely based on symptoms or ECG abnormalities is unreliable, in contrast to control subjects.

3.4 Cardiac MRI and Associated Abnormalities

The fairly recent introduction of cardiac MRI has led to numerous case reports and a few larger studies evaluating its role in the detection myocardial damage in AAV patients. Noninvasive tissue characterization has been made possible by cardiac MRI, enabling detection and differentiation of reversible and irreversible myocardial damage, often preceding hypocontractility seen on echocardiography and therefore particularly useful in conditions such as myocarditis and myocardial vasculitis [130,152–155]. These conditions are frequently undetected by standard imaging procedures (echocardiography or nuclear imaging techniques) during early stages of disease, as these techniques are unable to distinguish slight tissue structure changes (edema, cell infiltration) that occur without associated changes in left ventricular ejection fraction. Cardiac MRI is the only noninvasive technique that can give early, reliable, and reproducible information about myocarditis and myocardial vasculitis, entities commonly seen in vasculitis patients [152]. A summary of cardiac MRI abnormalities found in AAV patients is shown in Tables 20.7 and 20.8

3.4.1 MPA

Cardiac MRI data is very limited in MPA. To date, only one recent study evaluated 36 AAV patients using cardiac MRI, including 16, 11, and 9 patients diagnosed with MPA, GPA, and EGPA, respectively [156]. Although focusing on coronary artery lesions in AAV patients without evidence of cardiac symptoms or signs, myocardial necrosis using late gadolinium enhancement (LGE)

was found in 2 (13%) and 3 (38%) of MPA and EGPA patients, respectively. Remarkably, no LGE was found in GPA patients.

3.4.2 GPA

Currently, the use of cardiac MRI to detect cardiac abnormalities in patients diagnosed with GPA is limited to case reports [157–159] and a few studies as described below.

As discussed in the previous section, Mavrogeni et al. was the first to evaluate 36 AAV patients, including 11 GPA patients. Although no cardiac functional measurements were performed, no GPA patient demonstrated LGE lesions in this study. This result is in contrast to other cardiac MRI studies in GPA patients. The lower disease extent, relatively shorter duration of disease, and no history of cardiac disease as compared to other studies might be an explanation of this discrepancy.

A group from Poland performed several studies evaluating cardiac involvement in both GPA and EGPA patients using echocardiography and cardiac MRI. Their group was one of the first to perform cardiac MRI functional measurements in a relatively larger group of 11 GPA patients [160]. Of note, one year later the same GPA patients were used in another article, adding 10 EGPA patients to the analysis [161]. As discussed previously, they also reported global left ventricular strain measured by speckle-tracking echocardiography in both EGPA and GPA patients. In this study, segmental peak-systolic myocardial strain using feature tracking cine-sequence based MRI was used to detect subclinical myocardial involvement. In addition, standard cardiac MRI functional and LGE measurements were performed and compared with 21 age- and gender-matched controls. Interestingly, despite clinical remission, normal ECG, and echocardiography, a high prevalence of decreased segmental myocardial strain and nonischemic LGE lesions were found in both GPA and EGPA patients. Herein, midmyocardial LGE lesion was the most predominant localization of LGE in both GPA and EGPA. Similar results were found in our recent study, where approximately 50% of GPA patients showed cardiac abnormalities, despite being asymptomatic and demonstrating a normal ECG [32]. Herein, a total 28 (65%) GPA patients underwent cardiac MRI, while the remaining patients had predominantly contraindications for cardiac MRI including impaired renal function and contrast allergy. A total of 12 (43%) GPA patients had abnormalities on cardiac MRI, with the most predominant finding being wall-motion abnormalities followed by LGE in 6 (21%) and 5 (19%) patients, respectively (Fig. 20.4).

3.4.3 EGPA

Several case reports, including one of the first from our group, had already demonstrated cardiac abnormalities

more than a decade ago [162–164]. One of the first larger studies evaluated 11 EGPA patients using cardiac MRI [165]. They found myocardial damage in all patients, predominantly LGE and pericardial effusion in 9 (82%) and 7 (64%), respectively (Tables 20.7 and 20.8). Importantly, in 8 of the 9 patients in whom lesions were identified, the lesions were not confined to a single territory. Moreover, 4 (36%) patients showed lesions stretched out in the right ventricle, illustrating the global cardiac involvement in a subset of these patients. Subendocardial fibrosis in the absence of coronary artery disease was seen in 4 (36%) patients. No significant correlation was found between systolic function and the presence or extent of fibrotic lesions. The authors suggested that EGPA patients might present with a somewhat unique LGE pattern, as subendocardial fibrosis was predominantly found in contrast to midmyocardial or subepicardial fibrosis in nonischemic cardiomyopathies [152]. Similar findings were published by Neumann et al. 1 year later, demonstrating global subendocardial fibrosis in the 59% of EGPA patients with cardiac involvement [123]. Moreover, cardiac abnormalities were associated with high eosinophil counts and negative ANCA test.

A group from Paris published several papers with respect to cardiac MRI in EGPA patients [166–168]. The first cross-sectional study demonstrated cardiac involvement in 13 (65%) EGPA patients using cardiac MRI, with predominantly LGE and pericarditis in 65% and 50% of patients, respectively [166]. Cardiovascular risk factors were not predictive for cardiac abnormalities in these patients and 4 of 13 asymptomatic patients still demonstrated cardiac involvement on cardiac MRI. One year later, 8 EGPA patients were prospectively evaluated using cardiac MRI both at baseline and at 6 months' follow-up, after initiating immunosuppressive therapy [167]. In 75% of patients LGE abnormalities were found at baseline, which normalized in 3 and showed marked attenuation in 1 after 6 months' follow-up. Moreover, perfusion defects markedly improved in 2 and completely normalized in 1 after 6 months of immunosuppressive therapy. Follow-up cardiac MRI could therefore be of value to evaluate the effect of immunosuppressive therapy. In 2013, another 20 EGPA patients underwent both cardiac MRI in combination with fluoro-2-deoxyglucose PET (FDG-PET) to distinguish the cardiac MRI-detected LGE from fibrosis or inflammation using glucose uptake. Herein, hypofixation corresponds with fibrosis and hyperfixation corresponds with active inflammation. All EGPA patients were in remission. Cardiac MRI detected LGE in 14 (70%) patients, including fibrosis in 10 (50%), active inflammation in 2 (10%), and normal FDG-PET in 2 (10%). Importantly, the frequently observed endomyocardial fibrosis in the previous studies [123,165] could not be confirmed in these three studies, as the most predominant localization was midmyocardial fibrosis.

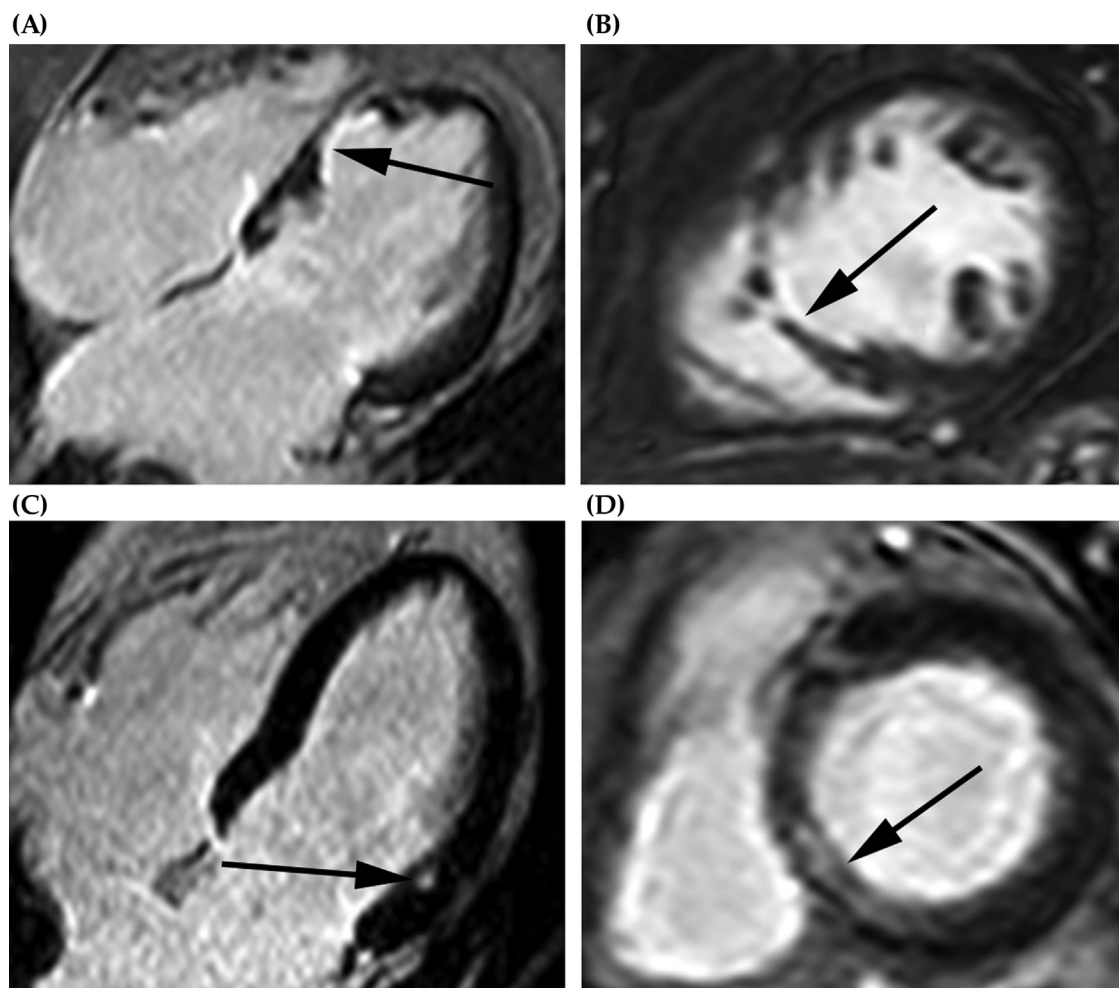


FIGURE 20.4 Cardiac MRI of two representative GPA patients and their LGE patterns. Subendocardial late gadolinium enhancement (LGE) with reduced wall thickness in a 64-year old GPA patient, demonstrated in 4-chamber (A) and short-axis plane (B). Small basal midmyocardial LGE in a 60-year old GPA patient, demonstrated in 4-chamber (C) and short-axis plane (D). *Adapted from the personal collection of the authors.*

In the same time period as the three previous studies, two other studies appeared: one from the group in Poland and one from Mavrogeni et al. [108,134]. The group from Poland published a multimodality approach in 20 EGPA patients using ECG, 24-h Holter registration, echocardiography, and cardiac MRI [134]. Again, LGE was the most prominent abnormality in 17 (85%) of patients in remission, whereas midmyocardial fibrosis was the most frequently observed localization. The results are in line with the three studies from the group in Paris. In contrast, Mavrogeni et al. demonstrated again predominantly diffuse subendocardial fibrosis in 10 (36%) of EGPA patients [108]. This phenomenon was associated with a decline in cardiac function after 2 years. In addition, more severe and clinically overt cardiac involvement was significantly higher in ANCA-negative EGPA patients.

Very recently, the same group from Paris retrospectively analyzed 42 EGPA patients who underwent cardiac MRI and assessed the impact on outcome after a

mean of 4.6 years follow-up [169]. The exact overlap with the three previously published articles remains unclear. Nevertheless, cardiac involvement was found in 60% of patients. The high prevalence of LGE in 82% of patients with cardiomyopathy was in line with studies discussed previously. In addition, no improvement on cardiac MRI was associated with increased cardiac event rate during follow-up, again suggesting a prognostic role for serial cardiac MRI evaluation as previously shown by another group [167]. The largest prospective study to date including 41 EGPA patients was performed by us [32]. Despite all being in remission, cardiac MRI still detected abnormalities in 20 (49%) patients (Fig. 20.5), irrespective of ECG abnormalities or asymptomatic presentation.

3.5 Pericardial Involvement

Involvement of the pericardium ranges from pericarditis and pericardial effusion to chronic constrictive

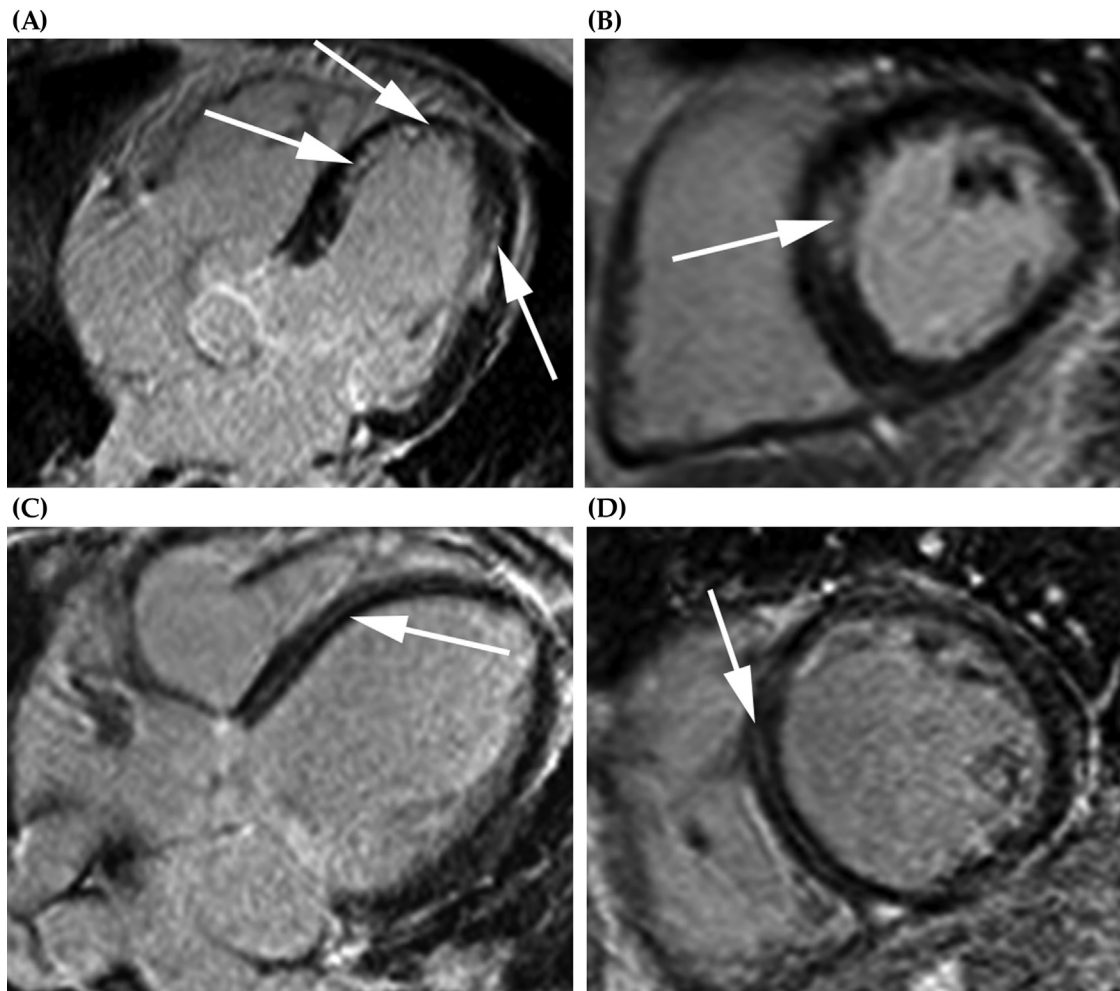


FIGURE 20.5 Cardiac MRI of two representative EGPA patients and their LGE patterns. Diffuse subendocardial LGE in a 56-year old EGPA patient, demonstrated in 4-chamber (A) and short-axis plane (B). Midmyocardial LGE in a 61-year old EGPA patient, demonstrated in 4-chamber (C) and short-axis plane (D). *Adapted from the personal collection of the authors.*

pericarditis, although the latter occurs rarely. In line with other cardiac abnormalities in AAV patients, the prevalence of pericardial involvement varies widely. The larger studies give insight into pericardial involvement, although only screen patients upon clinical indication. Therefore numbers might be underestimated. Differences in prevalence between imaging studies, also including asymptomatic patients, is most likely due to differences in disease state. Nevertheless, pericardial involvement seems to be one of the most prevalent abnormalities within AAV patients (Tables 20.6 and 20.8), which fortunately tends to respond well to immunosuppressive therapy.

3.5.1 MPA

One of the first autopsy studies already demonstrated the high prevalence of pericardial involvement in GPA patients, showing postmortem signs of pericarditis in

50% of cases [124]. One of the larger datasets with associated cardiac involvement is from the French Vasculitis network, demonstrating pericarditis in 9 (11%) patients (Tables 20.6 and 20.8). The same group published data of 36 MPA patients a few years later, demonstrating pericarditis as the most prevalent abnormality (6%), including one recurrent pericarditis patient [139]. Of note, other larger studies besides the French network did not demonstrate data regarding pericardial involvement. However, the recent meta-analysis including 673 patients, 277 (41%) with MPA, and 396 (59%) with GPA showed pericarditis and heart failure to be the most prevalent cardiac abnormalities in MPA patients, 3% and 2%, respectively. In addition, 4 patients demonstrated signs of pericardial rub or complaints of pericardial pain [14].

Unfortunately, prospective imaging studies in MPA patients that provide data on pericardial involvement are currently lacking.

3.5.2 GPA

The prevalence of pericardial involvement in GPA is most likely between 2% and 6% (Tables 20.6 and 20.8). Although two smaller studies demonstrated pericardial effusions in 55% [150] and 20% [160], the larger studies demonstrated lower, and probably more reliable, prevalences [32,144,146]. Despite the fact that the largest studies did not provide details regarding cardiac involvement, a fairly large retrospective study of 85 GPA patients demonstrated pericardial effusion in 7 (8%) patients, of whom 5 (6%) could be attributed to GPA [144]. Of these 5 patients, 1 had severe pericardial effusion, which resolved with immunosuppressive therapy. Moreover, no patient demonstrated pericardial tamponade or effusions that required therapeutic pericardiocentesis. Recently, a large study from Northern America including 517 GPA patients found that pericarditis was the most common abnormality found in these patients (1%) [146]. However, their overall rate of cardiac involvement (3.3%) was very low and is most likely due to the lack of screening of (asymptomatic) patients and characterizing involvement solely on patient history or medical files, as mentioned above.

Only three prospective studies have evaluated cardiac involvement in GPA patients. One study found no pericardial abnormalities in 22 GPA patients [147]. Of note, all patients in this study had normal cardiac function (LVEF > 50%). The same research group performed CMR in 10 GPA patients, demonstrating pericardial effusion in 2 (20%) GPA patients [161]. The largest prospective study, which was recently published by our research group, demonstrated pericardial effusion in 1 (2%) of 41 GPA patients.[32]

3.5.3 EGPA

The prevalence of pericardial involvement in EGPA is around 20% (Tables 20.6 and 20.8). Indeed, the prevalence of all studies ranges from 4% to 85%, but most of the larger studies demonstrate rates between 15 and 22%.

The first larger series in 1989 using echocardiography in 12 EGPA patients already showed pericardial involvement in 20% of patients, reflecting the prevalent role of the pericardium as a target in EGPA patients. Ten years later this was confirmed by Guillevin et al. in a larger patient population, demonstrating pericardial effusion in 21 (22%) of 96 EGPA patients. Similar rates were found in 2009, showing 9 (19%) of 49 EGPA patients. The French Vasculitis Study group published recently a large dataset of 383 EGPA patients, yet again demonstrating pericarditis in 58 (15%) patients. Finally, the recently published prospective study by our research group demonstrated pericardial effusion in 9 (22%) of 41 EGPA patients using CMR.

3.6 Valvular Abnormalities

3.6.1 Valvular Involvement

Valvular involvement, similarly to pericardial involvement, is a frequent observation in AAV patients. It can affect virtually all valves, although mainly involves the mitral and aortic valves. Valvular dysfunction may occur due to perforation and/or diffuse thickening and inflammation of valve leaflets, also known as valvulitis and/or endocarditis. Of note, false-positive ANCA antibodies may occur in the setting of bacterial endocarditis [170], making distinction between the two diseases challenging though of utmost importance to initiate the proper medical management. The prevalence of valvular involvement predominantly depends on which grading is classified as involvement. Unfortunately, the largest studies of AAV patients did not provide details regarding valvular involvement, but relatively large imaging studies are still available.

3.6.2 MPA

Despite the fact that the largest studies on AAV patients predominantly include MPA patients, imaging studies providing details regarding valvular abnormalities in MPA patients are currently lacking. Only a handful of case reports have described aortic valve dysfunction or large vessel involvement such as the aorta. A recent case report describes an MPA patient with acute progression toward aortic valve insufficiency within 2 months [114]. The authors postulated that anti-MPO antibodies induced vasa vasorum vasculitis and this, in turn, produced pathology of the aortic valve. This hypothesis was generated based on the histological observations of another MPA patient presenting with an aortic aneurysm, showing severe stenosis of the vasa vasorum due to intimal hyperplasia and decreased numbers of elastic fibers in the media [171]. Although rather rare, these cases should be taken into consideration, yet again emphasizing the need for imaging studies, not only to detect valvular abnormalities but also other cardiac pathologies.

3.6.3 GPA

Over the years, several case reports have been published demonstrating valvular involvement in GPA patients [172–174]. Of note, in a recent case series of 19 GPA patients, 17 (89%) presented with aortic regurgitation, 7 (37%) with mitral regurgitation, and 1 with aortic stenosis [174]. Valve replacement was required in most cases, whereas immunosuppressive therapy allowed complete resolution of valvular lesions in only 2 patients. This reflects the severity of initial valvular damage, illustrated by the low probability of complete resolution of the valvular defects.

Currently, only four larger studies have provided details regarding valve dysfunction, of which half was

prospectively assessed. Of note, the two retrospective studies from Morelli et al. [150] and Oliveira et al. [144] demonstrated the highest rates of valve dysfunction, including aortic regurgitation in 9 (100%) and 11 (13%), mitral regurgitation 3 (33%) and 38 (45%), and tricuspid regurgitation in 3 (33%), and 23 (27%) patients, respectively. With respect to the prospective studies from Miszalski-Jamka et al. [147] and Hazebroek et al. [32], lower rates of valvular involvement were shown, including aortic regurgitation in zero and 2 (5%), mitral regurgitation 6 (27%) and 1 (2%), and tricuspid regurgitation in no patients, respectively. These lower rates are most likely related to a different study population and grading of dysfunction, as Miszalski-Jamka et al. included patients with normal cardiac function and our research group included GPA patients in clinical remission with considering only moderate to severe dysfunction as cardiac involvement.

3.6.4 EGPA

Of the three AAV diseases, EGPA has been investigated most thoroughly using cardiac imaging techniques. Currently, at least eight original studies provide details regarding valvular involvement, ranging from 0% to 45% (Tables 20.6 and 20.8). Although the actual rate of valvular involvement is most likely around 4–6%, given the results of the largest studies. Overall, mitral regurgitation occurs most frequently, with the first echocardiographic study published in 1989 demonstrating mitral regurgitation in 4 (40%) patients without dysfunction of other valves. Strikingly, the five following echocardiographic studies showed lower rates of mitral valve dysfunction, ranging from 0% to 6% [32,123,134,148,149,151]. The two largest echocardiographic studies also showed involvement in either the tricuspid valve or the aortic valve (6% and 4%, respectively) [32,123].

Similar results are found in the six studies using cardiac MRI data. Of note, two of these five studies used the same patients as the previous echocardiographic data [32,123], and one likely overlapped the previous work [161]. Of the two remaining independent studies, the smallest study demonstrated 5 (45%) EGPA patients with mitral dysfunction without any other valve affected [165], whereas the largest study showed no valvular involvement in 28 EGPA patients [108].

3.7 Coronary Artery Disease

Surprisingly, although the occurrence of accelerated atherosclerosis in AAV patients is well established, currently only one study focused on properly visualizing coronary arteries in these patients. This study focused on magnetic resonance angiography (MRA) in 36 AAV patients using both diseased and healthy controls to evaluate differences [156]. Interestingly, MPA and GPA

demonstrated significantly increased maximal diameters of coronary arteries compared with healthy controls and with diseased controls, but not EGPA patients. In addition, fusiform coronary aneurysms were only detected in patients with MPA (25%) and coronary ectatic disease was found in the majority of MPA (88%) and GPA (18%). In addition, several cases have been described of patients presenting with acute coronary syndrome (ACS), including GPA [130,159,175,176] or EGPA patients [177]. Although the latter mimicked ACS and no significant CAD was detected, transient coronary spasm may occur in EGPA patients [178].

Unfortunately, most larger studies characterize CAD purely based on previous history and/or cardiac symptoms suggestive for ischemia, with only a minority performing CAG or other objective diagnostic tests to evaluate CAD. Moreover, those studies objectively evaluating CAD merely performed diagnostic tests in case of symptoms or other abnormalities suggesting ischemic heart disease. Therefore the following studies should be considered with caution as selection bias might have occurred [179,180]. Two large studies evaluated AAV patients and the incidence of cardiovascular events. Morgan et al. conducted a retrospective study comparing the incidence of cardiovascular events in a cohort of AAV patients with that in a matched cohort of patients with chronic kidney disease (CKD) [179]. This matched cohort was selected to correct for renal impairment as well as other traditional risk factors, finally identifying any potential excess cardiovascular risk in AAV patients. A total of 113 of 131 patients diagnosed with AAV from a vasculitis clinic registry were matched 1:1 for renal function, age at diagnosis, sex, smoking status, and previous history of a cardiovascular disease to patients with noninflammatory CKD. Herein, cardiovascular events were defined as acute coronary syndrome, new-onset angina, symptomatic peripheral vascular disease, stroke, and transient ischemic attack. Interestingly, a significant increased risk of a cardiovascular event for AAV patients as compared to CKD was found (hazard ratio (HR) 2.23 [95% confidence interval (CI): 1.1–4.4], p -value=0.017). The strongest predictors of adverse outcome after a median follow-up of 3.4 years were previous history of cardiovascular disease (HR 4 [95% CI 1.7–9.8]), history of dialysis dependency (HR 4.3 [95% CI 1.5–12.1]), history of smoking (HR 3.9 [95% CI 1.5–10]), age at diagnosis (HR 1.038 [95% CI 1.006–1.072]), estimated glomerular filtration rate at remission (HR 0.977 [95% CI 0.957–0.998]), and serum cholesterol concentration at presentation (HR 0.637 [95% CI 0.441–0.92]). Of note, only two patients with EGPA were included, while the majority were patients diagnosed as GPA ($n=65$) and MPA ($n=46$). Unfortunately, the cohort was underpowered for multivariable analysis to test for independent risk factors for cardiovascular events. In the same year,

Faurschou et al. performed a similar retrospective study including only GPA patients [180]. A total of 293 GPA patients were included and details regarding hospitalizations from 1977 to 2006 in Denmark were obtained from a national registry. These GPA patients were compared with the Danish background population with respect to rates of hospitalization for clinical manifestations of ischemic heart disease (IHD) after the date of vasculitis diagnosis by calculating standardized ratios of observed to expected (O:E) events. Although matched for age, gender, and calendar-year specific IHD events, GPA patients demonstrated a significantly increased O:E for IHD of 1.9 [95% CI 1.4–2.4]. A significantly increased risk was found for acute myocardial infarction (MI) (O:E ratio 2.5 [95% CI 1.6–3.7]), in particular in men aged >50 years at the time of diagnosis and with a cumulative dose of cyclophosphamide >36 g. Interestingly, this GPA population had an increased risk of cardiovascular events both in the early (within 5 years of diagnosis) and in the late (after 10 years of diagnosis) phases of the disease, suggesting that not only acute, but also chronic inflammation may be implicated in this process.

As noted above, studies actually performing imaging of the coronary arteries are scarce. Neumann et al. performed coronary angiogram (CAG) in 13 (26%) of EGPA patients with symptoms, of whom 12 (92%) had completely inconspicuous coronary arteries, whereas one had pre-existing CAD with two-vessel disease [123]. In our study, we evaluated CAD in 65 (71%) of our patients including 41 CT angiographies and 32 CAG (11 without prior CT angiography). Again, only in those patients with suggestive symptoms and/or other imaging abnormalities suggesting possible CAD. Using

CT-angiography, no significant difference in calcium score was found between EGPA and GPA patients (74 [0–512] vs. 21 [0–383]; $p=0.59$). However, a significant coronary stenosis (>70%) was found in 4 EGPA and 3 GPA patients (Fig. 20.6). Moreover, CAG confirmed significant stenosis in 6 EGPA and four GPA patients. This further demonstrates the high prevalence of affected coronary arteries in AAV patients, even though not all patients were screened for CAD.

In summary, AAV patients demonstrate a four-fold increase in coronary artery disease and the relapsing and remitting nature of AAV in combination with immunosuppression may accelerate atherogenesis.

3.8 Cardiac Histology

In the earlier years, several postmortem studies evaluated histological signs of AAV. The first histological description of EGPA was by Churg and Strauss in 1951, clearly demonstrating that the heart was most frequently involved in these patients (49%) [119]. The epicardium was the most common affected location showing multiple granulomatous nodules. The most constant lesion of the myocardium was interstitial eosinophilic inflammation, varying in extent from an occasional focus to diffuse myocarditis. Myocardial fibrosis was common, most probably resulting from anoxia secondary to vascular lesions, as well as from scarring of inflammatory foci. In a systemic review involving 27 histologically documented cases of cardiac involvement in GPA patients, the most common manifestations were pericarditis (50%) and coronary arteritis (50%). Histologic-proven myocarditis was less common (25%) [124].

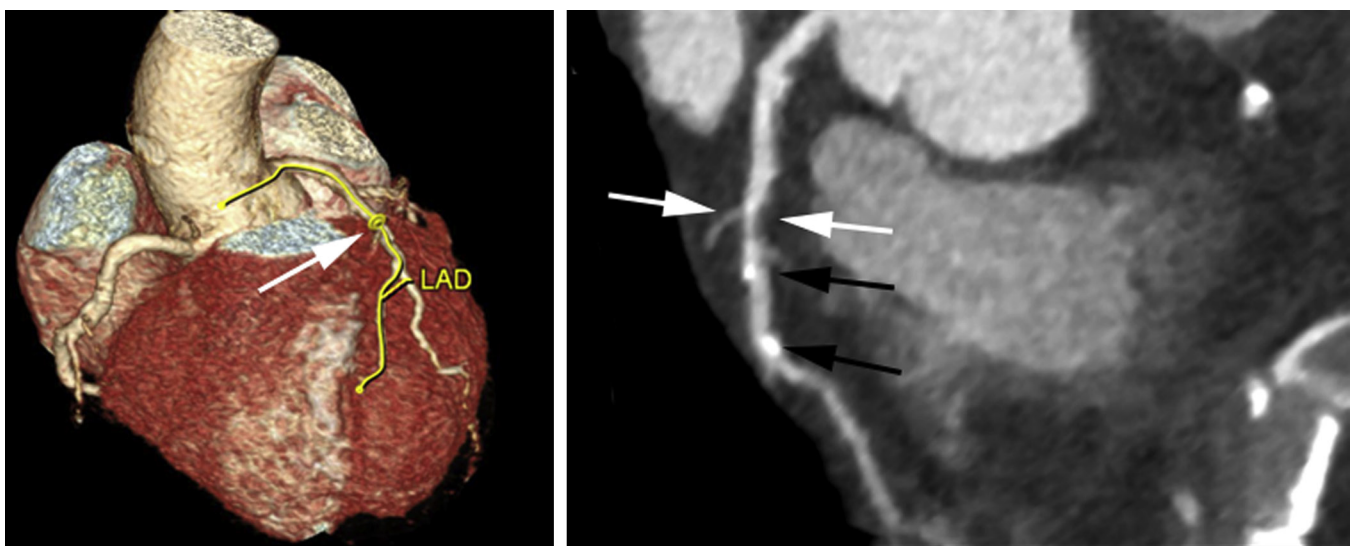


FIGURE 20.6 CT-coronary angiogram of a 57-year old GPA patient. A 3D reconstruction of the CT-coronary angiogram demonstrating the location of a significant lesion in the left anterior descending (LAD) coronary artery (left panel, *white arrow*). A 2D reconstruction showing significant stenosis (right panel, *white arrows*) and atherosclerotic plaque (right panel, *black arrows*). Adapted from the personal collection of the authors.

However, only a few studies evaluated endomyocardial biopsies (EMB) in living patients. In part, this may be due to the fairly limited use of this technique in general hospitals and a relatively high likelihood of sampling error. Wassmuth et al. performed EMB in 6 of 11 EGPA patients, confirming extensive fibrosis previously characterized using cardiac MRI and identified additional destruction of small myocardial vessels [165]. In addition, Neumann et al. performed EMB in 11 (22%) EGPA patients to confirm suspected Löffler endocarditis [123]. Löffler endocarditis is a form of restrictive cardiomyopathy with severe clinical manifestations, including a grossly thickened endomyocardium with intraventricular mural thrombosis [181]. This disease is associated with other forms of hypereosinophilic disorders such as tropical endomyocardial

fibrosis and idiopathic hypereosinophilic syndromes (HES). It consists of three stages including acute necrotic stage, a thrombotic necrotic stage, and a late fibrotic stage. Although the systemic hypereosinophilia may cause serious dysfunction in other organs it primarily infiltrates the myocardium, as indicated by the severe heart failure prevalence of over 75% in patients with HES [181]. In the study of Neumann et al., acute eosinophilic endomyocarditis was seen in 2 patients, and biopsy was nondiagnostic in 2 patients. Interestingly, 7 patients demonstrated typical histologic signs of Löffler endocarditis (Fig. 20.7). Of note, these patients demonstrated little or no pericardial effusions, more severe left heart failure, elevated cardiac enzymes, and thrombus formation. In our study, we performed EMB in 13 of 91 included AAV patients [32].

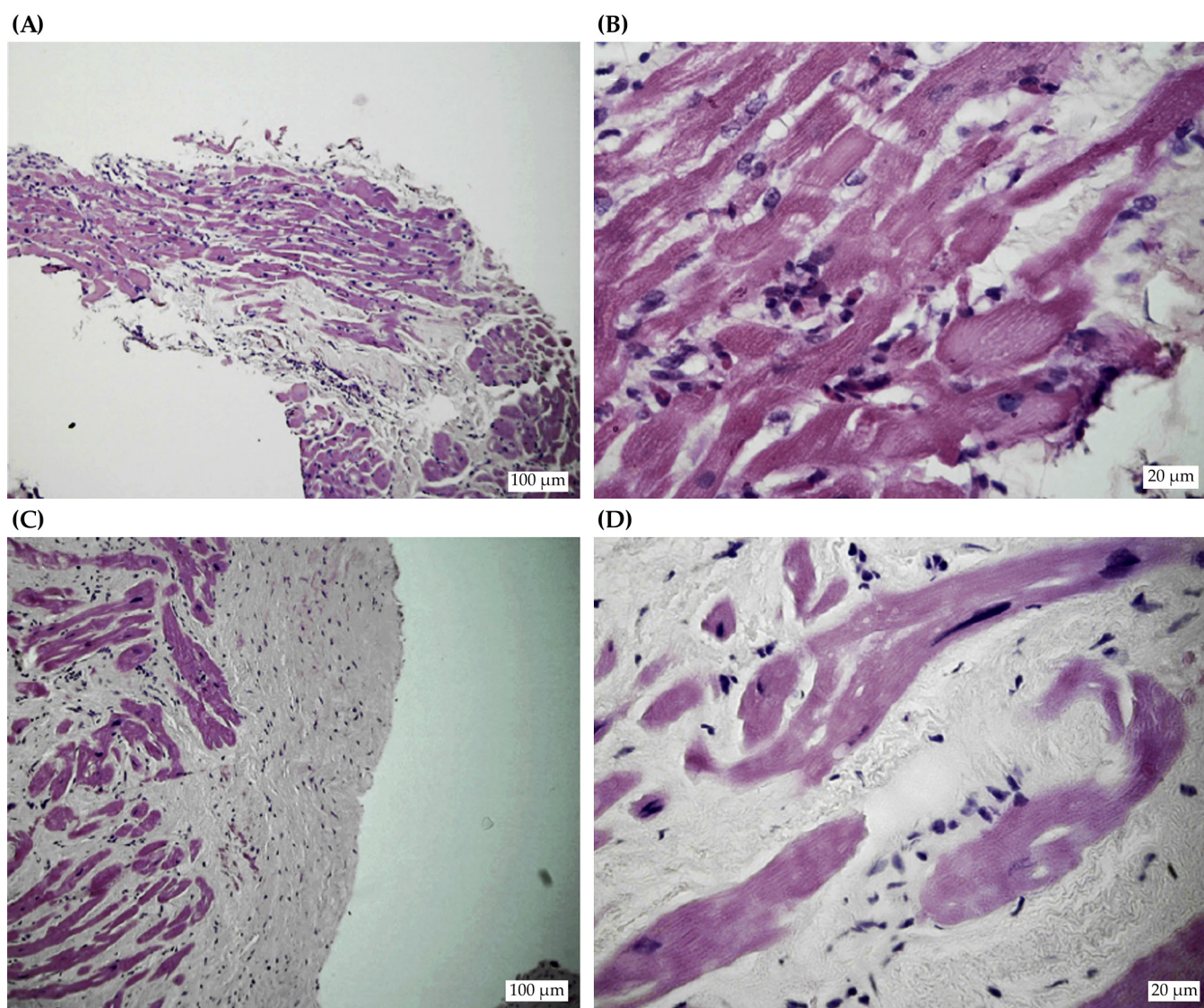


FIGURE 20.7 Left ventricular endomyocardial biopsy of EGPA. Hematoxylin and eosin (HE) staining of left ventricular endomyocardial biopsy performed in EGPA patient, primary magnification 25× and 20×: active myocardial inflammation with eosinophils illustrating the inflammatory stage of Löffler endomyocarditis (A and B). Severe endomyocardial fibrosis corresponding to the fibrotic stage of Löffler endomyocarditis (C and D). Adapted from Neumann et al. [123].

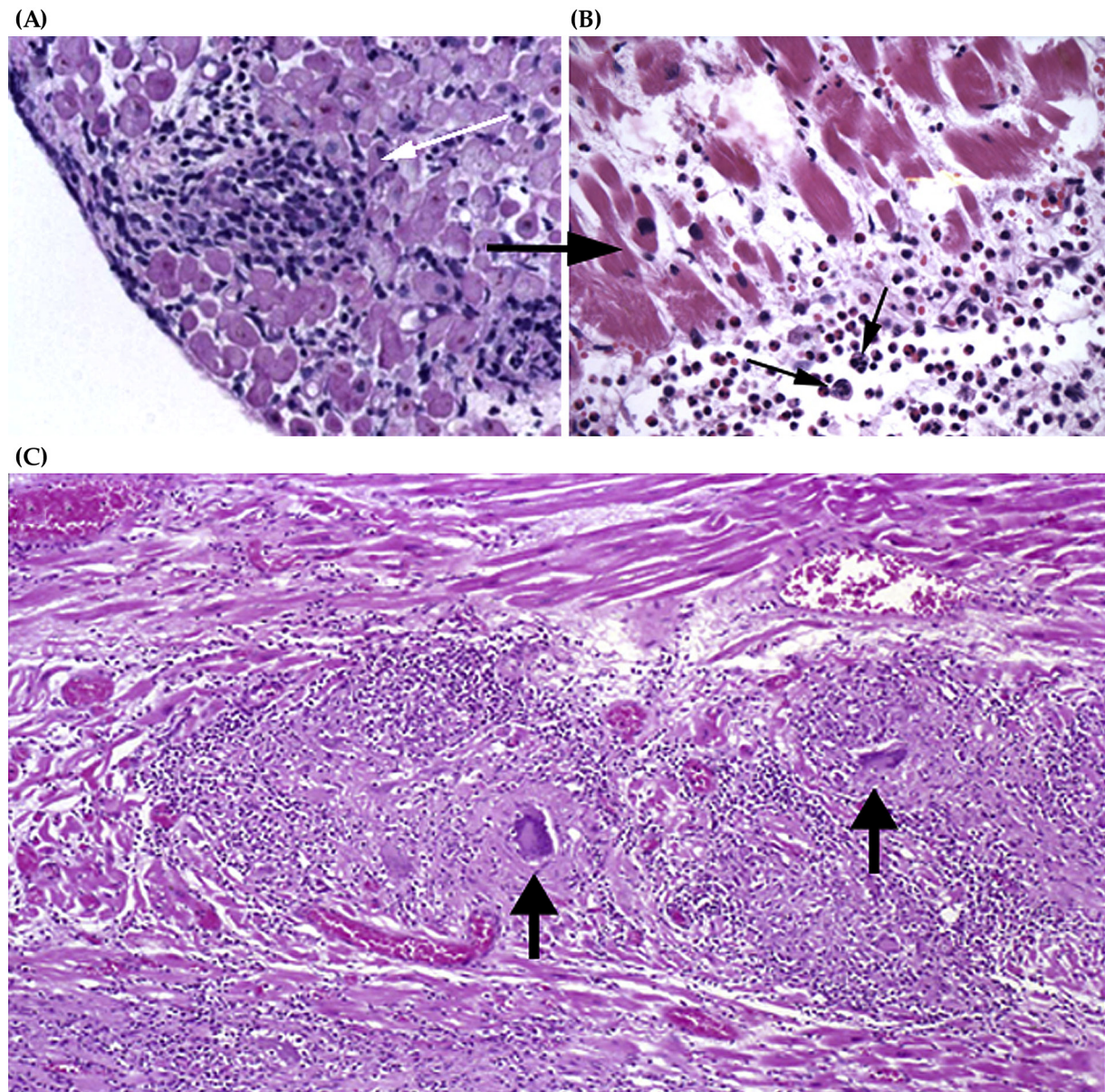


FIGURE 20.8 Right ventricular septal endomyocardial biopsy of a 65-year old patient diagnosed with EGPA. Hematoxylin and eosin (HE) stain of endomyocardial biopsy performed in EGPA patient, demonstrating infiltrates of inflammatory cells (A, *white arrow*), including eosinophils (B, *small black arrows*) and necrotizing granulomas (C, *big black arrows*). Original magnification: A&B $\times 200$ and C $\times 100$. Adapted from the personal collection of the authors.

Cardiac biopsies were performed in 11 EGPA and 2 GPA patients, based upon clinical indication for their unexplained heart failure. Herein, infiltration of leukocytes was the predominant finding, with 1 EGPA patient demonstrating eosinophilic infiltrates and granulomas (Fig. 20.8).

3.9 Heart Transplantation

To date, only one relatively large study investigating post-transplantation outcome has been conducted in EGPA patients [182]. As previously mentioned, heart involvement is the leading cause of death in EGPA patients, already demonstrated in earlier years [118].

As disease remission is not achieved in all patients, and relapses are frequent [183], the possibility of orthotopic heart transplantation (OHT) in these refractory patients arises. The first case report in 1989 describes a 22-year-old man with severe cardiomegalie and progressive cardiac symptoms and signs, despite increasing immunosuppressive treatment. One year later, after being anuric and with a mean blood pressure of 30 mmHg, he underwent OHT. Three weeks after transplantation, he experienced a relapse of EGPA. An EMB did not show rejection or signs of EGPA-related cardiac involvement, and after increasing his steroids his condition improved and eosinophil count returned to normal. After 2 years, this

patient was doing fine without the heart being affected by EGPA. However, others believe EGPA is a limitation of OHT due to their recurrences [184].

Although the International Society for Heart and Lung Transplantation (ISHLT) does not consider systemic diseases contraindications for OHT [185], the relapse-remitting nature and the high risk of post-transplant disease recurrence often disqualifies patients from the procedure. The largest study to date involves 9 post-transplant EGPA patients from 8 transplant centers in 6 different countries between 1987 and 2009. The authors retrospectively assessed their outcomes. Their median age was 36 years (range 22–62) and all presented with severe acute eosinophilic myocarditis, abnormal ECG findings, and severely diminished LVEF (mean $24\% \pm 6\%$) due to active EGPA. Seven (78%) patients had dilated cardiomyopathy including 2 with intracavitary thromboses. Of the 6 patients who underwent CAG, only 1 patient had an abnormal CAG revealing one-vessel disease with 40% stenosis. Histologically, eosinophil-rich infiltrate was the predominant finding followed by nonspecific myocardial fibrosis. A post-transplant relapse of EGPA was observed in one-third of patients (range 2–48 months), while another third suffered from postgraft asthma and/or sinusitis flares that required increased corticosteroid doses. Importantly, all patients had post-transplant complications, with infection and cell-mediated rejection being the most frequent complication, occurring in 6 (67%) patients. The transplantation-to-heart-rejection interval ranged from as little as 1 to 74 (mean 23 ± 28) months, illustrating more frequent late cell-mediated rejection as compared to similar disease, ie, hypersensitivity myocarditis [186] or lymphocytic myocarditis [187]. Importantly, 4 (44%) of 9 patients died suddenly, with a post-transplant survival of 3–60 (mean 32 ± 29) months. The remaining 5 (56%) survivors had a follow-up 55 to 102 (mean 74 ± 23) months. The poor 5-year post-transplant survival rate of 57% is most likely due to lack of optimal treatment, in particular of historical patients and transplantation during the active phase of EGPA. Considering only the 6 patients transplanted during the last decade, the 5-year post-transplant survival rate increases to a fairly good prognosis of 80%.

In summary, the previous data illustrates OHT is feasible, albeit with high late-rejection rate and only a fair prognosis (considering the patients transplanted the last decade), and very rare in EGPA patients. Thus more prospective data is needed to improve patient care in these relatively young patients.

4. PROGNOSIS

If untreated, AAV will result in death within weeks to months. Since the introduction of prednisolone and cyclophosphamide as standard therapy, the overall

prognosis of AAV has improved from a 1-year survival of <20% to a 1- and 5-year survival of 88% and 78%, respectively [13]. With prolonged survival, patients may experience long-term sequelae as a result of vasculitis or its treatment [1]. Over the past 40 years, major improvements in therapeutic strategies for AAV patients have been developed to refine immunosuppressive regimens and minimize toxicity. Importantly, the treatment is not curative but aims to control disease activity in a 3- to 6-month induction phase followed by maintenance therapy [188]. With current knowledge, patients with AAV clearly have increased mortality as a consequence of cardiovascular disease. Generally, early death in patients with AAV is due to the disease itself and/or infectious complications of immunosuppressive drugs [189]. Late death, however, is either due to cardiovascular disease and/or malignancies [16,190].

In 1996, Guillevin et al. performed a prospective study of 342 EGPA patients and MPA (as a subgroup of polyarteritis nodosa (PAN)) patients and identified five factors associated with poor prognosis (Five-Factor Score, FFS) and requiring intensive immunosuppression in these patients [15]. One of these factors included cardiomyopathy, illustrating the importance of cardiac involvement on prognosis. Of note, the majority of patients (61%) were diagnosed with PAN, whereas only 52 (15%) MPA patients and 82 (24%) EGPA patients were included. In addition, the diagnostic method(s) used for the detection of cardiac abnormalities was not provided. Nonetheless, subsequent studies demonstrated similar findings, indicating that approximately 50% of deaths in EGPA patients can be directly related to cardiac involvement [128,140]. In line with previous results, Suppiah et al. pooled the available data of four studies, finally including 535 MPA and GPA patients to predict cardiovascular events. Herein, cardiac events were characterized as death from any cardiovascular cause, nonfatal stroke, nonfatal myocardial infarction, and coronary artery bypass graft or percutaneous coronary intervention. A total of 74 (15%) had at least one cardiovascular event after 5 years' follow-up. The final model showed diastolic hypertension and older age as independent predictors of adverse outcome. In contrast, positive PR3 ANCA was associated with reduced risk of cardiovascular events. Finally, a prospective evaluation of the prognostic relevance of cardiac involvement detected by screening both EGPA and GPA patients was recently published by our research group. Herein, cardiac involvement was associated with increased all-cause and cardiovascular mortality, irrespective of the diagnostic method used for detection of cardiac involvement (Fig. 20.9). This confirms the previous results described above and again emphasizes the importance of cardiac screening in AAV patients.

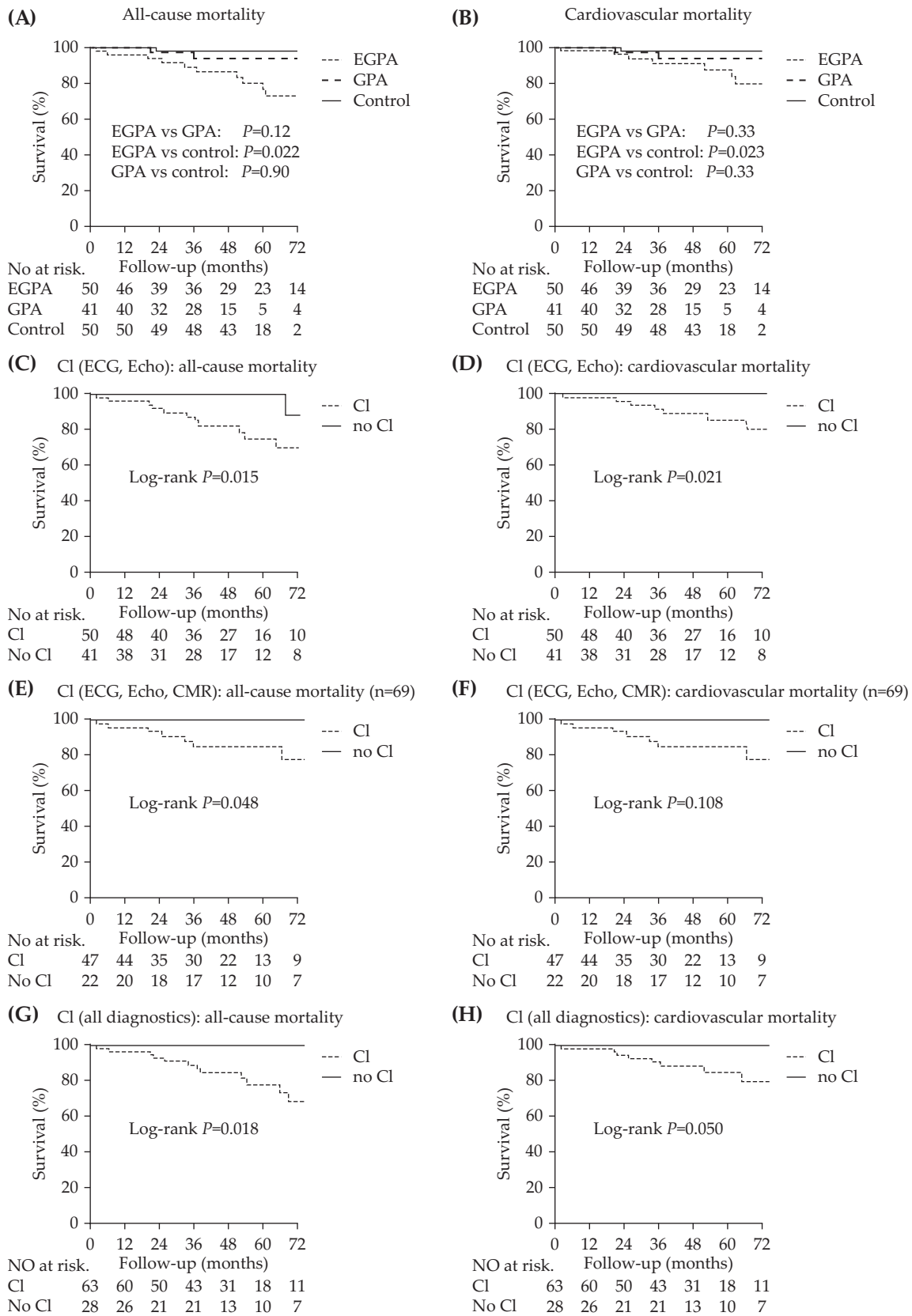


FIGURE 20.9 Survival curves for AAV patients and control subjects, including subgroup survival curves of cardiac involvement in AAV patients. Survival curves for EGPA, GPA, and control patients using all-cause mortality and cardiovascular mortality (Panels A and B, respectively). Survival curves for cardiac involvement based on ECG and/or echocardiographic involvement in AAV patients (all patients) using all-cause mortality and cardiovascular mortality (Panels C and D, respectively). Survival curves for AAV patients who underwent ECG, echocardiography, and CMR ($n=69$) using all-cause mortality and cardiovascular mortality (Panels E and F, respectively). Survival curves for using all diagnostic methods for AAV patients (clinical setting) using all-cause mortality and cardiovascular mortality. EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; AAV, ANCA-associated vasculitis; CI, cardiac involvement; CMR, cardiac magnetic resonance imaging. Adapted from Hazebroek et al. [32].

5. TREATMENT OF AAV AND CARDIAC IMPLICATIONS OF THERAPY

In the following sections, we discuss the most important treatment strategies (Tables 20.9 and 20.10), but detailed discussion of all emerging and future immunosuppressive regimens is beyond the scope of this chapter. A flow diagram for current treatment in AAV is shown in Fig. 20.10.

5.1 Induction of Remission

Current remission induction strategies include the use of high-dose glucocorticoids together with daily oral or intravenous-pulsed cyclophosphamide or rituximab, as well as plasma exchange for individuals with vital organ- or life-threatening disease. The conventional treatment of glucocorticosteroids and cyclophosphamide has shown to be effective in 80–90% of patients with multisystem AAV [22]. On the contrary, methotrexate and possibly mycophenolate mofetil are also options for the induction of remission in nonorgan or nonlife-threatening AAV [188].

Glucocorticosteroids produce rapid improvement in genomic effects on the cytosolic and more rapid nongenomic effects on the membrane bound glucocorticoid receptor, although these effects are only shortlived in AAV patients [198]. Interestingly, despite the introduction of glucocorticoids into treatment strategies for vasculitis over 50 years ago, no randomized controlled trials exist to date to support their use. In addition, evidence is also lacking to guide dosage and overall duration of therapy [199]. Nevertheless, glucocorticoids remain an integral part of induction regimens in AAV, although increasing evidence shows that high-dose steroids contribute significantly to the morbidity in AAV patients [200]. Therefore it is important to tailor the dose of steroids, often used together with an immunosuppressive agent, to minimize the harm, while still controlling disease. The PEXIVAS trial, currently recruiting 500 patients worldwide, aims to address the efficacy of a rapidly reducing glucocorticoid regimen, as well as the place of plasma exchange in severe AAV [201].

Cyclophosphamide is one of the two recommended induction strategies (alongside rituximab) for multisystem AAV patients in combination with high-dose steroids [188]. Cyclophosphamide, a cyclic nitrogen mustard phosphamide ester, was first used as a chemotherapeutic agent in the 1950s [202]. It is a cytotoxic alkylating agent capable of killing B and T cells. Although the drug is life-saving in AAV patients, the toxicity is considerable when used as continuous daily oral therapy for up to 2.7 years, providing over 100 g lifetime exposure in some patients [203,204]. Due to its cytotoxic effects on rapidly dividing normal cells, predicted and observed side effects include

reversible nausea, vomiting, diarrhea, and hair loss. In addition, permanent infertility and malignancy occur with increasing cumulative doses, with an incidence of 5% at 10 years and 16% after 15 years [205]. Although no absolute cutoff dose to avoid toxicity exists, the British Society for Rheumatology guidelines for management of AAV recommend restricting total exposure to <25 g [188]. The current protocols including cyclophosphamide use a much lower cumulative dose, mainly due to refinement of cyclophosphamide by reducing exposure and cumulative toxicity. From the sequential replacement of cyclophosphamide by azathioprine in the CYCAZAREM trial [22], to replacement of cyclophosphamide by methotrexate for early systemic disease without critical organ manifestations in the NORAM trial [23], to the use of pulsed intravenous rather than daily oral cyclophosphamide in the CYCLOPS study [24], cyclophosphamide has become a much safer drug to use. A serious downside of administering less cyclophosphamide is suggested by long-term follow-up of patients in the CYCLOPS and CYCAZAREM studies, as reduced cyclophosphamide exposure was associated with a higher risk of relapse [206,207]. In addition, methotrexate was associated with less effective disease control in the NORAM study, although ultimately no increase in mortality or long-term morbidity was observed [208].

To decrease toxicity rates and to improve mortality and morbidity, several novel treatment strategies have entered the field. With ongoing identification of molecular pathways that play a pivotal role in disease development, specific targeting of inflammatory immune mechanisms becomes increasingly realistic. A rationale for B-cell targeted therapy in AAV has emerged from the presence of B cells at sites of inflammation, correlation of B-cell activation with disease activity in GPA, the efficacy of cyclophosphamide, which is a relatively B cell-specific immunosuppressant, and the contribution of ANCA to the pathogenesis. Several case series and small prospective studies have highlighted the efficacy of the anti-CD20 B cell-depleting monoclonal antibody rituximab in refractory AAV. Subsequently, two randomized trials evaluated rituximab for induction of remission in new and relapsing AAV patients [194,195]. The Rituximab versus Cyclophosphamide for ANCA-associated Vasculitis (RAVE) trial included 197 patients with severe AAV, whereas the Rituximab versus Cyclophosphamide in ANCA-Associated Renal Vasculitis (RITUXVAS) trial recruited 44 patients with newly diagnosed renal AAV. The results of the two trials showed that rituximab was not inferior to daily oral or pulse intravenous cyclophosphamide for the induction of complete remission by 6 months and was associated with similar rates of adverse events. The RAVE trial also demonstrated superiority of rituximab to cyclophosphamide for the subgroup of 101 patients treated for relapsing disease (67% vs. 42%, respectively). Of note, no

TABLE 20.9 Completed Clinical Trials in ANCA-Associated Vasculitis

Study	Name	Treatment studied	Patients	Primary outcomes
A	Stassen et al. [191]	Mycophenolate mofetil in patients intolerant for cyclophosphamide for remission induction	GPA and MPA	Pilot trial. Induction of remission achieved in 78% at the expense of acceptable side effects. Prospective randomized trials needed
A	Silva et al. [192]	Mycophenolate mofetil in patients with renal involvement	MPA	Pilot trial. Induction of remission in 76% of patients achieved with mild side effects. Prospective randomized trials needed
B	NORAM [23]	Methotrexate versus cyclophosphamide for remission induction	GPA and MPA	Methotrexate is less effective than cyclophosphamide in patients with nonrenal AAV for disease control
C	MEPEX [25]	Plasma exchange versus methylprednisolone in remission induction	GPA and MPA	Renal outcome is better when plasma exchange was performed in patients with severe renal disease but patient survival is similar
D	RAVE [193,194]	Rituximab versus cyclophosphamide in remission induction	GPA and MPA	A single course of rituximab is as effective and as safe as treatment with cyclophosphamide followed by azathioprine
D	RITUXVAS [195]	Rituximab with cyclophosphamide versus cyclophosphamide in remission induction	GPA and MPA	In patients with severe renal disease, rituximab in combination with two pulses of cyclophosphamide is as effective and as safe for remission induction as cyclophosphamide pulse therapy
E	CYCLOPS [24]	IV cyclophosphamide versus oral cyclophosphamide in remission induction	GPA, MPA and RLV	IV cyclophosphamide is as effective as oral cyclophosphamide and reduces cumulative cyclophosphamide doses for induction therapy
F	GC with 6 versus 12 CYC pulses [183]	Glucocorticoids and either 6 or 12 intravenous cyclophosphamide pulses in remission induction and maintenance	EGPA FFS ≥ 1	Complete remission rates and severe side effects comparable for both groups. Superiority of 12-pulse regimen, mainly in reducing mild relapses
F	AZA versus 6 CYC pulses [196]	Azathioprine versus 6 intravenous cyclophosphamide pulses in remission induction and maintenance	EGPA FFS < 1	In majority of EGPA patients remission can be achieved with glucocorticoids alone. In patients who are refractory to glucocorticoids or had relapses, treatment with intravenous pulse CYC or oral AZA was fairly effective
V	WEGENT [26]	Methotrexate versus azathioprine in remission maintenance	GPA and MPA	Methotrexate is as effective and as safe for remission maintenance as azathioprine
W	CYCAZAREM [22]	Azathioprine versus cyclophosphamide in remission maintenance	GPA and MPA	Azathioprine is as effective as cyclophosphamide and reduces cumulative cyclophosphamide doses for maintenance of remission
X	IMPROVE [197]	Mycophenolate mofetil versus azathioprine in remission maintenance	GPA and MPA	Mycophenolate mofetil is less effective than azathioprine for maintenance of remission
Y	MAINRITSAN [27]	Rituximab versus azathioprine in remission maintenance	GPA and MPA	Rituximab is more effective to prevent relapse compared with azathioprine whereas adverse events are similarly frequent
-	MTX versus LEF [222]	Oral methotrexate versus leflunomide for remission maintenance	GPA	Less relapses occur with leflunomide as compared with methotrexate for remission maintenance but more adverse events occur with leflunomide
-	WGET [92]	Etanercept with standard therapy vs. standard therapy in remission induction and maintenance	GPA	Etanercept is not effective for maintenance of remission and when combined with standard therapy results in a high rate of treatment related complications (eg, malignancies)

IV, Intravenous; RLV, renal-limited vasculitis. Adapted from Hilhorst et al. [38].

TABLE 20.10 Recommendations for Treatment Strategies for ANCA Vasculitis and Associated Level of Evidence

Recommendations	Evidence level/ strength
Remission Induction for Newly Diagnosed Disease	
<i>Remission induction for limited or nonsevere (nonorgan- and nonlife-threatening) newly diagnosed disease</i>	
In GPA, remission induction regimen with MTX in combination with GC can be used.	B/I
In EGPA and MPA, remission induction can be achieved with GC alone. At present, no consensus on the use of any immunosuppressant agents in combination with GC in patients with EGPA or MPA that is nonsevere exists.	B/IIa
<i>Remission induction for severe (organ- and/or life-threatening) newly diagnosed disease</i>	
In GPA, MPA, or EGPA, remission induction with a combination of high-dose GC and CYC is recommended.	B/I
In GPA and MPA, remission induction with high-dose GC and RTX as first-line therapy is recommended in those whom CYC is contraindicated or in those whom CYC presents an unacceptable risk of infertility.	B/I
CYC dose should be adjusted in patients >60 years of age and in those with renal impairment.	B/I
We recommend that the remission induction therapy with CYC, combined with GC, last a minimum of 3 to a maximum of 6 months. Once remission is achieved, CYC should be stopped and the patient switched to a different maintenance therapy.	B/I
We recommend that GC be given in adults at an initial dose of 1 mg/kg/day PRED-equivalent for remission induction purposes. This may be preceded by pulsed methylprednisolone (0.5 g/day to 1 g/day for 1–3 days) in patients with life-threatening disease and/or major organ involvement.	B/I
Prophylaxis against <i>Pneumocystis jirovecii</i> infection should be given to patients receiving CYC or RTX. This prophylaxis consists, in the absence of allergy, of trimethoprim/sulfamethoxazole compounds (800/160 mg 1 tablet 3 times per week or 400/80 mg daily).	C/IIa
There is insufficient evidence to support a recommendation that plasma exchange can be used as first-line therapy in any patient with AAV. Plasma exchange may be a reasonable adjuvant therapy for AAV patients who clinically deteriorate because of active vasculitis despite ongoing remission induction therapy with high-dose GC and CYC or RTX.	C/IIb
Remission Maintenance Therapy	
In patients with severe AAV in remission after a combined CYC-GC-based induction treatment, maintenance therapy can be based on AZA or MTX, initially in combination with low-dose GC. LEF or mycophenolate mofetil may be alternative agents in patients not tolerating or with contraindications to AZA and MTX.	B/IIa
In patients with severe AAV in remission after a combined CYC-GC-based induction treatment, maintenance therapy with RTX infusions is an alternative to AZA, especially for those patients with PR3-ANCA-positive GPA.	B/I
We recommend the use of AZA, MTX, or their alternatives for remission maintenance therapy to be continued for a minimum of 18 months after successful remission induction. There is not yet enough evidence to support further recommendations on the optimal duration of their use for maintenance.	C/IIa
The use of trimethoprim/sulfamethoxazole (800/160 mg twice daily) as remission maintenance therapy can be considered in GPA as an adjuvant to immunosuppressant or after the cessation of maintenance immunosuppressive treatment.	C/IIa
Topical therapies may be considered, in combination with the systemic therapy and in collaboration with ENT subspecialists, to alleviate the symptoms of upper airway and ENT disease.	C/IIa
Relapsing Disease	
In AAV patients with a major organ- or life-threatening relapse, either CYC or RTX in conjunction with high-dose GC is recommended. In whom CYC was already given for initial remission induction or a previous disease flare, we recommend using RTX for remission reinduction.	B/I
Plasma exchange can be used as first-line therapy in all patients with relapsing AAV with severe renal (GFR < 50 mL/min) or pulmonary hemorrhage. Plasma exchange may be a reasonable adjuvant therapy for patients who clinically deteriorate because of active relapsing vasculitis despite ongoing remission induction therapy with high-dose GC and CYC or RTX.	C/IIa
In nonsevere relapses patients should be treated with an increase in GC dose in addition to optimizing patient's concurrent immunosuppressant agent.	C/IIa
Refractory Disease	
In patients with severe GPA or MPA who fail to respond to CYC as remission induction therapy, the use of RTX in combination with GC is recommended.	C/IIa

ANCA, Antineutrophil cytoplasm antibody; AAV, ANCA-associated vasculitides; GC, glucocorticoids; CYC, cyclophosphamide; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; EGPA, eosinophilic GPA; RTX, rituximab; MTX, methotrexate; AZA, azathioprine; PR3, proteinase 3; GFR, glomerular filtration rate. Adapted from McGeoch et al. [223].

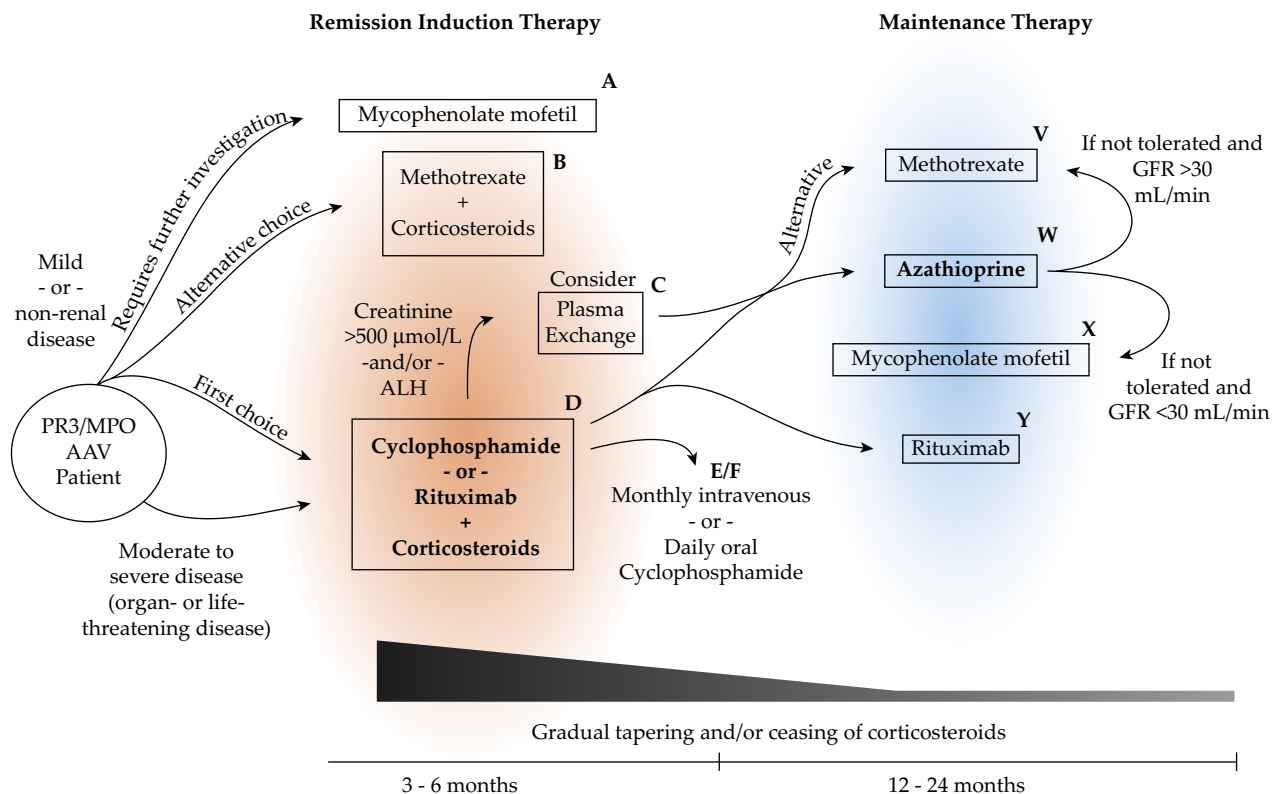


FIGURE 20.10 Flow diagram for treatment in AAV. Evidence for every step is given as follows, linked to symbols in the diagram. (A) Stassen et al. [191] and Silva et al. [192]; (B) NORAM trial [23]; (C) MEPEX trial [25]; (D) RAVE trial [193,194] and RITUXVAS trial [195]; (E) CYCLOPS trial [24]; (F) Ribi et al. [196] and Cohen et al. [183]; (V) WEGENT trial [26]; (W) CYCAZAREM trial [22]; (X) IMPROVE trial [197]; (Y) MAINRITSAN trial [27]. Adapted from Hillhorst et al. [38].

difference in safety was observed between the treatment groups, suggesting glucocorticoid therapy rather than cyclophosphamide is the major treatment-related cause of toxicity in these patients.

According to the role of T regulatory cells in AAV pathogenesis, another novel promising therapy is currently being validated, called Abatacept. Abatacept comprises the ligand-binding domain CTLA4 plus modified Fc domain derived from IgG1. By containing CTLA4, abatacept is able to block the engagement of CD28 with its ligand, thereby inhibiting T-cell activation. The drug has shown to be efficacious in 20 patients with limited relapsing GPA. It was well tolerated on top of glucocorticosteroids and an immunosuppressant (either azathioprine, methotrexate, or mycophenolate mofetil) with 90% of patients experiencing disease improvement and 80% achieving remission. In addition, glucocorticosteroids discontinuation was possible in a high percentage of patients [209].

5.2 Maintenance of Remission

Despite the successful introduction of glucocorticosteroids and cyclophosphamide into treatment regimens making AAV a relapsing-remitting disease,

maintaining the state of remission remains a major challenge as at least 10% of AAV patients relapse each year [210,211]. Several factors are associated with higher relapse rates, including upper respiratory involvement, nasal carriage of *S. aureus* infection, absence of renal involvement, persistent ANCA positivity during remission, and withdrawal of immunosuppression or glucocorticosteroids [22,23,49,199,212]. Importantly, the majority of treatment strategies is associated with early and late toxicities and fail to prevent vital organ damage, such as to the heart.

Several randomized controlled trials have been conducted to evaluate optimal maintenance therapy, including CYCAZAREM where continuous cyclophosphamide was compared with azathioprine. No difference in relapse rates was observed, with 13.7% in the cyclophosphamide group and 15.5% in the azathioprine group at 18 months after diagnosis [22]. However, long-term follow-up has suggested poorer outcomes for those switched to azathioprine, instigating again the question of optimal timing of transition from induction to maintenance therapy [207]. In the WEGENT trial, azathioprine was compared with methotrexate for maintenance therapy [26]. Similar to CYCAZAREM, no difference in relapse rate was observed with 36% in the azathioprine

group and 33% in the methotrexate group after a mean follow-up of 29 months after remission. The IMPROVE trial found mycophenolate mofetil, a prodrug of mycophenolic acid that inhibits proliferation of B and T cells, to be less effective compared with azathioprine for prevention of relapse (55% vs. 38% after 39 months, with similar adverse events rates, despite its success in the field of transplantation [197]). Thus mycophenolate mofetil is not recommended as a first-line remission maintenance agent in AAV but may play a role for patients intolerant of azathioprine or for whom methotrexate is contraindicated (creatinine > 150 $\mu\text{mol/L}$).

Of note, in the previously mentioned RAVE study where patients in rituximab-induced remission received no maintenance therapy, and those in cyclophosphamide-induced remission received azathioprine similar relapse rates (32% and 29%, respectively) and severity of disease flares were observed at 18 months between the groups [194]. In line with these results, two retrospective studies suggested that successive rituximab infusions could be effective in maintaining remission in AAV patients [213,214]. Recently, the MAINRITSAN trial proved superiority of rituximab to azathioprine in maintaining remission in 115 AAV patients, illustrated by only 5% as compared to 29% of patients relapsing after 28 months using rituximab or azathioprine, respectively [27]. This difference was maintained during extended follow-up, with 12.7% vs. 48.1% after 39 months' follow-up in rituximab or azathioprine limb, respectively [215].

Note that EGPA patients have been largely excluded from AAV trials, as the clinical phenotype and treatment response has a number of distinct features. As mentioned before, EGPA patients are stratified according to prognostic tool called the Five-Factors Score (FFS), which also includes cardiac involvement [15]. The French group conducted two studies, including nonsevere (FFS of 0) [196] and severe (FFS ≥ 1) EGPA patients [183]. Interestingly, a high percentage (93%) of EGPA patients with an FFS of 0 achieves remission using glucocorticosteroids alone, but relapse rates are high of up to 35%. For those with any adverse prognostic features, cyclophosphamide remains the first-line therapy, with maintenance strategies following those of GPA and MPA [183]. Although no randomized controlled trials are available to support this, the EGPA taskforce does recommend the latter treatment strategy. In addition, adding immunosuppressants (including cyclophosphamide) to glucocorticosteroids should be considered in those with severe alveolar hemorrhage, eye involvement, and fulminant mononeuritis multiplex, although not part of the FFS they can be life-threatening [216]. More recently, smaller studies suggested a potential favorable effect of rituximab, with the largest case series demonstrating 83% improvement after 6 months and 34% reaching complete remission that was sustained for

12 months [217–219]. However, only 6% of patients were able to completely withdraw from glucocorticosteroids by 12 months, suggesting that rituximab in EGPA has a limited steroid-sparing effect. Interestingly, two small open-label studies using an IgG monoclonal antibody specific for interleukin-5 (IL-5), mepolizumab, reported the efficacy and potential corticosteroid-sparing effect of this novel drug in a limited number of patients [220,221]. Nevertheless, these results have led to the launch of the first randomized placebo-controlled trial specifically for EGPA patients. Herein, the efficacy and safety of 300 mg monthly subcutaneous mepolizumab over 52 weeks in 130 patients with relapsing or refractory EGPA receiving background glucocorticosteroids will be tested ([ClinicalTrials.gov](https://clinicaltrials.gov) identifier NCT02020889).

5.3 Cardiac Implications of Therapy

As the previous sections indicated, AAV is currently a chronic relapsing rather than a fatal condition. Therefore it is important to consider the impact of damage as a result of disease activity and the cumulative effects of immunosuppression on patients' long-term health and quality of life. This is illustrated by cardiac involvement, which remains the major determinant of mortality and usually occurs late in the course of the disease [189]. Although studies evaluating the effect of therapy in reducing cardiac involvement are very limited and most evidence is based on case reports, the evidence generally does suggest some favorable effects on cardiac function [18,19,124,224–227].

Three studies have demonstrated that endothelial dysfunction may be reversible with immunosuppressive or TNF- α blocker treatment [106,228,229]. In addition, rituximab may reverse cardiac involvement in GPA patients as suggested by a more recent cardiac MRI case report, showing reversed myocarditis and AV block in one patient [230]. A comprehensive case report involving an EGPA patient presenting with progressive heart failure due to pericarditis, eosinophilic myocarditis, and myocardial necrotizing vasculitis was published in 1998. They demonstrated that combination therapy of glucocorticosteroids and cyclophosphamide resulted in both a clinical (regression of pericardial effusion, normalization of systolic and diastolic dysfunction, and increase of cardiac index to 2.8 L/min/m²) and histologic (sequential endomyocardial biopsies at 1, 3, and 6 months of follow-up) resolution of cardiac involvement. Moreover, no recurrences were registered at 12-month follow-up with the patient receiving a maintenance drug regimen. Recently, similar results on a larger scale were demonstrated in a retrospective study. A total of 51 patients with EGPA diagnosed and treated in four tertiary centers between 1990 and 2013 were enrolled. All patients were scheduled for follow-up including cardiac MRI to

assess LVEF and myocardial damage depicted by LGE. Adverse cardiac events were defined as cardiac death and/or hospitalization due to decompensated heart failure. At diagnosis, 15 (29%) patients showed cardiac involvement, all presenting with heart failure, including 13 (25%) with systolic heart failure (LVEF<50%). Two patients had suspected acute coronary syndrome although later excluded by CAG, 1 presented with sudden cardiac arrest successfully resuscitated, 4 demonstrated myocarditis, another 4 perimyocarditis, and 1 patient had pericarditis. All patients received glucocorticosteroids and 18 (35%) received additional immunosuppressive therapy at diagnosis. During the disease course, an additional 6 (12%) received immunosuppressive therapy on top of glucocorticosteroids therapy due to relapse. Of note, 29 (57%) received ACE inhibitors or ARB, 25 (49%) betablockade and 7 (14%) an MRA. After a mean follow-up of 39 ± 39 months, all patients were in clinical remission, 29 (57%) had cardiac involvement of which 25 (49%) heart failure symptoms, and none had symptoms of angina. Although no patient died during the follow-up period, 10 (20%) patients were hospitalized for decompensated heart failure. The interesting finding was that those patients receiving additional immunosuppressive therapy at diagnosis demonstrated a significantly lower frequency of new onset or progression of heart failure or heart failure hospitalization as compared to those who did not receive additional immunosuppressant (12 [36%] vs. 1 [6%]; $p=0.02$ and 10 [30%] vs. 0 [0%]; $p=0.009$, respectively). In addition, initiating additional immunosuppressant at diagnoses was associated with prolonged cardiac event-free survival (Log-rank $p=0.049$). Finally, cardiac function improved in those receiving additional immunosuppressive therapy at diagnosis as compared to no cardiac improvement in those who did not receive this therapy. The authors suggested that the lack of or inadequate duration of additional immunosuppressive therapy is an independent determinant of cardiac involvement and the extent of myocardial damage in EGPA, although the study was actually underpowered to evaluate independent predictors of outcome.

In summary, the effects of treatment with glucocorticosteroids and/or immunosuppressive therapy on cardiac manifestations are currently not well known, but nevertheless serve as an avenue for future research endeavors.

6. CONCLUSION

The large body of evidence clearly demonstrates that AAV patients both have an increased risk of cardiovascular disease and more importantly, that cardiac involvement is shown to be an independent predictor

of mortality. The most predominantly found abnormalities are pericarditis and cardiomyopathy. Despite the latter evidence, cardiac screening is not advocated in current guidelines, most likely leading to underestimating the true scale of this problem. This is illustrated by the high prevalence of cardiac involvement in AAV patients in the few studies performing cardiac screening, irrespective of suggestive symptoms or signs. The increasing amount of novel imaging studies using cardiac MRI to evaluate cardiac involvement in AAV patients demonstrates even higher than expected prevalence of cardiac abnormalities, even patients in clinical remission presenting with normal ECG and echocardiography. Several studies suggested a worse outcome in those with cardiac involvement assessed by cardiac MRI, although lacked sufficient power and follow-up duration to prove these hypotheses. Our group was the first to prospectively demonstrate that cardiac involvement assessed by different modalities, including cardiac MRI, is associated with poor outcome. However, the AAV population was still relatively small, making prediction of independent predictors impossible. Nevertheless, the substantial evidence emphasizes the need for larger international, multicenter trials to prospectively assess independent predictors of outcome, including the potential benefit of cardiac MRI abnormalities, and to further improve prognostic models to guide treatment strategies.

To summarize, the heart may be involved in many ways in AAV patients making multimodality assessment obligatory to improve patient care and outcome in these patients [132,218].

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Behçet's Disease

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1. INTRODUCTION

Behçet's disease (BD) is a systemic inflammatory disorder whose clinical hallmark is recurrent oral and genital ulcers variably associated with skin and organ involvement. BD has worldwide distribution and both genders can be affected. Male preponderance in the Turkish and Arab [1] populations has been described by some authors, while in Europe the male-to-female ratio is approximately the same [2–5]. A more severe course has been reported in young males with increased morbidity and mortality [4]. BD can affect all ages, but onset is more common in the third decade of life [6].

BD was originally described in 1937 by a Turkish dermatologist, Hulusi Behçet (1889–1948), as a triad of recurrent oral and genital ulcers and iritis. A few years earlier (1930–1931) the Greek ophthalmologist Benediktos Adamantiades (1875–1962) reported a similar case of relapsing iritis and hypopion associated with leg ulcerations and thrombophlebitis [7]. Therefore some authors have proposed the alternative term “Adamantiades-Behçet's disease” to denote BD [8,9].

Another term sometimes used to indicate BD is the “Silk Route disease,” pointing to the geographic distribution of this condition, which appears to have spread over the ancient “Silk Route” over the centuries. Historically, the Silk Road was a route of commerce that followed the eastern shores of the Mediterranean Sea, which corresponds to the 30th and 45th degrees of Northern latitudes [8].

Given its protean clinical manifestations, BD has been classified within different disease frames in the past two decades. It is currently mostly considered systemic vasculitis, although histology it is actually consistent with periphlebitis rather than vasculitis proper because in BD lesions the inflammatory infiltrate surrounds blood vessels (mainly venules) rather than invading and

destroying the vessel wall [10]. Other authors consider BD a polygenic autoinflammatory disease according to recent insights into its pathogenic mechanisms [11].

1.1 Epidemiology

The highest prevalence of the disease is found in the countries along the ancient Silk Route, namely Turkey, the Middle East, Iran, Saudi Arabia, China, Korea, and Japan (11.9–370 per 100,000 population) [9]. In Western countries the prevalence has wide variability, ranging between 0.12 and 7.5 per 100,000 people, with the lowest prevalence rates in Northern Europe and higher rates in countries on the Mediterranean Basin [8].

In the United States, a population-based cohort study performed in Olmsted County, Minnesota, over 45 years (1960–2005) showed an overall incidence of 0.38 per 100,000 and a prevalence of 5.2 per 100,000 [8]. These data are similar to the estimated prevalence reported in France (2.4/100,000) [2] and Northern Italy (3.8/100,000) [5].

The migration of the Middle and Far East populations to the Mediterranean Basin and increased disease recognition may partially justify the increased prevalence recorded in some countries, such as Germany and Italy, in recent years [5,12]. Different clinical phenotypes and prognosis of BD patients according to their ethnic background have been reported. In their cohort analysis of 369 European, 350 North African, and 50 Sub-Saharan African patients, Savey et al. [13] observed a male preponderance in Sub-Saharan patients who showed more frequent cardiovascular and central nervous system involvement, with higher mortality.

Interestingly, the frequency of cardiovascular involvement reached 54% in the Sub-Saharan African group, whereas the rate of immunosuppressive use was not correspondingly higher. These data support the hypothesis

that lower socioeconomic conditions and suboptimal healthcare may influence mortality rate [14]. Sibley et al. [15] compared the clinical manifestations and activity of BD patients followed at two tertiary centers in the United States (National Institutes of Health and New York University) and at the Turkish Tertiary Referral Center of Istanbul University, Cerraphasa Medical School.

American patients showed more frequent gastrointestinal and neurologic disease, were more likely female, and had longer disease duration. Eye and vascular disease frequency rates were similar in both US and Turkish patients.

Finally, a change in disease expression has been noted in recent years, which is attributed to increased awareness of the disease and greater accessibility to hospitals, together with improvements in both hygienic conditions and therapeutic strategies.

Kim et al. [16] retrospectively evaluated 3674 patients divided into two decades and found a significant decline in rates of complete phenotypes of the disease, especially with regard to the major presenting features, such as genital ulcers, ocular involvement, and skin lesions. In addition, the mean patient age has increased progressively over the past three decades, together with joint, gastrointestinal, and central nervous system manifestations.

1.2 Genetics

Evidence for the relevance of genetic make-up to the susceptibility of developing BD derives from both inheritance and genetic studies performed in various countries. Familial aggregation is usually construed as evidence supporting genetic predisposition to disease. Significant familial clustering has been reported in 1–18% of BD patients, mostly of Turkish, Israeli, and Korean origin, especially in families of probands carrying the HLA B51 allele. The familial aggregation was higher in Turks (18.2%), Koreans (15.4%), and Jews (13.2%) than in Chinese (2.6%), Japanese (2.2%), and Europeans (1%) [17].

Likewise, twin concordance studies are frequently used to estimate the role of genetic factors in the pathogenesis of multifactorial diseases. In a recent twin study by Masatlioglu et al. [18], the pairwise concordance rate for BD was 2/6 for monozygotic twins and 1/8 for dizygotic twins, accounting for 41% of the phenotypic variance for BD among twins [17]. These data point to an interplay of genetic and as yet poorly characterized environmental factors in producing the clinical phenotype of BD.

Among the genes, the human leukocyte antigen (HLA) class I allele HLA-B51 is the genetic factor with the strongest association with BD susceptibility, independent of the different ethnicities [19]. The relationship between HLA-B51 and BD was first identified four decades ago and then replicated in nearly every genetic study on BD [20]. However, the pathogenic role of

HLA-B51 is still poorly elucidated. Neutrophil hyperfunction and presentation of specific antigens to CD8⁺ cytotoxic lymphocytes have been proposed as possible mechanisms, but much remains to be explained, not least the fact that the HLA-B51 allele is quite common in the general population, yet BD is a rare disease. Studies looking at associations between HLA-B51 subtypes and BD in different populations have generated discordant results, *de facto* failing to link one or few subtypes to disease susceptibility [4,21].

Whether HLA-B51 modifies the clinical phenotype of BD is still debated. In this regard, HLA-B51 has been linked to male gender, ocular and skin disease, and genital ulcers as well as to a decreased risk of gastrointestinal disease, but the respective relative risks are fairly weak [22].

In addition to HLA-B51, some HLA-I residues have been shown to influence antigen binding and regulation of cell-mediated cytotoxicity, supporting the role of one or more pathogenic peptides in BD [20]. In particular, such molecules could heighten the risk of BD through the regulation of both natural killer (NK) cells and CD8⁺ cytotoxic T lymphocytes activation [20]. Other genes and loci localized outside the HLA region may also be involved in the pathophysiology of BD [20,23–26].

GIMAP genes encode evolutionary conserved GTP-binding proteins that are preferentially expressed in immune cells. The GIMAP proteins represent a specific protein family (GTPase immune-associated proteins) which has been shown to play a role in modulating peripheral T-cell function and T-cell development and selection [27]. A lower level of GIMAP4 mRNA has been found in CD4 T cells from BD patients, which could contribute to resistance to T-cell apoptosis in BD [28]. However, thus far, the association described in Asian populations between the GIMAP region and BD has not been replicated in Europeans [29].

Targeted resequencing genetic studies have also found that the familial Mediterranean fever gene MEFV and the toll-like receptor 4 gene TLR4 are implicated in the pathophysiology of the disease, supporting at least a partial contribution of innate immunity and autoinflammation mechanisms to its pathogenesis [26].

2. CLINICAL PRESENTATION AND DIAGNOSTIC CRITERIA

2.1 Clinical Presentation

Recurrent oral ulcers are one of the hallmarks of BD, often representing the first clinical sign of the disease. BD-related aphthosis is sometimes referred to as “complex aphthosis” to distinguish it from the more common simple aphthosis, which affects roughly 15% of the general population. In contrast to simple aphthosis,

complex aphthosis is characterized by frequent recurrences of ulcers, which are usually particularly painful and take longer to heal. Moreover, in complex aphthosis genital ulcers may also coexist with oral ulcers. The most frequent sites of genital ulcers in BD are the major and minor labia in females and the scrotum and the shaft of the penis in males.

Aphtha can be categorized as minor, major, or herpetiform. Minor aphtha are usually small (<1 cm in diameter), superficial, and located on the buccal and labial mucosa; they heal within days and do not cause scarring. Major aphthous ulcers are larger (>1 cm), deeper, persist for weeks to months, and cause scars. Herpetiform ulcers are small (medium diameter, 1–3 mm), usually manifest as a group of coalescent lesions around a larger plaque, and heal spontaneously in 1–4 weeks [30].

The pathergy phenomenon is nearly unique to BD. It is induced by a 20–22 gauge needle prick in the dermis (usually of the forearm); the test is considered positive if an erythematous papule or pustule greater than 2 mm develops within 48 h. Skin pathergy reaction remains the most diagnostically relevant test in BD patients, although its prevalence varies according to different ethnicities.

Papulopustular lesions are the most common skin manifestation of BD. When papulopustular lesions occur around a hair follicle they are sometimes called “pseudofolliculitis,” whereas when they resemble acne vulgaris they are called “acneiform lesions.” Erythema nodosum-like lesions are the second most common skin manifestation of BD; they affect women in particular and should be differentiated from superficial thrombophlebitis. Histology may show vasculitis, but may also be nonspecific [31,32].

Uveitis and retinal vasculitis are among the most common organ manifestations, occurring in 60–80% of patients. Intraocular inflammation may involve the anterior or posterior segment, or both [33].

Gastrointestinal, neurologic, and cardiovascular complications are present in variable numbers of patients. The frequency of gastrointestinal involvement has been reported as low in the Middle and Far East (2.8% in Turkey, 4% in Saudi Arabia), moderately high in China (10%) and Taiwan (32%), and higher in the United Kingdom (38–53%) and Japan (50–60%).

Ulcers involving the gastrointestinal tract typically appear irregular, round or oval, punched-out, deep, and larger than 1 cm; they can be single or a few. The majority of patients with BD have ileocecal localization of the disease. Colonic and rectal involvement are even more rare. Colonic ulcers are described as “volcano-type” due to the nodular margins and aspects of deeply penetrating lesions at endoscopic evaluation.

Nervous system involvement, also known as “neuro-Behçet’s disease” (NBD), is observed in approximately

10–15% of cases and may be subclassified into two major forms: a parenchymal form, which often presents as aseptic meningoencephalitis (“neuro-Behçet’s” stricto sensu), or an isolated cerebral venous sinus thrombosis often complicated by intracranial hypertension [34]. Cerebral (typically dural) sinus thrombosis is associated with other vascular manifestations, such as deep-vein thrombosis, superficial thrombosis, and pulmonary artery aneurysms.

Vascular involvement has an estimated occurrence rate varying from 10% to 50%. Studies from Morocco, Saudi Arabia, Lebanon, and Turkey reported increased prevalence of venous lesions as compared to Korean populations [35]. Venous thrombosis most commonly involves popliteal and femoral veins. Thrombosis of superior or inferior vena cava and of upper extremity veins are also observed. Caval involvement rates vary from 2% to 13%. Thrombosis of the hepatic veins (Budd-Chiari syndrome) may occur as an extension of thrombosis from inferior vena cava.

The main features of pulmonary involvement are pulmonary artery aneurysms, arterial and venous thrombosis, pulmonary infarctions, recurrent pneumonia, and pleurisy. These are all considered rare manifestations, with an estimated prevalence of about 1%, according to different studies [36].

The most common pulmonary findings are pulmonary artery aneurysms; given their frequent association with cardiac involvement, they will be discussed in the [Section 4](#) of this chapter, in the discussion of cardiac manifestations of BD.

2.2 Diagnostic Criteria

The diagnosis of BD is based on a combination of clinical symptoms and signs, since there is no specific histologic, laboratory, or radiologic finding. Several sets of diagnostic and classification criteria have been proposed, following the disease definition by Hulusi Behçet. However, such criteria were not built on consensus, have not been validated, and are now basically only of historical interest [37].

In 1990 the International Study Group on Behçet’s Disease (ISGB) composed of experts from seven countries (Turkey, Iran, Japan, Tunisia, France, UK, and United States) presented the ISGB criteria, which soon became the most commonly used criteria [38] ([Table 21.1](#)).

It is worth noting that in the ISGB criteria oral ulcers represent a prerequisite to classify a patient as having BD. Therefore such criteria are by definition inapplicable to the odd BD patient with no oral ulcers (<1–2% of the entire BD population). In addition, vascular and cardiac manifestations have not been included in the ISGB criteria because of their poor specificity. Lastly, although the authors explicitly defined the ISGB

criteria as “diagnostic criteria,” they are in fact “classification criteria.” In this regard, in a longitudinal study from our group using the specialist diagnosis as the gold standard, we showed that 87% of patients with BD fulfilled the ISGB criteria at 10 years from diagnosis, while the percentage of patients with early BD who met the criteria was as low as 23% (unpublished data). Therefore the ISGB criteria should not be used to diagnose BD in the individual patient, but they are useful – because of their high specificity – to enroll patients in clinical trials [39].

In 2006 the new International Criteria for Behçet’s Disease (ICBD) were presented; these criteria were subsequently validated in some countries, and recently revised (2010) [38] (Table 21.2).

Differing from the ISGB criteria, the ICBD criteria includes as items both vascular and neurological manifestations.

To summarize, the ISGB criteria have very good specificity at the expense of sensitivity and accuracy, while the ICBD have better sensitivity (97% vs 77%), lesser specificity (97% vs 99%), and better accuracy (97% vs 87%).

TABLE 21.1 International Study Group for Behçet’s Disease (ISGB) – 1990 Criteria [38]

1. Recurrent oral ulceration (aphthous or herpetiform) observed by the physician or patient recurring at least three times in one 12-month period plus two of the following:
2. Recurrent genital ulceration
3. Eye lesions: anterior uveitis, posterior uveitis, cells in the vitreous by slit lamp examination or retinal vasculitis observed by an ophthalmologist
4. Skin lesions: erythema nodosum, pseudofolliculitis, papulopustular lesions, or acneiform nodules in postadolescent patients not on corticosteroids
5. Pathergy, read by a physician at 24–48 h
Sensitivity 90%, specificity 95% – but not at onset [39]

TABLE 21.2 International Criteria for Behçet’s Disease (ICBD) [40]

Ocular lesions 2 points
Oral aphthosis 2 points
Genital aphthosis 2 points
Skin lesions 1 point
Central nervous system involvement 1 point
Vascular manifestations 1 point
Optional: pathergy test, when used, 1 point
A patient scoring ≥4 points is classified as having active Behçet’s disease
In the validation set, ICBD had a sensitivity of 95% versus the International Study Group for Behçet’s Disease (ISGB) 1990 criteria at 85%, and specificity 91% versus ISGB 96.0%. Pathergy test (assessed when done in >90% of patients and controls) increased sensitivity from 96% to 98.5% and slightly decreased specificity from 92.1% to 91.6% [38,40,41].

Nevertheless, the performance of the ICBD criteria needs to be validated in different populations; thus additional validation studies are required [42].

3. PATHOPHYSIOLOGY OF THROMBOSIS AND VASCULAR DAMAGE IN BEHÇET’S DISEASE

BD is a systemic inflammatory disease that may involve both arterial and venous blood vessels of any size. Immunohistochemical analysis of BD inflammatory vascular lesions typically shows a perivascular infiltrate of neutrophils and mononuclear cells, with few B lymphocytes, NK and macrophage cells, and a high preponderance of T (CD3⁺) lymphocytes (mostly CD4⁺ cells) [43–45]. T cells are enriched in $\gamma\delta$ and Th1/Th17 lymphocytes in the peripheral blood and at sites of active inflammation. In contrast, decreased frequency of CD4⁺ FOXP3⁺ Tregs has been found in the peripheral blood of patients with BD. However, the pathogenic mechanisms underlying the histological changes observed in BD still remain largely elusive.

An infectious trigger for BD was advocated by both Adamantiades (who postulated a bacterial cause) and Behçet (who hypothesized a viral cause). The trigger hypothesis is not yet out of fashion, but conclusive evidence is still lacking. The animal model that perhaps comes closest to mimicking BD is herpes simplex virus-induced vasculitis, in which 258 ICR strain mice after repeated inoculation of the virus developed arthritis, oral and genital ulcers, and keratitis clinically resembling BD [46]. However, in humans, no single pathogenic agent has consistently been singled out as a trigger of BD.

Regardless of the putative enticing event, a perturbed immunological homeostasis in BD is demonstrated by the activation of both innate and acquired immunity. With regard to innate immunity, a primed state of neutrophils in BD has been related to their hyperactivation, as confirmed by an increased expression of neutrophil activation markers CD10, CD11a, and CD14 on neutrophil cell surface [47]. A boosted chemotaxis, phagocytosis, reactive oxygen species (ROS) and superoxide generation, as well as increased myeloperoxidase levels have also been reported [43,47,48]. Increased levels of IL-18, IFN- γ , and TNF- α as well as Th17 cells can all contribute to prime the neutrophils [49], while circulating cytokines can concomitantly activate the endothelium [50] and shift it to a procoagulant state.

Similarly, the increased $\gamma\delta$ /Th1/Th17 activity and the decreased Treg activity consistently point to an orchestrated response of the acquired immunity. In particular, $\gamma\delta$ T cells might be a key link between innate and acquired immunity. Normally, $\gamma\delta$ T cells patrol mucosal sites and are able to recognize antigens or pathogens,

especially heat shock proteins (HSP) from *Streptococcus* and *Mycobacterium* species, without HLA restriction. They are strong inducers of Th1 and Th17 responses in experimental models, are expanded in patients with active BD, and are suppressed by biological agents. Therefore this subgroup of T cells may be of special relevance to the pathogenesis of BD [51].

The pathogenesis of cardiovascular lesions stricto sensu in BD is still a matter of debate. There is good evidence that prothrombotic factors including anti-cardiolipin antibodies do not explain the propensity of patients with BD to develop thrombotic events. In contrast, the most widely held belief is that in some patients an inflamed endothelium shifts from an anticoagulant to a procoagulant state. In this regard, Schmitz-Huebner and Knop [52] found that ischemia of endothelial cells was able to enhance platelet aggregation. In keeping with this notion, increased levels of von Willebrand factor and thrombomodulin have been found in patients with BD, suggesting endothelial cell damage. On the other hand, increased levels of platelet-activating factor and P-selectin activation markers on platelets in BD patients with thrombosis may represent a link between endothelial activation/damage and thrombosis [53].

Autoinflammatory mechanisms may contribute to the prothrombotic risk of patients with BD, as demonstrated by some studies showing a higher frequency of thrombosis development in BD patients carrying mutations in the MEFV gene, which is responsible for familial Mediterranean fever [54].

4. CARDIAC INVOLVEMENT IN BEHÇET'S DISEASE

The spectrum of cardiac involvement in the course of BD is protean and includes valvular disease, coronary arteritis with or without myocardial infarction, aneurysms of the coronary arteries, of the heart chambers, or of the aortic sinus (sinus of Valsalva), intracardiac thrombus (IT) formation, pericardial effusion, myocarditis, arrhythmias, pulmonary artery hypertension, and endomyocardial fibrosis (EMF).

According to the literature, the frequency of cardiovascular involvement in BD is highly variable, ranging between 7% and 46% with a lethal outcome in about 20% of cases [55–57]. On the other hand, the frequency of cardiac involvement proper is lower, ranging from 1–6% to 16.5% as reported by case-series and autopsy registry data based on a few tens of cases [58–60]. The discrepancies in the reported frequencies may be explained by referral and ascertainment biases as well as by varying degrees of skill in performing and interpreting clinical investigations, such as echocardiography.

In one of the largest published series [60], 52 (6%) of 807 consecutive patients fulfilling the international criteria for BD [38] had evidence of symptomatic cardiac involvement. The authors considered the cardiac manifestations to be related to BD when they occurred during disease flares and when other causes of heart disease were excluded. Cardiac involvement represented the first clinical disease manifestation in 17 (33%) patients. In this case series, pericarditis was the most common detected manifestation (39% of cases), followed by endocardial disease including valvulopathy (27%), IT (19%), and EMF (8%), while other manifestations were rare. The mean age at diagnosis was relatively young (29 ± 9.9 years), although not significantly different from that of patients with BD without cardiac disease. Patients with cardiac lesions were more commonly males (87% vs 65% of those without cardiac lesions), less likely to be of European origin (33% vs 50%), and less frequently HLA-B5 positive (35% vs 53%). Arterial and/or venous involvement were overrepresented, suggesting that cardiac and vascular manifestations are part of the same disease spectrum. However, despite being one of the largest and more recent case series on cardiac involvement in BD, this study did not address the question of subclinical heart involvement.

In this regard, a subclinical contractile dysfunction has been observed in other studies, mainly by using conventional and tissue Doppler echocardiography [61,62].

Histopathologic findings of acute vasculitis alongside degenerative changes are considered typical in the setting of cardiac BD, different from the scarring and thickening commonly observed in other large-vessel vasculitides, such as Takayasu arteritis, which is characterized by more smoldering inflammation [63].

Diffuse myxoid degeneration has been observed in the histologic exam of the aortic valves in an operative series of nine patients undergoing surgery [64]. In another series of seven patients, central necrosis with granulation tissue was found at valvular histology, while in the aortic wall medial necrosis and inflammatory infiltration of the adventitia were described [65].

In this section, the main issues concerning cardiac involvement in the course of BD are discussed. Echocardiographic abnormalities have been examined in-depth in the setting of BD-related valvular and aortic disease. More rare entities, such as EMF, endocardial fibroelastosis, pericarditis, myocarditis, intracardiac thrombosis, and pulmonary artery aneurysms have also been reviewed. Subclinical involvement, such as silent myocardial ischemia and diastolic dysfunction as well as the controversial issue of accelerated atherosclerosis are discussed. Electrocardiographic findings and conduction disorders are also analyzed.

4.1 Valvular Disease

Increased prevalence of valvular disease in BD has been documented by several case-control studies, both in symptomatic patients and in those without clinical evidence of cardiac involvement by echocardiography.

Echocardiography is a useful diagnostic tool to detect valvular abnormalities in BD [66]. Most of the data comes from studies that used transthoracic echocardiography (TTE), while data from transesophageal echocardiography (TEE) are more limited. The wide variability of valvular abnormalities in BD detected by echocardiographic investigations (eg, from 6% up to 50% of frequency of mitral and aortic prolapse detection has been reported in different studies) is probably attributable to differences in the criteria for diagnosing valve defects, to the heterogeneity of study populations, and to varying degrees of skills in performing and interpreting echocardiography.

The most common findings detectable in the course of BD are mitral valve regurgitation and prolapse, dilation of proximal aorta, and – according to some authors – interatrial septal aneurysms [67,68]. Aortic abnormalities are also common, but will be discussed separately. Mitral valve prolapse is usually associated with mitral insufficiency in BD; the rates of both abnormalities have been found higher than controls in some series [67,68]. Specifically, using TEE, a significantly higher incidence of mitral valve prolapse (25% vs 3%) and mitral regurgitation (40% vs 6%) was found in the case-control study performed by Gürgün et al. on patients with BD without cardiac symptoms [67]. The frequency of mitral valve prolapse reached 50% in the study by Morelli et al. [69]. These data are in contrast with that of subsequent studies in which mitral prolapse and regurgitation showed lower prevalence. In particular, Bozkurt et al. [70] found rates of mitral valve prolapse and regurgitation of 1.8% and 3.7%, respectively. Selection criteria might explain the differences of reported frequencies of mitral valve disease in BD (Fig. 21.1).

Interatrial septal aneurysms were disclosed in 10 out of 30 Turkish BD patients studied by Heper et al. [68] using a combination of TTE and TEE. The increased prevalence (approximately 30%) was in line with the previous observations by Gürgün et al. [67], but these findings, both from Turkish series of BD patients, have not been confirmed by other studies [66].

With regard to the histopathology, hyperplasia and sclerosis are common findings in the affected valves. Neutrophil and plasma cells infiltration is detected, though the amount of plasma cells is usually less as compared to the infiltrate observed in other vasculitides, such as Takayasu's arteritis. Light microscopy shows mucoid degeneration and fibrinoid necrosis. Such changes can eventually cause weakening of, and damage to, endocardial valves, resulting in overt valvulopathy.

Mitral valve involvement in BD may be associated with endocarditis verrucosa ulcerosa. A massive perivascular infiltration of lymphocytes and plasma cells in adventitia and vasa vasorum along with the fibrous proliferation of the vasa vasorum is a common finding in this context [71].

4.2 Aortic Abnormalities, Including Valvulopathy

Aortic valve prolapse and regurgitation have been found in the Turkish cohort of Gürgün et al. [67] in 5% of patients versus none of unaffected controls, a difference that did not reach statistical significance, similar to other studies [66]. In some cases, aortic valves may have normal morphology, and regurgitation may develop progressively in the context of left ventricle enlargement.

On the other hand, when aortic insufficiency is acute, a marked thinning and redundancy of aortic leaflets, with or without mobile masses and/or free echo space within the annulus and/or ventricular septum assessed by TEE, may be considered pathognomonic

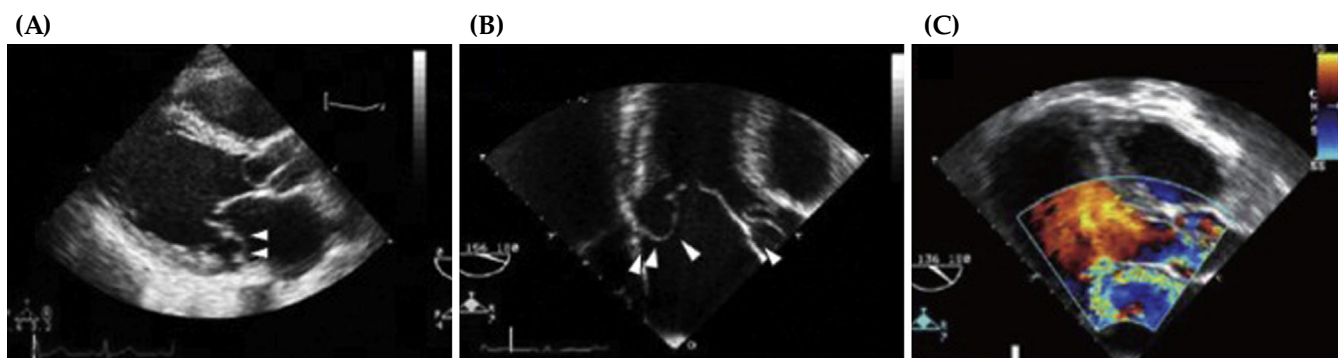


FIGURE 21.1 A case of mitral valve prolapse in the basal portion with severe mitral regurgitation in a 42-year-old man with BD [169]. (A) Transthoracic echocardiography showing mitral valve prolapse, predominantly involving the basal portion of the posterior mitral leaflet (white arrowheads), together with aneurysmal changes of the aortic valve. (B) Transesophageal echocardiography (156 degree) showing mitral valve prolapse in basal portion of the mitral leaflet (white arrowheads). (C) Transesophageal echocardiography (136 degree) showing severe mitral regurgitation. Adapted from Yoon et al. [169].

of BD [72,73]. However, these findings may resemble infectious endocarditis and even meet the major Duke criteria. Specifically, aortic regurgitation due to BD can be misdiagnosed as an infective endocarditis when an echo free space mimics aortic root abscess, producing a peculiar image of “vegetation-like” mobile lesions. To semiquantify the severity of the lesions it may be useful to focus the echocardiographic study on aortic cusp and on adjacent segments of the ascending aorta and inter-ventricular septum.

The majority of surgical cases for the treatment of severe aortic insufficiency showed a male predominance (93%, 79%, and 57% in [73–75], respectively), confirming the higher propensity of BD in manifesting as a systemic disease in men [63].

The aorta itself does appear to be abnormal in several patients, although aortic aneurysms are considered very rare in BD [63]. Aortic root dilatation, which is thought to precede aneurysm formation, is one of the findings commonly reported in echocardiographic studies in asymptomatic BD patients [59,60,92]. An aorta dilation >4cm was found in 48% of BD patients randomly selected for a Turkish TEE study [67], while in another Italian study 30% of subjects undergoing TTE had dilatation of the ascending aorta [69] (Fig. 21.2).

However, some authors did not find statistically significant enlargement of aortic root diameter in BD patients as compared to controls [70,76]. One of the reasons for the discrepancy of these data might be the different mean ages of the study patients and the different disease durations [63,70].

In addition to aortic dilatation, echocardiographic studies have shown reduced aortic distensibility, which may help explain the propensity of aneurysm formation in the course of the disease as well as the increased incidence of aneurysmal dilatation of Valsalva sinus [67]. As previously stated, the underlying vasculitic process may indeed weaken the aortic wall and the structures near the aortic sinus, contributing to aneurysmal dilatation [77].

Cases of cardiac BD complicated with rupture of Valsalva sinus aneurysm have been reported [78]; aortic root dissection associated with perforation of the left Valsalva sinus into the left ventricular outflow tract was described in one of them [77]. In the latter case, pathology of the aortic wall and valves revealed focal fibrinoid necrosis and myxoid degeneration with inflammatory cell infiltration [77] (Fig. 21.3).

The vasculitic process of the aortic wall determining adherence and fistula formation to the right atrium was the most probable underlying cause in another case of aorto-atrial fistula without concomitant aneurysm [79].

4.3 Endomyocardial Fibrosis, Endocardial Fibroelastosis, and Cardiomyopathies

Cases of EMF in BD have very rarely been reported. EMF has been interpreted as a long-term complication of vasculitic process that may involve endocardium, myocardium, or both. Mural thrombus may complicate the clinical picture. In their case report and literature review updated in 2012, Buturak et al. [80] found an

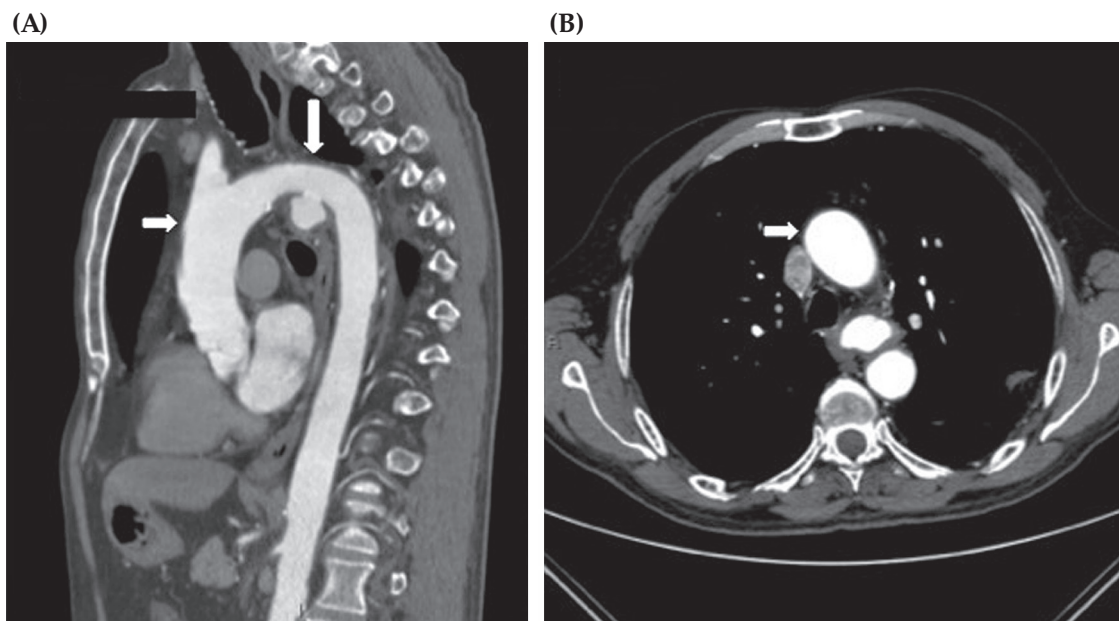


FIGURE 21.2 CT angiography of a 54-year-old man affected with BD, with mucocutaneous and vascular involvement. Sagittal (A) and transverse (B) sections showing aneurysmatic dilatation of ascending aorta with parietal thickening (white arrows). Adapted from the personal collection of Dr. Salvarani, Arcispedale Santa Maria Nuova – IRCCS, Reggio Emilia, Italy.

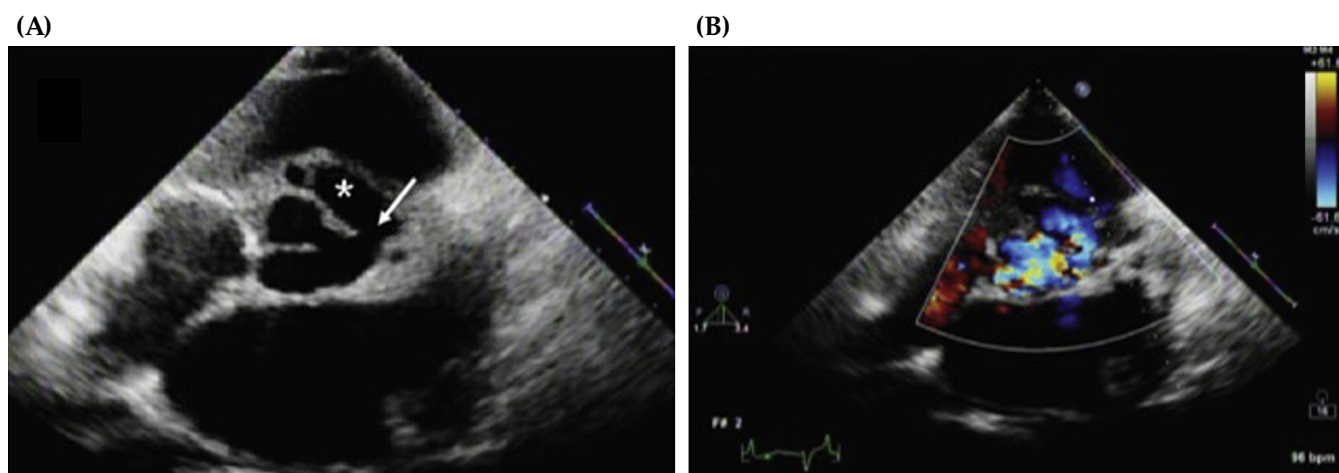


FIGURE 21.3 A case of aortic root dissection associated with perforation of the left Valsalva sinus into the left ventricular outflow tract in a 49-year-old woman affected with BD [77]. (A) Parasternal short axis view at the aortic level shows the echolucent cavity (*) between the aortic root and the left and right Valsalva sinus, and a perforation (arrow) of the left Valsalva sinus. (B) Short-axis view of the aortic root with color-flow imaging demonstrating blood flow streaming through the perforation of the left Valsalva sinus into the dissection between the aortic root and the Valsalva sinus. Adapted from Zhao et al. [77].

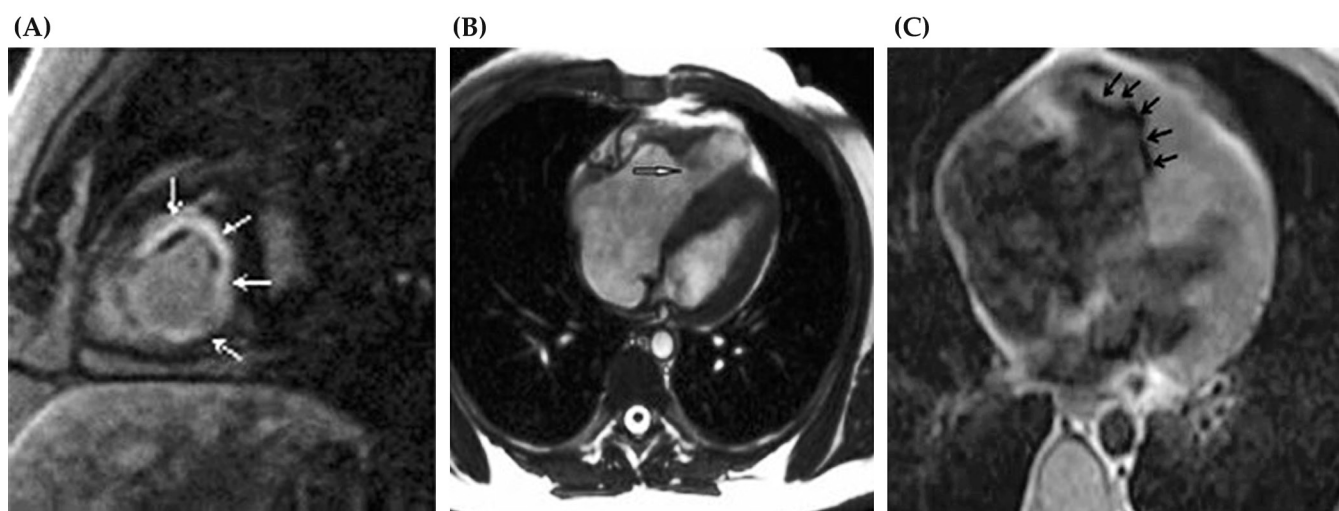


FIGURE 21.4 A case report of right ventricular endomyocardial fibrosis mimicking Ebstein anomaly in a 26-year-old Turkish male with Behçet disease [80]. (A) Turbo spin echo (TSE) T1 weighted axial magnetic resonance (MR) image showing low signal intensity (arrows) along the right ventricle endomyocardium indicating endomyocardial fibrosis. (B) Four-chamber cine MR denoting the downward displacement of the tricuspid leaflets (arrow), mimicking Ebstein anomaly. (C) Delayed-enhancement MR image of short axis showing subendocardial hyperenhancement of the right ventricle, suggestive of fibrosis. Adapted from Buturak et al. [80].

additional 15 cases of endomyocardial fibrosis with BD since 1977, when it was first described at necropsy [81] (Fig. 21.4).

Analysis of the main features of the reported cases revealed a Mediterranean dominance, and predominant tendency to involve the right ventricle and tricuspid valve, with heart failure being present in most of them.

Pulmonary aneurysm and cerebral sinus thrombosis have been reported as the main features of vascular involvement complicating EMF [82].

When EMF extends to the leaflets and papillary muscles, insufficiency of atrioventricular valves develops.

The histological examination available for some of the cases showed fibrous tissue with granulocytes, neovessels, mononuclear cells, and fibroblast infiltrates (Fig. 21.5). Necropsy studies showed that intraventricular thrombi covered EMF.

The detection of the lesions was incidental, during routine echocardiography or surgery procedures. Typical echocardiographic features are a bright echogenic aspect resembling pseudotumor, the displacement of the leaflets of the tricuspid or mitral valves, and right atrial enlargement, with or without narrowing of the ventricles. In the case of Buturak et al. [80] the aspect of abnormal tricuspid valve mimicked an Ebstein anomaly,

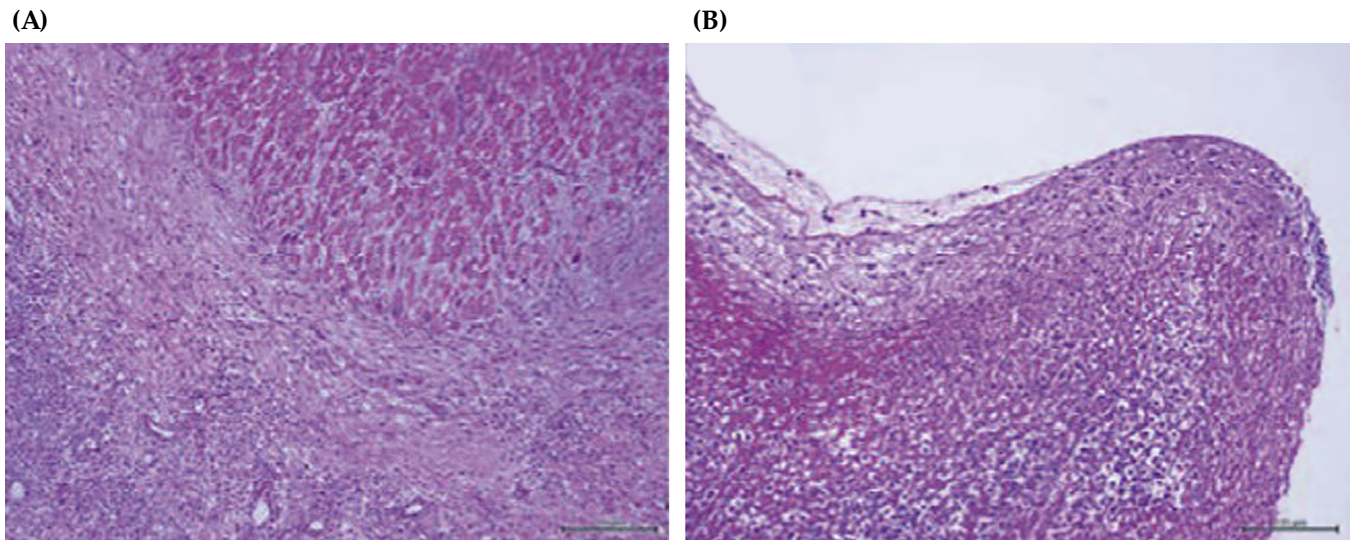


FIGURE 21.5 A case report of right ventricular endomyocardial fibrosis mimicking Ebstein anomaly in a 26-year-old Turkish male with BD [80]. Panel A shows interstitial fibrosis, fibrosis among the myocardial fibers and myocyte hypertrophy. Panel B cell shows debris and fibrin accumulation on endocardial and myocardial surfaces. Adapted from Buturak et al. [80].

because of the distortion of the leaflets by the fibrous endomyocardial mass.

In another case, a presumptive diagnosis of a tumor mass of the right ventricle involving the tricuspid valve was made in a BD patient who had suffered from a previous right ventricle thrombus, which resolved after immunosuppressive treatment [83]. Histological examination of the mass showed lymphocyte infiltration and fibroelastic changes within the lesion entirely restricted to endocardium and typically not involving the subendocardial myocardium, suggestive for endocardial fibroelastosis.

In addition, linear calcifications surrounding fibrosis area can be visualized at the CT or MRI. A case of endocardial calcification in a Japanese man with a 30-year history of BD was recently reported on the midlateral and apical wall [84]. The authors documented the extension of endocardial calcification over a period of 11 years, as well as the dilatation of both the atria and right ventricle. A diagnosis of EMF-related restrictive cardiomyopathy was also made on the basis of echocardiographic findings, catheterization, and finally endomyocardial biopsy (Fig. 21.6).

Apart from restrictive cardiomyopathy, which in BD is strictly linked to right-sided EMF, other forms of cardiomyopathies have sporadically been reported in the literature. In some cases, an underlying myocarditis may have a silent clinical course and progress to dilated cardiomyopathy with frank clinical presentation [85]. A fatal form of symptomatic dilated cardiomyopathy was reported by Kaatz et al. [86] in a 67-year-old man with a diagnosis of late onset BD; two other cases of dilated cardiomyopathy with symptomatic left ventricular dysfunction and normal coronary arteries were described by Schueble et al. [87]. Al Izzi et al. [88] recently reported the case of a 33-year-old Arab male with dilated cardiomyopathy presenting as

severe congestive cardiac failure with paroxysmal atrial fibrillation, requiring implantable cardiac defibrillator. In this case a 6-year history of recurrent mouth ulcers followed by genital ulcers and pustular folliculitis was present. Although the occurrence of both disorders could be interpreted as coincidence, such a cardiomyopathy could also be interpreted as the first manifestation of BD. Of note, in the latter case several endomyocardial biopsies showed degenerative changes and lysis of monocytes with mild interstitial fibrosis and cytoplasmic vacuolization, a pathologic picture not conclusive with regards to the etiology.

4.4 Silent Myocardial Ischemia and Acute Myocardial Infarction

Silent myocardial ischemia and acute myocardial infarction (AMI) in BD are more frequently due to coronary vasculitis with normal coronary arteries in the absence of coronary atherosclerosis. The pathological findings of coronary involvement in BD include arteritis and inflammatory obliterative endoarteritis of the vasa vasorum, which causes destruction of the media and fibrosis, with ensuing weakening of the arterial wall and tendency of aneurysm formation [89–91]. Some authors have also hypothesized a coronary microvascular dysfunction, which might impair coronary flow reserve (CFR) [92].

In a survey by Geri et al. [60] of 52 BD patients with symptomatic heart disease, 17% had myocardial infarction. Recently, Gullu et al. [92] examined 40 healthy subjects and 33 BD patients without any previous vascular involvement and without any identifiable risk factor for coronary microvascular dysfunction, such as diabetes mellitus, hypertension, hyperlipidemia, smoking, alcohol use, and concomitant corticosteroids/vasoactive

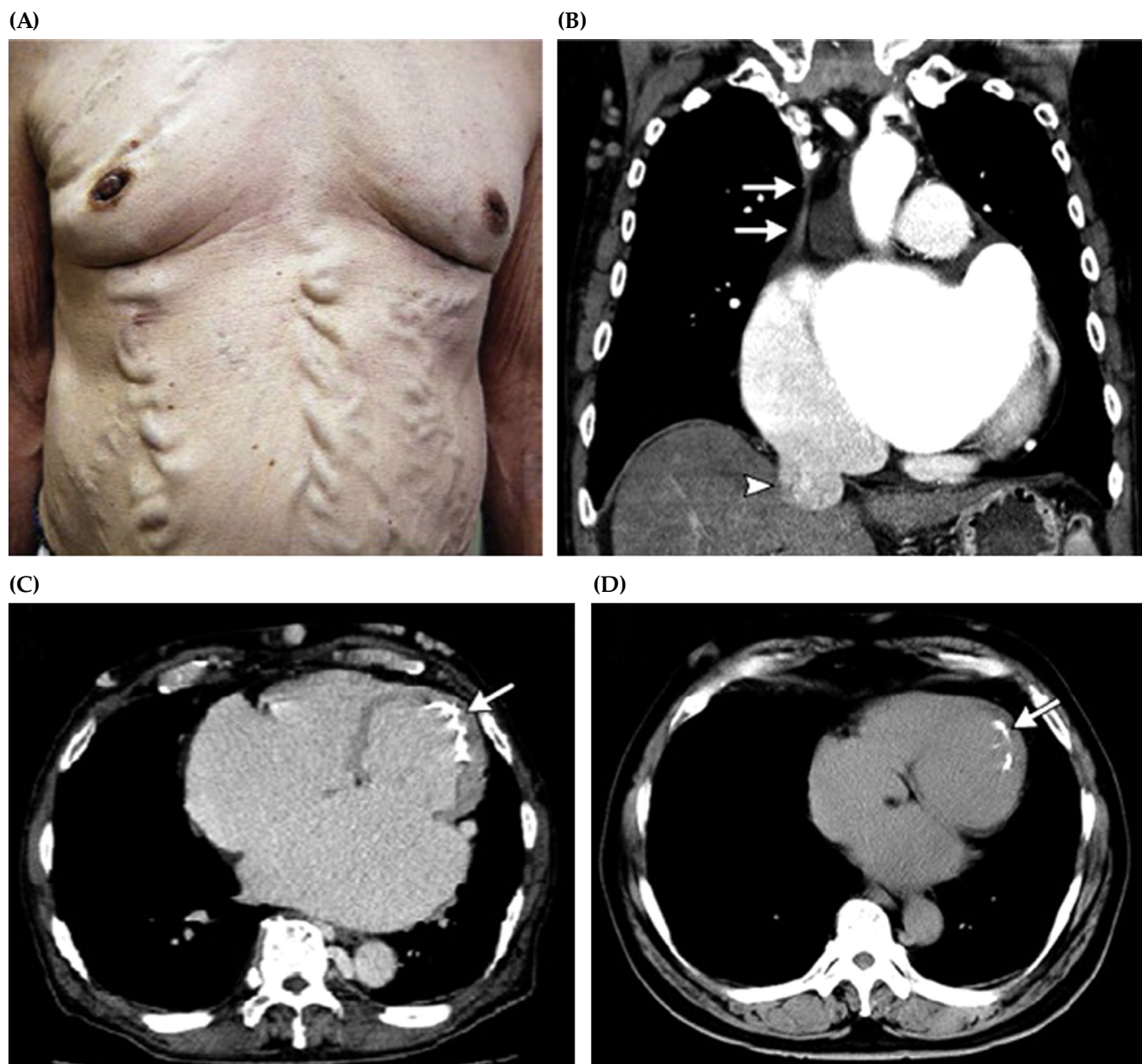


FIGURE 21.6 Nishida and Hakuno [84] reported the case of a 68-year-old man who presented with varices of the chest and abdominal wall (Panel A) due to superior vena cava obstruction (Panel B, *arrows*). Chest CT showed also dilatation of inferior vena cava (Panel B, *arrowhead*). CT scan detected endocardial calcification on the midlateral and apical wall (Panel C, *arrow*), more extensive than that seen on previous imaging performed 11 years earlier (Panel D, *arrow*). Adapted from the personal collection of the authors.

drug use. The authors identified eight patients with silent myocardial ischemia, without any significant coronary stenosis at coronary angiography. The study confirmed the results of Turkometz et al. [93] who previously found ischemic changes on stress ECG in 8 out of 41 BD patients (as compared to 1 out of 35 controls).

4.5 Accelerated Atherosclerosis

Preliminary reports on accelerated atherosclerosis in BD produced conflicting results, different from what was previously established for rheumatoid arthritis and systemic

lupus erythematosus, or in other types of vasculitis, such as Takayasu's arteritis. In one of the largest studies on the issue, Seyahi et al. [94] evaluated the presence of subclinical atherosclerosis in 239 BD patients, among which 167 had major organ involvement. One hundred patients with rheumatoid arthritis, 74 with ankylosing spondylitis, and 156 healthy controls were concomitantly evaluated. The authors determined traditional atherosclerotic risk factors in all groups and assessed the frequency of plaques and intima-media thickness (IMT) in the carotid and femoral arteries. The frequency of plaques and the mean IMT in both districts appeared similar between the three groups

TABLE 21.3 The Main Case–Control Studies Assessing Intima-media Thickness in Behçet's Disease (BD) Patients

Study	Country	Controls N	IMT	BD patients N	Disease status	IMT
Caldas et al. [96]	Brazil	23	0.561 ± 0.134	23	Syst/MC	0.594 ± 0.138
Can et al. [97] ^a	Turkey	51	0.39 ± 0.09	36	AD/ID	0.56 ± 0.122
Hassan et al. [98]	Egypt	20	0.4 ± 0.1	30	ID	0.72 ± 0.4
Yurdakul et al. [99]	Turkey	20	0.59 ± 0.09	40	Non sys/non V	0.69 ± 0.15
Ozgen et al. [100]	Turkey	29	0.547 ± 0.04	37	AD/ID	0.675 ± 0.07
Messedi et al. [101]	Tunisia	50	0.581 ± 0.087	50	ID	0.658 ± 0.112
Hong et al. [102]	Korea	20	0.59 ± 0.11	40	AD/ID	0.71 ± 0.17
Caliskan et al. [103]	Turkey	35	0.46 ± 0.82	53	V/non V	0.515 ± 0.012
Ozturk et al. [104]	Turkey	21	0.57 ± 0.14	21	ID	0.86 ± 0.18
Seyahi et al. [94] ^a	Turkey	156	0.68 ± 0.08	239	Syst/MC	0.71 ± 0.09
Rhee et al. [105]	Korea	53	0.52 ± 0.06	41	No CVD risk	0.52 ± 0.09
Ozturk et al. [106]	Turkey	34	0.54 ± 0.13	34	Non V	0.81 ± 0.17
Oflaz et al. [107]	Turkey	46	0.55 ± 0.14	50	V/non V	0.69 ± 0.15
Keser et al. [108] ^a	Turkey	77	0.48 ± 0.09	114	No CVD risk	0.55 ± 0.14
Alan et al. [109]	Turkey	42	0.59 ± 0.12	40	V/non V	0.81 ± 0.12

Apart from the study by Seyahi et al. [94] disease status has been specified.

N, numbers; Syst, systemic disease; MC, only mucocutaneous manifestations; AD, active disease; ID, inactive disease; V, vascular; CVD, cardiovascular; IMT, intima-media thickness.

^aStudies evaluating larger populations and yielding the most significant results on the issue.

Adapted from Merashli et al. [95].

and the authors concluded that increased atherosclerosis was not a prominent feature in BD, even in patients with major organ involvement. Similarly, an excess of atherosclerotic cardiovascular disease burden or mortality in BD patients has not been documented [94]. However, other studies and a recent *meta*-analysis appear to question the above-mentioned results.

Merashli et al. [95] recently performed a systematic review and *meta*-analysis of studies where atherosclerosis was determined by flow-mediated dilatation (FMD) endothelial-mediated dilatation (EMD) and IMT of carotid arteries, taking into account the relevant literature from January 2000 to January 2014 (see Table 21.3). The designated outcomes were the differences in FMD/EMD measured at the brachial artery in BD patients versus healthy controls. Nine studies met the inclusion criteria on FMD/EMD, 11 on IMT, and 4 on both. FMD was impaired in BD, even in inactive state; IMT and plaque frequency were also found with greater frequency in the fourth decade of life. However, as causality cannot be proven by cross-sectional studies, the authors concluded that further studies should be prospectively designed with serial FMD and carotid ultrasound measurements. This could be helpful in better defining whether risk factor modification can lead to early prevention of endothelial dysfunction, which is currently not established.

Arterial stiffness is another determinant of cardiovascular risk, with a direct role in atherosclerosis development.

Yilmaz et al. [110] assessed arterial stiffness of 96 BD patients, in active and inactive disease periods, and compared them with a control population of 54 healthy age- and sex-matched subjects.

Night pulse-wave velocity (PWV) values, indicators of arterial stiffness, were higher in patients with active BD than in those with inactive disease. After linear regression analysis, 24h PWV positively correlated with age and duration of the disease. In addition, cardiac output values and day central diastolic blood pressure were higher in BD patients than in the control group. The authors argued that these results may be attributed to more prominent inflammatory changes in the vascular wall during active phases of the disease.

Aortic stiffness was evaluated by transthoracic echocardiography by Tunc et al. [111], given the good correlation of this method with other more invasive techniques [112]. Tunc et al. [111] found significantly low values of aortic strain and distensibility and decreased aortic diameter change in the BD group as compared with controls. The authors' findings were in agreement with the results of Ikonomidis et al. [113], which showed an association between aortic stiffness and disease duration as well as left ventricular diastolic dysfunction.

Endothelial dysfunction (ED) can be defined as the shift of the properties of the endothelium toward a phenotype characterized by impaired vasodilation and a

TABLE 21.4 Principal Serum Biomarkers of Endothelial Dysfunction in Patients With Behçet's Disease

Serum biomarker	Function	Findings in Behçet's disease (BD)
Adrenomedullin	Induces vascular relaxation; angiogenic and cardioprotective factor; increases in vascular diseases, such as hypertension	Increased [119] relative to unaffected controls
Asymmetric dimethylarginine (ADMA)	Product of metabolism of L-arginine; inhibits nitric oxide synthesis and therefore impairs endothelial function	Increased in active BD, especially those with vascular involvement [120]
Homocysteine	Induces endothelial dysfunction by multiple mechanisms	Increased in active BD [114,121]
Malondialdehyde (MDA)	Indicator of lipid peroxidation and oxidative stress	Increased [122,123] relative to unaffected controls
Nitric oxide (NO)	Mediates endothelium-dependent vasodilation	Lower levels in patients with active BD [122] and relative to unaffected controls [120]
Thrombomodulin (TM)	Mediate endothelial thromboresistance	Increased in active BD [124]

proinflammatory and prothrombic status. ED is reportedly a feature of systemic vasculitis, and evidence for ED has specifically been provided for patients with BD. In particular, patients with BD have been shown to have impaired FMD relative to unaffected controls [107,114,115] as well as changes in serum biomarkers pointing to ED (Table 21.4). Pharmacological treatment with nebivolol [116], atorvastatin, lisinopril [117], and glucocorticoids [118] can reverse, at least in part, BD-related ED.

4.6 Diastolic Dysfunction

Similar to other “hot topics” in the setting of BD-related cardiac involvement, the issue of left ventricular diastolic dysfunction is still debated. Mitral E/A ratios <1 were found to be more frequent in BD patients than in age-matched controls in two studies [67,125] (22% vs 6% and 31% vs 5%, respectively), when pulse-wave Doppler of the mitral inflow was used. In contrast, no difference in the E/A ratio was found by the other two groups, although some abnormalities in isovolumic relaxation time and deceleration time [111,113] were reported.

By using Doppler tissue imaging of the lateral mitral annulus controversial results have similarly been found [61,70,111]. Gullu et al. [92] performed echocardiographic evaluation of the coronary diastolic peak velocities of the left anterior descending coronary artery at baseline and after dipyridamole infusion (0.84 mg/kg over 6 min). Mitral E velocity and mitral A velocity appeared similar between the two groups, while the mitral E/A ratio was lower in BD patients, suggesting a left ventricular diastolic dysfunction. The CFR was significantly impaired in BD patients as compared with controls, but no statistical significance in terms of CFR values was found in the BD patients group when stratified according to the ongoing colchicine therapy at the time of the evaluation.

Koc et al. [62] investigated parameters of left ventricular diastolic function with conventional and tissue

Doppler echocardiography as well as their relationship with P-wave dispersion (PD) in 31 BD patients and 31 healthy controls. Apart from the lower mitral inflow E/A ratio and the diastolic myocardial velocity ratio (Em/Am) in BD group, the authors found higher P-max and P-wave dispersion, which correlated positively with the duration of the disease, with the left atrial volume index (LAVi) and with E/Em ratio. A negative correlation was additionally found between PD and the E/A and E/Em ratios.

Demirelli et al. [126] recently used the speckle tracking technique in an echocardiographic study for subclinical left ventricular dysfunction assessment. Data from 30 BD patients and 25 controls showed that measurements of left ventricular longitudinal strain and strain rate were lower in the BD group, while the left ventricular basal rotation (at the apex) and left ventricular torsion were higher than in the control group. The authors concluded that combined assessment of left ventricular longitudinal strain and strain rate, together with torsion and basal rotation values at the apex, could be useful parameters to indicate subclinical dysfunction.

4.7 Intracardiac Thrombi

Intracardiac thrombi (IT) are a relatively uncommon cardiac manifestation of BD. In a survey by Geri et al. [60] on 52 BD patients with symptomatic heart involvement, 10 (19%) had IT, 5 in the right ventricle and 5 in the right atrium. The predilection of IT for the right heart chambers has been confirmed by a review of the literature, which demonstrated that in 95% of cases IT are found in the right heart, especially the right ventricle (74% of cases), while the second most common site is the right atrium (43% of cases) [127]. In contrast, the left chambers have been shown to be affected in only 4% of cases. Some patients have multiple lesions in one or more chambers. The thrombi are typically adherent to the free wall of the tricuspid valve and their presence

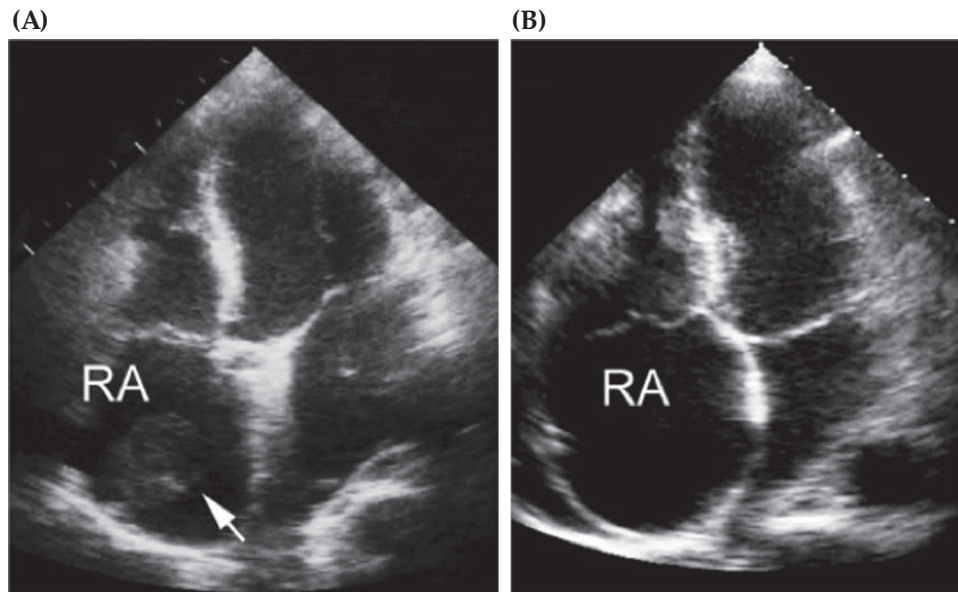


FIGURE 21.7 Right atrial thrombus showed by echocardiography in a 45-year-old BD man with concomitant deep-vein thrombosis [170] (Panel A). Panel B shows the complete resolution of the thrombus 2 months after initiation of immunosuppressive therapy with high-dose steroids and cyclophosphamide. Adapted from Kuno et al. [170].

is associated with pulmonary thromboembolism (56% of cases), arterial involvement (38% of cases), or venous thrombosis (42% of cases), while in a minority of cases (3%) IT coexist with endocarditis. Patients with IT are more often male (86%) and young (mean age 27 years).

The exact pathogenesis of IT in the course of BD is unclear. Right ventricular dysfunction is not a prerequisite for IT, in contrast to left-sided IT in subjects not affected by BD; in BD IT usually overlay a kinetic myocardial segment. Several mechanisms have been proposed as predisposing factors [1]: the detection of high levels of endothelial products such as von Willebrand factor antigen may support the hypothesis of endothelial ischemia and damage due to an underlying vasculitic process [2,128]; factor V Leiden and prothrombin 20210G-A gene mutations have been found to be markedly increased in BD patients with thrombosis, including IT [3,129]; concomitant hyperhomocysteinemia might play a role, as it has been linked to thrombosis in a more recent *meta-analysis* [4,130]; and EMF may represent the predisposing histopathological lesion for IT at least in some cases, although it is difficult to prove [131].

Similarly, it is difficult to establish whether IT are the direct consequence of deep-vein thromboembolism or a distinct manifestation in the setting of vascular BD, when both conditions occur concomitantly. However, thromboembolism remains exceptional in the course of BD, probably because of the tight adhesion of the thrombi to the inflamed vessel walls. Therefore it is more likely that a primary endothelial injury is implicated in the pathogenesis of IT. Consistent with this notion, resolution of IT has been documented with immunosuppressive treatment with and without anticoagulation [127] (Fig. 21.7).

Pathological findings following surgical resection of IT have documented variable pictures: inflammatory infiltrate and EMF were found in some cases, while normal myocardium was sampled in others [66,90].

The differential diagnosis of BD-related IT includes large endocardial vegetations and intracardiac tumors, especially myxoma.

4.8 Pericarditis and Myocarditis

In the largest series on clinical characterization of BD phenotype to date, pericarditis and myocarditis were considered rare manifestations [132]. In a recent analysis of 6075 Iranian patients by Davatchi et al. [132] cardiac manifestations were seen in 0.6% of cases, with a frequency of pericarditis of 0.08%. In contrast, in the Geri et al. series of 52 patients with symptomatic heart involvement, pericarditis was diagnosed in 39% of patients [60]. Such a high frequency had been reported in a few series dating back to the 1980s [133–135].

BD-related pericarditis is usually benign and presents with chest pain and sometimes fever, but it can also be wholly asymptomatic. When performed, pericardial biopsy typically shows lymphoplasmacytic or histiocytic vasculitis. Many patients can be managed with colchicine or nonsteroidal antiinflammatory drugs (NSAID) alone, and complications such as constrictive pericarditis or heart tamponade are exceedingly rare. However, up to one-third of patients have a relapsing course.

Of relevance, apart from a single case of myocarditis cited by Geri et al. [60], myocarditis is not mentioned in the largest studies, including the Iranian series

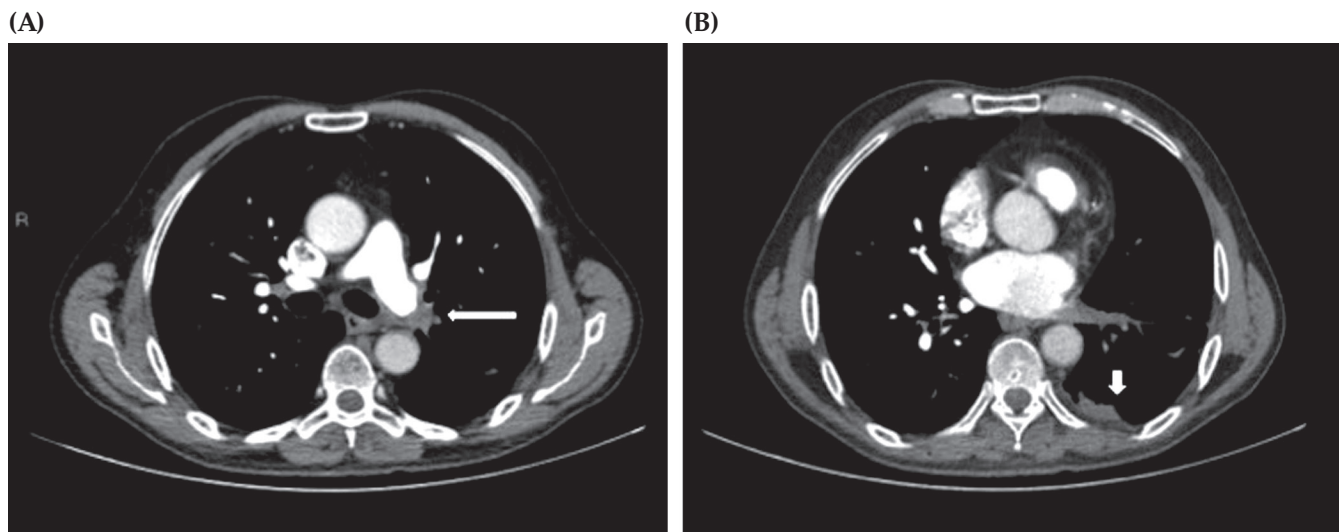


FIGURE 21.8 Transverse section showing thrombosis of the left inferior pulmonary vein (A, long arrow). The parenchymal consolidation detected in the left lower lobe is attributable to a concomitant pulmonary infarction (B, white arrow). Adapted from the personal collection of Dr. Salvarani, Arcispedale Santa Maria Nuova – IRCCS, Reggio Emilia, Italy.

[132]. One case of giant cell myocarditis was recently described [136].

4.9 Pulmonary Artery Aneurysms

BD is the most common cause of pulmonary artery aneurysms (PAA), which occur almost exclusively in men, often of young age, and represent one of the leading causes of mortality of BD. PAA are mainly found in the right lower lobe, although the right and left main pulmonary arteries may also be affected. The events leading to PAA have not been fully clarified, but formation of an inflammatory thrombus in the pulmonary arteries followed by recanalization and excessive angiogenesis spreading into the arterial wall have been proposed as causative mechanisms. Perivascular inflammation, which has been documented in resected specimens of aneurysmatic pulmonary arteries, may be a contributing factor to the formation of pseudoaneurysms via weakening of the arterial wall [137]. The concomitant mural thrombus of pseudoaneurysms is another potential cause of ischemia and infarction in the lung parenchyma (Fig. 21.8). Hemoptysis is the presenting symptom in most cases and should be carefully evaluated when occurring in male BD patients, particularly those with evidence of peripheral venous thrombosis.

The prevalence of PAA in BD patients is not known but is considered rare. In 2003 Hamuryudan et al. [137] found 26 PAA cases in a cohort of 4400 patients diagnosed with BD since 1992 in Turkey. Twenty-one patients had concomitant deep vein thrombosis of the lower extremities, four had vena cava thrombosis, three had IT, and four had arterial aneurysms at other sites.

The prognosis of untreated PAA is poor. Uzun et al. [138] reported in an earlier cohort of Turkish patients a mortality of 50% within 10 months after the onset of the hemoptysis.

4.10 Cardiovascular Complications and Surgical Implications

In general, conservative treatment of arterial occlusion is preferred to surgery due to the risk of aneurysms and pseudoaneurysms, clots, or occlusions related to arterial manipulation. In fact, in the acute phases of the disease, surgery is accompanied by high morbidity and disruption of suture lines, resulting in very early bleeding and, in the later phases, pseudoaneurysm formation [85]. AMI may be complicated by late stent thrombosis and ventricular and femoral artery pseudoaneurysms, the latter was described at the site of the collagen-plug vascular closure device used during the catheterization procedure [89].

Another case of subepicardial hematoma presumably occurred following microrupture of the right coronary artery has been reported. In this case, the patient was taking warfarin and immunosuppressive therapy, due to previous deep-vein thrombosis. Coronary angiography showed total occlusion of the right coronary artery with abundant collateral circulation from the diffusely enlarged left coronary artery [139], suggesting a chronic process rather than acute thrombosis. However, aortitis remains one of the most challenging problems for cardiac and vascular surgeons. When involved by the disease, as in the case of severe aortic insufficiency, aortic tissue becomes inflamed and very fragile. Apart from pseudoaneurysm formation, postoperative complications

following surgical repairs include hemorrhage, valve detachment, and paravalvular leakage [63].

In regards to IT-related complications, pulmonary occlusion, pulmonary artery thrombosis, or pulmonary arterial aneurysms can be concomitantly detected. In the series of Emmungil et al. [140] characterizing 22 cases of IT among a total of 2216 BD patients, 16 had pulmonary occlusion or pulmonary artery thrombus simultaneously with cardiac thrombus; pulmonary arterial aneurysms was detected in 4 cases. When the authors performed analysis, dividing patients by those with early and late disease, respectively, they could not find any relationship with pulmonary vascular complications. However, in late disease (longer than 1 year), they observed a trend showing more nonpulmonary vascular complications.

4.11 The Role of Imaging

As previously mentioned regarding valvular abnormalities, echocardiography plays a pivotal role in the assessment of cardiac BD, especially for aortic valve and aortic root involvement. The main indications for performing an echocardiographic assessment of BD patients can be summarized as follows:

- In patients with peripheral vascular involvement and/or cardiac symptoms, search for valvular abnormalities, aortic root dilation, aneurysms, IT, and signs of pericarditis;
- Monitor cardiac lesions in patients undergoing treatment;
- Characterize cardiac lesions when surgery is planned; and
- Detect pulmonary artery aneurysms involving the main trunk and its major branches as well as indirectly determine systolic pulmonary artery pressure (sPAP).

In this regard, Sehaya et al. [141] recently investigated the frequency of elevated sPAP estimated by echocardiography in BD patients with pulmonary artery involvement (PAI), as compared to BD patients without PAI involvement, systemic sclerosis patients, and healthy controls. The authors found a modest elevation of the estimated sPAP in BD patients with PAI (cutoff levels were set at 35 mmHg), mild decrease in diffusing capacity of lung for carbon monoxide (DLCO), mild cardiac dysfunction, and increase in pro-BNP levels. They suggested “cardiac microvessel vasculitis,” in addition to the well-recognized large vessel disease in this specific subgroup of patients. Interestingly, in a previous study performed by the same group increased sPAP estimated by echocardiography was reported in a proportion of patients who developed PAI involvement later on, with fatal outcome [142].

With regard to the accuracy of MRI in diagnosing BD-related cardiac involvement, adequate evidence from the literature is still lacking. Cardiac MRI is an advanced diagnostic imaging technique that may have a role in providing further details when first- and second-level investigations have already supported the clinical suspicion of one of the following conditions:

- Pericarditis, endocarditis, EMF;
- Interatrial septal aneurysms, left atrial dilatation, and coronary aneurysms;
- Aneurysmatic enlargement of sinus Valsalvae and ascending aorta;
- Mitral prolapse, with or without regurgitation; and
- Left ventricular diastolic and systolic dysfunction.

In routine surveillance of coronary aneurysms, an excellent correlation between CT angiography and the more invasive procedure of cardiac catheterization has been reported in terms of number, size, and location of the aneurysms. In addition, CT angiography has the advantage of being able to disclose extraluminal changes [143].

4.12 Electrocardiographic Findings and Conduction Disorders

The spectrum of ECG abnormalities described in the course of BD is broad and includes P-wave abnormalities, atrioventricular conduction disturbances, left and right bundle branch blocks, abnormal late potentials, QT prolongation, and ectopic arrhythmias. QT abnormalities and arrhythmias are discussed separately.

P-wave abnormalities are recognized markers of potential left atrial enlargement, left atrial hypertension, or altered conduction. In particular, P-wave dispersion is a well-known electrophysiologic feature of proneness to atrial fibrillation. Dogan et al. [144] were the first to investigate P-wave abnormalities in BD. They analyzed the ECG findings in 29 affected subjects and 45 controls matched for age, gender, and prevalence of hypertension, diabetes mellitus, hyperlipidemia, and cigarette smoking. The authors found a significantly higher P-wave duration and P-wave dispersion in the BD group, which also correlated with the duration of the disease. Nebivolol was recently shown to cause a significant decrease in maximum P-wave duration and dispersion in BD patients [145].

Conduction disturbances, such as right bundle branch block and T-wave changes were the most common abnormalities in BD patients with pulmonary artery involvement, according to a study by Seyahi et al. [141]. The authors found a mildly increased sPAP in BD; ECG signs of left and right ventricular hypertrophy and right axis deviation were also present in a smaller percentage of patients. In contrast, there were no differences

in terms of heart rate in the BD cohort as compared to systemic sclerosis and healthy subjects.

Cansel et al. [146] found both prolonged interatrial and intraatrial electromechanical delay measured by tissue Doppler imaging and conventional echocardiography, suggesting a possible correlation between these findings and changes in structure and electrophysiological properties of the atrial myocardium in active BD.

Sayin et al. [147] evaluated a total of 50 patients diagnosed with BD for QRS prolongation and fragmentation (fQRS, or QRS complexes), which are usually associated with increased cardiovascular mortality and sudden cardiac death. They found significantly longer QRS duration and corrected QT duration in BD patients compared with controls; in addition, an fQRS pattern was more common in BD patients than in controls. Interestingly, an association of these findings with longer disease duration was also reported.

4.13 Markers of Increased Risk for Ventricular Arrhythmias

Increased incidence of ventricular arrhythmias and sudden cardiac deaths was reported in BD as early as 50 years ago [148,149] – yet the pathophysiology of these events remains unclear. QT intervals and ventricular arrhythmias were determined in 73 BD patients and compared to those of 51 healthy controls without a history of cardiac disease [150]. QT dispersion was significantly greater in BD patients than in controls, and there was a trend for greater QT dispersion and corrected QT dispersion in BD patients than in those without ventricular arrhythmias. The authors interpreted the dispersion as a consequence of the combination of short minimum and long maximum QT intervals rather than exclusive QT prolongation. The results were in line with previous findings by Goldeli et al. [149] who found that mean QT dispersion and corrected QT dispersion were significantly more frequent in BD patients as compared to unaffected controls.

Increased dispersion of refractoriness was proposed as one of the arrhythmogenic mechanisms in BD, in addition to the autonomic dysfunction and the concomitant presence of myocardial disease. Autonomic nervous system dysfunction has been described in BD patients, and is potentially responsible for the loss of balance in cardiac innervation. In turn, this may influence cardiac repolarization and favor ventricular arrhythmias. On the other hand, when small areas of myocardial fibrosis develop, the presence of early changes affecting the coronary microcirculation without involvement of epicardial vessels may be considered.

Heper et al. [68] found higher QT dispersion in their series of 30 Turkish patients as compared to controls, in the absence of cardiac ischemic disease and mitral inflow

E/A inversion determined though echocardiographic study. On the basis of these findings, possible myocardial structural involvement has been suggested by the authors. Similarly, significantly increased QT dispersion, high incidence of late potentials, and more complex ventricular arrhythmias were found in 28 BD patients as compared with age- and sex-matched control subjects by Kirimli et al. [171], postulating the existence of an arrhythmogenic substrate in cardiac BD.

5. TREATMENT OPTIONS AND CARDIAC IMPLICATIONS OF THERAPY

The therapeutic approach to BD is determined by the organs involved and the severity of the disease. To date, no randomized clinical trials have been carried out to establish the best therapeutic scheme in BD with cardiovascular involvement [151]. According to the 2008 recommendations by the European League Against Rheumatism (EULAR) for the management of BD [11], it has to be noted that the category of evidence and the strength of recommendation for the management of major vessel involvement in BD are still III and C, respectively. As a rule, major organ involvement – especially cardiac involvement – requires aggressive treatment with high-dose glucocorticoids and immunosuppressive agents.

The first evidence that early recognition and aggressive treatment of cardiovascular involvement in the course of BD improved outcomes came from the studies by Hamuryudan et al. [152], which mainly included patients presenting with pulmonary artery aneurysms. More specifically, their suggested strategy consisted of (1) three pulses of 1 g methylprednisolone followed by prednisolone 1 mg/kg/day, tapered over 2–5 months (2); monthly pulses of 1 g cyclophosphamide for the first year and every other month for the second year. As maintenance therapy following cyclophosphamide, azathioprine was used at the dosage of 2.5 mg/kg/day.

More recently, with the advent of anti-TNF- α biologic agents, a number of case reports and case series have been published attesting to the efficacy and safety of such medications (mainly infliximab and to some extent adalimumab) in treating cardiovascular manifestations of BD. In particular, a BD patient with aseptic endocarditis and aortitis was reported as having a favorable response to infliximab [153]. The same authors reported additional three cases with severe vascular manifestations including thoraco-abdominal aortic dissection, internal carotid artery dissection, and iliac vein thrombosis [153]. Schreiber et al. [154] recently described the successful treatment of PAA with infliximab in a 43-year-old BD patient with arterial aneurysms in the common carotid and common iliac arteries, and thrombosis in a femoral vein and pulmonary arteries. In their literature review, the authors found four other

reported cases of PAA successfully treated with infliximab as well as adalimumab in one case [153].

IT is usually treated with glucocorticoids and cyclophosphamide, often in association with anticoagulants, although the rationale for use anticoagulation is debated (see in the following) [127,155].

As already noted, surgical options have a very limited indication in the case of BD with vascular involvement, due to the high risk of perioperative complications including a vascular pathergy phenomenon. Favorable results have been reported using the endovascular embolization approach to the thrombose bleeding pulmonary artery aneurysms, while the classical surgical approach is usually not recommended due to high perioperative mortality [156]. The surgical approach has a rationale for replacing damaged heart valves, although close monitoring for late complications, such as dehiscence of the prosthetic valve and paravalvular leakage is mandatory [71].

These complications occur more frequently after aortic valve replacement than after a composite valve graft insertion [65,157]. Standard anticoagulation protocol is usually instituted in such cases with a target international normalized ratio (INR) between 3 and 3.5, according to current guidelines [158]. With regard to cardiac and surgical implications of immunosuppressive therapy, the standard recommendations issued for immunosuppressive agents and anti-TNF agents in rheumatic diseases also apply to BD [159,160]. Cardiac toxicity in the form of arrhythmias, toxic myocarditis, and even cardiogenic shock has been associated with colchicine poisoning (toxicity rate >5 µg/L); indeed, colchicine has a peculiarly narrow therapeutic window and interacts with a wide spectrum of other common drugs that may alter its metabolism and increase its plasmatic levels.

5.1 Anticoagulation in Vascular Behçet's Disease

No large controlled studies are available on the best approach to treating thrombosis in BD patients, and the exact role of long-term anticoagulation in such patients is still debated, but the bulk of evidence militates against the use of anticoagulants in cardiovascular BD.

The main evidence from the last 10 years suggests avoiding anticoagulants. At the American College of Rheumatology 2003 annual meeting [161] the results of long-term course of deep-vein thrombosis in BD patients were presented. The authors showed that the risk for recurrence as well as of post-thrombotic syndrome was significantly lower in patients taking immunosuppressants than in those taking anticoagulants. These results were in line with previous evidence, starting from the first controlled trial on azathioprine in BD in 1990 [162], which showed a significantly lower rate of deep-vein thrombosis in patients receiving azathioprine regardless of anticoagulation.

Several years later, Sarica-Kucukoglu et al. [163] performed a retrospective analysis of 32 BD subjects with thrombotic events in a total of 2319 cases. Recurrence or progression was observed in thrombus size despite the administration of heparin or warfarin. Ahn et al. [164] found no difference concerning thrombosis recurrence rates between a treatment schedule based on immunosuppressants alone and a combination with anticoagulants in their retrospective analysis of 37 cases.

On the basis of the above-mentioned and other additional results, the EULAR recommendations on the management of BD have excluded the use of anticoagulants, antiplatelet, and antifibrinolytic agents for the treatment of BD vascular events, with the risk of fatal bleeding outweighing any putative advantage deriving from that therapy. The authors instead recommended intensifying the immunosuppressive treatment [11]; nevertheless, the category of evidence and strength of recommendation remain IV and D, respectively. On the other hand, it should be taken into account that the odd BD patient may have concomitant prothrombotic risk factors, which may make the case for anticoagulants in selected cases. Thus an accurate work-up for prothrombotic tendencies should be performed before starting or intensifying an immunosuppressive regimen alone [165].

Meanwhile, in the literature a wide variability in the therapeutic approach is observed, according to the clinical experience from the different BD clinics over the world. In this regard, two examples come from recent surveys based on expert opinion, conducted through questionnaires in order to evaluate the physicians' approach to some vascular BD complex cases. Tayer-Shifman et al. [166] recently conducted a survey among 33 rheumatologists from Israel, 55 from Turkey, and 25 from the United States asking whether they would initiate anticoagulants and when to stop the treatment in three paradigmatic examples of vascular BD, one of them with IT and pulmonary embolus in the right lower lobe.

Interestingly, the survey produced different answers. While Israeli and American groups considered this case similar to the other two (sagittal sinus thrombosis plus left arm superficial vein thrombosis, case #2; azygos vein thrombosis, case #3) in terms of therapeutic approach, 60% of the Turkish rheumatologists considered the case with IT more severe than the other two and initiated anticoagulation in addition to immunosuppressive treatment at the time of diagnosis.

The second interesting finding was that only 40–44% of the Turkish physicians would give anticoagulation at diagnosis of a venous thrombotic event, as compared to 97% and 87% of the Israeli and American physicians, respectively. Finally, in the case of IT, life-long warfarin treatment was the choice of about one-third of Israeli and Turkish physicians, as compared to 70% of Americans.

In 2010, a questionnaire was administered to all participants of the meeting of the International Society for Behçet's Disease [165]. The answer to the question about the preferred treatment in a patient with BD and proven deep-vein thrombosis was to intensify immunosuppression in 90% of the responders. However, about 52% of the physicians would add anticoagulants as well.

Approximately the same results have been reported by one of the widest and most recent surveys published by Alibaz-Oner et al. [167], who aimed to investigate the therapeutic approaches that Turkish physicians would take during the initial event and relapse of vascular BD, as well as the association of different treatment protocols with relapse. The study retrospectively evaluated a cohort of 936 BD patients. Of relevance, cardiac involvement was present in 3.1% of the entire cohort (for a total of 8 cases), arterial disease in 8.1%, and pulmonary aneurysms in 11.2%. The authors did not observe any additional positive effect of anticoagulation added to immunosuppressants to prevent relapse in the course of vascular involvement. However, a significant subset (30–60%) of patients with vascular BD in Turkey still receives anticoagulation.

6. CONCLUSIONS

Rates and types of heart involvement in BD range widely in the literature. When present, cardiac BD increases mortality rate. Valvular disease, mainly interesting mitral and aortic valve and usually causing insufficiency, is considered the most common manifestation. The exact pathophysiology of valvular abnormalities remains unclear, although several lines of evidence support a major role for the vasculitic process in causing destruction of the valve tissue, dilatation of the ascending aorta, and sinus Valsalva aneurysm formation.

Histopathologic features usually reveal mononuclear and neutrophilic infiltration, endothelial proliferation, destruction of the elastic lamina, fibrinoid necrosis, less frequently histiocytes and eosinophils, and sporadically giant cells [168]. EMF could be interpreted as a rare and long-term complication of such a vasculitic process, which may involve endocardium, myocardium, or both, sometimes associated with IT.

Functionally, the long-term complications detectable through echocardiography are EMF-related restrictive cardiomyopathy or, more often, a dilated cardiomyopathy in the setting of progressive ventricular dysfunction. Silent myocardial ischemia and AMI are more frequently due to coronary vasculitis with normal coronary arteries, in the absence of atherosclerosis. However, more recent evidence is in favor of an accelerated atherosclerotic process even in BD, similar to what has already been clearly established in rheumatoid arthritis and systemic lupus erythematosus.

The spectrum of ECG abnormalities in the course of BD is broad as well, including P-wave abnormalities, atrioventricular conduction disturbances, QT prolongation, and ectopic arrhythmias, possibly related to the myocardial structural changes, such as the development of some fibrotic areas.

IT formation is a rare but serious manifestation of cardiac BD [140] and represents one of the leading causes of death. Cardiac thrombi are mainly located in the right ventricle and often associated with pulmonary arteritis. The reason for right ventricle predilection remains unclear: based on autopsy findings it seems that EMF may play a role.

Right heart thrombosis is highly specific of BD, and should be taken into consideration in the differential diagnosis in any patient with such a finding.

Pulmonary arterial aneurysm occurs most exclusively in men of young age, and together with IT are the leading causes of mortality in BD.

No large controlled studies are available on the best approach to treating thrombosis in BD patients, and the exact role of long-term anticoagulation in such patients is still debated, but the bulk of the evidence militates against the use of anticoagulants in cardiovascular BD (2008 EULAR recommendations, category of evidence IV, strength of recommendation D). Most experts recommend aggressive treatment with high-dose glucocorticoids and immunosuppressive agents (2008 EULAR recommendations, category of evidence III, strength of recommendation C).

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Rheumatic Fever and Rheumatic Heart Disease

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1. INTRODUCTION

Nontreated streptococcal group A throat infections in children and teenagers with genetic predisposition can result in the development of rheumatic fever (RF), an autoimmune disease. This condition can cause serious damage of the heart valves, leading to rheumatic heart disease, as well as poststreptococcal glomerulonephritis and other invasive diseases.

The knowledge of symptoms compatible with those of RF date from the fifteenth century in some European countries, and the disease was considered as a common condition around the nineteenth century in Europe and North America and after World War II in other parts of the world [1].

The studies done by R. Lancefield in the beginning of twentieth century on streptococcal antigens especially on the M protein have allowed better comprehension of the mechanisms that lead to the autoimmune lesions that result in both RF and rheumatic heart disease (RHD).

Several genes, primarily those that regulate the immune response, play a role in the development of the disease. In fact, a network of genetically controlled reactions that is mediated by both humoral and cellular immune responses leads to the development of autoimmune reactions.

In the present chapter the group A streptococci is briefly described followed by the epidemiology of RF and RHD, the genes that predispose the development of the disease as well as the diverse molecules and their receptors on the bacterial surface.

In the following, the major mechanisms of the immune response that lead to RF and RHD (molecular mimicry, epitope spreading, and degeneracy) as well as clinical

data, treatment, and the possibilities of vaccine development against *S. pyogenes* are presented.

1.1 *Streptococcus pyogenes* Colonization

S. pyogenes is also known as group A streptococci, as proposed by R. Lancefield in 1941. This species is classified as β -hemolytic based on its ability to completely lyse red blood cells when cultivated in blood agar plate. The bacteria are characterized by their cell wall, which is composed of N-acetyl- β -D-glucosamine and rhamnose carbohydrates.

For intracellular persistence, streptococci have developed a battery of proteins for adhesion and invasion into host cells (Fig. 22.1).

The cell wall of group A streptococci (GAS) contains the M protein, lipoteichoic acid (LTA), GAS pili, and fibronectin binding protein (FBP) and are considered major components contributing to bacterial attachment to host epithelial cells. LTA provides weak adhesive hydrophobic interactions between bacterial cells and host components [2]. GAS pili mediate the attachment and contribute to microcolony and biofilm formation [2]. FBP affixes to extracellular matrix (ECM) components, providing more secure connections. The M protein is the major antigen of bacteria and plays an important role in the adhesion to and invasion of epithelial cells. M protein binds directly to components of the ECM such as fibronectin and is considered a virulence factor associated with resistance to phagocytosis. The capsule is composed of hyaluronic acid, which is chemically similar to that of host connective tissue.

GAS also secretes several products, such as (a) pyrogenic toxin, which causes the rash of scarlet fever; (b)

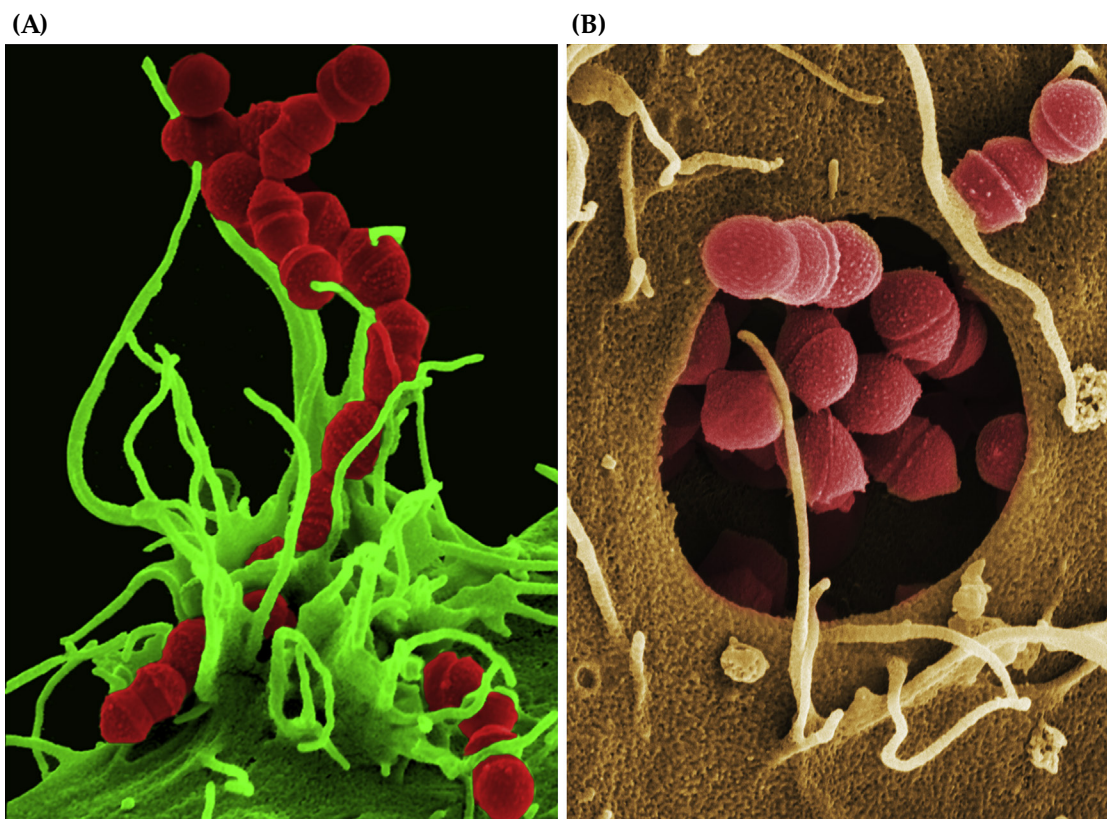


FIGURE 22.1 *Streptococcus pyogenes* throat colonization. *S. pyogenes* adhesion (A) and invasion (B) into oropharynx cells lead human throat infection. Images by emission scanning EM, adapted from the personal collection of Prof. Dr. M. Rhode, HZI, Braunschweig, Germany.

streptokinases; (c) DNases; (d) hyaluronidases, which are enzymes that contribute to cell invasion by destroying connective tissues; and (e) streptolysins O (SLO) and S (SLS), which mediate neutrophil platelet aggregation, triggering apoptosis of host cells. Superantigens (Sags, Spes and smeZ) have the property of binding to the β chain of antigen-presenting cells (APCs), triggering activation of T-CD4⁺ cells, the production of proinflammatory cytokines, and overstimulation of the immune response [3].

1.2 Epidemiology

Classically, GAS isolates were identified using serotype specific antisera against the M protein that is a fibrillar surface protein and an important antigen of *S. pyogenes*. It inhibits phagocytosis of GAS in the absence of opsonizing antibodies, promotes adherence to human epithelial cells, and helps to overcome the innate immunity [4].

The M protein consists of two polypeptide chains in a double-helix coiled coil that form fibrils extending up to 600nm away from the bacterial surface. It is approximately 450 amino acid residues and is divided into tandem repeat blocks distributed in four regions (A, B, C, and D).

The amino-terminal (N-terminal) portion is composed of regions A and B, which present variable numbers of amino acid residues. The A region shows high variability and defines the different M types, identified through *emm* gene polymorphism genotyping. Currently more than 220 strains are known according to CDC (Centers for Disease Control and Prevention) [5,6].

The C-terminal portion is highly conserved and is responsible for the binding of the bacteria to the oropharynx mucosa and presents antiphagocytic properties [4,5].

Based in distinguishes different chromosomal organizations, presence and arrangement of *emm* and *emm*-like genes, a complementary typing system, called *emm*-pattern typing was proposed. The A-C *emm*-pattern isolates are usually recovered from the throat, the D *emm*-pattern strains from the skin, and the E *emm*-patterns from both biological sites [7–9].

Certain GAS strains are historically known as rheumatogenic (*emm* 1,3,5,6,18,19 e 24) [10]; however, epidemiological features in tropical regions challenge the concept of association between pharyngitis resulting from known rheumatic GAS and RF/RHD, suggesting that the link between a defined *emm*-type and determined disease is not universal [11].

TABLE 22.1 The Most Frequent *emm* Types Identified in Different Countries

Country	Year	More frequent <i>emm</i> types	References
USA	2000–2007	1, 12, 28, 4, 3, 2	[13]
Canada	2000–2007	12, 1, 28, 4, 3, 77	[13]
North America	2000–2004	1, 3, 28, 12, 89	[14]
North America	2000 2001	12, 1, 28, 4, 3, 2 1, 12, 4, 28, 3, 2	[15]
Australia	2001–2002	1, 4, 12, 28, 75	[16]
Germany	2003–2007	1, 28, 3, 12, 89, 4, 77, 6, 75, 11, 118, 2, 83	[17]
Norway	2006–2007	28, 1, 82, 12, 4, 3, 87, 89, 6	[18]
Denmark	2003–2004	28, 1, 3, 89, 12	[19]
Hungary	2004–2005	1, 80, 4, 28, 66, 81.1, 82, 84	[20]
Barcelona	1999–2003	1, 3, 4, 12, 28, 11, 77	[21]
Brazil	2004–2008	1, 87, 22, 12, 77, 6, 89, 33, 75, 3	[22]

Systematic epidemiological reviews have shown significant differences in *emm*-type distribution in geographically and socioeconomically different regions of the world. Relatively limited numbers of *emm*-type strains are recovered from high-income settings, while a much higher diversity of strains circulate in low-income settings [3,11,12]. Forty-eight *emm*-types in 229 samples were identified from Brazilian patients treated at Clinical Hospital and São Paulo Hospital, which are affiliated with the University of São Paulo (USP) and Federal University (UNIFESP), respectively. The most common *emm* types were *emm1*, *emm87*, *emm22*, *emm12*, *emm77*, *emm6*, *emm89*, *emm33*, *emm75*, and *emm3*. These types are comparable to those found in other countries, particularly in high-income countries, in which *emm1* and *emm12* were the most common types. Table 22.1 summarizes all of the data [13–22].

GAS is an exclusively human pathogen responsible for a broad variety of clinical manifestations, ranging from pharyngitis and impetigo to invasive diseases, such as necrotizing fasciitis and toxic shock syndrome. Some strains can also trigger autoimmune sequelae, such as ARF, RHD, and glomerulonephritis.

GAS infections are a major cause of morbidity and mortality worldwide. The prevalence of severe GAS diseases is at least 18.1 million cases, which are responsible for approximately 517,000 deaths per year [23].

Most cases of ARF occur in children aged 5–15 years. However, it may eventually occur in young adults [24,25]. In Latin America, in 2003, 136,971 children between 5

and 14 years of age were found to have RHD [26]. In Brazil, RF is the leading cause of valvular heart disease [27]. According to the World Health Organization (WHO) epidemiological model and data from IBGE (Brazilian Institute of Geography and Statistics), the number of streptococcal pharyngitis infections is approximately 10 million cases. This prevalence could lead to 30,000 new cases of RF, of which approximately 15,000 could develop cardiac lesions [27]. In spite of a reduction in the incidence in the world the global incidence is still high, particularly in low-income countries. Therefore RF remains a leading cause of acquired heart disease in Brazil and many regions of Africa, India, and Oceania (Fig. 22.2) [28]. Some outbreaks have been reported in Utah and Colorado or in remote areas in Canada, as well as in other developed countries. Reporting incidences in American, Canadian, and Western European countries are 0.1–2.0 cases per 100,000 persons. In low-income countries or emerging economies the general incidence can be as high as 10 to 20 cases per 100,000 persons in tropical regions, or even more than 50 cases per 100,000 in Northern Australia [28–31].

Rheumatic fever is presently responsible for significant morbidity and mortality, particularly in underdeveloped and “emerging countries” as the cause of 90% of cardiac surgeries in children and over 30% of cardiac surgeries in adults, most of them being young [32]. The acute phase of RF is frequently asymptomatic, especially rheumatic myocarditis (RM). The most common symptoms of acute rheumatic fever (ARF) are arthritis and Sydenham’s chorea, with carditis being more commonly asymptomatic [33].

1.3 Genetic Susceptibility to RF/RHD

Not all individuals affected by streptococcal pharyngotonsillitis develop RF/RHD. Whether the condition progresses to this point depends on many factors, genetic susceptibility being one of the most important.

Pathogen recognition and phagocytosis lead to activation of costimulatory molecules and specific T lymphocytes. Phagocytosis occurs via pathogen recognition receptors (PRRs), which are associated with antigen processing and presentation to T cells via molecules of the Major Histocompatibility Complex (MHC). The innate and adaptive immune responses are closely connected and interact to eliminate pathogens.

Several polymorphisms in genes related to both the innate and adaptive immune response and that code for molecules involved in the immune response have been described to be associated with susceptibility to RF/RHD. Notably, some molecules of the MHC class II genes (HLA class II alleles) appear to be dominant contributors to the development of the disease (Table 22.2) [34–49]. Other genes involved in the host defense and

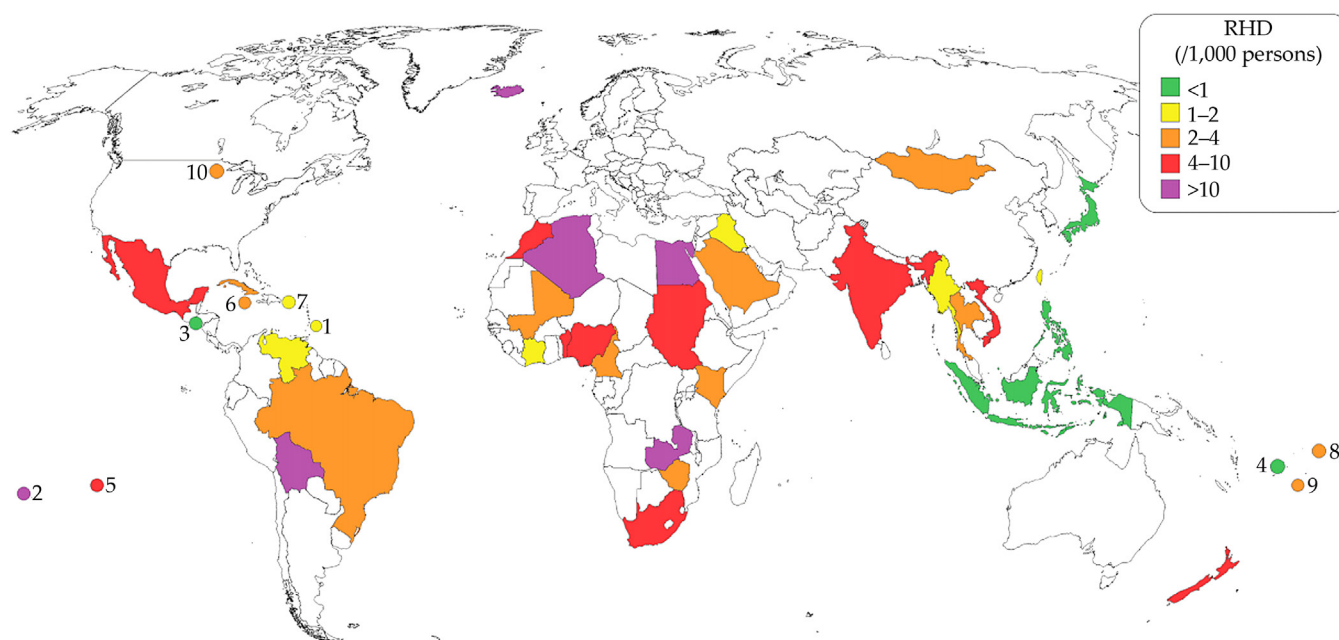


FIGURE 22.2 Map showing reported worldwide prevalence of RHD from 1970 through 1990. Adapted from Seckeler and Hoke [28].

inflammatory process (Fig. 22.3) also contribute to the development of the cardiac tissue damage and can determine whether the rheumatic valve lesions will present regurgitation, stenosis, or both, in RHD patients [50–64]. One example is the amount of production of an acute phase inflammatory protein MBL (mannan-binding lectin), which plays a role in innate immunity [65]. Variants of the promoter and exon 1 regions of the *MBL2* gene have been reported in patients with RF/RHD, in which the A allele is associated with the development of mitral stenosis (MS) and the O allele with aortic regurgitation (AR). Interestingly, these alleles code for high and low production of MBL, respectively [53,66].

In addition, a polymorphism in the *ACE* (angiotensin I converting enzyme) gene, recently described in association with RHD, likely increases the possibility of developing valvular fibrosis and calcification [67].

2. CLINICAL PRESENTATION AND DIAGNOSTIC CRITERIA

The diagnostic criteria for ARF were first developed by Jones in 1944, and were modified in 1965, 1984, 1992, 2002, and 2015 [68–73].

The criteria are divided into major and minor criteria (Fig. 22.4). Diagnosis is made by the presence of either two major or one major and two minor criteria, *plus* evidence of recent streptococcal infection, either by a pharyngeal swab culture positive for GAS, positive rapid GAS antigen test, or rising serologic antibody titers of

antistreptolysin O (ASO), DNase B, or streptokinase. New echocardiography data have recently been added for the diagnosis of ARF (Fig. 22.4).

Nevertheless, the diagnosis of RF is still a challenge—the most common manifestations, such as arthritis, have a broad differential diagnosis, while the manifestations that may lead to severe heart disease, ie, carditis, are primarily asymptomatic. Sydenham’s chorea is the only acute manifestation of RF that can lead to a definitive diagnosis of ARF, as there are few similar diseases to consider as a differential diagnoses.

2.1 Valvular Lesions and Arthritis

The cellular and humoral immune responses are defined as Th1 and Th2, respectively [74].

Episodes of ARF are mediated by an antibody (Th2-type) affecting mainly joint tissue and the central nervous system, whereas cellular immune response (Th1-type) is more present in rheumatic carditis [74].

The Th1 or cellular immune response usually leads to severe carditis and subcutaneous nodules. Subcutaneous nodules in ARF are a sign of severe carditis, as both clinical manifestations are consequences of a Th1 response. Cellular response in ARF also leads to severe valvular sequelae.

The Th2 episodes as mentioned above are caused by a predominantly antibody-mediated response and lead to clinically significant symptoms such as arthritis, chorea, and *erythema marginatum*. The humoral response usually does not lead to severe valvular sequelae, thus a patient

TABLE 22.2 Alleles of HLA Class II Genes Associated With RF/RHD Susceptibility

Gene	Allele	Population	RR	References
HLA-DRB1	DR1	South African	5.2	[34]
		Martinican–admixed		[35]
		Brazilian–Mulatto		[36]
	DR2	African American	3.7	[37]
	DRB1*1602	Mexican–Mestizo	5.3	[38]
	DR3	Indian		[39]
		Turkish		[40]
	DR4	Caucasian American	3.5, 2.3	[37,41]
		Saudi Arabian	13.6	[42]
	DR5 (DR11)	Turkish		[49]
	DR6	South African	2.6	[34]
	DRB1*13	Egyptian		[43]
	DR7	Turkish		[40]
		Brazilian–Mulatto	3.8	[44]
		Brazilian–Caucasian	2.4	[45]
HLA-DRB4	DR53♦	Turkish	2.8	[46]
		Egyptian	3.0	[43]
		Latvian	4.2	[47]
	DQA1*0104	Brazilian–Mulatto		[44]
		Japanese	2.8	[48]
		Egyptian		[43]
	DQA1*0201	Egyptian		[43]
		Egyptian/		[43,38]
		Mexican–Mestizo		
	DQA1*0301	Mexican–Mestizo		[38]
		Latvian	3.1	[47]
		Latvian	4.3	[47]
	DQA1*0501	Japanese	3.2	[48]
HLA-DQA1	DQA1*0701			
HLA-DQB1	DQB1*0302			

HLA class II alleles were defined by serology (DR) or molecular biology (DRB1, DRB4, DQA1, DQB1); ♦ association with DR4, DR7, and DR9.

that has had only one episode of ARF with arthritis or Sydenhan's chorea usually has mild valve sequelae. Subsequent episodes of ARF may not have the same type of response as the previous episodes, thus a patient that had a Th2-predominant response in a first episode may have a Th1 type-response in a recurrent episode. This type of immune response may lead to severe cardiac valvular sequelae, as mentioned above, and arouses adherence to

secondary prophylaxis, even in patients with mild valvular sequelae [75].

The Jones criteria establish the diagnosis of ARF. However, few patients exhibit a symptomatic acute phase of disease. Thus the majority of the RF diagnoses are made by finding compatible cardiac sequelae such as MS and mitral-aortic disease. To increase the sensitivity of the Jones criteria, the American Heart Association recently proposed revised criteria associating clinical features with Doppler echocardiography data (Table 22.3) [73]. Echocardiographic criteria for the diagnosis of RF, even though they not extensively validated, are a promising step toward faster and simpler diagnosis of RF, particularly for subclinical carditis or valvular damage [73].

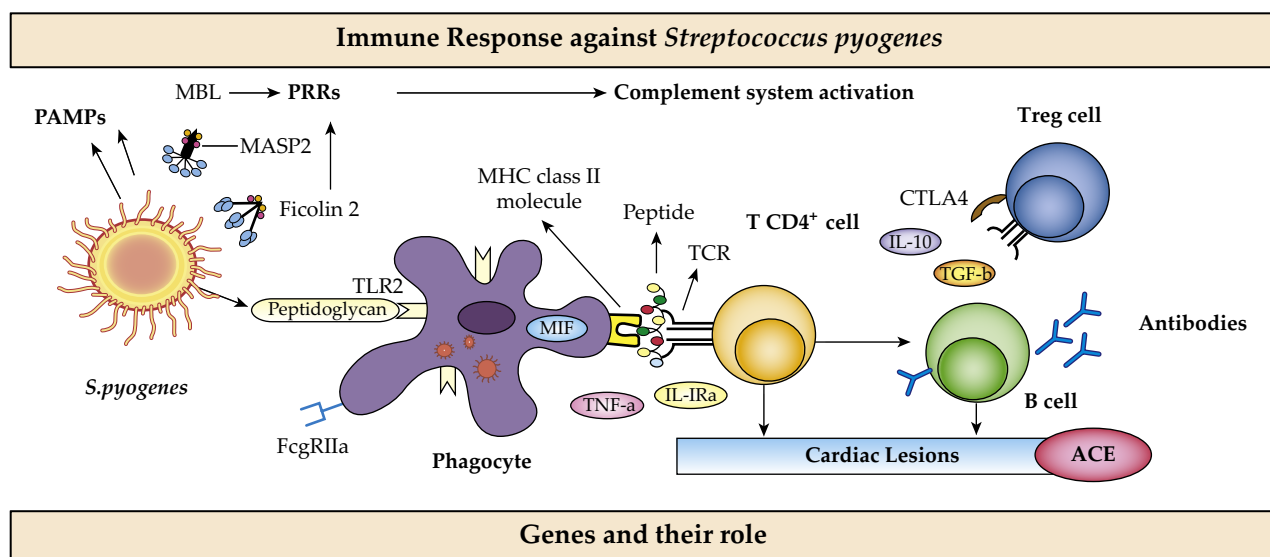
A retrospective study performed in the State of São Paulo, in Brazil, including 786 children and adolescents diagnosed with symptomatic ARF by modified Jones criteria [71] described the most common clinical symptoms. Of these children, there were 404 (51.3%) males and 382 (48.7%) females, with a mean age of 9.4 years. Arthritis was present in 453 (57.6%) patients, carditis in 396 (50.4%), and chorea in 274 (34.8%). *Erythema marginatum* and subcutaneous nodules were observed in only 13 (1.65%) and 12 (1.5%) of patients, respectively. Typical migratory polyarthritis occurred in 290 (64%) patients with joint involvement [71]. Among the 54% children with rheumatic carditis diagnosis, the type of valvular damage most frequently observed was mitral regurgitation (75%), followed by aortic regurgitation (25%), tricuspid regurgitation (9%), and aortic stenosis (0.1%) [71]. A recent report showed high incidence of associated diseases caused by RHD, such as stroke, infective endocarditis, valvular heart surgery, and major bleeding due to anticoagulant treatment for atrial fibrillation or after replacement of a native valve by a valvular prosthesis [76].

Classically, the symptoms of ARF start 2 to 4 weeks after a streptococcal infection. The clinical manifestations of ARF are detailed individually in the following.

2.2 Arthritis

Arthritis is the acute manifestation of RF that more commonly follows the 2–4 week interval after the streptococcal infection. Carditis is generally asymptomatic during this period, and chorea occurs several weeks or months after the streptococcal infection. Thus arthritis is the major common clinical aspect of the Jones's criteria [68–71].

The arthritis associated with RF as seen today is quite different from classical descriptions (migratory polyarthritis). It is more frequently polyarthritis of the large joints, more commonly additive (not migratory) and asymmetric [25,77,78]. These characteristics may be different according to the population studied and in particular with the streptococcal strain prevalent in the



Pathogen Recognition and Complement System Activation

MBL2 - recognizes mannose and N-acetylglucosamine in pathogens
MASP2 - protease; lectin pathway
FCN2 - codifies Ficolin 2; carbohydrate binding; opsonic activities

Pathogen Recognition and Phagocytosis

TLR2 - pathogen recognition (lipoproteins); activation of innate immunity
FCGR2A - phagocytosis and clearing of immune complexes
MIF - macrophage migration inhibitory factor; macrophage function

Antigen presentation to T cells

HLA-DRB1, *HLA-DQA1*, *HLA-DQB1* - presenting peptides derived from extracellular proteins

Proinflammatory Cytokines

TNFA - proinflammatory cytokine
IL-1RA - inhibits the IL-1 function ; IL-1 inflammatory responses

Immune Response Control

CTLA4 - inhibitory signal to T cells
IL-10 - immunoregulation
TGFB1 - regulates cell proliferation, differentiation, migration

Cardiac Tissue Damage Progression

ACE - enzyme; conversion of angiotensin I into a physiologically active peptide angiotensin II.

FIGURE 22.3 Genetic susceptibility to RF/RHD. *S. pyogenes* throat and/or skin infections lead to immune activation to eliminate the bacteria. Several polymorphisms in genes that code for molecules that control the immune response are involved with the pathogenesis of RF/RHD. *MBL2*, Mannose-binding lectin 2; *MASP2*, Mannan-binding lectin serine peptidase 2; *FCN2*, Ficolin 2; *TLR2*, Toll like receptor 2; *FCGR2A*, Fc fragment of IgG, low affinity IIa, receptor (CD32); *MIF*, Macrophage migration inhibitory factor; *HLA*, Human leukocyte antigen; *TNFA*, Tumor necrosis factor α ; *IL-1RA*, Interleukin-1 receptor antagonist; *CTLA4*, Cytotoxic T-lymphocyte-associated protein 4; *IL-10*, Interleukin 10; *TGFB1*, Transforming growth factor, β 1; *ACE*, Angiotensin I converting enzyme; *PAMPs*, Pathogen-associated molecular pattern; *PRRs*, Pathogen recognition receptors; the functional role of these molecules is described. Adapted from the personal collection of the authors.

community [79,80]. Polyarthralgia has recently been considered as major Jones criteria, after exclusion of other causes of arthralgia in moderate- and high-risk populations (Fig. 22.4) [73]. Characteristically, there is intense articular pain but mild inflammatory signs. Acute RF arthritis generally does not lead to articular sequelae, Jaccoud's arthritis being a rare exception. A history of rapid improvement with salicylates or NSAIDs drugs is characteristic [73]. Acute RF arthritis can persist for 4 weeks to several months (rarely), particularly in persons over 18 years old. These adult patients often have the same asymmetric involvement of large joints as observed in children and may require long-term nonsteroidal anti-inflammatory use [80].

The patient with arthritis presents a diagnostic challenge, as multiple etiologies can be responsible for articular inflammation. It is not uncommon for the

rheumatic patient to have overlapping autoimmune diseases [81,82], thus all arthritis in RF patients must be examined with careful clinical and laboratorial evaluations. The main differential diagnosis includes infective endocarditis (common in patients with chronic RHD) and other autoimmune diseases, the most common being rheumatoid arthritis and lupus erythematosus [73].

RF arthritis can be extremely painful. Nonsteroid anti-inflammatory drugs (NSAIDs) can relieve signs of ARF, and some authors recommend paracetamol as the preferred symptomatic treatment until the diagnosis is established. Once diagnosis is well established the preferred NSAIDs is Naproxen twice daily 10–20 mg/kg/day, or other nonsteroidal anti-inflammatory drugs like Ibuprofen [25,78]. The acetylsalicylic acid (ASA) anti-inflammatory dose is 80–100 mg per

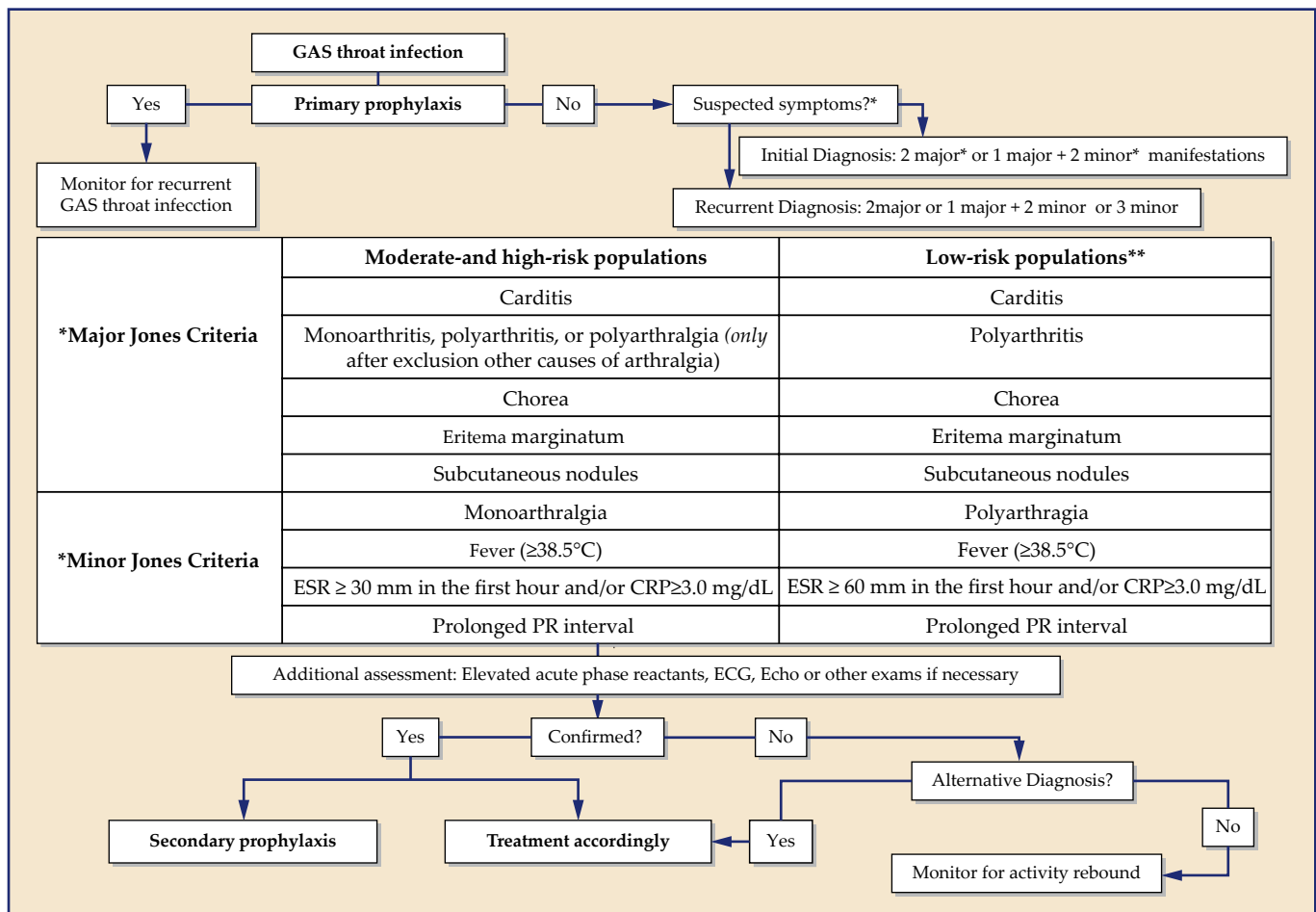


FIGURE 22.4 Major and minor diagnosis criteria. **Low-risk populations are those with ARF incidence ≤ 2 per 100,000 school-aged children or all-age rheumatic heart disease prevalence of ≤ 1 per 1000 population per year. Adapted from the personal collection of the authors.

TABLE 22.3 Doppler Findings in Rheumatic Valvulites

Pathological mitral regurgitation	Pathological aortic regurgitation
Seen at least two views	Seen at least two views
Jet length ≥ 2 cm in at least one view	Jet length ≥ 1 cm in at least one view
Peak velocity > 3 m/s	Peak velocity > 3 m/s
Pansystolic jet in at least one envelope	Pandistolic jet in at least one envelope

Adapted from Gewitz et al. [73].

kilogram of body weight daily in 4–6 divided doses. Adult doses may be as high as 4–8 g/day. Because of stomach irritation and upset, among other side effects, an enteric-coated tablet can be used. ASA and other NSAIDs should be taken with food to prevent stomach pain. In spite of the need for high doses and common side effects, ASA is still recommended as the first-line treatment for RF arthritis by many authors [83], which was recently reviewed by Gewitz et al. [73]. Fig. 22.5 summarizes the recommended treatment regimes.

The majority of cases need at least 2 weeks of regular treatment to relieve symptoms and inflammation, and sometimes up to 6 weeks of treatment is necessary to resolve the symptoms of arthritis [25].

2.3 Carditis

Acute rheumatic carditis is the most severe manifestation of RF. This condition can be fatal [77,84] as it leads to valvular sequelae that are characteristic of chronic RHD. Even so, most acute rheumatic carditis is asymptomatic, and chronic RHD can be asymptomatic for years or decades before heart failure ensues. Approximately 40–60% of ARF episodes result in RHD [23].

Rheumatic carditis is a pancarditis affecting the pericardium, myocardium, and endocardium. Pericarditis can be the most characteristic manifestation of acute rheumatic carditis, causing precordial or retrosternal chest pain that is relieved when the patient sits or leans forward. It is frequently associated with a pericardial friction rub. Rheumatic pericarditis usually does not cause large pericardial effusions, and there are few reports regarding cardiac

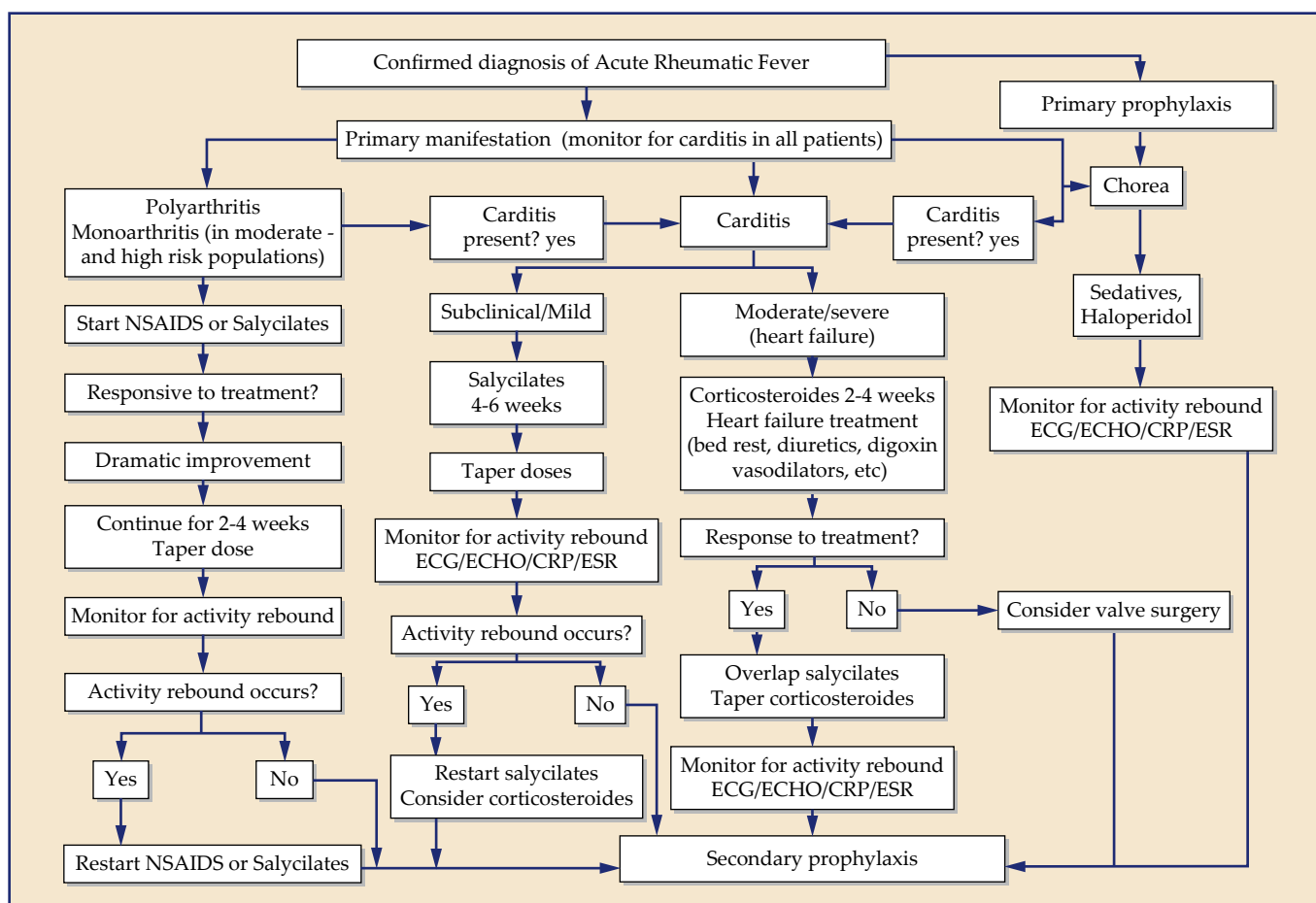


FIGURE 22.5 Recommended treatment for acute rheumatic fever. Adapted from the personal collection of the authors.

tamponade due to ARF [85,86]. Typically, pericarditis and myocarditis have full completely recovery after an acute episode of RF. However, during carditis episode, a valvulitis, mainly detected by the presence of mitral regurgitation or, less commonly, aortic regurgitation may not have a completely recovery, resulting in future RHD (Table 22.3).

Macroscopic histopathological findings are fibrinous pericarditis or “bread and butter pericarditis,” a macroscopic feature that is occasionally observed during cardiac surgery. There are no described sequelae of rheumatic pericarditis.

As acute RM is frequently asymptomatic [87], its diagnosis is difficult and requires a high suspicion rate. Rheumatic patients with acute myocarditis often present with mild symptoms, such as tachycardia or worsening of heart failure symptoms. These symptoms are frequently attributed to a worsening of valvular heart disease or another cause of imbalance, such as blood volume or salt overload. Myocarditis can also lead to conduction disturbances, such as first-degree AV block, which is a minor Jones criteria (Fig. 22.6). These conduction disturbances may also be observed in conditions with similar signs and symptoms such as infective endocarditis, thus a through

differential diagnosis is essential in these patients. The upper limits of normal PR intervals (according to age groups 3–12 years old: 0.16ms, 12–16 years old: 0.18ms, and over 17 years old: 0.20ms) should be verified to correct diagnosis of prolonged PR interval [88,89].

It is interesting that troponin elevations in RM are mild, within the nonpathological range [90], or even nonexistent is high-sensitivity troponin dosage is not used [91]. This behavior of troponins in RM is expected of a kind of myocardial inflammation that does not lead to sequelae and causes transient, albeit sometimes important, myocardial dysfunction. Even patients with important myocardial dysfunction during RM can experience ventricular function recovery after proper treatment with corticosteroids [92]. Even though prognosis of rheumatic myocarditis with regards to ventricular function is good, it can prove fatal, mainly in patients with previous severe rheumatic valvular heart disease [84]. Acute RM can also be fatal in patients with severe left ventricular end-diastolic pressure or volume overload due to valvular heart disease [84]. Thus early diagnosis and treatment with salicylates or immunosuppressant are essential.

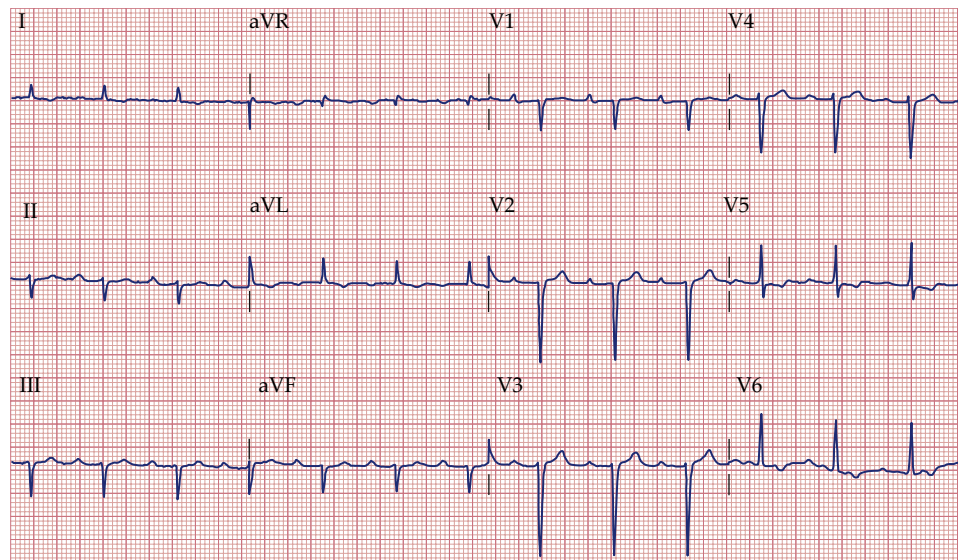


FIGURE 22.6 Prolonged PR interval is showed on electrocardiogram of a patient with acute rheumatic fever. Adapted from the personal collection of Dr. Nelson Samesina from the Eletrocardiography Unit from Heart Institute (InCor), São Paulo, Brazil.

Acute rheumatic carditis can be divided in four categories, as follows:

- a. **Subclinical carditis:** This category is found in patients with isolated arthritis and/or pure chorea; subclinical carditis is not necessarily associated with the auscultatory findings of a valve lesion and can be observed only as an abnormal pattern on the echocardiogram [93]. Two situations may occur in predisposed patients: (1) normal cardiovascular examination, radiologic and Doppler echocardiography despite ECG changes, particularly PR interval prolongation; (2) identifying Doppler echocardiography mitral and/or mild, nonphysiological aortic regurgitation.
- b. **Mild carditis:** The clinical observations include the presence of tachycardia disproportionate to fever, a softer first heart sound, and/or mitral systolic murmur. Moreover, there is a normal clinical cardiac examination and normal radiological and electrocardiographic examinations, or a potentially altered PR interval and mild or both mild/moderate regurgitation on Doppler echocardiography (echo). The left ventricle dimensions are normal on echo [94]. Subclinical to mild rheumatic carditis is estimated to be around 15–30% of all cases [95,96]. We must keep in mind that the echocardiogram can result in excessive diagnosis of RHD and strict criteria should be used to avoid overdiagnosis (Table 22.3) [73].
- c. **Moderate carditis:** More prominent clinical manifestations are found than for mild carditis, as persistent tachycardia and more intense mitral regurgitation is observed; however, there is no precordial thrill. Moderate carditis may be associated with an aortic diastolic murmur and/or a Carey Coombs murmur that refers to a short mid-diastolic murmur that occurs in patients with acute mitral rheumatic valvulitis. Sometimes it is followed by a third gallop. It can be distinguished from MS by the absence of an opening snap. Austin Flint is a low-pitched, mid-diastolic murmur best heard at the cardiac apex associated with severe aortic regurgitation. Early signs of heart failure, such as a slight increase in heart size and pulmonary congestion (also mild), may be observed on chest radiography. There are premature ST-T changes on the electrocardiogram. Doppler echocardiography shows isolated mild/moderate mitral regurgitation or is associated with mild/moderate aortic regurgitation. A mild to moderate increase in the left chambers can also be observed [94].
- d. **Severe carditis:** In this situation, additional findings are signs and symptoms of heart failure, arrhythmias, pericarditis, and severe murmurs related to mitral and/or aortic valves. A radiological examination shows significant cardiomegaly and pulmonary congestion signals. The electrocardiogram shows signs of left ventricular hypertrophy and, occasionally, right ventricular hypertrophy. Moderate mitral and/or aortic regurgitation and moderate/major left chamber enlargement are observed on echo [89].

It is noteworthy, however, that these subdivisions are in some way empirical. In particular, carditis may also be subdivided in two subtypes: subclinical to mild and moderate to severe. This subdivision may have therapeutic implications. Cases of mild carditis often respond

quickly to the use of salicylates, despite the frequency of adverse side effects, particularly gastrointestinal [83]. Moderate to severe carditis usually responds more quickly to corticosteroids [73].

Some series have reported that up to 90% of all rheumatic carditis are asymptomatic [94], only being diagnosed by the finding of a later typical rheumatic sequelae and/or by specific exams such as myocardial biopsy during cardiac surgery. However, globally, about 50–65% of people with RF have clinically detectable carditis [25,97]. Some authors argue that all symptomatic carditis should be classified as “severe,” because the majority of rheumatic carditis is asymptomatic, and can cause valvular sequel [84,93].

The minor manifestations of ARF (Fig. 22.4) consist of fever, arthralgia, elevated sedimentation rate, C-reactive protein, and prolonged PR interval in the electrocardiogram. In teenagers with slower heart rates the upper limit of normal PR interval would be around 180ms. Ambulatory ECG monitoring shows that at all ages some individuals with a normal resting PR interval will from time to time have periods when the PR interval becomes prolonged to >200ms [98]. Nonetheless, a suspected ARF case with prolonged PR interval should be considered as a minor manifestation sign until an alternative diagnosis is made [99].

Cardiac arrhythmias observed in RF are usually supraventricular and most commonly related to the intensity of the valvular heart disease and the degree of cardiac inflammation and pericarditis. Newly onset atrial fibrillation may happen as a manifestation of ARF [68]. In adult patients a recurrence of RM can be manifested as a worsening in New York Heart Association functional class and appearance of atrial fibrillation. As the natural history of valvular heart disease leads to atrial dilatation and eventually atrial fibrillation there is a difficult differential diagnosis between rheumatic inflammation and natural progression of the disease. In spite of general complacency about first-degree heart block in ARF, abnormal conduction with dysrhythmias, occasional complete heart block, and, rarely, Stokes–Adams attacks are important early signs of ARF and may precede other signs. Every person, particularly children, with episodic fainting is entitled to an ECG, and frequent ECGs are imperative in any case of RF with signs of arrhythmias [100,101]. Malignant ventricular arrhythmias have rarely been described [102]. Thus recognition of arrhythmias during an ARF episode emphasizes the need for continuous cardiac monitoring in select patients.

The Aschoff bodies were described in 1904 and their origin has long been debated. Evidence suggests that they are derived from cardiac mesenchyme. These nodules are composed of cells in the process of regeneration of cardiac muscle fibers in a rather atypical way [103,104]. The prevalence of Aschoff bodies in left atrial appendages (35%) that are collected after elective surgery for

valvular heart disease has been well documented in the literature since 1939.

The histological finding of Aschoff bodies is the most characteristic finding of rheumatic inflammation in the heart [93,94] and effectively makes the diagnosis of acute RM. Rheumatic myocarditis is a particular feature of acute rheumatic carditis that also encompasses rheumatic pericarditis and rheumatic valvulitis. Even in more modern studies, the prevalence of Aschoff bodies after elective surgery is as high as 30%. The finding of an Aschoff body in a myocardial specimen obtained after elective cardiac surgery can be the sole sign of ARF (Fig. 22.7) [82].

The diagnosis of acute RM is difficult and frequently requires the use of multiple imaging techniques. Echocardiography can reveal mild to moderate pericardial effusion, and rarely pericardial effusion or even pericardial tamponade. Transesophageal echocardiography can occasionally show small multiple nodules on the edge of native valves, representing the rheumatic *verrucae* that characterize the acute phase of the disease [93].

Laboratory exams (Fig. 22.4) usually show low levels of inflammatory markers as levels of C-reactive protein or erythrocyte sedimentation rate [105]. Other exams, like white blood cell count and blood cultures, if febrile, should be performed to help in differential diagnosis [73]. Characteristically, as stated before, RM does not raise troponin levels, and almost never leads to permanent myocardial damage or ventricular dysfunction. Evidence of preceding GAS infection (Serum streptococcal antibody: antideoxyribonuclease B (anti-DNAse B) and anti-streptolysin O (ALSO) should also be done in all suspected cases of ARF). If the initial titer is below the upper limit of normal, repeat testing after 10–14 days [89].

Imaging techniques that highlight inflammation in the heart are particularly useful for the diagnosis of acute rheumatic carditis. Gallium-67 myocardium scintigraphy can be used to demonstrate myocarditis and has been studied in the diagnosis of acute rheumatic patients [106]. A good correlation has been shown between positive scintigraphy and myocardial biopsy for the diagnosis of active myocarditis [107]. Positron-emission scintigraphy associated with tomography (PET-CT) is currently being evaluated and appears to have better sensitivity than a Gallium scan, in addition to much better spatial resolution. This technique may prove to be an important diagnostic tool for the detection of RM (Fig. 22.8A and B).

It is interesting to note that cardiac magnetic resonance imaging shows very mild abnormalities in patients with acute rheumatic carditis [92]. The alterations observed are usually mild edema in the myocardium and a general absence of fibrosis detected by late enhancement. The lack of significant troponin elevation and a generally good prognosis of left ventricular function suggests that there is no extensive myocardial destruction in RM, and this fact

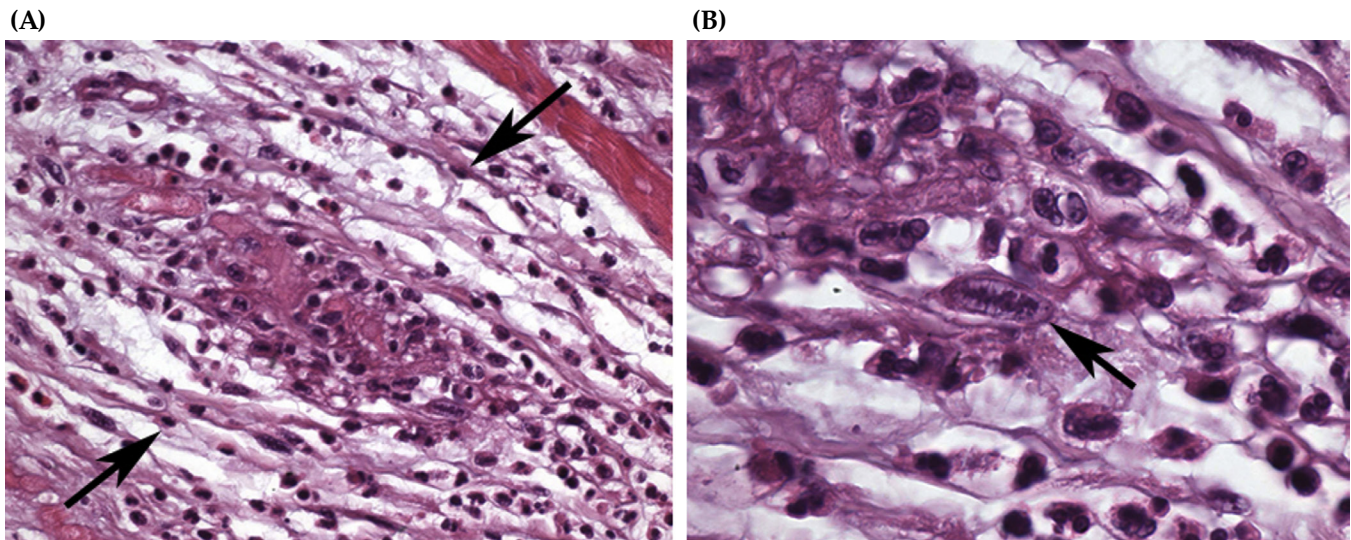


FIGURE 22.7 Light photomicrographs of left atrium surgical sample. Myocardial Aschoff body (arrows) showing central area of degenerating collagen surrounded by lymphocytes, macrophages, and few eosinophils (hematoxylin-eosin stain; $\times 400$). Some plump macrophages, called Anitschkow cells, are also observed in the inflammatory infiltrate. They have abundant cytoplasm and central nuclei with a caterpillar appearance, right photomicrograph (arrow) (Hematoxylin-eosin stain; $\times 1000$). Adapted from the personal collection of Dr. Lea DeMarchi, from Heart Institute (InCor), São Paulo, Brazil.

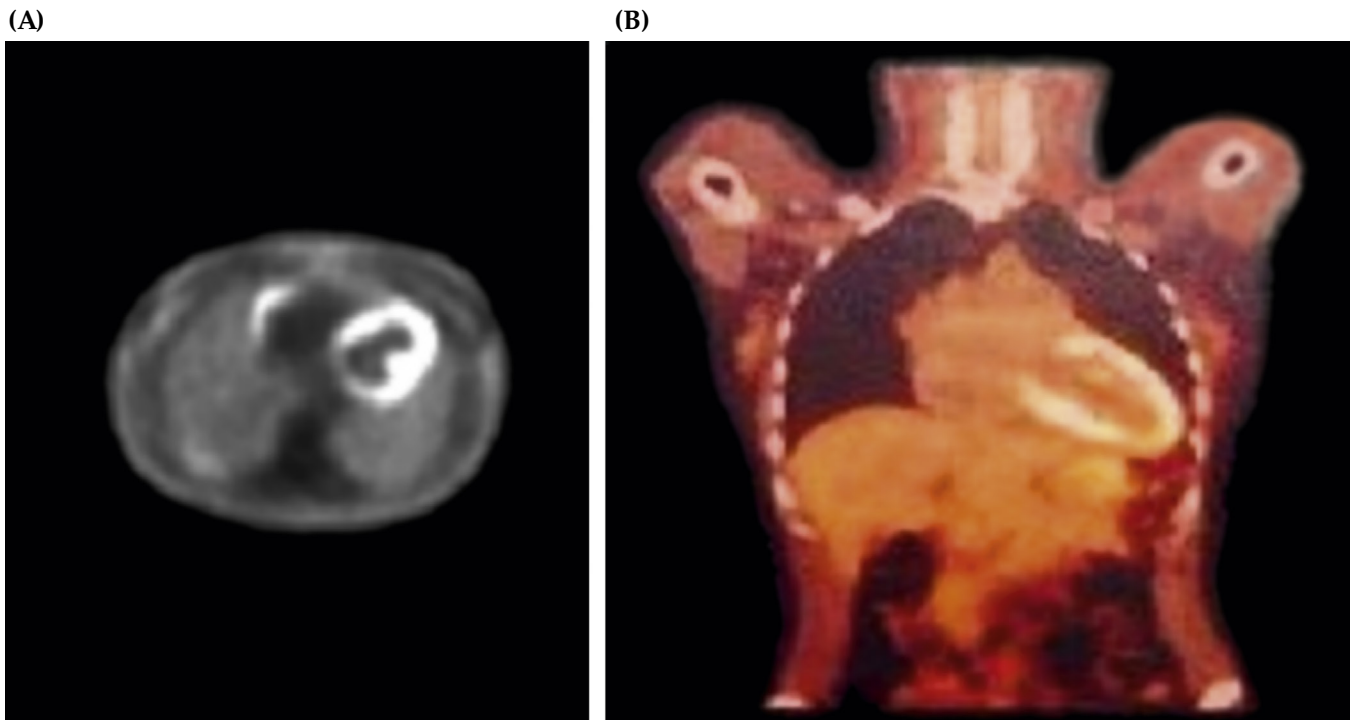


FIGURE 22.8 Pet CT image showing increased 18-fluorodeoxyglucose (FDG) uptake in the myocardium in a patient with acute rheumatic fever. (A) Transversal view; (B) Coronal view. Adapted from Nuclear Medicine Department, from Heart Institute (InCor), São Paulo, Brazil.

may justify the relatively poor MRI findings in this disease, when compared to the myocarditis of other etiologies [108].

Some old and more recent studies have found no difference among the efficacy of aspirin, cortisone, and adrenocorticotrophic hormone for the acute treatment of the symptoms of ARF [109,110]. However, the hormonal treatments caused faster resolution of symptoms. Some

groups continue to recommend the use of ASA as the first-line treatment of uncomplicated ARF [28,83]. However, gastrointestinal side effects are not uncommon. The use of corticosteroids for carditis in patients who are in severe heart failure is the preferred choice of treatment. Anti-inflammatory medications are given until the inflammatory markers normalize, usually 4–6 weeks (Fig. 22.4).

2.4 Sydenham's Chorea

Sydenham chorea is the commonest form of acquired chorea [111,112]. Large series over prolonged periods have noted a decline in the number of cases [113]. Chorea remains a major manifestation in 20–40% of cases of ARF, and there are reports of increasing numbers of sporadic cases in the United States [114].

Sydenham's chorea is the most characteristic manifestation of ARF. There are few other diseases that cause chorea in young patients, and these include *Lupus erythematosus* and Wilson's disease [73]. Thus Sydenham's chorea can solely establish the diagnosis of ARF. Chorea affects younger patients and more commonly young females.

The clinical manifestations include hypotonia, involuntary movements, and emotional instability, leading to physical incoordination or difficulties in walking and performing movements. Choreic movements are common in these patients and may worsen with stress and completely disappear in sleep. This clinical feature can be important in the characterization of rheumatic chorea. Severe chorea can affect deglutition and speaking because of involuntary tongue movements. A tendency to drop objects may also be noted, and facial movements may become apparent. The fasciculation and erratic movement of the tongue in acute chorea has been described in the literature as resembling a “bag of worms.” All of these symptoms are commonly apparent in only half of the body, a condition characterized as hemichorea.

Chorea is caused by an imbalance of neurotransmitters and is characterized by an excess of dopamine and reduced levels of γ -aminobutyric acid (GABA). This imbalance is caused by anticaudate antibodies, and the treatment is based on physiopathology.

Sydenham's chorea can lead to subtle sequelae given that these patients are more likely to develop psychiatric symptoms of the obsessive-compulsive spectrum [115]. Women that suffered from chorea in childhood can also experience a recurrence of symptoms in pregnancy (so-called *chorea gravidarum*) or when using oral contraceptive drugs.

2.5 Cutaneous Manifestations

Cutaneous manifestations are rare in ARF. *Erythema marginatum* is a manifestation clinically evidenced by pink circular nonpruriginous lesions. They occur as circular patterns of bright pink, macules or papules on the trunk and proximal extremities (Fig. 22.9). The lesions start as solid erythema, which may be slightly raised, appear and disappear within hours, and frequently go unnoticed by the patient. The erythema extends outward while the skin in the center returns to normal, hence the name “erythema marginatum.” The rash varied in duration from 2 days to 2.5 years in a dated series [116]. Apparently, treatment



FIGURE 22.9 Erythema marginatum, arm skin lesions image. Courtesy of League of Prevention and Treatment of Rheumatic Fever—(Liga de Combate à Febre Reumática) of Clinical Hospital, School of Medicine, University of São Paulo, Brazil.

with salicylates and corticosteroids has no effect on the duration of *erythema marginatum*.

Subcutaneous nodules are a rare, but highly specific manifestation of ARF and strongly associated with carditis. They present as crops of small, round painless nodules on the extensor surfaces of the elbows, wrists, knees, ankles, Achilles tendon, occiput, and posterior spinal processes of vertebrae [89]. The nodules are associated with severe cardiac disease as both are caused by cellular autoimmune aggressions. Both cutaneous manifestations require no specific treatment.

2.6 Additional Assessment

The stethoscope is commonly the only noninvasive tool available to doctors in low-income countries or in remote locations, where the RF and RHD are more prevalent. However, the detection frequency is usually low. Doppler echocardiography has shown to be more sensitive and specific than cardiac auscultation in the early detection of RF and RHD, as shown in some studies in Africa and other parts of the world [96].

After the first episode of carditis, cardiac auscultation may fail to identify murmurs in up to one-third of cases. These children can progress to rheumatic valve disease in the following years. A study conducted by highly trained clinicians in New Zealand showed that the clinical diagnosis of heart valve lesions, even among cardiologists, is often inaccurate [117]. Consequently, it is unlikely that the accuracy of clinical diagnosis by the medical community in other countries is more reliable if echocardiographic criteria are not used.

Many cases of ARF are clinically silent and occur in asymptomatic children without cardiac murmurs; this

fact suggests that echocardiographic screening is desirable to improve the identification of new cases and thus initiate early secondary prevention measures. Since 2004, the WHO has recommended echocardiography screening in high-prevalence regions.

Marijon et al. (2007) conducted a portable echocardiography study in school children in Mozambique and Cambodia that showed higher detection rates for RHD than for auscultation [118,119]. The prevalence of RHD identified early was then documented in several studies around the world. The highest cited prevalence was among children of school age in the country of Togo (3.3%), followed by Mozambique (3.0%), Cambodia (2.2%), Australian Aborigines (2.2%), Zaire (1.4%), and Zambia (1.2%), as well as a prevalence of 6.7% in Vietnam and nearly 10% in a region of Brazil [117,118,120–123].

Mitral valvulitis is the most characteristic component of RHD. It is frequently associated with regurgitation. The aortic valvulitis is less common and is usually associated with mitral valve disease. The pulmonary and tricuspid valves are rarely involved. Residual valve damage is a major concern in patients with RF and can cause intractable heart failure, often requiring surgical intervention.

The prevalence of RHD in asymptomatic patients as detected by Doppler echocardiography has not been well estimated, and can vary from 0% to 53% (weight pooled prevalence is around 16.8–18.1%) depending on the echocardiography criteria used [73]. It is possible through early detection to begin secondary prophylaxis and prevent these children from recurrent infections, thus improving prognosis.

2.7 Echocardiography in Early and Late Diagnosis—WHF Criteria and Revised Jones Criteria

The echocardiogram can demonstrate valvular thickening, valve failures, as well as small rheumatic verrucous lesions on the edge of the valves, which are characteristics of RF (Figs. 22.10 and 22.11).

In 2012 the World Heart Federation Guidelines defined echocardiographic diagnostic criteria. The echocardiographic evaluation can separate the diagnosis among three categories: definite RHD, borderline or absence of RHD [71,89,118,124].

When interpreting the echocardiogram, the RHD pretest probability should always be considered.

Pathological mitral regurgitation and at least two morphological characteristics of RHD of the mitral valve define the subcategory of “definite RHD,” according to the WHF. Mitral valve regurgitation is the most common manifestation of RHD in young people. Echocardiographic (Figs. 22.10 and 22.11), surgical, and postmortem anatomical data have demonstrated that this combination of morphological features is present in advanced disease. Colloquial

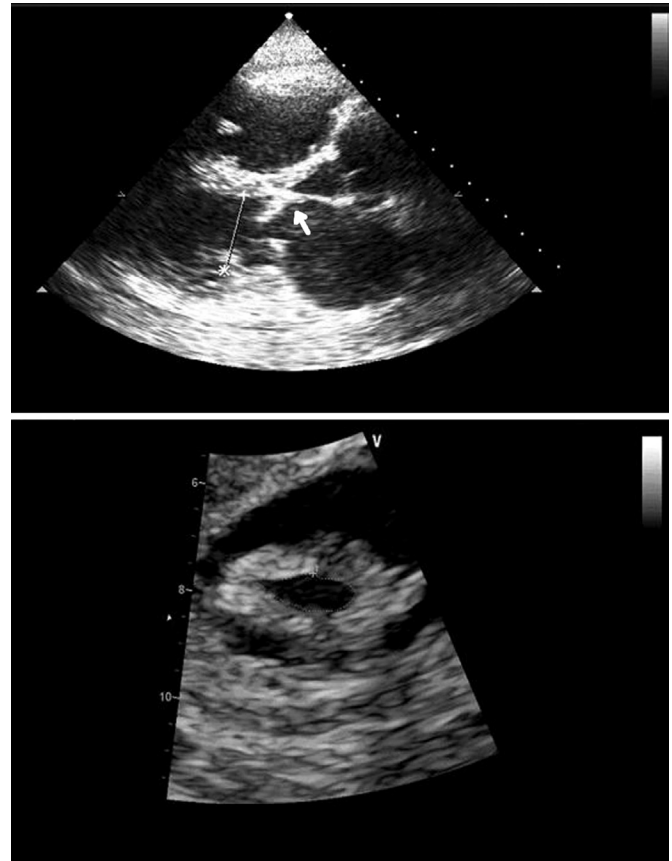


FIGURE 22.10 Echocardiogram. Parasternal long-axis echocardiographic view of a mitral valve of an RHD patient with valve regurgitation. Adapted from Echocardiography Department, from Heart Institute (InCor), São Paulo, Brazil.

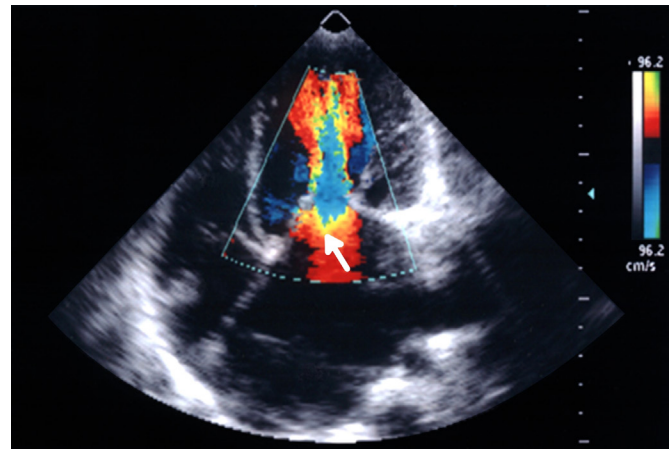


FIGURE 22.11 Echocardiogram. Apical four-chamber echocardiographic view of a patient with rheumatic mitral valve regurgitation (green color flow). Adapted from Echocardiogram Department, from Heart Institute (InCor), São Paulo, Brazil.

descriptions of the mitral valve deformity include “elbow” or “hockey stick” and indicate morphological changes ie, a thickening and restriction of the motion of the anterior leaflet of the mitral valve (Fig. 22.12).

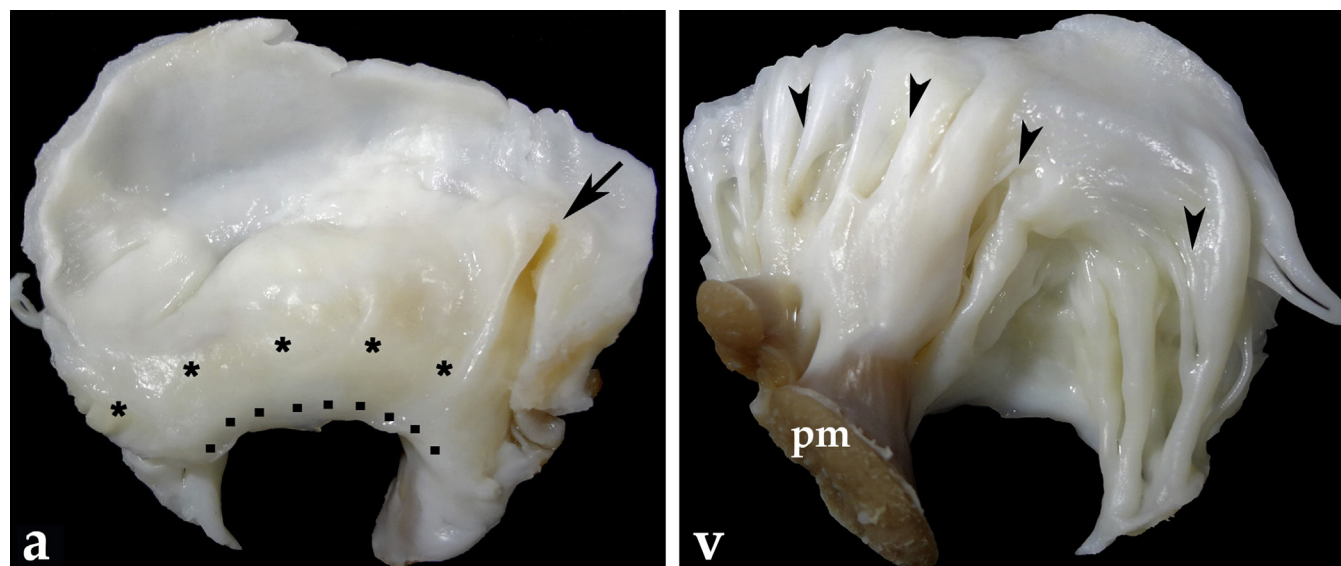


FIGURE 22.12 Post-rheumatic mitral stenosis, surgical excision. Atrial (a) and ventricular (v) aspects of anterior mitral cusp showing thickened and distorted leaflet with commissural adherence (arrow), retraction at the closure line (*), and crescentic notching of the free edge (■). There is marked thickening, fusion, and retraction of the chords (arrowheads) and fibrosis in the papillary muscle tips (pm). Adapted from the personal collection of Dr. Lea DeMarchi, from Heart Institute (InCor), São Paulo, Brazil.

2.8 Mitral Valve Stenosis

Stenosis with an average gradient equal to or greater than 4mm Hg and at least two morphological mitral valve RHD characteristic findings define mitral valve stenosis [73].

Worldwide, the most common cause of mitral valve stenosis is RHD. This condition is associated with at least two morphological changes of RHD. Typically, the leaflets are thickened, and the posterior leaflet is relatively immobile and moves parallel to the anterior leaflet of the mitral valve during systole. The differential diagnosis is congenital mitral valve stenosis, which is easily distinguished from RHD as it is often associated with abnormal formation of the papillary muscle. Ninety-nine to 99.3% of all cases of MS in individuals younger than 50 years are due to RHD. Annular calcification is a differential diagnosis that must be considered in individuals older than 50 years [89,93,118,124].

2.9 Aortic Valve Disease

Isolated aortic valve disease is recognized as a manifestation of RHD; however, this condition is not common. A large study of 10,000 consecutive RHD patients showed that isolated rheumatic aortic valve disease occurred in 4.5% of patients aged 18 years or younger; 2.8% of the identified patients were older than 18 years. Isolated rheumatic mitral valve disease is much more common. In aortic valve disease, the presence of multiple morphological characteristics allows for the confirmation of rheumatic disease (Table 22.4). For consistency with the criteria for

mitral valve disease and to increase specificity, the ultimate criterion for aortic valve RHD requires the presence of two morphological characteristics of rheumatic aortic valve in addition to pathological aortic regurgitation (Table 22.4). The most common differential diagnoses are congenital aortic stenosis, root dilation of the aorta, and other forms of inflammatory carditis, as well as systemic lupus erythematosus and ankylosing spondylitis.

2.10 Multivalvular Disease

Worldwide, RHD remains the most common cause of combined aortic and mitral valve disease. Pathological mitral and aortic regurgitation or pathological aortic regurgitation associated with mitral valve rheumatic disease is characteristic of this category as there are few alternative explanations for these valve diseases.

2.11 Borderline RHD

The category of “borderline RHD” only applies to persons aged less than or equal to 20 years. In this age group, it is less likely that sufficient echocardiographic characteristics will manifest to allow for the finding of “definitive RHD” because it can take some time to develop chronic disease. The classification “borderline RHD” was established to increase the sensitivity of the echocardiography criteria (although reducing specificity) in individuals aged less than or equal to 20 years as this age group benefits the most from early detection and secondary prevention of RHD [89,118,124,125].

TABLE 22.4 Rheumatic Heart Disease: Echocardiographic Criteria

RHD ≤20 years old	RHD ≥20 years old
A. Pathological mitral regurgitation, and at least two morphological characteristics of RHD in the mitral valve	A. Pathological mitral regurgitation and at least two morphological features of RHD of the mitral valve
B. Mean gradient ≥4 mm Hg	B. Mean gradient ≥4 mm Hg
C. Pathological aortic regurgitation, and at least two morphological characteristics of RHD in the aortic valve	C. Pathological aortic regurgitation, and at least two morphological characteristics of RHD in the aortic valve in individuals <35 years of age only (hypertension, bicuspid aortic valve, and dilated aortic root must be excluded)
D. Borderline disease in both mitral and aortic valves	D. Pathological aortic regurgitation and at least two morphological characteristics of RHD of the mitral valve
Borderline RHD (or A, B, or C)	
A. At least two morphological characteristics of RHD in the mitral valve, without mitral regurgitation or stenosis	
B. Pathological mitral regurgitation	
C. Pathological aortic regurgitation	

Pathological mitral regurgitation: (1) regurgitation is seen in two views, (2) in at least one view jet length more than 2 cm, (3) peak velocity ≥3 m/s, (4) pan-systolic jet in at least one envelope.

Pathological aortic regurgitation: (1) regurgitation is seen in two views, (2) in at least one view jet length ≥1 cm, (3) peak velocity ≥3 m/s, (4) pan-diastolic jet in at least one envelope.

Adapted from Reményi et al. [124] and RHD Australia (ARF/RHD Writing Group) National Heart Foundation of Australia and the Cardiac Society of Australia and New Zealand [89].

Above the age of 20 years, moderate valve regurgitation is more common, and the use of the category “borderline RHD” is not recommended. Minimum echocardiography changes that are criteria for “borderline RHD” may represent early RHD in some individuals and normal findings in others. Thus we must consider the clinical picture and epidemiology (primarily the prevalence of RF in the region) when interpreting echocardiographic findings. If the pretest probability (or risk) of RHD is high, the presence of the disease should be considered.

In summary, the diagnosis of ARF and RHD depends on specific training, high clinical suspicion, and the use of various tools. On one hand, the lack of diagnosis may result in severe heart valve damage in the future. On the other, overdiagnosis can result in unnecessary treatment. Similarly, the diagnosis of recurrent rheumatic activity remains a challenge for health professionals. The rapid recognition of rheumatic activity allows for appropriate treatment. Currently, there is no diagnostic laboratory test for ARF, so diagnosis remains a clinical decision, based on recognition of high-risk groups, evidence of recent GAS infection, identification of major and minor manifestations associated with diagnosis tests, as acute phase reactants (CRP), electrocardiogram (prolonged PR interval), and echocardiography (carditis) (Fig. 22.4).

3. PATHOPHYSIOLOGY

3.1 Autoimmune Reactions

The humoral and cellular hyperimmune responses that follow *S. pyogenes* infections are related to the

autoimmune reactions observed in susceptible individuals due to molecular mimicry. The term of “molecular mimicry” was introduced in 1964 by Damian RT to define the mechanism by which self-antigens are recognized by cross reactivity after an infection [126]. Cross-reactivity with pathogen antigens, *S. pyogenes*, in the case of RF/RHD is responsible for the damage in the articulations (joints), brain (gangliosides), and heart (myocardium and valves proteins). Several proteins are the target of B lymphocytes (antibodies) and T lymphocytes. As polyarthritis is the first episode, it is likely that antinovium circulating antibodies subsequently react by cross-reactivity with proteins with some identity or conformational similarities with heart-tissue proteins, leading to carditis [75].

The presence of autoantibodies against human tissue proteins in the articulations, brain, and heart were first described by Cavelti PA in 1945 [127], followed by several works and extensively reviewed by Cunningham [128]. Of note, the presence of streptococcal and human tissue cross-reactive antibodies against joint synovium, myosin, tropomyosin, vimentin, and keratin was related to the development of arthritis, carditis, erythema marginatum, and subcutaneous nodules [128,129].

Antibodies or immune complexes are proposed to target the valve surface and to increase the expression of adhesion molecules, such as VCAM, favoring inflammation and cellular infiltration into the heart tissue [130]. Chemokines are molecules that play an important role in cellular traffic into tissue. CCL1/I-309 and CXCL9/Mig chemokines were observed to be upregulated in

rheumatic heart valvular lesions. Through an in vitro assay, it was demonstrated that memory T lymphocytes (CD4⁺CD45RO⁺) preferentially migrated to the valve tissue toward the CXCL9/Mig gradient and were conclusively shown to contribute to permanent valvular damage [131]. The combination of these events is implicated in the cellular infiltration of leukocytes (monocytes, macrophages, T and B lymphocytes) into heart tissue. This infiltration occurs throughout the inflammatory and autoimmune reactions in heart tissue, leading to permanent valve damage and, consequently, heart dysfunction.

3.2 Molecular Mimicry Mechanism

The immune system, through the action of T and B lymphocytes, recognizes pathogenic and self-antigenic epitopes that share (1) identical amino acid sequences; (2) homologous but nonidentical sequences; (3) common or similar amino acid sequences of different molecules (proteins, carbohydrates); and (4) structural similarities between the microbe or environmental agent and its host [132,133].

In the case of RF, Sydenham chorea, and RHD, all of these criteria for mimicry of streptococcal epitopes are met, and cross-reactivity occurs a few weeks after *S. pyogenes* infection and during the progress of the disease. In addition, it is possible that during the clearance of the bacteria by antibiotic therapy, an uptake of streptococcal antigens occurred by antigen presenting cells (monocytes/macrophages) in order to eliminate the pathogen, thus activating autoimmune reactions, but there is no evidence that confirms this hypothesis.

The N-terminal portion of the M protein is the major target of cross-reactive reactions. Several works have noted that these cross-reactive epitopes are recognized by antibodies [128] (reviewed by Cunningham, 2000) and/or T cells [129].

3.3 Antibody-Mediated Molecular Mimicry

Cross-reactive antibodies targeting the polysaccharide and N-terminal region of the streptococcal M protein and human proteins have been described (Table 22.5). The generation of immune complexes occurs through the

interaction between antigen and specific antibodies and facilitates the clearance of foreign antigens by phagocytic cells.

During polyarthritis episodes antibodies against streptococcal group A carbohydrates precipitate to form immune complexes. This event is followed by complement cascade activation and consequently to the induction of transitory arthritis.

Cross-reactive antibodies also play an important role in Sydenham chorea by targeting neurons in the human basal ganglia. N-acetyl- β -D-glucosamine, a group A streptococcal carbohydrate, cross-reacts with some brain antigens from the basal ganglia (lysoganglioside and tubulin), as described by Cunningham's group [134,135]. Cardiac myosin is the most abundant protein in the heart. By using affinity purified antimyosin antibodies, Cunningham's group identified a five-amino acid residue (Gln-Lys-Ser-Lys-Gln) of an epitope of the N-terminal M5 and M6 proteins as being cross-reactive with cardiac myosin [136].

N-acetyl β -D-glucosamine is present in both the streptococcal cell wall and heart valvular tissue, leading to cross-reactive antibodies against laminin, an extracellular matrix α -helical coiled-coil protein that surrounds heart cells and that is also present in the valves [128]. The immunization of mice with recombinant SPE-B (streptococcal pyrogenic toxin B) induces both the deposition of IgG and complement activation in mouse heart valves triggering apoptosis in heart cells. These data suggest a role for SPE-B in the generation of heart lesions [137].

Using a proteomic approach, other valve proteins that presented increased or diminished expression such as vimentin, lumican, and collagen-VI were recently described and are likely target-proteins of cross-reactive antibodies and/or cellular reactivity [138].

Anitcollagen antibodies seem to be involved with the valve damage in RF/RHD, but the mechanism that leads alteration in the structure of the valves is controversial. Some researchers found that proteins of rheumatogenic streptococcal strains (M types 3 and 18) form complexes with human collagen IV that could trigger antibody response against the valve, similar to how it occurs in other diseases such as Goodpasture

TABLE 22.5 Human and Murine B-Cell Reactivity Against *S. pyogenes* Antigens and Human Proteins

Origin of antibodies	Cross reactive antigens	References
Murine and rabbits sera of <i>S. pyogenes</i> immunized animals	N-acetylglucosamine, laminin, brain, vimentin	Reviewed by Cunningham et al. [128] and Guilherme et al. [129]
Human polyclonal (sera) and murine monoclonal antibodies (hybridomas) against <i>S. pyogenes</i> antigens	N-acetylglucosamine, cardiac myosin, keratin, vimentin, sarcolemma, valve proteins, glomerular protein, tropomyosin	Reviewed by Cunningham et al. [128] and Guilherme et al. [129]

and Alport syndromes as reviewed by Tandon et al. [139]. It was shown that collagen VI presents diminished expression in the RHD valves. However, the mechanism(s) leading these alterations are not known. A speculative hypothesis is that the inflammatory process mediated by macrophages/monocytes, dendritic cells, and B and T lymphocytes exposes both forms of collagen (IV and VI) in diverse valve sites (IV-basement membrane and VI-extracellular matrix) [138,139].

3.4 T Cell-Mediated Molecular Mimicry

The role of T lymphocytes in the development of ARF and RHD was only described in the 1980s after the discovery of anti-CD3, -CD4 (helper activity) and -CD8 (cytotoxic activity) monoclonal antibodies. The presence of T lymphocytes in heart tissue suggested their possible role in the pathogenesis of RHD as described by Raizada et al. and Kemeny et al. [140,141]. The T-cell immune response was also evaluated in the context of RHD for its ability to recognize in vitro the streptococcal M protein and/or heart tissue-derived proteins, likely via molecular mimicry. An early study showed that M protein-stimulated T cells from RF/RHD patients could recognize a 50- to 54-kDa myocardial protein fraction, indicating that auto reactivity to heart antigens was probably caused by streptococcal infection [142]. The functional role of these antigens, however, was later demonstrated by isolating these cells from human myocardium and valves of severe RHD patients who underwent surgery for valve correction. This approach allowed the first demonstration of the reactivity of these cells against streptococcal-derived M protein peptides and both human myocardium and valve tissue proteins obtained from ex vivo specimens during surgical valve corrections [143]. Table 22.6 summarizes the T-cell autoimmune reactivity [143–147].

M5 epitopes and several heart tissue-derived proteins (from the myocardium and valvular tissue) were also preferentially recognized by peripheral T lymphocytes from RHD patients when compared with normal individuals, primarily in the context of HLA-DR7. This phenotype, as mentioned above, is a susceptibility factor predisposing to the disease [146].

Both of the studies mentioned above also allowed the demonstration that streptococcal-primed peripheral cells expand as oligoclonal populations in the heart tissue [148] and secrete primarily inflammatory cytokines (Th1-type) (IFN γ and TNF α). Only a small number of cells producing IL-4 (a regulatory cytokine) were found in the valvular tissue. This is in contrast to the myocardium, thus indicating that the lack of autoimmune reactivity regulation likely contributes to the observed permanent valve damage. The Th17 subset of cytokines is also frequently involved with inflammatory reactions. We observed large numbers of IL-17 and IL-23-producing cells in the valves of RHD children. These cytokines are frequently involved in the development of autoimmune diseases. A Turkish RHD cohort study recently reported increased numbers of Th17 cells in peripheral blood that correlated with IL-17A cytokine levels in the sera and decreased numbers of T regulatory cells [149]. These data together indicate how the autoreactive immune response can be deleterious to heart tissue, consequently leading to valve/heart dysfunctions.

4. TREATMENT AND VACCINE PERSPECTIVE

4.1 Treatment

Most patients with ARF carditis respond very well to general measures used in acute heart failure, such as bed rest, salt and water restriction, and drugs, such as diuretics, digitalis, and vasodilators. As mentioned before, severe RM should be considered for immunosuppressive therapy. The most common immunosuppressant drug studied and used in these patients is the corticosteroid prednisone [150], which is administered in doses of 1–2 mg/kg/day. High-dose corticotherapy must be maintained for at least 6 weeks, with gradual weaning off the medication. Patients with refractory heart failure eventually require emergency surgery for repair or replacement of severely damaged valves, although they receive a higher-dose immunosuppressant treatment, with intravenous methyl-prednisolone at doses up to 1 g/day (Fig. 22.5) [83].

TABLE 22.6 Human and Murine T-cell Reactivity Against *S. pyogenes* N-Terminal Amino Acid Residues of M Protein [144,145]

Origin of T cells	M-protein amino acid residues	References
Human heart tissue infiltrating T-cell clones and peripheral blood T cells	DKLKQQRDTLSTQKET LKQQRDTLSTQKETLEREVQN	Guilherme et al. [143] and Guilherme et al. [146]
Murine lymph node T cells	KKEHEAENDDKLKQQRDTL DKLKQQRDTL	Cunningham et al. [147]

Underlined—identical amino acid residues.

The anti-inflammatory treatment of RM with corticosteroids is not made with the objective of preventing rheumatic valvular sequelae. The main objective of corticosteroid therapy is to limit the duration of the myocardial inflammation, mainly in symptomatic patients. As left ventricular function normalizes when rheumatic carditis subsides, reducing the period of inflammation and ventricular dysfunction is beneficial for the patient. Thus anti-inflammatory treatment is given only to symptomatic patients with signs of heart failure in order to shorten the duration of the disease. Clinical practice shows dramatic improvements in ventricular function and heart failure control with corticosteroids in rheumatic carditis patients.

Unfortunately, most clinical trials involving anti-inflammatory treatment for RF are from the 1950s and 1960s [150]. These studies focused mainly on anti-inflammatory treatment as a way to reduce the cardiac sequelae of RF. It is unlikely that these clinical trials would be repeated today as most physicians that care for ARF patients consider corticosteroids as an essential drug and find it unethical to treat patients without them. Little evidence of benefit was found when corticosteroids or intravenous immunoglobulin were used to reduce the risk of heart valve lesions in patients with ARF [150]. As salicylates are weak anti-inflammatory drugs, at least when compared to corticosteroids, most clinicians do not find these drugs adequate or useful for treating severe RM (Fig. 22.5).

4.1.1 Primary and Secondary Prophylaxis

Rheumatic fever is the most preventable of all heart diseases [87,125], and the correct treatment of streptococcal pharyngitis is satisfactory to prevent the development of the disease. Primary prophylaxis has been used to dramatically lower the incidence of RHD in countries such as Cuba and in most countries of the developed world.

Streptococcal pharyngitis is a common diagnosed and treatable illness but has a feature that greatly hinders adherence to treatment, which makes it a self-limiting disease. Even without antimicrobial therapy, many cases of sore throat resolve spontaneously in approximately 7–10 days, leading to inadequate treatment with over-the-counter analgesics and antipyretics. This phenomenon causes discontinuation of prescribed antibiotic therapy, failure to seek medical attention to treat pharyngitis, and consequential exposure of predisposed individuals to untreated streptococcal infection. This pattern of events causes subsequent episodes in patients who often do not seek medical attention.

Families with many children at home, especially those of low socioeconomic status, also learn that the

“sore throat symptoms” are self-limiting and do not seek health services. The correct treatment taken to the seventh day of streptococcal tonsillitis prevents the development of RF [87].

The self-limiting characteristic of the disease makes the treatment of choice for tonsillitis a single dose. Even today, benzathine benzylpenicillin G is the antibiotic of choice and is given at a dose of 600,000 IU for patients up to 27 kg weight and 1,200,000 IU for those over 27 kg (Fig. 22.5) [151,152]. These medications were given as single intramuscular doses. A single dose is sufficient for complete treatment of the disease and eradication of streptococci, reliably preventing the development of RF [87]. Contributing to its usefulness is the fact that there is no group A β -hemolytic streptococci resistant to penicillin [153]. The discomfort of the intramuscular application, which can be minimized with proper application technique, should not be an obstacle to the use of this medication as a first choice in most patients.

Oral therapy should not be used routinely [154], as 10 days of therapy is generally necessary for the complete eradication of GAS throat. When using, for example, amoxicillin at 500 mg, 3 times a day for 10 days, as is commonly prescribed in primary care units for tonsillitis, there is a very high risk of nonadherence to complete treatment [87]. Noncompliance with antibiotic therapy increases the risk of developing ARF. Oral regimens of alternative antibiotic regimes should be reserved only for patients who are allergic to penicillin. The importance of penicillin in the management of RF is based on well-documented efficacy for eradication of streptococci using penicillin, which is widely reported in the literature [89,151,152]. Cephalosporins, clarithromycin, and clindamycin may be used, but their effectiveness has been demonstrated in only a few studies, and their efficacy has been tested in far fewer patients than those involving penicillin derivatives (Fig. 22.5) [89,151,152]. Primary prophylaxis should be performed in all patients with suspected streptococcal tonsillitis, and this course of action does not require diagnostic confirmation. The oropharynx culture is only used to epidemiologically trace the various serotypes of *streptococcus*. Rapid tests for streptococci are rarely used in low- and middle-income countries as these tests only add unnecessary cost and complication in a context of limited resources [155]. In highly endemic situations, the WHO recommends that for simple tonsillitis, clinical suspicion is sufficient for starting antibiotic therapy. This therapy is preferably benzathine benzylpenicillin as a single intramuscular dose, thereby eliminating the possibility of non-compliance [125].

The United States and Europe have eradicated RF through aggressive detection programs and early treatment of streptococcal pharyngitis with intramuscular benzathine penicillin in children and adolescents. Some

authors argue that primary prophylaxis is unnecessary and that only secondary prophylaxis should be performed [155]. The logic proposed by these authors is that performing the screening by echocardiography in the entire population allows the identification and treatment with secondary prophylaxis of all RF carriers in a given population. These authors believe that this approach would be more cost effective than continuing to use the inexpensive and available penicillin G benzathine in all cases of streptococcal infection [156]. However, there is also evidence that primary prophylaxis may be the more cost-effective regimen [155].

Secondary prophylaxis should be performed in those patients who are diagnosed with RF to prevent new infections that can lead to disease progression [157]. Serious cardiac lesions are not commonly established after the first attack of RF. Consequently a single episode of ARF has a low probability of developing serious rheumatic sequelae. The prevention of further episodes of ARF, even those that are asymptomatic, may prevent the development of severe valvular lesions. Even patients with severe valvular heart disease if correctly managed using secondary prophylaxis may have a reduction in the progression of valve diseases and also prevent the onset of rheumatic sequelae in other valves [89,151]. The medication of choice for secondary prophylaxis (Fig. 22.5) is benzathine benzylpenicillin G, administered intramuscularly at a dose of 600,000 IU for patients up to 27 kg and 1,200,000 IU for patients over 27 kg. These treatments are given every 15 days over the first 2 years after the outbreak and every 3 to 4 weeks according to residual lesions [89,151,152]. In situations of high incidence and endemic cases of RF, the prophylaxis regimen with benzathine penicillin every 4 weeks seems to be inadequate [157,158]. The risk of recurrence of RF is greater in the first 2 years after an ARF event. Prophylaxis in this period (first 2 years after ARF episode) should be done with penicillin injections every 15 days, in order to reduce RF recurrence [158]. The last American Heart Association statement has recommended secondary prophylaxis every 4 weeks in the United States [152]. The same statement has recommended long-term benzathine penicillin G prophylaxis every 3 weeks in those who have recurrent RF despite good adherence to an every 4-week regimen. Serum drug levels may fall below the protective level before the fourth week. In high-risk populations, mainly in low-income countries, we do recommend the every 3 weeks regime due to higher recurrence rates of the 4-week regimes [157,158].

Other optional treatments are penicillin V 250 mg twice daily, which has a higher RF recurrence rate. For allergic patients, the most appropriate scheme is sulfadiazine 500 mg by mouth 2 times a day. However, this medication should not be maintained for too long due to its low effectiveness and risk of side effects, such as

leucopenia. For those patients allergic to penicillin and sulfadiazine, macrolides or azalide may be an option [152].

Secondary prophylaxis should be prescribed continuously after surgical correction of valvular heart disease, even if the mitral and aortic valves have been replaced by bioprostheses. Even if the valve prostheses themselves cannot be affected by the immune response causing RF the patient may still develop RM that can have serious consequences, even leading to death. Other sequelae such as arthritis, which can be extremely painful and limiting, especially in adults, can be avoided.

Pregnant patients also deserve special attention given that the immunological changes that occur during pregnancy predispose patients to new episodes of RF. Fortunately, benzathine benzylpenicillin G can be safely used from the first trimester of pregnancy.

The treatment of secondary prophylaxis should take into consideration the time and duration of the manifestation of RF with carditis as previously described [152]. It should be noted that patients who have occupational exposure to streptococci (eg, health professionals, workers in kindergartens and schools, primary school teachers) should continue secondary prophylaxis while occupational exposure persists, regardless of the cardiac sequelae.

4.2 Vaccine Perspective

As mentioned in the Introduction, streptococcal infections have had a significant impact on health for more than 150 years, primarily due to the autoimmune sequelae of RF/RHD. The disease has remained a health problem in several countries, and the development of a safe vaccine remains a challenge.

Several vaccine development studies have been proposed in recent decades, with no satisfactory results. However, in the last 20 years, the introduction of new methodologies, such as the synthesis of peptides and molecular biological techniques, has allowed the development of new proposals. Some models of the anti-group A *streptococcus* (GAS) vaccine candidates based on the M protein and other alternative streptococcal antigens are being designed [12].

Briefly, a strain-specific vaccine based on recombinant N-terminal portions of the M protein of the 26 and 30 most prevalent serotypes in the US has entered into phase I clinical trials [159,160].

Two other models of vaccines that incorporate C-terminal protective epitopes are in development. One is based on a minimal protective epitope identified as J8 that induces protective antibodies in mouse models [161]. StreptInCor, another candidate vaccine, is composed of 55 amino acid residues [162]. This vaccine epitope was selected by the analysis of a large panel of human sera and peripheral blood cells. This epitope can

undergo processing by antigen-presenting cells (monocytes and/or macrophages) and generate a universal and robust and safe immune response [163]. Experimental assays using several animal models showed that the candidate vaccine induced high titers of opsonic, neutralizing, and protective antibodies [164,165]. The immunogenicity and safety of the StrepInCor vaccine epitope was also evaluated for a period of 1 year in a model of HLA class II transgenic mice. Specific and nonauto reactive antibodies were produced without autoimmune or pathological reactions in the heart or other organs [166].

Although the development of a vaccine to protect against *S. pyogenes* without triggering autoimmune reactions remains a challenge, knowledge of the mechanisms that lead to RF and/or RHD allows and favors the construction of a safe and efficacious anti-*S. pyogenes* vaccine.

5. CONCLUSIONS

Rheumatic fever and RHD were identified more than 200 years ago and are still considered health problems in several countries, affecting millions of children around the world.

Fortunately, the efforts of several clinicians and researchers in the last 50–70 years have provided an increased understanding of *S. pyogenes* and infectious diseases caused by this bacterium. The identification of several strains around the world also allows the definition of strains that cause RF and RHD, which are considered the major sequelae of *S. pyogenes* infections.

The new tools of molecular biology have led to the identification of several genetic markers that predispose humans to the disease. These advances have also led to the identification of several molecules that play important roles in the immune response, with these functions either being protective or leading to autoimmune reactions and disease.

The diagnostic criteria established by Jones are still useful for diagnosis. Heart-imaging technologies and echocardiograms have improved treatment and follow-up of patients. Penicillin benzathine is still the treatment of choice, and other drugs are used in individuals who are allergic to penicillin benzathine. Several models of anti-*S. pyogenes* vaccines are currently being developed and it will perhaps be possible in the coming years to prevent the development of new cases of RF and RHD.

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Chagas Cardiomyopathy

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1. INTRODUCTION

Chagas disease (ChD) is one of the 17 WHO-defined neglected tropical diseases (NTDs) [1]. The disease is one of the most important parasitic infections in South America. The lifecycle of the parasite and the associated disease were described by the Brazilian physician Carlos Ribeiro Justiniano das Chagas (1879–1934) in 1909 [2]. He identified the parasite in the blood of a child with “fever, anemia, edema and generalized lymphadenopathy,” described the lifecycle of *Trypanosoma cruzi* [3] and determined its clinical manifestations, epidemiology, and entire lifecycle in vectors, wild reservoirs, and human hosts [4–6].

Although human infection has been found in 4000–9000-year-old mummies [7,8], endemic ChD was caused by deforestation by human actions over the last two to three centuries [9].

Chagas’ heart disease is the leading cause of infectious myocarditis worldwide [10] and is the most serious and frequent manifestation of chronic ChD, with 20–30% of infected individuals affected 10–30 years after infection. Persistent low-grade parasitemia, accompanied by an unbalanced immune response, appears to be the main pathogenic mechanism underlying myocardial damage, microvascular dysfunction, and neuronal cell loss [11].

2. EPIDEMIOLOGY

Historically, ChD was associated with impoverished populations in rural areas of countries (ie, Mexico and other countries in Central and South America) where

clay and shoddily constructed domiciles allowed the vector to thrive and perpetuate its lifecycle.

In the late 1970s, approximately one quarter of the Latin America population was under constant threat of infection. Countries such as Brazil had an overall estimated prevalence of 4.2% of the population, corresponding to 6.5 million infected individuals [12,13]. At this time, an annual incidence of 700,000 newly infected subjects and more than 45,000 fatalities were calculated in Latin America [14]. The institution of vector and parasite control programs, concomitant with blood-bank screening, achieved a reduction in the annual incidence to 41,200 cases and fatalities to 12,500 in 2006; moreover, there was a decrease in the number of countries considered to be endemic areas (from 22 to 18) [15].

However, a new scenario emerged in the context of the globalization of ChD, due to the emigration of people infected with *T. cruzi* from endemic to nonendemic countries in North America, Europe, Asia, and Oceania, including the United States, Canada, Spain, France, Switzerland, Japan, emerging Asian countries, and Australia [16]. This changing context created new epidemiological and public health issues for these countries, including management of the risks of transfusion, tissue transplantation, and congenital transmission, as well as the need for medical care for the type of patient who, until recently, had not been on the local health system agenda. Therefore areas with high prevalence in some populations were created over the past 20 years due to immigration waves, consequently increasing the prevalence of this condition in these locations, which attracted the attention of public health systems and contributed to the increased visibility of the disease outside of the historically endemic areas.

Due to this changing context, it is estimated that more than 300,000 individuals currently residing in the United States are infected with *T. cruzi* [17]. Although most of these cases are attributed to infections that occurred outside the country, at least 31 people have been infected inside the continental United States via vectorial transmission or the transfusion of infected blood products [16]. An ecological study in Texas found that 61.5% of the collected bugs were infected with *T. cruzi* [18].

The transmission of *T. cruzi* can occur through congenital, blood-borne, organ-derived, and oral route in non-endemic areas [19,20]. Since 2007, blood-donor screening has been performed in the United States [21]. In Spain, a regulatory law passed in 2005 requires that all at-risk donors (persons born in endemic areas, born of mothers native to endemic areas, and who have undergone transfusion in endemic areas) be screened for *T. cruzi* infection or otherwise be excluded from donation [22].

An epidemiological transition was noted due to a trend in the aging of patients with ChD, with the highest prevalence and mortality detected in the more advanced age groups [23]. This transition can be explained chiefly as a cohort effect as a consequence of exposure to *T. cruzi* infection in the past [6], prior to the control of vectorial transmission by the enforcement of public health institutions in endemic countries. The chagasic elderly comprise a vulnerable group of patients who exhibit an association between the risk of morbimortality for Chagas' heart disease and other comorbidities, often present in this age group, including hypertension, coronary disease, diabetes mellitus, and thyroid dysfunction [24–26].

3. ETIOLOGY

Trypanosoma cruzi is the protozoan implicated in the development of ChD. Taxonomically, the parasite belongs to the *Kinetoplastida* order and the Trypanosomatidae family. It has a flagellum and its single mitochondrion contains a kinetoplast, which is an extranuclear DNA network corresponding to the parasite's mitochondrial genome localized near the flagellate's basal body [27].

The disease can be transmitted to a broad spectrum of domestic and wild mammals, in addition to humans, by bloodsucking insects of the family Reduviidae and the subfamily Triatominae. There are approximately 140 species of triatomines, but only a few are capable of transmitting the disease [28]. Both female and male insects can transmit *T. cruzi* throughout their lifetimes (up to 2 years). Birds and reptiles do not carry the parasite but may be important sources of blood meals [27,29].

Chagas disease is autochthonous to 22 countries in the continental Western Hemisphere and is mainly

transmitted by triatomines, also called the kissing bug, barbeiro, bicudo, or vinchuca, which inhabit the American continent from the Southern United States and Mexico in the north to Argentina and Chile in the south [16]. Notably, only a small number of autochthonous vector-borne cases of infection have been reported in the United States [29]. Although Europe is colonized by different subfamilies of triatomines, there has been no indication of *T. cruzi* transmission by these insects in this area [30].

The disease was initially enzootic, maintained among wild animals and vectors, and it was transmitted accidentally to humans when they invaded the wild ecotope or when vectors invaded human homes [31–33]. With deforestation, the vector (*Triatoma infestans*) adapted to human houses, facilitating transmission to humans.

Several conditions have been correlated with the possibility of infection by the parasite, including the time elapsed between the bite and defecation, the number of evacuations and amount of feces produced during this interval, the number of parasites eliminated, and the percentage of infecting forms and their capacity to penetrate the damaged skin after the bite [34].

Therefore the likelihood of natural infection of humans by *T. cruzi* would not be very high if it were not in long and intense contact with infected triatomines [35]. The probability of infection increases throughout the lifetime if these and others determinants persist such as the quantity of parasites in the initial inoculation, lineage of the inoculated *T. cruzi*, the reinfections, and the host response [35–39].

4. TRANSMISSION

The insects become infected by sucking blood from animals or humans who have circulating parasites (trypomastigote forms). In the digestive tracts of triatomines, the parasites differentiate into epimastigotes and then into metacyclic trypomastigote forms in the final portion of the intestine. The mammals become infected after contact with metacyclic forms eliminated, after a blood meal, with the feces of triatomines through the injured skin or mucosa. Once in the vertebrate host, the metacyclic trypomastigote parasite is caught by macrophages from the local reticuloendothelial and connective cells, and it differentiates into amastigotes that begin to replicate by binary fission. When the cell is swollen with amastigotes, they transform back into trypomastigotes. Then, the parasites lyse the cell, invade adjacent tissues, and spread by the lymphatics and the bloodstream to distant sites, which are mainly muscle cells (cardiac, smooth, and skeletal) and ganglion cells, where they undergo further cycles of intracellular multiplication [40–42].

4.1 Blood Transfusion and Organ Transplantation

The risk of ChD after transfusion of one unit of blood from an infected donor is as high as 10–20%, with a higher risk with the transfusion of platelets [40]. The risk depends on the concentration of parasites in the components after fractionation.

With few exceptions, blood screening for *T. cruzi* is mandatory in most endemic regions, but this route of transmission remains an issue in nonendemic areas due to the immigration of infected people [31].

4.2 Congenital

The *T. cruzi*-infection prevalence among pregnant women varies widely between different countries, distinct geographical areas, and rural or urban localities [35], and is estimated to be approximately 4.7% (range 3.9–5.6) [43]. The prevalence is significantly higher for mothers with detectable parasitemia measured by (polymerase chain reaction) PCR, thus suggesting a strong relationship between maternal parasite load and vertical transmission [44,45].

At least 2 million women in Latin America of fertile age are estimated to be chronically infected with *T. cruzi*, resulting in an incidence of congenital infection of at least 15,000 cases/year (WHO/PAHO 2006). In nonendemic areas, the incidence is 66–638 cases/year in the United States and approximately 20–183 per year in Europe [46].

Congenital transmission could be prevented through universal treatment of infected women of fertile age. Its diagnosis among the pregnant and the newborn is mandatory, considering that the etiological treatment of the child has high rates of cure and minimal side effects [47].

4.3 Oral Transmission

The oral transmission of *T. cruzi* was reported for the first time in 1968 [48]. Outbreaks occur due to the consumption of food or beverages contaminated with the vector's feces or food contaminated with the urine or scent gland secretions from marsupials, containing metacyclic trypomastigotes of the parasite [49]. The occurrence of oral transmission in urban areas often occurs due to the delivery of traveling infected triatomines in food and beverages, such as sugarcane and juices. An outbreak with great repercussions was described in Venezuela in 2010, with a total of 103 individuals infected by the ingestion of guava juice [50]. In addition, oral transmission of this disease in the Amazon region has been reported since the 1960s [51].

5. DIAGNOSIS

The diagnosis of acute *T. cruzi* infection can be accomplished by the direct detection of parasites in the blood or by PCR. There is no standardized test to detect anti-*T. cruzi* IgM antibodies [52,53].

During the chronic phase, diagnosis is based on serological assays [53,54]. Several techniques are used for antibody detection, including indirect immunofluorescence, enzyme immunoassays (EIAs), hemagglutination, and rapid tests provided by different companies [55]. Their sensitivity can vary significantly, but the currently available tests used in blood banks are highly sensitive, and the current consensus is that a single highly sensitive EIA can be used for *T. cruzi* screening [55]. A second assay is necessary to confirm the initial positive result. The World Health Organization recommends that samples be tested in two assays based on different formats before confirming the diagnosis [56].

Most of the commercially available antibody-based assays use crude parasite extracts or subcellular fractions of cultured parasites as antigen preparations [55]. More recently, assays using recombinant antigens have been developed; these assays are less reactive with the serum of patients with other diseases, such as *Leishmania* [57,58] and *Trypanosoma rangeli* infection [59].

There are a few confirmatory assays, such as western blotting [60], immunoblotting [61,62], or radioimmuno-precipitation assay (RIPA) [62]; however, these assays are expensive and are rarely available in Latin America. In this region, immunofluorescence assay is the method of choice for *T. cruzi* confirmation.

Samples with low reactivity to *T. cruzi* have represented approximately 30% of the reactive samples screened by blood banks [63,64]. These samples are challenging, especially for donor counseling. They might represent previous exposure to *T. cruzi* with a self-limited infection. Supporting this idea is the clear association between antibody levels and PCR reactivity [65]. Case reports of seroreversion in the absence of treatment have previously been reported [66–68], as well as seroreversion among untreated controls in Benznidazole (nitroheterocyclic compound with trimanomicidal activity) clinical trials [69,70].

In general, *T. cruzi* PCR is not used for diagnostic purposes. The parasitemia is very low and likely accounts for discrepancies between assays [71]. Even in samples with high antibody titers, the frequency of PCR positivity was low and, in most recent reports, the rate of positivity in chronic phase has been 60%, but its sensitivity can vary widely as up to 90% [72–74]. The PCR positive rates were demonstrated to vary in different regions of Latin America. Furthermore, this reaction has been used in clinical trials to include patients and as an endpoint

criterion for a cure [75]. On the other hand, quantitative PCR assays have been used in monitoring for reactivation [76].

Thereby to deal with more precise PCR results and make them comparable between laboratories, the main constraint until now has been the lack of a universal reagent presenting accurately quantified *T. cruzi* DNA samples to be used as standards in all quantification assays [77].

6. NATURAL HISTORY AND PATHOGENESIS

After inoculation, there is an incubation period of 1–3 weeks. The acute phase can be asymptomatic or can present with a fever, rash, lymphadenopathy, and hepatosplenomegaly that lasts for 2–4 months. Some patients present with an inflammatory nodule, at the site of inoculation (chagoma), or with unilateral painless periorbital edema (Romana sign). In a few cases, the clinical presentation is more severe, resulting in acute myocarditis and/or meningoencephalitis mainly in children and death.

During the acute phase, the parasitemia is high, and the parasite can be directly detected in peripheral blood smears.

Trypomastigotes can infect different cells (ie, cardiac myocytes, muscle cells, endothelial cells of the nervous system, cells of the reticuloendothelial systems, and adipose tissue). The mechanism of cell entry is complex and involves host cell recognition, attachment, and signaling, which are required to initiate invasion [78]. Recently, the host-cell LDL receptor was demonstrated to be necessary for parasite entry [79]. Passage into the host-cell lysosomal compartment is required to establish an effective intracellular infection [78].

The innate and adaptive immune responses are important for the decrease in the levels of parasitemia during the acute phase of infection. Although the exact mechanism is not fully understood [80], IL-12 seems to play a key role in the process by driving the type 1 T-lymphocyte response with IFN- γ production. Lytic antibodies are also important for reducing parasite levels. It is possible that the type of immunological response established during the acute phase influences the disease outcome [81].

After acute infection, the patient becomes asymptomatic and enters the indeterminate phase. Approximately 50% of individuals will remain asymptomatic for life. New theories have suggested that adipose tissue could serve as a long-term reservoir for parasites, from which relapse of infection could occur [82]. Recent studies using very sensitive bioluminescence in in vivo animal models have suggested that *T. cruzi* remains in the gut during the chronic phase [83].

During the chronic phase, the parasite is barely detectable in muscles (eg, the heart tissue). Therefore it is difficult to understand how it can drive such an important inflammatory response there. Nevertheless, the persistence of *T. cruzi* seems to be important for the development of the disease, and cardiomyopathy is associated with parasite detection in the peripheral blood by PCR [72]. The pathogenesis is complex and likely represents an imbalance of the immune response to the parasite, leading to inflammation, oxidative stress, microvascular damage, and fibrosis (Fig. 23.1) [84].

Several cross-reactive autoantibodies and T-cell clones that recognize *T. cruzi* and host antigens have been described; these factors most likely aggravate the inflammatory process in the heart [84]. Inflammatory activity plays a key role as a pathophysiological

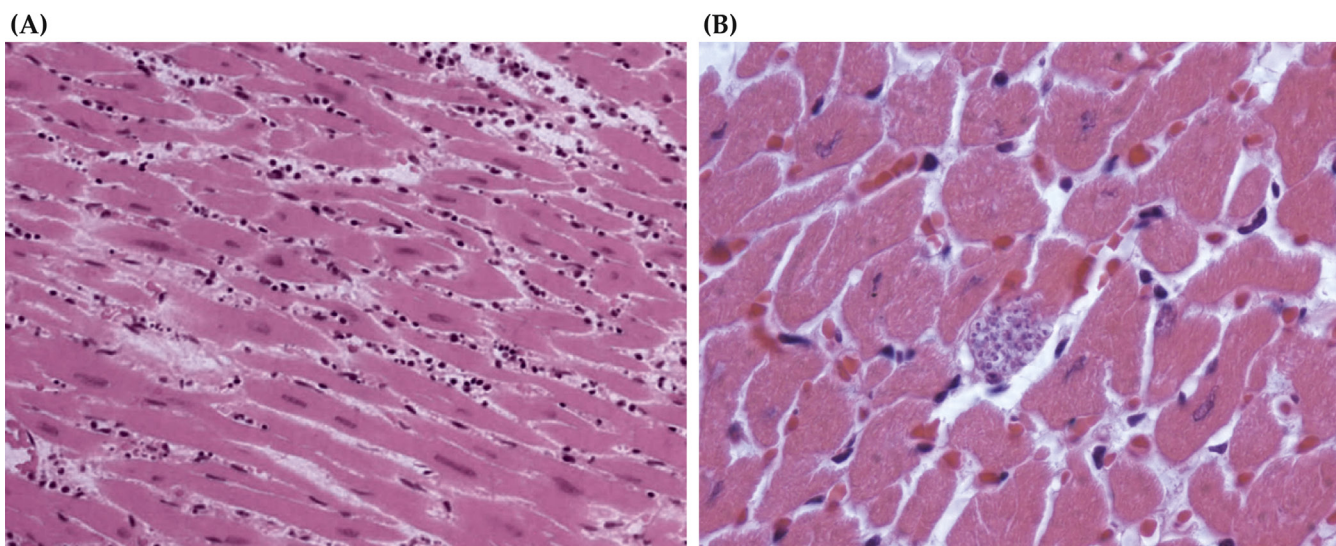


FIGURE 23.1 Inflammatory myocardial damage seen in patients with Chagas' heart disease. (A) HE 200x (B) HE 1000x – nest of amastigotes of *T. cruzi* in the infected group. Adapted from the personal collection of Dr. Fabio Fernandes, University of São Paulo Medical School, São Paulo, SP, Brazil.

mechanism for ChD [85]. It has been established that autoimmunity may be a contributing factor in the pathogenesis of heart disease. There is an antimyosin-specific autoimmune response both in humans with ChD and in animal models. It was observed that *T. cruzi* antigens (eg, B13 protein) are homologous to proteins of the heart (particularly myosin), causing cross-reactivity that sensitizes CD4⁺ T lymphocytes. The role of these autoantibodies in the pathogenesis of Chagas' heart disease requires further investigation [85]. Chagas' heart disease is also characterized by elevated sera levels of anti- β -1 adrenergic receptor autoantibodies (anti- β 1AR-AABs) and anti-M2 muscarinic acetylcholine receptors (anti-M2R-AABs). Anti- β 1AR-AABs are present in about 30% and anti-M2R-AABs in about 40–77% of patients with Chagas' heart disease. The circulating levels of these autoantibodies do not correlate with the grade of ChD and with the severity of contractile dysfunction. Therefore the role of these autoantibodies in the pathogenesis of Chagas' heart disease and the utility of them in therapy needs further research [86,87].

Megaesophagus and megacolon syndromes occur in consequence to inflammation and fibrosis of these viscera, which results in destruction of the autonomic nervous system and subsequent organ dysfunction [24].

Parasympathetic denervation also occurs in the heart, and autonomic dysfunction is detected during all phases of the disease. However, it is not clear how important dysautonomia is to the pathogenesis of the cardiac form of ChD [88].

7. INFECTION IN IMMUNOSUPPRESSED PATIENTS

Since 2004, infection by *T. cruzi* has been classified as an opportunistic infection for AIDS patients [89]. Reactivation of ChD usually occurs in immunosuppressed patients with HIV infection when their CD4 cell count falls to less than 200 cells per μ L [90]. The parasite commonly involves the central nervous system (CNS), causing meningoencephalitis or a mass effect that causes

symptoms similar to those of toxoplasmosis reactivation, which also has a high prevalence in the same endemic areas [91]. Myocarditis can also occur, although less frequently. The manifestations of heart disease are mostly limited to heart failure and arrhythmias [92].

In recent years, transplantation has become available in Chagas endemic areas. As a result, organ transplantation in *T. cruzi*-infected individuals has become more common [93].

In solid organ transplantation, the incidence of reactivation has varied greatly among transplant centers and has been documented to be 8.3–17% [93], depending on the organ and the protocol of the immunosuppression proposed. Sequential monitoring for any clinical evidence of reactivation or parasitemia is indispensable for justifying treatment [93,94].

The development of several chronic chagasic cardiomyopathies can lead to heart transplantation as the treatment of choice, although reactivation has been reported to occur in 26.5–42.9% of patients. Mortality related to ChD reactivation has been reported to be 0.3%; thus the survival rates are no different from those of other heart transplant recipients [95,96].

8. CHAGAS' HEART DISEASE: DIAGNOSIS AND TREATMENT

8.1 Clinical Presentation

According to the *I Latin American Guideline for the Diagnosis and Treatment of Chagas' Heart Disease* [25], the chronic phase can be divided into four clinical forms: indeterminate, cardiac, digestive, and mixed (cardiac and digestive impairment in the same patient). The cardiac form can occur with or without global ventricular dysfunction (usually called the arrhythmogenic form) [Table 23.1](#).

The patient with ChD is classified as having the indeterminate form if the electrocardiogram (ECG), barium swallow, and barium enema are normal in addition to the absence of previous or current symptoms of heart

TABLE 23.1 Clinical Classification of Left Ventricular Dysfunction in Chronic Chagasic Cardiopathy

Stage A	Stage B1	Stage B2	Stage C	Stage D
Indeterminate form with neither current nor previous heart failure symptoms and with normal ECG and chest radiography findings	Patients with electrocardiographic changes (conduction disorders or arrhythmias) and no ventricular dysfunction; the patient can have mild echocardiographic changes (regional contractility abnormalities) but normal global ventricular function	Patients with global ventricular dysfunction with reduced LV ejection fraction (LVEF) and neither current nor previous signs or symptoms of HF	Patients with current or previous HF symptoms and ventricular dysfunction	Patients with HF symptoms at rest that are refractory to maximized clinical treatment (NYHA FC IV) and require specialized and intensive interventions

ECG, Electrocardiogram; HF, heart failure; LVEF, Left ventricular ejection fraction; NYHA FC, New York Heart Association functional class. Adapted from Andrade et al. [25].

failure. Analyzing 160 patients with the indeterminate form based on ECG findings for 8 years it was concluded that the indeterminate form of ChD represents a benign condition with a favorable long-term prognosis [97].

However, the evolution from indeterminate status to clinical chronic Chagas' cardiomyopathy (CCC) or gastrointestinal disease is thought to occur 10–20 years after acute infection in a slow and progressive fashion. Previous studies suggest that up to 5% of patients will evolve each year from the indeterminate form to a clinical form of the disease. So it is important to identify clinical predictors for severe clinical disease that could guide early treatment [97]. A 10-year retrospective cohort study suggested a moderate rate of progression to cardiomyopathy (1.85%/y) in blood donors infected with *T. cruzi* but without cardiomyopathy at baseline [98].

The cardiac form can present as arrhythmogenic, thromboembolic, dilated, mixed, or silent. The extent of myocardial involvement and its functional consequences are the fundamental determinants of the natural history [99].

Thromboembolism can occur in both pulmonary and systemic circulation. Pulmonary embolism is more frequent, appearing in the more advanced phases of heart disease. Some authors have defined Chagas as a disease independently associated with ischemic stroke [100]. The apical region of the left ventricle is a critical region in the chagasic heart where aneurysms, thrombi, or both occur with high frequency. Cardioembolism occurs in 56% of Chagas patients presenting with stroke [101].

Cardiac damage manifests later with the emergence of heart failure. Chronic Chagas' cardiomyopathy is essentially a dilated cardiomyopathy in which chronic inflammation, usually mild and continuous, causes progressive tissue destruction and extensive heart fibrosis. Similar to other forms of heart failure, mortality increases as myocardial function deteriorates. Left ventricular ejection fraction (LVEF) is a valuable index for estimating the outcomes in patients with heart failure and significant associations of LVEF with both functional class and exercise capacity have been demonstrated [102].

The initial evaluation of a patient with Chagas disease consists of the medical history, ECG, and chest radiography. Patients with symptoms, signs, or abnormal ECG findings should undergo a comprehensive cardiac evaluation, including echocardiography, ambulatory 24-h Holter, and exercise testing. In special situations, patients could be submitted to electrophysiological study, cardiac catheterization, nuclear medicine, or cardiac magnetic resonance [52].

8.2 Electrocardiography

Electrocardiography is one of the most important tests in the evaluation of patients with ChD and is used

to define the clinical stage of the disease with potential prognostic implications. The presence of either major or minor ECG abnormalities is therefore a sensitive marker of the presence of LV dysfunction in this disease. Electrocardiographic changes are frequently the first indicators of the cardiac form. Initially, the changes are characterized by transient or fixed atrioventricular conduction delays, right bundle-branch conduction delays, ventricular repolarization changes, and ventricular ectopies [25]. In Chagas' heart disease, a complete right bundle-branch block associated with a left anterior hemiblock (Fig. 23.2) is the most frequent abnormal change (>50% of patients) [52].

In a study of the frequency of ECG abnormalities in *T. cruzi*-seropositive patients, compared to seronegative blood donors, right bundle-branch block and left anterior fascicular block, isolated or in association, were more frequently found in seropositive cases. Several ECG abnormalities were more commonly found in seropositive donors with depressed LVEF, including rhythm disorders (frequent supraventricular ectopic beats, atrial fibrillation, or flutter and pacemaker), intraventricular blocks (right bundle-branch block and left anterior fascicular block), and ischemic abnormalities (possible old myocardial infarction and major or minor ST abnormalities) [102,103]. Seropositive donors with LV dysfunction also showed longer PR, QRS, and corrected QT intervals/durations. Both the QRS and QTc durations were inversely associated with LVEF values, and they showed moderate accuracy in the detection of reduced LVEF. QTc interval dispersion is also associated with some prognostic indicators in patients with heart failure (HF) secondary to Chagas' cardiomyopathy [102].

Dynamic electrocardiography (Holter) is indicated for assessing the chagasic patient with syncope due to ventricular bradyarrhythmia or tachyarrhythmia [103].

Ventricular premature beats are present in 15–55% of individuals with positive serology for ChD, including those with no evidence of structural heart disease. The presence of numerous polymorphic and complex extrasystoles is associated with more severe heart disease [104]. The occurrence of ventricular premature beats with multiple morphologies is a relatively common finding that is attributed to extensive myocardial damage and is correlated with the presence of late potentials, which is observed using signal-averaged ECG [105]. When ChD patients with abnormal ECGs at rest and during heart failure are studied by dynamic electrocardiography, virtually all of them (99%) present with ventricular premature beats (VPBs), and 87% have multiform VPBs or repetitive forms, such as nonsustained ventricular tachycardia (NSVT) [106].

In a retrospective and prospective series and in a systematic review, nonsustained ventricular tachycardia was demonstrated to worsen the predicted prognosis [107].

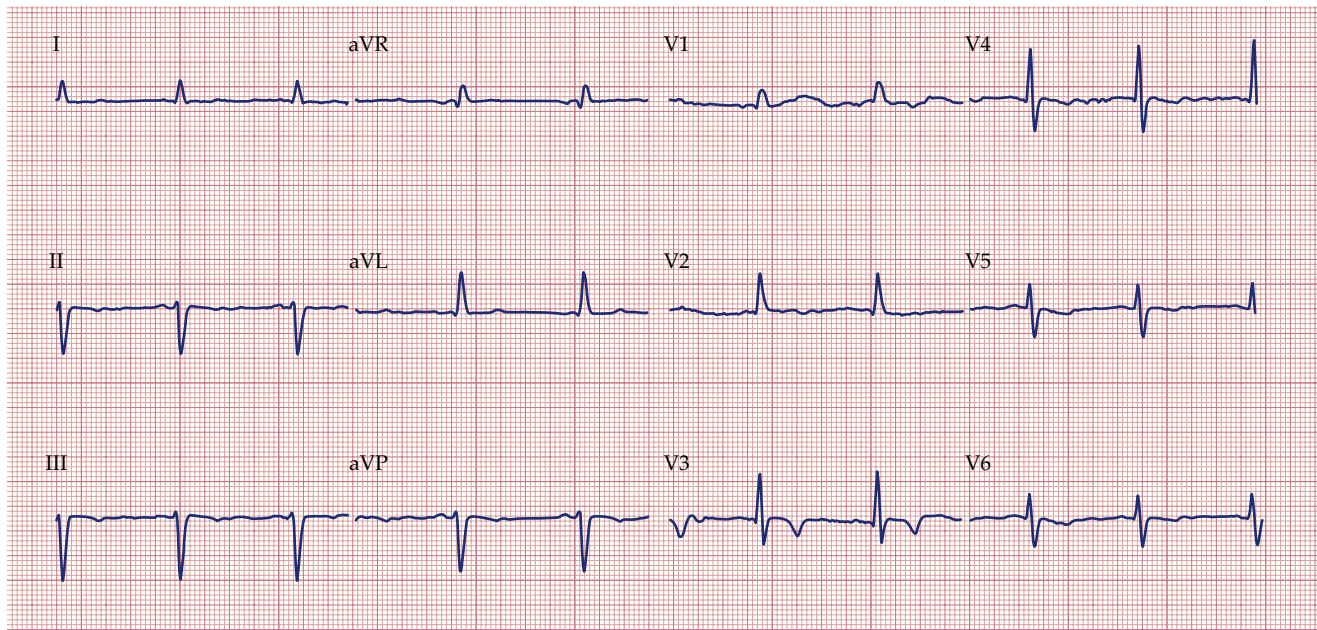


FIGURE 23.2 Right bundle-branch block associated with left anterior hemiblock in a patient with Chagas heart disease. Adapted from the personal collection of João Marcos Barbosa-Ferreira, University of the State of Amazonas, Manaus, AM, Brazil.

Autonomic involvement is a well-established feature of ChD, in which anatomic and functional abnormalities have been extensively described. The majority of studies have observed parasympathetic dysautonomia preceding LV systolic dysfunction and progressive involvement of the sympathetic system in advanced Chagas' heart disease. Various physiological and pharmacological tests show impaired cardiac autonomic regulation in patients with ChD. These tests show autonomic changes in both the parasympathetic branch and the sympathetic branch, with commitment even in the early stages without the presence of ventricular dysfunction [108].

8.3 Electrophysiological Study

Sudden death is a dramatic outcome in patients with CCC because it often occurs in an optimally productive stage of their lives. The mechanism of sudden death is complex and still not completely understood. Sustained ventricular tachycardia degenerating into ventricular fibrillation probably plays an important role in this setting. Chagas' myocarditis can alter the myocardial substrate in a manner that facilitates the emergence of fatal ventricular tachycardia, similar to the long-term consequences of myocardial infarction. Ventricular tachycardia can arise from various regions in both ventricles, but LV inferolateral scarring is the main source of sustained ventricular tachycardia reentrant circuits. Developing methods that could identify patients who are at risk of dying remains a challenge [104,105].

Electrophysiological studies (EPS) are indicated to map ventricular tachycardia and also to investigate sinus functions, atrioventricular conduction, and to clarify syncope of undetermined origin [25].

8.4 Exercise Testing

Exercise testing is rarely utilized in Chagas' heart disease. It could be useful to clarify chest pain in chagasic patients and to detect exertion-induced arrhythmias in CCC [25].

In their seminal study published in 1922, Chagas and Villela described a peculiar absence of chronotropic response to atropine in Chagas cardiopathy patients. Further studies confirmed that chagasic patients might present chronotropic incompetence during dynamic and isometric exercise. Patients with chronotropic incompetence exhibited a significantly impaired exercise capacity, as reflected by a reduced duration of exercise and VO₂ max, compared to Chagas patients with a normal heart rate response [109].

8.5 Echocardiography

In the cardiac form of ChD, echocardiography (ECHO) allows for the assessment of regional and global LV contractility, RV impairment, the presence of apical or submitral aneurysms (Fig. 23.3), intracavitary thrombi, and diastolic function changes [110]. The classical echocardiographic aspect of advanced CCC is a large dilation of the atrial and ventricular cavities with diffuse

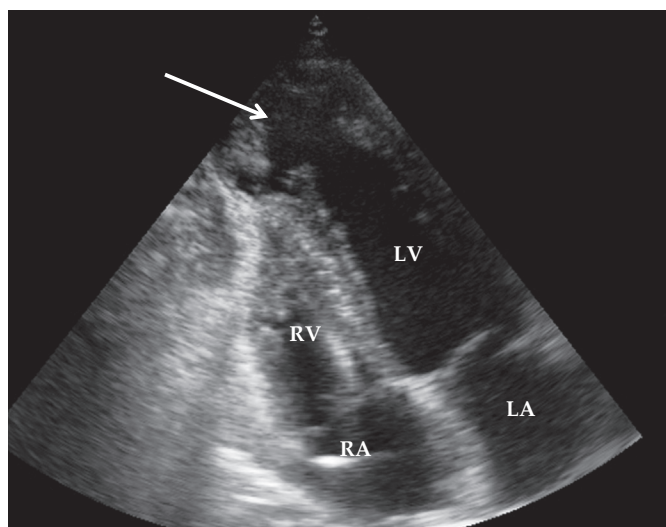


FIGURE 23.3 Echocardiography study demonstrating apical aneurysm of the left ventricle (arrow) in a patient with Chagas heart disease. RA, Right atrium; LA, Left atrium; RV, Right ventricle; LV, Left ventricle. Adapted from the personal collection of João Marcos Barbosa-Ferreira, University of the State of Amazonas, Manaus, AM, Brazil.

biventricular hypokinesia. Additionally, atrioventricular valve regurgitation secondary to dilation of the valvar rings is observed. Despite the predominance of diffuse contractile deficits, ventricular aneurysms detected by ECHO (Fig. 23.3) in patients are characteristic of CCC and are associated with a higher thromboembolic risk (apical position) and with malignant ventricular arrhythmias (basal inferior or posterior lateral wall). Echocardiography can also visualize intramural thrombi in the atria, especially in the presence of atrial fibrillation [101]. Survival analyses have indicated that impaired systolic function and increased ventricular dimensions have significant value in predicting cardiac morbidity and mortality. Echocardiography is also commonly used in the follow-up of patients and in the assessment of various therapeutic modalities [25].

Echocardiographic data in patients with the indeterminate disease form have been numerous but sometimes conflicting, due to a generalized lack of strict criteria used to characterize this latent form of the disease. Many reports have included patients with only minor ECG changes, likely representing mild, but true myocardial damage [111,112]. This fact may explain some earlier reports of either segmental or diffuse impairment of LV contractility [113]. To date, the presence of diastolic dysfunction does not exclude a diagnosis of the indeterminate form of Chagas' disease, which is based on electrocardiographic criteria, as already stated. Indeed, it is conceivable that diastolic dysfunction could represent the initial stage of myocardial damage and could antedate global LV systolic dysfunction [25]. In the indeterminate form, although most echocardiogram tests are normal, we can observe subtle changes in systolic and diastolic function. Delay in

isovolumetric contraction time in the interventricular septa of patients with the indeterminate form on tissue Doppler was demonstrated [110]. Another study evaluated the strain rate in patients with the indeterminate form and observed early changes in radial and longitudinal shortening percentages that were smaller than in control group. Two-dimensional longitudinal strain in the basal inferior and inferoseptal walls, as well as the apical segment of the inferolateral wall, was less in patients than in controls. Two-dimensional radial strain was reduced in several segments of the LV walls, as well as in the global radial strain. Two-dimensional circumferential strain at the basal segment of the anterior wall showed a lower value in patients than in controls, whereas global circumferential strain was similar between patients and controls [107].

8.6 Nuclear Medicine

Myocardial perfusion scanning can be used to assess biventricular function, abnormal segmental motion, and autonomic function, mainly of the sympathetic branch. This method can also reveal both transient and irreversible perfusion defects in patients with Chagas cardiomyopathy, and coronary catheterization might be needed to exclude obstructive coronary artery disease [25].

In individuals with the indeterminate form of ChD, who have abnormal segmental motion determined by tissue Doppler imaging (TDI)-derived strain, cases with the presence of perfusion defects (8%) were demonstrated using myocardial perfusion scanning, as well as poststress LVEF reduction (28%). They also observed the presence of some degree of intraventricular dyssynchrony at rest, which was normalized poststress [114].

By myocardial perfusion scintigraphy, a high prevalence of reversible perfusion defects in patients with Chagas cardiomyopathy in the presence of angiographically normal subepicardial coronary arteries was demonstrated, suggesting the presence of abnormalities in the regulation of myocardial blood flow at the coronary microvascular level [115].

Patients in various phases of Chagas' heart disease using iodine-123 (I-123) *meta*-iodobenzylguanidine (MIBG) and thallium-201 myocardium segmental uptake in correlation with the severity of LV dysfunction were studied. Group I consisted of 12 subjects (43 ± 4 -years-old, 7 men) with no symptoms and no cardiac involvement on electrocardiography (ECG) or echocardiography; group II consisted of 13 patients (48 ± 3 -years-old, 9 men) with abnormal resting ECG and/or echocardiographic segmental abnormalities and an LV ejection fraction of $> \text{or} = 0.5$; group III consisted of 12 patients (59 ± 3 -years-old, 10 men) with more severe heart disease, LV dilation, and an LV ejection fraction of < 0.5 . I-123 MIBG single-photon emission computed tomographic (SPECT) segmental uptake defects were

observed in group I (33%), group II (77%), and group III (92%). Quantitative analysis showed the mean areas of reduced LV I-123-MIBG uptake: in group I it was $3.7 \pm 2.1\%$; in group II it was $8.3 \pm 2.3\%$; and in group III it was $19.0 \pm 3.3\%$ ($p < .05$ for differences between groups II and III and $p < .01$ for difference between groups I and III). A marked topographic association between perfusion, innervation, and wall-motion abnormalities (assessed by gated-SPECT perfusion studies) was observed in all groups. Defects predominated in the inferior, posterior lateral, and apical LV regions. Thus extensive impairment of cardiac sympathetic function at the ventricular level occurred early in the course of Chagas' cardiomyopathy and was related to regional myocardial perfusion disturbances, before wall-motion abnormalities. Both conditions were associated with the progression of ventricular dysfunction [116].

In a prospective study, CCC patients were analysed ($n = 36$, age 57-years-old, 17 men) who had previously undergone myocardial perfusion scintigraphy and two-dimensional echocardiography and who had undergone a new evaluation after an interval of 5.6 ± 1.5 years. The aim of this study was to analyze the association between myocardial perfusion changes and the progression of left ventricular systolic dysfunction. Between the first and final evaluations, a significant reduction of LVEF was observed

($55 \pm 11\%$ and $50 \pm 13\%$), as well as an increase in the area of the perfusion defect at rest ($18.8 \pm 14.1\%$ and $26.5 \pm 19.1\%$), respectively. Twenty patients with normal coronary arteries (56%) showed reversible perfusion defects involving $10.2 \pm 9.7\%$ of the left ventricle. A significant topographic correlation was found between reversible defects and the appearance of new rest perfusion defects at the final evaluation. The authors concluded that the progression of LV systolic dysfunction was associated with both the presence of reversible perfusion defects and an increase in perfusion defects at rest. These results supported the notion that myocardial perfusion disturbances participate in the pathogenesis of myocardial injury in Chagas cardiomyopathy [117].

8.7 Magnetic Resonance

Cardiovascular magnetic resonance imaging (MRI) accurately identifies myocardial fibrosis in patients with ChD. Late myocardial enhancement is the best noninvasive method for assessing fibrosis or myocardial necrosis. Magnetic resonance imaging has also been shown to be effective for assessing myocardial edema, a marker of inflammation, and it is highly sensitive for the detection of thrombi, especially in the left ventricle, and in other pathologies such as myocarditis and infarct [118].

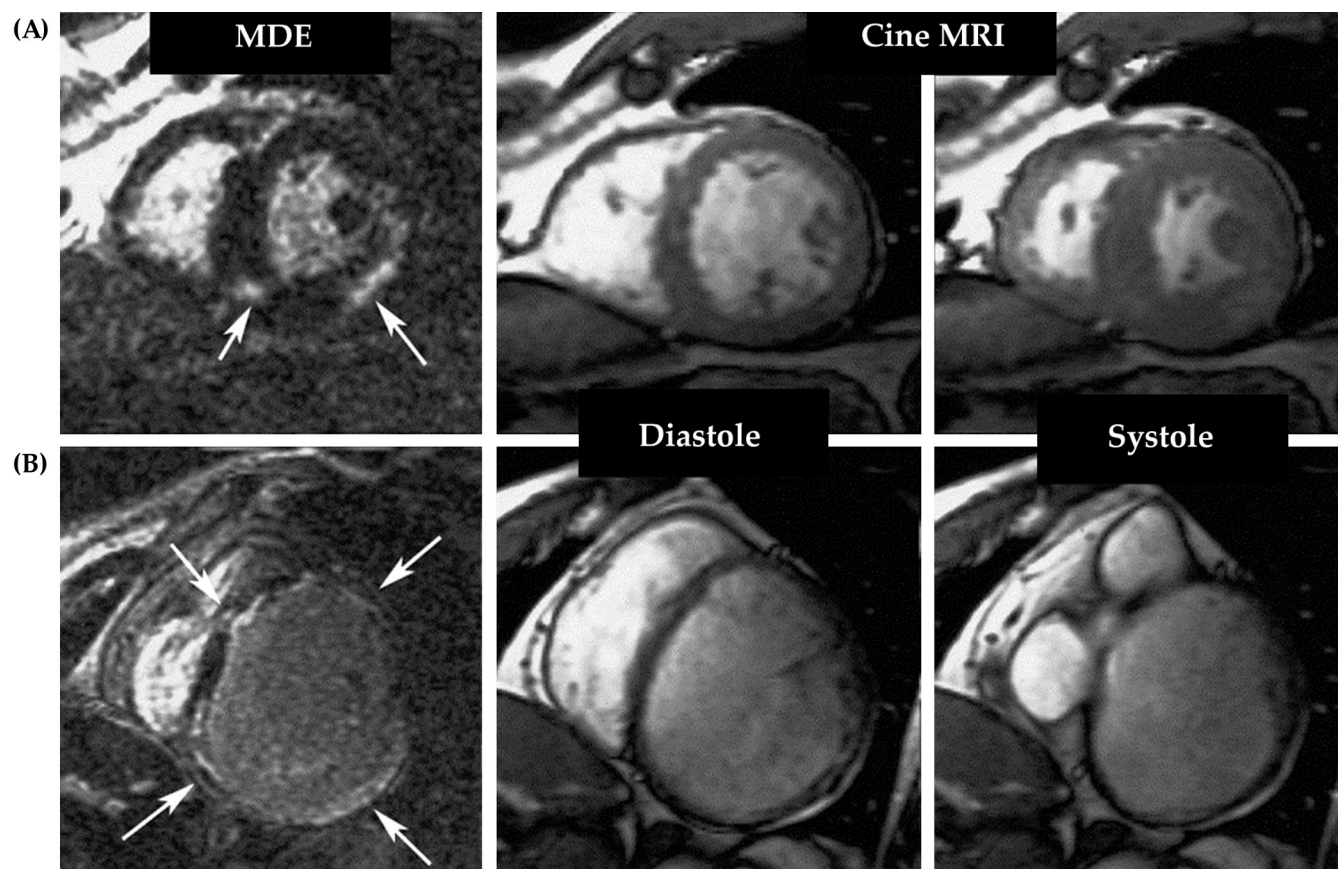


FIGURE 23.4 Extent of myocardial fibrosis (MF) (arrows) and left ventricular function. (A) Patient with small area of MF (8.2%) and normal left ventricular ejection fraction (65.5%). (B) Patient with large area of MF (23.8%) and severe left ventricular dysfunction. Adapted from Rochitte et al. [118].

The degree of myocardial fibrosis indetermined form to the most severe disease stages (LV dysfunction) (Fig. 23.4). Myocardial fibrosis on MRI was present in 68.6% of all of the patients, in 20% of those with an indeterminate form, in 84.6% of patients with Chagas' heart disease, and in 100% of patients with Chagas disease and ventricular tachycardia. Quantified myocardial fibrosis increased progressively across disease severity subgroups ($0.9 \pm 2.3\%$ in the indeterminate form; $16.0 \pm 12.3\%$ in Chagas' heart disease; and $25.4 \pm 9.8\%$ in ventricular tachycardia) and New York Heart Association functional classes (I: $7.5 \pm 9.5\%$; II: $21.9 \pm 13.8\%$; and III: $25.3 \pm 9.9\%$ of LV mass). Left ventricular ejection fraction and myocardial fibrosis had significant negative correlation, similar to segmental myocardial fibrosis and systolic function (Fig. 23.5). Additionally, myocardial fibrosis was inversely correlated with LVEF and clinical status, in agreement with a previous biopsy study that correlated interstitial collagen deposition to LV dysfunction. Moreover, MRI provides evidence of myocardial involvement in seropositive patients without clinical symptoms or wall-motion abnormalities, further supporting the use of MRI-defined myocardial fibrosis as a subclinical marker of disease severity. Segmental myocardial fibrosis analysis indicates the LV apex and inferolateral regions as preferable sites for myocardial fibrosis, in accordance with previous pathological studies [118].

Magnetic resonance imaging is also capable of showing wall thinning and wall-motion abnormalities, enabling easy visualization of the typical narrow-neck aneurysm at the apex of the LV and of aneurysms in other locations as well. The apical aneurysm is a common place for the

formation of thrombi, although they can occur at other sites. Magnetic resonance imaging clearly depicts the thrombi in both ventricles [119].

8.8 Cardiac Catheterization

Cardiac catheterization may be indicated to exclude the presence of obstructive coronary disease in patients with symptoms of chest pain or risk factors for coronary artery disease [25].

8.9 Biochemical Markers

In patients with ChD, the peptides atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and N-terminal pro-BNP have been described as markers with good accuracy for detecting dysfunction [120]. Previous studies have shown that even in asymptomatic patients, ANP and BNP are elevated in ChD, showing high predictive value for mortality and the need for heart transplantation. Therefore these markers are good for identifying patients at higher risk who require more intensive and earlier treatment [121]. In a 10-year follow-up study of 1398 patients (37.5% of whom had ChD), it was demonstrated that BNP is a strong predictor of mortality in patients with ChD. Infected persons with baseline BNP levels in the top quartile had a risk of death twice that of persons in the bottom quartile (hazard ratio 2.07, 95% CI 1.29, 3.32). The discriminatory ability of BNP in predicting mortality was similar to that of an electrocardiogram [122]. A previous study also observed that BNP measurement was more accurate than conventional methods (ECG and chest radiography) for screening patients with ventricular dysfunction [123]. Our group detected that NT pro-BNP was increased in different cardiomyopathies, including ChD, showing a positive correlation with the degree of dysfunction. In another study, we found that patients with the indeterminate form had a mean level of NT pro-BNP of 53.2 pg/mL, patients with abnormal electrocardiograms had a mean level of 83.3 pg/mL, and those with myocardial dysfunction had a mean of 831 pg/mL ($p < .001$, compared with the control group). There was no statistically significant difference between patients with the indeterminate form and abnormal electrocardiograms relative to control group with a mean of 32 pg/mL [124].

Additionally, Troponin levels have been also used as surrogate for myocardial involvement, more so in the acute phase of the infection in animal studies [125].

Recent research has led to a growing appreciation of the complexity of the metabolic aspects of heart failure pathophysiology. Metabolic failure, resulting in a global imbalance between catabolic and anabolic signals affects, not only the myocardium, but also peripheral tissues and organs. Metabolic feedback signals from

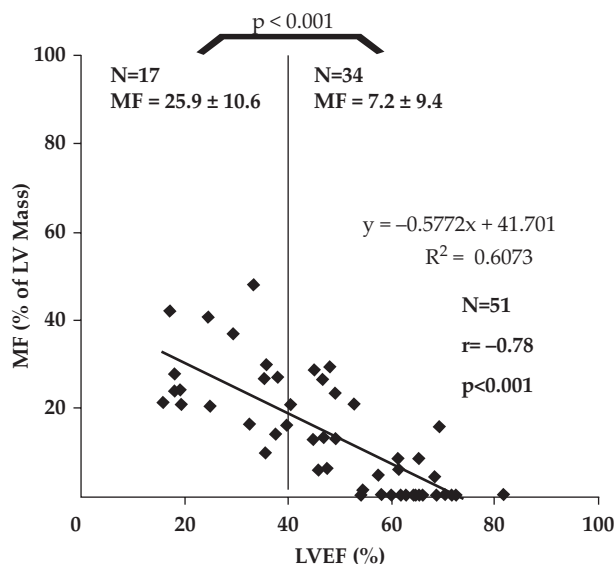


FIGURE 23.5 Good correlation between left ventricular ejection fraction (LVEF) and myocardial fibrosis (MF). Patients with LVEF >40% had less quantified myocardial fibrosis than those with LVEF ≤40% (Student *t* test). Adapted from Rochitte et al. [118].

muscle and fat actively contribute to disease progression. The adipocytokines are bioactive mediators produced by adipose tissue. The main adipocytokines are adiponectin and leptin. Increased adiponectin levels in patients with Chagas' heart disease were found. Furthermore, a relationship was observed between adipocytokine levels and autonomic nervous system (ANS) function in Chagas' heart disease. The levels of adiponectin were associated with reduced sympathetic activity and increased parasympathetic activity, and the levels of leptin were associated with increased sympathetic activity and reduced parasympathetic activity in subjects with Chagas' disease and cardiac involvement [106]. Published results found reduced leptin levels in patients with HF, compared with a control group and with patients with other forms of ChD [124].

Inflammatory activity plays a key role as a pathophysiological mechanism for CCC. It is considered as the main mechanism responsible for myocardial injury and ventricular dysfunction. The lack of correlation between parasitism in cardiac tissue and intensity of inflammatory activity suggests that the autoimmunity process is very important in this immune response [126]. Activated lymphocytes initiate a reaction of delayed hypersensitivity type in the cardiac tissue through the production of inflammatory cytokines [127,128]. Studies in heart tissue of patients with CCC have identified the presence of inflammatory cytokines such as interferon, tumor-necrosis factor α (TNF- α), interleukin-2, interleukin-4, and interleukin-6 (IL-6) [129]. In peripheral blood it was also demonstrated that the chronic infection induces a shift of the immune response to a proinflammatory profile with the production of Th1 cytokines. In addition, there is suppression of cytokines with antiinflammatory TH2-type, such as interleukins 4 and 10 [130–132]. Elevated serum levels of inflammatory cytokines such as interleukin-6 and TNF- α were demonstrated in the group of CCC patients compared to patients with idiopathic-dilated cardiomyopathy. These higher levels of IL-6 were associated with worse outcomes. These findings suggested that specific inflammatory pathways are particularly active (locally or systemically) in patients with Chagas' cardiomyopathy and may be responsible for differences in the clinical course of the disease compared to other etiologies [119]. Chemokines and their receptors also play an important role in the immune response. Previous studies have shown increased levels of CCL2 in patients with CCC and severe systolic dysfunction and increased CCR5 expression in CCC with mild dysfunction [133].

8.10 Clinical Parameters and Prognosis

Patients with Chagas' cardiomyopathy have the worst prognosis compared to all other cardiomyopathies. The presence of serious ventricular arrhythmias, conduction disturbances on ECG, and heart failure provide an

unfavorable prognosis. Ejection fraction (EF), functional class, and maximal oxygen consumption (VO₂ max) were effective predictors of survival in chagasic patients in previous studies. It is noteworthy that EF and VO₂ max were strong predictors of survival in cardiomyopathy due to ChD [119].

The association between the impairment of VO₂ max and poor long-term prognosis has been debated. Strikingly limited short-term survival has been demonstrated in patients with marked exercise limitations [119]. The prognostic significance of VO₂ max remained strong even when ChD patients with only mild LV dysfunctions were included. A previous study showed that the functional capacity of patients in the initial phase of chronic ChD was higher than that in patients in advanced phases and that this functional capacity decreased following myocardial functional impairment [119].

A simple risk score to predict death in CCC was validated in an independent cohort when six independent prognostic factors were identified and each was assigned a number of points proportional to its regression coefficient: New York Heart Association class III or IV (5 points), evidence of cardiomegaly on radiography (5 points), LV systolic dysfunction on echocardiography (3 points), non-sustained ventricular tachycardia on 24-h Holter monitoring (3 points), low QRS voltage on electrocardiography (2 points), and male sex (2 points; Fig. 23.5). The authors calculated risk scores for each patient and defined three risk groups: low risk (0–6 points), intermediate risk (7–11 points), and high risk (12–20 points). In the development cohort, the 10-year mortality rates for these three groups were 10%, 44%, and 84%, respectively. In the validation cohort, the corresponding mortality rates were 9%, 37%, and 85%, respectively (Fig. 23.6) [134].

An algorithm was also proposed to guide mortality risk assessment and therapeutic decision-making in patients with ChD. For patients with an abnormal ECG, the evaluation of functional capacity by NYHA classification would provide the first line of risk assessment. Left ventricular function indices, obtained ideally by two techniques, chest radiography and ECHO, would represent the second step. The third method of evaluation would be Holter monitoring to analyze for the presence of NSVT [134] (Fig. 23.7).

9. TREATMENT OF CHAGAS CARDIOMYOPATHY

Management consists of treating the different clinical manifestations of the disease, such as ventricular dysfunction and heart failure (HF), thromboembolic phenomena, and rhythm disorders [25].

Although CCC is an important cause of HF in Latin America and is a unique clinical entity with unique

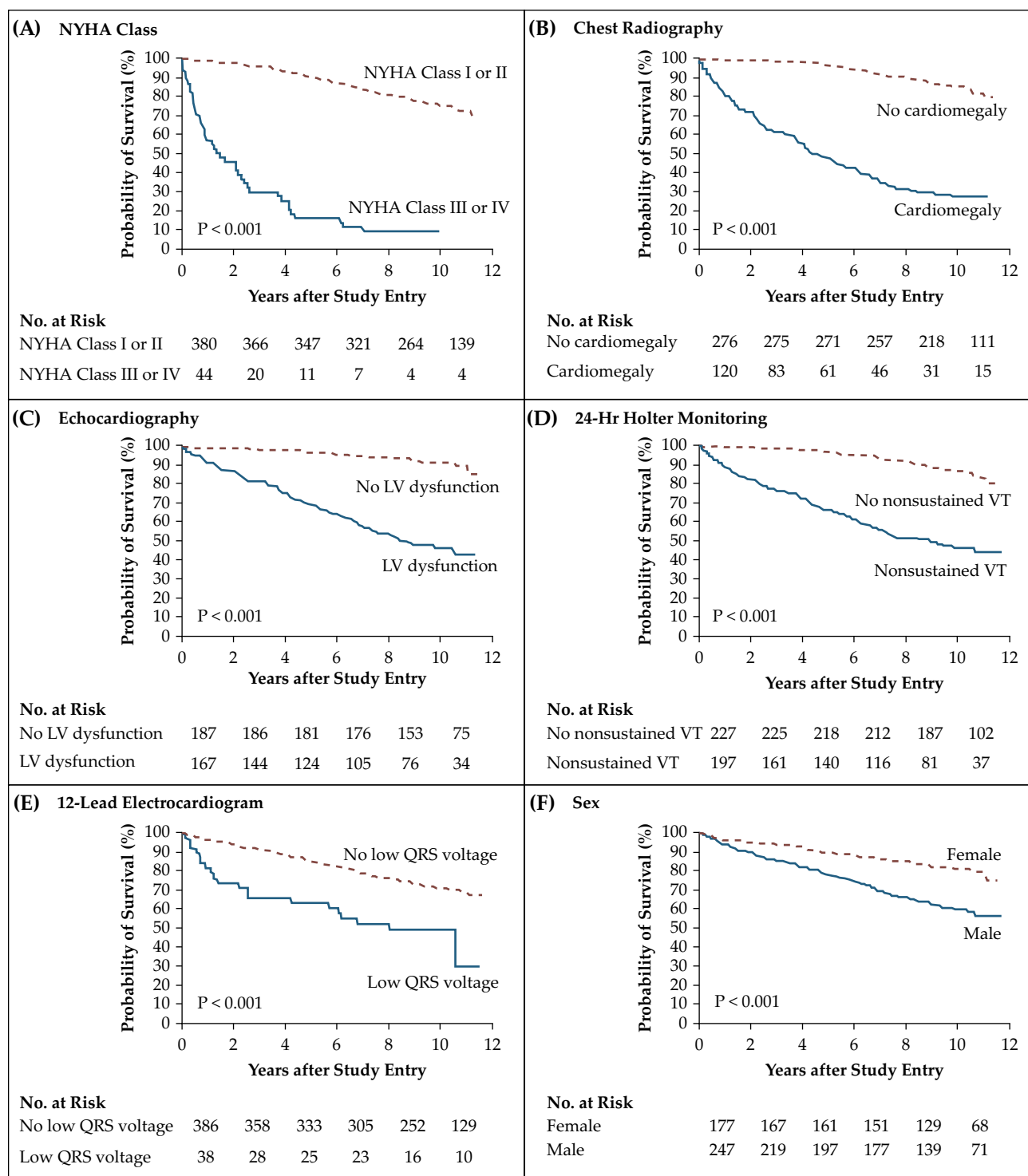


FIGURE 23.6 Kaplan-Meier survival curves for six variables that were significantly associated with outcome in multivariate analysis. The dichotomized variables were NYHA class III or IV (vs class I or II) (Panel A), presence (vs absence) of cardiomegaly on chest radiography (Panel B), presence (vs absence) of segmental or global wall-motion abnormality on echocardiography (Panel C), presence (vs absence) of nonsustained ventricular tachycardia on 24-h Holter monitoring (Panel D), presence (vs absence) of low QRS voltage on electrocardiography (Panel E), and male (vs female) sex (Panel F). LV denotes left ventricular and VT ventricular tachycardia. Adapted from Rassi et al. [134].

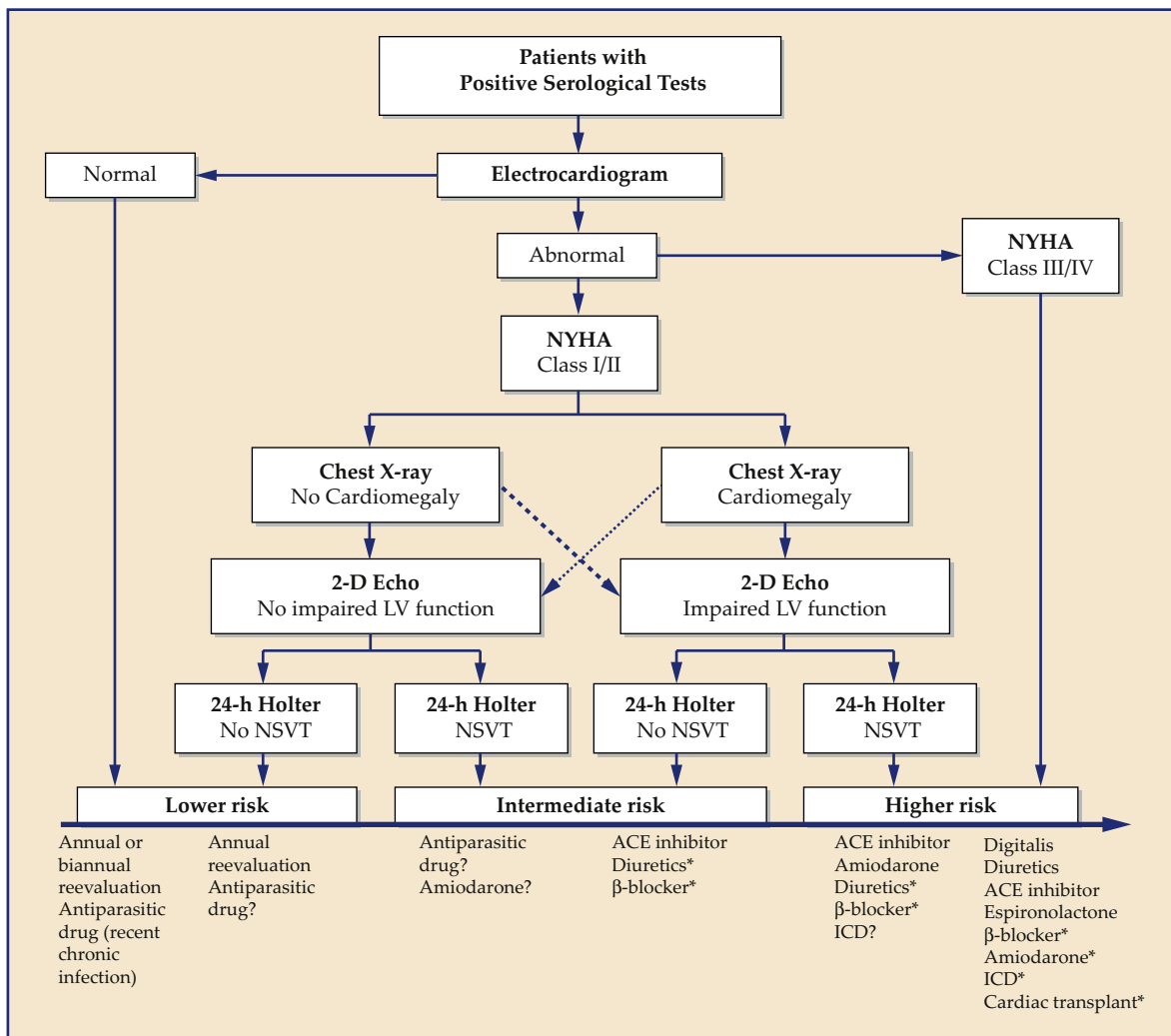


FIGURE 23.7 Proposed algorithm to guide mortality risk assessment and therapeutic decision-making in patients with Chagas disease. Adapted from Rassi et al. [176].

therapeutic characteristics, these patients have not been included in large-scale studies assessing drugs to treat HF. Thus the real efficacy and tolerability of these drugs in these patients have not been scientifically established and their use has been extrapolated empirically from the results obtained for HF due to other etiologies. Despite the few specific studies, treatment of CCC is similar to that of other etiologies. Therefore the treatment of chagasic HF is based on the routine combination of three types of drugs: diuretics, angiotensin-converting-enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), and adrenergic beta-blockers (BBs). Digitalis, antiarrhythmic drugs, and oral anticoagulation drugs can be used in special situations [107]. In a review of treatments for Chagas' heart disease, it was observed that the dosages of some medications used for routine heart failure treatment were similar between chagasic patients and those with other etiologies of HF [135]. The

recommendations and levels of evidence for the treatment of heart failure in chronic chagasic cardiopathy are summarized in Table 23.2.

9.1 Angiotensin-Converting-Enzyme Inhibitors/Angiotensin Receptor Blockers

The effects of captopril treatment on the outcomes of *T. cruzi* infection in mice were investigated and it was concluded that captopril administration significantly decreased cardiac necrosis and fibrosis without affecting mortality or host-parasite burden [136]. Enalapril has also showed some decreased cytokine profile, heart fibrosis, and in vitro activity against *T. cruzi* in an acute mice model [137].

A clinical study including 17% of patients with CCC and assessing the action of captopril in 115 patients with HF showed benefits with improvement in functional

TABLE 23.2 Recommendations and Levels of Evidence for the Treatment of Heart Failure in Chronic Chagasic Cardiopathy

Therapies	Indications	Level of evidence	Strength of recommendation
Angiotensin-converting-enzyme inhibitors/ angiotensin receptor blockers	ACEI or ARB (if intolerant to the former) to patients with LV systolic dysfunction, LVEF <45%, and FC I/II/III/IV HF	C	I
Spironolactone	Patients with LV systolic dysfunction, LVEF <35%, and FC III/IV HF	B	I
Diuretics	Patients with congestive signs and symptoms (FC II-IV HF)	C	I
Beta-blockers	Patients with asymptomatic LV systolic dysfunction (FC I HF) or hypovolemic	B	IIa
Amiodarone	Patients with ventricular ectopies, symptomatic NSVT, and LV dysfunction	B	I
Amiodarone	Patients with symptomatic or asymptomatic SVT, with or without LV dysfunction, not treated with ICD	C	I
Cardiac transplantation	Refractory HF, depending on inotropic drugs and/or circulatory support and/or mechanical ventilation VO ₂ peak ≤10 mL/kg/min Ventricular fibrillation or refractory sustained ventricular tachycardia Persistent FC III/IV HF with optimal therapy	C	I

ACEI, angiotensin-converting-enzyme inhibitors; ARB, angiotensin receptor blockers; FC, functional class; HF, heart failure; ICD, implantable cardioverter defibrillator; LV, left ventricular; LVEF, left ventricular ejection fraction; NSVT, nonsustained ventricular tachycardia; SVT, sustained ventricular tachycardia.

Adapted from Andrade et al. [25].

class, but it did not separate the effects according to the etiologies [138]. The use of ACEIs in CCC is recommended for all patients with ventricular dysfunction, from NYHA functional class (FC) I to IV. When ACEIs are not tolerated, the use of ARBs is recommended [25].

9.2 Beta-Blockers

Although scarce, there is direct evidence that the use of BBs is beneficial for the treatment of HF and specifically of HF of chagasic etiology. Extrapolating the recommendations regarding the treatment of patients with HF of other etiologies, carvedilol, bisoprolol, or metoprolol succinate should be used to treat chagasic patients with previous or current HF symptoms and/or signs and with an LVEF ≤45%. The daily dose should be slowly titrated, aiming to avoid a heart rate <50/min at rest. These drugs can also be indicated in the absence of HF symptoms and signs when there is LV dysfunction or remodeling. However, these drugs are contraindicated in patients with bradycardia ≤50 bpm or with AV conduction disorders (PR > 280 ms) [25,139].

9.3 Amiodarone

Two randomized trials have supported the use of amiodarone in patients with NSVT and Chagas disease: Grupo de Estudio de la Sobrevida en la Insuficiencia Cardíaca en Argentina (GESICA) (*n*=516, 9.5% with Chagas disease)

and Argentine Pilot Study of Sudden Death and Amiodarone (EPAMSA) (*n*=127, 22% with Chagas disease). Amiodarone was compared with a control group (no amiodarone) in GESICA and with placebo in EPAMSA. It significantly reduced overall mortality after mean follow-up of 12 and 13 months, respectively. Notably, in the two trials, 80–90% of patients had complex ventricular arrhythmias (couplets or NSVT episodes or both). In GESICA, amiodarone had a greater benefit in patients with NSVT at baseline (mortality reduced from 57% to 44%) than in those without NSVT (mortality reduced from 34% to 28%) [140,141].

Although the antiarrhythmic effects of amiodarone are well known, there has been some evidence that it also has anti-*T. cruzi* activity. However, when evaluated whether patients using amiodarone had lower parasite loads than matched infected subjects when mean daily dose of amiodarone was 205 ± 54 mg, the authors observed that the qualitative analysis of PCR showed positive results in 86% of patients on amiodarone treatment and in 58% of control patients. After matching, PCR was positive in 69% of paired control subjects (*p* = .13), thus no difference between the groups was demonstrated [142].

9.4 Diuretics

Similar to other HF etiologies, diuretics such as furosemide should be used in chagasic patients to relieve congestive symptoms and signs. Regarding aldosterone blockade, the use of spironolactone has demonstrated

some benefit to survival and myocardial fibrosis reduction in *T. cruzi*-infected hamsters [123].

The largest randomized, double-blind, and placebo-controlled study assessing spironolactone in the chronic phase of HF included etiologies other than ischemia, such as ChD (including some patients with CCC). Thus spironolactone is indicated in patients with LV systolic dysfunction, LVEF $\leq 35\%$, and NYHA FC III/IV HF [123].

9.5 Implantable Electronic Cardiac Devices

ChD can cause conduction disturbances with significant bradyarrhythmias, such as advanced atrioventricular blocks with cardiac pacemaker indications. The scientific evidence regarding indications for an implantable cardioverter defibrillator (ICD) in CCC has been limited to publications of case series, retrospective cohorts, or registries involving only secondary prevention of cardiac sudden death. There is no scientific evidence supporting the indication for ICD in the primary prevention of cardiac sudden death; thus there is currently no recommendation for it. With regard to cardiac resynchronization therapy (CRT), it is worth noting that complete left bundle-branch block, the major electrocardiographic indication for that procedure, is rare in CCC. Despite the lack of consistent scientific evidence, the criteria for CRT indication in CCC were extrapolated from those used for ischemia and idiopathic dilated cardiomyopathy [25].

9.6 Cellular Transplantation

A group demonstrated, in a mouse model of chagasic cardiomyopathy, that bone-marrow cells injected intravenously migrated to the heart and induced significant reductions in inflammatory infiltrates and interstitial fibrosis [143]. Another group evaluated the coculture of skeletal muscle and mesenchymal stem cells for cell therapy of heart failure in ChD in rats. Histopathological analysis of the animals receiving cocultured cells demonstrated the presence of myogenesis and angiogenesis. The authors also observed significant remodeling and improvement of LVEF; these data could reflect the antiinflammatory effects of bone-marrow cells. Importantly, the variability in cell numbers in the ChD group was due to individual variability in each animal (autogenous transplant) [144]. In other study, it was concluded that the cotransplantation of stem cells and skeletal myoblasts was functionally effective in ChD ventricular dysfunction [145]. The early effects of bone-marrow cell transplantation to the myocardium of 28 patients with heart failure due to ChD were evaluated in a procedure consisting of the aspiration of 50 mL of bone marrow, separation of the mononuclear fraction, and intracoronary injection. Evaluation of ventricular function, based on the LVEF and functional

capacity evaluated in 6-min tests, showed significant improvement, which was maintained for 2 months [37]. Quality-of-life assessment by the Minnesota Living with Heart Failure questionnaire revealed significant improvement in the global score after 1 month; this improvement was maintained throughout the follow-up period. The authors concluded that intracoronary injection of bone-marrow mononuclear cells (BMNCs) was feasible, and they suggested that it may potentially be safe and effective in patients with heart failure due to ChD [146].

However, another study with multicenter, double-blind, placebo-controlled randomized trial to assess the efficacy of BMNC therapy observed that this therapy did not improve LV function or quality of life in patients with CCC. Therefore this therapy should be further studied for the group of patients with Chagas disease including new cell types, injection routes, and time windows for the start of therapy [147].

9.7 Cardiac Transplantation

Cardiac transplantation in ChD is controversial because it is an infectious disease that seems to be difficult to confirm the eradication of the etiologic agent and can be reactivated by immunosuppression in up to 20% of patients. ChD reactivation is diagnosed when parasites are detected in tissues (usually the myocardium) or when parasites are detected in the blood in association with symptoms or signs attributable to acute infection by *T. cruzi*. Parasites were detected in endomyocardial biopsies of a postheart-transplant chagasic patient by conventional histological examination, immunohistochemistry, and PCR, thereby allowing for the diagnosis of reactivation. Immunosuppressive and therapeutic plans to control disease reactivation have been intensively discussed and have motivated the use of various protocols. Therefore patients with ChD require additional care, compared to other cardiac transplant patients [148]. To compare ChD reactivation rate in patients under two different immunosuppression protocols a study was developed with chagasic patients divided into two groups: one taking azathioprine and the other group taking mycophenolate mofetil in equivalent doses, in addition to prednisone and cyclosporine. They concluded that mycophenolate mofetil showed high reactivation rates and suggested that lowering mycophenolate mofetil doses or even substituting it for azathioprine in this special group of transplanted patients could represent a better current regimen for transplanted patients. Immunosuppression adaptation in these patients could result in lower infection, rejection, and chagasic reactivation rates and could lead to a very good survival rate after transplantation [149]. Despite this specific care, the survival of patients who undergo heart transplantation for end-stage

Chagas' cardiomyopathy is equal to or longer than that of patients who undergo transplantation for idiopathic or ischemic cardiomyopathy, and in prospectively monitored patients who have undergone transplantation, *T. cruzi* reactivation is a rare cause of death [95].

9.8 Immunomodulation

Ultimately, given the recent literature overwhelmingly noting the importance of parasite persistence in promoting progression of ChD and cardiomyopathy, a therapeutic vaccine offers great promise for complementing or possibly replacing current drug therapy, in a context of its low efficacy in the majority of ChD forms and intolerance due to unacceptable side effects [150]. Although there is now a considerable body of evidence and broad consensus that parasite persistence is requisite for pathogenesis and that antiparasitic immunity can be protective against *T. cruzi* pathogenesis without eliciting autoimmune pathology. Nevertheless, a rigorous monitor for any evidence of "triggering" this autoimmunity process by the vaccine must be evaluated by biomarkers for disease progression to allow for extensive vaccine evaluation both in animal models as in humans [150,151].

A vaccine would be a therapeutic vaccine to prevent or delay the onset of CCC in patients with indeterminate ChD or in patients with early-stage evidence of clinical Chagas cardiomyopathy, with initial clinical manifestations, ECG, or echocardiographic alterations [152].

9.9 Antitrypanosomal Therapy

Two drugs were introduced for treatment during the 1960s and 1970s and remained the only therapeutic options: nifurtimox (Nfx; 8–10 mg/kg daily) administered every 8 h for 90 days and benznidazole (BZN; 5 mg/kg daily) administered two to three times daily, with a maximum dose of 300 mg for 60–90 days [25,52,153].

Specific antitrypanosomal treatment is indicated during acute infection, in children younger than 18-years-old, with congenital infections, in patients with immunosuppression conditions that lead to reactivation of the disease, and in individuals exposed to the parasite by laboratory accident [154].

There has been debate regarding the use and effectiveness of Nfx and BZN during the chronic phase of the disease, but currently, most experts believe that drug therapy should be offered to the majority of the Chagas patients [27,155,156]. The treatment can lead to benefits such as reduction in parasite load and avoidance of the formation of new inflammatory foci and the extension of tissue damage [157]. Observational clinical studies have shown that chronic patients subjected to antiparasitic treatment with BZN exhibited significant reduction in the occurrence of

electrocardiographic changes and a lower frequency of deterioration of their clinical condition [70,158].

To date, only a few clinical trials have been performed to investigate the specific therapeutic value of these drugs on ChD by analyzing parasite- and patient-related outcomes. Studies regarding patient outcomes remain lacking, and there is no reasonable amount of evidence to support the treatment of the chronic indeterminate form [70,159–161].

Some ongoing trials have been addressing the paucity of options for the treatment of the infection, as well as the controversy regarding the evidence allowing for the treatment of subjects with the chronic indeterminate form.

A multicenter trial (the BENEFIT [Benznidazole Evaluation for Interrupting Trypanosomiasis] study) to assess the effects of etiological therapy on cardiac outcomes in patients with CCC was developed. The study showed, that among patients with established cardiomyopathy and, despite BZN treatment significantly reducing detection of circulating parasites, it did not reduce cardiac clinical progression in patients affected by this clinical form [73].

Two trials comparing azolics and BZN have been concluded. In CHAGASAZOL trial, it was evaluated efficacy, safety, and side-effect profile of posaconazole compared with BZN in patients with chronic ChD. A significantly larger percentage of treatment failures for azole than for BZN was reported [153]. Other azolics have been under investigation (such as the prodrug of ravuconazole [E1224]) to assess their efficacy compared to placebo and BZN (NCT01489228). The results obtained from these trials indicated that the azolics are not as efficacious as monotherapy for the treatment of ChD in the indeterminate form. Benznidazole has been shown to be an efficacious drug to maintain sustained clearance of the parasite even after 1 year [162].

The problem with the current drugs is that they are very toxic. Approximately 1 in 5 individuals abandon treatment and 1 in 40 treated individuals has a severe reaction requiring hospitalization, additional treatments, or interruption of the treatment [163].

The main adverse drug reactions (ADRs) due to the use of Nfx are anorexia, weight loss, abdominal pain, nausea, emesis, vertigo, mood changes, myalgia, and polyneuropathy. For BZN, the most frequent ADRs are rash and pruritus, myalgia, nausea, dyspepsia, dysgeusia, and peripheral neuropathy. Only up to 7.4% of all of these symptoms could be deemed severe [164]. The rate of drug interruption due to side effects was about 24% for the BZN in the most recent trial [73].

Dermatological ADRs can occur in up to 30% of patients using BZN beginning 8–10 days after the initiation of treatment. The dose does not need to be modified or interrupted in most patients and the symptoms

should be managed with topical antihistamines or a short course of systemic corticosteroids. Other cutaneous reactions can occur, such as nonbullous polymorphous erythematous rashes, often followed by desquamation [165].

Neuropathy might be present during the use of both drugs; this symptom often occurs at the end of the treatment, is dose-dependent, and requires the immediate interruption of the drug. Although bone suppression is rare, patients should have their peripheral blood counts monitored every 2 weeks and after the treatment course [25,52,166].

Once an adverse event is detected, symptomatic treatment is an option in patients with mild toxic drug reactions, whereas interruption of treatment should be considered when the toxic effects are severe. Benznidazole serum concentrations do not appear to be related to the appearance of serious ADRs [46,167].

The evaluation of a cure for ChD is currently difficult due to the lack of validated biomarkers for the modification of parasite loads and eventual parasitological cures in chronically infected individuals [168,169]. The low degree of parasitemia and the persistence of positive immunologic reactions represent some of the difficulties involved in addressing therapeutic efficacy [170].

A therapeutic failure is defined by the persistence of the parasite detected using parasitological methods or the detection of parasite DNA (ie, PCR), while treatment success would be measured by the reduction of antibody titers. However, a reduction in *T. cruzi*-specific antibody titers often requires many years, rendering measurement of treatment success insensitive and lengthy [69,158].

In the early chronic phase, treatment efficacy, as shown by negative seroconversion, is between 60% and 94% in children up to 13 years of age given BZN [69]. In contrast, during the late chronic phase, the serological cure rate with BZN is estimated to be only 8–37%, although this value increases the longer the patients are followed up with [70,171].

Treatment has been shown to inhibit the progression of cardiomyopathy [70] and to lower the frequency of fatal outcomes [56].

Thus based on general consensus and evidence-based standards, treatment should be administered to patients with congenital, acute infection, reactivation, or early chronic infection and, bearing in mind the risk–benefit ratio, it should be offered in the chronic phase. It should be avoided in some groups of patients, such as those with advanced cardiomyopathy, those suffering from kidney or liver failure, and those who are pregnant [46].

Etiological treatment should be considered with the goal of elimination or at least reduction of the parasite burden; reducing this stimulus should be the goal of the

currently available drugs. It is hoped that more efficacious drugs with fewer side effects will emerge as cures or treatment options with improved treatment efficacy [162,172,173].

10. CONCLUSIONS

Chagas disease is still a serious public health problem. Inflammatory activity plays a key role as a pathophysiological mechanism for CCC. It is considered the main mechanism responsible for the myocardial injury and ventricular dysfunction. It's essentially a myocarditis and inflammatory process, although more intense in the acute phase, is clinically silent but incessant in patients in the indeterminate and chronic phases of the disease, leading to a continued inflammatory response and consequently to progressive fibrotic cardiomyopathy [88,174].

Progression to myocardial dysfunction represents a major cause of morbidity and mortality. In addition, some studies suggest that heart failure due to Chagas disease has a worse prognosis compared to other etiologies, such as ischemic heart disease and idiopathic dilated cardiomyopathy.

Therefore investigations in relation to the pathogenesis and pathophysiology of development and progression of Chagas' heart disease are important in the proposed new therapies in an attempt to minimize this mortality.

Although it seems to be difficult to eradicate, it's possible to control, through elimination of domestic vectors, control over blood donors all over the world, education for the population to avoid oral transmission of the infection, and maintenance of a permanent surveillance to avoid the reintroduction of the infection into already controlled transmission areas [175].

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Familial Mediterranean Fever

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1. INTRODUCTION

1.1 Perspective

Familial Mediterranean fever (FMF) is a recessively inherited disease characterized by recurrent attacks of fever and sterile polyserositis. It is associated with mutations in the MEditerranean FeVer (MEFV) gene, located on the short arm of chromosome 16. MEFV encodes for pyrin, a protein with immune-regulatory properties [1]. It has been speculated that the FMF-related abnormal inflammatory response may predispose FMF patients (or mutated MEFV carriers) to develop inflammation-related cardiac complications. This notion is based on the increased rate of atherosclerotic cardiovascular disease demonstrated in many other inflammatory conditions, like rheumatoid arthritis, and on the premise that similar to other rheumatic diseases, FMF patients are also exposed to chronic inflammation during and in between attacks, culminating in an increased risk for atherosclerosis [2]. Nevertheless, the association between FMF and increased rate of cardiac disease is controversial, as adjudged from the conflicting results for both, risk for coronary disease and for arrhythmias yielded by several studies in phenotypically and genotypically different subsets of FMF patients.

Importantly, cardiac involvement in FMF may result from inflammation of the pericardium, which may rarely be complicated by large pericardial effusions and constrictive pericarditis. In addition, amyloidosis, which is the most devastating complication of FMF and appears almost exclusively in colchicine untreated patients, may, on rare occasions, involve the heart [3]. In this chapter, we review the data on the epidemiology of cardiac involvement in FMF, cardiac markers, predicting a risk to develop ischemic heart disease and arrhythmias in FMF, the association between MEFV mutations and the predisposition to different cardiac diseases, FMF-related cardiac amyloidosis and pericarditis, and the apparent

role of colchicine treatment in preventing cardiac manifestations in FMF.

1.2 Epidemiology of Cardiac Involvement in FMF

FMF primarily affects Sephardi Jews, Arabs, Turks, and Armenians [4] but has also been reported in Ashkenazi Jews, Druzes, individuals from other peoples living along the Mediterranean coasts and patients with a mixed ethnic background [5]. Its prevalence (as its name suggests) is highest in the Mediterranean area, although cases have been reported worldwide, including the Far East [6]. It is estimated that 100,000 individuals are affected by FMF in these regions [1]. The prevalence of FMF in Turkey is reported to be within the range of 1:400 to 1:1000 [7]. In Israel, the overall FMF disease burden rate was reported to be about 1:500, although specific ethnicity may be associated with higher prevalence [8].

It may be speculated that the high mutation carriage rate in certain regions provided a survival advantage, as observed in other genetically inherited diseases (ie, the sickle cell anemia trait, which provides protection against malaria). It was proposed that the mutated MEFV gene could result in an improved immune response to certain lethal infections, such as *Mycobacterium tuberculosis* [7,8] and *Clostridium difficile*, however, no such association has been proven as yet. Therefore, the high prevalence of the FMF gene and its possible clinical benefits should be further investigated.

1.3 Genetics of FMF and Association with Cardiac Diseases

The mapping of the MEFV gene to chromosome 16 was first discovered by Pras et al. [9]. Pyrin is a 781-amino acid protein, mainly expressed in the cytosol

of neutrophils, activated monocytes and eosinophils [10], as well as in synovial fibroblasts [11]. Its expression influences the circulating level of pro- and anti-inflammatory proteins. The mutated pyrin protein causes higher inflammasome activity, thereby leading to increased IL-1 β production [8,10], and NF κ B activation [12]. Currently, around 300 different genetic variations of the MEFV gene have been identified (according to the International Society for Systemic Auto-Inflammatory Diseases online genetic database) [13].

While the MEFV gene is highly polymorphic among Mediterranean populations, a certain number of MEFV mutations were found accountable for most FMF cases reported in the region. The Turkish FMF Study Group investigated genetic mutations in 1090 FMF patients. The most commonly found mutation was *M694V*, found in 51.4% of alleles (1121/2180), followed by *M680I* in 14.4% (313/2180), and *V726A* in 8.6% (188/2180). Homozygosity for *M694V* was found in 306 patients (28.1%), 42 patients were homozygous for *M680I* (3.8%), and 509 patients carried the *M694V* alone or with another mutated allele (46.7%). The *M694V/M694V* genotype was found to be associated with an earlier age of onset and higher frequency of arthritis and arthralgia compared with other genotypes ($p < 0.001$ for both associations) [14]. The correlation between homozygous *M694V* genotype and specific phenotypes has also been reported by other groups [5,15,16].

The possible role of carrying a mutated MEFV gene (without the development of an overt FMF) in the pathogenesis of heart disease in the general population has been evaluated by several groups. Accordingly, Basar et al. studied 91 patients with early onset coronary disease (age <45 years), 106 patients with coronary disease (patients' age above the threshold, set to define early onset disease), and 119 healthy controls, and evaluated the prevalence of MEFV mutations. Notably, none of the patients were diagnosed with FMF. At least one mutated MEFV allele was found in 41.8% of patients with early coronary disease, 16% of patients with non-early coronary disease, and 20.2% of controls. The *R202Q* variation in heterozygous form was the most commonly found sequence variation in both groups of patients with coronary disease. Overall, it was concluded that the presence of mutations in the MEFV gene may predispose for the development of coronary disease in young subjects compared with healthy controls (OR=2.838, 95% CI 1.540–5.232) [17]. Of note, however, the *R202Q* variation is considered a nonpathogenic MEFV polymorphism. Thus the significance of this finding is questionable.

Grimaldi et al. also reported that MEFV mutations in non-FMF patients are associated with myocardial infarction (MI). This time the culprit was the *M694V* mutation, which was detected at a higher rate in young MI patients (12% vs 6% in healthy controls, $p < 0.01$), giving an odd

ratio (OR) of 2.2 (CI of 1.3–4.1, $p < 0.01$) for developing an acute myocardial infarction [18]. These results are in line with the more severe FMF phenotype found in carriers of *M694V*, which may predispose to increased inflammatory burden and, assumingly, increased atherosclerosis. Nevertheless, the MI patients were largely affected by several other risk factors that could have led to the patients' MI. Also, the difference in the rate of MI between the study and control groups was very low (6%). Thus, more studies are still required to determine whether carriage of MEFV mutations increases the risk for development of IHD.

Somewhat contrasting data was published by our research group. We found that carriage of the MEFV *E148Q* mutation is associated with longevity in Ashkenazi Jewish subjects. In this study, the prevalence of MEFV mutations in 200 nonagenarians (>90 years of age) was compared to the known prevalence, by ethnic origin, in the Israeli population. One-fifth of the studied population carried an MEFV mutation, most commonly *E148Q* (73% of total mutations), a frequency (for *E148Q*) much higher than expected in the general Ashkenazi population (19.8% vs 2.6%, $p < 0.0001$) [19]. No difference was noted between carriers and noncarriers in the rates of ischemic heart disease, diabetes, stroke, and a wide range of other serious conditions. These findings go along with selective advantage that might be conferred by high frequency of carriage of MEFV mutations in the at-risk population of the Mediterranean basin and Far East, where carriage of the MEFV *E148Q* mutation is very common. This study, however, could not clearly relate the advantage conferred by the mutation to protection from a specific cardiac disorder.

The local expression of MEFV in myocardial tissue in health and cardiac disease was investigated only in one study. Hermansson et al. evaluated gene expression in atrial myocardial tissue of ischemic ($n = 5$) and nonischemic ($n = 3$) heart tissues. The atrial tissue was extracted (in accordance with strict ethical guidelines) from patients undergoing CABG surgery, while the control tissue was purchased from Invitrogen and BioChain (CA, USA). Total cellular RNA was extracted from human heart tissue, and inflammasome mRNA levels were measured by Quantitative-PCR. MEFV gene expression was found to be significantly lower in tissue extracted from the atrium of patients with ischemic heart disease. Moreover, upon sequencing, they found that one of the patients who developed ischemic heart disease was homozygous for the *K671M* mutation. The authors suggested that the MEFV mutation and abnormally low pyrin expression may contribute to the inflammatory process occurring in ischemic cardiomyopathy [20]. Although very interesting, lack of pathophysiologic inference and small study sample make these findings of yet uncertain significance.

2. CLINICAL PRESENTATION AND DIAGNOSTIC CRITERIA

2.1 Clinical Presentation

FMF is characterized by recurrent and self-limited attacks of fever and localized pain (as a manifestation of sterile serosal inflammation), although the clinical spectrum may also include nonserosal manifestations such as exertional leg pain, skin lesions, particularly erysipelas-like eruption in the calf, and short- or long-term attacks of myalgia [4]. Other more peculiar manifestations with neurological, hepatic, and nephrological findings may also exist [21].

Attacks may differ in their length, usually lasting one to three days, but may be shorter or longer (from several hours up to one week). The attacks may also diverge in their frequency, occurring between once every few years, to 3 attacks per week [12]. A severity scoring tool for FMF has been suggested by several groups, although Mor et al.'s system has been widely adopted and has been used to date by several groups (Tables 24.1 and 24.2) [22].

This scoring system allows to score severity, by one of two sets. The first set defines the severity of FMF based **only** on the expression of the attacks (site, duration, and frequency), and is independent of features such as colchicine dose and age of disease onset, while the other one makes use of many features of the

disease, in addition to attack characteristics. Mor et al. criteria were found to have a specificity and sensitivity of around 90%.

There are no specific signs or symptoms suggesting cardiac involvement in FMF. Chest pain is among the most common clinical symptom found in FMF, occurring in 13.7–25.7% of FMF patients [4,23]. Importantly, although the typical FMF-related chest pain is pleuritic chest pain, it is still nonspecific for FMF and may arise from pneumonia, musculoskeletal pain, recurrent benign pericarditis, pericarditis associated with a myriad of other autoimmune or inflammatory diseases and pulmonary emboli [4]. Even within the spectrum of FMF manifestations, pleuritic chest pain does not necessarily suggest cardiac involvement, as it is more often caused by inflammation of pleura rather than pericard [24]. On the other hand, the diagnosis of FMF is often missed due to the absence of other typical symptoms. This might occur when recurrent pericardial inflammation is the only sign of FMF.

2.2 Diagnostic Criteria

FMF diagnosis is entirely clinical, although genetic analysis might assist in the diagnosis of uncertain cases. Prior to 1997, several sets of criteria were available for diagnosing FMF, however, they were not based on statistical evaluation and their sensitivity and specificity were obscure [25,26]. In 1997, Livneh et al. suggested a novel set of clinical criteria, which soon became widely accepted by the FMF community (Table 24.3). The presence of one major criterion (history of a typical attack) is sufficient for diagnosing FMF. Alternatively, in cases with incomplete attacks, a combination of incomplete attack with another minor criterion or with several supportive criteria as defined in the foot note of Table 24.3 is required for diagnosis. Despite the usefulness of the criteria, FMF diagnosis requires a high index of suspicion, particularly in nonendemic regions.

TABLE 24.1 Determination of Degree of Severity in FMF Patients

First step—Determination of severe disease	Presence of ≥ 2 of the following: 1. >24 attacks/year (>2 per month) 2. >1 site/attack ^a 3. >2 sites/course of disease
Second step—Determination of disease with intermediate severity (in a disease not defined as severe by the above criteria)	Presence of ≥ 1 of the following: 1. >18 attacks/year 2. Duration of attack >4 days ^b
Third step—Determination of mild disease	Absence of criteria defining severe or intermediate forms of FMF.

^aIn at least 25% of the attacks.

^bIn most attacks.

Adapted from Mor et al. [5].

TABLE 24.2 Criteria for FMF Disease Severity^{a,b}

- >1 site in a single attack in at least 25% of attacks
- >2 sites in the course of the disease
- ≥ 2 mg/day colchicine to achieve remission
- ≥ 2 pleuritic attacks during the course of the disease
- ≥ 2 Erysipelas-like erythema attacks during the course of the disease
- Age of onset ≤ 10 years

^aSevere disease ≥ 3 criteria; intermediate disease = 2 criteria; mild disease ≤ 1 criterion.

^bThe above-mentioned Table 24.2 is specifically useful for patients who are chronically treated with colchicine.

Adapted from Mor et al. [5].

3. PROPOSED PATHOPHYSIOLOGY FOR NONAMYLOIDOSIS CARDIAC INVOLVEMENT IN FMF

3.1 Inflammatory Markers

FMF attacks are characterized by increased circulating levels of acute phase reactants, such as fibrinogen, C-reactive protein (CRP), serum amyloid A (SAA), and phospholipase A2, and inflammation cytokines such as IL-6, IL-1 β , and TNF α [2,12,27]. During clinical remission, the inflammatory markers may normalize or remain high (in 30% of patients), especially in those untreated [2]. In one study [28], attack-free and amyloidosis-free, colchicine-treated FMF patients, were found to have significantly

TABLE 24.3 Criteria for Diagnosing FMF**Major Criteria**

Typical attacks of:

1. Peritonitis (generalized)
2. Pleuritis (unilateral) or pericarditis
3. Monoarthritis (hip, knee, ankle)
4. Fever alone

Minor Criteria

1–3. Incomplete attacks involving one or more of the following sites:

1. Abdomen
2. Chest
3. Joint
4. Exertional leg pain
5. Favorable response to colchicines

Supportive Criteria

1. Family history of FMF
2. Appropriate ethnic origin
3. Age <20 years at disease onset
- 4–7. Features of attacks
4. Severe, requiring bed rest
5. Spontaneous remission
6. Symptom-free interval
7. Transient inflammatory response, with one or more abnormal test result(s) for white blood cell count, erythrocyte sedimentation rate, serum amyloid A, and/or fibrinogen
8. Episodic proteinuria/hematuria
9. Unproductive laparotomy or removal of white appendix
10. Consanguinity of parents

“Typical attack” requires the presence of all the following features:

1. Recurrence of attacks (≥ 3 of the same type)
2. Fever (rectal temperature of $\geq 38^\circ\text{C}$) in most attacks
3. Short duration (12h–3 days)

“Incomplete attack” is defined as painful and recurrent attack that differs from “typical attacks” in one or two of the following ways:

1. The rectal temperature was normal or lower than 38°C
2. The attacks are longer or shorter than specified (but not shorter than six hours or longer than a week)
3. No signs of peritonitis are recorded during the abdominal attacks
4. The abdominal attacks are localized
5. The arthritis is in joints other than those specified.

*Attacks not fulfilling the requirements for definition of either typical or incomplete attacks are disregarded.

*FMF is defined by the presence of (1) ≥ 1 major criteria; or (2) ≥ 2 minor criteria; or (3) 1 minor criterion plus ≥ 5 supportive criteria; or (4) 1 minor criterion plus ≥ 4 of the first 5 supportive criteria.

Adapted from Livneh et al. [4].

increased levels of high-sensitive CRP (hs-CRP, median of 0.78 vs 0.15 $\mu\text{g/mL}$, $p=0.03$) and IL-17 (median of 1.29 vs 1.08 pg/mL ; $p<0.01$) compared with controls. In the same study, however, the mean values of IL-6 and IL-23 were similar in FMF patients and in control subjects [28]. Also, other studies have shown that IL-17 and IL-18 levels were higher in FMF subjects regardless of disease activity [29]. In contrast, it was reported that the levels of IL-1 β , IL-6, and TNF α were comparable in FMF and control subjects [30]. Thus, with respect to various inflammatory cytokines, the evidence for their serum levels, as compared to unaffected subjects, is strongly

inconsistent, while for acute phase reactant it is usually in agreement.

Increased CRP levels, commonly encountered in FMF patients, is a well-recognized risk marker for increased cardiovascular morbidity and mortality in the general population [31,32]. CRP itself was reported to have a deleterious effect on vascular disease, as it directly increases inflammatory cell infiltration, is associated with impaired endothelial function, decreased secretion of vasodilatory substances, and overall contributes to the acceleration of the formation and disruption of atherosclerotic plaque [32]. It may be inferred that the chronically elevated CRP found in a subset of FMF patients with subclinical inflammation, particularly those unresponsive to or untreated with colchicine, will have cardiovascular effect such as accelerated atherosclerosis and cause increased cardiac morbidity.

3.2 Markers for Endothelial Injury and Endothelial Dysfunction

Atherosclerosis may be mediated by vascular endothelial dysfunction, due to the inflammatory process involving the vascular wall and manifested as decreased production of vasoprotective substances by the injured endothelium [10]. There are several markers of endothelial injury that may be influenced by inflammation and were evaluated in FMF, including asymmetric dimethylarginine (ADMA), nitric oxide (NO), osteoprotegerin (OPG), thrombomodulin (TM), and adrenomedullin (AM). ADMA is a proteolytic residue of arginine-methylated proteins, acting as an endogenous competitive inhibitor of NO synthase, thereby preventing vasodilation. OPG, an osteoclastogenesis inhibitor, and TM are two proteins produced by the endothelium. The detection in the serum of these two proteins has been considered to indicate vascular injury [28].

Pumak et al. reported significantly lower levels of circulating markers of vascular injury in uncomplicated attack-free colchicine-treated FMF patients: ADMA (median of 2.56 vs 3.26 $\mu\text{mol/L}$ in control individuals; $p=0.04$), OPG and TM [28]. In contrast, Terekeci et al. reported that in their hands, ADMA levels were higher in 38 attack-free FMF patients (10 of whom were recently diagnosed and hence were not treated with colchicine at inclusion) compared with control subjects (0.54 ± 0.10 vs $0.31 \pm 0.07 \mu\text{mol/L}$; $p<0.001$) [33]. The latter values are lower than those of Pumak et al., both for control subjects and FMF patients, and may be due to the different measurement techniques [28]. The authors speculated that increased ADMA levels in FMF may explain the decreased NO levels, formerly observed by Panossian et al. [34]. Yilmaz et al. reported in patients with FMF-related amyloidosis and proteinuria high levels of ADMA and a significantly lower level of flow-mediated dilation (FMD; an ultrasonography marker for

endothelial function) compared to a patients with non-diabetic glomerulopathies, further supporting endothelial dysfunction in FMF, yet in complicated FMF. In a study by Yilmaz et al. [35], elevated levels of ADMA were found in FMF-related amyloidosis compared with patients with nondiabetic related nephrotic-range proteinuria and normal glomerular filtration rate (median of 3.9 vs 2.5 $\mu\text{mol/L}$, $p < 0.001$). Of note, there is no consent between different laboratories on the normal range of ADMA, a problem complicating the interpretation of the results. Thus, ADMA levels and their role in cardiovascular disease of FMF is yet to be determined.

As noted previously, Pumak et al. reported lower than normal OPG values in FMF patients (attack free, all treated with colchicine, median of 361.5 vs 548.9 pg/mL; $p = 0.01$) [28]. In contrast, Yuksel et al. evaluated 31 FMF patients during an attack-free period (14.3% of whom had amyloidosis; 61.3% were treated with colchicine) and 18 control subjects. In that study, OPG values were found to be higher in the FMF group (median 1.9 vs 0.3 pmol/L, $p = 0.016$) [30]. However, the mixed population in the Yuksel et al. study and the conflicting results with the Pumak et al. study preclude any meaningful conclusion for the role of OPG as cardiac marker in FMF.

In contrast to Pumak et al.'s finding of lower than normal TM levels in FMF (median of 2.69 vs 3.59 ng/mL, $p < 0.01$) [28], Ozbalkan et al. reported an increased TM in 25 FMF patients (five during an attack) compared to those found in 25 healthy control individuals (20.9 ± 12.1 vs 14.1 ± 8.4 ng/mL; $p < 0.05$). Unexpectedly, the TM of FMF patients during an attack-free period (22.4 ± 12.9 ng/mL) were higher than those during an attack (14.8 ± 5.4 ng/mL) and comparable to those of healthy control subjects [36]. The latter results are difficult to explain, and the role of TM in FMF as a possible marker for endothelial injury remains elusive. Also, the major differences in the results of TM in FMF patients and control subjects is not surprising given the large differences reported in the results of TM when using different ELISA kits [37].

Nitrate is a stable end product of NO metabolism and therefore can be used for nondirect quantification of NO production by the endothelium. AM is a vasodilator known to be secreted by the vascular endothelium [38], among many other cellular sources. Balat et al. reported higher urinary and serum levels of nitrate and AM in young FMF patients, compared to control subjects [39]. Although this result does not necessarily reflect normal endothelial function in FMF patients and may be explained by its secretion from other sources, the net effect is a protective one. In another study, Üreten et al. found AM to be similar in FMF patients and healthy control subjects, regardless of attacks or CRP levels [40], further opposing a harmful net effect on endothelial function by various endothelial factors possibly released in FMF patients.

In conclusion, different soluble markers of atherosclerosis and endothelial function have been investigated in FMF patients. The results of studies on the five markers tested in FMF are inconclusive, yet the net outcome of endothelial cell activity seems to remain within normal boundaries, as inferred from normal or higher levels of vasodilators released in FMF.

3.3 Markers for Increased Thrombogenicity

It has been suggested that FMF patients have elevated thrombogenicity, thus, increasing their susceptibility to develop vascular events. However, even to date, it remains unknown whether increased thrombogenicity occurs in FMF in the absence of amyloidosis-related nephrotic syndrome. If indeed present in FMF, this flaw may be explained by the interplay between inflammation and coagulation mediators. In fact, pro-inflammatory states may be associated with procoagulation due to increased levels of procoagulation factors and decreased levels of intrinsic anticoagulants and fibrinolysis [41,42]. Importantly, there is no evidence for high rate of clinically overt thromboembolism in FMF. Most anecdotal case reports are restricted to patients with renal disorders or primary hypercoagulability, or may be attributed to coincidence [42–47]. Absence of reported propensity for thromboembolism is in line with our experience in FMF patients, although a specific evaluation of the rate of thromboembolic events in FMF patients has neither been explored nor reported in the literature.

Nonetheless, evaluation of the coagulation system in FMF patients has been reported by several research groups. Aksu et al. performed coagulation tests in 27 FMF patients and 26 healthy individuals and reported a shortened prothrombin time (PT) and thrombin time (TT), decreased protein C activity, and increased levels of human prothrombin fragment F 1+2 [41]. In contrast, Demirel et al. reported normal PT values in FMF patients, during attack free periods, and increased PT during attacks, compared with control subjects [48]. They also reported that the P-selectin, an adhesion molecule causing platelet recruitment, was significantly lower and the tissue plasminogen activator (t-PA) was significantly higher during attacks (but not during the attack-free interval) [48]. Therefore, evaluation of PT in FMF patients yielded conflicting results and merits further research.

Pumak et al. also reported that the von Willebrand factor (vWF; 71.6 vs 68.8 mU/mL), tissue factor (TF; 194.3 vs 212.9 pg/mL), and t-PA (1.7 vs 1.84 ng/mL) were similar in FMF patients and control subjects, respectively ($p > 0.05$ for all) [28]. Lack of increased vWF in FMF patients who were continuously treated with colchicine (both during an attack and an attack-free period) was also reported by Demirel et al. [48].

Thrombin-activated fibrinolysis inhibitor (TAFI) is zymogen, which inhibits the interaction between plasminogen and fibrin, thereby decreasing fibrinolysis and promoting thrombogenesis. Bavbek et al. reported that serum TAFI levels in FMF patients (in between attacks) was significantly increased compared with healthy individuals (116.64 ± 21.8 vs 78.48 ± 19.7 mg/mL, $p < 0.001$). Also, a positive correlation was found between TAFI and CRP levels [49]. Regrettably, TAFI levels in FMF were not investigated by other research groups, and its contribution to the yet unproven association between FMF and procoagulation remains unknown.

In conclusion, based on the absence of reports on increased rate of thromboembolism in FMF, and the conflicting results with regard to the underlying mechanisms that may lead to hypercoagulability, the data are in favor of normal functioning of coagulation pathways in FMF. Of note, primary thrombophilia (activated protein C resistance, presence of anticardiolipin antibodies, etc.) were not reported to be more prevalent in FMF patients [42].

Increased mean platelet volume (MPV) has been linked to increased platelet aggregation, and synthesis and release by platelets of thrombogenic mediators (ie, thromboxane A₂, serotonin, platelet factor-4) and adhesion molecule. Also, increased MPV was found to be associated with increased β -thromboglobulin (β -TG), another marker of platelet activation. It has been suggested that increased MPV may be associated with a prothrombotic risk, with increased rate of coronary disease and myocardial infarction, and occurs more often in patients with traditional cardiovascular risk factors [50–52]. Overall, there are nine publications reporting on MPV values in FMF patients (Table 24.4). Five have found increased MPV values compared with control subjects [53–58], while three have not [59–61]. There was a single study that reported lower than normal MPV [62]. Two studies reported on MPV in FMF-amyloidosis patients with conflicting results (one found higher and the other one lower than normal MPV). Coban et al., who were the first to report increased MPV in FMF, also found a negative correlation between MPV and duration of colchicine treatment ($r = 0.40$, $p = 0.017$) and diagnosis delay ($r = 0.58$, $p = 0.001$) [53]. Arica et al., who reported an increased MPV and platelet count in children, also found that MPV and platelet count during attacks, and remission, are statistically comparable [54]. Makay et al., who found normal MPV during the attack-free interval, reported lower than normal MPV during attacks [60]. This finding is surprising as it contrasts the paradigm of inflammation-associated thrombogenicity.

Abanonu et al., who reported normal MPV in FMF, also found lower than normal β -TG in FMF (111.06 ± 52.56 vs 162.89 ± 91.52 ng/mL; $p < 0.05$). No correlation was found between β -TG and age at FMF onset/diagnosis, colchicine dose, or disease severity. They suggested that a beneficial

effect of colchicine treatment on platelet function may underlie their finding [59]. Uluca et al. conducted one of the biggest studies on MPV in children with FMF (175 with and 77 without FMF), and no difference was found in platelet count and MPV between the groups [61].

The patient population of Sakalli et al., who reported higher than normal MPV in adults and children with FMF, consisted in part of patients with proteinuria, in some of whom due to amyloidosis. They reported that the presence of proteinuria was associated with increased MPV values, regardless of age [55]. In contrast, Ozkayar et al., who reported that uncomplicated FMF was associated with a significantly higher than normal MPV, also found that FMF-amyloidosis was associated with a significantly lower than normal MPV [58].

When interpreting all the aforementioned results of MPV in FMF, it should be kept in mind that MPV can be highly affected by technical aspects of blood drawing, collection, and analysis. In particular, the time interval from blood collection to MPV measurement, the temperature at which the tubes are kept before and during MPV measurement, and the laboratory machinery used, may all affect results. Also, EDTA, commonly used for its antiaggregant properties, can affect the platelet's structure and cause platelet swelling (K-EDTA causes more swelling than Na-EDTA). Notably, the different studies on MPV in FMF patients have utilized different anticoagulants. The exact used collection tubes and measurement lag time are not specified by any study, nor standardized. Therefore, limiting any credible comparisons between studies. Lancé et al. proposed that MPV should be evaluated 120 min after blood collection when using K2-EDTA (resulting in normal MPV results within the range of 7.2–10.8 fL) or 60 min following blood collection in a tube containing sodium citrate (the proposed normal MPV values within the range of 6.1–9.5 fL) [63]. Other essential points are that none of the studies reported on the proportion of patients with abnormal MPV values, and all mean/median results are within the proposed range for normal values, while only MPV higher than 11.01 fL might be considered a risk for increased cardiovascular mortality [64]. Lower cutoff values for hazardous MPV, which have been proposed, are not yet acceptable [64]. Finally, the wide variation of MPV values reported in different studies may stem from different genetic backgrounds of patients.

4. CARDIAC INVOLVEMENT IN FMF

4.1 Cardiac Amyloidosis

Continuous inflammatory burden is associated with increased production of IL-1, IL-6, and TNF α , thus facilitating the production of the acute phase reactant SAA by the liver. Abnormal processing of SAA by mononuclear phagocytes and matrix metalloproteases may result in

TABLE 24.4A Clinical Characteristics of Investigated Patient Groups in Studies Evaluating MPV in FMF

Study #	Group	n	Gender (F/M)	Age (years)	Colchicine resistance (%)	Amyloidosis (%)	Disease duration (years)	Colchicine treatment (years)	Colchicine dosage (mg/day)	Disease severity score	Attacks/year	BMI (kg/m ²)	Other CV comorbidities ^b	References
1	FMF	35	16/19	34.0±9.0	NA	0	NA	9.9±9.7	1–2	NA	NA	24.1±3.0	None	[53]
	Control	35	17/18	35.0±9.0								25.3±2.3	None	
2	FMF	84	39/45	0–15	NA	NA	NA	NA	1.1±0.5	NA	NA	NA	NA	[54]
	Control	57	NA	NA								NA	NA	
3	FMF 1	63	37/26	10.3±4.1 ^a	NA	4.7	NA	4.0±2.8	NA	NA	NA	NA	NA	[55]
	Control 1	50	29/21	8.4±3.7								NA	NA	
	FMF 2	50	30/20	33.1±9.0	NA	36	NA	12.7±9.6	NA	NA	NA	NA	NA	
	Control 2	43	24/19	31.8±7.0								NA	NA	
4,5	FMF	40	27/13	30.8±12.2	NA	NA	NA	NA	75% 1–1.5. 7.5% untreated	NA	NA	NA	NA	[56,57]
	Control	20	12/8	29.2±10.0								NA	NA	
6	FMF 1	74	34/40	30.9±9.3	NA	0	NA	NA	1.5	NA	NA	NA	None	[58]
	FMF 2	29	10/19	35.5±16.3	NA	100	NA	NA	1.5	NA	NA	NA	None	
	Control	180	87/93	33.7±9.5								NA	None	
7	FMF	25	16/9	35.7±12.3	0	NA	NA	NA	0–3 (one untreated pt)	68% mild 28% mod 4% severe	NA	25.4±4.4	20% smokers	[59]
	Control	28	22/6	31.8±10.3								25.7±4.6	10.7% smokers	
8	FMF	63	29/34	NA	NA	NA	3.5±3.3	NA	1.1±0.3	NA	NA	NA	NA	[60]
	Control	49	21/28	10.5±4.0								NA	NA	
9	FMF	157	84/73	9.1±3.6	NA	0	NA	NA	0.5–2	NA	NA	NA	NA	[61]
	Control	77	39/38	8.3±3.5								NA	NA	
10	FMF	120	64/56	29.5±11.0	NA	0	NA	NA	NA	NA	NA	NA	NA	[62]
	Control	75	NA	33.0±8.0								NA	NA	

BMI, body mass index; CV, cardiovascular; NA, not available.

^a $p < 0.05$.

^bNA - in cases not all risk factors (including smoking) were specified/excluded & corresponds to for an analysis of.

TABLE 24.4B Laboratory Findings and Technical Aspects of Diagnostics in Studies Evaluating MPV in FMF

Study #	Group	FMF genotype	CRP (mg/dL)	SAA (mcg/mL)	Testing method	Platelet counts (×10 ⁹ /L)	MPV (flv)	β-TG (ng/mL)	References
STUDIES REPORTING ON INCREASED MPV VALUES IN FMF									
1	FMF	NA	NA	NA	Blood was collected in citrate (1:4, v/v) and analyzed within 1 h	257.0±61.0	8.6±0.9 ^a	NA	[53]
	Control		NA	NA		284.0±64.0	7.8±0.5	NA	
2	FMF	56% M694V HoZ-M	0.41±0.34	NA	Retrospective. EDTA tube (analysis conducted for 64 selected children with unspecified characteristics)	347.0±48.0 ^a	8.1±0.4 ^a	NA	[54]
	Control		NA	NA		265.0±97.0	7.4±0.3	NA	
3	FMF 1	46.9% M694V	0.39±0.34	NA	EDTA tube	336.0±73.0	7.2±1.0 ^a	NA	[55]
	Control 1	16.8% V726A 15.04% E148Q	NA	NA		328.0±72.0	6.6±0.4	NA	
	FMF 2	14.1% M680I 1.76% M694I ^b	0.49±0.35	NA		266.0±72.0 ^a	8.5±1.3 ^a	NA	
	Control 2		NA	NA		325.0±78.0	7.1±0.4	NA	
4,5	FMF	NA	Median 1.98 ^a	NA	Unspecified tube and collection method	259.5±82.3	8.9±1.0 ^a	NA	[56,57]
	Control		Median 0.28	NA		251.8±32.5	8.2±0.4	NA	
6	FMF 1	NA	Median 0.05	NA	Within 2 h after collection, unspecified tube	Median 229.5	Median 10.2 ^a	NA	[58]
	FMF 2		Median 0.31	NA		Median 369.0	Median 6.9 ^a	NA	
	Control		NA	NA		Median 239.0	Median 9.2	NA	
STUDIES REPORTING ON SIMILAR MPV IN FMF AND IN CONTROLS									
7	FMF	NA	Median 0.36	NA	Citrate tube	195.3±74.7	8.5±1.1	111.1±52.6 ^a	[59]
	Control		Median 0.17	NA		203.4±73.4	8.7±1.2	162.9±91.5	
8	FMF	33% M694V HoZ-M	0.37±0.31	NA	Retrospective. EDTA tube	303.0±76.0	7.9±0.9	NA	[60]
	Control		NA	NA		281.0±72.0	8.1±0.6	NA	
9	FMF	NA	2.0±1.5	NA	EDTA tube	302.0±59.0	8.0±1.1	NA	[61]
	Control		NA	NA		312.0±59.0	7.8±1.2	NA	
STUDIES REPORTING ON LOWER MPV VALUES IN FMF COMPARED TO CONTROLS									
10	FMF	NA	0.29±0.18	NA	Retrospective. EDTA tube	282.0±72.0 ^a	7.9±0.9 ^a	NA	[62]
	Control		NA	NA		275.0±68.0	8.3±0.8	NA	

NA, not available; TG, Thromboglobulin; HoZ-M, homozygote mutations; CHtZ-M, compound heterozygote mutations; HtZ-M, heterozygote mutations; HR, heart rate; CRP, C-reactive protein; SAA, serum amyloid A.

^a*p* < 0.05; note that CRP and SAA levels were mentioned only if they were examined in attack-free period.

^bThe occurrence of other mutations (A744S, H2O2Q, K695R, P369S, R761H) seems not to be explicitly specified.

an extracellular deposition of N-terminal SAA segments arranged in an insoluble cross β -sheet structure, called amyloid A. Early manifestations of the resulting disease, reactive (or AA) amyloidosis, usually involve the kidneys, liver, and spleen. In a more progressive stage, amyloidosis may also involve the autonomic nervous system (ANS), the endocrine organs, and the heart. In the absence of treatment, 50–60% of FMF patients may develop amyloidosis, systemic complications, and amyloidosis-associated death, although risk is associated with ethnicity, genotype, family history of amyloidosis, gender, FMF phenotype, SAA isotype, and other not fully understood environmental factors [12,65]. The highest risk is in patients homozygous to the MEFV M694V mutation and to the complex allele V726A-E148Q and carrying the α/α genotype of the serum amyloid A1 (SAA1) gene [1]. According to other reports, the rate of amyloidosis in untreated patients may be as high as 90% [3].

Even today, in the colchicine era, amyloidosis continues to occur, mainly in those untreated, affecting 11.4–12.9% of FMF patients in some series [12]. Importantly, amyloidosis may develop at a young age in FMF patients, especially in those untreated and undiagnosed. In some series of patients with amyloidosis of FMF, more than 70% developed some degree of systemic amyloidosis before the age of 18 years [35]. Patients with chronic renal disease due to amyloidosis have a poor prognosis compared to patients with chronic renal disease due to other reasons. It has been suggested that cardiovascular factors contribute to the poor prognosis in the presence of renal amyloidosis [66]. Although this hypothesis may be true in general, its relevance to amyloidosis of FMF remains unknown.

Unlike AL amyloidosis, where cardiac involvement is commonly found and is a leading cause of mortality, cardiac involvement in AA amyloidosis is rare [67]. FMF in that regard seems to make no exception. Nevertheless, the exact rate of cardiac amyloidosis in FMF patients remains unknown and is thought to be extremely low in the current colchicine era. Lachmann et al. evaluated the natural history of 374 patients with AA amyloidosis (six of whom had FMF) and reported that cardiac failure due to amyloidosis was found in only one (0.3%). Findings consistent with cardiac amyloidosis were reported in 2/224 patients with AA amyloidosis undergoing echocardiography (0.9%) [68]. Hamer et al. reported 30 patients with AA amyloidosis (due to various etiologies), of whom one patient (3.3%) died of cardiac dysfunction. They also reported in their series a patient with first-degree AV block and postmortem findings of perivascular amyloid deposits, despite normal cardiac chamber thickness and motion observed on echocardiography before death [67]. These findings refer to AA amyloidosis in general and may not be relevant to AA amyloidosis of FMF.

Ambartsyian et al. [69] evaluated tissue specimens taken from 260 FMF patients (60 of whom died from complications of amyloidosis). Cardiac amyloidosis was less common than kidney, spleen, digestive tract, and endocrine system amyloidosis. Cardiac amyloidosis may involve the endocard, myocard, and the periadventitial tissue (Fig. 24.1). In some cases, amyloid deposits were found to narrow or occlude the vascular lumen. Large amyloid deposits may also cause local displacement of the cardiac cells, resulting in cardiomyocyte atrophy. Reactive connective tissue growth and lymphoplasmocytic infiltration were also seen [69,70]. It was reported that cardiac amyloidosis is commonly found with lung amyloidosis, and that involvement of kidneys was not mandatory.

In another study by the same author, 68 patients who died from presumable FMF complications were evaluated [71]. Eleven had cardiac clinical manifestations and microscopic amyloidosis; while nine others had cardiac histopathology findings without clinically overt cardiac disease (overall 29.4% of FMF patients who died due to FMF-related complications had cardiac amyloidosis). Clinically, patients with cardiac amyloidosis were diagnosed as having ischemic heart disease, cardiomyopathy, and valvular disease. Macroscopically, amyloidosis-affected hearts were thicker (ie, cardiomegaly) with thickened valves. Cardiac amyloidosis was found to be associated with myocyte atrophy and clinical progression into heart failure [71]. In a different work by the same author, 105 autopsies (FMF-related deaths) were evaluated. Twenty-one cases had morphologic cardiac changes, 10 of whom were symptomatic [70].

Yilmaz et al. reported the rate of cardiovascular complication in 98 FMF patients with renal amyloidosis for a time interval with a median of 40 months. Seven patients (7.1%) developed acute myocardial infarction, nine (9.2%) reported symptoms consistent with unstable angina, two underwent coronary angiography (2%), and 4 (4.1%) developed heart failure. Moreover, nine deaths (9.2%) were attributed to cardiovascular disease: six to coronary disease and three to sudden death. It remains unclear, however, as to the proportion of patients with cardiac amyloid deposition. Yilmaz et al. also reported that cardiovascular events were more common in FMF amyloidosis than in proteinuria unrelated to FMF [35].

It should be concluded that although rare in the colchicine era, cardiac amyloidosis should be suspected in FMF-amyloidosis patients with cardiac structure and function abnormalities, yet definite diagnosis must rely on cardiac biopsy. Also, lack of gross cardiac anatomy and function abnormalities does not exclude the presence of microscopic cardiac amyloidosis, although the clinical significance of this finding remains unknown.

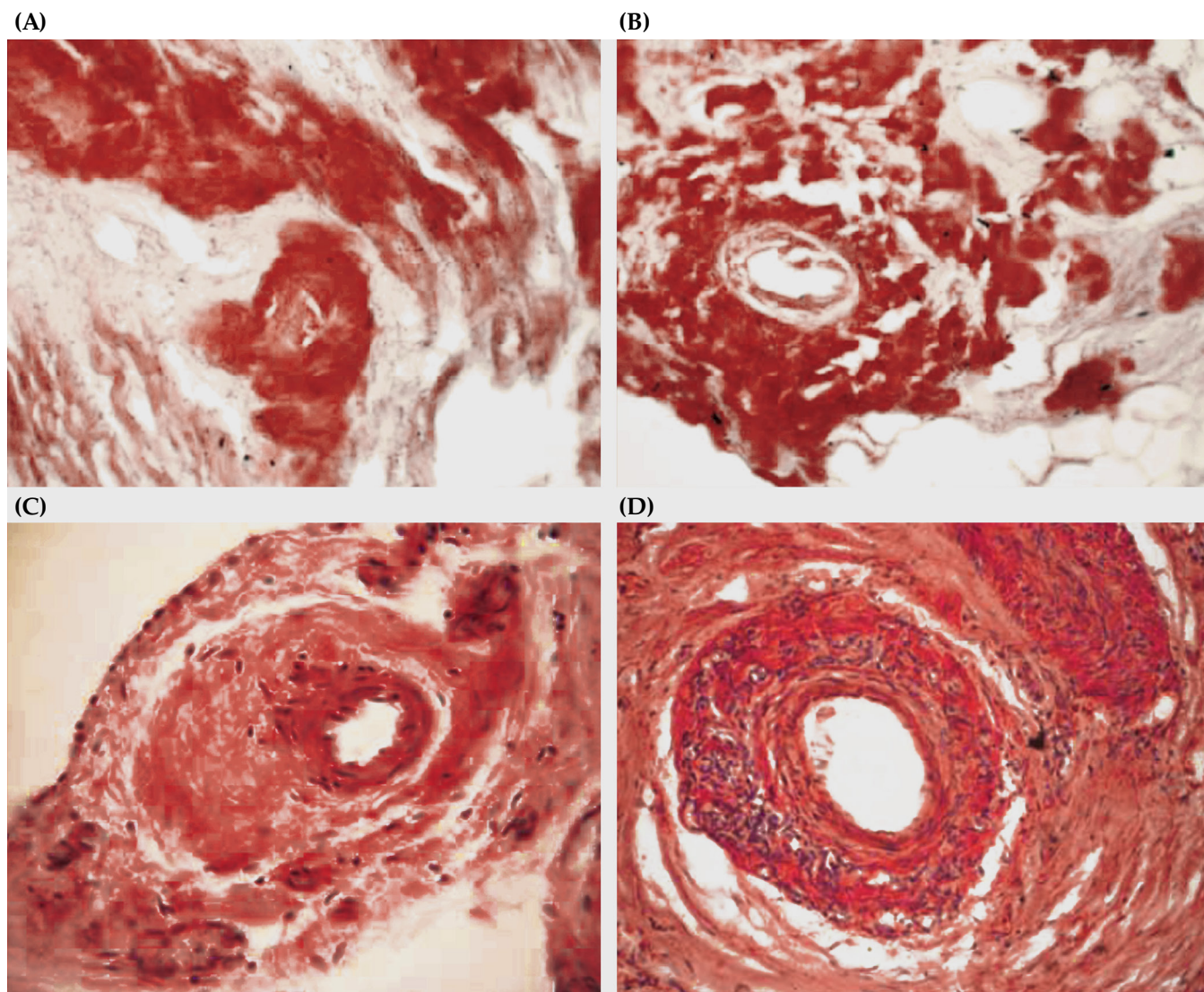


FIGURE 24.1 Congo red staining for cardiac amyloid demonstrates accumulation of amyloid in the stroma of myocardium (A), myocardium coronary artery (B), periadventitium (C), and myocardial arterial wall, accompanied by proliferation of adventitia (D). Adapted from Ambartsyan [69] and Hambardzumyan [70].

4.2 Prevalence of Traditional Risk Factors for Accelerated Atherosclerosis

It was speculated that FMF patients may have an increased risk to develop atherosclerotic cardiovascular disease due to traditional risk factors. This possible association was studied in several studies. Langevitz et al. evaluated the prevalence of IHD risk factors and actual cardiovascular disease in 290 FMF patients (all treated with colchicine) and their 233 spouses. FMF patients and their spouses had similar rates of smoking (36.6% vs 38.6%, respectively) and DM (11% vs 8.2%), but a higher rate of hypertension (27.9% vs 17.2%, $p < 0.05$). In contrast, obesity was found to be at a lower rate in FMF patients (10% vs 18.9%, $p < 0.05$), as well as the rates of sedentary lifestyle (70% vs 92.7%, $p < 0.05$) [27]. A subgroup analysis

of 45 FMF patients and 26 spouses who developed IHD found a similar rate of CV risk factors, with the exception of obesity, which was less common in FMF patients compared with the control subjects (9% vs 35%, $p < 0.05$). The median number of cardiovascular risk factors for patients with FMF who developed IHD and spouses who developed IHD was similar (two to three risk factors) [27].

In a different study by Akar et al., they reported that the rate of hypertension (14.3%) and DM (4.4%) in 587 young FMF patients (mean age of 32.4 ± 12.3 at the beginning of the follow-up) were similar to the reported national frequencies for the same age group [7]. Lidar et al. also reported that the prevalence of cardiovascular risk factors (ie, hypertension, DM, and smoking rate) were similar in colchicine responders and colchicine nonresponders [1].

Acay et al., who investigated the lipid profile in 60 FMF patients and 60 control subjects [72], reported significantly higher triglyceride (TG) levels in FMF patients (117.64 ± 59.55 vs 81.82 ± 14.25 , $p=0.003$) and lower high-density lipoprotein (HDL) levels (43.69 ± 14.97 vs 46.14 ± 8.15 mg/dL, $p=0.01$) compared with controls. They also found a higher atherogenic index (the ratio between TG to HDL levels), thought to reflect atherosclerosis risk (not formally), in FMF (3.15 ± 2.38 vs 1.86 ± 0.43 , $p=0.005$) [72]. An abnormal lipid profile was also reported by other groups, but mostly in small patient samples [59,73].

In contrast to these reports, Twig et al. evaluated cardiovascular risk factors in FMF patients and controls at ages 17 and 45 in a large cohort [74]. At age 17, the characteristics of 745 FMF patients were compared to those of 787,714 young adults without FMF. Following a multivariable regression analysis, it was found that FMF was associated with a lower risk for developing overweight (OR 0.65, 95% CI 0.44–0.96, $p=0.03$) and hypertension (OR 0.66, 95% CI 0.48–0.92, $p=0.012$) at that age. Also, 57 FMF patients and 1568 control army personnel aged 45 were compared. A hazard ratio (HR) of 0.32 (95% CI 0.10–0.82, $p=0.002$) for obesity, 0.49 (95% CI 0.25–0.95, $p=0.037$) for TG levels ≥ 150 mg/dL, 0.56 (95% CI 0.31–0.98, $p=0.048$) for LDL ≥ 130 mg/dL, and 0.49 (95% CI 0.23–1.00, $p=0.05$) and for elevated BP values were found in FMF patients, following a cox-regression multivariate analysis. In contrast, the HR for HDL < 40 mg/dL was 2.14 (1.368–3.359, $p=0.001$) in FMF patients, while DM was similarly found in both groups [74]. Bilginer et al. reported similar LDL, TG, and total cholesterol levels in 70 FMF patients and 50 control subjects, as well as increased HDL levels in the FMF patients (53.29 ± 17.9 vs 43.4 ± 8.48 mg/dL, $p=0.029$) [75]. A similar lipid profile in FMF patients compared with controls was also reported by several other authors in various age groups [76,77]. In conclusion, there is cumulating evidence for the lack of association between FMF and dyslipidemia, DM, and other traditional cardiovascular risk factors.

Homocysteine levels in FMF patients were also studied by several groups. Bilginer et al. found that the homocysteine levels were similar in FMF patients and control subjects [75]. In contrast, Peru et al. reported increased homocysteine (10.36 ± 3.36 vs 8.64 ± 3.15 μ mol/L, $p=0.035$) and lipoprotein-a (Lp-a) (20.84 ± 23.89 vs 8.56 ± 7.48 mg/dL, $p=0.013$) levels in FMF patients compared with control subjects [76]. Similarly, Karatay et al. also found homocysteine levels higher than 15μ g/dL in 55.8% of FMF patients compared to 26.7% of control subjects ($p=0.011$) and Lp-a higher than 30 mg/dL in 71.2% of FMF patients compared with 46.7% of control subjects ($p=0.028$) [78]. Therefore, it seems unsettled yet as for whether FMF patients have increased homocysteine and Lp-a levels, and whether this abnormality, if it exists, is FMF related, and has any clinical significance. These questions merit further investigations.

4.3 Markers for Atherosclerosis and Endothelial Dysfunction

Intima media thickness (IMT), flow-mediated dilation (FMD) of the brachial artery, and nitroglycerine-mediated dilation (NMD) were investigated in six studies (Table 24.5). IMT reflects the lumen narrowing attributed to atherosclerosis as measured at several anatomic landmarks along the carotid artery. In contrast, FMD and NMD are functional tests, in which the vasodilation induced by periodic ischemia (FMD as a reflection of endothelial function) or by exogenous NO (NMD) is measured by ultrasound imaging.

Akdogan et al. evaluated IMT and FMD in 43 FMF patients (11.6% of whom were colchicine resistant, with a high prevalence of the M694V mutation) and 29 healthy controls [79]. They reported increased IMT and decreased FMD values in FMF patients compared with the controls. Interestingly, M694V homozygous genetic background (compared with other mutations) was associated with more prominent FMD impairment as reflected by lower FMD values ($4.3 \pm 1.5\%$ vs $6.5 \pm 2.2\%$, $p=0.005$; Fig. 24.2). Not unexpectedly, a positive correlation was found between the maximum IMT of carotid arteries and age ($r=0.29$; $p=0.014$). Maximum IMT > 1.3 mm (referred to as atherosclerotic plaque in this particular study) was found in two FMF patients and none of the control group. FMD and IMT were not found to be influenced by the response to colchicine [79]. Yet, the results should be interpreted with caution due to unspecified patients' age, smoking status, presence of family history of ischemic heart disease, age at diagnosis, and length of colchicine treatment.

Bilginer et al. also reported on increased IMT in young adolescents with FMF (median age 14, 65% M694V homozygous) [75]. A positive correlation was found between the common carotid artery (CCA)-IMT and SAA levels ($r=0.24$, $p=0.04$). They also reported a positive correlation between the internal carotid artery (ICA)-IMT, erythrocyte sedimentation rate ($r=0.31$, $p=0.008$), and fibrinogen levels ($r=0.30$, $p=0.012$) [75]. Ugurlu et al. also evaluated IMT in a large group of 100 FMF patients (5% amyloidosis, 5% DM, 15% hypertension, 34% smoking, 5% other diseases, and obesity), comparing the results with 103 controls [80]. They found a significantly higher IMT both in the carotid artery and in the femoral artery in FMF. Exclusion of patients who had amyloidosis and concomitant diseases (18 patients) did not significantly alter the results. Surprisingly, femoral artery IMT was significantly lower in FMF patients with amyloidosis and concomitant cardiovascular risk factors compared with those without (0.47 ± 0.10 vs 0.58 ± 0.17 , $p=0.016$). This may be partially explained by a lower mean BMI in these patients.

Atherosclerotic plaque was defined in this study as a protrusion into the vessel lumen of $> 50\%$ of the surrounding wall. Interestingly, FMF patients had statistically

TABLE 24.5A Clinical Characteristics of Investigated Patient Groups in Studies Investigating IMT, FMD, and NMD

Study #	Group	n	Gender (F/M)	Age (years)	Colchicine resistance (%)	Amyloidosis (%)	Disease duration (years)	Colchicine treatment (years)	Colchicine dosage (mg/day)	Disease severity score	Attacks/year	BMI (kg/m ²)	Other CV comorbidities ^b	References
1	FMF	43	NA	NA	11.6	0	NA	NA	NA	NA	NA	NA	NA	[79]
	Control	29	NA	NA								NA	NA	
2	FMF	70	30/40	14.0 median	NA	0	6.9±3.0	5.7±3.5	1–1.5	NA	70% None 20% 2/year 10% >4/year	21.9±4.1	NA	[75]
	Control	50	24/26	14.0 median								21.5±2.6	NA	
3	FMF	49	26/23	10.7±3.7	NA	NA	2.5±1.8	NA	NA	NA	NA	17.7±3.0	None	[76]
	Control	26	13/13	10.5±3.9								18.1±3.1	None	
4	FMF	61	31/30	31.5 median	NA	0	16 median	NA	1.5 median	NA	2 median	24.8 median	34.4% smoking	[81]
	Control	31	15/16	31.0 median								24.3 median	29% smoking	
5	FMF	100	54/46	40.0±6.0	NA	5% with no CRF	21.5±9.3	10.7±7.0	NA	NA	NA	27.0±5.0	5% DM 15% HTN ^a 34% smoking 5% other diseases	[80]
	Control	103	59/44	40.0±5.0								27.0±5.0	44% smoking	
6	FMF	98	37/61	32.0 median	NA	100%, all with NA nephrotic syndrome		NA	NA	NA	NA	26.0 median	NA	[35]

BMI, body mass index; CV, cardiovascular; CRF, chronic renal failure; NA, not available; DM, diabetes mellitus; HTN, hypertension.

^a $p < 0.05$.

^bNA - in cases not all risk factors (including smoking) were specified/excluded.

TABLE 24.5B Laboratory Findings and Technical Aspects of Diagnostics in Studies Investigating IMT, FMD, and NMD

Study #	Group	FMF genotype	CRP (mg/dL)	SAA (mcg/mL)	Testing protocol and method	Mean-IMT CCA (mm)	Max-IMT Rt ICA (mm)	Mean IMT Rt ICA (mm)	Mean IMT Lt ICA (mm)	FMD (%)	NMD (%)	References
1	FMF	Of 28 patients:	0.3 ± NA ^a	21.82 U/L	Max-IMT - the highest value of 6 measurements	NA	0.79 ± 0.18 ^a	0.62 ± 0.08 ^a	0.61 ± 0.07 ^a	5.7 ± 2.4 ^a	NA	[79]
	Control	M694V (53.6%) V726A (14.9%) M680I (14.9%) 32.1% HoZ-M 46.4% CHtZ-M 7.1% HtZ-M M694V/M694V-E148Q (3.5%)	0.07 ± NA	10.33 U/L		NA	0.61 ± 0.11	0.53 ± 0.07	0.53 ± 0.07	10.8 ± 1.9	NA	
2	FMF	65% M694V HoZ-M 15% M694V/V726A	0.79 ± 0.41 ^a	55.9 ± 51.6 ^a	10 mm proximal and distal to the carotid bifurcation, average of 4 measurements	0.37 median ^{a, b}	NA	0.25 median ^{a, b}	NA	NA	NA	[75]
	Control	15% M694V/M680I 3.5% M694V/E148Q 1.5% M680I/V726A	0.40 ± 0.00	14.0 ± 2.6		0.28 median	NA	0.22 median	NA	NA	NA	
3	FMF	M694V (62%) M680I (15%)	10.7 ± 15.3 ^a	23.2 ± 41.9 ^a	20 mm below the carotid bulb, average of 3 measurements of the maximal IMT	0.038 ± 0.007 ^{a, b}	NA	NA	NA	NA	NA	[76]
	Control	V726A (10%) 52% HoZ-M 19% CHtZ-M 18% HtZ-M 4% DHoZ-M	4.0 ± 1.2	3.5 ± 1.0		0.032 ± 0.004	NA	NA	NA	NA	NA	
4	FMF	NA	0.218 median ^a	±	IMT - lower 1/3 cervical region proximally and 1 cm above the carotid bulb distally, averaged 3 measurements FMD - Cuff inflation to 200 mmHg, NMD - 5 mg nitroglycerin	0.50 median ^c	NA	NA	NA	18.2 median	24.2 median	[81]
	Control		0.102 median	±		0.51 median ^c	NA	NA	NA	20.6 median	22.5 median	
5	FMF	NA	0.43 median ^a	6.0 median ^{a, d}	IMT - measured at both the right and left sides of the near and far walls of the distal CCA, carotid bulbs and ICA. Averaged 8 measurements	0.57 ± 0.15 ^a	NA	NA	NA	NA	NA	[80]
	Control		0.31 median	3.0 median		0.48 ± 0.15	NA	NA	NA	NA	NA	
6	FMF	NA	1.43 median	NA	NA	NA	NA	NA	NA	6.0 median	NA	[35]

NA, not available; HoZ-M, homozygote mutations; DHoZ-M, double homozygote mutations; CHtZ-M, compound heterozygote mutations; HtZ-M, heterozygote mutations; CRP, C-reactive protein; SAA, serum amyloid A; IMT, intima-media thickness; Rt, right; Lt, left; CCA, common carotid artery; ICA, internal carotid artery; FMD, flow-mediated dilation; NMD, nitroglycerin-mediated dilation.

^a*p* < 0.05.

^bMeasured side not specified.

^cAveraged result of right and left measurements.

^dUnits not provided. Note that CRP and SAA levels were mentioned only if they were examined in attack-free period.

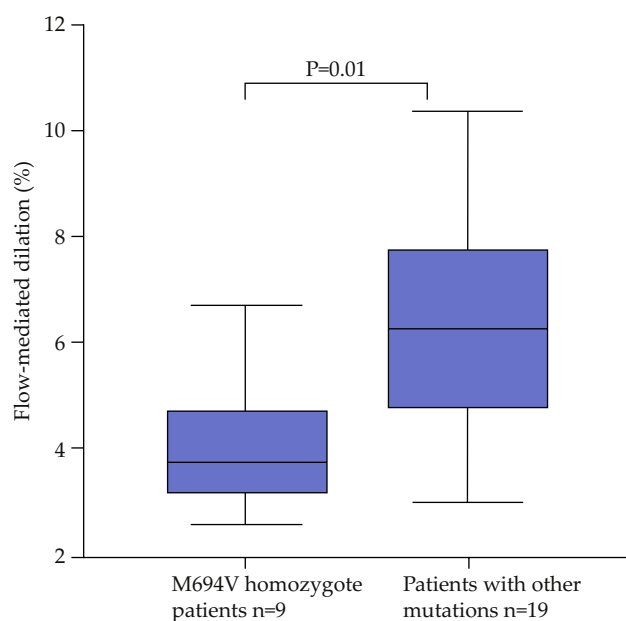


FIGURE 24.2 Comparison of flow-mediated dilation (FMD) between M694V homozygous FMF patients and FMF patients with other mutations. Adapted from Akdogan et al. [79].

similar rates of carotid artery atherosclerotic plaque (14% vs 9%, $p > 0.05$) and femoral artery atherosclerotic plaque (1% vs 5%, $p > 0.05$, respectively) compared with controls. There was a nonsignificant ($p = 0.07$) difference in plaque distribution in FMF patients vs controls: carotid bulb (69% vs 33%) ICA (29% vs 54%) and CCA (12% vs 13%). Following multiple regression analysis, the presence of atherosclerotic plaque in FMF patients was found to be associated only with diabetes. A correlation was found between carotid IMT values and age, BMI, and fasting glucose level in FMF patients. Femoral IMT values in FMF were found to be correlated with age and BMI [80].

In contrast to these studies, Sari et al., who evaluated IMT, FMD, and NMD in 61 FMF patients and 31 control subjects, found no statistically significant differences in any of the measured parameters. They also reported that none of the subjects had carotid artery plaque [81]. Yilmaz et al., who evaluated FMD in 98 patients with amyloidosis complicated with nephrotic syndrome, found that the median FMD was 6%, but the results were not compared to healthy controls [35]. Overall, FMD was found to be lower in FMF patients in two studies; statistical significance was found in one. Also, increased IMT was reported in four studies, while in a single study, no significant difference was found (Table 24.5). It is conceivable that certain genetic backgrounds (such as M694V homozygote) and treatment issues will affect endothelial function (as manifested by FMD), however, whether such an association is translated into a clinical outcome needs to be further explored.

4.4 Evaluation of Coronary Flow and Markers for Cardiac Ischemia

Myocardial ischemia can be diagnosed via biochemical blood testing (eg, troponin in the setting of acute myocardial infarction) and advanced invasive or non-invasive radiological cardiac studies. Karakurt Arıtürk et al. reported that the level of heart-type fatty acid-binding protein (h-FABP), a new small myocytic protein, recently proposed as a marker of acute coronary syndrome [82–84], was higher in FMF patients than in control subjects during remission (4.89 ± 0.83 vs 3.06 ± 2.13 ng/mL, $p < 0.01$), but not during attacks [57]. No subsequent studies have evaluated h-FABP in FMF patients. The cause for the peculiar finding, that there is constantly low-level myocardial damage, remains unclear.

Caliskan et al. evaluated coronary flow reserve (CFR) in 33 FMF patients (57.6% were M694V carriers) and 35 healthy control subjects [85]. Diastolic peak flow velocities (DPFV) at the distal left anterior descending (LAD) artery were measured, using color Doppler flow mapping at baseline and following dipyridamole infusion (0.56 mg/kg over 4 min). CFR was calculated by dividing hyperemic DPFV with baseline DPFV. They found that resting DPFV values were similar in the two groups (24.2 ± 3.7 vs 23.3 ± 3.2 , $p = 0.26$). However, FMF patients had significantly lower hyperemic DPFV (54.8 ± 9.9 vs 70.0 ± 12.4 , cm/sp $p < 0.001$) and CFR (2.27 ± 0.38 vs 3.02 ± 0.50 , $p < 0.001$). CFR was found to inversely correlate with hsCRP levels ($r = -0.426$) and FMF disease duration ($r = -0.429$). Also, a positive correlation was found with HDL levels ($r = 0.402$). Importantly, certain genotypes (M694V/M694V and 680I/680I) were found to associate with the lowest CFR (Fig. 24.3). The authors suggested that impaired CFR in FMF may reflect coronary vascular involvement in FMF [85]. Nonetheless, these results were neither investigated nor confirmed by subsequent studies.

4.4.1 Stress Test

Canpolat et al. conducted exercise stress tests and reported that FMF patients were asymptomatic during the postexercise period [86]. Completion of exercise stress tests by small groups of FMF patients without rhythm abnormalities, ECG changes, major physical complaints or other complications were also reported by Ardic et al. [87] and Sahin et al. [88]. Although exercise stress tests may be of low sensitivity in certain patient populations, the lack of abnormal results in the few studies that reported on exercise tests in FMF patients are in line with the view that FMF is not associated with increased cardiovascular risk. Nevertheless, these studies were not specifically aiming at the evaluation of intolerance to a stress but rather at other parameters during and following exercise, such as heart rate response to physical activity. Therefore, no definitive conclusions can be drawn from these studies on coronary blood vessel function.

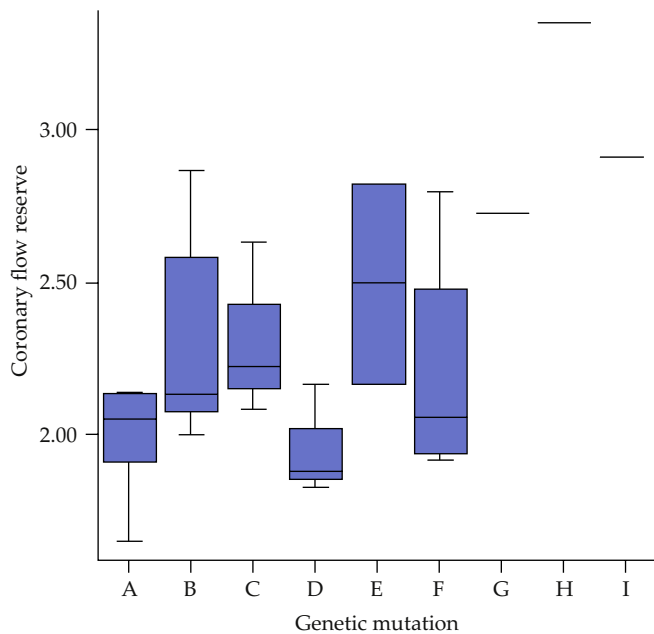


FIGURE 24.3 Coronary flow reserve (CFR) values of the subjects with each genetic mutation seen in the study population. A, M694V-M694V (n=8); B, M694V- (n=7); C, M694V-M680I (n=4); D, M680I-M680I (n=4); E, M680I- (n=2); F, M680I-V726A (n=5); G, M680I-A744S (n=1); H, V726A- (n=1); I, without genetic mutation (n=1). Adapted from Caliskan et al. [85].

4.5 Prevalence of Ischemic Heart Disease and Cardiac Functional Abnormalities in FMF Patients

There are anecdotal reports in the literature as to the occurrence of myocardial infarction (MI) in FMF patients, including in very young adults [71,89,90]. We reported a rate of 10% (2 of 20 patients) MI in FMF-amyloidosis. However, these patients had multiple cardiovascular risk factors, including hypertension, renal failure, and various medications administered to prevent renal transplant rejection [91].

Akar et al. evaluated the outcome of 587 FMF patients, who were followed in their medical facility. The mean age at the beginning of the follow-up was 32.4 ± 12.3 years. Most patients were treated with colchicine, although only 61.4% used it regularly. IHD occurred in 2.4% of the patients, a rate similar to that reported in the same age group in the general population [7].

In another study, Langevitz et al. included 290 colchicine-treated FMF patients above 40 years (mean age 55.0 ± 8.8 years, F:M ratio 1) and 233 spouses of the FMF patients (mean age 53.8 ± 10.0 years, $p > 0.05$, F:M ratio 1.24), allegedly with similar environmental exposure. The rate of IHD was found to be comparable in FMF patients, their spouses (15.5% vs 11.2%, $p > 0.05$), and in individuals of the general Israeli population, matched for age and gender, obtained from population reference data of ministry of health (16%, $p > 0.05$) [27]. Importantly, the prevalence of IHD in FMF patients was

significantly lower compared with the rate of IHD in patients with other chronic inflammatory conditions (ie, rheumatoid and psoriatic arthritis, etc. 15.5% vs 30.2%, $p < 0.05$) [27]. Thus, the only two existing large cohorts that evaluated IHD did not find an increased rate of IHD in FMF patients.

4.6 Echocardiographic Findings in FMF

Echocardiographic parameters in FMF were reported in 17 studies (Table 24.6). All studies but one reported on normal ejection function in FMF. Terekci et al. investigated the effect of FMF attacks on echocardiographic parameters. Thirty-four young males with FMF were studied during an attack and during the remission phase of the FMF disease. They found no effects during an FMF attack on tissue Doppler imaging (TDI) echocardiographic parameters compared with the results during the attack-free period [92].

Acar et al. conducted an atrial TDI study and evaluated PA (ie, the time interval from the onset of P-wave on surface electrocardiogram to the beginning of a late diastolic Am wave, hence representing atrial electromechanical coupling) [93]. In their study, PA was obtained from the lateral mitral annulus (PA lateral), septal mitral annulus (PA septum), and right ventricular tricuspid annulus (PA tricuspid). PA lateral was found to be significantly higher in FMF patients compared with controls (58.0 ± 9.0 vs 51.0 ± 5.8 ms, $p < 0.001$). PA septum and PA tricuspid were similar in both groups. Thus, the difference between PA lateral and PA tricuspid (which is assumed to represent interatrial electromechanical delay) and the difference between PA septum and PA tricuspid (assumed to represent intra-atrial electromechanical delay) were significantly higher in FMF patients compared with controls [93]. Notably, abnormal interatrial electromechanical delay was reported to be linked to increased risk for stroke [94]. Yet, the later association was not investigated in FMF.

Arslan et al. reported that pediatric patients with FMF had similar cardiac anatomy, compared with control patients, adjusted for age and blood pressure. However, the isovolemic contraction and relaxation time of the ventricles were significantly higher in FMF [95].

Tavil et al. also investigated left and right ventricular structure and function in FMF patients and controls [96]. They reported that the LV diameters, ejection fraction, and LV diastolic filling velocities were similar in both groups. However, compared to normal control subjects, in FMF, mitral relaxation time was increased (107 ± 25 vs 85 ± 10 ms; $p < 0.001$), early diastolic peak velocity at mitral annulus was lower ($E'm$; 12.6 ± 3.4 vs 14.7 ± 3.3 cm/s, $p = 0.04$), late diastolic peak velocity was higher ($A'm$; 10.1 ± 2.6 vs 8.6 ± 2.0 cm/s, $p = 0.015$), and $E'm/A'm$ ratio was lower (1.24 ± 0.4 vs 1.71 ± 0.5 , $p = 0.012$). According to the authors, these findings suggest left ventricular diastolic dysfunction

TABLE 24.6A Clinical Characteristics of Investigated FMF Patient Groups Evaluated for Their Echocardiographic Findings

Study #	Group	n	Gender (F/M)	Age (years)	Colchicine resistance (%)	Amyloidosis (%)	Disease duration (years)	Colchicine treatment (years)	Colchicine dosage (mg/day)	Disease severity score	Attacks/year	BMI (kg/m ²)	Other CV comorbidities ^b	References
1	FMF	38	20/18	34.4±10.2	10.4	0	9.8±4.2	8.6±3.5	1–1.5	6.6±2.4	12.4±8.8	21.2±2.1	NA	[86]
	Control	34	18/16	33.2±9.3								22.3±1.8	NA	
2	FMF	38	24/14	36.2±12.1	NA	0	11.3±6.5	6.7±3.1	NA	NA	NA	24.0±3.5	None	[87]
	Control	35	23/13	34.1±9.9								23.4±2.9	None	
3	FMF	34	0/34	22.0±2.0	NA	NA	5.0±3.0	NA	NA	NA	NA	23.0±2.0	None	[92]
4	FMF	50	20/30	29.4±11.8	NA	0	6.5±7.1	NA	NA	NA	NA	24.2±4.8	10% Smoking	[103]
	Control	50	20/30	31.3±11.9								24.6±4.7	14% Smoking	
5	FMF	33	20/13	28.4±12.5	NA	NA	6.8±7.7	NA	1.5 in all patients	NA	NA	23.7±5.4	12% Smoking	[93]
	Control	33	20/13	28.5±12.1								24.3±5.0	12% Smoking	
6	FMF	26	10/16	9.8±3.8	NA	0	3.0±2.0	NA	1–2.5	NA	NA	NA	None	[95]
	Control	25	11/14	9.3±2.9								NA	None	
7	FMF	30	19/11	35.0±8.0	NA	NA	15.4±7.6	NA	1–1.5	NA	NA	24.0±6.0	37% Smoking	[96]
	Control	30	18/12	33.0±7.0								23.0±5.0	30% Smoking	
8	FMF	31	19/12	36.0±8.0	NA	NA	12.4±7.6	5.6±2.7	NA	NA	NA	24.0±6.0	35% Smoking. UKR DysLip	[97]
	Control	27	17/10	34.0±7.0								23.0±5.0	33% Smoking. UKR DysLip	
9	FMF	70	43/27	11.1±3.5	NA	0	3.8±0.8	NA	NA	NA	NA	NA	None	[104]
	Control	50	30/20	10.7±3.1								NA	None	
10	FMF	38	20/18	32.8±11.0 ^a	NA	10.3 Proteinuria	6 - median	6 - median	NA	NA	NA	NA	37% Smoking	[105]
	Control	35	16/19	28.1±9.1								NA	34% Smoking	

11	FMF	23	13/10	32.3±9.4	NA	0	9.4±6.5	NA	1–1.5	NA	NA	25.1±3.8	47.8% Smoking	[106]
	Control	22	12/10	32.5±7.0								24.4±3.5	50% Smoking	
12	FMF	33	17/16	36.7±12.0	NA	NA	NA	NA	NA	NA	NA	25.8±4.5	None	[85]
	Control	35	15/20	36.8±5.2								26.9±2.1	None	
13	FMF	29	16/13	10.1±3.8	NA	NA	2.4±1.8	NA	NA	NA	NA	17.7±3.8	None	[77]
	Control	30	16/14	10.7±3.4								18.1±3.1	None	
14	FMF	44	23/21	Median 30.0	NA	0	Median 15.5	NA	0.5–2	NA	Median 2	Median 23.4	30% Smoking	[98]
	Control	27	15/12	Median 29.5								Median 22.2	30% Smoking	
15	FMF 1	14	7/7	35.5±16.6	0%	Nonuremic amyloidosis, 8/14 pts FMF	NA	NA	NA	NA	NA	NA	NA	[107]
	FMF 2	9	6/3	32.7±12.9	0%	Uremic amyloidosis, 7/9 pts FMF	NA	NA	NA	NA	NA	NA	NA	
	Control	19	11/8	41.6±10.8								NA	NA	
16	FMF	25	12/13	11.8±5.3	NA	NA	5.9±3.8	1.9±1.7	0.81±0.28	NA	2.7±3.2	21.5±2.5	NA	[99]
	Control	23	13/10	9.9±3.7								17.8±2.9	NA	
17	FMF	69	33/36	10.8±2.8	NA	0	4.6±3.2	NA	NA	NA	NA	NA	None	[100]
	Control	71	34/37	9.9±2.8								NA	None	

NA, not available; BMI, body mass index; CV, cardiovascular; UKR, unknown rate; DysLip, dyslipidemia; NA, not available.

^a*p* < 0.05.

^bNA - in cases not all risk factors (including smoking) were specified\excluded.

TABLE 24.6B Echocardiographic Findings in FMF Patients

Study #	Group	FMF genotype	CRP (mg/dL)	SAA (mcg/mL)	LVEF (%)	LVEDD (mm)	LVESD (mm)	IVSd (mm)	LVPW (mm)	LVMI (g/m ²)	LA-D (mm)	Mitral E/A ratio	References
1	FMF	M694V (57.8%) V726A (10.5%) M680I (10.5%)	3.2±2.2 ^a	48.5±21.4 ^a	64.4±4.1	48.0±4.4	28.6±2.2	NA	NA	NA	NA	NA	[86]
	Control	36.8% HoZ-M 52.6% CHtZ-M 10.5% HtZ-M	0.8±0.2	10.4±4.6	67.1±4.8	46.8±3.8	28.5±3.3	NA	NA	NA	NA	NA	
2	FMF	NA	NA	NA	65.3±6.7	47.7±5.0	30.4±4.5	9.1±1.2	8.4±1.3	NA	NA	NA	[87]
	Control		NA	NA	64.9±6.5	48.0±4.3	30.9±3.6	9.2±1.3	8.3±1.0	NA	NA	NA	
3	FMF	NA	NA	NA	NA	51.0±5.0	32.0±5.5	9.0±1.0	8.0±2.0	NA	30.0±6.0	NA	[92]
4	FMF	NA	25.4±30.8 ^a	NA	66.6±5.6	46.6±3.9	29.4±3.3	9.1±1.3	8.3±1.1	89.3±16.1	31.3±5.0	1.4±0.4	[103]
	Control		3.4±1.4	NA	66.9±4.9	46.3±4.5	29.3±3.9	9.0±1.2	8.1±1.2	88.8±17.3	32.1±4.8	1.5±0.4	
5	FMF	NA	1.95±1.79	NA	66.6±6.0	46.2±3.9	29.1±3.6	9.1±1.5	8.3±1.2	89.8±16.3	30.3±5.3	NA	[93]
	Control		0.34±0.05	NA	66.1±4.9	46.5±4.7	29.5±3.8	8.6±1.2	7.9±1.1	85.3±17.5	31.4±4.8	NA	
6	FMF	M694V (42.3%) V726A (26.9%) M680J (23.1%)	7.5±1.1	NA	70.6±6.1	36.4±7.1	21.8±3.6	7.0±1.4	6.8±1.6	NA	25.5±4.3	1.5±0.2	[95]
	Control	19.2% HoZ-M 38.5% CHtZ-M 42.3% HtZ-M	3.4±0.9	NA	71.0±5.5	38.1±6.0	21.4±3.5	7.2±1.2	6.9±1.3	NA	25.4±3.1	1.5±0.1	
7	FMF	NA	NA	NA	68.6±8.1	46.7±3.9	28.4±3.7	9.4±1.2	8.8±1.1	NA	NA	1.24±0.4 ^a	[96]
	Control		NA	NA	69.5±8.0	43.8±8.3	26.2±5.2	9.3±0.8	8.4±1.1	NA	NA	1.71±0.5	
8	FMF	NA	NA	NA	73.0±8.0	46.0±5.0	28.0±4.0	9.0±0.8	9.0±1.2	82.0±24.0	NA	1.23±0.31	[97]
	Control		NA	NA	74.0±5.0	43.0±4.0	26.0±4.0	9.0±0.8	8.0±0.9	76.0±16.0	NA	1.39±0.26	
9	FMF	NA	0.04±0.07	NA	71.5±4.6	37.5±5.1	22.4±3.7	6.1±1.4	6.5±1.7	NA	22.6±4.1	NA	[104]
	Control		0.03±0.02	NA	71.2±4.3	37.7±5.5	22.6±3.8	6.1±1.5	6.6±1.9	NA	23.4±4.1	NA	
10	FMF	NA	2.6±11.3	NA	60.4±4.0 ^a	NA	NA	NA	NA	NA	NA	NA	[105]
	Control		3.0±4.9	NA	63.0±5.0	NA	NA	NA	NA	NA	NA	NA	
11	FMF	NA	NA	NA	69.0±4.4	47.4±3.8	28.6±2.2	9.1±1.0	9.3±1.0	NA	33.0±2.7 ^a	1.33±0.30	[106]
	Control		NA	NA	67.0±4.2	47.9±3.9	28.5±3.3	8.9±1.0	9.1±0.9	NA	31.0±3.3	1.47±0.42	

12	FMF	24.2% M694V–M694V 21.1% M694V 12.1% M694V–M680I 12.1% M680I–M680I	0.88±0.36 ^a	NA	67.0±4.5	45.3±3.5	28.6±3.1	9.2±1.2	8.8±0.7	75.8±12.8	NA	1.11±0.23 ^a	[85]
	Control	6.1% M680I– 15.1% M680I–V726A 3.0% M680I–A744S 3.0% V726A–	0.21±0.36	NA	67.0±2.3	45.6±4.2	28.5±3.0	9.2±1.3	9.1±1.3	80.4±12.4	NA	1.34±0.25	
13	FMF	In 26 pts: M694V (62% of mutations, 71% of pts)	10.8±4.7 ^a	22.3±39.4 ^a	68.3±4.9	39.3±5.2	24.8±4.3	NA	NA	NA	NA	1.86±0.41 ^a	[77]
	Control	M680I (15%) V726A (10%). 53.8% HoZ-M	4.1±1.2	3.6±1.1	67.9±4.9	39.8±5.8	23.9±4.1	NA	NA	NA	NA	1.16±0.20	
14	FMF	NA	Median 0.250	NA	Median 66.0	Median 46.0	Median 31.0	Median 8.0	Median	Median 82.8	Median 3.4	Median 1.40	[98]
	Control		Median 0.093	NA	Median 65.0	Median 47.0	Median 29.0	Median 8.0	Median	Median 74.3	Median 3.2	Median 1.47	
15	FMF 1	NA	NA	NA	72.1±7.8	49.6±4.4	NA	8.9±1.2	8.8±2.1	NA	NA	1.29±0.23	[107]
	FMF 2		NA	NA	70.3±11.3	49.4±5.2	NA	8.2±1.4	9.2±0.9	NA	NA	1.25±0.14	
	Control		NA	NA	68.6±8.3	49.4±4.9	NA	8.6±1.3	8.3±1.5	NA	NA	1.21±0.14	
16	FMF	All were homozygote M694V (56%)	1.06±1.32 ^a	NA	70.8±4.4	36.1±5.7	21.8±3.0	7.2±1.2	7.4±1.1	NA	25.5±3.3	2.03±0.45	[99]
	Control	M680I (28%) V726A (16%)	0.18±0.15	NA	71.7±4.8	34.5±5.3	20.6±3.1	7.4±1.3	7.0±1.1	NA	24.8±3.5	1.85±0.50	
17	FMF	NA	NA	NA	65.5±7.2	39.3±5.1	23.7±3.6	6.8±1.2	6.8±1.1	NA	25.7±3.4	1.99±0.43	[100]
	Control		NA	NA	66.2±6.5	38.8±6.2	24.4±2.9	7.1±0.9	6.8±1.2	NA	25.4±2.4	1.98±0.27	

NA, not available; *pts*, patients; *HoZ-M*, homozygote mutations; *CHtZ-M*, compound heterozygote mutations; *HtZ-M*, heterozygote mutations; *HR*, heart rate; *CRP*, C-reactive protein; *SAA*, serum amyloid A; *LVEF*, left ventricle ejection fraction; *LVEDD*, left ventricle end-diastolic diameter; *LVESD*, left ventricle end-systolic diameter; *IVSd*, interventricular septum thickness during diastole; *LVPW*, left ventricle posterior wall thickness; *LVMI*, left ventricular mass index; *LA-D*, left atrial dimension.

^a*p* < 0.05; note that CRP and SAA levels were mentioned only if examined during an attack-free period.

in the investigated FMF patients. Interestingly, no correlation was found between any clinical feature and the aforementioned echocardiographic findings. In contrast, a TDI study of the right ventricle did not reveal any difference in the investigated parameters between the groups, thus excluding right-heart diastolic failure [96].

In another study by the same authors (with a similar study design), Tavil et al. [97] did not find a significant difference in A and E values, as well as in the A/E ratio, compared with controls. Nevertheless, they reported a prolonged left ventricular isovolumic relaxation time (IVRT) in FMF patients compared with controls (103 ± 14 vs 91 ± 13 ms, $p=0.01$) [97]. Caliskan et al. who studied 33 FMF patients and 35 controls [85] found that anatomical dimensions and function were similar in both groups. Nevertheless, mitral A-wave duration was significantly increased in FMF patients (70.7 ± 14.4 vs 59.7 ± 11.6 cm/s, $p=0.001$), the E/A ratio was significantly lower (1.11 ± 0.23 vs 1.34 ± 0.25 , $p<0.001$), and mitral E-wave deceleration was significantly increased (205.9 ± 28.1 vs 187.2 ± 16.7 ms, $p=0.002$). Lateral Em and lateral Em/Am ratios were statistically similar in both groups, yet, values for lateral Am (16.6 ± 4.0 vs 14.2 ± 2.6 cm/s, $p=0.006$) and lateral IVRT were significantly increased in FMF patients (91.7 ± 16.6 vs 84.4 ± 8.4 ms, $p=0.030$). The authors concluded that the results could be attributed to diastolic dysfunction in FMF [85].

Baysal et al., who evaluated echocardiographic parameters in 29 children with FMF and 30 controls [77], found that anatomical parameters were similar in both groups. Nevertheless, FMF patients has a significantly higher mitral A velocity (7 ± 1.54 vs 6.55 ± 0.55 cm/s, $p=0.001$) and a significantly higher mitral E/A ratio (1.86 ± 0.41 vs 1.68 ± 0.20 , $p=0.001$). Using TDI, they found that left ventricular IVRT and left ventricular isovolumetric contraction time (IVCT) were statistically similar in both groups. However, FMF patients had lower peak early mitral diastolic velocity ($E'm$, 90.20 ± 14.23 vs 97.51 ± 6.07 cm/s, $p=0.010$), higher peak late diastolic velocity ($A'm$, 55.49 ± 8.35 vs 51.82 ± 4.30 cm/s, $p=0.037$) and lower $E'm/A'm$ ratio (1.66 ± 0.36 vs 1.89 ± 0.15 , $p=0.002$). They also concluded that left ventricular diastolic dysfunction is common in children with FMF. Notably, the diastolic function of the right ventricle was not evaluated in the present study [77].

Sari et al. who also attempted to address echocardiographic changes in FMF, included in their study 44 patients (none of whom had amyloidosis) and 27 control subjects [98], and found a significantly higher tricuspid annular plane systolic excursion <2.0 cm in FMF (8/44) compared with controls (1/27, $p=0.025$). The mitral E and A velocity were statistically similar in both groups as well as E/A ratio (median of 1.40 vs 1.47, $p>0.05$). IVRT was statistically similar (median of 75 vs 77 ms, $p>0.05$), although E wave deceleration time was significantly

lower in FMF patients (median of 133 vs 145 ms, $p=0.04$). TDI testing demonstrated similar mitral $E'm$ peak velocity and $S'm$ peak velocity, but borderline $A'm$ peak velocity (median of 10 vs 8 cm/s, $p=0.05$). The $E'm/A'm$ ratio was significantly lower in FMF compared with controls (median of 1.77 vs 1.79, $p=0.02$). The authors concluded that left ventricular diastolic dysfunction is common in FMF. Also, based on the presence of lower tricuspid $E'm/A'm$ ratio in FMF patients (median of 1.1 vs 1.4, $p<0.001$) and the fact that more FMF patients had an $E'm/A'm$ ratio <1.0 (19/44 vs 0/27, $p<0.001$), the authors concluded that FMF patients generally have right ventricle diastolic dysfunction [98]. The results on the diastolic function of the right ventricle contrast those published by Tavil et al. [96].

Ozdemir et al. evaluated 25 children with FMF (mean age 11.8 ± 5.3 years) and 23 control subjects (9.9 ± 3.7 years, $p>0.05$) [99]. They found no significant difference in any of the anatomical cardiac parameters. In a TDI study, a significantly lower $E't/A't$ ratio (early to late diastolic peak velocity of tricuspid annulus) was found in FMF patients, compared with control subjects (1.94 ± 0.81 vs 2.19 ± 0.59 , $p=0.03$). All other TDI results were similar in both groups including $E'm$ peak mitral velocity (16.2 ± 2.56 cm/s in FMF patients vs 15.6 ± 3.45 in controls, $p>0.05$), $A'm$ peak mitral velocity (5.51 ± 1.31 vs 6.22 ± 2.20 , $p>0.05$), $E'm/A'm$ ratio (3.04 ± 0.68 vs 2.66 ± 0.68 , $p>0.05$), $E'm$ wave deceleration time (84.1 ± 13.3 vs 83.7 ± 12.8 , $p>0.05$), and isovolumic relaxation time (49.7 ± 4.47 vs 54.3 ± 6.38 , $p>0.05$). Also, $E't$ peak velocity and $A't$ peak velocity differences in both groups did not reach statistical significance. The authors concluded that children with FMF have impaired right ventricular diastolic dysfunction [99].

In contrast to these results, Koca et al. evaluated 69 young children with FMF (10.8 ± 2.8 years) and 71 age- and sex-matched healthy control subjects. They found no difference in anatomical cardiac systolic and diastolic parameters [100]. No TDI measurements were performed.

In conclusion, most studies reported abnormal echocardiographic parameters suggestive of diastolic dysfunction in FMF patients. The pathology behind diastolic dysfunction in FMF remains obscure. If it indeed exists, it seems to be subclinical, as to date there is no evidence for increased rate of congestive heart failure (CHF) in FMF. It is known that cardiac inflammation and secondary fibrosis is associated with diastolic dysfunction [101,102]. Nevertheless, myocardial leukocyte infiltration does not seem to exist in FMF.

4.7 Coronary Vasculitis

Although overall rare, FMF patients have an increased risk for developing systemic vasculitis, such as polyarteritis nodosa (PAN, was reported in

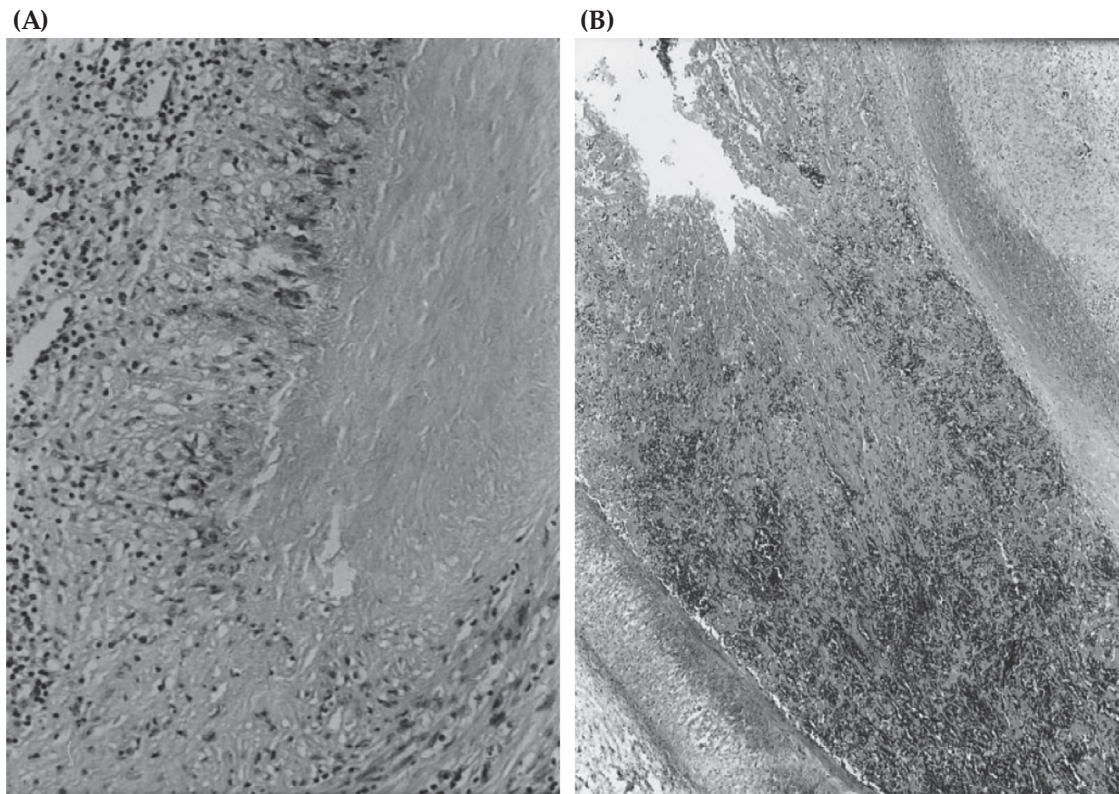


FIGURE 24.4 Histological findings in the left coronary artery (palisading granuloma; panel A) and right coronary artery (lumen occlusion; panel B). Adapted from Serrano et al. [89].

about 1% of FMF patients), Henoch-Schönlein purpura (HSP, reported in 2.6–7% of FMF patients), and Behcet's disease (BD, reported in 0.8% of FMF patients in the Israeli population). Also, *MEFV* mutations were reported to occur at a high rate in PAN and HSP (27–34% and 38% of patients, respectively) [108]. There are also case reports of FMF patients who developed Takayasu's arteritis [109] and other forms of arteritis. Rarely, systemic vasculitis may be complicated by coronary vasculitis.

Serrano et al. described a 29-year-old FMF-amyloidosis patient who received a kidney transplant at age 24 [89]. The patient presented with clinical and laboratory signs of myocardial infarction, treated with balloon angioplasty to an occluded left anterior descending artery, but died a few hours later due to cardiogenic shock. Histological evaluation of the heart found a few amyloid deposits in the subendocardium, left coronary artery perivascular lymphocyte infiltrate, and a formation of palisading granuloma (Fig. 24.4, panel A). In addition, an examination of the right coronary artery demonstrated complete occlusion by an intravascular thrombus (Fig. 24.4, panel B). Vasculitis was restricted to the large epicardial arteries and was not associated with fibrinoid necrosis, arterial microaneurysms, or vasculitis in other organs, thus making PAN less likely [89].

Ambartsymian also suggested that coronary vasculitis may underlie cardiac ischemia in amyloidosis of FMF (a conclusion supported by histopathologic findings in the hearts of FMF patients who died from amyloidosis complications) [71]. In conclusion, coronary vasculitis may occur in FMF in association with vasculitides, commonly occurring in FMF, but not as a manifestation of FMF itself.

4.8 Electrocardiographic Findings and Conduction Disorders

Eliakim et al. were the first to report on ECG changes in 12 patients during and in between FMF attacks [110]. They reported that 3/12 (25%) patients exhibited ECG changes during FMF attacks including a slight ST segment elevation, transient T wave inversion, and other nonspecific ST-T changes (Fig. 24.5). The authors also recorded resting ECG of 18 other patients in between attacks and found no abnormalities. Eliakim et al. concluded that ECG changes could be attributed to pericarditis in at least two of the patients [110]. Characteristic ECG changes related to acute pericarditis were reported by Erol et al. [111] and Kees et al. [24] while normal ECG at baseline were reported by Şahin et al. [88], Canpolat et al. [86], and Ardic et al. [87].

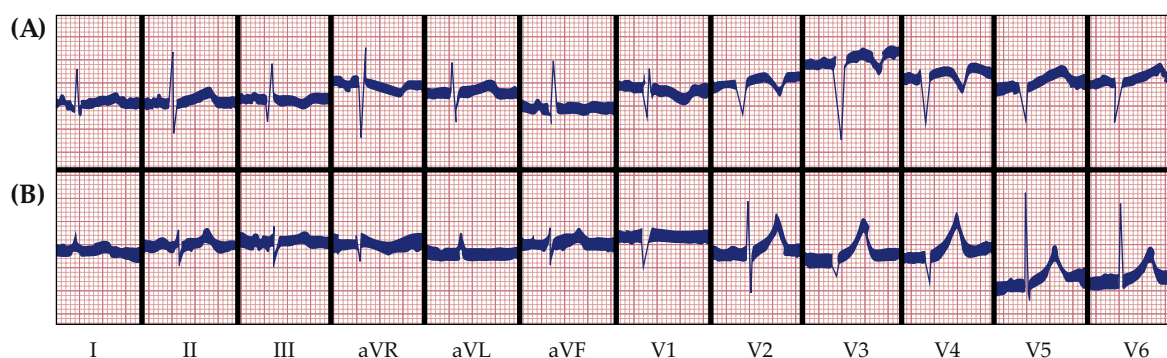


FIGURE 24.5 ECG changes in a 14-year-old boy with FMF during an attack (panel A), and in between attacks (panel B). Note anterolateral T wave inversion during an attack that normalized thereafter. Adapted from Eliakim and Ehrenfeld [110].

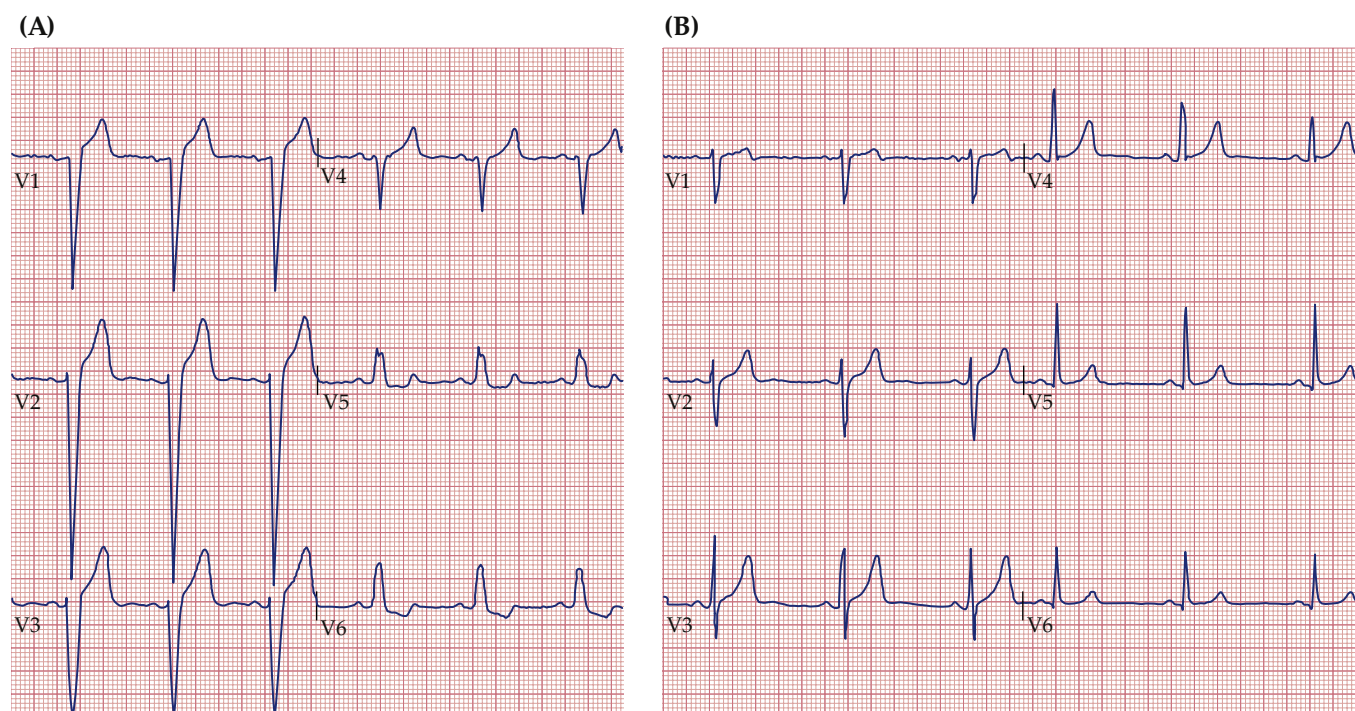


FIGURE 24.6 CLBBB in an FMF patient during an attack (panel A) and ECG normalization following colchicine initiation (panel B). Adapted from Cocco [112].

Cocco [112] reported a 53-year-old patient with systemic vasculitis Raynaud's phenomenon, high levels of antineutrophil antibodies, and histologic findings consistent with microscopic polyangiitis. The patient also had two MEFV mutations. He presented with FMF attacks during which CLBBB on ECG was observed (Fig. 24.6, panel A), but troponin test was negative. The patient responded well to colchicine therapy, including with a normalization of his ECG changes (Fig. 24.6, panel B). The authors thought that ECG changes could have stemmed from coronary vasculitis, but, good response to colchicine, without

coadministration of steroids or immunosuppression and absence of a rise in troponin levels made this suggestion less likely [112]. Also, and in the context of FMF, other options for transient ECG changes such as atherosclerosis related complications, or pericarditis are plausible [113].

Based on published data, but not on our experience, it can be concluded that ECG changes during FMF attacks may occur. Surprisingly, the correlation between ECG changes and echocardiographic and laboratory results has not been systematically investigated in FMF during and in between attacks.

4.9 Prevalence of Ventricular Arrhythmias

In a 24-h Holter ECG conducted on 38 FMF patients, grade 2 ventricular premature complexes (according to the Lown grading system) were reported in three patients (7.89%). No association with disease duration and severity could have been established [86]. In contrast, Fidanci et al. did not document any ventricular arrhythmias in 70 pediatric patients with FMF using Holter ECG [104]. Also, Şahin et al., who conducted a 24-h Holter ECG in 35 young FMF patients and 35 healthy controls, found a similar rate of ventricular ectopic stimuli in both groups ($p=0.299$) [88]. In our experience, ventricular arrhythmias are uncommonly encountered in FMF, and when occurring can usually be attributed to other reasons rather than to FMF.

4.10 Markers of Increased Risk for Ventricular Arrhythmias

4.10.1 QT Dispersion

QT dispersion (QTd) is an electrocardiographic marker that was suggested to reflect on repolarization heterogeneity and its measurement is based on the assumption that each electrocardiographic lead measures regional repolarization. Dispersion value represents the largest difference of repolarization parameters (either QT, QTc, JT, or others) where measurements are taken from a 12-lead ECG. The presence of such electrophysiological heterogeneity is assumed to facilitate the occurrence of reentrant cardiac arrhythmia.

QT dispersion parameters in FMF patients were evaluated in nine studies (Table 24.7), two reported increased QT parameters in FMF patients, while seven found similar values in FMF patients and control subjects. Neither colchicine nonresponsiveness nor the presence of amyloidosis was associated with increased QT dispersion in FMF. This finding is in line with the lack of increased reported rates of ventricular arrhythmias in FMF.

Interestingly, Canpolat et al. reported a correlation between QTcd and CRP/ESR ($r=0.34$, $p<0.001$; $r=0.42$, $p<0.001$, respectively) [86]. Akcay et al. also found a positive correlation between QTcd and CRP ($r=0.30$, $p<0.001$) or ESR ($r=0.40$, $p<0.001$) in FMF patients [103]. It should be noted that a wide range of QTd values were reported in the healthy and in patients with different types of cardiac diseases (with a large overlapping between the two). There is no agreed QTd threshold for increased risk of developing arrhythmias [114]. In addition, the prognostic value of QTd measurements in some subsets of patients was questioned recently [115], further complicating the interpretation of the aforementioned results.

4.10.2 QT Variability Index

QT variability reflects the changes in duration of repolarization along time. QT variability index (QTVI) in FMF patients was assessed in two studies. QTVI was computed by a log ratio adjusted to RR interval according to the following equation:

$$QTVI_{(RR)} = \log_{10} \left[\frac{QT_v / QT_m^2}{RR_v / RR_m^2} \right]$$

Mean QT (QT_m) and QT variability (QT_v), as well as mean RR (RR_m) and variability (RR_v), are required for QTVI calculation. Normalized QT variability (QTVN), unlike QTVI, is not adjusted either to heart rate variability or to RR interval changes. Therefore, QTVN was calculated for the mean QT:

$$QTVN = \frac{QT_v}{QT_m^2}$$

In the first study, QTVI results were found to be similar in 53 FMF patients (50.9% were colchicine nonresponders) and 53 controls. A separate comparison of FMF patients who responded well to colchicine and those who were colchicine resistant revealed both groups with a similar QTc, QTVI, and QTN [120]. In a subsequent study, 12 FMF patients who developed amyloidosis were investigated [121]. QTVI results were significantly higher in FMF-amyloidosis compared with healthy controls (Fig. 24.7). A history of renal transplantation was associated with lower QTVI results compared with patients who had developed end-stage renal disease and remained on dialysis. However, the results did not reach statistical significance (-1.15 ± 0.31 , vs -0.96 ± 0.48 , $p>0.05$). Therefore, it can be concluded that QT variability increases in amyloidosis of FMF but not in uncomplicated FMF, regardless of the response to colchicine. Notably, the investigated FMF-amyloidosis patients had other cardiovascular risk factors that may be associated with arrhythmogenicity.

In the later study, the involvement of the heart itself with amyloidosis was not directly sampled and therefore it remains unknown whether increased QTVI in FMF-amyloidosis (supposedly associated with an increased risk for arrhythmias) might be attributed to cardiac amyloidosis or to other causes such as autonomic nervous system involvement. Similarly to QTd, a lack of consensus exists as for the threshold value above which QTVI is arrhythmogenic. Also, the reported QTVI in FMF-amyloidosis was lower (and thus less indicative of an adverse cardiovascular prognosis) than that of patients with a high rate of ventricular arrhythmias reported in other studies (-0.8

TABLE 24.7A Clinical Characteristics of Investigated FMF Patient Groups Evaluated for Their QT Dispersion Values

Study #	Group	n	Gender (F/M)	Age (years)	Colchicine resistance (%)	Amyloidosis (%)	Disease duration (years)	Colchicine treatment (years)	Colchicine dosage (mg/day)	Disease severity score	Attacks/year	BMI (kg/m ²)	Other CV comorbidities ^b	References
1	FMF	50	20/30	29.4±11.8	NA	0	6.5±7.1	NA	NA	NA	NA	24.2±4.8	10% Smoking	[103]
	Control	50	20/30	31.3±11.9								24.6±4.7	14% Smoking	
2	FMF	38	20/18	34.4±10.2	10.4	0	9.8±4.2	8.6±3.5	1–1.5	6.6±2.4	12.4±8.8	21.2±2.1	NA	[86]
	Control	34	18/16	33.2±9.3								22.3±1.8	NA	
3	FMF	30	16/14	33.5±10.2	NA	NA	21.7±9.3	10±NA, 4 untreated	1.22±0.72	6.9±2.0	NA	26.2±3.9	NA	[116]
	Control	37	20/17	33.7±10.6								27.3±4.8	NA	
4	FMF	35	17/18	11.6±3.5	0	0	5.6±2.8	3.1±2.1	0.96±0.29	6.2±1.5	NA	20.0±4.3	NA	[88]
	Control	35	15/20	12.4±3.2								20.2±3.8	NA	
5	FMF	38	20/18	32.8±11.0 ^a	NA	10.3 Proteinuria	6 - median	6 - median	NA	NA	NA	NA	37% Smoking	[105]
	Control	35	16/19	28.1±9.1								NA	34% Smoking	
6	FMF	32	18/14	35.3±16.9	0	0	NA	NA	NA	NA	NA	NA	NA	[117]
	Control	37	NA	34.0±13.7								NA	NA	
7	FMF	22	8/14	36.3±10.5	100	0	NA	NA	NA	NA	>12	23.5±5.6	36.4% Smoking 9.1% HTN 13.6% dyslipidemia	[118]
	Control	22	8/14	34.0±13.3								22.2±1.9	18.2% Smoking 9.1% dyslipidemia	
8	FMF	18	7/11	50.2±14.2	0	100 8/18 ESRD 6/18 KTp	NA	NA	1.7±0.5	NA	NA	24.8±5.1	22.2% Smoking 33.3% HTN ^a 22.2% dyslipidemia	[119]
	Control	18	7/11	44.0±13.9								23.6±2.2	11.1% Smoking 33.3% dyslipidemia	
9	FMF	69	33/36	10.8±2.8	NA	0	4.6±3.2	NA	NA	NA	NA	NA	None	[100]
	Control	71	34/37	9.9±2.8								NA	None	

BMI, body mass index; CV, cardiovascular; ESRD, end-stage renal disease; KTp, status post kidney transplantation; HTN, hypertension; NA, not available.

^a*p* < 0.05.

^bNA - in cases not all risk factors (including smoking) were specified/excluded.

TABLE 24.7B QT Dispersion Values and Technical Aspects of Measurements in FMF Patients

Study #	Group	FMF genotype	CRP (mg/dL)	SAA (mcg/mL)	Testing protocol and method	QTc (ms)	QTd (ms)	QTcd (ms)	QTdr (%)	TDR (ms)	QTac/RR slope	QTec/RR slope	References
STUDIES REPORTING ON INCREASED QTD PARAMETERS IN FMF													
1	FMF	NA	25.4±30.8 ^a	NA	Manual, 50 mm/s, 2 mV/cm, average of 3 complexes, 8 analyzable leads or more	NA	36.0±11.4 ^a	40.4±13.5 ^a	NA	60.2±16.0 ^a	NA	NA	[103]
	Control		3.4±1.4	NA		NA	20.0±11.2	21.9±12.4	NA	49.0±9.5	NA	NA	
2	FMF	M694V (57.8%) V726A (10.5%) M680I (10.5%)	3.2±2.2 ^a	48.5±21.4 ^a	Unspecified method, 25 mm/s	NA	78.4±16.4 ^a	76.6±12.6 ^a	NA	NA	0.28±0.08	0.25±0.06	[86]
	Control	36.8% HoZ-M 52.6% CHtZ-M 10.5% HtZ-M	0.8±0.2	10.4±4.6		NA	42.8±12.8	NA	NA	NA	0.13±0.02	0.11±0.03	
STUDIES REPORTING ON SIMILAR QTD PARAMETERS IN FMF AND IN CONTROLS													
3	FMF	NA	0.73±0.92 ^a	3.14±4.82 ^a	Manual, 50 mm/s, 1 mV/cm, average of 3 complexes, and 2 examinees	NA	65.9±12.3	73.9±15.0	NA	NA	NA	NA	[116]
	Control		0.26±0.41	0.37±0.26		NA	67.6±12.7	76.0±13.3	NA	NA	NA	NA	
4	FMF	17% M694V HoZ-M	0.24±0.43	NA	Unspecified, 25 mm/s	421.0±16.0	29.0±9.0	NA	NA	NA	NA	NA	[88]
	Control		NA	NA		414.0±14.0	27.0±8.0	NA	NA	NA	NA	NA	
5	FMF	NA	2.6±11.3	NA	Manual, 25 mm/s, 2 examinees, average of 3 complexes	NA	20 median	33.6±15.7	NA	NA	NA	NA	[105]
	Control		3.0±4.9	NA		NA	20 median	35.5±19.2	NA	NA	NA	NA	
6	FMF	NA	NA	NA	Automated software, average of 5 complexes	419.2±20.9	48.0±12.5	51.4±12.0	5.5±1.3	NA	NA	NA	[117]
	Control		NA	NA		414.9±23.3	46.7±9.7	49.7±10.5	5.3±1.2	NA	NA	NA	
7	FMF	NA	NA	NA	Automated software, average of 5 complexes	421.1±20.8	42.0±11.9	46.0±13.5	5.0±1.6	NA	NA	NA	[118]
	Control		NA	NA		419.1±20.0	42.8±7.3	45.3±7.9	4.8±1.0	NA	NA	NA	
8	FMF	NA	NA	NA	Automated software, average of 5 complexes	423.8±25.4	39.0±17.2	40.6±16.4	4.2±1.6	NA	NA	NA	[119]
	Control		NA	NA		415.9±24.8	38.4±12.3	40.7±13.2	4.3±1.5	NA	NA	NA	
9	FMF	NA	NA	NA	Manual, 25 mm/s, average of 3 measurements, 8 analyzable leads or more	NA	44.4±16.7	64.0±21.4	NA	NA	NA	NA	[100]
	Control		NA	NA		NA	42.6±33.6	59.7±24.0	NA	NA	NA	NA	

NA, not available; HoZ-M, homozygote mutations; CHtZ-M, compound heterozygote mutations; HtZ-M, heterozygote mutations; HR, heart rate; TDR, total dispersion of repolarization (ie, using the interval between the peak and the end of a T wave); CRP, C-reactive protein; SAA, serum amyloid A.

^a*p*<0.05; note that CRP and SAA levels were mentioned only if were examined in attack-free period.

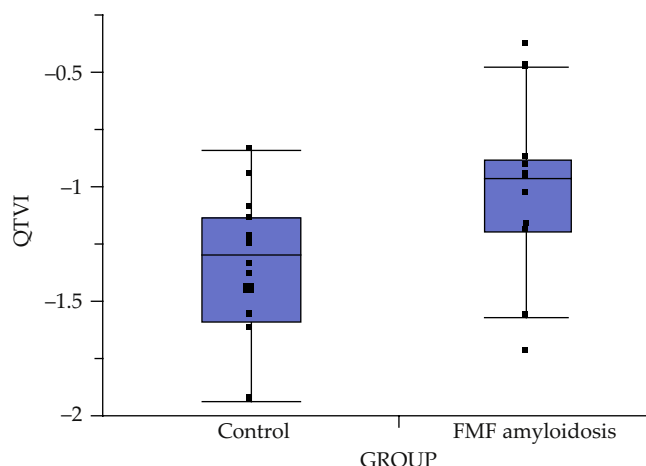


FIGURE 24.7 QT variability index (QTVI) distributions in control subjects and FMF-amyloidosis patients. The *black dots* represent raw values. In these and subsequent box plots, the *central line* represents the distribution median; the box spans from 25 to 75 percentile points. Adapted from Nussinovitch et al. [121].

according to Haigney et al. [122], and -0.47 according to Piccirillo et al. [123]. Finally, QTVI results measured in FMF-amyloidosis patients were similar to those reported for healthy controls in other studies, further complicating the interpretation of the current results [124,125]. Thus, the QTVI and QTN values implying arrhythmogenicity in FMF or FMF-amyloidosis need to be determined.

4.11 Prevalence of Supraventricular Arrhythmias

In 1963, Gale et al. published the first case report on atrial fibrillation in a 30 year old FMF patient with acute pericarditis [126]. Canpolat et al. conducted a 24-h Holter ECG on 38 patients with FMF, reporting that paroxysmal AF was found in two patients (5.2%) [86]. Fidanci et al., in contrast, performed Holter ECG in 70 pediatric FMF patients and found no tracing of supraventricular arrhythmias [104]. Şahin et al. reported that the rate of supraventricular ectopic stimuli is similar in young FMF and control patients [88]. Salah et reported that palpitations were a common complaint among young children with FMF (59% of 55 included patients), but Holter ECG was not performed [127].

In our experience, supraventricular arrhythmias are as common in FMF patients as in the general population. Despite absence of increased rate of atrial arrhythmias, there are several studies evaluating anatomic and electrocardiographic markers for increased risk of supraventricular arrhythmias in FMF patients.

4.12 Markers of Increased Risk for Supraventricular Arrhythmias

4.12.1 Atrial Anatomy

Increased atrial size may predispose to the development of supraventricular arrhythmias. Atrial size was assessed in many studies, but only a single study (Kalkan et al.) reported differences in left atrial parameters between FMF patients and control subjects. The authors found that the left atrium diameter was significantly higher in FMF patients (33 ± 2.7 vs 31 ± 3.3 mm; $p=0.04$) and the mitral E wave velocity was significantly lower (0.81 ± 0.12 vs 1.1 ± 1.09 cm/s; $p=0.039$) [106]. However, atrial dimensions were within normal limits in both groups. They also found significantly lower strain values and strain rate values in several cardiac segments, not in correlation with coronary anatomy, and concluded that these results may suggest subclinical cardiac involvement in FMF [106]. The possible contribution of these findings to increased risk for arrhythmias seems however unlikely.

4.12.2 P-Wave Dispersion

Similar to QT dispersion, P-wave dispersion (Pd) measured by a 12-lead ECG is thought to represent heterogeneous atrial activation. Technically, minimal P-wave duration is subtracted from the maximal P-wave duration. According to some investigators, increased values are thought to suggest an increased arrhythmogenic state. Pd in FMF patients was investigated in six studies (Table 24.8). Increased Pd values were found in two studies, while in the other four similar results were found in FMF patients and control subjects.

Acar et al. reported increased Pd values in FMF patients, which were found to correlate with markers of interatrial electromechanical delay, according to TDI ($r=0.622$, $p<0.001$) and with CRP levels ($r=0.427$, $p<0.001$) [93]. Notably, in that particular study, the Pd results of both FMF patients and control subjects were within the range of those reported in healthy individuals (reported Pd range for healthy controls ranged from 7 to 58.6 ms) [128]. There are also methodological issues, limiting the comparison of different studies (automated vs manual, difference in scales, etc.). Substantially high Pd values in FMF patients (61.9 ± 20.8 ms) were reported only in one study by Arslan et al [95]. Yet, most data favor normal Pd values in FMF. These results are also in line with the lack of increased rates of supraventricular arrhythmias in FMF.

4.13 Pericardial Involvement

In early studies on FMF, pericarditis attacks were reported as uncertain manifestation of FMF [132]. Later studies, however, included pericarditis attacks within the spectrum of FMF. In fact, nowadays, the presence

TABLE 24.8A Clinical Characteristics of Investigated FMF Patient Groups Measured for Their P-Wave Dispersion Values

Study #	Group	N	Gender (F/M)	Age (years)	Colchicine resistance (%)	Amyloidosis (%)	Disease duration (years)	Colchicine treatment (years)	Colchicine dosage (mg/day)	Disease severity score	Attacks/year	BMI (kg/m ²)	Other CV comorbidities ^b	References
1	FMF	33	20/13	28.4±12.5	NA	NA	6.8±7.7	NA	1.5 in all patients	NA	NA	23.7±5.4	12% Smoking	[93]
	Control	33	20/13	28.5±12.1								24.3±5.0	12% Smoking	
2	FMF	26	10/16	9.8±3.8	NA	0	3.0±2.0	NA	1–2.5	NA	NA	NA	None	[95]
	Control	25	11/14	9.3±2.9								NA	None	
3	FMF	30	16/14	33.5±10.2	NA	NA	21.7±9.3	10±NA, 4 untreated	1.22±0.72	6.9±2.0	NA	26.2±3.9	NA	[116]
	Control	37	20/17	33.7±10.6								27.3±4.8	NA	
4	FMF	26	15/11	32.2±13.8	0	0	19.6±NA	NA	1–2.5	NA	NA	22.6±4.7	11.5% Smoking	[129]
	Control	28	NA	32.3±14.1								22.6±2.6	7.1% Smoking 7.1% hyperlipidemia	
5	FMF	22	13/9	38.0±11.1	100	0	NA	NA	NA	NA	>12	24.0±5.6	13.6% Obesity 13.6% smoking 13.6% HTN 9.1% hyperlipidemia	[130]
	Control	22	13/9	32.3±13.3								22.0±2.2	36.4% Smoking 4.5% hyperlipidemia	
6	FMF	16	5/11	49.7±11.9	0	100 7/16 ESRD 6/16 KTp	NA	NA	1–2.5	NA	NA	23.8±5.0	12.5% Smoking 12.5% hyperlipidemia 31.2% HTN ^a	[131]
	Control	16	5/11	43.8±14.1								23.3±2.1	12.5% Smoking 12.5% hyperlipidemia	

BMI, body mass index; ESRD, end-stage renal disease; KTp, status post kidney transplantation; CV, cardiovascular; HTN, hypertension; NA, not available.

^a*p* < 0.05.

^bNA - in cases not all risk factors (including smoking) were specified/excluded.

TABLE 24.8B P-Wave Dispersion and Technical Aspects of Diagnostics in FMF Patients

Study #	Group	FMF genotype	CRP (mg/dL)	SAA (mcg/mL)	Testing protocol and method	Pmax (ms)	Pmin (ms)	Pd (ms)	PA lateral-tricuspid (ms)	PA septum-tricuspid (ms)	References
STUDIES REPORTING ON INCREASED PD PARAMETERS IN FMF											
1	FMF	NA	1.95±1.79	NA	Manual, 50 mm/s, 2 mV/cm, average of ≥3 complexes, 8 analyzable leads or more	98.6±9.0 ^a	55.9±7.3	42.8±7.9 ^a	21.3±7.4 ^a	4.7±5.5 ^a	[93]
	Control		0.34±0.05	NA		93.1±8.5	57.8±7.1	35.3±6.1	12.9±4.6	2.1±1.7	
2	FMF	M694V (42.3%) V726A (26.9%) M680J (23.1%)	7.5±1.1	NA	Automated, unclear whether presented results are averaged	NA	NA	61.9±20.8 ^a	NA	NA	[95]
	Control	19.2% HoZ-M 38.5% CHtZ-M 42.3% HtZ-M	3.4±0.9	NA		NA	NA	32.6±10.2	NA	NA	
STUDIES REPORTING ON SIMILAR PD PARAMETERS IN FMF AND IN CONTROLS											
3	FMF	NA	0.73±0.92 ^a	3.14±4.82 ^a	Manual, 50 mm/s, 1 mV/cm, average of 3 complexes, and 2 examinees	114.7±10.7	71.1±9.5	43.7±11.9	NA	NA	[116]
	Control		0.26±0.41	0.37±0.26		113.5±10.1	66.4±11.7	47.1±11.2	NA	NA	
4	FMF	NA	NA	NA	Automated, averaging of 7–12 beats	107.9±11.0	79.2±14.9	28.8±9.5	NA	NA	[129]
	Control		NA	NA		110.4±9.3	81.6±11.3	28.9±10.0	NA	NA	
5	FMF	NA	NA	NA	Automated, averaging of 7–12 beats	109.9±9.4	84.2±8.6	25.7±6.6	NA	NA	[130]
	Control		NA	NA		110.9±9.8	83.2±11.3	27.4±10.6	NA	NA	
6	FMF	NA	NA	NA	Automated, averaging of 7–12 beats	118.2±10.5	84.0±13.6	34.2±10.2	NA	NA	[131]
	Control		NA	NA		113.3±11.0	82.0±12.0	31.2±9.8	NA	NA	

NA, not available; HoZ-M, homozygote mutations; CHtZ-M, compound heterozygote mutations; HtZ-M, heterozygote mutations; HR, heart rate; CRP, C-reactive protein; SAA, serum amyloid A; P_{max}, maximal P-wave duration; P_{min}, minimal P-wave duration; Pd, P-wave dispersion; PA, time interval from the onset of P-wave on surface ECG to the beginning of A wave interval with tissue Doppler echocardiography, PA lateral-tricuspid represents interatrial electromechanical delay, PA septum-tricuspid represents intra-atrial electromechanical delay.

^ap<0.05; note that CRP and SAA levels were mentioned only if were examined in attack-free period.

of typical attacks of pericarditis, although rare are considered among the major criteria used for diagnosing FMF [4].

Pericarditis usually appears concurrently with other classic FMF attacks, such as peritonitis or arthritis, but on rare occasion it may be the only FMF manifestation [133]. Classic manifestations of pericarditis of any reason are found in FMF as well, including fever, orthopnea, pleuritic chest pain, pericardial friction rub, and transient repolarization changes found on an electrocardiogram. Complications include a large pericardial effusion that may necessitate pericardiocentesis and constrictive pericarditis [24]. A single study reported a large hemorrhagic pericardial effusion (1000mL; treated with pericardiocentesis) suspected to be caused by FMF exacerbation (however, the patient was also on Coumadin and aspirin, which could increase the risk for hemorrhage) [134].

Terekci et al., in a cross-sectional study performed during an FMF attack, found pericardial effusion by echocardiogram in 23.3% of 34 patients. They also reported that the pericardial effusion was unrelated to disease duration, family history, and physical findings [92]. In a different study by the Turkish FMF Study Group, comprising 2468 FMF patients, only 60 (2.4%) were suspected of having one attack or more of pericarditis during the course of their disease. Definitive diagnosis of pericarditis was made in only 34 patients (1.37%) [14]. According to another report, the prevalence of pericarditis was about 0.7% and only in two it was recurrent [135] (0.1% of the study group). In these two patients pericardial tamponade, requiring urgent pericardiocentesis, developed. Constrictive pericarditis (some of the cases were treated with pericardiectomy) [14,136], and cardiac tamponade [137] were also reported by other groups, but seems to represent rare complications.

Ozdemir et al.'s cross-sectional echocardiographic study of 25 children with FMF did not find any evidence of pericardial effusion during an attack-free period [99]. This result might be related to the small sample size. Tutar et al. evaluated the presence of pericardial involvement by echocardiography in 55 attacks occurring in 42 FMF patients. Only 27 were treated with colchicine. They found minimal pericardial effusion in two patients during attacks (4.7% of the study group, and 3.6% of attacks), which resolved spontaneously at the end of the attack [138]. Dabestani et al. reported on echocardiographic findings in 30 FMF patients. In both symptomatic (4/30 who experienced chest pain during the study) and asymptomatic patients, pericardial effusion and/or thickening were noted (50% and 23%, respectively) [139]. They found that the duration of FMF was longer in patients with pericardial involvement (28.9 ± 12.2 years vs 18.5 ± 10.6 years, $p < 0.02$). Moreover, since 50% of the patients who experienced chest pain did not have pericardial involvement, chest pain in the

context of FMF may not be sensitive enough to yield a diagnosis [139].

Kees et al. from our research group evaluated the medical records of 1553 FMF patients who reported pleuritic chest pain, and identified 27 patients diagnosed with pericarditis during a 20-year follow-up [24]. In 3/27 patients, pericarditis was the first manifestation of FMF. Also, 12/27 (44.4%) had recurrent events of pericarditis during follow-up. Mean attack duration was 4.2 ± 3.5 days. Seventeen patients underwent echocardiography revealing pericardial effusion in 11 patients. Characteristic pericarditis-related ECG changes were found in 22 patients (ECG was performed on 25). Cardiac enlargement on chest X-ray was found in 8/21 patients. Pericardial friction rub was found in 2/27 patients. The following symptoms/conditions accompanied the pericarditis attacks: fever (92.6%), peritonitis (51.8%), arthritis (51.8%), and pleuritis (48.1%). Pericarditis was self-limited and without long-term complications in all patients. It can be concluded that pericarditis is uncommon in FMF, appearing in <1% of FMF patients [24]. Nevertheless, subclinical pericardial involvement may occur occasionally as well [140]. Although chronic pericardial involvement may occur, it is rare, and may include chronic and large pericardial effusions (Fig. 24.8) [137,141], or constrictive pericarditis (Fig. 24.9) [24,136]. On rare occasions, pericardial involvement may be the first (or even the only) clinical manifestation of FMF, thus FMF should be considered in patients with pericardial effusion or recurrent pericarditis (unresponsive to treatments other than colchicine), especially when they belong to a high-risk ethnic group [135,141].

4.14 Aortic Abnormalities

Aortic anatomy and distensibility in FMF has been evaluated by several studies (Table 24.9). Tavit et al. evaluated the aortic diameter in FMF patients and in control subjects throughout the cardiac cycle [97]. Aortic strain was calculated by subtracting the aortic diastolic diameter from the systolic diameter and dividing the result by the diastolic diameter. Aortic distensibility was calculated by multiplying the aortic strain by two and dividing by the pulse pressure. The two study groups had comparable systolic and diastolic blood pressure values, and similar systolic aortic diameters (32.6 ± 2.8 vs 31.4 ± 2.0 mm, $p > 0.05$). Nevertheless, the diastolic aortic diameter was higher in FMF patients (31.2 ± 2.9 vs 29.6 ± 2.7 mm, $p = 0.03$), resulting in a lower pulsatile diameter change (1.4 ± 0.6 vs 1.8 ± 0.5 mm, $p = 0.04$). As a result, FMF was associated with lower aortic strain (4.91 ± 1.66 vs $7.23 \pm 2.14\%$, $p = 0.01$) and lower distensibility ($2.84 \pm 1.46 \cdot 10$ vs $4.02 \pm 1.42 \cdot 10^{-6} \text{ cm}^2$, $p = 0.001$). A significant correlation was found between

(A)



(B)

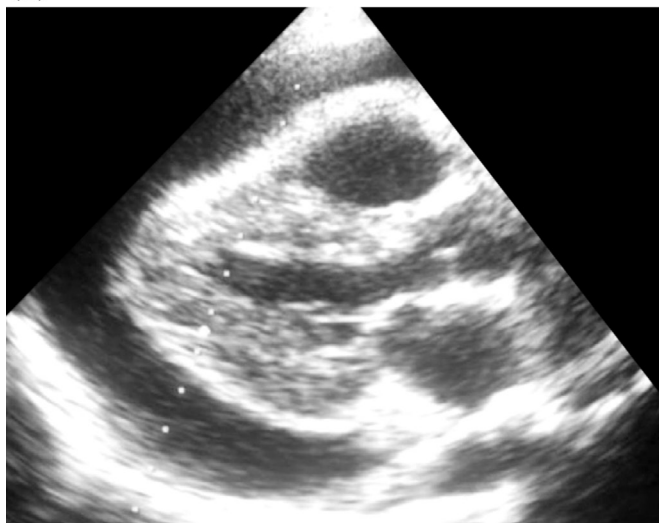


FIGURE 24.8 Chest X-ray in a 13-year-old boy with FMF (Panel A) and an echocardiogram study of the same patient (Panel B) demonstrating an expanded cardiac silhouette (A) and abundant pericardial fluids compressing the myocardium (B), suggestive of cardiac tamponade. Adapted from Sanchez Ferrer et al. [137].

disease duration and aortic strain ($r = -0.29$, $p < 0.001$) and between disease duration and aortic distensibility ($r = -0.32$, $p < 0.001$) [97].

Sari et al. also conducted a study attempting to evaluate aortic characteristics in FMF patients [98]. They included 44 FMF patients (none with amyloidosis) and 27 healthy control subjects. Similar values in FMF patients and control subjects were found regarding systolic diameter of the aorta (median of 3.1 vs 3.0, respectively, $p > 0.05$) and diastolic diameters of the aorta (median of 2.9 vs 2.7, $p > 0.05$). Also, the aortic strain was similar in FMF patients and controls (median of 7.5% vs

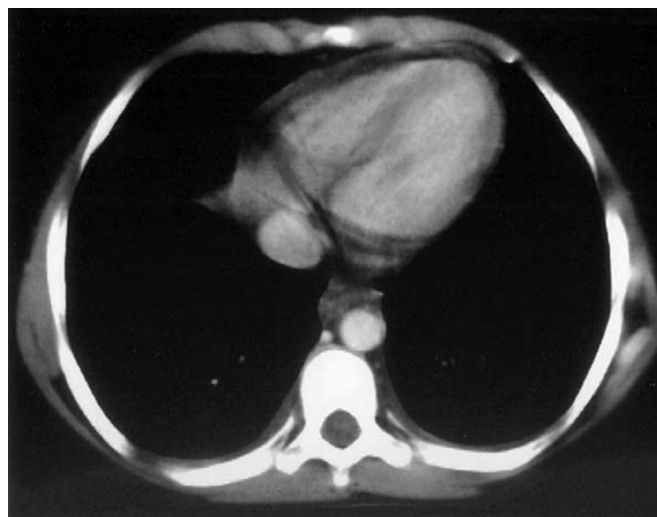


FIGURE 24.9 CT scan of an FMF patient demonstrating pericardial thickening and bilateral small pleural effusions. Adapted from Ishak et al. [142].

6.4%, respectively, $p > 0.05$). The distensibility index was calculated by the following equation: $\ln(\text{systolic blood pressure}/\text{diastolic blood pressure})/\text{aortic strain}$. Results in FMF patients and controls were similar regarding the distensibility index (median of 5.4 vs 7.9, respectively, $p > 0.05$) and distensibility (30.8 vs $19.7 \times 10^{-3}/\text{kPa}$, $p > 0.05$) [98].

Vascular distensibility can also be evaluated by the quantification of pulse wave propagation along the vascular tree. Pulse wave velocity (PWV) is calculated by dividing the distance between the two landmarks used for measurements (in meters) and transmit time (in seconds). Clinically, it serves as an indirect marker for atherosclerosis but may also be abnormal when the composition of the vessel wall is modified (ie, smooth muscle hypertrophy, increased collagen content, decreased content of extracellular matrix, etc.). In a study that included 23 FMF patients and 23 controls, PWV was found to be increased ($p = 0.05$) in FMF patients compared to controls, but no numerical data was given in order to quantify the magnitude of the increased PWV. Also, the authors found that PWV is correlated with BMI and age. No multivariate analysis was conducted.

Despite these limitations, the authors suggested that the inflammatory process in FMF might contribute to increased arterial stiffness and that colchicine may prevent these changes [143]. Later on the missing numerical data was reported in a review by the same author: $8.53 \pm 1.65 \text{ m/s}$ in FMF vs $7.75 \pm 0.86 \text{ m/s}$ in controls [144].

Although it was suggested that increased inflammatory burden of patients in Yildiz et al.'s study [143] (as manifested by increased CRP levels) may explain the difference between Sari et al. and Yildiz et al. studies

TABLE 24.9A Clinical Characteristics of Investigated FMF Patient Groups Evaluated for Their Aortic Anatomy and Distensability

Study #	Group	n	Gender (F/M)	Age (years)	Colchicine resistance (%)	Amyloidosis (%)	Disease duration (years)	Colchicine treatment (years)	Colchicine dosage (mg/day)	Disease severity score	Attacks/year	BMI (kg/m ²)	Other CV comorbidities ^a	References
1	FMF	31	19/12	36.0±8.0	NA	NA	12.4±7.6	5.6±2.7	NA	NA	NA	24.0±6.0	35% Smoking. UKR DysLip	[97]
	Control	27	17/10	34.0±7.0								23.0±5.0	33% Smoking. UKR DysLip	
2	FMF	44	23/21	Median 30.0	NA	0	Median 15.5	NA	0.5–2	NA	Median 2	Median 23.4	30% Smoking	[98]
	Control	27	15/12	Median 29.5								Median 22.2	30% Smoking	
3	FMF	23	17/6	29.4±8.7	NA	0	8.0±8.4	NA	1–2	NA	NA	23.29±3.53	13% Smoking	[143,144]
	Control	23	17/6	29.2±9.0								23.47±4.10	NA	

BMI, body mass index; CV, cardiovascular; NA, not available.

^aNA - in cases not all risk factors (including smoking) were specified/excluded.

TABLE 24.9B Laboratory Findings and Technical Aspects of Measuring Aortic Parameters in FMF and in Control Patients

Study #	Group	FMF genotype	CRP (mg/dL)	SAA (mcg/mL)	Pulse wave velocity (m/s)	Aortic systolic diametion (mm)	Aortic diastolic diametion (mm)	Pulsatile diameter change (mm)	Aortic strain (%)	Distensibility (10 ⁻⁶ cm ²)	Distensability index	References
1	FMF	NA	NA	NA	NA	32.6±2.8	31.2±2.9 ^a	1.4±0.6 ^a	4.91±1.66 ^a	2.84±1.46 ^a	NA	[97]
	Control		NA	NA	NA	31.4±2.0	29.6±2.7	1.8±0.5	7.23±2.14	4.02±1.42	NA	
2	FMF	NA	Median 0.250	NA	NA	Median 31.0	Median 29.0	NA	Median 7.5	NA	Median 5.4	[98]
	Control		Median 0.093	NA	NA	Median 30.0	Median 27.0	NA	Median 6.4	NA	Median 7.9	
3	FMF	NA	1.35±2.26	NA	8.53±1.65 ^a	NA	NA	NA	NA	NA	NA	[143,144]
	Control	NA	0.27±0.11	NA	7.75±0.86	NA	NA	NA	NA	NA	NA	

NA, not available; HoZ-M, homozygote mutations; ChH-Z-M, compound heterozygote mutations; HiZ-M, heterozygote mutations; HR, heart rate; CRP, C-reactive protein; SAA, serum amyloid A.

^ap≤0.05; note that CRP and SAA levels were mentioned only if were examined in attack-free period.

[145], the difference between the results cannot be easily explained. However, a recent study by our research group (in preparation for publication) found that PWV in FMF is normal. The distribution of the PWV values (standardized for BP and age, designated hereafter PWV_Z) of 80 FMF patients, fall well within the limits of 90th percentile of normal control subjects with even much less dispersion in the range of results in FMF (one-sided 95% CI 1%, 5%, $p=0.02$). Subgroup analysis, for the type of mutations, patient origin, number of sites involved in the disease, or disease activity, showed no statistically significant differences between the PWV_Z of the subgroups ($p=0.631$, $p=0.230$, $p=0.086$, $p=0.669$). Moreover, there was clear (but insignificant) trend for lower PWV_Z in patients with higher and longer exposures to colchicine. The best was seen with the correlation between PWV_Z and the mean colchicine dose where the correlation coefficient was -0.185 ($p=0.053$). Altogether, two studies of four favor normal parameters of aorta elasticity in colchicine-treated FMF patients. Our study even brought forward evidence supporting a role for colchicine, a factor not well studied by others and may be the missing link explaining conflicting results.

4.15 Association between FMF and Rheumatic Heart Disease

In 1961, Heller et al., described six autopsied patients with FMF and amyloidosis. One patient had rheumatic mitral stenosis [146]. Shapiro et al.'s study in 1962 [147], suggested that elevated antistreptolysin-O (ASLO) titers may be found in some FMF patients and that this finding might not be specific to acute rheumatic fever (ARF). They noted that some of the classic FMF symptoms might be misinterpreted as ARF [147]. In fact, ARF is one of the leading misdiagnoses in FMF patients presenting with arthritis [148]. Majeed et al. reported on articular presentations of 133 children with FMF and found that 4% of included children had asymmetric oligoarticular arthritis, similar to the expected presentation in ARF [149].

In 1999, Tekin et al. [150] suggested that the coexistence of FMF and ARF is not coincidental and reported that a possible relationship exists between ARF and FMF. Included in the study were 162 FMF patients, 5.2% had a history consistent with ARF and 1.85% had a history of rheumatic carditis (compared with a reported rate of 0.65% in the general Turkish population). They also reported elevated ASLO titers in 75% of patients during an arthritis attack (903 ± 597 IU/mL), in 42% of patients during an attack in another site (742 ± 433 IU/mL), and in 38% of patients during an attack-free period (720 ± 310 IU/mL). It was suggested that streptococcal infections may precipitate FMF attacks [150].

In order to evaluate an antistreptococcal response in FMF, Yalçinkaya et al. [148] studied ASLO and anti-DNase B titer in 44 children with FMF and 165 healthy controls. They found no clinical signs (by physical examination) of streptococcal infections, but nevertheless abnormally high ASLO titer was detected in 36.4% of FMF patients and increased anti-DNase B titer in 40.9%. Also, a confirmed diagnosis of group A β -hemolytic streptococcal infection was made by throat culture in 15/44 (34.1%) of FMF patients compared with 22/165 (13.3%) of the control group, with higher ASLO and anti-DNase B titers in the affected FMF patients than in the control subjects (470 ± 275 vs 240 ± 183 Todd units, $p<0.05$, and 608 ± 429 vs 464 ± 572 units, $p<0.001$, respectively). The authors suggested that an exaggerated immunological response to streptococcal pharyngitis may predispose FMF patients to develop ARF. Importantly, all culture-positive patients were treated with oral penicillin, and it was not reported whether ARF was developed in any of these patients [148].

In a subsequent study, comprising 2838 FMF patients, a history of ARF was found in 139 patients (4.9%). However, the authors suggested that some of the cases could be attributed to the misdiagnosis of FMF articular involvement as ARF [14]. Ozen et al. reported that carriage of MEFV mutations was associated with ARF in 4/70 of the investigated patients (5.71%) [151]. Kalyoncu et al. surveyed 676 parents of 440 children with FMF (mostly obligatory carriers) and 770 controls (without a known family history of FMF or rheumatologic disorders). They reported a history of ARF in 3.3% of parents, compared with 1.7% of the control group ($p=0.05$) [152]. Tutar et al. included 27 pediatric patients with rheumatic heart disease (RHD) and reported seven MEFV mutations among 54 analyzed chromosomes, a mutated gene frequency of 1:7.7, compared with 1:32.7 in the general Turkish population. The authors concluded that MEFV mutations are almost four times more prevalent in RHD patients compared with the general population [153].

In contrast to the aforementioned reports, the most extensive evaluation of genetic background in RHD was performed by Simsek et al., who undertook a genetic analysis of 100 adult patients with documented RHD compared with 100 healthy controls. Mutations in the MEFV genes were found in 22% of RHD patients and 24% of healthy control subjects [154]. The conflicting results are not easy to interpret due to the inclusion of different sample size, genetic backgrounds, and the fact that ARF susceptibility is probably multifactorial, with strong environmental effect. Also, if a single MEFV mutation results in increased susceptibility to RHD, it might be reasonable to expect higher risk for developing RHD in FMF than in mutated gene carriers, a difference not yet observed. Thus, whether FMF predisposes to ARF or to RHD is yet to be determined.

5. TREATMENT OF FMF AND CARDIAC IMPLICATIONS OF THERAPY

5.1 Colchicine Treatment

Colchicine is an alkaloid, extracted from the plant *Colchicum autumnale*, which inhibits the polymerization of cytoskeletal tubulin, thereby microtubules disruption [10]. Also, colchicine decreases the degranulation of neutrophils, enhances the synthesis of stable prostaglandins, and alters the function and expression of certain adhesive molecules (E-selectin) and thereby the interaction of endothelial cells with neutrophils [155]. The idea of using colchicine for treating FMF was first suggested by Goldfinger in 1972 [156]. Colchicine treatment has dramatically changed the natural history of FMF and is considered to be the mainstay treatment of FMF, due to its proven effects of decreasing attack rates and preventing the development of amyloidosis (level of evidence A, strength of recommendation I) [1]. In fact, complete cessation of attacks throughout several years was observed in more than 60% of treated patients, while a substantial decrease of attack frequency was observed in about 30% of patients [157]. Before the colchicine era, patients generally developed systemic amyloidosis before their fourth decade and few survived beyond their fifties [7]. Surprisingly, some patients with FMF chose not to use colchicine (11.4–12.9% of patients are with low compliance), which predisposed them to amyloidosis. Colchicine was reported to resolve or stop the progression of proteinuria when amyloidosis-related renal injury started to develop, especially when administered in a dose higher than 1.5 mg/d. Treatment was effective (likely to prevent further decline in renal function) only in patients with creatinine levels lower than 1.5 mg/dL [3]. Side effects are more frequent with higher dose (≥ 1 mg/day). Nevertheless, it is estimated that 5–10% of FMF patients respond poorly to colchicine, even in high dose (≥ 2 mg/day). The definition of colchicine nonresponsiveness is somewhat arbitrary. It has been defined as having typical attack at least once in three months [1] or once every month [120] despite colchicine dose of ≥ 2 mg/day. The Turkish FMF Study Group found in 2258 FMF patients (80% with regular and 17% with irregular use of colchicine) that 51.2% responded completely, 46% experienced occasional attacks, and 2.8% were nonresponsive [14].

It has been suggested that colchicine plays an important cardiac protective role. Nonetheless, there are no controlled studies that demonstrate an increased rate of ischemic cardiovascular disease (ICVD) in untreated FMF patients. Importantly, early studies, before the colchicine era, did not report high occurrence of myocardial infarction in FMF. It might be expected that colchicine nonresponders will suffer from an increased rate of ICVD, if colchicine indeed possesses cardioprotective

properties. Nevertheless, in a study by Lidar et al., no statistical difference was found in the rate of CVD between colchicine responders and colchicine nonresponders (0% vs 5%, $p > 0.05$). Thus, it might be suggested that the presence of a chronic inflammatory state (which is more pronounced in colchicine nonresponders) does not increase the risk for ICVD disease in FMF. Alternatively, since colchicine treatment was continued in the colchicine refractory group, it might be that a cardiovascular protective effect of colchicine is the factor that normalizes the risk for ICVD in these patients.

In conclusion, there is evidence supporting a cardiovascular protective role for colchicine in FMF. The beneficial effects might be via a decrease of inflammatory burden or direct effect of colchicine on other systems such as inhibition of platelet aggregation and secretion, as demonstrated in some studies [158,159]. Moreover, the controversial results on the involvement of the heart in FMF (and in particular the question of an increased rate of atherosclerosis) may stem from a difference in compliance with colchicine treatment in the different study groups. It even has been suggested that colchicine should be given for treatment of non-FMF patients who have high CRP levels, atherosclerosis, or cardiac ischemia, but these research objectives have been expanded into clinical practice only in a very limited manner, as described in the following [10].

Much prior to the insight offered by FMF on the cardioprotective effect of colchicine, it has been shown that colchicine may prevent building of atheromatous plaques in animal models of atherosclerosis [160]. Based on such experiments and the emerging understanding of the importance of inflammation in atherosclerosis, a trial with colchicine in angina pectoris, myocardial infarction, and prevention of restenosis after angioplasty and bypass grafting was proposed already in 1992 [161]. However, in a prospective trial, conducted for three months after elective angioplasty, oral colchicine in a dose of 0.6 mg bid failed to prevent restenosis in 130 patients of the study group compared to 67 control subjects receiving placebo. Angiograms performed at six months following the procedure revealed similar narrowing of the treated lesions in the two groups (47% vs 46% of lumen diameter). Restenosis in at least one lesion was found at a similar rate in the two groups (41% vs 45%) [162].

Twenty years later the study was repeated, this time in diabetic patients. Colchicine 0.5 mg bid or placebo were given to 112 or 110 diabetic patients undergoing PCI with bare metal stent (eluting drug stents were contraindicated). The trial lasted for six months, after which restenosis was determined through angiography and intravascular ultrasound. Restenosis was much lower in colchicine-treated patients (16% vs 33%, $p = 0.007$; odds ratio: 0.38, 95% CI: 0.18 to 0.79, number needed to treat 6. Lumen area loss was 1.6 mm² vs 2.9 mm² ($p = 0.002$) [163].

Encouraging data regarding the prevention of IHD arrived from two additional studies. One retrospective study compared colchicine effect in large cohorts of gout patients either receiving ($n=576$) or not receiving ($n=712$) colchicine. Allocation to one of the study groups was based on prescription. Baseline characteristics were comparable for the groups. Prevalence of MI was lower in colchicine receivers (1.2% vs 2.6%, $p=0.03$). Colchicine effect persisted when allopurinol users were excluded from the analysis [164]. In another study, this time a randomized controlled trial, conducted prospectively, in 532 patients with stable IHD, the primary outcome, consisting of various expressions of coronary disease occurred after a median follow-up of three years, in 5% of 282 patients, on colchicine 0.5mg/day and in 16% of the 250 patients, who were not (hazard ratio: 0.33; 95% confidence interval [CI] 0.18 to 0.59; $p<0.001$; number needed to treat: 11) [165].

The mechanism of colchicine effect is unknown but could be related to disruption of atheroma building as seen in animal models [166], effect on macrophages, neutrophils, and endothelial cells, all of which are implicated in the pathogenesis of cardiovascular disease, or indirectly by affecting other risk factors such as CRP. The latter was researched in two studies. In one, colchicine in a dose of 0.5mg bid was given to 44 patients with stable coronary artery disease but with elevated plasma levels of high-sensitivity C-reactive protein (hs-CRP, ≥ 2.0 mg/L), a predictor of future vascular events. This was an open-labeled study. Twenty adjusted control subjects received no drug. After four weeks, colchicine effectively reduced CRP levels in the study compared to no change in the control group (60% vs 11%, $p<0.001$) [167]. However, these successful results could not be repeated in a study prescribing colchicine 0.5mg bid to 80 patients with acute coronary syndrome or acute ischemic stroke who were followed for 30 days. The difference from baseline values to 30-day values was around 7mg/L for patients and control subjects. Importantly, platelet function was also tested in this study but no effect of colchicine could be shown on platelet aggregation with ADP, arachidonic acid, and collagen [168].

In summary, there are only three studies proving that colchicine may be effective in preventing IHD. This is a nice beginning that should stimulate further studies. Also the pathogenesis of this effect needs also to be investigated.

6. CONCLUSIONS

The only inherent cardiac involvement in FMF is pericarditis. Clinically overt pericarditis is uncommon, of short duration, recurs along the course of the disease, and usually is accompanied by an attack in another site. Occasionally, FMF patients have subclinical

long-standing pericardial effusion, which rarely may develop to a large function impeding effusion.

Other forms of cardiac involvement in FMF arise from complications; the first is amyloidosis, in which inflammation-induced elevated SAA is incompletely metabolized and deposited in tissues, including the heart. As is the case in many other organs, amyloid deposits in the heart may remain asymptomatic for many years. They involve all cardiac layers and structures, including the endocard, myocard, valves, and blood vessels. In the colchicine era, clinically overt cardiac amyloidosis is rare, occurring in late stages of AA amyloidosis. It should be suspected in an already established FMF-amyloidosis, with progressive kidney disease, in which CHF (usually diastolic), valvular disease, anginal syndrome, arrhythmia, or conduction disturbances were developed. Although definite diagnosis must rely on cardiac biopsy, to date cardiac amyloidosis may be inferred clinically from patient status and imaging.

The second complication, also related to the inflammatory burden of FMF, is ICVD. Distinct from amyloidosis, where no doubt is raised as for its existence in FMF, the occurrence of ICVD in FMF and whether FMF constitutes a risk factor for the development of ICVD is under considerable debate, as more than 100 studies conducted to date on various aspects of cardiovascular involvement in FMF gave conflicting results.

Many studies evaluating pathogenic factors possibly underlying the development of ICVD in FMF were performed, including on the genetic propensity linked to carriage of MEFV mutations, five different markers suggestive of endothelial injury, coagulation factors leading to increased thrombogenicity, platelet volume and function, and the frequency of traditional risk factors. Two-thirds of 56 studies on these subjects yielded normal results, while one-third suggested that FMF predisposes to develop ICVD. All abnormal results were equally balanced with normal findings in the same topic. The net result is that none of the pathogenic items studied favored abnormality in FMF.

Studies evaluating the presence of actual atherosclerosis in FMF, assessing vascular wall thickness and flow, coronary flow and function, and aortic stiffness, were more balanced. There were 23 such studies, only half of which favored increased risk for ICVD in FMF. Again, each studied item with abnormal findings was balanced by normal findings in the same topic, leading to the net result that in FMF uncomplicated with amyloidosis, various vascular parameters appear to be normal.

Similarly, with regard to studies on atrial size, ECG changes, and arrhythmogenicity, where no increased abnormalities rate, or risk to develop, changes were detected. The only exception was that of echocardiogram, where most studies (yet, not all of them) suggested that there is FMF-associated diastolic dysfunction. Yet,

even under these circumstances the diastolic dysfunction remains subclinical.

Indeed, in real life, there is no a single study that claims increased occurrence of ICVD in FMF. On the contrary, the only two studies on this subject reject the option that FMF patients suffer more often from ICVD. The reason for the deviation in FMF from the paradigm that inflammation may cause ICVD is unknown, but colchicine, a drug exclusively used in FMF, was suggested as the cause. This suggestion is supported by findings in FMF and in patients with gout and anginal syndrome. More studies are required to better define the cardioprotective role of colchicine and whether it induces its protective effect directly or through suppression of inflammatory mediators.

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Desirable and Adverse Effects of Antiinflammatory Agents on the Heart

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1. INTRODUCTION

Antiinflammatory drugs have greatly improved the quality of life and management of several rheumatic conditions. However, the benefits of antiinflammatory therapies should be weighed against the potential harms with careful evaluation of side effects.

Although not very common, there are potential side effects that may involve the cardiovascular system and increase the risk of myocardial infarction. Such effects are especially increased in patients at moderate-to-high risk of cardiovascular disease and thus the clinical evaluation should not only consider the indications, the potential side effects, but also the risk profile of the patient in order to select the best therapeutic option minimizing as much as possible the risks.

The aim of the present chapter is to summarize current evidence on the potential cardiovascular effects of antiinflammatory therapies including either adverse or potentially desirable effects. Nonsteroidal antiinflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs), with a focus on hydroxychloroquine, methotrexate, sulfasalazine, minocycline, varespladib, leflunomide, and colchicine, are discussed.

2. NONSTEROIDAL ANTIINFLAMMATORY DRUGS (NSAIDs)

NSAIDs have greatly improved the quality of life and morbidity of patients with rheumatologic diseases, which has also prevented or limited the use of other drugs (eg, corticosteroids and more potent analgesics such as opiates), with potentially more severe or

different side effects. However, their long-term use may be complicated by gastrointestinal and renal side effects as well as potential harmful effects on the cardiovascular (CV) system. Such harmful effects have been especially reported for cyclooxygenase-2 (COX-2) inhibitors [1,2], but also for nonselective NSAIDs, such as ibuprofen and diclofenac [2–5].

2.1 NSAID Mechanism of Action and Potential Adverse Cardiovascular Side Effects

NSAIDs inhibit the COX enzyme, which transforms phospholipid-derived arachidonic acid into prostaglandins (Fig. 25.1).

There are two main COX enzymes: the COX-1 is constitutively expressed by platelets and gastrointestinal mucosa mediating both platelets aggregation and protection of gastric mucosa; the COX-2 is expressed during inflammatory conditions (Table 25.1).

Nonselective NSAIDs (eg, Ibuprofen and naproxen) inhibit both COX-1 and COX-2 and may have gastrointestinal side effects.

Aspirin is more COX-1 selective, and is a pivotal antiplatelet agent, since it irreversibly blocks the platelet thromboxane-synthetase. Historically, the aim was to define the minimal dosage of aspirin that maintained the antiplatelet effects, without the side effects associated with the much higher dosages required to reach the antiinflammatory action. Therefore studies have tried to identify progressively lower dosages while retaining the antiplatelet effect, and several randomized trials demonstrated that aspirin's antithrombotic activity is present at doses between 50 and 1500mg/day [6–8]. Its effectiveness as antithrombotic agent (COX-1 inactivation) has

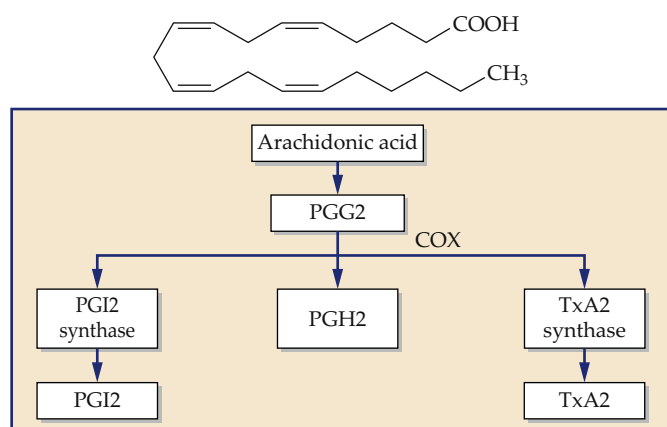


FIGURE 25.1 Prostaglandins synthesis by COX and the pathways to PGI₂ synthesis and TxA₂ synthesis. Adapted from the personal collection of the authors.

TABLE 25.1 Physiological Actions and Effects of Inhibition of COX-1 and COX-2

	COX-1	COX-2
Physiological effects	Maintenance and protection of gastrointestinal mucosa. Platelet aggregation.	Induction by inflammatory cytokines. Proinflammatory effects.
Inhibition effects	Decreased production of mucous and bicarbonate. Antiplatelet effects.	Antiinflammatory and analgesic effects.

also been suggested for dosages as low as 30mg daily [9–11]. Aspirin's ability to protect from vascular disorders depends mainly on COX-1-suppression even if other minor effects not related to TXA-2 were reported, as inhibition of platelet function or plasma coagulation and an increased fibrinolysis [6]. Much higher concentration of the drug and thus higher doses are need to have antiinflammatory and analgesic effects (COX-2 inactivation).

There is no evidence that lower doses are less effective as antiplatelet agent than higher ones. Conversely, some doubts have emerged about its antiplatelet activity at higher doses because of the possible concomitant inhibition of PGI-2 at these concentrations of the drug. PGI-2 can derive both from COX-1 and COX-2 stimulation and can inhibit platelet aggregation and induce vasodilatation. Thus hypothetically significant suppression of PGI-2 could reduce the antithrombotic effect of aspirin and increase the thromboresistance. However, these data are controversial and not adequately established [6–8,12].

Doses of aspirin up to 1500mg/day have been shown to be effective as antiplatelet agent, but an attenuated

antiplatelet efficacy at higher daily doses remains to be convincingly demonstrated [6–8].

These considerations are relevant when an antiinflammatory agent is required in patients who need antiplatelet agents, such as in some cases of pericarditis, myocarditis, and other inflammatory conditions [13].

COX-2 selective drugs have been developed since the 1990s to provide more antiinflammatory effects without COX-1 inhibition. The introduction of COX-2 inhibitors (the "coxibs") changed antiinflammatory therapies [14]. However, the coxibs increased cardiovascular risk and reports were published from 2001 indicating an increased risk of myocardial infarction in patients treated with COX-2 inhibitors [15,16].

Use of NSAID is associated with increased risk of CV events and reduced aspirin-associated cardioprotection. In a systematic meta-analysis of 138 randomized trials to assess the risk of vascular events comparing COX-2 inhibitors with traditional NSAIDs, a statistically significant difference for serious vascular events [relative risk (RR), 1.57; 99% confidence interval (CI), 1.21–2.03; $p=0.0006$] and myocardial infarction (RR, 2.04; 99% CI, 1.41–2.96; $p=0.0002$) was noted between COX-2 inhibitors and naproxen [17]. Further studies were consistent with these findings [18–20]. In a multicenter, retrospective cohort data of approximately 49,000 patients (mean age, 65 years) with more than 111,000 person-years of follow-up and commonly prescribed NSAIDs, naproxen was the safest [18]. The cohort included patients that were recently hospitalized for myocardial infarction, unstable angina, or revascularization. Comparing naproxen to NSAID nonusers, the adjusted incidence rate ratios (IRR) for serious coronary heart disease (myocardial infarction, coronary heart disease, death) was lowest at 0.88 (95% CI, 0.66–1.17; $p=0.3940$), while IRR for serious CV disease (myocardial infarction, stroke, or death) from any cause was reported at 0.91 (95% CI, 0.78–1.06; $p=0.2346$). Risk was independent of naproxen doses of more than 1000mg. Compared with naproxen, current use of diclofenac was associated with a 44% increased risk of serious coronary heart disease (RR, 1.44; 95% CI, 0.96–2.15; $p=0.076$) and 52% risk serious CV disease/death (RR, 1.52; 95% CI, 1.22–1.89; $p=0.0002$). Those taking ibuprofen had a 25% increased risk (RR, 1.25; 95% CI, 1.02–1.53; $p=0.032$). Serious coronary heart disease incidence rate ratios were much higher for rofecoxib (RR, 2.29; 95% CI, 1.24–4.22; $p=0.008$) at a dosage higher than 25mg and celecoxib (RR, 1.61; 95% CI, 1.01–2.57; $p=0.046$) at a dosage higher than 200mg when compared with naproxen at doses higher than 1000mg daily. Similar results on naproxen safety were reported in others meta-analyses and reviews [19–22].

In the CNT (Coxib and traditional NSAID Trialists) Collaboration's meta-analysis [21] researchers collected data on vascular and gastrointestinal effects of coxibs and

traditional NSAIDs, focusing on the settings of patients at increased risk of vascular diseases. They undertook *meta*-analyses of 280 trials of NSAIDs versus placebo and 474 trials of one NSAID versus another NSAID. Major vascular events were increased by about one third by a coxib (RR, 1.37, 95% CI, 1.14–1.66; $p=0.0009$) or diclofenac (RR, 1.41; 95% CI, 1.12–1.78; $p=0.0036$), mainly due to an increase in major coronary events (coxibs 1.76, 1.31–2.37; $p=0.0001$; diclofenac 1.70, 1.19–2.41; $p=0.0032$). Ibuprofen also significantly increased major coronary events (2.22, 1.10–4.48; $p=0.0253$), but not major vascular events (1.44, 0.89–2.33). Naproxen did not significantly increase major vascular events (0.93, 0.69–1.27). Vascular death was increased significantly by coxibs (1.58, 99% CI, 1.00–2.49; $p=0.0103$) and diclofenac (1.65, 0.95–2.85, $p=0.0187$), nonsignificantly by ibuprofen (1.90, 0.56–6.41; $p=0.17$), but not by naproxen (1.08, 0.48–2.47, $p=0.80$). The proportional effects on major vascular events were independent of baseline characteristics, including vascular risk. Heart failure risk was approximately doubled by all NSAIDs. All NSAID regimens increased upper gastrointestinal complications (coxibs 1.81, 1.17–2.81, $p=0.0070$; diclofenac 1.89, 1.16–3.09, $p=0.0106$; ibuprofen 3.97, 2.22–7.10, $p<0.0001$; and naproxen 4.22, 2.71–6.56, $p<0.0001$). The analyses did not provide definite conclusions about the dose-dependent risk and time-dependent risk. At the most common daily doses the vascular risks of different coxib regimens seemed similar. The traditional NSAID regimens studied were all high-dose, with little variation between trials, so comparable analyses were not possible. There was no clear evidence of increased vascular risk immediately after starting treatment of a specific NSAID.

The risk associated with the duration of treatment with NSAIDs in patients with cardiovascular disease has only been specifically investigated in a few studies. Nussmeier et al. [23] administered COX-2 (intravenous parecoxib for 3 days, followed by oral valdecoxib for 7 days) or placebo for 10 days in patients after coronary bypass and found increased cardiovascular risk in treated patients after only 10 days. Similar results were also obtained in a population-based cohort study of elderly people starting rofecoxib [24]: the risk of myocardial infarction was highest following first-time use (adjusted RR, 1.67, 95% CI, 1.21–2.30), with events occurring within a median of 9 days of therapy.

Rofecoxib's dangerousness was confirmed in the APPROVE trial [22], in which approximately half ($n=1287$) of the participants were randomized to receive 25 mg of rofecoxib daily, while the remainder ($n=1299$) received placebo for over 3 years in patients with recurrent neoplastic polyps. Compared with placebo, patients taking rofecoxib were found to have increased risk of CV events (31 vs. 12 per 100 patient years; hazard ratio, 2.80;

95% CI, 1.44–5.45), which included myocardial infarction (21 vs. 9), cerebrovascular events (15 vs. 7 per 100 patient years; hazard ratio [HR], 2.32; 95% CI, 0.89–6.74), and nonadjudicated CV event including chronic heart failure (CHF), pulmonary edema, or cardiac failure (17 vs. 4 per 100 patient years; HR, 4.61; 95% CI, 1.50–18.83). The risk was not apparent until after 18 months of therapy. APPROVE was stopped prematurely and Merck took rofecoxib off the market.

In 2011 Schjerning Olsen et al. [25] published a national cohort study in which a time-stratified analysis of cardiovascular risks in patients with prior myocardial infarction starting therapy with coxibs or traditional NSAIDs was reported. NSAID treatment was significantly associated with increased risk of death/recurrent myocardial infarction (MI) (hazard ratio 1.45; 95% CI, 1.29–1.62) at the beginning of the treatment, and the risk persisted throughout the treatment course (hazard ratio 1.55; 95% CI, 1.46–1.64 after 90 days). Analyses of individual NSAIDs showed that among traditional NSAIDs diclofenac was associated with the highest risk (hazard ratio 3.26; 95% CI, 2.57–3.86 for death/MI at day 1–7 of treatment). These data were consistent with increased cardiovascular risk even during short-term treatment.

The same Danish team of researchers also recently showed that the risk of bleeding and cardiovascular events related to the concomitant use of antithrombotic therapy (aspirin, clopidogrel, anticoagulants, or their combination) and NSAIDs was significantly increased in patients after the first myocardial infarction [26]. A nationwide cohort study included more than 60,000 patients; of these 34% received at least one NSAID. The median follow-up was 3.5 years. Roughly 18,000 deaths occurred (29.2%) and approximately 5,000 bleedings (8.5%) and 18,000 cardiovascular events (30%), with an evident higher risk in patients treated with NSAIDs (bleeding 4.2 vs. 2.2 per 100-person-years; HR 2.02 [95% CI, 3.8–4.6], cardiovascular events 11.2 vs. 8.3 per 100-person-years; HR 1.4 [95% CI, 1.30–1.49]).

A similar investigation assessed the bleeding and thromboembolism risk related to ongoing therapy with NSAID and antithrombotic treatment for atrial fibrillation [27]. Use of NSAIDs even in this setting was associated with increased absolute risk of serious bleeding and thromboembolic events. The study involved more than 150,000 patients. The median follow-up was 6.3 years. There were approximately 17,000 (11.4%) and 19,000 (13.0%) occurrences of serious bleeding and thromboembolism, respectively. At 3 months, absolute risk for serious bleeding within 14 days of NSAID exposure was 3.5 events per 1000 patients compared with 1.5 events per 1000 patients without NSAID exposure. In patients treated with oral anticoagulants the absolute risk difference was 2.5 events per 1000 patients. In both settings, the events prior to myocardial infarction and atrial

fibrillation occurred regardless of type of NSAID and even in short-term treatment.

Today data on the cardiovascular safety of NSAIDs are incomplete and most studies have focused only on a few compounds.

McGettigan et al. [20] performed a large systematic review of controlled observational studies concerning major cardiovascular events associated with use of individual NSAIDs, in different doses and in populations with low and high background risks of cardiovascular events, including data on less investigated drugs. The database comprised 51 studies and 43 unique datasets. Of the most studied drugs (10 or more studies), the highest overall risks were seen with rofecoxib, 1.45 (95% CI, 1.33–1.59) and diclofenac 1.40 (1.27–1.55), and the lowest with ibuprofen, 1.18 (1.11–1.25) and naproxen, 1.09 (1.02–1.16). Of the less studied drugs etoricoxib, 2.05 (1.45–2.88), etodolac, 1.55 (1.28–1.87), and indomethacin, 1.30 (1.19–1.41) had the highest risks. In pair-wise comparisons, etoricoxib had a higher RR than ibuprofen, RRR=1.68 (99% CI, 1.14–2.49) and naproxen, RRR=1.75 (1.16–2.64); etodolac was not significantly different from naproxen and ibuprofen.

Although not confirmed by all experimental and clinical evidence, the best pathophysiological explanation of the increased CV risk in patients on NSAID, especially COX-2 inhibitors, is the theory of the imbalance of prostaglandins (Fig. 25.1). COX-2-inhibitor-associated thrombosis may be mediated by the opposing effects of TXA2 and PGI2. These selective NSAIDs may increase CV risk at high doses by promoting thrombosis via decreased PGI2 production in the endothelium and unchecked production of TXA2 by COX-1. The imbalance in circulating levels of PGI2 and TXA2 results in increased vascular tone, platelet aggregation, and vascular smooth muscle proliferation due to the unopposed TXA2 effects [28].

2.2 Interactions Between Aspirin and Other NSAIDs

In patients with a known CV disease who are already on aspirin, NSAIDs may interfere with the antiplatelet effect of the drug. NSAIDs inhibit the cardioprotective effects of aspirin, a finding that has been consistently seen in several randomized controlled trials [4,5,29,30]. Subgroup analysis of data shows more than a twofold increase in the risk of myocardial infarction in patients randomized to receive aspirin who also took an NSAID for more than 60 days [4,5,29,30].

This interaction can be clarified by the molecular mechanisms: COX-1 and COX-2 are homodimeric peroxidase enzymes. Each dimer has three folding units: an epidermal growth factor-like domain, a membrane-binding domain, and an enzymatic domain. The enzymatic domain contains a peroxidase catalytic site and an

adjacent site for COX activity at the apex of a hydrophobic channel [31]. Aspirin blocks the access of arachidonic acid to the catalytic site by irreversibly acetylating a serine residue at position 529 in platelet COX-1, near but not within the catalytic site.

Nonaspirin NSAIDs can bind reversibly either within the hydrophobic channel or the catalytic site. The half-life of aspirin in blood plasma is much shorter than the half-life of most other NSAIDs and its initial binding affinity is relatively weak. This may result in prior occupancy of the catalytic site by NSAIDs. The steric change of the enzyme caused by this chemical bond prevents aspirin from gaining access to its target serine and from permanently inactivating the enzyme. NSAIDs achieve only a reversible inhibition of thromboxane A2 (TXA2) formation as long as they are there in sufficient amounts, as can be seen in Fig. 25.2, which shows the mechanisms of competition between aspirin and ibuprofen [30].

This interaction between aspirin and nonaspirin NSAIDs has been demonstrated in *in vitro* as in *in vivo* studies [32]. *In vitro* many NSAIDs (such as ibuprofen, piroxicam, etc.) may reduce the antiplatelet effects of aspirin, which can be seen at therapeutically relevant concentration, but there are significant differences between the compounds. Some authors explained this by interactions at different binding sites [33]. A recent molecular analysis by Saxena et al. [34] confirmed that the attenuation of antiplatelet action of aspirin is not a class effect of all NSAIDs and suggested that it depends on the ability of forming hydrogen bond with some amino acids of the hydrophobic channel. This study showed that celecoxib, dipyron (active metabolite), ibuprofen, flufenamic acid, naproxen, nimesulide, oxaprozin, and piroxicam significantly interfered with the antiplatelet activity of aspirin, while diclofenac, ketorolac, and acetaminophen did not. However, among the interfering compounds the specific pharmacokinetic properties, the timing of administration, and their dosing intervals remain of critical importance for the potential interaction with aspirin, as is clear from the clinical studies.

In this regard the most remarkable evidence is with ibuprofen. In 2001 Catella-Lawson et al. [4] found that ibuprofen counteracted the antiplatelet effects of aspirin if it was given 2h before aspirin or in multiple daily doses. Moreover, they reported that no interference was noted in patients who had taken aspirin before ibuprofen. These results were confirmed by several studies [29,35–40]. Two recent studies focused on time doses depending interactions: a single-blind placebo-controlled trial in healthy volunteers [41] evaluated the interaction on acetylsalicylic acid (ASA)'s effect by naproxen, ibuprofen, meloxicam, and etoricoxib taken 2h before ASA. *Ex vivo* thrombocyte function was measured using the platelet function analyzer 100 (PFA-100), which showed that only ibuprofen and naproxen significantly inhibited ASA.

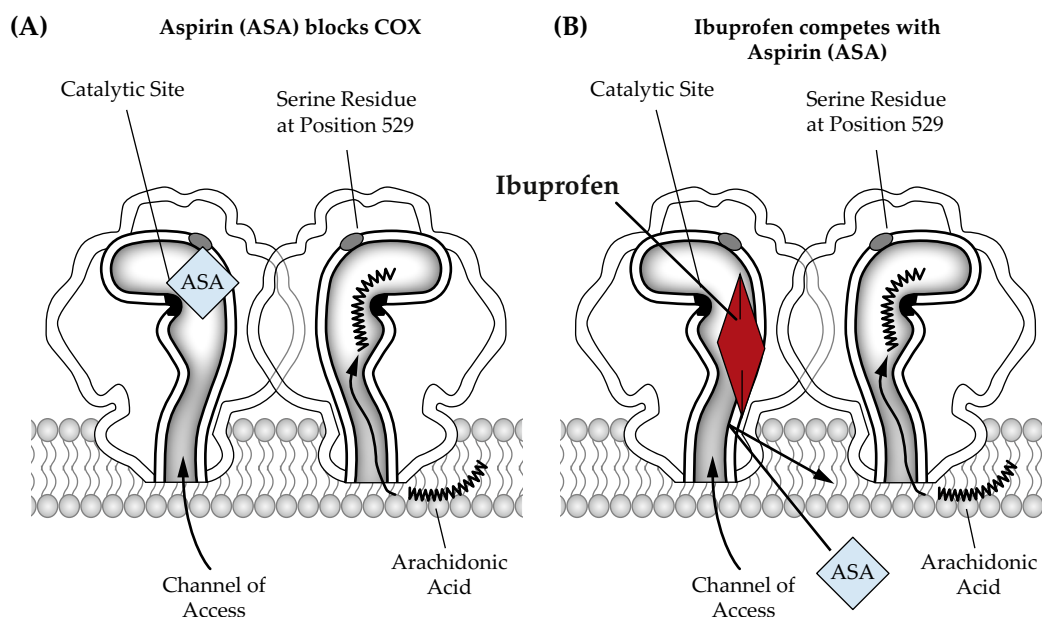


FIGURE 25.2 The mechanisms of aspirin action on COX-1 (panel A) and how ibuprofen may interfere with aspirin action (panel B). *Adapted from the personal collection of the authors.*

Yokoyama et al. [42] investigated the in vitro antiplatelet effect of NSAID alone, aspirin alone, and aspirin before and after NSAID addition to platelet-rich plasma. This model confirmed that the antiplatelet effect of aspirin is significantly diminished when taken after, but not before, ibuprofen or mefenamic acid. None of the other NSAIDs tested had any effect irrespective of the timing of dosing, and naproxen only showed a mild thrombocyte inhibitory effect. However, data on naproxen are controversial: some studies showed negative interference with aspirin's effects [4,43] and others an ASA-like activity [5,44,45].

2.3 Balancing Gastrointestinal and Cardiovascular Side Effects

Current guidelines on NSAID use have been developed by rheumatologists [46], gastroenterologists [47–50], cardiologists [51,52], or multidisciplinary teams of experts [53–57]. Rheumatologists were first concerned with safety, thus recommending paracetamol (acetaminophen) as a first-line analgesic. Gastroenterologists dealt mainly with gastrointestinal (GI) risk factors and gastroprotection, while cardiologists were worried about CV safety and suggested naproxen use in patients with CV risk factors. Some multidisciplinary consensus papers discussed both GI and CV risks and put forward evidence-based proposals on how to balance the benefits and risks of antiinflammatory therapy [53–57].

For patients with both low GI and CV risks, any non-selective NSAID (ns-NSAID) alone may be acceptable. For those with low GI and high CV risk, naproxen may be preferred because of its potential to lower CV risk compared

with other ns-NSAIDs or COX-2 selective inhibitors. In patients with high GI risk, if CV risk is low, a COX-2 selective inhibitor alone or a nonselective NSAID with a proton pump inhibitor appears to offer similar protection from upper GI events. However, only celecoxib will reduce mucosal harm throughout the entire GI tract. When both GI and CV risks are high, the optimal strategy is to avoid NSAID therapy, if at all possible (Figs. 25.3 and 25.4) [58].

Additional potential adverse CV events include new or worsening hypertension and congestive heart failure [28,59,60]. Although prostaglandins have both vasodilator and vasoconstrictor effects, the overall effects of NSAIDs are to raise systemic vascular resistance and to reduce renal perfusion in susceptible individuals [61]. These

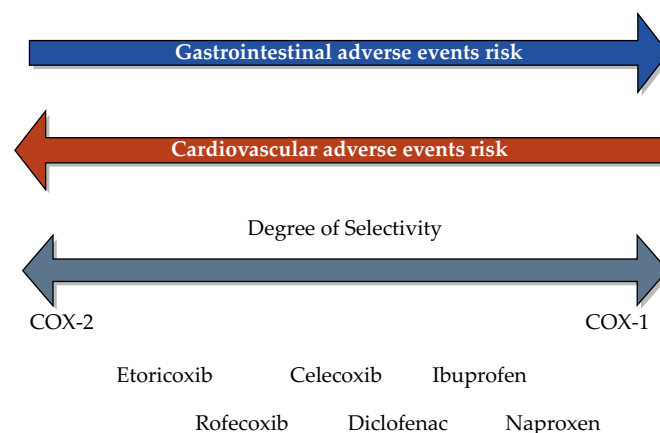


FIGURE 25.3 The spectrum of COX inhibitors and increased risk of CV adverse events (see text for additional explanation). *Adapted from the personal collection of the authors.*

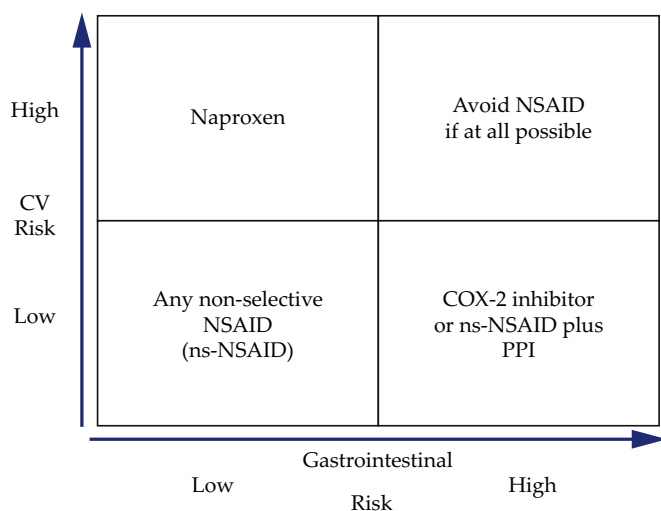


FIGURE 25.4 Management of antiinflammatory therapies with NSAIDs according to gastrointestinal (GI) and cardiovascular (CV) risks. Adapted from the personal collection of the authors.

effects explain how NSAIDs can raise blood pressure, antagonize effects of antihypertensive drugs, and increase hypertension-related morbidity. Moreover, in predisposed patients NSAIDs can exacerbate their tendency to develop symptomatic congestive heart failure [28,59–62].

3. HYDROXYCHLOROQUINE

Hydroxychloroquine (HCQ) is a 4-aminoquinoline that differs from chloroquine (CQ) only by the addition of a hydroxyl group decreasing its toxicity while conserving its efficacy. Both drugs belong to the group of the antimalarial agents, but they have now become mainstays in the management of rheumatic diseases, principally systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) [63]. Due to its lower toxicity, HCQ is the form used predominantly today.

Hydroxychloroquine has numerous known immunomodulatory effects but its specific mechanism in individual diseases is not clear. The major proposed mechanism of action of antimalarials on the immune system include: (1) interference with lysosomal acidification and inhibition of proteolysis, chemotaxis, phagocytosis, and antigen presentation; (2) decreasing macrophage-mediated cytokine production, especially interleukin (IL)-1 and IL-6; (3) inhibition of phospholipase A2 and thereby antagonizing the effects of prostaglandins; (4) absorption and blocking of UV light cutaneous reactions; (5) binding and stabilizing DNA; (6) inhibition of T- and B-cell receptors calcium signaling; (7) inhibition of matrix metalloproteinases and inhibition of toll-like receptors signaling [64].

Recently, antimalarials have been shown to have potential beneficial effects in many other immunological diseases, including Sjögren's syndrome, antiphospholipid

syndrome, undifferentiated connective tissue diseases, etc. In addition there is growing evidence of their beneficial impact on cardiovascular risk [64].

3.1 Antithrombotic Effect

Several studies indicate that CQ analogues have an effect in the prevention of thrombotic phenomena [65]. The antithrombotic effect of CQ analogues has been attributed to a range of mechanisms, including reduction in red blood cell aggregation, inhibition of platelet aggregation and adhesion, reduction in blood viscosity, and enhancement of antiplatelet activity with consequent secretion of arachidonic acid [66–68]. In clinical studies of thrombosis, HCQ was originally used as antithrombotic prophylaxis treatment in orthopedic surgery and has been shown to be effective in several studies [69,70]; today HCQ prevents significant thromboembolic events in the postoperative period following total hip arthroplasty or during pregnancy [71,72]. In SLE, several studies have demonstrated the decreased risk of thrombosis by HCQ, which has also been associated with better survival in a multivariate analysis [73–75].

Hydroxychloroquine has also been shown to reduce antiphospholipid-antibodies titers in the plasma of SLE patients with associated antiphospholipid syndrome (APS) [76]. In mice with antiphospholipid-induced thrombosis, HCQ reduces the extent and size of the thrombus [77].

3.2 Hypoglycemic Effect

The mechanism by which HCQ exerts a hypoglycemic effect is not entirely clear. Inflammation is known to be associated with impaired glucose control and for this reason the antiinflammatory effect of this therapy could be an explanation. Chloroquine analogues has been shown to increase C-peptide response, which Gerstein et al. cite as a potential effect of improved β cell functioning with decreased blood glucose [78,79]. Furthermore, HCQ's inhibitory effect on insulin metabolism has also been demonstrated in animal models, with effects including reductions in lysosomal intracellular insulin degradation and increases in insulin accumulation [80–82]. This results in an elevation of insulin blood concentration with consequent reduced glucose levels in a concentration-dependent fashion in diabetic rats [83]. This effect on insulin metabolism appears to have pharmacologic significance based on several lines of evidence. Case reports have documented patients developing hypoglycemia while using HCQ [84]. Several randomized controlled trials among patients with poorly controlled diabetes demonstrated that HCQ significantly reduces levels of glycated hemoglobin, on the order of what is observed with sulfonylureas in diabetic patients with and without concomitant rheumatic disease [78,85,86].

When HCQ was combined with insulin for the treatment of diabetes mellitus, glycated haemoglobin decreased significantly compared with patients receiving placebo, and the insulin dose had to be reduced by 30% in the HCQ group [87]. Large epidemiologic studies among patients with RA have shown that HCQ use is associated with significant reductions in the risk of diabetes [88,89] and the risk reduction was dose-dependent as demonstrated in a nationwide, population-based cohort study that was conducted using the Taiwan National Health Insurance Research Database of SLE patients [90].

Only a double-blind cross-over study by Solomon et al. seems to disagree with the prior clinical studies of diabetes prevention as well as insulin and glucose metabolism involving HCQ and patients with RA. These findings may be due to the shortness of HCQ treatment, which did not allow an effect on insulin and glucose metabolism to be observed. Instead, this study showed small and statistically significant improvements in total and LDL cholesterol during HCQ treatment [91].

3.3 Antilipidaemic Effects

There is increasing evidence demonstrating the beneficial impact of antimalarial agents on lipids in particular in reducing the levels of total cholesterol, triglycerides, and LDL while increases levels of HDL cholesterol irrespective of concomitant steroid administration, diet, or weight [92,93]. Chloroquine analogues have plasma lipid-lowering effects both in RA and in SLE, which is therapeutically relevant due to the increased risks of premature atherosclerosis in these diseases [71,94–96]. In fact, dyslipidaemias are very frequent in SLE and certainly play a pivotal role in the 50-fold greater risk of developing coronary artery disease, which is an important cause of mortality in SLE patients [97].

The mechanism by which this effect is mediated is uncertain. Hydroxychloroquine has a relatively weak antiinflammatory effect; therefore, it is unlikely that the beneficial effect on lipids is conferred solely by controlling systemic inflammation. In cultured human fibroblasts and mouse adrenal cells, CQ has multiple effects and it has been reported that it is an inhibitor of cholesterol biosynthesis in rat hepatocytes [98]. Hydroxychloroquine also inhibits lysosomal hydrolysis of cholesteryl esters through an increase in the pH within lysosomes and inactivates acid proteases [99].

Furthermore, HCQ upregulated LDL-C receptors, enhancing the plasma removal of this lipoprotein and resulting in lowering of its serum levels [100], and also increased activity of HMG-CoA reductase and slowed degradation of the enzyme [64].

Interestingly, changes in lipoproteins have been detected as early as 3 months after antimalarial therapy in 24 patients with SLE [101]. Finally, in a large longitudinal

study involving 1260 patients with SLE, the use of anti-malarials was negatively correlated with total cholesterol levels and was also associated with lower levels of blood pressure, both systolic and diastolic. However, a reduction in blood pressure is not known to be a direct pharmacologic effect of this class of drugs [102].

3.4 Adverse Events

Retinopathy as a toxic result of long-term use of this medication has traditionally been underlined, while cardiac side effects are rarely reported, but in some cases can be severe and irreversible.

Retinopathy is the most important ophthalmologic complication of antimalarial therapy. Antimalarials bind to melanin in the pigmented epithelial layer of the retina, an effect that may damage rods and cones, which may lead to permanent vision loss. The exact incidence of retinopathy is uncertain; with at least 10 years of use, it may occur in up to 3% to 4% of patients taking HCQ, in up to 10% of those taking chloroquine, and never in those taking quinacrine [103,104].

Estimates of risk depend on the sensitivity of the screening method, and risk increases with higher doses and a greater duration of therapy [104,105].

A registry-based study of 3,995 patients with RA or systemic lupus (SLE) who used hydroxychloroquine found that the risk of toxicity was low in the first 5–7 years of exposure (0.29–0.33%, respectively) [104]. The point estimates of risk rose steadily thereafter; risks at 10, 15, and 20 years were 1%, 2.1%, and 3.1%, respectively. Similarly, risk was significantly greater for patients treated with a cumulative dose of greater than 1000g of HCQ, compared with those treated with less (odds ratio 4.5, 95% CI, 1.4–14.5).

The earliest retinal abnormalities are asymptomatic and can only be detected by ophthalmologic examination. These “premaculopathy” changes consist of macular edema, increased pigmentation, increased granularity, and loss of the foveal reflex. Subtle functional loss in the paracentral retina can occur before biomicroscopic changes in the retinal pigment epithelium [106–108]. Detection of changes at this stage, using techniques such as spectral-domain optical coherence tomography (SD-OCT) and multifocal electroretinography, is desirable since such changes are likely to stabilize without loss of visual acuity, and in some cases retinopathy may be completely reversible on discontinuation of the medication [109,110]. With advanced electrophysiological screening methods, up to 7% of patients taking HCQ have retinal changes after 5 years of use. These are rarely symptomatic, but may require alterations in dosing regimen and drug discontinuation.

Monitoring is necessary to avoid permanent vision loss, although incidence of retinal toxicity is reduced

through the use of lower doses of medication. Several expert groups have issued recommendations for screening for antimalarial ocular toxicity [111,112].

The following is a brief summary of the monitoring of ocular health suggested for patients receiving hydroxychloroquine (HCQ) or chloroquine, based on the revised recommendations of the American Academy of Ophthalmology and the recommendations of the American College of Rheumatology [111,112]. All patients should undergo a baseline eye examination before or within a year of beginning treatment with an antimalarial drug; the examination should include a dilated retinal examination, automated visual field testing, and, if available, additional objective testing. The frequency of follow-up examinations range from every 6 months to 5 years, depending on age, hepatic and renal function, the presence of obesity or of concomitant retinal disease, duration of therapy, and cumulative dose (usual doses of HCQ are up to 200–400 mg/day and of chloroquine are 250 mg/day). A second eye examination is acceptable after 5 years in patients with normal eye examination and normal liver and kidney function at baseline. After 5 years of therapy, all patients should be rescreened at least annually. Retinal changes with HCQ use may be rapidly reversible in patients with very early changes, but significant clinical recovery is not generally seen once bilateral paracentral scotomas or visible bull's eye maculopathy is present.

Up to 70 cases of cardiotoxicity have been reported in the literature, although less than half of these have been proven on endomyocardial biopsy [113]. In the past, CQ has been predominantly implicated, but more recently several reports of HCQ-induced cardiomyopathy have emerged, likely reflecting its increased prevalence of use [113–118]. Reported cases have occurred predominantly in SLE including discoid lupus but also in RA and scleroderma [119]. Cardiac toxicity remains controversial and includes conduction disturbances and cardiomyopathy. In the largest prospective study conducted to date on 85 patients treated with HCQ, the rate of heart conduction disorders during the 12-month follow-up was similar to what is expected in the general population [120]. Cardiac side effects comprise conduction disturbances and cardiomyopathy. At present there are about 30 reports of patients with conduction disorders such as bundle-branch and atrioventricular block. The drug has a quinidine-like effect with possible QT prolongation, which may predispose to arrhythmias [121]. The evolution of the conduction disorder follows a typical pattern with development of complete right bundle branch block and left fascicular block, which may progress to complete heart block [121–123]. Overall conduction defects have been reported in 18 of 112 patients with SLE (16%) [122]. A case report suggested that CQ cardiotoxicity manifested suddenly as atrioventricular

block with QT(c) interval prolongation and short torsade de pointes [124].

The second most common cardiac adverse event is the development of cardiomyopathy—often with hypertrophy, restrictive physiology, and congestive heart failure. To date there are about 50 published cases. These patients are especially women aged between 30 and 81 years, a cumulative dose from 15 to 5040 g, and 2–35 years of use [118]. Overall this adverse event is reported in less than 1% of treated patients [122]. Chloroquine and HCQ may induce lysosomal dysfunction with an accumulation of pathologic metabolic products, which can be seen in ultrastructural histology as pathognomonic cytoplasmic inclusion bodies [125,126]: the usual phenotypic presentation is with cardiac hypertrophy, restrictive physiology, and possible evolution toward congestive heart failure [118,127,128]. The outcome is variable with usual reversibility, but worsening cases leading to cardiac transplantation have also been reported in isolated case reports [128].

Cardiotoxicity is difficult to diagnose. Many cases may be asymptomatic at the beginning, and initial manifestations may be masqueraded by the concomitant signs and symptoms of the rheumatologic disease that is treated. The most frequent presenting symptoms relate to decompensate left or biventricular failure but nonspecific chest discomfort may be a presenting or coexistent feature [102]. Clinical features of other toxicities may also be present [120].

Since, at present, there are no well-established risk factors and limit values for dosages and duration of therapy, it is important to adequately monitor these patients. On this basis, it is recommended to perform an annual follow-up, which should include physical examination (assessment for congestive heart failure signs and symptoms, accompanying visual dysfunction, or neuromyopathy), an electrocardiogram (assessment for conduction disorder or abnormality of repolarization), and a serum enzyme count (creatinase, troponin). Echocardiography plays a key adjunctive role in the diagnosis of HCQ cardiotoxicity. Diffusely thickened ventricular walls on transthoracic echocardiography are one of the hallmarks of this form of cardiomyopathy, biatrial enlargement and restrictive physiology are frequently associated [118]. Such findings, in the absence of significant systolic dysfunction, may be the predominant structural abnormalities seen on echocardiogram or may precede the development of systolic dysfunction [118] but also assessment for hypertrophy, abnormal myocardial texture, and restrictive physiology are important findings.

If myocardial damage is suspected, further diagnostic measures are essential to provide an accurate diagnosis and exclude differential diagnoses, such as amyloidosis, myocarditis, and sarcoidosis: (1) Cardiac magnetic

resonance (CMR) also has a prognostic value through its assessment of the degree of fibrosis as determined by delayed gadolinium enhancement imaging. In fact, the presence of delayed gadolinium enhancement in nonischemic cardiomyopathies predicts an eightfold increased risk of an adverse cardiac outcome [129]. (2) Endomyocardial biopsy with an electron microscopic examination; histopathological findings in HCQ cardiotoxicity are typical and include enlarged and vacuolated cells on light microscopy and the presence of myelinoid and curvilinear bodies, thought to represent abnormal lysosomes, within cardiac myocytes on transmission electron microscopy; and curvilinear bodies in particular appear to be the most specific histological indicator of antimalarial-related cardiotoxicity and were first reported by Piette et al. [130].

The CQ/HCQ medication should be discontinued immediately if cardiac toxicity is suspected because of the early reversibility of cardiomyopathy [118].

4. METHOTREXATE

Methotrexate (MTX) is an analogue folic acid (folate) with similar structural and physicochemical properties of folate. It was first introduced as a chemotherapeutic agent to inhibit nucleotide biosynthesis in various cancers. A low dose of MTX was later found to be effective for the treatment of psoriatic arthritis and of RA, and it is now recognized as a DMARD [131].

The pharmacological action is primarily attributed to its ability to inhibit multiple enzymes that are involved in *de novo* biosynthesis of nucleotides such as dihydrofolate reductase, thymidylate synthase, aminimidazole carboxamide ribonucleotide transformylase (AICART), and amido-phosphoribosyl-transferase, resulting in inhibition of purine and thymidylate synthesis, thus interfering with DNA synthesis, repair, and cellular replication. Methotrexate is cell cycle specific for the S phase of the cycle. Actively proliferative tissues are more susceptible to its effects [131]. Although the mechanism involved in the antiinflammatory effect of MTX in RA therapy remains controversial, AICART has been proposed as the potential target of this therapy [132]. More recently, inhibition of the mammalian target of the rapamycin pathway (mTOR) has been proposed as a potential mechanism of action of MTX [133].

Due to increased risk of cardiovascular (CV) diseases related to autoimmune diseases, many studies have investigated the negative or positive relationship between different therapies and cardiovascular events. Methotrexate has two properties that may have an opposite effect on vascular disease and the prolonged use of this drug may, therefore, have significant clinical implications: it affects inflammation and homocysteinemia.

Long-term MTX therapy may promote hyperhomocysteinemia, adding to the already elevated baseline serum homocysteine levels observed in some patients with RA and, in so doing, may increase the risk of vascular disease [134]. However, MTX also decreases inflammation and thus seems to have a vasculoprotective effect. The first study that investigated the impact of these potentially paradoxical actions of MTX was done by Prodanovich et al. [135]. The study, a retrospective cohort study, analyzed computerized records of 7615 outpatients diagnosed with psoriasis and 6707 with RA at the Veterans Integrated Service Network and showed that MTX therapy reduced the incidence of vascular disease in this populations [135].

Low-to-moderate cumulative dose appears more beneficial than the higher dose, probably related to the fact that patients who had higher doses of MTX possibly had more severe and/or prolonged inflammatory state increased by their disease, which may not have been optimally controlled by the drug. In addition, a combination of MTX and folic acid led to further reduction in the incidence of vascular disease [135]. A very important prospective study showed that MTX therapy was associated with significantly increased survival for patients with RA. Choi et al. [136] observed a 60% survival benefit from all causes mortality and a 70% decrease in mortality from cardiovascular diseases in patients treated with MTX when compared with those who were not despite worse prognostic factors for mortality in treated patients at baseline [136]. Another study on data from the Veterans Affairs Rheumatoid Arthritis (VARA) registry investigated potential associations of methylenetetrahydrofolate reductase polymorphisms and use of MTX with time-to-cardiovascular event and showed that traditional risk factors conferred substantial CV risk, while MTX use and increasing years of education were protective [137,138].

Antiinflammatory properties of MTX were also used in another cardiac disease: chronic heart failure (CHF). In a prospective, randomized, placebo-controlled, single-blind study, conducted on 71 patients with chronic heart failure by Gong et al., MTX was added to the conventional therapy in 35 patients to modulate the expression of numerous inflammatory cytokines involved in the pathogenesis of chronic heart failure. The results suggest that the addition of MTX to conventional therapy for chronic heart failure has significant antiinflammatory effects and improved several indices of functional status such as New York Heart Association (NYHA) functional class, 6-min walk test distance, and quality of life (QOL) [139].

Classical cardiovascular risk factors, in particular dyslipidemia and diabetes, have been studied in order to assess the influence of MTX on their metabolism. The investigation by Geraminova et al. enrolled 193 patients (168 women and 25 men) less than 60 years of age with RA

studied for cardiovascular risk, divided in MTX-treated and untreated group. Dyslipidemia was significantly less frequently identified in long-term MTX-treated patients, in particular the serum of treated-patients exhibited higher high-density lipoprotein cholesterol (HDL) concentrations, but MTX failed to affect the incidence of cardiovascular diseases in these population [140].

Discordant results from Navarro–Millán and colleagues found a robust increase in total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) after treatment for 6 months with MTX in mono-therapy, or associated with other therapies (etanercept, or combination therapy with sulfasalazine and HCQ). Despite the increase in total cholesterol, LDL-C, and HDL-C, the TC/HDL-C ratio (ie, atherogenic index) decreased slightly in all treatment arms [141].

It should be noted that in autoimmune disease the lipid association with cardiovascular disease may be paradoxical. In fact, raised total cholesterol and reduced HDL cholesterol levels are established cardiovascular disease risk factors; however, in autoimmune conditions the lipid-association appears paradoxical, with inflammation as a potential confounding factor. In fact, inflammation induces an inverse association between raised C-reactive protein (CRP) levels and both total cholesterol and HDL cholesterol levels. Johnson studied a total of 11,437 blood samples and identified a significant ($p < 0.001$) biphasic relationship between total cholesterol and CRP: total cholesterol increased within the healthy CRP range of less than 5 mg/L, but decreased with CRP levels above 10 mg/L. The two effects approximately cancelled each other out in the intermediate CRP range of 5–10 mg/L. There was an inverse relationship between HDL cholesterol and CRP. These authors concluded that lipid levels change significantly during inflammatory illness in a population with both acute and chronic conditions, providing a strong epidemiological basis for the better understanding of lipid changes in inflammatory conditions and with antiinflammatory therapies [142].

The impact of MTX glycemic metabolism has also been investigated. A study on diabetic patients with concomitant rheumatic diseases showed efficacy of HCQ, but not of MTX, in reducing glycated hemoglobin levels [85]. More recently de Rotte et al. showed that triple DMARD therapy or MTX monotherapy and increased erythrocyte methotrexate polyglutamate (MTXGlu) concentrations in RA patients are associated with reduced levels of glycosylated hemoglobin (HbA1c) after 3 months of treatment [143]. The mechanism was partly mediated through a decrease in inflammation, which explains why treatment with other DMARDs also resulted in reduced HbA1c levels. But there may also be an additional direct effect of MTX on glucose metabolism. Actually

prolonged treatment with low doses of MTX increased skeletal muscle glucose transporter 4 expression in mice with experimentally induced diabetes and was also associated with significant reduction of glucose and insulin serum concentrations in control mice and diabetic mice [144].

Major reported side effects of MTX have been hepatotoxicity, bone-marrow suppression, osteoporosis, and renal toxicity. Serositis has also been reported; in particular, pleurisy was the most common form [145,146]. Although pericarditis is a rare event several cases have been described, and few also occurred in patients with RA treated with MTX monotherapy [147–149]. Pericarditis usually occurred weeks to months after initial exposure to MTX and always had a close temporal relationship to MTX ingestion. The pathogenesis of MTX-induced serositis is not understood. MTX-induced pneumonitis, felt by some to be related to pericarditis, has been presumed to result from immunologic mechanisms based on the observation of MTX-induced leukocyte inhibitor factor production by peripheral blood lymphocytes and on the predominance of helper T lymphocytes in bronchoalveolar lavage fluid [150,151]. The presence of a recurrent right eosinophilic pleural effusion and pleuropericarditis associated with MTX treatment was also described in a psoriatic arthritis even if MTX had not previously been described as drug-induced eosinophilic effusions [152].

5. SULFASALAZINE

Salicylazosulfapyridine (sulfasalazine, SSZ) was originally proposed as a treatment for RA because of its antiinflammatory and antimicrobial activities [153,154]. Although early studies suggested a beneficial effect, the drug's efficacy was challenged by the findings of a negative 1948 report, which was influential despite a severely flawed study design [155,156]. The introduction of corticosteroids during this period, an event hailed as a modern medical miracle, further dampened enthusiasm for the use of SSZ in RA.

Salicylazosulfapyridine was resurrected as a therapeutic agent for rheumatic disorders after beneficial results were reported in a trial performed in the late 1970s and in the first placebo-controlled trial in 1983, and it is widely available [157,158].

Approximately 30% of SSZ is rapidly absorbed by the small bowel and is then returned, largely unaltered, via the enterohepatic circulation into the bile. Thus approximately 90% of the ingested drug reaches the large intestine as an intact molecule [155,159]. In the colon, SSZ is reduced by the bacterial enzyme azoreductase to sulfapyridine and 5-aminosalicylic acid (5-ASA). Coliform bacteria are, therefore, necessary to reduce the relatively

inactive parent drug to its active moieties. Nearly all of the sulfapyridine is absorbed, while 5-ASA is largely excreted in the feces, thereby explaining its utility in inflammatory bowel disease.

Sulfapyridine is subsequently metabolized in the liver via hydroxylation and acetylation. The half-life of these components is prolonged in slow acetylators, a property that may affect toxicity but not efficacy. No major drug-drug interactions have been reported [155,159].

Mechanism of Action—Studies directly comparing the efficacy of sulfapyridine with 5-aminosalicylic acid (5-ASA) suggest that sulfapyridine is the active moiety in patients with RA, unlike in inflammatory bowel disease in which 5-ASA is the active metabolite [160–163]. Sulfapyridine may have disease-modifying effects since its administration is associated with decreases in the erythrocyte sedimentation rate (ESR) and serum concentrations of CRP [161,162].

The mechanism of action of sulfapyridine in RA has not been identified [159]. Since sulfapyridine is a sulfonamide, an antibacterial effect has been entertained; however, the observation that other sulfonamides are ineffective in RA makes this hypothesis unlikely. For example, one 24-week study directly compared sulfapyridine (1.25 g per day) with cotrimoxazole (trimethoprim-sulfamethoxazole, 480 mg three times daily) in patients with RA [164]. Disease activity either was unchanged or had worsened among patients treated with cotrimoxazole; by comparison, improvement was observed in those administered sulfapyridine. Sulfapyridine may also reduce secretions of inflammatory cytokines such as interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1 [163].

Furthermore, a positive effect due to the parent molecule alone cannot be entirely excluded. Several investigators have studied the parent molecule and have determined several plausible mechanisms of action. As an example, increased production of adenosine at sites of inflammation has been suggested to be an antiinflammatory property of SSZ that it shares with another effective antirheumatic drug, MTX [165,166].

Another possible mode of action is the inhibition of nuclear factor-kappa B (NF- κ B). This protein, which is also suppressed by glucocorticoids, induces the transcription of central mediators of the immune response. An in vitro study found that the administration of SSZ strongly inhibited NF- κ B-dependent transcription in colonic cells [167]. Interestingly, these actions were unique to SSZ and were not observed with sulfapyridine or with 5-ASA, thereby suggesting that the parent molecule may have important antiinflammatory properties. Salicylazosulfapyridine may also inhibit osteoclast formation via modulatory effects on the receptor activator of NF- κ B (RANK), osteoprotegerin (OPG), and RANK-ligand [168]. There may be additional immunosuppressive effects. For example, SSZ inhibits tumor necrosis

factor (TNF)- α expression via apoptosis of macrophages [169]. Finally, SSZ and its metabolites appear to suppress B-cell function but not T-cell function [170].

6. MINOCYCLINE

Minocycline (and doxycycline) is a semisynthetic tetracycline antibiotic with antiinflammatory properties used to treat multiple inflammatory diseases, including RA. Tetracyclines exhibit multiple antiinflammatory properties, including the inhibition of T-cell activation and chemotaxis, the downregulation of proinflammatory cytokines, including TNF α and IL-1 β , and the inhibition of matrix metalloproteinases [171].

Minocycline has proven to be a very safe and moderately effective DMARD in the treatment of RA, but its efficacy appears to vary greatly depending on the patient population in which it is used. Although an initial open-label study using minocycline in treatment-resistant RA was encouraging, two subsequent double-blind, placebo-controlled studies from the 1990s found only modest, although statistically significant, clinical improvement. The participants in these latter two trials had long-standing, DMARD-refractory disease. In contrast, more recent trials examining minocycline in DMARD-naïve, early RA yielded more impressive results. In separate studies, minocycline showed superior efficacy and similar tolerability to placebo and hydroxychloroquine [171].

Sulfasalazine and minocycline have been demonstrated to be possible rare causes of drug-induced hypersensitivity syndrome (DIHS) with eosinophilic myocarditis [172,173].

Drug-induced hypersensitivity syndrome is a rare but serious adverse drug reaction. It is characterized by a delayed onset, variable clinical symptoms, and prolonged disease course. Anticonvulsants, diaphenyl-sulfone, salazosulfapyridine, allopurinol, minocycline, calcium blockers, terbinafine, and mexiletine are the main drugs that cause this syndrome.

Visceral organ involvement is common and may manifest as hepatitis, nephrotoxicity, cerebral edema, pneumonitis, pericardial effusion, hypereosinophilia, leukocytosis, myocarditis, and/or thyroiditis. The mortality rate is approximately 10% and is usually due to hepatotoxicity or myocarditis [174,175].

Myocarditis associated with DIHS often occurs one to several months later and even after the offending drug has been discontinued. Myocardial injury is mediated by toxic cationic proteins, oxygen metabolites, and lipid mediators produced by eosinophils [172].

Acute necrotizing eosinophilic myocarditis is a rare and severe complication of drug-induced hypersensitivity syndrome that usually presents with acute chest pain, ST-segment elevation, and an increase in cardiac

enzymes, frequently with rapid deterioration of systolic function and a mortality rate above 50%. Pericarditis and cardiac arrhythmias are also possible presentation. In most cases the diagnosis is established on autopsy [174].

Necrotizing eosinophilic myocarditis, presenting as acute myocardial infarction, has been described in a patient with ankylosing spondylitis 6 weeks after starting sulfasalazine therapy [176].

Variability in clinical and laboratory presentation and concomitant use of antiinflammatory drugs may delay the diagnosis of drug-induced hypersensitivity syndrome in patients. The absence of blood eosinophilia does not rule out the diagnosis of drug-induced eosinophilic myocarditis [172]. A positive *in vitro* lymphocyte transformation test may be helpful in the diagnosis of sulfasalazine-induced drug-induced hypersensitivity syndrome. After 6 days of culture, the lymphocyte transformation test measures drug-specific proliferation (^3H -thymidine incorporation) in comparison with negative and positive controls [177].

The diagnosis of eosinophilic myocarditis is based on clinical criteria including electrocardiography, echocardiography, and cardiac enzymes. The diagnosis is confirmed by endomyocardial biopsy. In subacute and chronic eosinophilic myocarditis, intracardiac thrombi, endomyocardial fibrosis, and restrictive cardiomyopathy can be found.

The autopsy revealed eosinophilic myocarditis with prominent interstitial edema and inflammatory infiltrate composed of eosinophils and mononuclear cells. Vascular congestion of alveolar wall capillaries and alveolar damage were present [174].

7. VARESPLADIB

Phospholipase A(2) (PLA(2)) are enzymes that hydrolyze the ester bond of glycerophospholipids releasing free fatty acids and lysophospholipids, including the arachidonic acid, the precursor of the eicosanoids, and the inflammatory cascades. PLA(2) are present in the atherosclerotic plaques and their direct involvement in the proatherogenic inflammatory response is well documented. Epidemiological and genetic studies have demonstrated the correlation of the PLA(2) mass and enzymatic activity with the incidence of cardiovascular diseases [178].

However, although some sPLA₂ isoforms are proatherogenic (groups IIA and V), other isoforms are protective (group X). Higher circulating levels of sPLA₂-IIA concentration and activity are associated with cardiovascular risk in asymptomatic individuals and in patients with established coronary disease [178].

Varespladib methyl is a nonspecific pan-sPLA₂ inhibitor with favorable effects on atherosclerotic lesions in animal studies. Initial studies demonstrated that

varespladib reduced levels of sPLA₂-IIA by more than 90%, in addition to lowering low-density lipoprotein cholesterol (LDL-C) and CRP in patients with stable coronary disease and acute coronary syndrome (ACS) [179–181]. The Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks (VISTA-16) study was designed to evaluate the effects of varespladib on cardiovascular risk in patients with ACS [182]. The VISTA-16 trial is a double-blind, randomized, multicenter trial of 5145 patients randomized within 96 h of presentation of an ACS to either varespladib ($n=2572$) or placebo. The primary efficacy measure was a composite of cardiovascular mortality, nonfatal myocardial infarction (MI), nonfatal stroke, or unstable angina with evidence of ischemia requiring hospitalization at 16 weeks. Six-month survival status was also evaluated. The primary endpoint occurred in 136 patients (6.1%) treated with varespladib compared with 109 patients (5.1%) treated with placebo (hazard ratio [HR], 1.25; 95% CI, 0.97–1.61; log-rank $p=0.08$). Varespladib was associated with a greater risk of MI (78 [3.4%] vs. 47 [2.2%]; HR, 1.66; 95% CI, 1.16–2.39; log-rank $p=0.005$). The composite secondary endpoint of cardiovascular mortality, MI, and stroke was observed in 107 patients (4.6%) in the varespladib group and 79 patients (3.8%) in the placebo group (HR, 1.36; 95% CI, 1.02–1.82; $p=0.04$). Despite experimental and observational clinical data suggesting that pan-inhibition of sPLA₂ would exert beneficial cardiovascular effect, the VISTA-16 trial provides evidence to the contrary. Despite lower achieved levels of LDL-C and CRP, there was no evidence of a beneficial reduction in the primary cardiovascular outcome. In contrast, treatment with varespladib caused an excess of MI and the composite of cardiovascular mortality, MI, and stroke. Consequently, these findings suggest that short-term sPLA₂ inhibition with varespladib is harmful following ACS. The precise mechanism underlying the adverse effect on the rate of MI with varespladib remains unknown. The study did not demonstrate an additional protective action of PLA₂ inhibitors over the standard of care treatment with statins, antiplatelet drugs, and coronary revascularization [182].

8. LEFLUNOMIDE

Leflunomide (LEF) is an isoxazole derivative used for the treatment of RA. Leflunomide is structurally unrelated to other immunomodulatory DMARDs and offers a unique mechanism for the therapy of RA.

Leflunomide has also been effective in patients with psoriatic arthritis [183], juvenile polyarthritis [184], refractory dermatomyositis [185], and systemic lupus erythematosus [186], but it has not been effective in ankylosing spondylitis [187]. Teriflunomide, the active

metabolite of LEF, was found effective in patients with multiple sclerosis and is available for use in that condition [188].

Leflunomide is an oral drug that is rapidly absorbed from the gastrointestinal tract. Once absorbed, LEF is converted to its active form, a malononitrilamide known as teriflunomide [189]. The major action of teriflunomide at the doses given for RA is inhibition of the synthesis of a pyrimidine known as ribonucleotide uridine monophosphate pyrimidine (rUMP).

Leflunomide decreases the synthesis of rUMP through inhibition of the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH). Inhibition of DHODH leads to inability of activated cells to move from G1 to the S phase by activating the p52 pathways of apoptotic selection. The inhibition of rUMP is thought to be the primary mechanism involved at the levels of drug reached in most patients [190,191].

One study has suggested that polymorphisms in the estrogen receptor allele, estrogen receptor 1 (ESR1), may be associated with differences in efficacy among women treated with LEF [192].

Leflunomide has effects on many components of the immune-inflammatory response, some of which may be relevant to its mode of action in RA:

- Leukocyte adhesion—LEF inhibits leukocyte adhesion to vascular endothelial cells [193,194]. This inhibition may be crucial during the “homing” of leukocytes to synovial high endothelial cells, which may occur early in the pathogenesis of RA [195,196].
- Memory T cells—LEF may have a preferential effect on self-reactive lymphocytes that comprise the memory cell population. Memory T cells have a greater tendency to undergo division arrest after restimulation with antigen than do lymphocytes undergoing a primary response [197]. Thus memory cells are more susceptible to dihydroorotate dehydrogenase (DHODH) inhibition.
- Dendritic cells—Teriflunomide interferes with dendritic cell function, thereby leading to disruption of antigen presentation.
- Synovial inflammatory cells—The administration of either LEF or methotrexate (MTX) significantly decreases levels of synovial infiltration of lymphocytes and type I synoviocytes [198]. This decreased synovial infiltration of inflammatory cells is accompanied by reductions in markers of vascular inflammation and metalloproteinase production, perhaps explaining the decreased rate of radiographic progression [199].
- NF- κ B—Teriflunomide blocks two steps essential for NF- κ B activation: the degradation of the inhibitor of kappa B α and the subsequent translocation of the p65 subunit to the nucleus. These effects block the proinflammatory consequences of NF- κ B activation in a dose- and time-dependent manner [200].
- Tyrosine kinases—Teriflunomide inhibits protein tyrosine kinases Janus kinase (Jak)1 and Jak3 [201]. These kinases influence the stimulation of T cells through their interleukin (IL)-2 receptors.
- Interleukins—Teriflunomide decreases the response to IL-4 by B cells, an effect that may augment the activity of hyaluronic acid synthetases [202–204]. Teriflunomide also inhibits IL-10 and IL-11 secretion and reduces the synthesis of IL-2 [205].
- Transforming growth factor (TGF)- β —Teriflunomide may increase the synthesis of TGF- β , a cytokine that exerts a dampening effect on the immune system in some settings [206].
- C-jun NH2-terminal protein kinase (JNK)—LEF protected against acetaminophen (APAP)-induced hepatotoxicity in an animal model by inhibiting APAP-induced activation of c-jun NH2-terminal protein kinase, JNK, thus preventing downstream Bcl-2 and Bcl-XL inactivation, mitochondrial permeabilization, and cytochrome c release [207].
- Other kinases—Inhibition of Jak3 and mitogen-activated protein kinase 2 (MAP3K2) [208].

Leflunomide was shown to have a potent antiviral and immunosuppressive activity in an allogeneic cardiac transplant model of cytomegalovirus infection [209] and proved to be effective in preventing cardiac allograft rejection when combined with rapamycin [210]. Leflunomide was shown to improve the clinical outcome of patients suffering from rheumatoid arthritis at an efficacy comparable to that of methotrexate (MTX) (level of evidence A) [190].

Besides diarrhea, alopecia, abdominal pain, nausea, and vomiting, hypertension is a common side effect, even if associations between leflunomide and cardiovascular risk factors are not well described [211].

A small percentage of patients with RA develop hypertension when taking LEF. Concurrent therapy with NSAIDs is a risk factor. The proposed mechanism is the displacement of NSAIDs from albumin by teriflunomide, thereby increasing NSAID activity. Blood-pressure monitoring is recommended during the first months of treatment and is also recommended if NSAID therapy is begun at a later date [211].

At the same time, the therapy with leflunomide was also shown to lower plasma glucose levels, possibly due to a decrease in body weight, a known side effect [212]. When compared to MTX monotherapy, treatment with leflunomide was found to be associated with an increase in cardiovascular events, which is suspected to be due either to the induction of hypertension as a known cardiovascular risk factor or the possible inferiority of leflunomide regarding inflammation control [213].

9. COLCHICINE

Colchicine, the extract from the *Colchicum autumnale* plant (autumn crocus), used by ancient Greeks more than 2000 years ago [214], is one of the oldest known drugs still prescribed today. In 1820, the French chemists P.S. Pelletier and J.B. Caventou were the first to extract colchicine as the active alkaloid of colchicum [215]. While colchicine in combination with probenecid was approved by the US Food and Drug Administration (FDA) prior to 1982, until recently colchicine was widely used for the treatment of gout without official FDA evaluation. On July 30, 2009, the FDA approved colchicine for the treatment of familial Mediterranean fever and acute gout and for prophylaxis against gouty arthritis [216]. Colchicine is also used today as a first-line treatment for pericarditis [13].

9.1 Pharmacokinetics

Colchicine is a lipid-soluble drug that can penetrate easily into body tissues after uptake in the jejunum and ileum [217]. After oral ingestion it has a 44% bioavailability and binds relatively weakly to albumin (32% bound). Its peak plasma concentration occurs in about 1 h, with a long terminal half-life [217]. Colchicine enters red blood cells and leucocytes where it remains in much higher concentrations than the plasma [218]. Colchicine can be found inside leukocytes for up to several days after administration [219], with this local pooling and long half-life explaining why a once- or twice-daily dosage scheme is enough for inflammation suppression. Colchicine is predominantly eliminated by the liver, through biliary excretion and metabolism by the hepatic cytochrome CYP3A4 [220], with about 10–20% of the drug secreted unchanged in urine. Peak plasma concentration and plasma half-life can be significantly prolonged in patients with liver or renal failure or in the elderly [221], with side effects appearing even at low doses.

Colchicine has a relatively low therapeutic index, with effective steady-state plasma concentrations ranging between 0.5 and 3 ng/mL.

9.2 Mechanisms of Action

Due to the rapid suppression of gouty arthritis without interfering with uric acid kinetics, colchicine is generally classified as an antiinflammatory agent in the medical literature. However, it should be noted that the characterization “antiinflammatory” defines only part of this drug’s actions. Colchicine is unique in that its mechanism of action does not involve the arachidonic acid pathway affected by NSAIDs and glucocorticosteroids. Colchicine binds to unpolymerized tubulin heterodimers, forming

a stable complex that effectively inhibits microtubule dynamics on binding to microtubule ends. It causes microtubule depolymerization by inhibiting lateral contacts between protofilaments [222], thus affecting any process that requires cytoskeletal changes, including cell mitosis, exocytosis, and neutrophil motility [220]. At low concentrations, colchicine inhibits the formation of microtubules while at higher concentrations it promotes their depolymerization [222]. Colchicine has been found to impair adhesion of neutrophils to the vascular endothelium. In vitro experiments have shown that colchicine diminishes endothelial selectin family-dependent adhesiveness, affecting both endothelial E-selectin and, at higher concentrations, neutrophil L-selectin surface expression [223]. It has also been found to increase leukocyte cyclic adenosine monophosphate levels, inhibit interleukin-1 (IL-1) production by activated neutrophils, and downregulate tumor necrosis factor α receptors in macrophages and endothelial cells [220].

Recently, colchicine was associated with another anti-inflammatory mechanism in gout. Colchicine appears to block the crystal-induced activation of the NLRP3 inflammasome protein complex, which proteolytically cleaves caspase-1 and leads to secretion of proinflammatory cytokines IL-1 β and IL-18 [224]. Other studies have shown the close interaction of microtubules and NLRP3 inflammasome activation [225]. Inflammasomes have recently been associated with atherosclerotic disease [226] and cardiac ischemia/reperfusion injury [227]. However, any direct effects of colchicine on inflammasome activation in the heart or vessels have not been studied.

Depending on concentration, colchicine could have other effects as well. A study by Ben-Chetrit et al. [228] showed that colchicine has the potential to induce changes at the transcriptional level (affecting cell-cycle-regulatory genes) in human umbilical vein endothelial cell line cells. However, the concentration used was almost 100-fold the average plasma concentration in patients under colchicine treatment, thus it is not clear if these results have any clinical meaning. In another recent in vitro study, colchicine prevented the contractile and mitochondrial dysfunction caused by the macrophage migration inhibitory factor in human atrial cells [229].

Colchicine is widely used in in vitro experiments requiring cytoskeleton disruption. Since colchicine was “officially” endorsed by the FDA, several human studies have emerged examining the possible potential of this low-cost drug for other clinical indications.

A significant percentage of patients report adverse effects, mainly diarrhea (approximately 7%) [230] and other gastrointestinal symptoms, serious enough to cause discontinuation of therapy even at low daily doses. Rare side effects of colchicine administration include liver failure, bone-marrow depression, which

may manifest as leucopenia or aplastic anemia, and rhabdomyolysis [230].

Overall, 1–2 mg/day of colchicine is safe even when given continuously for decades, as learned in patients with FMF (who usually now continue this drug also in pregnancy and lactation) and in patients with Behcet's disease, without any notable long-term side effects, contrasting sharply with NSAIDs and glucocorticoids. In FMF patients even prolonged exposure to colchicines seems to have no effects on male or female fertility and pregnancy outcomes [13].

The dose should be adjusted according to age and renal or hepatic dysfunction (Table 25.2).

Colchicine interacts with macrolides antibiotics (that decrease colchicine metabolism), statins (that increase the risk of myotoxicity), cyclosporine (reciprocal enhancement of adverse/toxic effects), and verapamil (increase verapamil serum concentration, enhances colchicines nephrotoxicity). When using these drugs, consider therapy modification or dose reduction [13].

9.3 Colchicine and Pericarditis

Following observational studies and multicenter randomized control trials [231–234], the new Guidelines for the Management of Pericardial Disease recommend colchicine as first-level drug for acute and recurrent pericarditis as adjunct to aspirin or NSAID in order to hasten the response to NSAID and reduce recurrence rates during follow-up [13].

TABLE 25.2 Dose of Colchicine According to Particular Condition

Condition Dose Adjustment	
Children	
≤5 years	0.5 mg/day
<5 years	1.0–1.5 mg/day in two or three divided doses
Elderly	
>70 years	Reduce dose by 50% and consider creatinine clearance (CrCl)
Renal Impairment	
Clcr 35–49 mL/min	0.5–0.6 mg once daily
Clcr 10–34 mL/min	0.5–0.6 mg every 2–3 days
Clcr <10 mL/min	Avoid chronic use of colchicine. Use in serious renal impairment is contraindicated by the manufacturer
Hepatic Dysfunction	Avoid in severe hepatobiliary dysfunction and in patients with hepatic disease
Pregnancy	There are no controlled data in human pregnancy. It has been assigned to pregnancy category C by the FDA

Colchicine halves the recurrences in acute pericarditis (first episode and recurrences) and is effective in the prevention of postpericardiotomy syndrome following cardiac surgery, as an adjunct to aspirin/NSAID therapy.

In “The Investigation on Colchicine in Acute Pericarditis (ICAP) [231],” eligible adult patients with acute pericarditis were randomly assigned to placebo or colchicine (0.5 mg twice daily for 3 months for patients >70 kg or 0.5 mg once daily if ≤70 kg) in addition to conventional antiinflammatory therapy with aspirin or ibuprofen in a multicenter, double-blind, placebo-controlled trial. Of the 240 randomly assigned participants (mean age 52.1 ± 16.9 years, 60% males), 65 patients (27.1%) reached the primary outcome: incessant/recurrent pericarditis within 18 months. The percentage was 16.7% in the colchicine group and 37.5% in the placebo group (relative risk reduction 0.56; 95% CI, 0.30–0.72; NNT 4). Colchicine reduced symptoms persistence at 72 h (respectively, 19.2% vs. 40.0%; $p=0.001$), number of recurrences, hospitalizations (respectively, 5.0% vs. 14.2%; $p=0.016$), and improved the remission rate at 1 week (respectively, 85.0% vs. 58.3%; $p<0.001$). Overall adverse effects and withdrawal rates were similar in the study groups. No serious adverse effects were observed.

Other randomized control trials obtained similar results: CORP trial (Colchicine for Recurrent Pericarditis) and CORP-2 trial (A Randomized Trial of Colchicine for Multiple Recurrences of Pericarditis) in which colchicine halved recurrences, even if it did not eliminate them [232,233].

In the CORP trial (120 patients with a first recurrence of pericarditis) at 18 months, the recurrence rate was 24% in the colchicine group and 55% in the placebo group [absolute risk reduction, 0.31 (95% CI, 0.13–0.46); relative risk reduction, 0.56 (CI, 0.27–0.73); number needed to treat, 3 (CI, 2–7)]. Colchicine reduced the persistence of symptoms at 72 h [absolute risk reduction, 0.30 (CI, 0.13–0.45); relative risk reduction, 0.56 (CI, 0.27–0.74)] and mean number of recurrences, increased the remission rate at 1 week, and prolonged the time to subsequent recurrence. The study groups had similar rates of side effects and drug withdrawal [232].

In the CORP-2 trial (240 patients with multiple recurrences of pericarditis) the proportion of patients who had recurrent pericarditis was 26 (21.6%) of 120 in the colchicine group and 51 (42.5%) of 120 in the placebo group (relative risk 0.49; 95% CI, 0.24–0.65; $p=0.0009$; number needed to treat 5). Adverse effects and discontinuation of study drug occurred in much the same proportions in each group [233].

The COPPS study [234] was instead a multicenter, double-blind, randomized trial for the prevention of the postpericardiotomy syndrome (PPS). On the third postoperative day, 360 patients (mean age 65.7 ± 12.3 years, 66% males), 180 in each treatment arm, were randomized to receive placebo or colchicine. The primary efficacy endpoint was the incidence of PPS at 12 months.

Secondary endpoint was the combined rate of disease-related hospitalization, cardiac tamponade, constrictive pericarditis, and relapses. Baseline characteristics were well balanced between the study groups. Colchicine significantly reduced the incidence of the PPS at 12 months compared with placebo (respectively, 8.9 vs. 21.1%; $p=0.002$; number needed to treat=8). Colchicine also reduced the secondary endpoint (respectively, 0.6 vs. 5.0%; $p=0.024$). The rate of side effects (mainly related to gastrointestinal intolerance) was similar in the colchicine and placebo groups (respectively, 8.9 vs. 5.0%; $p=0.212$).

In the trials mentioned earlier and in the new guidelines for the management of pericardial disease [13], colchicine is recommended at low, weight-adjusted doses to improve the response to medical therapy and prevent recurrences. The dose is 0.5mg once for patients <70kg or 0.5mg b.i.d. for ≥ 70 kg (1mg daily may improve the compliance, if tolerated).

Tapering of colchicine is not mandatory but may be considered to prevent persistence of symptoms and recurrence: alternatively 0.5mg every other day (<70kg) or 0.5mg once (≥ 70 kg) in the last weeks [13]. A loading dose was initially used but is now avoided to reduce potential gastrointestinal side effects and improve patient compliance.

Common side effects are gastrointestinal (up to 10% of cases) including nausea, vomiting, diarrhea, and abdominal pain, usually being a common cause of drug withdrawal; generally mild, they may resolve with dose reduction. Weight-adjusted doses may reduce these side effects. Notably diarrhea may be exacerbated by the common concomitant use of antibiotics and proton pump inhibitors. Less common side effects include elevation of transaminases and reversible alopecia. In <1% of cases, other side effects are reported, including bone-marrow suppression and myotoxicity.

In published trials colchicine has been used for 3 months after the initial episode of acute pericarditis and for 6 months after a recurrence. In recurrent more severe cases, some authors advocate a longer use of the drug: up to 12–24 months after the last recurrence, tailored to the individual patient and with gradual tapering, considering that the recurrences have been described after colchicine discontinuation. Most of the experts prefer to discontinue it as the last drug, after having discontinued first corticosteroids and secondly NSAIDs [13,235].

It's important to remember that colchicine halves but does not eliminate all recurrences. It generally fails in monotherapy: efficacy has been demonstrated almost exclusively for combination therapy with NSAID and/or corticosteroids. In chronic pericardial effusions with normal CRP it is generally not efficacious. It may be efficacious in pericardial effusion with elevation of inflammatory indexes.

9.4 Colchicine and Postoperative Atrial Fibrillation

Atrial fibrillation (AF) after cardiac surgery (postoperative atrial fibrillation [POAF]), occurring in 10–65% of all cardiac surgery patients [236], is a significant problem, in view of the associated increased morbidity and mortality and lengthening of hospital stays [237]. Besides the obvious atrial trauma, other factors have also been implicated in its pathogenesis, including surgery-related pericardial inflammatory processes, autonomic disturbance, and changes in plasma volume regulation [238].

Colchicine came to the forefront in a pivotal substudy of the COPPS (Colchicine for the Prevention of the Postpericardiotomy Syndrome) trial [239], a multicenter, double-blind, randomized trial. The administration of colchicine from postoperative day 3 and for up to 1 month was associated with 45% reduction in the incidence of POAF, followed not unexpectedly by reduced in-hospital and rehabilitation stay, while halving the mean duration of POAF episodes. There has been some controversy [240] as to whether postoperative day 3 is the optimum time for treatment initiation. The COPPS main study was designed to test colchicine efficacy in preventing postpericardiotomy syndrome and not POAF. The incidence of POAF is high on the first few postoperative days, which was reflected in the fact that 43% of the POAF episodes documented in the COPPS substudy occurred before the onset of colchicine treatment. It is reasonable to assume that an earlier (maybe even preoperative) initiation of treatment would be even more effective. Based on this hypothesis, the COPPS-2 trial (Colchicine for the Prevention of Postpericardiotomy syndrome and postoperative atrial fibrillation) [241] was a multicenter study including 360 cardiac surgery patients. Patients were randomized to receive placebo ($n=180$) or colchicine (0.5mg twice daily in patients ≥ 70 kg or 0.5mg once daily in patients <70kg; $n=180$) starting between 48 and 72h before surgery and continued for 1 month after surgery. The primary endpoint of postpericardiotomy syndrome occurred in 35 patients (19.4%) assigned to colchicine and in 53 (29.4%) assigned to placebo (absolute difference, 10.0%; 95% CI, 1.1–18.7%; number needed to treat=10). There were no significant differences between the colchicine and placebo groups for the secondary endpoints of postoperative AF (colchicine, 61 patients; placebo, 75 patients; 95% CI, –2.2 to 17.6%) or postoperative pericardial/pleural effusion (colchicine, 103 patients; placebo, 106 patients, CI, –8.5 to 11.7%), although there was a reduction in postoperative AF in the prespecified on-treatment analysis (placebo, 61/148 patients [41.2%]; colchicine, 38/141 patients [27.0%]). Adverse events occurred in 21 patients (11.7%) in the placebo group versus 36 (20.0%) in the colchicine group, but discontinuation rates were similar. No

serious adverse events were observed. Overall, among patients undergoing cardiac surgery, perioperative use of colchicine compared with placebo reduced the incidence of postpericardiotomy syndrome but not of postoperative AF or postoperative pericardial/pleural effusion. The increased risk of gastrointestinal adverse effects reduced the potential benefits of colchicine in this setting, with a high rate of perioperative discontinuation of both the active drug and the placebo [241].

A plausible hypothesis is that colchicine exerted its prophylactic action, at least in part, thanks to its anti-inflammatory properties. Although the COPPS substudy did not correlate POAF reduction with the fall of inflammatory markers, atrial or possibly pericardial inflammation seems to have a causative relationship with POAF. The peak incidence of POAF has been correlated with the peak concentration rise of CRP in cardiac surgery patients [242] and elevated white blood cell counts [243]. However, the strongest evidence in favor of this proposition comes from the fact that glucocorticoids significantly reduced POAF incidence in animal models [244] and clinical studies [245].

9.5 Colchicine and Atrial Fibrillation Recurrence After Ablation Therapy

The positive results of the COPPS substudy were followed by an investigation of Deftereos et al. into the possible role of colchicine administration in another population at risk for AF, namely patients who have undergone pulmonary vein isolation as part of AF ablation therapy [246]. In this prospective randomized double-blind study, the administration of colchicine monotherapy for 3 months was associated with preventing AF recurrences after pulmonary vein isolation within that timeframe (16% colchicine vs. 33.5% for the placebo group).

This effect was accompanied by a significant decrease in inflammatory mediators, IL-6 and CRP. This study was prompted by the strong link between inflammation and early postablation AF recurrence [247], with persistently elevated CRP for up to 3 months after AF ablation [248], and followed another study, by Koyama et al. [249]. In that study, patients undergoing pulmonary vein isolation received hydrocortisone (2 mg/kg) on the day of the procedure and prednisolone (0.5 mg/kg/day) per os for the following 3 days. The prevalence of immediate postablation AF (<3 days after ablation) was significantly lower in the corticosteroid (7%) than in the placebo group (31%). However, the prevalence of early postablation AF (4–30 days after ablation), when patients were not receiving corticosteroids, was the same between the two groups.

In retrospect, it could be expected that patients undergoing AF ablation therapy would benefit from

inflammation suppression, similarly to postcardiac surgery patients, although early postablation AF occurrence does not necessarily have the same causes as POAF. Atrial fibrillation ablation is a minimally invasive procedure where there is local inflammation, but no excessive catecholamine production, fluid shift, or major surgery trauma. Thus the key mechanism is probably inflammation, either alone or together with the remodeling of ablated areas. Inflammation has been shown to be a cause of AF [250], leading to adverse atrial remodeling and making the atria substrate vulnerable to AF. However, each episode of AF may become responsible for more adverse structural and electrical remodeling, creating a vicious circle [251]. In the 5A (Antiarrhythmics After Ablation of Atrial Fibrillation) study, the only predictor of a 6-month free AF period was the absence of early (<6 weeks) postablation AF recurrence [252]. Therefore although Deftereos et al. administered colchicine for 3 months postablation [246], positive results may persist for a longer period of time. While inflammation seems to be causatively linked to early postablation AF and colchicine administration is a promising prevention strategy, the optimum duration and dose of treatment have not been defined. A shorter colchicine treatment period has not been tested but, if effective, would certainly have advantages over a 90-day treatment, especially since a considerable proportion of patients (12.3%) in the study [246] discontinued colchicine, mainly due to gastrointestinal complaints.

The antiinflammatory properties of colchicine do not preclude other mechanisms playing a role in the reduction of POAF or postablation AF, besides inflammation suppression. In several *in vitro* or animal studies, colchicine administration has been shown to exert electrophysiological effects related to cytoskeletal disruption. In rat atrial fibroblasts, colchicine completely blocked both whole-cell and single-channel currents of mechano-gated channels, activated by cell mechanical deformations. Mechanically induced potentials in rat atrial fibroblasts have also been shown by the same researchers to depend on actin and tubulin polymerization. In another study, the modulation of L-type I (Ca) current by muscarinic and β -adrenergic agonists required an intact microtubule network and was affected in a colchicine environment. Abnormal Ca(2+) handling plays an important role in the induction and maintenance of AF [253].

9.6 Colchicine and Coronary Artery Disease

Traditionally, ACS's were considered the clinical manifestation of an intracoronary thrombotic event, usually resulting from erosion or rupture of an unstable atherosclerotic plaque that caused platelet aggregation and partial or total vessel occlusion. However, over

the past years this has proven an incomplete depiction, with more attention being given to the role of inflammation [253,254], both during plaque formation [255] and after plaque rupture [256]. One of the realizations of this notion has been the addition of high-dose statin therapy to standard ACS treatment, believed to be beneficial not only thanks to its lipid-lowering effects but also due to its antiinflammatory properties [257]. While research for better antiplatelet drugs has been extensive, with new drugs such as ticagrelor and prasugrel being approved for use in ACS, antiinflammatory treatment research has not progressed, especially as corticosteroids and NSAIDs have proved to be harmful in the setting of ACS [258]. Colchicine with its unique antiinflammatory mechanism and potential for long-term use could theoretically be a candidate to fill this gap, especially after it was found that colchicine treatment was accompanied by a reduction in high-sensitivity CRP independent of aspirin and atorvastatin in patients with stable coronary artery disease (CAD) [259].

A large prospective, randomized, observer-blinded endpoint trial by Nidorf et al. [260] (LoDoCo; low-dose colchicine study) examined the efficacy of continuous low-dose colchicine treatment in patients with stable CAD. The administration of 0.5 mg colchicine per day in addition to standard CAD therapy proved effective in reducing the composite of ACS, out-of-hospital cardiac arrest or noncardioembolic ischemic stroke (5.3% vs. 16%, $p < 0.001$; number needed to treat: 11). The difference was predominantly driven by a marked decrease in ACS in a median follow-up of 3 years. When excluding patients who did not tolerate or refused to take the drug, results were even more in favor of colchicine (4.5% vs. 16%).

In another study by Crittenden et al. [261], patients under treatment with colchicine for gout prophylaxis had a lower risk for myocardial infarction in comparison to gout patients who were not treated with colchicine. Although results were close to borderline in terms of statistical significance in favor of colchicine (myocardial infarction 1.2% in the colchicine vs. 2.6% in the no-colchicine group, $p = 0.03$), this is to be expected as this retrospective study spanned 1 year only and was not limited to patients with known CAD, who would potentially draw the most benefit.

Only one relatively small study examined the effects of colchicine in the setting of ACS [262]. In this pilot study, 80 patients were followed up on for 30 days after an ACS or acute ischemic stroke episode. The study examined whether patients on colchicine showed any decrease in high-sensitivity CRP and platelet aggregation compared with patients on placebo. No difference was shown, even though the colchicine dose was moderate (1 mg/day). There were no deaths during follow-up. Potential explanations for these results are that

inflammation suppression by colchicine may be less pronounced in the setting of an ACS, inflammation may play a relatively minor role after plaque rupture in contrast to plaque formation and destabilization, or that colchicine was unnecessary for inflammation suppression in patients taking high doses of statins as part of ACS treatment. However, the authors did not specify how many hours after ACS diagnosis colchicine was administered or what treatment ACS patients were subjected to (percutaneous coronary intervention [PCI] or thrombolysis). Patient numbers were also inadequate to detect evidence of clinical benefit or harm, therefore leaving the role of colchicine in ACS undecided [253].

In another study [263], patients were prospectively randomized to colchicine or placebo starting 48 h before scheduled coronary artery bypass grafting and for 8 days thereafter (0.5 mg twice daily). The primary outcome parameter was maximal high-sensitivity troponin T (hsTnT) concentration within 48 h after surgery. Secondary outcome measures were maximal creatine kinase-myocardial brain fraction (CK-MB) levels and area under the curve (AUC) of hsTnT and CK-MB concentrations; 59 patients were included. In conclusion, a short perioperative course of colchicine was effective in attenuating postoperative increases of hsTnT and CK-MB compared with placebo. This finding, which needs confirmation in a larger clinical trial powered to assess clinical endpoints, suggests a potential role for this agent in reducing cardiac surgery-related myocardial damage.

The effects of colchicine on stable CAD may not be completely attributable to inflammation suppression. Patients with familial Mediterranean fever who were treated with colchicine showed a reduction in biomarkers related with vascular injury independently of decreased inflammatory activity, as assessed by high-sensitivity CRP [264]. In *in vitro* experiments, colchicine has also been shown to reduce thrombin-induced platelet aggregation in a concentration-dependent manner [265]. Mean platelet volume (MPV), a marker of platelet activity and predictor of cardiovascular risk [266], was reduced in patients taking colchicine [267] and was negatively correlated with the duration of colchicine treatment [268] in patients with familial Mediterranean fever. The same results were seen in pediatric patients [269]. However, it is not clear if colchicine effects on MPV are direct or the result of inflammation suppression or even if there is any clinical significance in these findings. The only study that directly assessed platelet aggregation 30 days after an ACS in patients taking colchicine showed no difference from the placebo group [262]. Although these patients were already under dual antiplatelet therapy, these results cannot be safely extrapolated in a population with stable CAD under aspirin only, who were shown to benefit from colchicine administration for primary prevention of ACS [260].

9.7 Colchicine and Angioplasty

Coronary artery restenosis after percutaneous coronary intervention (PCI) remains a serious problem. Neointimal hyperplasia and local inflammation are key components of the restenosis process [270]. Colchicine, with its antimitotic and antiinflammatory properties [222], is theoretically attractive as an agent that could prevent restenosis. Although a relatively recent study in a dog model had encouraging results [271], experiments in the present era were disappointing. Two clinical studies evaluating colchicine failed to show benefit. O'Keefe et al. [272] studied patients who underwent plain balloon angioplasty and found restenosis in 45% of patients in the placebo-treated group compared with 41% in the colchicine-treated group (nonsignificant difference), and similar results were reported by Freed et al. [273].

The advent of bare-metal and especially drug-eluting stents marked a new era for angioplasty, with substantially lower rates of restenosis [274]. However, even in the modern era there are patient populations that are still plagued by high restenosis rates.

Deftereos et al. set out to study the effect of colchicine in diabetic patients who underwent PCI and could not, for various reasons, have a drug-eluting stent implanted and received a bare-metal stent instead [275]. In-stent restenosis rate was shown to be less than one-half in the colchicine group (16% vs. 33%) compared with placebo at 6 months after PCI, with a similar benefit in terms of lumen area loss, as determined by intravascular ultrasound. The authors suggested that the discrepancy with previous studies was due to the difference in mechanisms of vessel restenosis after plain old balloon angioplasty and in-stent restenosis. In balloon-only angioplasty, neointimal hyperplasia is only partly responsible for restenosis, while artery elastic recoil and remodeling play a substantial role in the restenotic process. Colchicine cannot be expected to have any effect on recoil or remodeling. In contrast, in-stent restenosis is almost exclusively due to neointima formation [276]. Although results were favorable for colchicine in this study, no clinical outcomes were measured. It is therefore too early to suggest a role for colchicine in post-PCI treatment [253].

9.8 Colchicine and Heart Failure

Chronic heart failure (CHF) has been shown to be associated with inflammatory activation, so inflammation has been designated as a therapeutic target in CHF. Deftereos et al. tried to test the efficacy of a 6-month course of antiinflammatory treatment with colchicine in improving functional status of patients with stable CHF [277]. They included patients with stable CHF randomly assigned to colchicine (0.5 mg twice daily) or placebo for

6 months. The primary endpoint was the proportion of patients achieving at least one-grade improvement in New York Heart Association class. Two hundred sixty-seven patients were available for final evaluation of the primary endpoint: its rate was 11% in the control group and 14% in the colchicine group (odds ratio: 1.40; 95% CI: 0.67–2.93; $p=0.365$).

The rate of the composite of death or hospital stay for heart failure was 9.4% in the control group, compared with 10.1% in the colchicine group ($p=0.839$). The changes in treadmill exercise time with treatment were insignificant and similar in the two groups ($p=0.938$). C-reactive protein and interleukin-6 were both significantly reduced in the colchicine group (-5.1 mg/L and -4.8 pg/mL, respectively; $p<0.001$ for both, compared with the control group).

In conclusion colchicine in patients with stable CHF, although effective in reducing inflammation biomarker levels, did not affect in any significant way patient functional status (in terms of New York Heart Association class and objective treadmill exercise tolerance) or the likelihood of death or hospital stay for heart failure.

In the end, in a recently published *meta*-analysis 15 randomized control trials (RCTs) ($n=3431$ patients, median treatment 3 and follow-up 15 months) were included [278]. All but two used colchicine 1 mg/day. In five trials ($n=1301$ at risk for cardiovascular disease: coronary artery disease, ACS or stroke, postangioplasty [2 RCTs], or congestive heart failure), colchicine reduced composite cardiovascular outcomes by ~60% (RR, 0.44, 95% CI, 0.28–0.69, $p=0.0004$; $I^2=0\%$) and showed a trend towards lower all-cause mortality (RR, 0.50, 95% CI 0.23–1.08, $p=0.08$; $I^2=0\%$). Treatment discontinuation overall and due to adverse events (RR, 4.34, 95% CI, 1.70–11.07, $p=0.002$; $I^2=29\%$; seven RCTs, 83/790 [10.5%] vs. 11/697 [1.6%]) was higher in colchicine-assigned patients [278].

Current RCT data suggests that colchicine may reduce the composite rate of cardiovascular adverse outcomes in a range of patients with established cardiovascular disease. The potential drug interactions of colchicine with statins, calcium antagonists, and other drugs, as well as the frequent comorbidities such as renal failure should be considered in this setting.

9.9 Colchicine and Preclinical Hints for Other Potential Uses

There are several *in vitro* and animal experiments where colchicine was shown to have important effects on myocardial cells in different settings, not related to inflammation but rather to microtubule disruption. Colchicine attenuates pressure-overload heart failure in dogs [279] and rats [280]. These results are relevant with the fact that microtubules seem to play a pivotal role

in cardiac hypertrophy progression [281]. At the cellular level, colchicine reduced myocardial cell stiffness in normal rat cardiomyocytes [282] and a hamster model of congestive heart failure [283]. Colchicine also increased Ca^{++} currents in rat cardiac cells [284].

All these results point toward novel possible areas of clinical studies for colchicine, namely arrhythmias, heart failure, particularly with preserved ejection fraction, and cardiac hypertrophy. In all these conditions, potential interactions of colchicine with other drugs, such as statins and calcium channel blockers, and other comorbidities (renal failure), should always be taken into account.

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The Effects of Immunosuppressive and Cytotoxic Drugs on the Heart

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1. INTRODUCTION

Due to the discovery of new drugs with targeted actions, the clinical manifestations and complications of some rheumatic diseases have dramatically changed over the past 20 years. As patients are surviving longer with fewer rheumatic side effects, focus has shifted to reducing the side effects related to the immunosuppressive therapies. Many of these agents have been shown to have cardiac side effects including hypertension, hypercholesterolemia, and cardiac toxicity. Conversely, these agents are also used in the therapy of cardiac diseases due to their immunosuppressive effects such as myocarditis, pericarditis, and cardiac sarcoid. Determining the direct toxic effects of the drugs is often complicated as many of the rheumatologic diseases also have cardiac effects that can mimic these toxicities. This chapter will review the positive and negative cardiac effects of the common immunosuppressive agents used to treat rheumatologic diseases.

2. GLUCOCORTICOIDS

Glucocorticoids are widely used to suppress inflammation, especially in the acute phase, of many rheumatologic illnesses. The mechanisms of immunosuppression of glucocorticoids are complex and not fully understood (Figs. 26.1 and 26.2). Glucocorticoids bind to the intracellular glucocorticoid receptor that resides in the cytoplasm of many tissues including bones, liver, brain, T and B cells, and macrophages [1,2]. Once activated, the receptor undergoes a series of conformational changes that result in nuclear translocation. In the nucleus, the glucocorticoid receptor interacts with glucocorticoid

response elements on the DNA of a number of glucocorticoid responsive genes. Based on the gene response, elements bound to the glucocorticoid/glucocorticoid receptor complex either activate transcription (transactivation) of DNA or inhibit transcription (transrepression). Additionally, transrepression can occur through two other mechanisms. The complex can be “tethered” by NF- κ B and not allowed to bind to the DNA or is not allowed to bind due to competition for the binding site with other nuclear coactivators. The result of both of these mechanisms is loss of transcription. Additionally, there are nongenomic mechanisms of glucocorticoids (Fig. 26.2) that occur within minutes or seconds of application to the cell. The mechanisms of these effects are less well understood but are thought to be mediated by affecting the cell membrane or the cytosolic glucocorticoid receptor [3,4]. These rapid cell processes may cause impairment of multiple cell functions including phagocytosis, cell migration, and antigen processing and presentation. Additionally, these nongenomic effects may block splicing, promoter switching, or posttranslational editing. The end result of these two processes is the increased or decreased synthesis of a number of antiinflammatory and inflammatory gene products. Antiinflammatory products that are increased include interleukin 10, I-kappa B, lipocortin-1, α -2 macroglobulin, secretory leukocyte-protease inhibitor, and IL-1 receptor-II [5,6,75]. Conversely, glucocorticoids will also decrease the synthesis of inflammatory cytokines including interleukins 1, 2, 3, 5, 5, 6, 8, 10, 13 and tumor necrosis factor α , interferon γ , and granulocyte macrophage colony stimulating factor [5–8].

Glucocorticoids are the mainstay of therapy for a number of rheumatologic diseases. Philip Hench won the 1950 Nobel Prize in Medicine for his work first

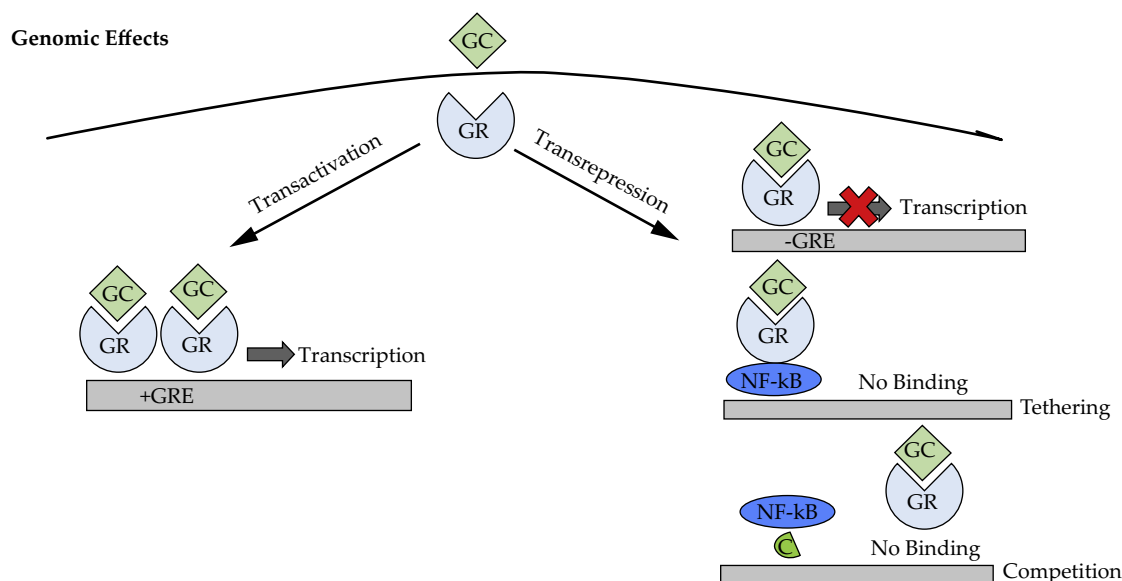


FIGURE 26.1 Diagram of the mechanism of the genomic effects of glucocorticoids in the cell. Glucocorticoid (GC) binds to the glucocorticoid receptor (GR) resulting in conformational changes to the receptor to an active state. That active glucocorticoid – glucocorticoid receptor complex then translocates into the nucleus and then either binds to positive or negative glucocorticoid response elements (GRE) which either activates or blocks transcription. Transrepression can also occur if the receptor complex instead binds to NF-κB or a nuclear coactivators (C) and doesn't allow transcription to occur. *Adapted and modified from Gessi et al. [2].*

Non Genomic Effects

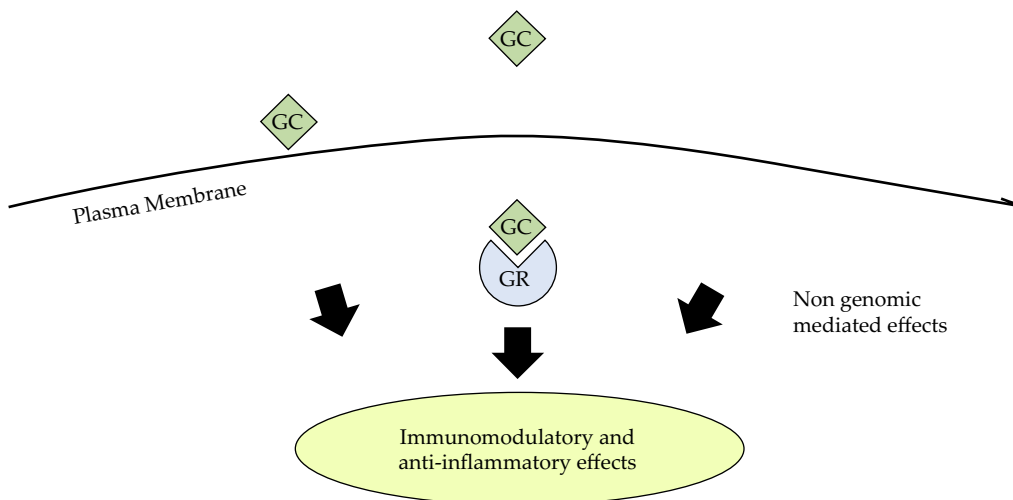


FIGURE 26.2 Diagram of the mechanism of the nongenomic effects of glucocorticoids. Glucocorticoid (GC) binds to the glucocorticoid receptor (GR) and has cytosolic immunomodulatory and antiinflammatory effects. *Adapted and modified from Gessi et al. [2].*

describing the benefits of steroids in rheumatoid arthritis [9]. Since then, they have been used for acute exacerbations, and in both short- and long-term settings for this therapy [10–13]. As shown in Table 26.1, glucocorticoids are also used in first-line therapy for a number of other rheumatologic diseases including juvenile arthritis, spondyloarthritis, polymyalgia rheumatic, systemic lupus erythematosus, poly- and dermato-myositis, gout,

giant-cell arteritis, Takayasu's arteritis, polyarteritis nodosa, microscopic polyangiitis, Churg–Strauss syndrome, Wegener's granulomatosis, and Behcet's disease [14–34]. Additionally, glucocorticoids are often used as second-line therapy for exacerbations in many of these same diseases, specifically for Kawasaki disease [27].

The cardiovascular side effects of glucocorticoids are related to their indirect effect on the heart by increasing

TABLE 26.1 Evidence Supporting the Use of Immunosuppressive Therapy in Rheumatologic Diseases

Disease	Glucocorticoids	Azathioprine	Methotrexate	Cyclosporine	Cyclophosphamide
Rheumatoid arthritis	[10–13]		[12]	[12]	
Juvenile idiopathic arthritis	[14,15]		[14,78]	[105,106]	[118]
Spondyloarthritis	[16]		[16]	[16]	
Polymyalgia rheumatica	[17]		[17,80]		
Systemic lupus erythematosus	[18]	[18]		[18]	[18]
Sjögren's syndrome			[81,82]		
Systemic sclerosis			[83,84]		[119,120]
Dermatomyositis	[19]	[19]	[19,85,86]		
Polymyositis	[19–21]	[19–21]	[19,85]		
Gout	[22]				
Giant cell arteritis	[23,24]		[87–89]		[121]
Takayasu's arteritis	[25]		[25,90]		
Polyarteritis nodosa	[26]	[66,67]	[66,91]		[91,122,123]
Kawasaki disease	[27]				
Microscopic polyangiitis	[28,29]		[94,95]		[28,29]
Churg-Strauss syndrome	[30,31]	[64]	[64,93]		[30,31,93]
Wegener's granulomatosis	[65]	[65]	[65]		[65]
Behçet's disease	[33,34]	[62]		[108,109]	

the incidence of many cardiac risk factors. Traditional risk factors for coronary artery disease include age, gender, total or LDL cholesterol, low HDL cholesterol, hypertension, diabetes, and tobacco abuse [35,36]. Many of these risk factors have been shown to be induced or worsened with the chronic use of glucocorticoid therapy including hypertension and diabetes.

The mechanism of glucocorticoid-induced hypertension is complex and thought to be related to increased extravascular volume, increased systemic vascular resistance, and nitric oxide deficiency [37]. The incidence of steroid-related hypertension has been shown to be related to dose and length of therapy [38,39]. Jackson et al. evaluated the pre- and postexposure to steroid blood pressure in 195 patients starting prednisone or prednisolone therapy [38]. They found that the incidence of hypertension defined as a systolic blood pressure over 160 mm Hg rose from 17.7% to 29.8% of the population. Similarly, the incidence of diastolic blood pressure >100 mm Hg rose from 15.3% to 20.2%, although this was not significant. The authors found no relationship between this increase and dose of drug. Additionally, they found that when age at the time of steroid initiation was included the effect of steroids on increasing blood pressure was no longer statistically significant. In contrast, Panoulas found that in a population of patients

with rheumatoid arthritis being treated with steroids the incidence of hypertension went from 67.3% on no steroids to 70% on <7.5 mg to 87% in those treated with more than 7.5 mg a day of prednisone [39].

Glucocorticoids have been associated with the development of diabetes, causing reduced β -cell insulin synthesis, increased hepatic gluconeogenesis, and inhibition of glucose uptake [37]. Panthakalam evaluated the effects of steroid therapy in 102 patients with rheumatoid arthritis [40]. Six patients had diabetes at baseline and all of them had worsening of their glycemic control. They found an 8.8% incidence of new onset diabetes in their population. Many others have evaluated the incidence of diabetes and have found between a two- and threefold increased relative risk for the development of either diabetes or hypoglycemia [41–45].

The correlation between steroids and hyperlipidemia is less clear. Svenson studied changes in lipoproteins in patients with rheumatoid arthritis being treated with a number of different immunomodulating regimens [46]. In 14 patients being treated with prednisone either alone or in combination with azathioprine or cyclophosphamide, they found that at baseline most patients had low cholesterol and LDL levels that normalized after therapy. Similarly, Boers found minimal changes in total cholesterol and only a significant increase in HDL [47].

Two studies have evaluated the overall effects of glucocorticoids on significant cardiac events. In a meta-analysis of six trials including 689 patients, Ravindran looked at the long-term safety of glucocorticoid therapy [48]. They evaluated the number of patients that were withdrawn from the trials due to either adverse or serious adverse events. In all studies, they found no difference in the withdrawal rate between the study and placebo arms. In contrast, Davis looked at a group of patients being treated with glucocorticoids for rheumatoid arthritis and found no difference in cardiac events in the group that was rheumatoid factor negative [49]. For those that were rheumatoid factor positive, there was a threefold increase incidence of the combined cardiovascular endpoint of myocardial infarction, heart failure, and death. Both the dose (>7.5 mg) and timing (recent compared to past use) were also associated with increased events.

Glucocorticoids have also been shown to have some efficacy for noninflammatory heart diseases, although their use in some of these areas remains controversial. One primary area is in the setting of myocardial infarction. Early studies in animals revealed a reduction in infarct size and prevention of myocyte necrosis [37]. However, others found a reduction in the wound-healing process [50,51]. Giugliano and colleagues performed a meta-analysis evaluating the use of steroids in patients with a myocardial infarction [52]. They reviewed a total of 186 articles and found 16 appropriate studies of which 11 were controlled trials. They found a 26% decrease in mortality with the use of corticosteroids overall, but no benefit in the controlled trials. Although not all studies reported the endpoint of cardiac rupture, there was also no difference in that endpoint between placebo and glucocorticoid therapy. It is important to note that the majority of these trials were performed in the era prior to the use of thrombolytic and primary angioplasty for the therapy of myocardial infarction. These therapies have been associated with a reduction in infarct size and mortality [53]. Additionally, medications such as angiotensin-converting enzyme inhibitors that have been shown to improve survival in patients with a low ejection fraction postmyocardial infarction were not used as routine therapy in these trials [54,55]. With current therapy, infarct size and remodeling should be less and therefore decrease the risk of myocardial rupture even further. At this time, therapy with glucocorticoids for the treatment of myocardial infarctions is not advised. However, for patients that present with myocardial infarction who are on chronic glucocorticoids for some other reason one can interpret this meta-analysis to mean that the risk of myocardial rupture is small and continued therapy is most likely safe.

Glucocorticoids have also been studied for the treatment of angina, cardiac protection during coronary interventions, and risk reduction after myocardial infarction.

Patients with vasospastic or Prinzmetal's angina caused by abrupt vasoconstriction are usually treated with nitrates or calcium-channel blockers. Patients unresponsive to those therapies were shown to benefit from glucocorticoid therapy with a reduction in the incidence of angina [56]. It should be noted that typical angina has not been shown to benefit from methylprednisolone therapy [57].

Acute myocardial infarction and chronic exertional angina are now commonly treated with direct angioplasty and stenting. This results in an inflammatory response to the vessel wall, which often in the long-term causes stent thrombosis. Prior to the development of antiinflammatory drug eluting stents, studies were conducted on the efficacy of glucocorticoids to prevent restenosis. Pepine et al. randomized 915 patients undergoing planned PTCA (percutaneous transluminal coronary angioplasty) to either 1 g methylprednisolone or placebo prior to the procedure [58]. They used a combined endpoint of angiographic restenosis, death, recurrent ischemia requiring catheterization, and coronary artery bypass grafting and found no benefit to therapy prior to the procedure. More recently, Ferrante et al. reported on the association between C-reactive protein (CRP) and restenosis rates in patients receiving bare-metal stents [59]. They reviewed a total of nine different studies and found that patients with a higher CRP level at baseline had an increased incidence of restenosis. They then hypothesized that these patients could be considered for antiinflammatory therapy, but there have been no randomized trials supporting this approach. It is also important to note that this research was done with bare-metal stents, which are not used as often in the current era of drug-eluting stents. The 4-year results of a trial comparing patients receiving bare-metal stents, bare-metal stents plus prednisone, or drug-eluting stents was recently published [60]. Three-hundred seventy-five patients were enrolled in a randomized trial and followed for the endpoint of cardiovascular death, myocardial infarction, and recurrent ischemia by 1 year. At 1447 days, the bare-metal stents had lower event-free survival (75.3%) than the other two arms (bare metal stent plus prednisone 84.1%, drug eluting stent 80.6%). This study demonstrates the superior results of drug-eluting stents, but also suggests the benefits of steroid therapy if a bare-metal stent is placed. For patients that can't tolerate long-term dual antiplatelet therapy adding steroid therapy could also be considered if a bare-metal stent is used.

Glucocorticoids are also commonly used for the treatment of pericarditis related to either cardiac surgery or inflammation of the pericardium (Table 26.2). Pericarditis has been shown to be the cause of 5% of emergency room visits for chest pain and has a recurrent rate of about 30% [61]. The most common

etiologies are viral, bacterial (*mycobacterium tuberculosis*), autoimmune, metabolic (uremia, myxedema), or surgical. Additionally, some malignancies will cause a

pericardial effusion but are less likely to cause pain and commonly breast, lung, and lymphoma will spread to the pericardial space. The usual therapies include aspirin, nonsteroidal agents, or colchicine but low-dose corticosteroids are recommended in patients that are refractory to these agents [61]. Imazio evaluated the retrospective use of corticosteroids in 100 patients with recurrent pericarditis in two centers [62]. One center routinely gave high-dose prednisone (1.0 mg/kg/day) and the other gave lower doses (0.2–0.5 mg/kg/day). Both tapered in the absence of chest pain or elevated CRP every 2 weeks. Of interest, patients treated with higher doses had more side effects related to the prednisone, but also had more adverse events including recurrences, cardiac tamponade, and constrictive pericarditis.

TABLE 26.2 Therapy for Pericarditis

Cause	Incidence	Usual therapy
Idiopathic	85–90%	Aspirin or NSAIDs plus colchicine
Viral	1–2%	Aspirin or NSAIDs plus colchicine
Bacterial	1–2%	Antibiotics
Tuberculous	4%	TB therapy, prednisone
Myocardial infarction	NA	Aspirin
Trauma	NA	NSAIDs
Neoplasm	7%	NSAIDs, glucocorticoids
Chest wall irradiation	Rare	NSAIDs
Post cardiotomy	Rare	Aspirin, NSAIDs

Consider prednisone course in addition to earlier therapy. Prednisone should not be first-line therapy.

Adapted from Adler et al. [61]; Lange and Hills [63].

3. AZATHIOPRINE

Azathioprine (Fig. 26.3) is a purine antagonist that interferes with the production of adenosine and guanine triphosphate, essential components of DNA [64]. It is the prodrug of 6-mercaptopurine, which was initially used in the 1950s to treat leukemia. Azathioprine was

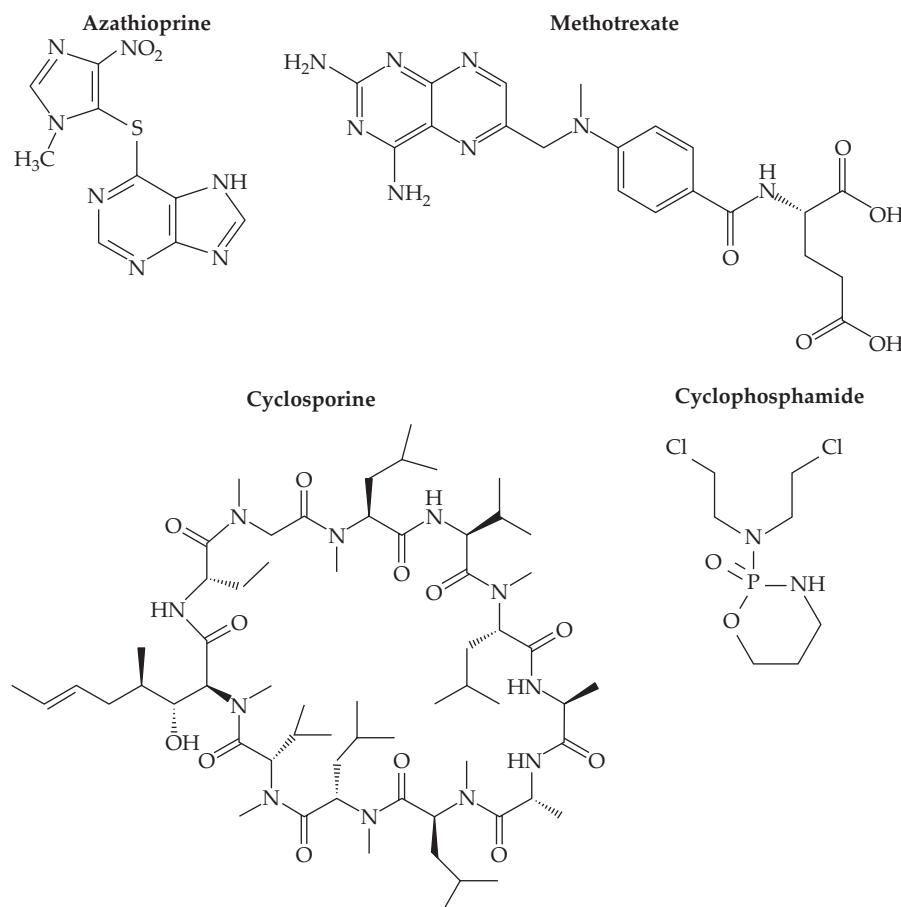


FIGURE 26.3 Diagram of the molecular structure of azathioprine, methotrexate, cyclosporine, and cyclophosphamide. Adapted from Ref. [139].

first studied in animals and found to be a more potent immunosuppressive agent than glucocorticoids and became the mainstay of immunosuppressant therapy for transplantation until the development of mycophenolate mofetil in the 1980s [65]. Azathioprine is converted by xanthine oxidase to 6-mercaptopurine, which is further metabolized into 6-thioguanosine-5'-phosphate and 6-thioinosine monophosphate, the two active agents. The mechanism of action for modifying immune responses with azathioprine is not entirely clear. Azathioprine tends to inhibit T lymphocytes more than B lymphocytes and may also be involved in the reduction of interleukin-2 [66,67].

Azathioprine is used as either first- or second-line therapy for a number of rheumatologic disorders including systemic lupus erythematosus [18], dermatomyositis [19] and polymyositis [19–21], and Behcet's disease [68]. Additionally, to avoid the side effects related to glucocorticoids it is often used as a maintenance agent for Churg–Strauss syndrome [69], sarcoidosis [69], and Wegener's granulomatosis [32]. Finally, for patients with refractory polyarteritis nodosa it will be used as rescue therapy [69,70].

The adverse effects of azathioprine are predominantly leukopenia or overt bone-marrow depression and gastrointestinal include vomiting and diarrhea, abdominal pain, and abnormal liver function [64]. There have been few reported cardiac side effects, but they include hypotension, cardiogenic shock, and atrial fibrillation. Brown reported a case of a patient on azathioprine who presented with hypotension that progressed to shock and requiring vasopressor therapy. His ejection fraction dropped to 20% initially, and all clinical symptoms slowly resolved over the next 20 days [71]. After infection and other etiologies had been ruled out they developed the hypothesis that the patient was hypersensitive to azathioprine. They also reviewed 27 other reports of hypotension and found that the syndrome of hypotension, decreased renal function, and hepatotoxicity often occurs and usually resolves over a 3–7 day period. There are also several case reports of azathioprine causing atrial fibrillation soon after the drug dose was given [72–75]. The mechanism of inducing this arrhythmia is unclear and is hypothesized to be related to an interaction with cardiac-ion channels, which could change atrial conduction or the refractory period, direct cardio toxicity, or ischemia [72].

Azathioprine has rarely been primarily used for the treatment of cardiac processes but is occasionally used as a secondary therapy for the treatment of recurrent pericarditis [61]. Vianello reported the results of a retrospective study of 46 patients with recurrent pericarditis that was refractory to steroids [76]. Oral prednisone was continued in all patients and azathioprine was started at a dose of 1.5–2.5 mg/kg/day. All patients had had at

least two recurrences of their pericarditis and almost half had also been treated with colchicine prior to initiating azathioprine therapy. After the initiation of azathioprine, 63% of patients had no more recurrences and only 15% were felt to be resistant to azathioprine. Eventually, 84.7% of patients were able to be weaned off steroids. Based on this study and other case reports, azathioprine is recommended as a third-line agent for refractory pericardial pain after the failure of nonsteroidal agents, colchicine, and glucocorticoids [61].

4. METHOTREXATE

Methotrexate is an antimetabolite agent that inhibits the enzyme dihydrofolate reductase. This results in inhibition of the synthesis of both purine and pyrimidine nucleotides and subsequently DNA and RNA [77,78]. This mechanism is primarily responsible for its action as a drug for the treatment of malignancies. Methotrexate also functions as an antiinflammatory drug, although the mechanisms of this are not as well understood. The primary mechanism is thought to be that low-dose methotrexate is responsible for the formation of adenosine, which has been shown to inhibit the proliferation of endothelial cells, fibroblasts, and also to block the adhesion and migration of white blood cells to inflamed tissues [77,79]. Similar to azathioprine and its effects on cytokines, methotrexate, by causing an increase in adenosine release through its polyglutamate metabolites, has been shown to reduce the synthesis of a number of proinflammatory molecules including interleukins-1, -6, -8, leukotrienes, and tumor necrosis factor α [80–82]. Additionally, methotrexate has been shown to increase the production of antiinflammatory cytokines including interleukin-4 and -10 [83].

As shown in Table 26.1, methotrexate is commonly used for a number of different rheumatologic disorders. It is a common second agent for the therapy of rheumatoid arthritis [12], juvenile idiopathic arthritis [14,84], spondyloarthritis [16], polymyalgia rheumatic [17,85], Sjögren's syndrome [86,87], systemic sclerosis [88,89], dermatomyositis [19,90,91], polymyositis [19,90], giant-cell arteritis [92–94], Takayasu's arteritis [25,95], and polyarteritis nodosa [69,96]. Additionally, it is often used as the sole maintenance agent in Churg–Strauss syndrome [69,96] and Wegener's granulomatosis [32]. Finally, it is the primary agent used for the therapy of mild microscopic polyangiitis [96,97].

There are rare reports of cardiac toxicities related to methotrexate including ventricular arrhythmias and ischemia. One case report is of a patient with psoriasis treated with methotrexate and a large anterior myocardial infarction that developed premature ventricular contractions that resolved when methotrexate was

discontinued [98]. Igawa reported on two cases of myocardial ischemia in patients being treated with methotrexate, etoposide, and cisplatin [99]. It is unclear if any of the drugs were related as the patients were elderly and had other risk factors for coronary disease. Forbat reported a case of a 22-year-old woman who developed pericarditis and a pericardial effusion when receiving treatment for a molar pregnancy. Of interest, in addition to pericarditis she had pleuritis and a pleural rub [100]. Finally, Urban reported on 18 patients who developed pleuritis that presented as chest pain after receiving high-dose methotrexate therapy [101]. This tended to resolve after a couple of days but resulted in most of those patients undergoing further evaluation due to suspicion of coronary artery disease. It is important to note that most of these side effects were reported in patients receiving high chemotherapy methotrexate doses and not the lower doses typically used for treatment of rheumatologic processes.

In contrast to most of the immunosuppressive agents, there is strong evidence that methotrexate might actually be cardiac protective, especially in patients with rheumatoid arthritis. Through its enhanced production of adenosine, which is a vasodilator, it is thought that methotrexate may result in coronary artery vasodilation and improved perfusion of the myocardium [77]. It has also been shown to improve cholesterol transport away from blood vessels to the liver [102]. A number of reviews of the literature have been performed analyzing the effects of methotrexate on cardiovascular disease. For patients with rheumatoid arthritis, Westlake reported the results of a review of 18 studies [103]. Two studies included an analysis of cardiovascular mortality, one showed a significant reduction, and the other showed a trend toward reduction in events. Similarly, four of the five studies evaluating cardiovascular morbidity (cardiovascular disease, cerebrovascular disease, and atherosclerosis) showed a significant reduction in events. Micha et al. performed a meta-analysis of studies evaluating the use of methotrexate in a variety of settings and reported on cardiovascular outcomes [104]. They found 10 studies in which methotrexate was being administered as the sole agent for rheumatoid arthritis, psoriasis, or polyarthritis and met their inclusion criteria. The use of methotrexate was associated with a 21% reduction in risk for overall cardiovascular disease and an 18% lower risk for myocardial infarction in the five studies that reported that outcome. This effect was maintained and even strengthened after adjusting for disease severity.

Based on the findings of a reduction in cardiac events with methotrexate and the basic science findings of improvement in coronary artery vasodilation and improved cholesterol transport to the liver and away from the vessels, the Cardiovascular Inflammation

Reduction Trial has been planned and is in process ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01594333) NCT01594333). This trial is randomizing 7000 patients with a prior history of myocardial infarction and either type 2 diabetes or the metabolic syndrome to either low-dose methotrexate (15–20 mg/week) or placebo [105]. The primary endpoint is a composite or nonfatal myocardial infarction, nonfatal stroke, and cardiovascular death. Important secondary endpoints include all-cause mortality and net clinical benefit or harm. The results of this trial may greatly expand the use of methotrexate into different populations of patients.

Finally, Wasko and colleagues recently reported on 5626 patients prospectively followed over a 25-year period and found that the use of methotrexate in 2920 of the patients was associated with a 30% reduction in the risk of death [106]. At baseline, many of the patients had comorbid conditions including hypertension in 20–26%, coronary artery disease in 4%, diabetes in 4–5%, and heart failure in 1–2% depending on the group. Additionally, between 32% and 53% were on prednisone at baseline. Significant predictors of mortality included age, male sex, higher BMI, use of prednisone, and the presence of lung disease, cancer, heart failure, and infection. However, they did not state if the use of methotrexate resulted in a reduction in heart-failure deaths. Myasoe-dova recently reported on 795 patients with rheumatoid arthritis that were identified over a period of 27 years [107]. Patients were followed for almost 10 years and during that time 92 of the patients developed heart failure. Risk factors for heart failure included being rheumatoid factor positive, having an elevated erythrocyte sedimentation rate (ESR) at diagnosis, and an ESR between 3 and 60 mm/h. Patients who were being treated with methotrexate had half the chance of developing heart failure compared to nonusers. In contrast, those taking corticosteroids had a two-fold increased risk of heart failure. Of interest, 76% of the methotrexate users were also taking corticosteroids and no increased risk of heart failure was found in that group.

5. CYCLOSPORINE

Cyclosporine A is a lipophilic cyclic peptide that was discovered in 1970 as part of a screening program for antifungal antibiotics [108]. Scientists isolated a fungus called *Tolypocladium inflatum* from a soil sample in Norway that synthesized a number of different cyclic peptides. One of these peptides was found to mediate immunosuppression by selectively inhibiting T lymphocyte proliferation. In 1983, it was approved by the US Food and Drug Administration to prevent graft rejection in transplantation. The use of this agent resulted in dramatic improvements in preventing rejection with

reduced side effects compared to the prior regimen of azathioprine and prednisone. For cardiac transplantation, the 50% survival posttransplant improved from 5 years in 1978 to 8.1 years by 1985 [109]. Cyclosporine A works by binding to cyclophilin and inhibiting the activity of calcineurin. This complex then inhibits the translocation of nuclear factor-activated T cells, which leads to a reduction in the activation of genes for interleukin-2, -3, -4, tumor necrosis factor α , CD40 ligand, granulocyte-macrophage colony stimulating factor, and interferon γ [108].

Cyclosporine is primarily used as a second-line or alternative drug for the treatment of many rheumatologic diseases (Table 26.1). It has been used in the treatment of rheumatoid arthritis [12], juvenile idiopathic arthritis [110,111], spondyloarthritis [16], lupus nephritis [18], and psoriatic arthritis [112]. It has also been shown to be effective for the ocular form of Behcet's disease [113,114].

The most common side effects of cyclosporine are renal insufficiency and hypertension [115,116]. Cyclosporine causes vasoconstriction, sympathetic excitation, and sodium retention. Additionally, it interferes with the balance of vasoactive substances including endothelin and nitric oxide with the end result being an increase in the incidence of hypertension [116]. In patients with rheumatoid arthritis, abnormal renal function has been reported in 20–30% of patients but only requiring withdrawal of the agent in 1% [117]. The incidence of hypertension has been reported to be about 10%.

Similar to methotrexate, ongoing research into the possible cardio-protective effects of cyclosporine is being performed. Schneider first reported the possible protective effects of cyclosporine A during ischemia in 2003 [118]. They removed slices of right atrium from patients undergoing cardiac surgery and placed the slices in a cyclosporine or insulin-containing solution for 30 min. They then analyzed the tissue for cell viability after 90 min of simulated ischemia followed by reoxygenation. The use of cyclosporine pretreatment resulted in an increase in viability from 30% to 53% and showed that cyclosporine could be used to reduce ischemia in patients suffering from a myocardial infarction. In 2008, Piot reported the results of a randomized study of 58 patients who presented with an ST-segment elevation myocardial infarction and underwent primary angioplasty [119]. Half of the patients received a bolus of cyclosporine and the other half received normal saline prior to inflating the angioplasty balloon. The cyclosporine group had a reduction in creatine kinase and a trend toward a significant reduction in troponin I. A subgroup of 27 patients underwent MRI on day 5 postinfarct and showed a reduction in the area of infarcted tissue.

The recent results of the larger recently completed CYCLE (Cyclosporine A in reperfused myocardial infarction) study were less impressive [120]. The authors randomly assigned 410 patients with an ST-segment myocardial infarction to 2.5 mg/kg cyclosporine IV or control. There was no difference in ST-segment resolution or median troponin T at day 4. Additionally, there were no differences in left ventricular ejection fraction at day 4 or 6 months. Finally, the results did not change based on the site of the infarct. The authors concluded that there was no clinical improvement in outcomes or left ventricular remodeling with the use of cyclosporine. Another similar study enrolling 972 patients is ongoing and will hopefully provide further guidance about the possible beneficial effects of cyclosporine on reperfusion (ClinicalTrials.gov NCT01502774).

6. CYCLOPHOSPHAMIDE

Cyclophosphamide (Fig. 26.3) is an alkylating agent that interferes with DNA base pairing leading to DNA strand breaks and blockade of replication [121]. It is primarily used for the therapy of malignancies including lymphoma, leukemia, neuroblastoma, retinoblastoma, and cancer of the ovary, breast, endometrium, and lung [122]. It is occasionally used as an immunosuppressant in the therapy of juvenile idiopathic arthritis [123], systemic lupus [18], systemic sclerosis [124,125], giant-cell arteritis [126], microscopic polyangiitis [28,29], Churg–Strauss syndrome [30,31,96], and Wegener's granulomatosis [32]. Additionally, cyclophosphamide is commonly used for rescue therapy in polyarteritis nodosa [28,96,127].

The toxicity of cyclophosphamide appears to be very dose-dependent. For the therapy of malignancy, doses typically range from 2 to 6 mg/kg body weight in contrast to the immunosuppressant doses of 100–200 mg a day [112]. There are no cardiac reported side effects at the lower doses typically used for immunosuppression. At higher doses, cyclophosphamide has been associated with arrhythmias, conduction disorders, acute heart failure, and hemorrhagic myopericarditis [123,128]. The risk of cardiac toxicity appears to be related to higher single doses rather than cumulative doses [129]. Risk factors for the development of heart failure include high doses infused slowly and lower levels of cardiac glutathione [122].

7. MYOCARDITIS

The principal use of antiinflammatory agents for the treatment of cardiac disease has been for the therapy of myocarditis. Myocarditis is an inflammatory disease of the

heart frequently caused by the immune-mediated responses to viral infections [130]. Cardiac damage is thought to be related to both the initial viral infection and the secondary immune response to that infection. Patients often present with symptoms of dyspnea and volume overload, but others will present with chest pain suggestive of myocardial infarction, arrhythmias, or cardiogenic shock. The therapy is primarily focused on treating the heart-failure symptoms with diuretics and reduced ejection fraction with angiotensin enzyme inhibitors and beta-blockers [131].

The use of antiinflammatory agents has been studied in a few different trials in patients with myocarditis. The myocarditis treatment trial (MITI) randomized 111 patients with biopsy-proven myocarditis to therapy with either prednisone/azathioprine or prednisone/cyclosporine versus placebo [132]. There was no difference in the primary endpoint of heart-transplant-free survival or the secondary endpoint of change in ejection fraction. This was followed by the Tailored Immunosuppression in Inflammatory Cardiomyopathy (TIMIC) study that evaluated 85 patients with myocarditis that had symptoms of heart failure for at least 6 months [133]. The investigators hypothesized that delaying therapy until the causative virus was no longer present might improve the efficacy of therapy. In fact, the presence of viral genome was an exclusion criterion for enrollment. In contrast to the MITI study, the authors found that therapy with prednisone (1 mg/kg) and azathioprine (2 mg/kg) versus placebo was associated with improved ejection fraction and a reduction in left ventricular size. In the placebo arm, there was further reduction in ejection fraction and increase in LV size. The final large randomized study compared the use of prednisone and azathioprine, again in patients with at least 6 months of heart-failure symptoms, to placebo [134]. Two hundred and two patients were enrolled and there was no difference in the primary endpoint was death, transplantation, or heart-failure readmission between the two groups. Of the 84 patients with increased HLA antibodies, the use of immunosuppressive therapy was associated with an increase in ejection fraction and reduction in left ventricular size.

Low-dose methotrexate was studied in one trial of patients with heart failure. Seventy-one patients with chronic heart failure were given low-dose methotrexate at 7.5 mg/week or placebo [135]. The authors reported an improvement in quality of life, 6 min walk distance, and New York Heart Association functional class. Additionally, they found reductions in interleukin-6 and tumor necrosis factor α . The only side effects noted were mild nausea and vomiting.

Finally, immunosuppressive therapy is felt to be the mainstay of therapy for giant-cell myocarditis. Giant-cell myocarditis is an autoimmune process diagnosed by the presence of heart failure and multinucleated

giant cells, lymphocytic infiltrate, and cellular necrosis on endomyocardial biopsy [136]. The disease is quite rare and often lethal. Cooper reported the results of a worldwide survey of patients with giant-cell myocarditis [137]. A total of 63 patients were reported on and 22 of them were treated with various combinations of prednisone, cyclosporine, and azathioprine. For patients who received any immunosuppressive therapy, survival was prolonged from 3.0 months to 12.3 months. This was followed by a small, observational trial of cyclosporine and steroids versus cyclosporine, steroids, and muromonab-CD3 [138]. Two patients underwent cardiac transplantation and one patient died at 178 days. The authors concluded that the use of immunosuppressive therapy resulted in low 1-year mortality compared to historical controls. All studies in patients with giant-cell myocarditis are limited due to the low occurrence of the disease process and the fact that the current suggested therapy is to proceed with immunosuppressive therapy or mechanical support.

8. CONCLUSIONS AND FUTURE DIRECTIONS

At this point, the cardiac side effects of glucocorticoids, azathioprine, methotrexate, cyclosporine, and cyclophosphamide are known and manageable. Many of the old teachings such as “don’t give steroids to patients with a new myocardial infarction because of the increased risk of adverse remodeling” have been shown to be false and potentially harmful. Patients who have appropriate reasons for their immunosuppressive agents should not have them abruptly discontinued in the setting of an acute cardiac illness.

Our understanding of the beneficial cardiac effects and side effects of these agents is still a work in progress. Coronary artery disease, one of the top causes of death, clearly has an inflammatory component and one can imagine that in the future patients at risk for coronary disease will be treated with antiinflammatory agents in addition to the antiplatelet agents, cholesterol-lowering agents, and other medications they currently receive. Methotrexate, and to a lesser extent, cyclosporine still hold promise as therapies for cardiac diseases and the next few years will be interesting to see the results of large clinical trials with these agents. These therapies may also be guided by inflammatory markers such as high-sensitivity CRP just like statin therapy is to some extent guided by low-density lipoprotein levels today.

Finally, further research into the therapy of myocarditis and the efficacy of immunosuppressant therapy versus the need for antiviral therapy or both still needs to be performed. Questions that need to be studied

include the benefits of antiinflammatory therapy in patients with myocarditis without active viral replication, and if there is a benefit, what is the best way to guide therapy. Since many of these patients recover back to normal is there a length of time that they need to be treated and what is the best method to follow the effects of therapy (ejection fraction, inflammation on MRI or endomyocardial biopsy, etc.)? All of these studies are limited by the low incidence of these diseases and therefore the difficulty of performing proper studies since any particular center will have a small number of patients. Clearly, there are many opportunities for future studies.

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Autoantibody-Directed Therapy in Cardiovascular Diseases

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1. INTRODUCTION

Autoimmunity is increasingly accepted as the origin or amplifier of cardiovascular disease or diseases in general affecting the cardiovascular system. Patients suffering from classic autoimmune disease frequently present with increased risk of cardiovascular disease [1]. Without claim to completeness, autoantibodies (AABs) found in patients with cardiovascular diseases are discussed here. With respect to atherosclerosis in general and specifically to acute cardiovascular disease and ischemic cardiomyopathy, several AABs have been identified in patients including antiphospholipid antibodies, antiheat shock protein antibodies, antimodified LDL antibodies, and anti-apoA-1 antibodies [2,3].

There are also additional AABs of interest for cardiovascular disease, as noted in Fig. 27.1.

Among the AABs found in patients with cardiomyopathies, some are primarily based on their negative chronotropic effect and/or reduced calcium transient measure by functional assays using rat cardiomyocytes or chick embryos and are often categorized as “cardiodepressant AABs” [4,5].

Cardiodepressant AABs have frequently been attributed to the IgG3 subclass, but, in general, the individual AABs representing this AABs class have been not characterized, nor have the specific myocardial targets of these cardiodepressant AABs [6]. However, anti-Na/K-ATPase AABs [7,8], anticardiac troponin I AABs [8–10], and antimyosin AABs [8,11] belong to the group of cardiodepressant AABs. Anti-Na/K-ATPase-, anticardiac troponin I, and antimyosin AABs have been found in small quantities in healthy subjects, but were up to 30% significantly more pronounced in patients with heart failure based on dilated and ischemic cardiomyopathy, indicating that

AABs generation is clearly more pronounced in idiopathic dilated cardiomyopathy (DCM) patients than in patients suffering from ischemic cardiomyopathy. The AABs were also frequently found in patients with myocarditis [9]. Cell experiments and animal studies using immunization with antigens or AABs exposure revealed the cardiopathogenic activities of AABs that ranged, in relation to the specific AABs, from Na/K-ATPase inhibition, altered intracellular Ca^{2+} handling and reduced contractility to myocarditis, fibrosis, and heart failure. In contrast, human studies analyzing the association between the presence of AABs and patients’ clinical symptoms and outcome are inconsistent [12,13]. It is also still not well understood which mechanisms enable the presentation of intracellular antigens such as myosin and troponin I as the target of immune response for AABs generation. Interestingly, antimyosin AABs, which have also been found in patients with Kawasaki disease and rheumatic fever, stimulate the β -adrenergic receptor mediated signaling in heart cells due to receptor cross-reacting [14,15]. Due to this function, antimyosin AABs may be partially discussed with respect to the new class of AABs directed against G-protein coupled receptors (GPCR-AABs), which are known as “functional antibodies.”

GPCR-AABs are also occasionally subsumed under the cardiodepressant AABs class [16]. Patients whose IgG-induced cardiodepressant activities in the above-indicated functional assays [4,5] are mostly carriers of AABs directed against the β 1-adrenergic (β 1-AABs) and muscarinic 2-receptor (M2-AABs) [5]. M2-AABs are often identified by their negative chronotropic activity, which was demonstrated in the bioassay of cultured spontaneously beating neonatal rat cardiomyocytes [17]. Via stimulation of their related receptor, M2-AABs

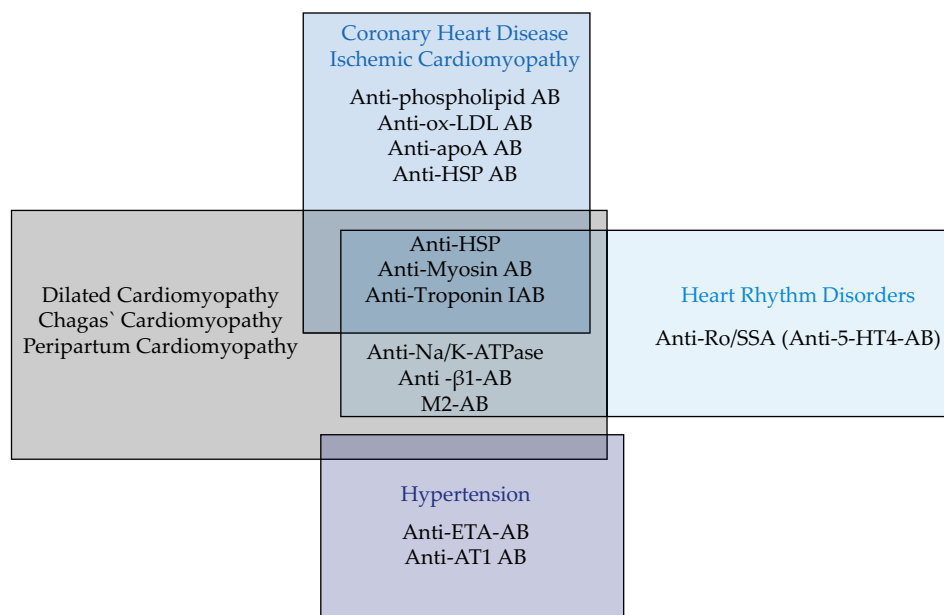


FIGURE 27.1 Autoantibodies relevant to cardiovascular diseases. *Adapted from the personal collection of the authors.*

decreased isoproterenol-stimulated cAMP accumulation in Guinea-pig ventricles, heart-beating frequency in cultured spontaneously beating neonatal rat cardiomyocytes, maximal rate of increase in ventricular pressure, and heart rate in rats in vivo [18]. Furthermore, M2-AABs purified from DCM patients induced bradycardia in chick embryos [19]. Therefore it seems reasonable that M2-AABs match the cardiodepressant AABs class. In contrast, the most obvious characteristic of β1-AABs is their positive chronotropic activity, which has been demonstrated in cultured spontaneously beating neonatal rat cardiomyocytes. In the presence of β1-AABs, cells are resistant to desensitization [20], which is associated with an AABs dose-dependent cAMP increase, as demonstrated in rabbit cardiac membrane [21]. However, data from experiments using myocytes and tissue preparation from animal and human hearts indicated the potential of β1-AABs for inhibiting the contraction force [5]. Together with the documented worsened cardiac function of patients with heart failure and positivity for β1-AABs [22], it is reasonable to assume that β1-AABs also match cardiodepressant AABs.

Since the mid-1970s [23], growing evidence has shown GPCR-AABs to be pathogenic drivers in cardiovascular disease (also see Chapter 3). As for other cardioactive AABs, GPCR-AABs were found in small quantities in healthy subjects. For the prevalence specifically of β1- and M2-AABs, as summarized from different studies [24,25] it was shown that up to 10% of the analyzed healthy subjects were positive for one of the two AABs, but more than 50% of the AABs-positive

individuals carried both β1- and M2-AABs. Due to the generally small number of healthy subjects analyzed in these studies combined with the different measuring methodologies used (bioassay, enzyme linked immunosorbent assay (ELISA))—whereby the ELISA for β1-AABs must especially be critically discussed ([26], see also Chapter 3)—a commonly accepted picture of the GPCR-AABs situation in healthy individuals cannot currently be presented. To the best of our knowledge the largest study including the largest number of individuals declared as being healthy (408 subjects, age 36.6 ± 21.5 ; sex (f/m) 177/231; EF not shown; NYHA class 1) [27], 10% of the healthy subjects were positive for β1-AABs and 11.3% for M2-AABs; among these, 63.4% carried both AABs with a sharper increase in the frequency of AABs positivity after 50 years of age. However, it is unusual that the authors classified healthy individuals as being in NYHA class I. In view of the AABs titers comparing healthy and diseased individuals, the titer was significantly lower in healthy individuals vs DCM patients ($n=53$; age 55.7 ± 15.5 ; sex (f/m) 19/34; EF 31.9 ± 12.2 ; NYHA class 3.15 ± 0.57) who were analyzed in comparison. In this study, 47.2% of DCM patients presented with β1-AABs and 50.9% with M2-AABs. The previously presented data were based on ELISA measurements. However, our own measurements using the bioassay of spontaneously beating neonatal rat cardiomyocytes confirmed the prevalence of β1- and M2-AABs, as well as the relatively low AABs titer—compared with those of diseased subjects—in healthy individuals visiting blood banks for blood donation. However, analyzing the blood for other cardiovascular-relevant GPCR-AABs, such

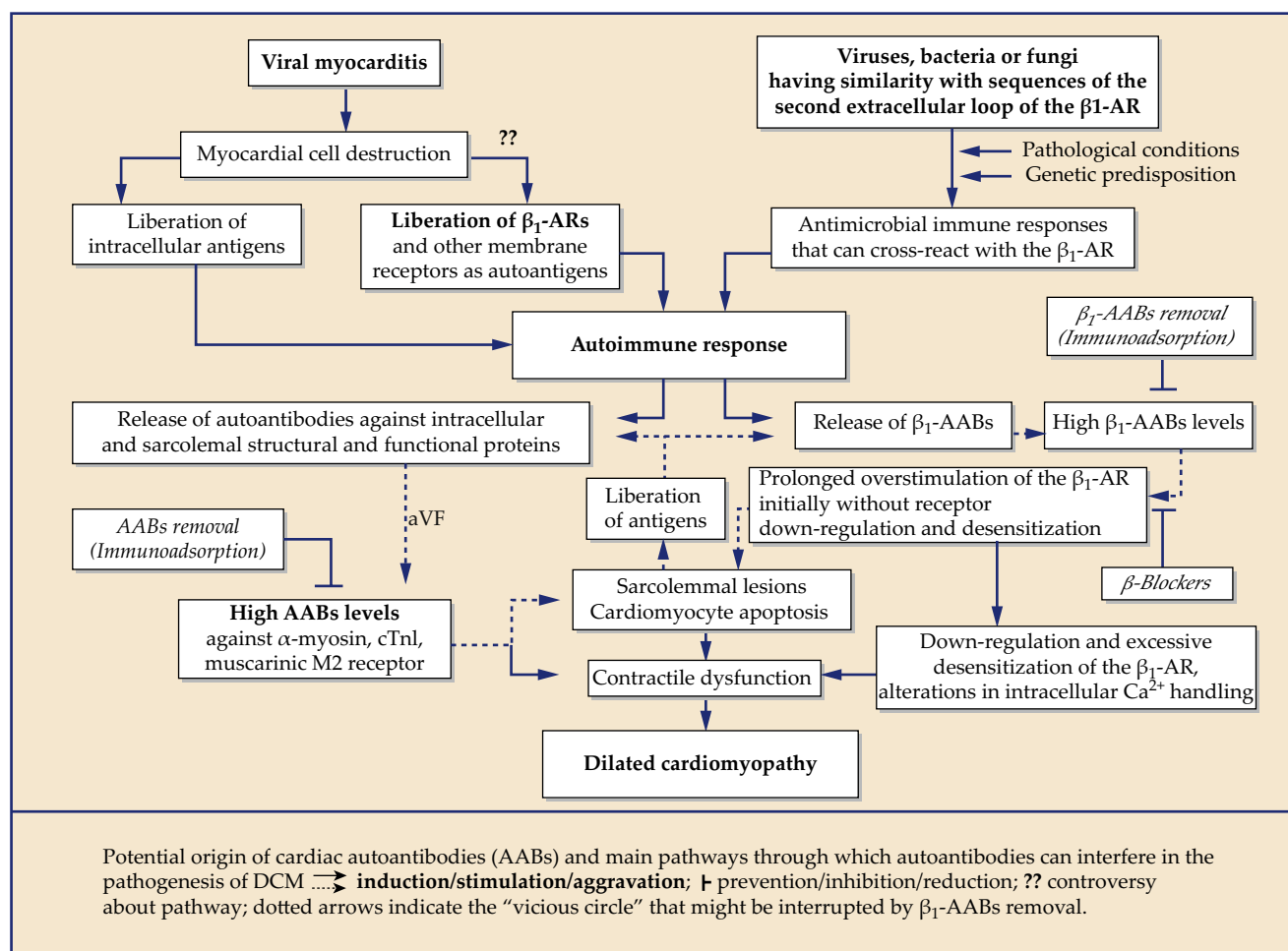


FIGURE 27.2 Pathogenesis of DCM in relation to autoimmunity and the generation of cardiopathogenic autoantibodies. Reproduced from Dandel et al. [33].

as AABs directed against β_2 -adrenergic (β_2 -AABs), α_1 -adrenergic (α_1 -AABs), endothelin A receptors (ETA-AABs), and angiotensin II receptor type 1 (AT1-AABs), we found significantly higher GPCR-AABs prevalence in cases where at least one of the GPCR-AABs was positive (unpublished data).

Among the cardiovascular diseases, AABs positivity in general and specifically GPCR-AABs positivity and titers are particularly pronounced in patients suffering from cardiomyopathies, such as dilated cardiomyopathy, Chagas' cardiomyopathy, and Peripartum cardiomyopathy, as well as in diseases associated with cardiovascular disorders (eg, forms of hypertension and Diabetes mellitus). These data combined with the discussed pathogenic consequences of GPCR-AABs are summarized in ([28–32]; see also Chapter 3).

The suggested involvement of autoimmunity connected with the generation of cardiopathogenic AABs in the pathogenesis of cardiomyopathies is clearly presented for DCM in Fig. 27.2.

In view of patients with cardiomyopathies, β_1 - and M2-AABs have mainly been detected. Based on functional assays (bioassay of spontaneously beating neonatal rat cardiomyocytes [34], Förster resonance energy transfer technique [35]), 70 to 80% of DCM patients carry β_1 -AABs among these with geographic variability such as directed against the first or second extracellular receptor loop [36,37]. Using the bioassay of spontaneously beating neonatal rat cardiomyocytes, nearly 100% of patients with Chagas' cardiomyopathy or Peripartum cardiomyopathy were positive for β_1 -AABs, but these were exclusively directed against the second receptor loop [34,38]. M2-AABs directed at the second extracellular loop were found in up to 40% of DCM patients, but in up to 100% in patients with Chagas' cardiomyopathy [34] and almost 45% of Peripartum cardiomyopathy patients [39].

Using data summarized in [40,41] concerning the occurrence as well as pathological and mechanistic aspects, β_1 - and M2-AABs were also seen in tight relation to cardiac electric abnormalities (Fig. 27.3).

The presence of β 1- and M2-AABs has also been frequently described in patients with primary electrical cardiac abnormalities, arrhythmias, ventricular tachycardia, and sudden death, as well as in patients with myocarditis [19,36,42–49]. In addition to β 1- and M2-AABs, other cardiopathogenic AABs such as anti-myosin and anti-Na/K-ATPase have also been discussed with regard to their place in cardiac electric abnormalities [41]. In addition, there is increasing interest in the role of anti-Ro/SSA antibodies in electric cardiac disturbances. Fig. 27.4 summarizes the suggested mechanisms by which AABs contribute to the development of electrical disturbances in the heart.

Anti-Ro/SSA antibodies are generated due to autoimmune responses against the Ro-antigen. Anti-Ro/SSA antibodies are antinuclear autoantibodies present in many autoimmune diseases, such as systemic lupus erythematosus, Sjögren's syndrome, polymyositis, dermatomyositis, systemic sclerosis, rheumatoid arthritis, and others [50]. It is commonly known that patients with autoimmune disease have a high risk of developing cardiovascular disease. Cardiac electric abnormalities are common for patients with autoimmune disease. An Ro/SSA autoantibody-dependent inhibition of specific K and/or Ca channels has been discussed as one reason [40,41,51]. However, it has been demonstrated that Ro/SSA

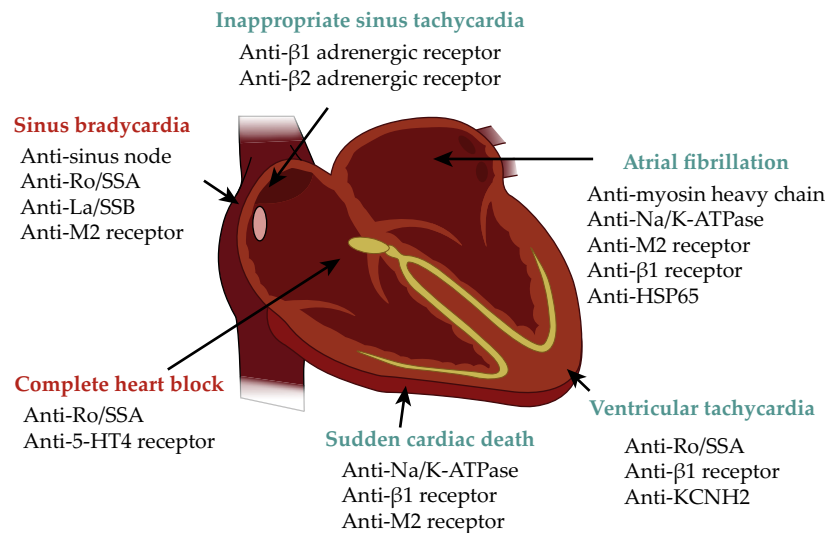


FIGURE 27.3 Autoantibodies related to cardiac abnormalities. Reproduced from Lee et al. [41].

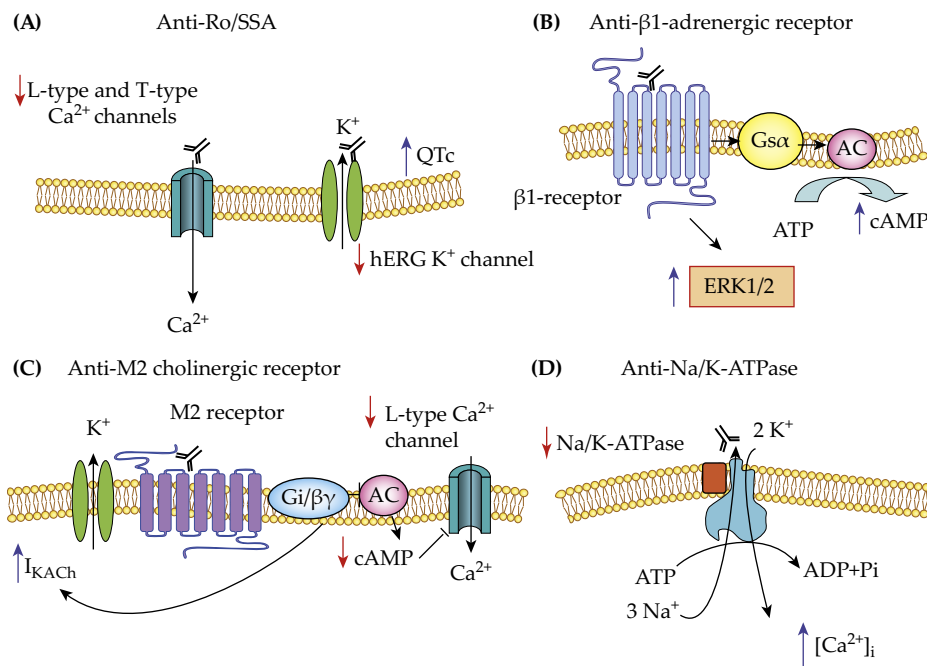


FIGURE 27.4 Suggested mechanisms of autoantibodies related to cardiac abnormalities. Reproduced from Lee et al. [41].

autoantibodies functioning as GPCR-AABs cross-react with the 5-hydroxytryptamine receptor 4 of human atrial cells. In this way, the serotonin-dependent activation of the L-Type Ca channel can be inhibited, which could explain the electric abnormalities in the heart seen in patients with autoimmune disease [52]. GPCR-AABs were also found in patients with different forms of hypertension and in other diseases associated with vascular alterations such as Diabetes mellitus and Alzheimer's disease, as commonly reported ([28–30,32]; also see Chapter 3). While classic autoantibodies induce immune responses resulting in destruction of the affected tissue, GPCR-AABs shows functional, most frequently agonistic activity, after binding their related receptors. These AABs are thus often named “agonist-like AABs,” but in our view would be better named “functional AABs” due to the recent finding of functionally active but antagonistic or inverse agonistic GPCR-AABs [52,53]. The related disease should consequently be named “functional antibody disease,” which would establish a new class of autoimmune disease.

Consequently, as suggested for the cardiodepressive AABs in general, GPCR-AABs and specifically β 1-AABs and M2-AABs have become therapeutic targets with the logical development of treatment strategies for patients with “functional antibody disease.” In view of these strategies, two different approaches are obvious. The first strategy includes the elimination of AABs from patients' circulation, as already indicated in Fig. 27.2; the second strategy is directed to in vivo AABs attack.

For AABs elimination from circulation, apheresis procedures have been applied based on unselective plasmapheresis (therapeutic plasma exchange; TPE) [54] or on apheresis technologies developed for the binding of immunoglobulins or more selective binding of specific IgG subclasses. By using these unselective apheresis technologies, GPCR-AABs together with all other cardiodepressant AABs indicated above can be removed from human circulation. For more specific blood clearance, such as directed to only the GPCR-AABs class, an apheresis technology is presently under development in which an aptamer, able to bind all of the currently known GPCR-AABs of interest for cardiovascular disease, is used to remove GPCR-AABs from circulation. In diseases where one specific GPCR-AABs was evidenced as the dominant pathogenic driver, highly selective apheresis columns may be applied. Such columns can carry either highly specific peptides or aptamers for the removal of only the specifically targeted GPCR-AABs. With respect to the blood clearance of only β 1-AABs, the technological basis of using either specific peptides or aptamers for β 1-AABs binding already exists and has been proven in animal experiments and in the case of peptides as binders in human studies [55,56].

In addition to blood-clearance technologies, there are currently therapeutic strategies under development directed at the in vivo attack of cardiopathogenic AABs by influencing their serum half-life as well as by binding the AABs and neutralizing their cardiopathogenic activities. Among these approaches, intravenous IgG treatment (IVIG) and B-cell depletion therapies, both unspecific, have been studied. Alternatively, strategies for in vivo AABs neutralization are seen as promising. Presently, concepts for the neutralization of the whole group of cardiorelevant GPCR-AABs or specifically for one of the GPCR-AABs are under investigation, in the last case specifically directed against β 1-AABs as the treatment target.

For further information concerning the treatment of diseases associated with autoantibodies directed against G-protein-coupled receptors see [57].

2. AUTOANTIBODY REMOVAL

2.1 Therapeutic Plasma Exchange (TPE) for GPCR-AABs Removal

Therapeutic plasma exchange (TPE) is an extracorporeal therapy for removing pathological substances from circulation. After transfer of the patient's blood to the TPE device, plasma and blood cells are separated. Thereafter, the blood cells are returned to the patient's circulation, while the plasma, which contains the harmful components, is discarded. The patient's plasma is then replaced by donor plasma or a combination of albumin and saline. TPE is a preferred therapeutic option in the therapy of autoimmune disorders and is, in general, substituted by immune-suppressive therapy for long-term benefits. TPE is used in a variety of diseases [54], mainly those with autoimmune background, eg, Guillain-Barré syndrome, lupus erythematosus, and thrombotic thrombocytopenic purpura.

In regards to cardiomyopathy, as documented in a case report of a 28-year old man positive for β 1-AABs suffering from heart failure NYHA class IV and on the heart transplantation list, TPE supplemented by immunosuppressive therapy was beneficial. After TPE, the patient improved in NYHA functional class, exercise test, and cardiac reserve, and removal from the transplant list resulted, which were all associated with the decrease of β 1-AABs. The presence or absence of additional cardiopathogenic AABs were not documented. One year after TPE, due to further dilatation and renewed reduction in cardiac reserve, a second course of TPE was performed with benefit shown to the patient, but no information was provided about the patient's follow-up [58]. Another case report described a 5-year old boy suffering from DCM from the age of

8 months who was positive for β 1-AABs and treated with TPE due to not having a donor heart. For 3 months, a clear benefit of TPE, visible among others by a strong decrease of B-type natriuretic peptide (BNP) associated with the β 1-AABs decrease was documented; however, unfortunately, the benefit was not maintained thereafter [59]. Moreover, in this case report, information about other cardiopathogenic AABs was missing. In patients with nonischemic heart failure ($n=9$, LVEF $<30\%$, NYHA II-IV), TPE resulted in improvement of LVEF from 22.7% at baseline to 30.8% after 3 months and 28.0% after 6 months and in parallel with quality of life. Reduced IgG immunostaining was demonstrated in five of seven pairs of myocardial biopsies sampled at baseline and 6 months after treatment. The specific type of anticardiac antibodies was not indicated [60]. TPE was suggested for kidney transplant recipients with refractory vascular rejection who were positive for AT1-AABs [61]. Seven of the 16 AT1-AABs-positive patients were treated with TPE combined with IVIG and AT1-receptor blockade and demonstrated significantly prolonged allograft survival compared with the patients treated with a standard therapy. Information about other GPCR-AABs present in patients was not given. However, AT1-AABs seem to be the dominant pathogenic GPCR-AABs in kidney transplant recipients suffering from refractory vascular rejection [62]. TPE was also applied to some patients, eg, those suffering from complex regional pain syndrome (CRPS) and presenting with GPCR-AABs. Summarizing case reports [63], three of the six patients reported meaningful improvements of mood and fatigue. For two patients showing no benefits from conventional treatment, case reports documented the loss of autoantibodies directed against the β 2-adrenergic receptor (β 2-AABs) after TEP, which was associated with strong improvement in pain and autonomic symptoms [64]. In a recent case report, a young woman with CRPS and positivity for β 2-AABs and M2-AABs benefited significantly from TPE, which was combined with Rituximab treatment in this case [65]. In conclusion, although TPE was indicated as first- and second-line therapy in many diseases with an autoimmune background [66], the currently available data for the benefit of TPE in patients with positivity of cardiovascular-pathogenic AABs autoantibodies is mainly from case studies, which means there is a level of evidence (C) and recommendation class (IIa-IIb) for the application of TPE in cardiovascular diseases associated with positivity for GPCR-AABs and other cardiovascular-pathogenic AABs.

2.2 Extracorporeal Adsorption (ECA) for the Removal of GPCR-AABs

In extracorporeal adsorption (ECA) for AABs removal from a patient's blood, the patient's circulation is connected with an extracorporeal machine in which the

blood is first separated into blood cells and plasma (Fig. 27.5). The antibody-containing plasma is then passed through the column carrying special ligands for antibody binding. In general, the two columns work in parallel. The first column is loaded with antibodies and, if saturated, the plasma is moved into the second column while the first is regenerated. The column's outflow, the autoantibody-free plasma, rejoins the blood cells and is infused back into the patient [67].

Depending on the column ligands used for the binding and consequent removal of the antibodies, there is one ECA technology, known as immunoabsorption (IA), that is currently the preferred technology for autoantibody removal. Among the commercially available columns for IA, there are, depending on the column ligands, such as for the removal of the total immune globulin fraction, a specific class of immune globulins or more specifically for IgG subfractions, as well as those designed to remove only specific antibodies (Table 27.1). Recently, aptamers, a new class of binders sometimes known as "chemical antibodies," have been designed, which may also be used as autoantibody binders in ECA [56,69,70].

Table 27.2 documents the IA treatment studies—with no claim to absolute completeness—performed between 1996 and 2016. The first evidence for IA benefit of β 1-AABs clearance in DCM patients was reported in 1996 [71], using the Ig-Therasorb (Baxter Corp., München, Germany; presently Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany). This column carries immobilized antihuman polyclonal IgG from sheep that recognizes kappa and lambda light chains as well as the heavy chains of human IgG. In this way, IgG-containing AABs present in patients can be cleared from patients' plasma. In this initial report, eight patients with DCM (NYHA classes II-IV) were treated for IgG removal to clear their blood of β 1-AABs. After a course of four and five sessions, respectively, of IA consecutively performed per week, β 1-AABs were strongly reduced to the range of healthy subjects. In seven out of the eight patients this was associated with a shifting

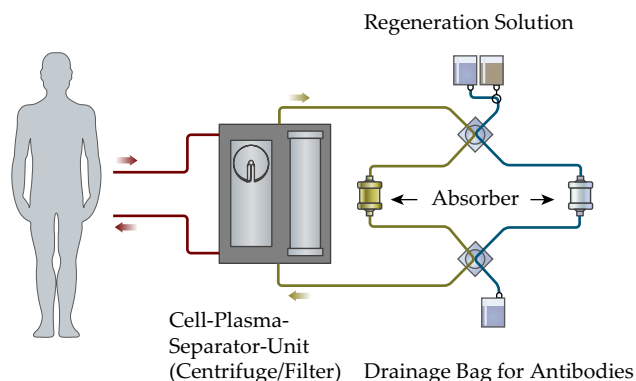


FIGURE 27.5 Principle of extracorporeal adsorption (modified). Reproduced from http://www.fmc.nl/media/1129/ia_brochure_algemeen_01_14_gb_w.pdf.

TABLE 27.1 Commercial Available Systems for Immunoabsorption (Suitable for the Therapy of Cardiovascular Diseases Associated With Autoantibodies)

Producer	Trade name	Specification	Binding ligand	Mechanism	Use
Miltenyi Biotec GmbH, Bergisch Gladbach Germany	TheraSorb—Ig pro adsorber TheraSorb—Ig flex adsorber	IgG 1-4, IgM, IgA,	Antihuman polyclonal Ig from sheep/Sepharose	Binding of κ and λ light chain and heavy chain of human Ig	M S
Fresenius Medical Care, Bad Homburg, Germany	Immunosorba Globaffin Ligasorb Coraffin (presently not available)	IgG1, 2, 4 (IgG3), IgM, IgA IgG 1, 2, 4 (IgG3), IgM, IgA IgG 1, 2, 4 (IgG 3), IgM, IgA Autoantibodies directed against the first and second loop of the β 1-adrenoceptor	Recombinant protein A from <i>Staphylococcus aureus</i> /Agarose Synthetic Peptid-GAM/Sepharose Recombinant protein A/Agarose Synthetic peptides representing the first (PDCM349; 14 mer) and second (PDCM075; 18 mer) extracellular loop of the β 1-adrenoceptor/Sepharose	Constant (Fc) region of Ig Constant (Fc) region of Ig Constant (Fc) region of Ig Antigen binding site of autoantibodies directed against the first and second loop of the β 1-adrenoceptor	M M S M
Asahi-Kasei Medical Co., Japan	Immusorba TR-350 Immusorba PH-350	IgG, Fibrinogen, CRP,	Tryptophan/Polyvinyl Alcohol Phenylalanin/Polyvinyl Alcohol	Ionic and hydrophobic interaction to Ig	S S
POCARD Ltd., Russia	IgAdsopak	IgG 1-4, IgM, IgA, IgE	Antihuman polyclonal Ig from sheep/Sepharose	Binding of κ and λ light chain and heavy chain of human Ig	M
Kaneka Corp. Japan	Selesorb	IgG 1-4, IgM IgA	Dextran sulfate/Cellulose	Ionic interaction to Ig	S

M, multiple use; S, single use.

to lower NYHA classes. In one patient, the β 1-AABs returned and the NYHA class deteriorated again, making heart transplantation necessary. Using the Ig-Therasorb column and adequate protocols, improvement in cardiovascular function was seen immediately after successful IA [72]. One day after finishing IA, invasively measured hemodynamic parameters were improved, while LVEF failed to show improvement. Insignificant LVEF changes immediately after finishing the IA course have also been confirmed in IA studies using other columns [73]. However, there have also been studies that demonstrated an LVEF increase together with increased cardiac index and stroke volume index acutely after IA [74–76]. With respect to the successful removal of IgG or more specifically IgG3 by Ig-Therasorb, even more importantly, IA blood clearing resulted in functional and clinical patient benefits after 3 months and prolonged based on the study design up to 6 months, 12 months, and 3 years, respectively [74,77–79]. In the 3-month study [76], IA was repeated monthly. Hemodynamic improvement seen after the first IA cycle persisted to the end of the study, where an improvement in LVEF was also indicated. Despite IA performance on only 5 consecutive days (one treatment session) without any repetition over the study time, a clear patient benefit was also seen after 12 months, which was detected by the continuous decrease of left ventricular internal dimension

in diastole (LVIDD) over the study time combined with NYHA class lowering visibly at the end of the study. In most of the patients treated with IA, a profound relapse in the β 1-AABs generation was not seen until the end of the study. With respect to the 12-month lack of β 1-AABs reincrease [77], the authors speculated that the ex juvantibus taking of antioxidants prevented the renewed formation of β 1-AABs. Indeed, a reduction of oxidative stress was demonstrated after IA of β 1-AABs-positive DCM patients [80].

After IA for whole IgG removal, the reduced IgG was mostly replaced. However, this was not always seen as necessary [77]. However, if patients are treated with Ig-Therasorb their blood is exposed to antihuman polyclonal IgG from sheep, which is under discussion for human therapy due to its animal origin.

As an alternative, a column (Immunosorba, Fresenius, Bad Homburg, Germany) using protein A of *Staphylococcus aureus* started in 2002 [81] for IgG removal is now frequently applied. This column preferentially binds IgG subclasses 1, 2, and 4, but also IgG3 and IgA- and IgM with lower affinity [82,83]. In the first study, due to minor IgG3 binding, a disadvantage for patients of the protein A column compared to Ig-Therasorb was demonstrated [81]. However, after IA protocol modification for improved IgG3 removal, IA with the protein

TABLE 27.2 Clinical Studies Using IA Treatment for Patients With Heart Failure

Trial (Ref.)	Study design	Intervention (immunoadsorber)	IA-treated patients/controls	Baseline characteristics	Follow-up	Results
Wallukat et al. [71]	CS	1 course of IA with 4/5 sessions + IgG substitution [A]	8/0	NYHA II-IV, β 1-AABs (+)	2.5 months	Pre-IA vs post-IA: day 5 n 7/8 patients (β 1-AABs (100 vs 8% LU); NYHA class reduction); 2.5 months (β 1-AABs return to β 1-AABs (+), pre-IA NYHA class)
Dorffel et al. [72]	CS	1 course of IA with 5 sessions [A]	9/0	NYHA III/IV, LVEF <25%, β 1-AABs (+)	5 days	Pre-IA vs post-IA: β 1-AABs (6.4 ± 1.3 vs 1.0 ± 0.5 , $p < 0.001$ LU); CO (3.7 ± 0.8 vs 5.5 ± 1.8 L/min, $p < 0.01$); MAP (76.0 ± 9.9 vs 65.0 ± 11.2 mm Hg, $p < 0.05$)
Müller et al. [77]	CC	1 course of IA with 5 sessions [A]	17/17 conventionally treated	NYHA II-IV, LVEF <30%, β 1-AABs (+)	12 months	Patients vs controls post-IA: β 1-AABs (<1.0 vs 5.0 ± 1.3 LU, $p < 0.001$); LVEF 37.9 ± 7.9 vs $25.2 \pm 5.9\%$, $p < 0.0001$; NYHA class improvement ($p < 0.001$)
Felix et al. 2000 [74]	RCT	1 course of IA with 3 sessions + IgG substitution [A]	9/9 conventionally treated)	NYHA III/IV, LVEF <30%, β -AABs (+)	3 months	Pre-IA vs post-IA: CI in the treatment group (2.3 ± 0.1 L/min/m ² vs 3.0 ± 0.3 L/min/m ² ; $p < 0.01$). β 1-AABs (>4 LU vs <2 LU) No change in the controls
Schimke et al. [80]	CC	1 course of IA with 5 sessions [A]	17/17 conventionally treated	NYHA II-IV, LVEF <30%, β 1-AABs (+)	12 months	Patients vs controls post-IA: β 1-AABs (<1.0 vs 5.0 ± 1.3 LU, $p < 0.001$); LVEF (37.9 ± 7.9 vs $25.2 \pm 5.9\%$, $p < 0.0001$); NYHA class improvement ($p < 0.001$), reduced serum markers for oxidative stress (TBARS ($p < 0.05$), LPO ($p < 0.05$) and antioxLDL-AB ($p < 0.05$))
Wallukat et al. [55]	CS	1 course of specific β 1-AABs IA with 5 sessions [B]	8/0	LVEF <35%, β 1-AABs (+)	12 months	Pre-IA vs post-IA: LVEF (28.5 ± 6.1 vs 36.6 ± 10.7 , $p < 0.05$); β 1-AABs (5.0 ± 0.5 vs $<1.2 \pm 0.6$ LU), serum oxidative stress markers (TBARS (8.4 ± 4.1 vs 3.7 ± 1.6 μ mol/L, $p < 0.05$))
Felix et al. 2002 [75]	CC	1 course of IA with 3 sessions + IgG substitution [A]	11/9 (healthy)	NYHA III/IV, LVEF <30%, cardiodepressant AABs (+)	3 days	Pre-IA vs post-IA CI (2.2 ± 0.1 vs 2.7 ± 0.2 L/min/m ² ; $p < 0.01$). Serum cardiodepressive AABs were found in the column eluate after IA
Mobini et al. [76]	CS	1 course of IA with 3 sessions + IgG substitution followed by 2 courses once per month for 3 months [A]	22/0	NYHA III-IV, LVEF <30%, β 1-AABs (+) and (-)	3 months	Pre-IA vs post-IA: LVEF (21.5 ± 6.4 vs $26.8 \pm 7.3\%$ ($p < 0.05$), CI: 2.2 ± 0.3 vs 2.7 ± 0.71 L/min/m ² , $p < 0.001$), No difference in CI and LVEF between β 1-AABs (+) and (-)
Knebel et al. [79]	RA	1 course of IA with 5 sessions + IgG substitution [AD]	17/17 conventionally treated	NYHA II/III, LVEF <35%, AABs (+) not declared	3 years (median 2.3 years)	Patients vs controls post-IA: reduced days of hospitalization ($p < 0.01$)

TABLE 27.2 Clinical Studies Using IA Treatment for Patients With Heart Failure—cont'd

Trial (Ref.)	Study design	Intervention (immunoadsorber)	IA-treated patients/controls	Baseline characteristics	Follow-up	Results
Hessel et al. 2004 [115]	CC	1 course of IA with 5 sessions [A]	17/17 conventionally treated	NYHA II-IV, LVEF <30%, β 1-AABs (+)	5 years	Post-IA patient vs controls: The 5-year mortality (10/17 vs 3/17), survival rates (82 vs 41%), Medical cost for annual treatment based on survival time and medical cost Germany 2000 (€24,900 vs €28,900) with resulted in incremental costs per life year gained for IA of about €35,000 seen as cost-effective due to the cited limit of US\$50,000 per quality-adjusted life-year gained
Staudt et al. 2005 [84]	CC	4 courses of IA with 2/3 sessions with improved IgG3 binding vs normal IA columns [C]	9/9	NYHA III/IV, LVEF 21.6 ± 2 vs $24.3 \pm 2\%$, AABs not declared	3 months	Pre-IA vs post-IA: IgG3 reduction (-65 ± 4 vs -36.4% , $p < 0.001$), LVEF (34.7 ± 4 vs $24.4\% \pm 2\%$, $p < 0.05$)
Schimke et al. [102]	CS	1 course of specific β 1-AABs IA with 5 sessions [B]	8/0	LVEF <35%, β 1-AABs (+)	12 months	Pre-IA vs post-IA: Oxidative stress: TBARS (LVEF (28.5 ± 6.1 vs 36.6 ± 10.7 , $p < 0.05$); β 1-AABs (5.0 ± 0.5 vs $<1.2 \pm 0.6$ LU)), serum oxidative stress markers (TBARS (8.4 ± 4.1 vs $3.7 \pm 1.6 \mu\text{mol/L}$, $p < 0.05$))
Staudt et al. 2006 [85]	RCS	1 course of IA with 5 sessions + IgG substitution vs 4 courses for 5 days + IgG over 3 months [C]	11/11	NYHA III/IV, LVEF <35%, AABs (+) not declared	6 months	Pre- vs post-IA: NYHA improvement (10/11 vs 11/11), LVEF improvement (26.5 ± 2.2 vs $34.8 \pm 2.9\%$, $p < 0.01$ and 28.1 ± 2.9 vs $37.0 \pm 1.6\%$, $p < 0.01$; no difference between groups
Staudt et al. 2006 [86]	CC	4 courses of IA with 5 sessions + IgG substitution over 3 months [C]	15/15 conventionally treated	NYHA III, IV, LVEF <35%, AABs (+) not declared	3 months	Pre-IA vs post-IA: NYHA improvement in patients, $p < 0.01$ vs unchanged NYHA in controls; LVEF patients (29.7 ± 1.0 vs 38.6%), controls (28.1 ± 1.0 vs $26.4 \pm 1.0\%$), $p < 0.001$; NT-ProBNP patients (>1400 vs $<800 \text{ pmol/L}$), controls (>1400 vs $>1500 \text{ pmol/L}$), $p < 0.001$
Cooper et al. [73]	CS	1 course of IA with 5 sessions [C]	4/0	NYHA II/III, LVEF $34.6 \pm 12.3\%$, AABs (+) not declared	6 months	Pre-IA vs post-IA: LVEF (26.3 ± 9.4 vs $28.7 \pm 11.4\%$, $p < 0.05$), exercise capacity (82.0 ± 30.8 vs 92.1 ± 34.3 Watt, $p < 0.01$), NT-proBNP (1230 vs 829 ng/L , $p < 0.001$)
Doesch et al. [91]	CS	1 courses of IA with 5 sessions [C]	27/0	NYHA II-IV, LVEF $24.1 \pm 7.8\%$, 6/27 TnI-AABs	6 months	Pre-IA vs post-IA: NYHA class improvement in 33%, LVEF improvement >5% in 33%, which were all diabetics and TnI (–), exercise capacity improvement >15 watt in 48%, in 5/6 TnI-AABs were cleared by IA but TnI-AABs returned
Doesch et al. [92]	CS	1 course of IA with 5 sessions [C]	51/0	NYHA II-IV, LVEF <50%	6 months	Pre- vs post-IA: LVEF (34.6 ± 12.3 vs $44.1 \pm 15.3\%$, n.s.)

Continued

TABLE 27.2 Clinical Studies Using IA Treatment for Patients With Heart Failure—cont'd

Trial (Ref.)	Study design	Intervention (immunoabsorber)	IA-treated patients/controls	Baseline characteristics	Follow-up	Results
Herda et al. 2010 [87]	CC	1 course of IA with 5 sessions + IgG substitution [C]	30/30 conventionally treated	NYHA II-IV, LVEF <45%, AABs (+) for cTnI-AABs and/or KChIP2-AABs in a subset of 14 patients and controls	3 months	Pre-IA vs post-IA: LVEF patients (33.0±1.2 vs 40.1±1.5, $p<0.001$) controls 30.1±1.2 vs 32.0±1.5, n.s.), exercise capacity improvement patients (114.2±7.4 vs 141.9±7.9 Watt, $p<0.05$), improvement of spiroergometry parameters, $p<0.05$ –0.01), no improvement in the controls; only improved peak VO ₂ , $p<0.01$ in patients with AABs (+)
Baba et al. 2010 [16]	CS	1 course of IA with 3/5 sessions, in 3 patients due to return of β 1- and/or M2-AABs second course after 3 months [D]	18/0	NYHA III/IV, LVEF <30%, β 1-AABs (+), M2-AABs (+), cardiodepressant AABs (+)	3 months	Pre-IA vs post-IA: Six-min walk test improved ($p<0.01$), LVEF improvement ($p<0.01$ only in patients with complete removal of AABs)
Trimpert et al. [93])	CS	1 course of IA with 5 sessions + IgG substitution [C]	17/0 (11 cardiodepressant AABs (+), 6 (–))	NYHA II-IV, LVEF <45%,	12 months	Pre-IA vs post-IA: LVEF AABs (+) (33.8±1.7 vs 51.8±1.7%, $p<0.001$, AABs (–) (no change); LVIDd AABs (+) (66.6±1.2 vs 61.2±2.2 mm, $p<0.05$); no return of the AABs (+)
Nagatomo et al. [99])	CS	1 course of IA with 2/5 sessions within 1 or 2 weeks [D]	16/0	NYHA III/IV, LVEF 18+2%, β 1-AABs (+), M-AABs (+)	3 months	Pre-IA vs post-IA: LVEF (18+2 vs 21+2%, $p<0.05$), BNP (752+156 vs 432+96 ng/L), 6 min walk distance (31+39 vs 369+30, $p<0.01$)
Dandel et al. [111]	RA	1 course with 5 sessions of unspecific or specific β 1-AABs IA [A,B,F]	216 (195 β 1-AABs (+), 140 IA, 116 unspecific IA, 24 specific β 1-AABs IA, 55 non-IA), (21 β 1-AABs (–), 21 unspecific IA)	NYHA II-IV, LVEF <30%, β 1-AABs (+)	5–14.5 years	Post-IA 5 years HTX/VAD free survival probabilities: β 1-AABs (+) vs (–) (69.4+4.4 vs 47.4+11.5%), β 1 AABs (+) with vs without IA (69.4+4.4 vs 25.5+11.4%), unspecific IA vs specific IA for β 1-AABs (+)(88.0+8.5 (column1) vs 78.8+8.4 (column3) vs 91.3+5.9 column2), IA responders vs nonresponders (89.3+3.6 vs 24.7.5+7.5%), post-IA 5 years HTX/VAD free survival probabilities tended to continue up to 10 years after IA
Bulut et al. 2013 [90]	CC	1 course of IA with 5 session + IgG substitution [C]	18/5 DCM conventionally treated /12 ischemic cardiomyopathy conventionally treated	NYHA II-IV, LVEF <35%, AABs (+) not declared	6 months	Pre-IA vs post-IA: LVEF (27.1+5.3 vs 36.8+8.2%, $p<0.05$, $n=12$ responder; 28.4+6.0 vs 28.2+5.8%, n.s., $n=6$ nonresponder; no change in conventional treated DCM and ischemic cardiomyopathy patients), regulatory T cells (2.32+0.22 vs 4.06+0.68%, $p<0.05$, $n=12$ responder; 4.86+0.28 vs 4.56+0.81%, n.s., $n=6$ nonresponder)

TABLE 27.2 Clinical Studies Using IA Treatment for Patients With Heart Failure—cont'd

Trial (Ref.)	Study design	Intervention (immunoadsorber)	IA-treated patients/controls	Baseline characteristics	Follow-up	Results
Pokrovsky et al. [101]	CC	1 course of IA with 5 sessions [E]	9/7	NYHA II-IV, LVEF <35%, β 1-AABs (+)	6 months	Pre-IA vs post-IA: LVEF (patients tended to improvement), BNP improved (507+279 ng/L vs 272+185 ng/L, $p < 0.05$, no change in the controls),
Reithaler et al. [94]	RA	2 courses of IA with 5 sessions, First course followed by second course after 41.7+27.4 months [A,C]	15/0	1st course: NYHA improvement \geq 1 class, LVEF improvement <5% but thereafter subsequent deterioration, AABs not declared	6 months	Pre-IA vs post-IA: first session NYHA (2.87 \pm 0.64 vs 2.33 \pm 0.72, $p < 0.05$), LVEF (33.0 \pm 0.4 vs 43 \pm 7.9%, $p < 0.001$); 2nd session NYHA 2.87 \pm 0.64 vs $p < 0.05$), LVEF (29.7 \pm 4.6 vs 34.9 \pm 8.3, $p < 0.05$)
Dandel et al. [98]	RA	1 course of IA with 4 sessions [F]	31/31 (DCM/DCM+Diabetes mellitus)	NYHA III/IV, LVEF <30%, β 1-AABs (+) 79%	5 years	Post-IA 3- and 5-years HTX-free survival probabilities for all patient (79.6+5.6 and 63.5+7.9%) without significant differences between the groups as it was also for post-IA 3-year freedom from β 1-AABs reappearance
Yoshikawa et al. [100]	RCS	1 course of IA with 5 sessions within 2 weeks followed by 1 course of IA (with 5 sessions within 2 weeks) [D]	22 with first and second IA courses/22 with only second IA course	NYHA III/IV, LVEF <30%, β 1-, M2-, Na/K-ATPase-, TnI-, and/or myosin-AABs (+)	12 months	Pre-IA vs post-IA: NYHA improvement ($p < 0.001$), (LVEF: 23.8+1.3 vs 25.9+1.3%) at 4 months after IA, no additional effect of the 2nd course of IA

[Used Apheresis Column]; A, Ig-Therasorb; B, Coraffin; C, Immunosorba; D, Immunosorba TR; E, IgAdsopak; F, Globaffin.

A column has shown to be adequate compared to Ig-Therasorb [84]. Subsequent studies have confirmed the persistent patient benefit of IA with this column for several months or even years, which was visible by increased cardiac function, decreased diastolic diameter, improved echocardiographic and cardiopulmonary exercise parameters, improved endothelial function, changed cell-mediated immunity, as well as decreased serum levels of natriuretic peptides [73,85–94]. The long-term patients benefit was associated with the increase in regulatory T cells (Tregs), improved endothelial function, combined with the decreased number of total and endothelial microparticles (MPs) in the blood [88–90].

However, as demonstrated for the Tregs, this increase was clearly associated with IA responders as indicated by their LVEF improvement [90].

Comparable patient benefit was demonstrated for the 6-month follow-up, regardless of whether a single course of IA was administered or IA was repeated monthly [85,94]. In 2007, the Multicenter, Randomized, Double-Blind, Prospective Investigation on the Effects

of Immunoadsorption on Cardiac Function in Patients with Dilated Cardiomyopathy began to verify the use of the protein A column for DCM treatment. Results of this study were expected in 2015 but are currently still not available [95]. In this study, IgG is replaced after IA, as was also reported in the majority of the above-indicated IA treatments. To date, there is no convincing published study that has shown the benefit of immunoglobulin replacement after IA. If replacement of immunoglobulins is considered, the fact that intravenous immunoglobulins contain foreign immunoglobulins that may induce a proinflammatory reaction in treated patients, which may attenuate the positive effect of IA, should be kept in mind. Consequently, immunoglobulin was not replaced in all IA studies, but any negative outcome after IA due to the lack of replacement has not been published so far [73,96].

There are further columns that can be used for IgG IA. Among these is one column carrying the synthetic peptide ligand Peptid-GAM [97] for IgG binding (Globaffin, Fresenius Medical Care, Bad Homburg, Germany), which

supplied a comparable long-term patient benefit as seen in patients treated with Ig-Therasorb; both patients suffering from DCM without and those with Diabetes mellitus benefited from treatment with Globaffin [98]. Another column carrying Tryptophan (Immunsorba TR; Asahi Kuraray Medical, Tokyo, Japan) was also used with success in DCM patients [16,99,100]. In the last of these studies enrolling patients from 10 sites in Japan, IA therapy was conducted in 40 patients with DCM (refractory to standard therapy for heart failure, New York Heart Association [NYHA] class III/IV, left ventricular ejection fraction (LVEF) < 30%). The mean echocardiographic LVEF was significantly improved ($23.8 \pm 1.3\%$ to $25.9 \pm 1.3\%$, $p = 0.0015$) after 3 months. However, subgroup analysis revealed improvement of echocardiographic LVEF in patients with higher baseline autoantibody scores but not in those with lower scores. Recently, IgAdsopak (POCARD Ltd., Russia) was introduced, which demonstrated patient benefit after 6 months mainly by BNP decrease [101].

For the highly specific removal of β_1 -AABs, Coraffin (Fresenius Medical Care, Bad Homburg, Germany) was designed. This adsorber uses two linear peptide ligands mimicking epitopes of the β_1 -adrenergic receptor and is able to bind β_1 -AABs directed to the first and second β_1 -receptor loops [97]. The successful application of this column in DCM patients was demonstrated in 2002 [55]. A strong reduction of the β_1 -AABs titer was seen after IA, which did not return to pathological levels within 1 year of follow-up. This was accompanied by clearly improved echocardiographic data comparing the pre-IA vs 1-year post-IA time. As already demonstrated [80], this patient benefit was associated with reduced oxidative stress [102]. Unfortunately, the Coraffin column is not currently available. As published in 2012 [103], IA using Immunsorba was performed in 2008 for the first time in a patient with pulmonary arterial hypertension (PAH) to remove α_1 -AABs and ETA-AABs, both thought to drive vascular alteration generally in hypertension and specifically in PAH [104–108]. For this patient, IA was seen as the last therapeutic option to avoid or delay lung transplantation. The IA protocol was comparable with the protocol used for DCM patients. All five of the treated patients thus far responded after IA with strong autoantibody reduction, reduction of the pulmonary arterial pressure, improvement of RV function, and exercise capacity. Finally, two patients without any autoantibody reappearance showed stable clinical improvement for more than 24 months. In the other patients, the autoantibodies returned and were associated with severe worsening of the clinical situation. After renewed IA, one patient recovered and was in stable clinical condition at the time of writing. The other two patients died due to ventricular arrhythmia and pulmonary embolism.

Thromboangiitis obliterans seems to be another field of IA treatment for GPCR-AABs removal. It was shown to

be beneficial in patients with thromboangiitis obliterans. Unfortunately, the patients were not characterized before IA (Ig-Therasorb) in view of positivity for any AABs [109]. However, using later findings, this benefit (in this study Globaffin was used) may have been related to the GPCR-AABs found in these patients [110].

Comparing the data of specific β_1 -AABs removal with those of unspecific IgG adsorption, the same high efficiency was obvious concerning the β_1 -AABs reduction combined with the absence of differences in long-term patient benefit [111]. This was interpreted by the authors as being strong evidence for the highly specific driving role of β_1 -AABs in the pathogenesis of DCM. This 2012 publication supplied the most convincing support for IA to date as a hopeful treatment strategy with clearly long-term patient benefit. In the retrospective analysis, all β_1 -AABs-positive and -negative HTx candidates with end-stage DCM of the German Heart Center Berlin (for inclusion criteria see Table 27.2) who were treated with IA between 1995 and 2005 were enrolled. The patients were evaluated for outcome (follow-up 5.3–14.7 years) and the efficiency of several IA technologies was assessed, mainly focused on unspecific vs specific IA. For control, β_1 -AABs-positive DCM patients referred to as HTx but who did not receive IA were used. The study design, which included 216 patients, is presented in Fig. 27.6.

Based on the survival rates at the 5-year follow-up, DCM patients who were previously IA positive for β_1 -AABs vs those who were negative may benefit significantly more from IA treatment. This was still evident one decade after IA treatment (Fig. 27.7A). Furthermore, the survival of patients who were positive for β_1 -AABs but did not receive IA vs those treated with IA was significantly reduced (Fig. 27.7B). Additionally, no different survival rates were evidenced for β_1 -AABs-positive DCM patients regardless of the IA system used.

Despite the absence of the results from the “Multicenter, Randomized, Double-Blind” study [95], the currently available study data point to a level of evidence B and class I recommendation (rather than class II) for IA treatment of DCM patients presenting with NYHA class II–IV.

Based on the in vitro experiment using Coraffin, which demonstrated ex vivo clearance of serum from β_1 -AABs of patients with Chagas’ cardiomyopathy, IA also was suggested as a treatment option for patients with Chagas’ disease, but mainly for those with Chagas’ heart disease [112,113]. In view of the specific situation for Chagas’ patients, with their multiple GPCR-AABs presence [34] (see also Chapter 3), we may assume that in these patients unspecific IgG adsorption for removal of all GPCR-AABs present may be superior to only β_1 -AABs removal. However, to the best of our knowledge, IA treatment has not been included in the therapy of Chagas’ disease.

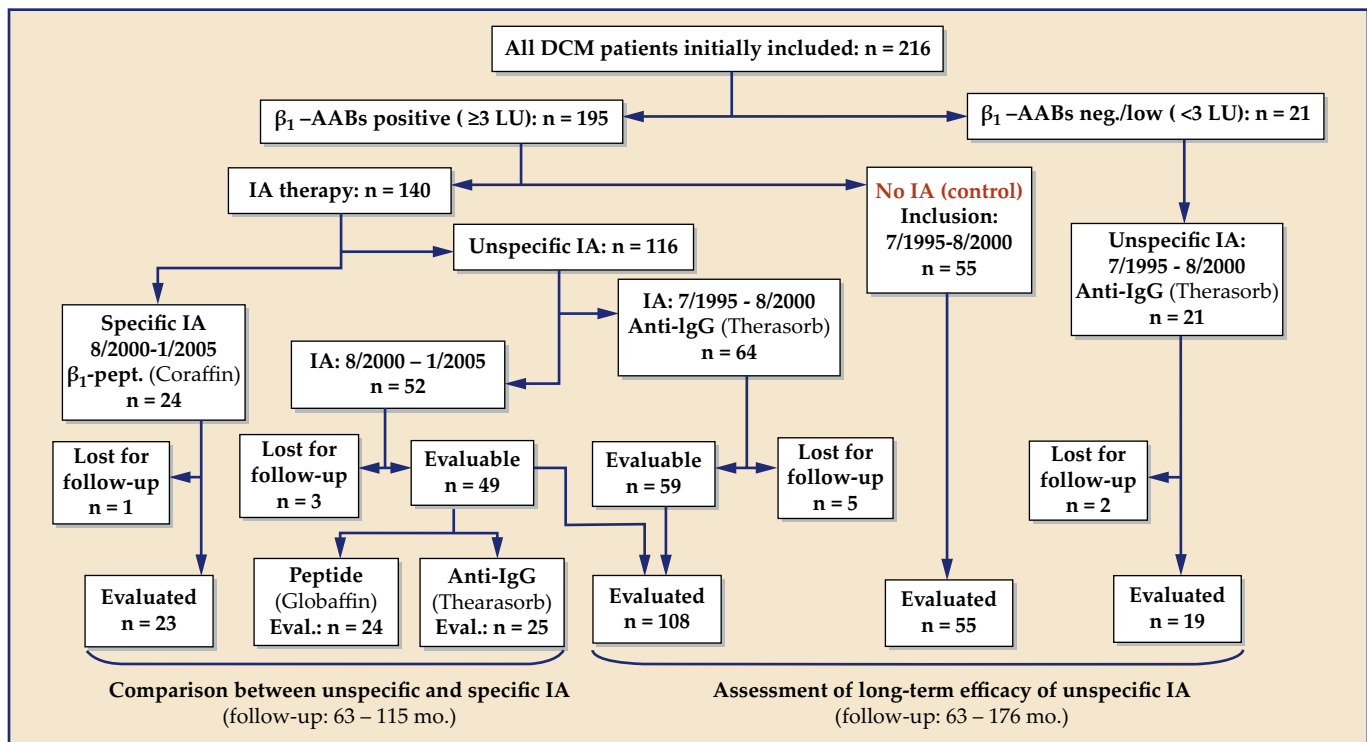


FIGURE 27.6 Study design for evaluation of immunoadsorption technologies with respect to patients' long-term outcome. Reproduced from Dandel et al. [111].

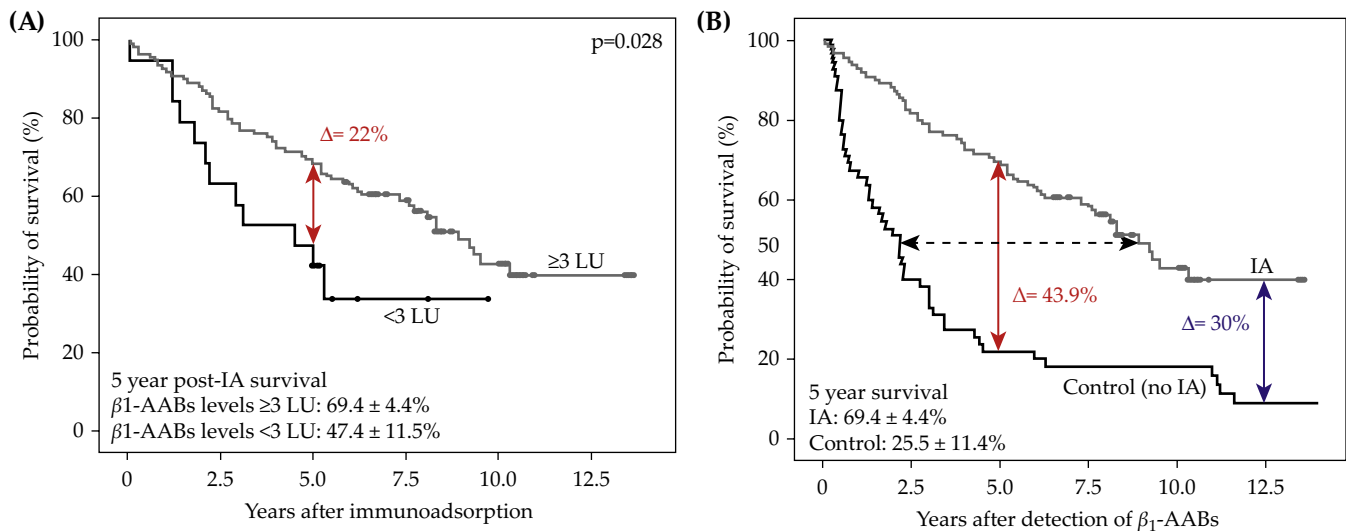


FIGURE 27.7 Kaplan-Meier estimates of heart transplantation/ventricular assist device (HTx/VAD) free survival for dilated cardiomyopathy (DCM) patients after immunoadsorption. (A) Comparison between β_1 -AABs-positive patients (≥ 3 laboratory units (LU)) and β_1 -AABs negative patients (< 3 laboratory units (LU)). (B) Comparison between the DCM patients positive for β_1 -AABs treated with IA and nontreated with IA (control). Adapted from Dandel et al. [111].

To complement existing IA technologies, columns could now be designed carrying aptamers for the removal of pathogenic serum components. Aptamers are synthetic, highly structured, preferentially single-stranded oligonucleotides, which, if well selected, bind to their corresponding target molecules with high specificity. Once selected and determined their exact nucleotide

composition, aptamers can be chemically and this way high-economically produced and in the same way easily modified related to certain specific tasks. With respect to use of aptamers in apheresis technology, their stability is another advantage that can be exploited at the production, processing, transportation, and storage stages and also enables their easy sterilization.

Recently, we supplied basic results for the introduction of aptamers in the apheresis technique applicable for GPCR-AABs removal. We selected a highly specific aptamer that binds β 1-AABs, such as those directed to the second loop of the human β 1-receptor [56,69,114]. Consequently, this aptamer may be used for β 1-AABs removal in DCM, Chagas' cardiomyopathy, and Peripartum cardiomyopathy. We also recently designed an apheresis column carrying this aptamer and tested this column in vitro for the clearance of human serum that was positive for β 1-AABs. Control sera were spiked with commercially available goat autoantibodies directed at the second loop of the human β 1-receptor and, last but not least, PBS-diluted IgG from DCM patients previously indicated as positive for β 1-AABs. After passing the β 1-AABs-containing preparations over the column, the flow-through was cleared from β 1-AABs, which was not the case for an aptamer-free control column. After column elution, the β 1-AABs were quantitatively found in the eluate. In the second part of this study, using a comparable column but with higher capacity, rats positive for β 1-AABs were cleared of the autoantibodies that did not return in the rats' blood until the end of the study [56], a situation that was seen in most human IA studies described above. However, for patients with multiple GPCR-AABs presence, a column would be clearly superior, containing an aptamer for that bind the whole class or even a significant part of GPCR-AABs, such as that which we recently identified and successfully tested using in vitro and animal experiments [70]. The apheresis procedures are initially cost intensive, but the patient benefit, which is mainly visible in significantly better survival rates, leads to reasonable costs per life-year gained [115]. After nearly 20 years of studying IA for autoantibody removal in DCM patients, IA has, in our view, impressively demonstrated long-term patient benefits, which enables the more extensive use than before in the treatment of DCM patients who are positive for GPCR-AABs. Beyond that, the possible benefit of IA should be more extensively evaluated in the near future in other diseases burdened with GPCR-AABs where a pathogenic impact for the diseases is evidenced. Prominent indications for IA could be hypertension, diabetes mellitus, and even Alzheimer's disease. Nevertheless, cost factors and logistical problems associated with IA may be important, especially in developing countries, where they could prevent the wider use of IA technology as a treatment option. This could explain why IA for GPCR-AABs removal has not entered the therapy of the millions of patients with Chagas' heart disease, which is one of the main causes of death in Latin America [116].

2.3 In Vivo Treatment for the Neutralization of GPCR-AABs

To overcome the problems limiting the extensive use of IA, it was logical to look for therapeutic approaches to directly fight against the generation of pathogenic AABs and/or their activity in patients' blood. Among these approaches, intravenous IgG treatment (IVIG), B-cell depletion therapies, and strategies for in vivo binding and neutralization of AABs may be promising.

2.3.1 Intravenous IgG Treatment (IVIG)

For IVIG, pooled plasma is prepared from several thousand healthy donors, which contains numerous antibodies directed at a wide range of antigens. The variable regions on the Ig Fab fragments in this preparation are diverse and can bind to a wide range of antigens such as nonself-antigens (foreign antigens), self-antigens, and antiidiotypic antibodies [117]. Among the multiple activities of the plasma preparation for immunomodulation, there is an influence of the complement activation, suppression of idiotypic antibodies, saturation of Fc receptors on macrophages, and suppression of various inflammatory mediators [118]. IgG interacts via its Fc region with Fc receptors on cells such as phagocytes and B cells and can bind with plasma proteins.

The clinical use of intravenous immunoglobulin began in 1952 as plasma protein replacement therapy for immune-deficient patients. However, IVIG has expanded beyond its traditional indication. In 1981 for diseases with an autoimmune background it was shown initially that IVIG was beneficial in patients with autoimmune idiopathic thrombocytopenic purpura (ITP) [119]. Due to its multiple antiinflammatory and immunomodulatory properties, IVIG is used successfully in a wide range of other autoimmune and inflammatory conditions, including Kawasaki disease, Guillain-Barré syndrome and other autoimmune neuropathies, myasthenia gravis, dermatomyositis, and several rare diseases.

Consequently, it was thought that IVIG may also be helpful in diseases with an immunological background based on the generation of cardiopathogenic AABs. As a result of the application of IVIG in AABs-positive patients, AABs half-life shortening would be expected. However, as summarized in [120], the pros and cons were seen after IVIG in patients with heart failure, in general and specifically in DCM patients, which presents a very confusing situation, particularly given the role that cardiopathogenic AABs play. Among 40 patients with chronic heart failure (NYHA II/III), LVEF <40% due to ischemic or idiopathic dilated cardiomyopathy, a significant benefit was seen in 19 IVIG treated patients (treatment time 5 months, follow-up 6 months)

(improvement of LVEF >5%, development of an anti-inflammatory cytokine pattern, NT-proANP decrease) [121]. However, differences between patients suffering from ischemic or idiopathic DCM were not seen, as was expected due to the more pronounced presence of AABs in the DCM patients, which are a more specific target of the treatment. In contrast, for 62 patients with DCM and LVEF <40%, no differences in benefits were seen for IVIG or placebo treatment [122], although cardiopathogenic AABs have been reported as sensitive to IVIG treatment [123]. Despite benefits of IVIG for DCM patients, an increase of β 1-AABs after IVIG [124] was seen, which could be in agreement with the above-mentioned proinflammatory side effects of IgG replacement after IA. Looking to other cardiomyopathies such as Peripartum cardiomyopathy, 6 women vs 11 conventionally treated patients demonstrated benefits that were visible by improved LVDD [125]. However, there are no indications for promoting this treatment strategy. With respect to myocarditis, the presently available data concerning IVIG treatment were reviewed in [126] as being confusing in view of patient benefits, as was exemplarily documented in [127–129]. Consequently there are no well-reasoned recommendations for the use of this treatment strategy [130,131]. Pericardial diseases were seen as another indication for IVIG. Reviewing the currently available data (18 citations retrieved, 17 reports [4 case series and 13 single case reports, with an overall population of 30 patients]), it was stated that IVIG in refractory pericarditis is a rapidly acting, well-tolerated, and efficacious steroid-sparing agents in refractory pericarditis [132]. Recently, for IVIG treatment in recurrent pericarditis, a level of evidence (C) and class IIb of recommendation was reported [133].

2.3.2 B-Cell Depletion

Rituximab, a chimeric monoclonal antibody against the protein CD20 presented by B cells, is the first and most prominent member of a group of agents that can be used for the selective depletion of CD20-positive B cells. The effect of Rituximab results from its regulatory effect on the cell cycle, including apoptosis stimulation, complement-dependent B-cell lysis, and antibody-dependent cell-mediated cytotoxicity [134].

As extensively discussed [135], Rituximab treatment was studied in a wide range of diseases with an autoimmune background and it was thought that patients who were positive for GPCR-AABs could also benefit from B-cell depletion by Rituximab. However, as already known, not all autoimmune diseases seem to benefit in the same manner from Rituximab [135]. Among the diseases associated with GPCR-AABs, Graves' disease was the only one where patients were frequently treated with Rituximab, but the study results were inconsistent regarding patient benefit [136,137]. Recently, for the

treatment of CRPS, Rituximab was given additionally after IA [65].

2.3.3 In Vivo Neutralization of GPCR-AABs

2.3.3.1 Peptide-Based Neutralization of GPCR-AABs

As already mentioned, peptides mimicking epitopes of the β 1-adrenergic receptor have been designed [97]. Such peptides may have the potential for in vivo competition with the cellular receptors for the related GPCR-AABs. In this way, treatment with peptides should reduce or abolish the pathogenic potency of GPCR-AABs, leading to patient benefit, just as IA does if the peptides are used as a binder for GPCR-AABs removal. For possible in vivo application, it was suggested to use the linear form, or for stability reasons the cyclized form of the peptides. In one of the key experiments for concept proof, rats were immunized with a glutathione S-transferase fusion protein containing the second extracellular domain of the human β 1-receptor. This resulted in the strong generation of β 1-AABs, which were, due to the homology of man and rat, directed against the second extracellular loop of the rats' β 1-receptor. The autoantibody serum presence was accompanied by the establishment of typical signs of heart failure in the rats. Treatment of these rats with COR-1, a cyclic peptide homolog for the second extracellular loop of the β 1-receptor, clearly diminished the rats' heart failure signs [138]. The concept was transferred to humans for clinical phase 1 trials [139,140] to start the development of a novel therapy for patients with DCM based on in vivo neutralization of β 1-AABs. In the first phase 1 trials, the drug's safety was demonstrated together with its in vivo efficacy for β 1-AABs neutralization in humans. Unfortunately, further trials presented adverse events. Among these were signs of the drug's immunogenicity, which discontinued further steps needed to bring this therapy concept to DCM patients [141,142]. Nevertheless, new experimental data for the heart failure protective potency of COR-1 found in animal experiments was recently published [143]. Besides the β 1-AABs neutralizing effect of COR-1, the depletion of memory B cells involved in the production of such antibodies was demonstrated. However, neutralization with COR-1 was directed exclusively to β 1-AABs. Consequently due to the finding of additional GPCR-AABs in DCM patients (eg, M2-AABs), or even more so if the DCM patients present with comorbidity (eg, hypertension, Diabetes mellitus), the benefit of COR-1 treatment in DCM patients could be limited.

2.3.3.2 Aptamer-Based Neutralization of GPCR-AABs

Based on the two aptamers already introduced [69,70], one specific for β 1-AABs directed at the second extracellular receptor loop and the other named

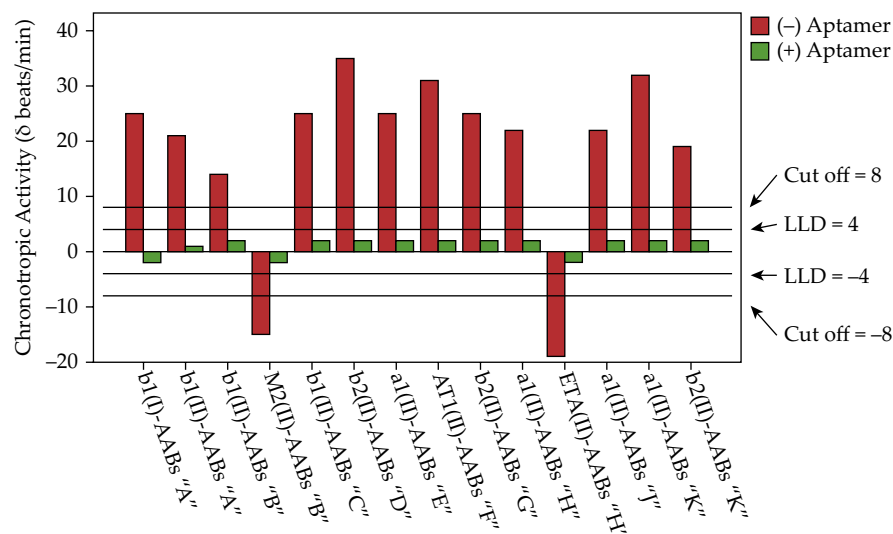


FIGURE 27.8 In vitro neutralization by the aptamer BC007 of autoantibodies directed against G-protein coupled receptors isolated from the serum of patients with different diseases. To demonstrate the autoantibodies' activity, patient IgG was prepared and, using the bioassay of cultured neonatal rat cardiomyocytes, the autoantibodies' chronotropic activity was analyzed in the absence and presence of the aptamer BC007. For experimental design see [114]. "A"—Dilated cardiomyopathy; "B"—Chagas' cardiomyopathy; "C"—Peripartum cardiomyopathy; "D"—Glaucoma; "E"—Hypertension; "F"—Malign hypertension; "G"—Chagas' megacolon; "H"—Pulmonary hypertension; "J"—Diabetes mellitus; "K"—Alzheimer's disease. Autoantibodies directed against the first extracellular loop of the: β 1-adrenergic receptor—b1(I)-AABs, β 2-adrenergic receptor—b2(I)-AABs; directed against the second extracellular loop of the: β 1-adrenergic receptor—b1(II)-AABs, β 2-adrenergic receptor—b2(II)-AABs, muscarinic2 receptor—M2(II)-AABs, α 1-adrenergic receptor— α 1(II)-AABs, angiotensin II receptor type I—AT1(II)-AABs, endothelin A receptor—ETA(II)—AABs. Reproduced from Wallukat et al. [148].

BC007, neutralizing all of the GPCR-AABs presently accepted as drivers of cardiovascular diseases, we considered another strategy for the in vivo neutralization of GPCR-AABs with the potency for transfer to humans [144]. While peptides used for in vivo neutralization carry all the advantages and disadvantages associated with their nature, aptamers, as oligonucleotides, show all of the characteristics of either short RNA or DNA sequences [145,146]. Most important is their reported low toxicity and lack of immunogenicity [147].

Fig. 27.8 shows an in vitro experiment clearly demonstrating the neutralizing potency of BC007 for several GPCR-AABs prepared from patients suffering from the indicated diseases and who carry these GPCR-AABs [148].

Bringing our concept forward step-by-step, and using a study design that was recently published in [149], spontaneously hypertensive rats (SHR) positive for β 1-AABs were treated with BC007 five times at weekly intervals (bolus application of 2mg/kg body weight followed by an infusion of the same amount over 20min). The SHR responded to BC007 treatment with a strong reduction in the cardiopathogenic β 1-AABs. The AABs did not substantially return within the study period. No signs for aptamer toxicity were observed by visual examination of the heart, liver, and kidney, or by the measurement of plasma CK, ALT, and creatinine (Fig. 27.9).

Fig. 27.10 demonstrates that in the serum of DCM patients, the same GPCR-AABs reduction can be achieved when patients were either immunoadsorbed or when the patient serum was ex vivo treated with BC007. Consequently in our view, BC007 treatment in GPCR-AAB-positive patients could potentially have a comparable benefit to that seen after IA.

Large chart: Human study for the removal of β 1-AABs; data shown are for β 1-AABs vs LVEF and oxidative stress (measured as thiobarbituric acid reactive substances (TBARS)) before and after immunoadsorption (for study design see [55,102]). Small chart: In vitro study demonstrating the concentration-dependent neutralization by BC007 of β 1-AABs in the serum sampled before immunoadsorption. For experimental details see [114].

Due to the demonstrated multifunctionality of BC007 for the neutralization of several GPCR-AABs (Fig. 27.8), evidence for the neutralizing potency of BC007 in animals (Fig. 27.9) and transferring this concept closer to humans (Fig. 27.10) suggests that patients may benefit from BC007 treatment in diseases that present preferentially with β 1-AABs, such as DCM and Peripartum cardiomyopathy, or present β 1-AABs alongside further pathogenic GPCR-AABs (sometimes DCM, Chagas' heart disease, DCM combined with hypertension and Diabetes mellitus), or were affected by only one or several other pathogenic GPCR-AABs (hypertension, Diabetes mellitus, and possibly also Alzheimer's disease) (see also Chapter 3).

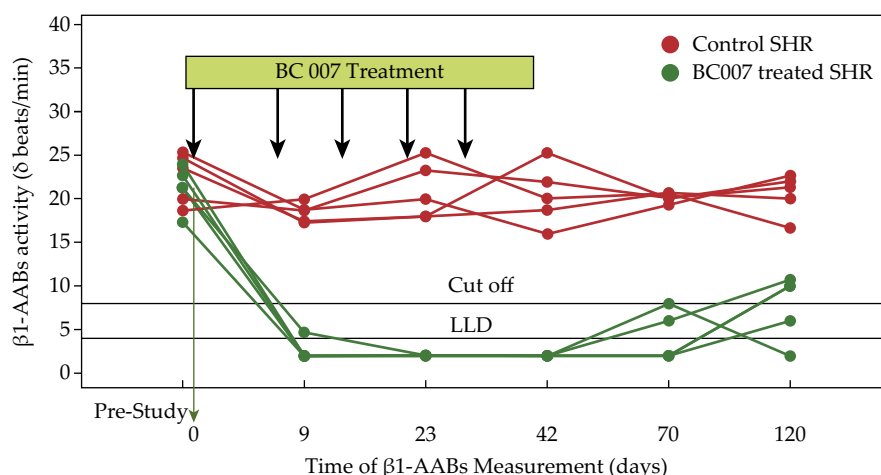


FIGURE 27.9 Elimination of β_1 -AR AABs from the blood of spontaneously hypertensive rats (SHR). BC007 was administered five times at weekly intervals (4 mg/kg BW IV) and was followed up for β_1 -AR AABs activity for 3 months after the last administration. Control rats received 0.9% NaCl solution. For experimental design see [148]. Rats treated with BC007 showed a continuous decrease of the AABs activity over time. (LLD=lower limit of determination; Cutoff=threshold for pathological β_1 -AR AABs activity). Reproduced from Wallukat et al. [148].

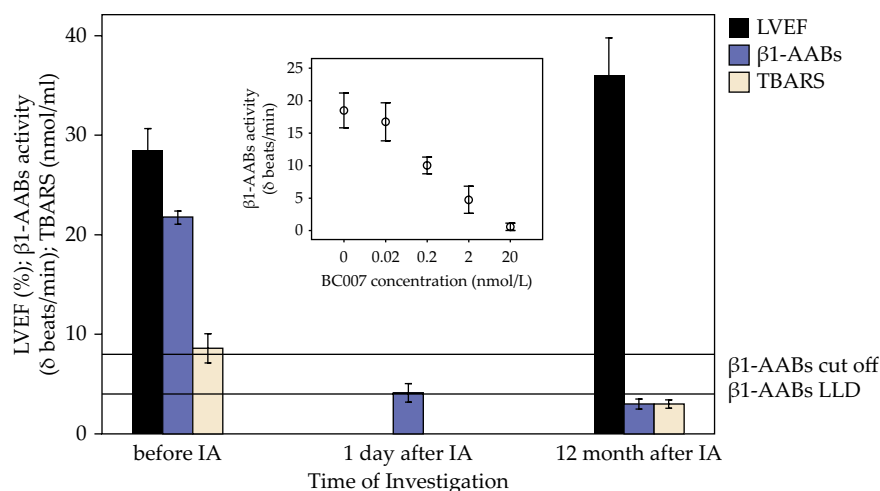


FIGURE 27.10 Lowering of serum autoantibodies directed against the β_1 -adrenergic receptor (β_1 -AABs) in patients with DCM ($n=7$). Reproduced from Wallukat et al. [148].

3. CONCLUSION

Therapeutic plasma exchange, extracorporeal adsorption, and concepts for in vivo neutralization were studied with respect to their application in patients suffering from diseases with an autoimmune background based on the presence of AABs and specifically of GPCR-AABs. At the time of writing and among these treatment options, only IA using peptides or proteins for binding of IgG and thus also of AABs such as GPCR-AABs, especially in patients with DCM, resulted in a clearly evidenced and significant long-term benefit. The future must show whether further binders for ECA, such as aptamers for GPCR-AABs

binding, will supplement this technique. Treatment strategies for the in vivo neutralization of GPCR-AABs would be superior with respect to patient burden, cost, and logistics. Such strategies using peptides or aptamers for the neutralization of the GPCR-AABs are being considered. The peptide concept is directed exclusively to the β_1 -AABs neutralization, limiting its applicability to only the disease affected by β_1 -AABs. Unfortunately, the evaluation of this concept was interrupted. In the aptamer concept, several GPCR-AABs can be neutralized in parallel. Consequently, not only patients whose diseases are driven exclusively by one, but also those patients with several pathogenic GPCR-AABs, would benefit.

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Cardiac Immunomodulation

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From Celsus' and Galenos' rather general description of inflammatory symptoms "rubor, dolor, tumor, calor and function laesa" and also still applicable therapeutic measures like "ubi pus ibi evacua" we have come to a more profound understanding of the underlying mechanisms of inflammatory processes opening new therapeutic options. At the same time more specific disease patterns have been defined. Cytokines play a central role in this new understanding of disease, and in recent years numerous immunomodulating therapies have evolved.

While the applications of some of the therapeutic options is limited by their cardiac side effects, others have become indispensable for the treatment of cardiac manifestations of autoimmune diseases, which is the case for coronary aneurysms in the medium-size vasculitis Kawasaki disease. Various agents are currently still under investigation as treatment options for cardiovascular diseases like heart failure and atherosclerosis, since existing results range from beneficial to harmful effects.

Most new immunomodulating strategies were initially developed and approved for the treatment of autoimmune disorders known to be associated with chronic inflammation like diseases from the circle of rheumatic forms or inflammatory bowel disease [1]. These diseases are characterized by an often overwhelming and uncontrolled immune response promoting disease progression and are related to increased cardiovascular morbidity and mortality [2,3]. According to the QUEST-RA study nearly half of all deaths in patients with rheumatoid arthritis (RA) are associated with cardiovascular diseases [4]. This underscores the tight relation between autoimmune diseases and cardiovascular diseases and complicates the evaluation of potential cardiovascular side effects of immunomodulating therapies [5].

1. INTERLEUKIN 1 β -SIGNALING PATHWAY

The proinflammatory cytokine interleukin-1 (IL-1) has been linked to a number of rheumatologic and inflammatory conditions. Of the 11 members of the IL-1 family, the ligands IL-1 and IL-1 β are of special clinical interest. As part of the IL-1 signaling system IL-1 β serves as an agonist, inducing proinflammatory effects by binding to the IL-1 type 1 receptor. This binding can be competitively blocked by the antagonist IL-1Ra [3], leading to an inhibition of IL-1 β action. The decoy receptor IL-1 receptor type II (IL-1RII) was shown to mediate neutralization and endocytosis of IL-1 β , while various soluble receptor forms are able to inhibit circulating IL-1 β [6]. The effect of the interleukin is also modulated by the regulation of its activation. The inactive pro-IL-1 β , which is produced mainly by mononuclear phagocytes in response to injury and infection, has to be activated via proteolytic cleavage involving caspase-1. The activation process is mediated by the NLRP3 inflammasome, also called cryopyrin (Fig. 28.1). Cryopyrin represents a complex of intracellular proteins and may be activated by exogenous signals [7]. While cryopyrin-associated periodic syndromes (CAPS) are associated with specific amino acid mutations altering the NLRP3 inflammasome, moderate imbalances in the IL-1/IL-1Ra system are suspected to be responsible for diseases that are associated with chronic inflammations like type 2 diabetes, RA, systemic onset juvenile idiopathic arthritis (SoJIA), gout arthritis, chronic obstructive pulmonary disease (COPD), and age-related macular disease [8–11]. More recently it was found that the development of atherosclerosis is also associated with alterations in the NLRP3 inflammasome, leading to increased IL-1 β levels. It was also shown that hyperlipidemia leads to IL-1 β caused inflammation via cholesterol crystal and minimally modified LDL mediated

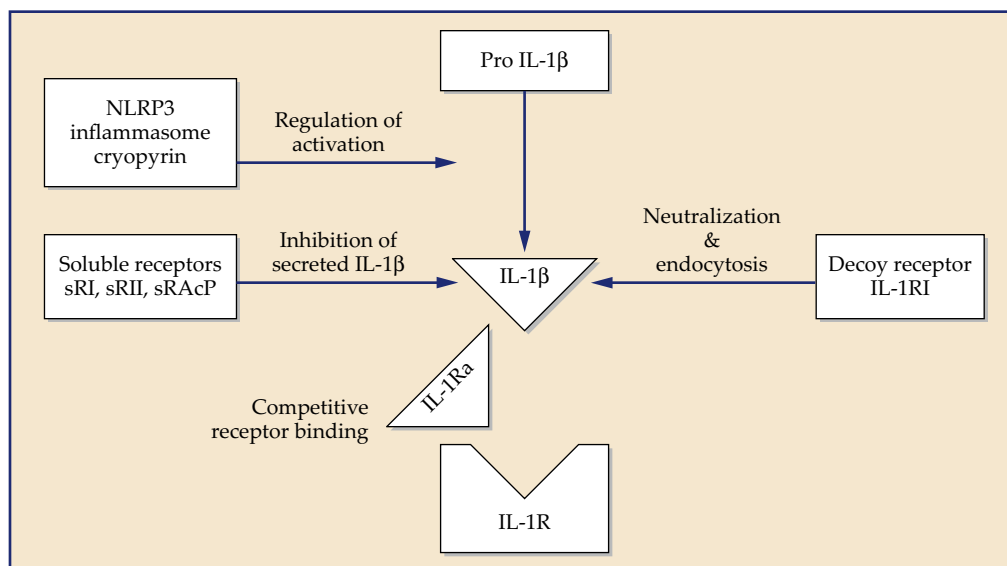


FIGURE 28.1 Regulation of IL-1 β signaling. Adapted from the personal collection of the authors.

activation of the NLRP3 inflammasome [12,13]. In addition, the NLRP3 locus is partly responsible for the regulation of plasma CRP levels [14].

1.1 Interleukin-1 β Modulating Substances

1.1.1 Canakinumab

Canakinumab (Ilaris) is a specific recombinant human antihuman IL-1 β monoclonal antibody developed by Novartis, Switzerland for the treatment of immune disorders. Chemically the drug substance is defined as an immunoglobulin G1, anti-(human interleukin-IL-1 β) human monoclonal, (1Glu>Glp)- γ 1 heavy chain (221-214)-disulfide with kappa light chain, dimmer (227-227":230-230")-bisdisulfide. The molecular formula for canakinumab is based on the amino acid composition without posttranslational glycosylation, but including N-terminal pyroglutamate formation and lysine residues at the C-terminals of the heavy chains [15]. The antigenic epitope includes Glu 64, which is essential for the recognition of human IL-1 β by the antibody. Although the epitope appears to be outside the IL-1 β /IL-1R interface the canakinumab/IL-1 β complex is unable to attach to the cell surface receptor and thus IL-1 β -dependent signaling is interrupted. While canakinumab binds to the human IL-1 β with high affinity it does not bind to human IL-1 α or the endogenous antagonist IL-1 Ra [15].

Canakinumab was granted orphan drug status by both the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). In 2009 the substance was approved by both agencies for the treatment of two CAPS associated with oversecretion of IL-1, the Muckle-Wells syndrome, and the familial cold autoinflammatory syndrome (FACS), and 4 years later the approval for systemic juvenile idiopathic

arthritis followed [16] in which canakinumab is recommended for patients with continued disease activity after treatment with glucocorticoid monotherapy, methotrexate, or leflunomide (level of evidence A). Canakinumab can also be given to patients following treatment with anakinra (level of evidence B) or tocilizumab (level of evidence C). Further recommendations depend on the clinical stage of the disease [17]. Clinical trials have shown encouraging results regarding the application of canakinumab in the treatment of gout arthritis [18–22], RA [23,24], and type 2 diabetes mellitus [25–28].

In addition to these findings relating IL-1 β to the development of atherosclerosis, adventitial capillary endothelial cells from atherosclerotic coronary arteries show an increase in IL-1 β protein levels compared to the corresponding cells of coronary arteries from patients with nonischemic cardiomyopathy that is directly proportional to the severity of the atheroma [29]. Therefore, it seems possible that IL-1 β blockers such as canakinumab may also be effective in the treatment of cardiovascular diseases. To test this hypothesis the canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS, NCT01327846) was designed. In this study, stable patients are included at least 1 month after myocardial infarction who are at high risk for recurrent cardiovascular events, defined by raised levels of the high-sensitivity C-reactive protein (hsCRP) (≥ 2 mg/L). They receive quarterly subcutaneous canakinumab injections. The primary end point of the main study is recurrent cardiovascular events including nonfatal myocardial infarction, nonfatal stroke, and cardiovascular death. In two subgroups the treatment with canakinumab on carotid plaque burden measured by integrated vascular MRI and on the incidence of new onset diabetes will

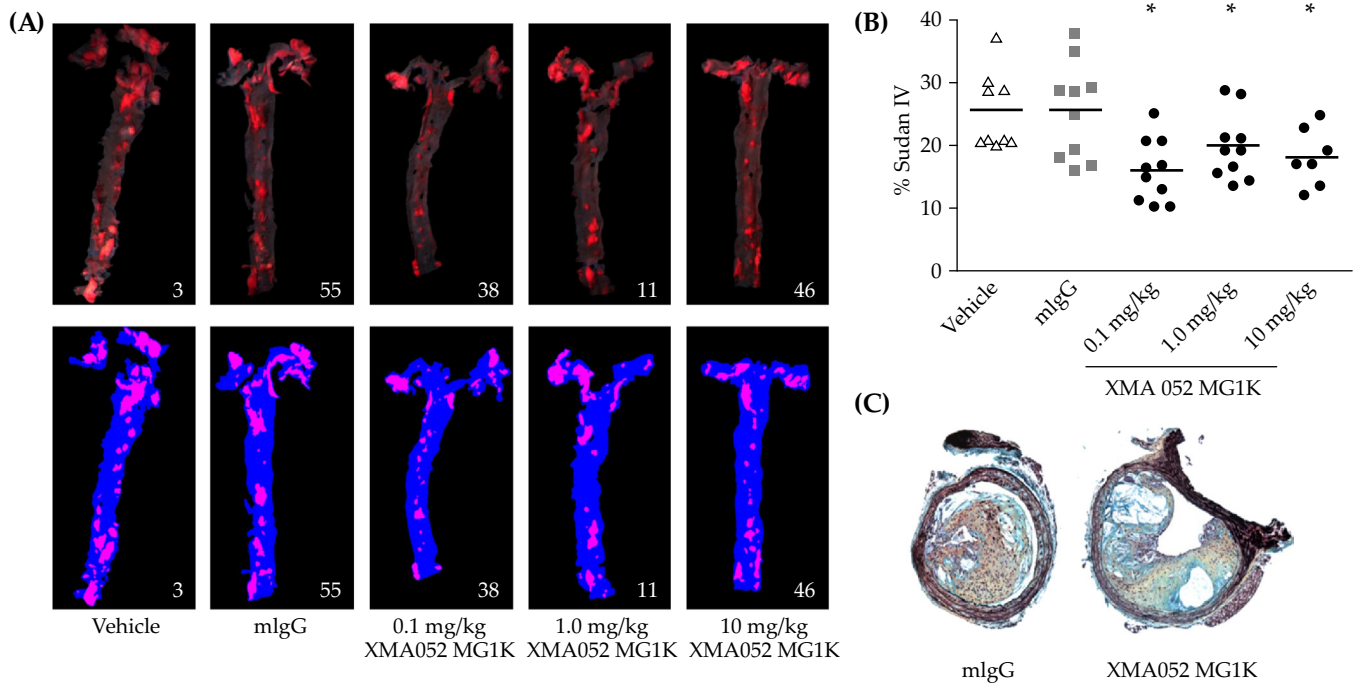


FIGURE 28.2 Effect of XMA052 MG1K on atherosclerotic lesions in the aortas and brachiocephalic arteries of ApoE-deficient mice. ApoE-deficient mice were fed an atherogenic diet for 16 weeks and dosed as indicated. Aortic lesion area was measured by en face analysis and expressed as percent Sudan IV positive pixels. En face images from representative individuals with lesion size approximating the mean are shown. (A) Lesion area was reduced by 37%, 22%, and 29% at the 0.1, 1.0, and 10 mg/kg doses, respectively. (B) Results are represented as mean \pm S.E.M ($n=10$). * $p < 0.05$ vs IgG and vehicle. (C) Representative images of brachiocephalic arteries for IgG and XMA052 (1.0 mg/kg) [34]. Adapted from the personal collection of the authors.

be evaluated, respectively [30,31]. The study started in April 2011 and is planned to be completed by April 2017.

1.1.2 Gevokizumab

Gevokizumab (XOMA-052) was developed by XOMA Ltd., USA and represents a humanized IgG-2kappa antibody. XOMA-052 binds to IL-1 β with high affinity leading to reduced binding affinity of the cytokine to its signaling receptor [32]. Since gevokizumab does not influence the affinity of IL-1 β for its decoy and soluble inhibitory receptors the endogenous regulatory mechanisms involving the clearance and inhibitory receptors also contribute to the effect of the antibody [6]. In August 2012 the FDA granted orphan drug status to gevokizumab for the treatment of noninfectious intermediate uveitis, posterior uveitis, panuveitis, and chronic noninfectious anterior uveitis [33]. A single intravenous injection of the substance was also shown to lead to complete resolution of intraocular inflammation in Behçet's uveitis [34,35].

In addition, the murine chimeric version of XOMA-052 (XMA052) has been shown to hinder the development and progression of atherosclerotic lesions in ApoE-deficient mice [34]. Mice were fed 0.1, 1.0, or 10 mg/kg XMA052 for 16 weeks. All three different doses led to a significant decrease in lesion area when compared to mouse IgG or vehicle as seen in

Fig. 28.2. A recently completed randomized, double-blind, parallel group, placebo-controlled, multicenter study examined the effects of gevokizumab on arterial wall inflammation in patients with a history of acute coronary syndrome within the previous 12 months (EudraCT Number: 2012-002677-53). The main objective of this trial is to evaluate the effect of 30 mg of gevokizumab given subcutaneously on the reduction of arterial wall inflammation compared to placebo. Secondary measures include the collection of information regarding the influence of the antibody on cardiac and vascular biological blood biomarkers, the safety profile of gevokizumab, and gevokizumab pharmacokinetics. The value of gevokizumab in the treatment of diabetes mellitus is also under clinical investigation. In a placebo-controlled trial (NCT00541983) gevokizumab treatment led to an improvement in glycaemia, possibly by improving insulin production and action, and reduced CRP levels in patients with type 2 diabetes [36].

1.1.3 Anakinra

Anakinra (Kineret) was developed by Amgen Inc., USA and is currently under the license of the Swedish orphan Biovitrum AB. Anakinra represents a recombinantly expressed IL-1R antagonist that competes with IL-1 α and IL-1 β for binding to the cell surface receptor.

Therefore unlike canakinumab and gevokizumab it does not specifically block IL-1 β but also the effects of IL-1 α . Since anakinra does not elicit a downstream response when binding to the receptor it leads to suppression of interleukin-induced inflammatory activity. Even though there has been no clinical study that directly compares the efficacy of anakinra to TNF- α inhibitors, a meta-analysis suggested that IL-1-antibodies are inferior to TNF- α inhibitors judging by their influence on the American College of Rheumatology scores of symptoms (ACR 20 and 50) [37], possibly due to its shorter half-life or more relevant involvement of TNF- α in the disease pathology [38].

In 1993, anakinra was first used for the therapy of RA and was approved by the FDA in 2001 for the reduction in signs and symptoms and slowing of the progression of structural damage in moderate to severe active RA in patients 18 years or older who have failed one or more disease modifying antirheumatic drugs (DMARDs). The only other approved indication pertains to the treatment of neonatal-onset multisystem inflammatory disease (NOMID). Off-label uses include use for adult-onset still's disease, gout, calcium pyrophosphate deposition (pseudogout), Behcet's disease, ankylosing spondylitis (AS), and uveitis. The number of potential clinical applications covers a broad spectrum of inflammatory conditions and diseases including type 2 diabetes, Sweet's syndrome, as well as cardiovascular diseases like non-ST-segment elevation myocardial infarction (NSTEMI), ST-segment elevation myocardial infarction (STEMI), and heart failure [39]. Thus numerous studies have shown significant reduction in inflammatory biomarkers after treatment with anakinra [40–44]. Even though this effect was detectable in patients with STEMI [40,41] and NSTEMI [42] as well as chronic systolic and diastolic heart failure [43,44] it is still unknown if blockage of IL-1 leads to improved outcome after acute myocardial infarction [45]. While the pilot MRC-ILA Heart Study (EudraCT number: 2006–001767–31) showed an increase in major adverse cardiac events (including death, stroke, and new myocardial infarction) in NSTEMI patients that had received anakinra compared to the placebo-control group at the 1-year follow up [42,45], the patient-level pooled analysis of the VCU-ART (NTC00789724) and VCU-ART2 (NTC01175018) pilot studies showed no significant difference in cardiac events when patients were treated with anakinra for 2 weeks [46].

More encouraging results were seen in studies investigating the use of anakinra in the therapy of chronic heart failure. Patients with a left ventricular (LV) ejection fraction below 40% and elevated hsCRP levels (2 mg/L) who received daily injections of anakinra (100 mg/day) showed improvement in cardiopulmonary exercise performance as indicated by

increase in peak oxygen consumption, ventilatory efficiency, and exercise time [44]. In a different setting, RA patients without known cardiovascular diseases who showed an inadequate response to DMARDs were first included in an acute randomized cross-over placebo controlled trial and received a single dose of anakinra (150 mg). After completion of the acute phase they took part in a chronic nonrandomized trial where one group received anakinra for 30 days (150 mg s.c. daily), while the other group was given an increased amount of prednisolone. The acute intervention was associated with improvement in coronary flow reserve, in LV function measured by systolic and diastolic velocity of the mitral annulus and the E to Em ratio, and improved endothelial vascular function as assessed by flow-mediated dilatation as well as aortic distensibility. At the same time a reduction in nitrooxidative stress as well as endothelin-1 (ET-1) and IL-6 levels was seen. In the chronic setting the group that had received anakinra showed greater improvement in LV and vascular function and disease-related biomarkers [47]. In the D-Hart pilot study (NCT01542502) patients with a preserved LV ejection fraction suffering from LV diastolic dysfunction (heart failure with preserved ejection fraction [HFpEF]) and plasma C-reactive protein levels of >2 mg/L received anakinra in a double-blind, randomized, placebo-controlled, cross-over trial. They received either first anakinra (100 mg/day) for 14 days and then placebo for 14 days or the other way round. In this study anakinra led to statistically significant improvement in peak oxygen uptake and significant reduction in plasma CRP levels [43]. Currently the D-HART2 trial (NTC02173548) that aims to determine the effects of IL-1 on the cardiovascular function of patients with HFpEF and evidence of systemic inflammation is underway. In this study, one group of patients will receive 100 mg anakinra s.c. once daily for 12 weeks, while the other group will be treated with placebo. The primary outcome measure will be aerobic exercise capacity as well as ventilator efficacy and the absolute changes of these parameters from baseline until week 12. The preliminary results of the study have yet to be published.

2. ROLE OF CD20 IN AUTOIMMUNE DISEASES

The different subsets of leukocyte-derived cells are characterized by specific clusters of differentiation markers (CD markers) on their surface, which serve as receptors or ligands in the pathway of cell signaling and cell adhesion. CD20 represents a B-cell specific antigen that is expressed during B-cell development starting at the pre-B-cell level. CD20 cannot be found on stem cells

or on early pre-B cells. It is still present through further B-cell differentiation and gets lost during terminal differentiation to plasma cells [48]. B cells contribute to the development of autoimmune diseases via secretion of autoantibodies, presentation of autoantigens, secretion of inflammatory cytokines, modulation of antigen processing, and presentation and generation of ectopic germinal centers [49] and are therefore a potential target for the treatment of autoimmune diseases.

2.1 Inhibition of CD20

2.1.1 Rituximab

The first B-cell targeting therapeutic antibody in clinical use was rituximab (MabThera), a mouse/human chimeric IgG1 mAb developed by Prof. Lee Nadler at the Dana-Farber Cancer Institute in Boston, MA, USA [50]. Rituximab was admitted to the European market in 1998 and is currently under the license of Roche (EU) and Biogen/Genentech (USA). It was originally developed and used for the treatment of chemotherapy-resistant B-cell malignancies. Since patients with autoimmune diseases like RA have an increased risk of developing B-cell non-Hodgkin's lymphomas [51] some patients who received rituximab also suffered from autoimmune diseases. Thus it became obvious that the anti-CD20 antibody also has positive effects on the progression of diseases like RA, which led to further clinical tests and eventually the use of MabThera for the therapy of autoimmune diseases [52]. Rituximab has a molecular weight of 145 kDa and is comprised of two heavy chains of 451 amino acids and two light chains of 213 amino acids. It targets the B-cell restricted surface antigen CD20, leading to rapid and profound B-cell depletion [53]. The elimination of B cells by rituximab is realized via different mechanisms including complement-dependent cellular cytotoxicity, antibody-dependent cytotoxicity, inhibition of cell proliferation, and direct induction of the apoptotic pathway as shown by *in vitro* studies [54,55]. Complement-dependent toxicity, activation of kinases, phosphokinases, and caspases as well as a downregulation of IL-10 were shown *in vivo* [56,57]. In 2006 rituximab was licensed for the therapy of moderate-to-severe RA not responding adequately to DMARDs including antitumor-necrosis factor biologics. According to the guidelines of the British Society for Rheumatology and the British Health Professionals in Rheumatology rituximab should be given to patients with active RA who have not responded to one or more biologics, or who are intolerant, or have contraindications, to anti-TNF therapy. It needs to be considered that patients who are rheumatic factor or anticitrullinated protein antibody positive are more likely to respond to rituximab than patients who are negative for both antibodies (level A evidence, strength of recommendation

I). It was shown to be as effective as adding a second TNF antibody to the therapy regime [58]. Rituximab can also be given before anti-TNF treatment, especially in patients who have an absolute or relative contraindication to anti-TNF-therapy (level A evidence, grade of recommendation I). If methotrexate is contraindicated, rituximab should be used either alone or in combination with leflunomide (level C evidence, grade of recommendation II) [59]. The effect of rituximab varies concerning the different B-cell subtypes, which has been the focus of many clinical studies whose aim was to explain the differences in therapy efficiency in different disease subgroups. In the above-mentioned guidelines it was shown that seropositive RA patients profit more from a therapy with the CD20-antibody than seronegative patients indicated by a lower disease activity score 28 and a longer time to relapse, possibly due to different cell characteristics [60].

Rituximab is also used for the therapy of systemic vasculitis where small-vessel vasculitis in particular responds well to the treatment [61]. According to the recommendations of the German Society for Rheumatology rituximab is as efficient and safe as cyclophosphamide in the initial treatment of severe, active ANCA-associated vasculitis (level of evidence A). Rituximab should not routinely be combined with cyclophosphamide, with the exception of extraordinary severe cases as studied in the RITUXVAS-study (EudraCT number 2005-003610-15, level of evidence B) [62]. Some patients with systemic lupus erythematosus (SLE) also profit from off-label rituximab applications, although great variations are seen in the relapse rates of affected patients [63]. It is likely that this phenomenon is also due to differences in disease characteristics, which underscores the necessity to identify biomarkers that are able to predict treatment outcome [64].

In regards to the heart rituximab is a double-edged sword. On the one hand it is beneficial in the treatment of the cardiac B-cell lymphomas [65] and recurrent giant-cell myocarditis [66], as well as in the treatment of antibody-mediated rejection or lymphoproliferative disorders after heart transplantation [67-69]. On the other hand, treatment with rituximab has not only been linked to arrhythmias but there are also case reports that associate the antibody with the development of Takotsubo cardiomyopathy, heart failure, and acute myocardial infarctions [70-76]. While arrhythmias are a frequent side effect of the therapy with this anti-CD20 antibody affecting about 8% of all treated patients [77], the incidence of myocardial infarctions is rather low. In most cases they pertain to patients who show cardiovascular risk factors or suffer from lymphoproliferative diseases [72,75]. In the only reported case of a myocardial infarction in a patient without known classical risk factors the development of myocardial infarctions may be associated with

concomitant corticosteroid treatment [72,78]. How rituximab promotes myocardial infarction is currently unclear but possible mechanisms involve vasoconstriction, platelet activation, rupture of atherosclerotic plaques, dissections of the coronary arteries [72], or alteration in blood pressure and heart rate as well as the inflammatory milieu [75]. Patients with a high burden of risk factors should be closely monitored and possible symptoms of a myocardial infarction should be taken very seriously. Kanamori and colleagues found that patients who gradually developed heart failure after rituximab treatment showed increased levels of the transforming growth factor (TGF)- β , possibly promoting the growth of reticulin fibers in the myocardium [76]. These fibers, which were found to be diffusely distributed in the cardiac muscle of patients examined, are thought to be at least partly responsible for the development of heart failure under rituximab treatment [76]. The arrhythmias that have been documented after rituximab infusion range from bradycardia, tachycardia, atrial fibrillation, and nonspecific dysrhythmias to polymorphic ventricular tachycardia [77,79]. While the cause of these arrhythmias is currently unknown one hypothesis suggests that reticulin fibers do not only have an influence on the contractility of the heart but also on myocardial conduction properties [79]. In addition, various results hint to a role of CD20 in the regulation of intracellular calcium concentration [80,81]. However, the exact electrophysiological basis for the rare presentation of arrhythmias following rituximab infusion remains to be explored.

3. INVOLVEMENT OF IgE IN ALLERGIC DISEASES

IgE plays a central role in the development of allergic diseases. In allergy-prone individuals exposure to allergen initiates a cascade of processes leading to the production of allergen-specific IgE. Initially, IgE connects with the Fc receptors of inflammatory cells like mast cells, basophils, and macrophages while eventually persistent antigen exposure will result in cross-bridging between allergen and IgE on the surface of these effector cells [82,83]. This will lead to degranulation of mast cells and basophils resulting in the release of proinflammatory mediators, such as histamine, prostaglandins, leukotrienes, chemokines, and cytokines.

3.1 IgE-Neutralizing Antibodies

3.1.1 Omalizumab

Omalizumab (Xolair) was developed by Novartis, Switzerland and represents a recombinant humanized monoclonal anti-IgE antibody, directed to an epitope expressed on the C3 domain of IgE that binds to

high- and low-affinity receptors [84]. Omalizumab has been shown to form trimeric and hexameric complexes with IgE, which leads to a decrease in circulating free IgE levels. The complexes are eventually cleared by the reticuloendothelial system without activation of a complement system [85]. In patients suffering from asthma omalizumab was shown to improve disease control [86] and to decrease eosinophil counts as well as the levels of interleukin (IL)-2+ and IL-13+ T-lymphocyte [87]. Omalizumab is usually given subcutaneously every 2–4 weeks at a dose that needs to be adapted to body weight and baseline total serum IgE levels [86,88]. In 2003 omalizumab was approved by the FDA for the treatment of moderate-to-severe persistent asthma that is not well controlled by inhaled corticosteroids and patients with chronic idiopathic urticaria who have persistent hives under H1 antihistamine treatment. In the stepwise approach for managing asthma in children ≥ 12 years of age and adults, omalizumab is provisioned in steps 5 and 6 with evidence level B. In step 5, it should be added to a high-dose of inhaled corticosteroids and long-acting β_2 agonists in patients who suffer from allergies, while step 6 envisions the additional administration of oral corticosteroids [89].

Since approval studies showed an increase in cancer rates in patients that had received Xolair most likely not related to the drug, the 5-year observational cohort study called the “Epidemiologic Study of Xolair (omalizumab): Evaluating Clinical Effectiveness and Long-Term Safety in Patients with Moderate to Severe Asthma” (EXCELS, NCT00252135) was performed [90]. Due to study limitations like the inclusion of patients with prevalent use of Xolair and a history of cancer or premalignant conditions as well as a high dropout rate the association between Xolair treatment and cancer could not be ruled out by this study [91,92], but it was seen that Xolair-treated patients had a higher incidence rate per 1000 patient years of overall cardiovascular and cerebrovascular serious adverse events, as well as for myocardial infarction, unstable angina, transient ischemic attack, pulmonary embolism/venous thrombosis, and pulmonary hypertension compared to non-Xolair-treated patients [93]. No increases in the rates of ischemic stroke or cardiovascular death were observed. The FDA found that these results suggest a potential increased risk of serious cardiovascular and cerebrovascular events in patients treated with Xolair. However, the magnitude of the risk could not be quantified as a result of the study limitations mentioned above plus the confounders due to the observational nature of the study. In addition, Xolair users showed higher baseline cardiovascular risk, and since the original focus of the study had not been on cardiovascular diseases, relevant cardiac risk factors were not measured and could therefore not be adjusted for. To further evaluate the cardiovascular and cerebrovascular

risks of Xolair, pooled analysis of 25 randomized double-blind, placebo-controlled clinical trials were reviewed by the FDA. The primary outcomes of interest included cardiovascular death, myocardial infarction, arrhythmias, heart failure, stroke, transient ischemic attack, pulmonary hypertension, pulmonary embolism, and unstable angina. Although no association between Xolair and an increase in these cardiovascular events was found in the examined group of relatively young patients, the FDA decided to add information describing the potential cardiovascular risk to the label [94]. A larger prospective study specifically focused on the potential cardiovascular side effects of Xolair is needed for clarification.

4. IL-6 SIGNALING

IL-6 is a glycosylated-circulating cytokine of 21–28 kDa that belongs to the IL-6 type cytokines and is secreted by a number of different cells including activated macrophages and lymphocytes. While it is mainly known to play an important role in the acute phase response to infection and injury it is also involved in hematopoiesis and immune response. In order to execute its actions it needs to bind to its high-affinity receptor complex, which consists of two membrane glycoproteins, in which the α -receptor IL-6R represents the low affinity-binding component, which has a size of 80 kDa. Only when IL-6 is bound to IL-6R is it able to bind with high affinity to gp130, the signal transducing subunit. IL-6-signal transduction leads to activation of the JAK/STAT, ERK, and PI3K signaling pathways [95]. While only a few cells including macrophages, neutrophils, some types of T-cells, and hepatocytes possess IL-6 receptors on their surface and may therefore be activated via the above-described classic-signaling, cells that express gp130 on their surface but lack IL-6R can be activated via trans-signaling involving the soluble IL-6R [96,97]. Soluble IL-6R (sIL-6R) is produced via proteolytic cleavage of the membrane bound IL-6R (mbIL-6R), a process that is induced during apoptosis and is also known as shedding or the transcription of an alternatively spliced IL-6R-mRNA lacking the transmembrane and cytosolic domains [98–101]. The sIL-6R binds to IL-6, and the resulting sIL-6R/IL-6 complex may then activate cells that express gp130 (Fig. 28.3). Trans-signaling plays a role in lymphocytes migration into inflamed regions and in the regulation of adhesion molecule expression on endothelial cells [98,102].

IL-6 has long been known as a predictive marker for both total and cardiovascular mortality independent of the traditional risk factors of atherosclerosis [103]. This cytokine not only plays a central role in the regulation of CRP and fibrinogen synthesis of the liver but is also involved in the migration of inflammatory cells [98],

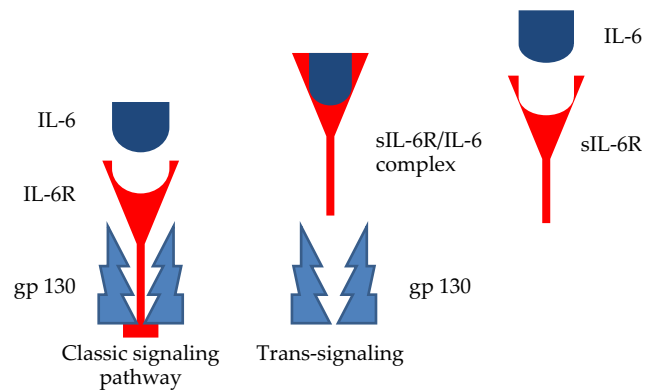


FIGURE 28.3 Interleukin-6 signaling pathways. Adapted from the personal collection of the authors.

the expression of adhesion molecules by endothelial cells [102,104], the activation of the hypothalamic–pituitary–adrenal (HPA) axis via peripheral synthesis of corticotropin-releasing factor [105–107], differentiation of T-lymphocytes into T-helper cells [108], and activation of coagulation via induced monocyte expression of tissue factor [109]. In patients suffering from acute myocardial infarction IL-6 blood levels were shown to be increased at the site of the ruptured plaques, while CRP levels in this area were lower than in the aorta [110]. Patients whose IL-6 receptor shows the variant, Asp358Ala, which leads to an impaired IL-6 signaling due to a decreased number of membrane-bound IL-6Rs either by increased receptor shedding or alternative splicing, had decreased risk of developing coronary heart disease. This IL-6 receptor polymorphism had no influence on other cardiac risk factors like lipid concentration, fasting glucose, systolic blood pressure, or body mass index. Even though there was an increase in the concentration of IL-6 and IL-6R for each Asp358Ala copy inherited, the decrease in downstream signaling resulted in attenuation of hepatic acute-phase reactant release, including decreases of CRP by 7.5% and fibrinogen by 1.0% [111,112]. These data indicate that atheroprotection may be achieved via targeting of the IL-6 signaling pathway [113].

4.1 IL-6 Antibodies

4.1.1 Tocilizumab

Tocilizumab is a genetically engineered humanized monoclonal antibody that was developed from a mouse antihuman IL-6 receptor antibody that competitively inhibits the IL-6 receptor by Chugai Pharmaceutical, Japan [114]. Since it was originally developed for the treatment of multiple myeloma it was initially called the *myeloma receptor antibody* before it was renamed tocilizumab. Humanization has resulted in reduced antigenicity in the human body, which led to a prolonged half-life of the drug. Thus even repetitive

treatment with the drug rarely causes production of neutralizing antibodies. Tocilizumab inhibits IL-6 signaling by competitive binding of the membrane-bound and soluble form of the IL-6R and thereby prevents IL-6 from binding to the receptor. The drug was approved for the therapy of RA in the EU in January 2009 and by the FDA in January 2010. The ACT-RAY study (NCT00810199, EudraCT no 2008-001847-20) aimed at comparing the clinical outcome of patients with RA and an inadequate response to methotrexate who are being treated with tocilizumab in combination to methotrexate with a group that was switched to tocilizumab monotherapy in combination with placebo [115]. The initial goal of the study was to show that the add-on strategy is superior to the tocilizumab monotherapy. This goal was not reached since the results showed no statistical significance in efficacy outcome, ie, in the DAS28 ESR remission rates between the two groups at week 24. As a result of this study, patients suffering from severe RA who are intolerant to methotrexate may be treated with tocilizumab monotherapy (8 mg/kg intravenously, level of evidence A, strength of recommendation II). However, the ACT-RAY study showed numerical superiority in efficacy outcome in the combination arm. Therefore patients with inadequate response to methotrexate who lack signs of intolerability of the substance are recommended to be kept on methotrexate treatment in addition to tocilizumab administration (level of evidence A, strength of recommendation II). While the above-mentioned links between IL-6 and the development of atherosclerosis and the positive effect of the IL-6R polymorphism make it tempting to extend the use of tocilizumab to the prevention of atherosclerosis the substance also seems to have a negative effect on lipid profile. Various studies showed a significant increase in total and LDL cholesterol levels as well as triglycerides under tocilizumab treatment [116–124], which was not seen in patients with the mentioned IL-6R polymorphism [111]. These unfavorable effects on an established cardiovascular risk factor are currently delaying the possible use of the IL-6R blocker in the therapy of atherosclerosis. However, this association was not observed consistently among all studies. Since patients suffering from RA were shown to have even lower LDL levels than controls the increase in cholesterol levels might just be a result of the decrease in disease activity [125]. Only high and normal dense LDL particles but not small-dense LDL particles, which are most important for atherosclerosis progression, were increased under tocilizumab therapy in RA patients [126]. It is recommended that all patients that are to be treated with tocilizumab should have a baseline fasting lipid profile and any abnormality should be treated in accordance with the corresponding

guidelines. The profile is to be repeated after 3 months of treatment (level of evidence B, strength of recommendation II). It should be noted that tocilizumab is able to decrease the bioavailability of certain statins (ie, simvastatin) by blocking the IL-6 associated inhibition of cytochrome P450 [127]. There are currently in vitro studies underway examining possible use of tocilizumab for the treatment of patients with high Lp(a) levels [128] since studies in patients with RA have also shown that tocilizumab leads to significant decrease in Lp(a) levels [126,129], shown only to be specific for the IL-6 antibody [128]. Further studies will be needed to ponder the favorable and harmful effects of tocilizumab. Currently, an open-label clinical trial (ENTRACTE, NCT01331837) is underway comparing the effects of tocilizumab and etanercept on the rate of nonfatal cardiovascular events and cardiovascular mortality in 3000 patients with moderate-to-severe RA and multiple cardiovascular risk factors or history of coronary heart disease [113]. Since all patients included in this study require antiinflammatory treatment there will be no placebo group included, which might limit the informative value of the study regarding the general effects of tocilizumab on the cardiovascular system. Investigation of the underlying mechanisms of the lipid-modulating effects of tocilizumab may enable the development of an optimized manner of IL-6 inhibition. In this regard targeting of subunit α of the IL-6R (sarilumab) and targeting IL-6 directly (sirukumab) present alternative options for targeting IL-6 effects.

4.1.2 Sarilumab

Sarilumab is a human anti-IL-6R α IgG1 antibody that was codeveloped by Regeneron, USA and Sanofi, France. It blocks IL-6-mediated inflammatory signaling by binding the subunit α of the membrane-bound and soluble human IL-6R with high affinity. Thus far there is no evidence of complement-dependent or antibody-dependent cell-mediated cytotoxicity [130]. In pre-clinical studies the antibody was shown to inhibit IL-6 signaling in a dose-dependent manner [131,132]. Using subcutaneously administered sarilumab for the treatment of RA in the setting of different phase I studies led to the reduction of acute-phase reactants including C-reactive protein (CRP), while being well tolerated by patients [133,134]. Initially it was also intended to be used in AS but further investigations regarding this use of sarilumab were stopped when a phase II clinical trial failed to show a clinical benefit for this indication. The randomized, multicenter, double-blind, parallel-group, placebo-controlled ALIGN trial (NCT01061723) aimed to assess the efficacy of the treatment with different concentrations of sarilumab, ie, 100 or 150 mg given weekly or every other week, and 200 mg given

every other week for 12 weeks, in patients with AS compared to a placebo arm [135]. Despite a marked reduction in hsCRP, the partial remission criteria developed by the Assessments in Ankylosing Spondylitis (ASAS) working group ASAS20 which were defined as primary efficacy end point, were not met at week 12 with either of the doses sarilumab that were investigated in the trial. In this trial, including the extension study (NTC01118728) where patients from either of the preceding groups were offered to continue sarilumab at a dose of 150 mg every week or if indicated for safety issues every other week in order to gain an additional overview of adverse events, no serious cardiovascular adverse events were reported. While other cardiovascular risk factors were not affected by the drug, there was a significant increase in total cholesterol as well as in LDL cholesterol levels. For treatment of RA, sarilumab was examined in the phase II/III MOBILITY study (NTC01061736), which used the same five doses as the ALING trial. The efficacy of sarilumab in addition to methotrexate (MTX) treatment in patients with active, moderate-to-severe RA who had shown inadequate response to MTX was proven in part A of this trial [136]. The SARIL-RA-EXTEND study (NTC01146652) is still underway to evaluate the safety and efficacy of sarilumab in patients with active RA. The trial is scheduled to be completed by December 2020.

4.1.3 Sirukumab

Sirukumab was developed by Janssen Research & Development LLC, USA and represents a human IgG1 κ antibody that targets IL-6 directly, thereby blocking its activity [137,138]. The phase II trial NCT00718718 demonstrated proof-of-concept that the direct IL-6 blockade provides a biologic therapy for RA. While no major adverse cardiac events occurred, the substance led to an increase in LDL levels after 2 weeks that was sustained until week 12 and normalized under cross-over placebo treatment. Sirukumab is currently under investigation in a phase 3 trial regarding its use in the therapy of MTX and sulfasalazine refractory RA (NTC01689532). In the frame of this study, patients receive either 100 mg sirukumab s.c. at week 0, week 2, and every second week through week 52 or 50 mg sirukumab s.c. at week 0, week 4 and every fourth week through week 52 and placebo injections between the sirukumab injections in week 2, week 6 and every fourth week through week 52. The primary outcome measures of this safety/efficacy study are the number of patients with adverse events, neutrophil count, platelet count, hepatobiliary abnormalities, and lipid parameter abnormalities, while clinical outcome measures like ACR20 response are marked as secondary outcome measures. The substance is also being investigated for a possible role in the therapy of lupus nephritis.

5. TNF- α AND ITS RECEPTORS

TNF- α is an inflammatory cytokine with a variety of functions. It is involved in tissue repair via enhancing fibroblast growth, plays a role in the immune response, promotes the growth of some tumors while it induces necrosis in others, and contributes to inflammatory and noninflammatory disorders [139–141]. TNF- α is mainly produced by monocytes, macrophages, B cells, T cells, and fibroblasts and is able to stimulate the production of other cytokines including IL-1. It binds to two different membrane-bound receptors, namely TNF receptor type 1 (TNFR1, p55) and TNF receptor 2 (TNFR2, p75) [142]. Both receptors also exist as soluble forms (sTNFR1, sTNFR2), which mainly exert antiinflammatory actions by binding to TNF- α and preventing the interaction with its membrane-bound receptors. However, sTNFR1 is also able to increase the half-life of TNF- α after binding to it in the bloodstream [143].

The heart is a TNF- α -producing and responsive organ. Oxidative stress, ischemia, dilated cardiomyopathy, heart failure, atherosclerosis, and myocardial infarction were shown to be associated with increased TNF- α levels [144–146]. While TNFR1 and TNFR2 are both expressed in the heart, the negative inotropic effect of TNF- α in adult cardiomyocytes resulting in decreased contractility has been shown to be mediated via activation of TNFR1 [147]. TNF- α and the soluble TNF receptors are associated with poorer short-term [148] and long-term prognosis of heart-failure patients [149,150]. Therefore several studies on the effects of inhibitors of TNF- α blockers in patients with heart failure were conducted.

5.1 Anti-TNF- α Antibodies

5.1.1 Adalimumab

Adalimumab (Humira) is currently under the license of AbbVie Ltd., USA and represents a fully humanized monoclonal antibody directed against TNF- α that is able to bind to both soluble and transmembrane TNF- α . It is administered subcutaneously every 2 weeks and was approved for the therapy of Crohn's disease in 2007. In order to evaluate the safety of adalimumab 71 global clinical studies involving 23,458 patients altogether were analyzed. While infections made up the most common side effect, ≤ 0.1 cases/100 patient years of cardiac adverse events were seen when all indications were included. With 0.2 cases/100 patient years the incidence was a little higher in patients with RA that were treated with adalimumab. Four severe cases of heart failure were reported in patients suffering from psoriasis who had received the TNF- α blocker [151]. The reported events and the results of studies involving other TNF- α blockers have led to the recommendation that patients

already suffering from moderate-to-severe heart failure (New York Heart Association (NYHA) functional class II–IV) should not be treated with adalimumab. Patients suffering from mild heart failure may receive adalimumab after careful consideration but need to be watched closely and the therapy terminated when new symptoms or signs for progression are reported [152]. Predisposition for arrhythmias should also be evaluated in patients before starting adalimumab treatment since Eder and colleagues reported the occurrence of ventricular arrhythmia after adalimumab treatment in a patient with Crohn's disease [153].

5.1.2 *Infliximab*

Infliximab (Remicade) was developed by Junming Le and Jan Vilcek at the New York School of Medicine, USA. The chimeric monoclonal antibody directed against TNF- α contains a human constant region of IgG1 and a variable murine binding site for TNF- α . It is able to bind to both soluble and transmembrane TNF- α [154]. Infliximab binds and neutralizes the soluble TNF- α homotrimer and its membrane-bound precursor with high specificity and affinity [155]. The antibody is given intravenously and its tissue penetration is higher than that of etanercept [156]. It has been proven to be effective in the therapy of Crohn's disease [157], RA [158], AS [159], psoriasis [160], and Behçet's disease [161]. It was approved by the FDA for the treatment of ulcerative colitis in children from 6 to 17 years, Crohn's disease in children over 6 years and adults, RA, AS, psoriatic arthritis, and plaque psoriasis in adults. The Anti-TNF Therapy Against Congestive Heart Failure trial (ATTACH) was designed to investigate whether TNF- α inhibition with infliximab might improve the clinical status of heart-failure patients (stage NYHA III and IV, EF \leq 35%), which was assessed by the clinical composite score at 14 weeks [162]. Even though infliximab led to a distinct decrease in CRP and IL-6 levels, patients who received infliximab did not show an improvement in the clinical parameters, and higher concentrations of the antibody were even associated with a worsening of heart failure [163].

There are various hypotheses concerning the underlying cause of the detrimental effect of infliximab on heart function. In vitro, infliximab was shown to induce antibody-dependent and complement-dependent cytotoxicity on cell lines expressing transmembrane-TNF α receptors [154]. Since TNF- α receptors are present on cardiomyocytes, cytotoxicity under infliximab treatment may add to the adverse effects of the drug. Another hypothesis states that a rebound phenomenon may play a role where TNF- α levels are increased after the treatment termination to levels above the baseline [163]. While TNF- α has a variety of detrimental effects on heart function as mentioned above it has been long known to exert cardioprotective effects during hypoxia

[164], a process that was recently shown to be mediated by prevention of calcium overload through phospholamban and PKA activation [165]. TNF- α also seems to be involved in ischemic preconditioning where it prevents mitochondrial damage [166,167]. Therefore, blocking TNF- α may also prevent the positive effects of cytokine. The achieved plasma levels of infliximab in the ATTACH trial (10–100 μ g) were also considerably above the levels recommended by the producing company (1–8 μ g/mL) [163,168]. Arrhythmias may represent another potential risk of infliximab therapy. A patient with RA was reported to have developed complete heart block after her third dose of infliximab [169]. On the other hand, using the TNF- α inhibitor in a mouse model for Chagas' heart disease led to a reduction in the occurrence of arrhythmias and second-degree atrioventricular blocks [170], and a patient with multisystem sarcoidosis involving the heart showed complete resolution of the heart block he had developed and regression of his pulmonary granulomas [171]. While Franco et al. found that infliximab and etanercept increase QT interval, QT dispersion, and heart rate corrected QT dispersion suggesting that ECGs of patients should be evaluated carefully before starting treatment with a TNF- α inhibitor [172], another group showed that the application of infliximab significantly reduced the QT corrected for heart rate in patients with AS, concluding that this substance may protect from fatal arrhythmias and sudden cardiac death [173]. In a swine model of ischemic ventricular fibrillation infliximab improved hemodynamic parameters and survival when given early after the return of spontaneous circulation [174]. These effects do not seem to be general for TNF- α inhibitors since etanercept did not prove to be protective in the same setting [175].

The patent protection for infliximab (Remicade) expired in February 2015, but the biosimilars Remisima (Mundipharma, Basel, Switzerland) and Inflectra (Hospira, Lake Forest, IL, USA) were approved by the EU in 2013 for the same applications as (Remicade) [176]. Remisima was proven to have a comparable pharmacokinetic profile and immunogenicity and an equivalent efficacy in patients suffering from RA (PLANETRA, NCT01217086) [177] and patients with AS (PLANETAS, NCT01220518) [178].

5.1.3 *Etanercept*

Etanercept (Enbrel) is a fusion protein consisting of the binding part of the human type II receptor for TNF- α (p75) linked to the Fc portion of IgG1. It is able to bind to both soluble and transmembrane TNF- α and is given subcutaneously. Etanercept is currently used in the therapy of active RA and AS. In patients with early RA treatment with etanercept has proven to be superior to MTX regarding disease progression including

radiographic structural damage (level of evidence A) [179,180]. With the help of a mouse model etanercept was shown to suppress oxidative stress in myocardial ischemia/reperfusion via Notch1 signaling [181]. In a recent study, etanercept led to slower progression of carotid intima-media thickness in patients with AS, possibly hinting at a positive influence on cardiovascular disease [182]. In the first randomized, double-blind, placebo-controlled multidose trial of etanercept it was proven to be safe and well tolerated, and significant dose-dependent improvement of LV-EF was seen [183]. A different study also suggested improvement in endothelial function [184]. Despite these promising results two large-scale placebo-controlled studies (ie, Research into Etanercept Cytokine Antagonism of Cytokines (RECOVER) and Randomized Etanercept North American Strategy to Study Antagonism of Cytokines (RENAISSANCE)) failed to produce significant results and were terminated prematurely. Results from both trials were analyzed in the Randomized Etanercept Worldwide Evaluation (RENEWAL) trial in order to receive longer-term information concerning morbidity and mortality. This study found that treatment with etanercept led to no clinical benefit in the rate of death or hospitalization due to chronic heart failure. The authors discuss various hypotheses concerning the reasons the TNF- α blocker did not fulfill the expectations. They suggested that proinflammatory cytokines may not play a deleterious role in the pathophysiology of CHF and speculated that physiological levels of TNF- α may even be necessary for cardiovascular homeostasis. Since there was no measurement of TNF- α levels or activity of the cytokine conducted in either study the doses of etanercept may have been too low to neutralize circulating and/or myocardial tissue levels of TNF sufficiently or the blockage of other inflammatory mediators might have been mandatory in order to see an effect. Since the study arm of RENAISSANCE that received etanercept for the longest time also showed a higher proportion of patients whose clinical state worsened it seems possible that the short-term benefits of etanercept observed in the earlier small phase I studies in CHF were offset in the long term by the ability of etanercept to stabilize biologically active (homotrimeric) TNF, thereby acting as an TNF agonist. The authors suggest that TNF- α inhibitors might be effective for a subgroup of patients that has not yet been defined [185]. While a patient with congestive heart failure due to giant-cell myocarditis was treated with etanercept [186] leading to complete clinical resolution and a patient with adult onset Still's disease suffering from congestive heart failure possibly associated with myocarditis was also successfully treated with the TNF- α inhibitor [187] it was reported that a patient with ankylosing spondylitis developed severe heart failure under treatment with the TNF- α

blocker [188]. The American College of Rheumatology recommends that RA patients with congestive heart failure NYHA III or IV or with an ejection fraction $\leq 50\%$ should not be treated with anti-TNF biologics (level of evidence C) [189]. Regarding its effect on the development of arrhythmias data are as contradictory as in the case of infliximab. One case report links etanercept to the development of atrial fibrillation in a patient with RA [190] since as mentioned above it was shown to increase QT, QT dispersion, and QT dispersion corrected [172]. On the other hand, etanercept led to a decrease in malignant ventricular tachyarrhythmias in canine models of myocardial infarction [191].

5.1.4 Golimumab

Golimumab (Simponi) is a fully humanized monoclonal IgG1k antibody developed by Centocor Inc., USA. It was approved in 2009 for the therapy of RA, AS, and psoriatic arthritis. Four years later it also received admission for therapy-refractory ulcerative colitis. It is given subcutaneously once a month. While the affinity of golimumab for soluble TNF- α is comparable to that of etanercept and greater than those of infliximab and adalimumab (2.4-fold and 7.1-fold, respectively) its affinity to the transmembrane form of TNF- α is 20 to 1400 fold lower. It was shown to have the potential of cell lysis via complement-dependent cytotoxicity [192]. A small randomized, double-blind, placebo-controlled trial of 41 patients suffering from AS showed that treatment with golimumab may have some beneficial effects on arterial stiffness. The data regarding intima-media thickness were not as nonambiguous but also showed a tendency toward a positive influence of the drug on the arterial dysfunction in patients with ankylosing arthritis [193]. Further studies will be necessary to characterize the effect of golimumab on the development of atherosclerosis in detail.

5.1.5 Certolizumab Pegol

Certolizumab pegol (Cimzia) developed by UCB, Belgium is a recombinant, humanized F(ab)' fragment of an antibody conjugated to polyethylene glycol to enhance its plasma half-life. It does not contain an FC region and therefore does not cause antibody-dependent cell-mediated or complement-dependent cytotoxicity as described for infliximab, etanercept, and adalimumab [194–196]. Another difference is that certolizumab pegol preferentially penetrates inflamed tissue compared to noninflamed tissue, and does so to a greater extent than adalimumab or infliximab [197,198]. It is directed against soluble and membrane-associated human TNF- α and was approved for the treatment of Crohn's disease that does not respond to conventional therapy in 2008 [196] and for RA in 2009.

6. SUBUNIT p40 AND ITS INFLUENCE ON CYTOKINE REGULATION

IL-12 and IL-23 are both heterodimers and have the subunit p40 in common. This subunit binds to the IL-12R β 1 subunit, which is part of the IL-12 receptor and IL-23 receptor. At the same time p35, the unshared subunit of IL-12 binds to the other part of its receptor called IL-12 β 2. On the other hand, IL-23R represents the part of the IL-23 receptor that binds to its unique subunit p19.

IL-12 was shown to induce the differentiation of CD4 naïve T cells to T helper 1 cells and the activation of natural killer cells. Both cell types then produce INF- γ and IL-2 among others. IL-23, on the other hand, stimulates the production of IL-17 by CD4⁺ T cells, which in turn leads to the production of proinflammatory cytokines by different cell types. Both interleukins have been shown to play a crucial role in the development of the clinical phenotype of psoriasis, which led to the development of anti-p40 antibodies [199].

6.1 Anti-p40 Antibodies (IL-12/IL-23 Antibodies)

6.1.1 Ustekinumab

Ustekinumab (Stelara) developed by Janssen-Cilag, Germany is a human monoclonal antibody that binds to the shared p40 protein subunit of the human interleukins 12 and 23, thereby preventing interaction with their cell surface IL-12-R β 1 receptor. In September 2013 ustekinumab was approved for the therapy of psoriasis and in January 2014 for the treatment of plaque psoriasis.

Patients suffering from psoriasis seem to have higher prevalence of cardiovascular risk factors and are more likely to experience heart disease, cerebrovascular disease, or arterial disease [200–202]. In 2011 a meta-analysis evaluated the number of major adverse cardiovascular events that were reported in studies with ustekinumab. However, in this meta-analysis neither a positive nor a negative effect of the anti-p40 antibody on cardiovascular events was found [203]. Of the patients with moderate-to-severe psoriasis that were treated with ustekinumab 0.3% suffered from myocardial infarction, stroke, or cardiovascular death.

6.1.2 Briakinumab (Ozespä)

Briakinumab represents another fully human monoclonal antibody (mAb) directed against the p40 subunit shared by IL-12 and IL-23, which was developed by Abbott Laboratories, USA. The substance proved to be effective in the treatment of moderate-to-severe chronic plaque psoriasis in one phase II and various phase III studies [204]. In this randomized, placebo-controlled trial

(NTC00570986) involving 1465 patients with moderate-to-severe psoriasis, one group received briakinumab 200 mg at weeks 0 and 4 and 100 mg at week 8 while the other group received placebo [205]. Of the 981 patients in the briakinumab treatment groups 236 of the patients withdrew within the first 12 weeks while 463 of the 484 patients in the placebo group did not enter the maintenance phase. The high dropout rates in both groups were mainly due to lack of efficacy defined as a physician's global assessment (PGA) score of "mild" or higher. The patients who remained in the study in week 12 were rerandomized to placebo, 100 mg briakinumab every 4 weeks, or briakinumab every 12 weeks until week 52. At week 12 the PGA "clear/minimal" response that was defined to be the primary endpoint was significantly higher in the treatment group. The effect was best maintained when briakinumab treatment was continued and repeated every 4 weeks. Major adverse cardiac events occurred in 7 briakinumab patients, 5 during the first 12 weeks and the others on days 131 and 225, respectively. At the same time 10 patients treated with the antibody developed nonmelanoma skin cancers while none was found in the placebo group. A higher rate of infections was also seen in the treatment group. Due to these severe adverse events Abbott Laboratories withdrew its application for briakinumab from the USA FDA and European Medicines Agency [206].

7. FUNCTION OF IL-17

IL-17 is part of the IL-17 cytokine family that includes IL-17A, IL-17B, IL-17C, IL-17D, IL-17E/IL-25, and IL-17F, which exert their different functions via their corresponding IL-17R subtype [207]. IL-17 mediates the recruitment of neutrophils to inflammatory sites via increased expression of proinflammatory cytokines [208], the induction of adhesion molecules, the upregulation of chemokines, and angiogenesis [206]. It was shown to act synergistically with TNF and IL-1 [209]. The levels of IL-17 are elevated in the blood and joints of patients with RA [210–212], and high concentrations of the interleukin can be found in skin affected by psoriasis, making it a possible target for RA and psoriasis therapy [213,214]. IL-17A was also shown to be involved in the development and progression of a number of cardiovascular diseases including atherosclerosis [215,216], hypertension [217], viral myocarditis [218,219], and dilated cardiomyopathy [220]. The role of IL-17 in atherosclerosis is still controversial [221–224]. While one study showed that the administration of a rat antimouse IL-17A antibody in ApoE^{-/-} mice led to a reduction in the atherosclerotic lesion area of more than 50% [221], another group that treated mice lacking the LDL-receptor with a mouse antimouse IL-17A antibody found no

effect on atherosclerosis [222]. Cheng et al. shed some light on this issue by testing a mouse antimouse and a rat antimouse antibody on ApoE^{-/-} mice [215]. Treatment with the rat antibody led to a marked decrease in plaque size but did not alter IL-17A signaling. In contrast, the antibody that was increased in mice caused a decrease in IL-17A production, which did not prove to have an influence on lesion size. The role of IL-17A in ischemia/reperfusion injury where the cytokine was shown to induce apoptosis and neutrophil infiltration seems to be clearer [225]. IL-17 knockout mice that were subjected to left coronary artery (LAD) ligation and subsequent reperfusion showed better cardiac function, smaller infarct size, and lower troponin T levels than wild type mice after I/R. These data were confirmed using a murine antiIL-17A antibody. At the same time the administration of exogenous IL-17A led to an increase in infarct size, cardiomyocyte apoptosis, and neutrophil infiltration. Recently IL-17A was also shown to be involved in early- and late-phase ventricular remodeling after myocardial infarction (MI) [226]. Myocardial infarction led to a significant increase in IL-17A mRNA and protein expression in the hearts of mice as early as 1 day after MI, peaking at day 7. The mRNA expression levels of the receptor subunits IL-17RA and IL-17RC involved in IL-17A signaling remained elevated until day 14 post-MI. Administration of IL-17A 10 min prior to LAD ligation caused an increase in infarct size, myocardial fibrosis, and cardiomyocyte apoptosis, resulting in a decrease of cardiac function. At the same time, genetic IL-17A deficiency had a protective effect. Considering the fact that post-MI levels of IL-17A were also shown to be increased in humans [227], the inhibition of IL-17A represents a promising target for therapeutic interventions in the field of cardiovascular diseases. While there are already some IL-17 antibodies available on the market, clinical studies regarding their use for indications like the prevention of I/R injury or heart failure are currently not available.

8. ROLE OF PHOSPHODIESTERASE 4 IN IMMUNE CELL RESPONSE

Members of the large family of phosphodiesterases (PDEs) are enzymes that participate in the regulation of the cellular levels of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) by catalyzing their hydrolysis into 5'-nucleotide monophosphates [228]. The second messengers, cAMP and cGMP, are involved in the mediation of the effects of cytokines, chemokines, neurotransmitters, and hormones. Increased levels of cAMP and cGMP lead to the activation of protein kinase A and protein kinase G, which phosphorylate various substrates including transcription factors and ion channels thereby contributing

to the regulation of immune responses, cardiac and smooth muscle contraction, glycogenolysis, platelet aggregation, apoptosis, and growth control [229]. Blocking phosphodiester hydrolysis results in higher levels of cyclic nucleotides and due to the many effects of cAMP and cGMP, PDE inhibitors are suspected to be effective antiinflammatory agents, antidepressants, supporters of improved memory and cognitive functions, antithrombotics, antiasthmatics, vasodilators, smooth muscle relaxants, and cardiostonic agents [230–232].

While some members of the 11 classes of cyclic nucleotide phosphodiesterases target both cAMP and cGMP others are selective for either one of the 2s messengers. Phosphodiesterase 4 (PDE4) solely terminates the downstream signaling of cAMP by catalyzing its hydrolysis [233–235]. To fulfill this function PDE4 contains an active site that consists of three pockets, a bivalent metal-binding M-pocket, which forms a complex with the phosphate moiety of cAMP, the glutamine containing the so-called Q-pocket, which forms hydrogen bonds with the nucleotide (purine) moiety of cAMP, as well as a solvent-filled side pocket (S-pocket) [236]. All known PDE-inhibitors contain a planar ring structure that is held tightly in the active site by a pair of hydrophobic residues [237] and show three main types of interaction with the PDEs: Interactions with the hydrophobic residues lining the cavity of the active site, water-mediated interactions with metal ions, and H-bond interactions with the protein residues essential for nucleotide recognition and selectivity [238]. The PDE4 subfamily is comprised of four members. PDE4B has a special function in inflammatory responses of lymphocytes, and targeted disruption of the PDE4B gene led to reduced production of cytokines in response to polysaccharides. Therefore, PDE4B represents an attractive target for antiinflammatory drugs and substances like roflumilast (Daxas, Tekeda, Japan) that are being used for the treatment of asthma and COPD [239,240]. Due to the high degree of sequence and structural similarity the design of an inhibitor that selectively inhibits one subtype of PDE4 is challenging, and currently inhibition of PDE4D in the brain is responsible for common side effects, such as nausea and emesis [241].

8.1 Phosphodiesterase Type 4 Inhibitor

8.1.1 Apremilast

Apremilast (Otezla, Celgene Corporation, USA) is a phosphodiesterase type 4 inhibitor that blocks the degradative action of PDE4 on cAMP. The substance was approved for the treatment of psoriasis and psoriatic arthritis by the FDA (2014) and the EMA (2015). It is the first substance that gained approval for the oral treatment of psoriasis since 1996 [242]. Its efficacy and safety

in the treatment of moderate-to-severe plaque psoriasis was shown by the ESTEEM 1 trial (NTC01194219), a phase III, multicenter, randomized, double-blind, placebo-controlled study that was divided into three treatment periods [242]. In the placebo-controlled phase A from week 0 to 16, patients were randomized to receive either 30 mg apremilast or placebo twice a day. In period B, which was considered the maintenance phase (week 16–32), placebo patients were switched to a starting dose of 10 mg once daily apremilast that was increased weekly up to 30 mg twice daily. In the last period from week 32–52 (period C) patients who had received apremilast in phase A and had achieved 75% or greater reduction in PASI score from baseline at week 32 were rerandomized to either continue apremilast, which was again titrated, or to switch to placebo. Patients that were rerandomized to placebo in phase C and lost PASI-75 response resumed apremilast without titration. In phase A the primary end point being the achievement of PASI-75 at week 16 was reached by 33.1% of patients in the treatment group vs 5.3% in the placebo group. Responses were maintained through week 52. Considering the timeframe from week 0 until week 52 two patients in the apremilast group experienced an MI equivalent to 0.4 events in 100 patient years vs no patient in the placebo group. Three patients in the treatment group were diagnosed with coronary artery disease (0.5 events in 100 patient years) vs 0 in the treatment group.

9. INVOLVEMENT OF IMMUNOGLOBULIN CTLA-4 IN T-CELL ACTIVATION

Following antigen recognition, T cells require costimulation to become fully activated. One of these costimulatory pathways involves the interaction of CD80 or CD86 on antigen-presenting cells with CD28 on T cells. It was recently shown that naïve as well as autoimmune effector T cells were shown to be dependent on these signals. The cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152, is a protein receptor that inhibits T-cell activation. It is found on the surface of T cells where its activation results in an inhibitory signal [243]. CTLA-4 has a greater affinity for CD80 or CD86 than CD28 and thereby outcompetes CD28 for CD80 or CD86 binding [244,245].

9.1 CTLA-4 Fusion Proteins

9.1.1 Abatacept

The costimulation modulator abatacept (Orencia), which was developed by Bristol-Myers Squibb, USA, represents a soluble recombinant fusion protein containing the extracellular domain of the human CTLA-4

and a fragment of the Fc domain of human IgG1, which was modified to prevent complement fixation [246]. It binds to the CD80 or CD86 molecule, thereby preventing the T-cell activation. It was approved in June 2007 for the treatment of patients with RA who are not responding well to common treatment options. While hypertension has been described as a common side effect of abatacept [247] blood pressure was not found to be increased in a recent trial that investigated the influence of abatacept on aortic stiffness as a marker of cardiovascular risk [248]. In this study, abatacept treatment led to an increase in all lipid parameters. Even though this increase was only significant for HDL it did not significantly decrease the atherogenic index. Abatacept did not have a positive influence on aortic stiffness possibly due to insufficient control of inflammation or the unfavorable change in lipid levels [248]. Abatacept is the basis for the second-generation belatacept, which is intended to be used for immune suppression after organ transplantation and which was shown to have a better cardiovascular and metabolic profile [249].

10. JAK-STAT PATHWAY

Since cytokine receptors lack intrinsic cytokine activity they rely on receptor-associated kinases to mediate the phosphorylation steps that are required for signaling, which is realized by signal transducers and activators of transcription (STATs) and receptor-associated kinases called Janus kinases (JAKs) [250,251]. All currently known JAKs, namely JAK-1, JAK-2, JAK-3, and tyrosine kinase 2 are receptor-associated kinases that are activated when cytokines bind to type I/II cytokine receptors inducing receptor oligomerization. Phosphorylation of the cytokine receptor by the activated JAKs induces the creation of docking sites for specific signaling proteins. At the same time STATs bind to the phosphorylated receptor and are phosphorylated themselves by the JAKs before they dissociate again from the receptor, dimerize, and translocate to the nucleus to induce gene transcription. Ligand specificity is realized by different combinations of JAKs, thus far with the known seven STATs. Therefore targeting particular JAKs or STATs may enable blocking of specific cytokines [252]. Special attention to the development of new therapeutic agents was focused on JAK3. It is mainly found in hematopoietic cells and has a crucial role in hematopoiesis [253]. JAK-3 associates specifically with the IL-2 receptor common γ chain (γ_c) used by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Patients showing mutations in the γ_c or lacking expression of JAK3 suffer from severe combined immunodeficiency (SCID) [251,254].

10.1 JAK-3 Inhibitor

10.1.1 Tofacitinib

Tofacitinib (Xeljanz, Jakvinus) was developed by Pfizer, USA and represents an oral JAK inhibitor approved by the FDA for the treatment of patients with moderate-to-severe active RA who have had an inadequate response to, or who are intolerant of, methotrexate [255]. It is rapidly absorbed and reaches its peak about 1 h after oral administration [256]. Among the known JAKs tofacitinib inhibits JAK-3 most effectively with an enzyme inhibitory potency of 1 nM [257] and is therefore classified as a JAK-3 blocker. Conversely, kinase assays revealed that tofacitinib also inhibits JAK-1 and JAK-2 but with 20–100-fold less potency and to an even lesser extent tyrosine kinase 2 [255,256]. The phase IIb trial NCT00550446 aimed at comparing the efficacy, safety, and tolerability of five oral doses of tofacitinib in patients with RA with inadequate response to disease-modifying antirheumatic drugs. This dose-ranging study tested different concentrations of tofacitinib from 1 to 15 mg and found that ≥ 3 mg of the JAK-3 inhibitor given twice daily led to significant improvement in ACR20 when compared to placebo. Infections represent the most common side effects of the JAK-3 inhibitor therapy. In the frame of the Oral Rheumatoid Arthritis Phase III Trials Standard (ORAL Standard, NCT00853385) 717 patients received either 5 or 10 mg tofacitinib twice daily, 40 mg of the TNF- α blocker adalimumab once every 2 weeks, or placebo in addition to a stable dose of methotrexate [258]. At month 6, ACR20 response rates were 51.5% for patients who had received 5 mg of tofacitinib twice daily, 52.6% for the group that was given 10 mg twice daily, 47.2% for patients under adalimumab treatment, and 28.3% for the placebo group. All three treatment groups had a significantly higher ACR20 response rate than the placebo group ($p < .001$). Adverse events that were more frequent in the tofacitinib group included infections and an increase in LDL and HDL levels as well as a decrease in leukocyte counts. Finally, in a double-blind, placebo-controlled, parallel-group 6-month study 611 patients with RA were randomly assigned to receive either 5 or 10 mg tofacitinib or placebo where 3 months of placebo treatment were followed by the administration of 5 or 10 mg of the JAK-3 inhibitor [259]. After 3 months the ACR20 response was significantly higher in patients of both treatment groups (59.8% and 65.7%) than in the placebo group (26.7%, $p < .001$). Serious infections developed in six patients. Headache and respiratory tract infections were other common adverse events. Recently Fukuyama et al. showed that mice treated with tofacitinib display aggressive behavior under stressful conditions, a side effect that had not been previously reported and will have to be evaluated further [260]. Tofacitinib significantly prolonged survival of mice that had gone

through heart transplantation [257], which may represent another potential application for the JAK-3 inhibitor.

11. p38 MITOGEN-ACTIVATED PROTEIN KINASE

The four isoforms of the p38 mitogen-activated protein kinases (p38s) called α , β , γ , and δ represent stress-activated serine/tyrosine kinases that play an essential role in the mitogen-activated protein kinase (MAPK) system. While p38 β was shown to confer a protective role during preconditioning [261,262], activation of the proinflammatory p38 α in cardiomyocytes seems to induce apoptosis [263]. Mice with reduced levels of p38 α were shown to be resistant to MI [264] and sustained activation of this isoform during ischemia aggravated the injury [265]. At the same time p38 α activity is required for the adaption to hypertrophic stress since myocyte-specific knockouts were shown to respond poorly to hemodynamic stress [266]. Even though all four p38 isoforms are found in the human heart, p38 α seems to present the most functionally relevant form [267] and has been the main target of new therapeutic interventions. Initially, p38 blockers were tested for potential use in RA and Crohn's disease, but the tested substances were not proven to be effective in the treatment of these inflammatory diseases when added to established therapeutic interventions [268,269]. Regarding the use of p38 blockers for cardioprotection the current study results look more promising and will be discussed in the following using losmapimod as an example.

11.1 p38 MAPK Inhibitor

11.1.1 Losmapimod

Losmapimod was developed by Glaxo Smith Kline, UK and inhibits the p38 MAPK α and β isoforms by competitively binding to the ATP binding site of p38MAPK [270]. In different settings of preclinical studies inhibition of p38MAPK was shown to reduce infarct size and inflammation, to increase cell viability, and to improve endothelial dysfunction, mean blood pressure as well as cardiac hypertrophy [271]. Recently, the phase II placebo-controlled study of LoSmapimod Treatment on Inflammation and Infarct Size (SOLSTICE, NCT00910962) examined the efficacy and safety of losmapimod in the treatment of NSTEMI patients [272]. Patients received either 7.5 or 15 mg losmapimod loading dose and then 7.5 mg orally twice daily for 12 weeks or placebo. For the calculation of safety and efficacy outcome the results from both treatment groups were combined. While the rate of treatment-related adverse events was higher in the treatment group (54 of 391 patients=14%) than in

the placebo group (13 of 135 patients=10%) and more patients discontinued treatment due to adverse events in the treatment group (9% versus 5%) the difference between the two groups did not reach significance. The biomarker-quantified infarct size, which was one of the primary efficacy end points, was compared between the treatment group and the placebo group at days 3–5 and week 12 and turned out to be similar in both groups at both time points. In addition, the infarct size of a small subpopulation was measured at days 3–5 and week 12 using MRI. At both time points the infarct size was smaller in the treatment group but did not attain significance. Regarding secondary end points a significant difference between the two groups was seen in cardiac function, LV-EF, LVEDV, and LVESV as well as in the concentration of the brain natriuretic peptide after 12 weeks of treatment favoring the losmapimod treatment group. While it might have been more informative to include a baseline measurement to ensure there was no initial difference in both groups and a comparison of the change in each parameter from days 3 to 5 to week 12, the authors also conclude that the study was not prepared to show the effectiveness of the p38 MAPK [272]. In addition to the lack of baseline measurements to exclude preexisting differences in the study groups, the outcome of SOLSTICE was limited by a high drop-out rate. About one-third of the patients did not complete the treatment phase mainly due to unspecified reasons (patients' decision). A proposed measure to increase the effectiveness of the losmapimod treatment is a change in formulation, enabling intravenous administration of the substance resulting in a more rapid inhibition of the p38 MAPK receptor [271].

12. ALTERNATIVE IMMUNOMODULATING STRATEGIES

12.1 Pyrimidine Synthesis Inhibitor Leflunomide

Leflunomide (Arava) is a synthetic isoxazole derivative with the chemical name *N*-[4-(trifluoromethylphenyl)-5-methylisoxazol-4-carboamide] that was developed by Sanofi-Aventis, Switzerland [273]. The pyrimidine synthesis inhibitor is considered to be a disease-modifying antirheumatic drug with antiproliferative characteristics [274]. Its mechanism of action and its chemical structure are different from other immunomodulatory drugs. Following oral application it is non-enzymatically converted into its active malononitrilamide metabolite A771726 (3 cyano-3-hydroxy-*N*-[4-(trifluoromethylphenyl)] crotonamide), also called triflunomide, by opening of the isoxazole ring (Fig. 28.4) [274]. The active metabolite selectively inhibits the enzyme

dihydroorotate dehydrogenase (DHODH), a mitochondrial enzyme (key enzyme in the synthesis of pyrimidine nucleotide triphosphates) critical for the *de novo* synthesis of pyrimidine ribonucleotides. The resulting lack of pyrimidine nucleotides results in an arrest of the cell cycle of activated T lymphocytes between the G1 and S phases, interrupting clonal expansion, which plays a role in the pathogenesis of RA [275]. The reduction in pyrimidine precursors may also lead to increased synthesis of immunosuppressive cytokines like the transforming growth factor β [276]. Possible secondary mechanisms include the inhibition of the expression of cell adhesion molecules, the blockage of the activation of the nuclear factor κ B as well as of matrix metalloproteinases and the modulation of IL-2 signaling [277]. In vitro Leflunomide was shown to reduce oxygen free-radical production, cyclooxygenase-2 activity, and neutrophil chemotaxis. Higher concentrations of triflunomide were shown to lead to an inhibition of tyrosine kinases. Since these concentrations are very seldom reached in patients with RA this effect is not considered to be of clinical relevance [278]. Leflunomide was shown to have potent antiviral and immunosuppressive activity in an allogeneic cardiac transplant model of RCMV infection [279] and proved to be effective in preventing cardiac allograft rejection when combined with rapamycin [280]. Leflunomide was shown to improve the clinical outcome of patients suffering from RA at an efficacy comparable to that of MTX (level of evidence A) [281]. In addition to diarrhea, alopecia, abdominal pain, nausea, and vomiting, hypertension is a common side effect [282]. At the same time, the therapy with leflunomide was also shown to lower plasma glucose levels possibly due to a decrease in body weight [283]. When compared to methotrexate monotherapy treatment with leflunomide was found to be associated with an increase in cardiovascular

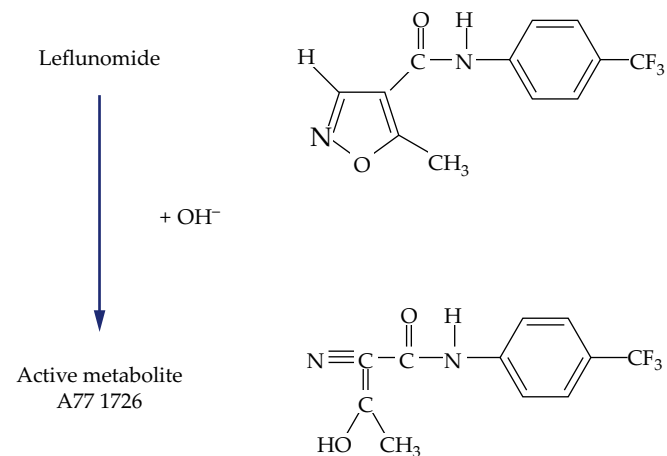


FIGURE 28.4 Conversion of the prodrug leflunomide into its active metabolite A771726 via opening of the isoxazole ring. Adapted from Breedveld and Dayer [277].

events, which is suspected to be due either to the induction of hypertension as a known cardiovascular risk factor or the possible inferiority of leflunomide regarding inflammation control [284].

12.2 Vitamin D

Vitamin D3 can be synthesized endogenously in cutaneous tissue after exposure to ultraviolet B irradiance followed by a thermal process or absorbed from the gastrointestinal tract after oral intake [285]. Independent of the source, vitamin D3 has to be metabolized in the liver to the still inert 25-hydroxyvitamin D (25(OH)D). Even though 25(OH)D requires further activation, it is measured in the clinical setting to represent the patient's vitamin D (VD) level [286]. Only 1,25-dihydroxyvitamin D (1,25 (OH)2D3), the biologically active derivative of VD is able to interact with the vitamin D receptor (VDR), which represents a nuclear, ligand-dependent transcription factor. Multiple cell lines were shown to express the VDR and to contain the key activating enzyme 25-OH-1 α hydroxylase (CYP27B1), that catalyzes the conversion of 25(OH)D to 1,25 (OH)2D3 [287]. Via paracrine and autocrine mechanisms VD is able to mediate a variety of effects. The VDR/VD complex participates in the transcription regulation of as much as 3% of the human genome including genes associated with inflammation and immune regulation [288–290]. While vitamin D has long been known for its central role in calcium and phosphate homeostasis and its lack with bone diseases, especially rickets, only recently has its role in the development and progress of chronic conditions like cardiovascular disease, diabetes, and autoimmune disorders been discovered [291]. Vitamin D was shown to regulate the immune response by T and B cells and to increase the effects of innate immune processes, while restraining the adaptive immune system, leading to improved outcome in autoimmune diseases [291]. A recent meta-analysis of case-control studies showed an association between VDR polymorphisms (TaqI and FokI) and the risk of developing RA [292]. In a prospective study of 1211 US physicians recruited in the setting of the Physician's Health Study (NTC00000500) higher 25(OH)D plasma levels were associated with lower risk of developing hypertension while certain polymorphisms of VDR led to increased risk of high blood pressure [293]. While Jorde et al. also demonstrated a relation between vitamin D levels and hypertension they did not find an association with a future risk of hypertension [294]. In a placebo-controlled trial in Denmark 130 hypertensive patients received either placebo or 3000 IU vitamin D per day for 20 weeks during winter (NTC01166165) [295]. Comparing the ambulatory blood pressure (24-h BP) at week 20 with the baseline results showed a non-significant 3/1 mmHg ($p=0.26/0.18$) reduction in blood

pressure. When considering only those 92 patients who suffered from vitamin insufficiency (<32 ng/mL), blood pressure under vitamin D treatment was decreased significantly (4/3 mmHg, $p=0.05/0.01$) indicating that a balanced vitamin D level might improve blood pressure. While it has been known for a long time that VD deficiency is associated with impaired glucose tolerance and reduced insulin turnover and insulin sensitivity [296–298] results from clinical trials were too inconsistent to show a relationship between VD supplementation and prevention of type 2 diabetes [299]. The RECORD trial (protocol 02PRT/35) involving 5292 people that were at least 70 years old and had previously suffered from low-trauma fracture was not able to show any influence of daily vitamin D or calcium supplementation on all-cause mortality, vascular disease mortality, cancer mortality, or cancer incidence [300]. The Copenhagen Vitamin D study (CopD study, no.2010-41–4826), an observational cohort study involving 247,574 patients from general practices in Copenhagen, was designed to determine the association between serum levels of 25(OH)D, calcium, and PHT and all-cause mortality [301]. This study showed that both very low (10 nmol/L) and very high (140 nmol/L) levels of 25(OH)D may be associated with an increase in mortality rate. Three years later the patient cohort was reanalyzed with respect to a possible relationship between vitamin D levels and cardiovascular disease mortality [302]. Compared to the 25(OH)D level, which was associated with the lowest cardiovascular disease mortality risk (70 nmol/L), this study showed a hazard ratio of 2.0 for cardiovascular mortality at low vitamin D levels (12.5 nmol/L) and a hazard ratio of 1.3 for high levels (125 nmol/L). Since cardiovascular risk factors like obesity were shown to be associated with low vitamin D levels it remains unclear if vitamin D deficiency is in part the cause of the consequences of cardiovascular diseases. At the same time this study stresses that an unreflected substitution of vitamin D resulting in high vitamin D levels may also cause adverse effects.

12.3 Doxycycline

Doxycycline is a semisynthetic substance that belongs to the family of tetracyclines known for their

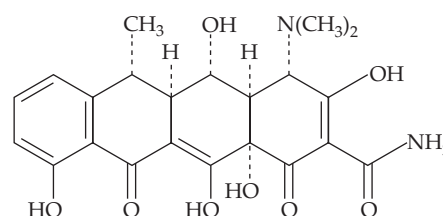


FIGURE 28.5 Chemical structure of doxycycline. Adapted from Griffin et al. [313].

antibiotic properties. Its parent compound chlortetracycline (Aureomycin) was first isolated from *Streptomyces aureofaciens* in 1947 [303]. Tetracyclines bind to the bacterial ribosome and allosterically inhibit the binding of the amino acyl-tRNA at the acceptor site, leading to the cessation of protein synthesis [304]. Doxycycline is composed of a four-ring core to which are attached various side groups (Fig. 28.5). The oxygen-rich lower half of the molecule is important for metal-ion chelation and essential for the binding of the substance to prokaryotic and eukaryotic targets [305]. The dimethylamino group at the C4 carbon on the upper half of the molecule is of special importance for the antimicrobial activity. Chemically modified tetracyclines (CMTs) whose dimethylamino group was removed lack antimicrobial activity but are still able to bind to other targets like matrix metalloproteinases (MMPs) [306]. MMPs are a family of zinc-dependent proteases including collagenases, gelatinases, stromelysins, and membrane-type MMPs [307]. Since they are involved in the proteolysis of the extracellular matrix (ECM) linked to heart remodeling tumor, invasion, and inflammation, the inhibition of MMPs can be beneficial in various pathophysiological processes [308–310]. Doxycycline does not only inhibit MMPs directly with different effectiveness depending on the MMP and the pH but also inhibits their synthesis [311]. It was shown to reduce the mRNA and protein expression of different MMPs in vitro [312]. Since MMP transcription is induced by proinflammatory proteins these upstream signaling cascades present possible targets for doxycycline [313]. Tetracycline was shown to inhibit IL-1-induced MMP-3 expression [314].

MMPs play an important role in LV remodeling after MI and their activation was shown to lead to ECM damage and cardiomyocyte injury [315]. Inhibition of MMPs causes a reduction in reperfusion injury [316,317] and post-MI remodeling [318–321]. In this context, doxycycline as the most potent MMP inhibitor among the tetracycline group was proven to have a cardioprotective effect on isolated animal hearts exposed to ischemia/reperfusion injury [317,322] and to attenuate postinfarct LV remodeling [323,324] at a much lower dose than needed for its antibacterial effects. Therefore an open-label, randomized phase II trial called the TIPTOP trial (NTC00469261) was designed to examine the effect of doxycycline on LV remodeling in patients with STEMI [325]. STEMI patients with an LV-EF <40% were randomly assigned to receive either standard treatment alone or in combination with doxycycline (100mg twice a day for 7 days) starting immediately after primary percutaneous coronary intervention. The percentage change from baseline to 6 months (%Δ) in echography LV end-diastolic volume index, which was considered the primary end point of the study, turned out to be significantly smaller in the doxycycline group (0.4%) than in the

control group (13.4%). Doxycycline also reduced infarct size and severity. While these results are in line with pre-clinical studies of broad-spectrum MMP inhibitors [318–321] they are contrary to the findings of the only other phase II clinical trial that examined the effect of a selective MMP-1 inhibitor (PG116800) on cardiac remodeling after acute myocardial infarction [326]. The Prevention of Myocardial Infarction Early Remodeling (PREMIER) trial, a multicenter, randomized, double-blind, placebo-controlled study had examined 253 patients with first STEMI and an EF between 15% and 40% who had received the selective MMP-1 inhibitor PG116800 or placebo on top of the standard treatment starting about 54h after MI for 90 days. In this setting the primary end point, change in ΔLVEDVI from baseline to 90 days, did not differ between the groups. The authors of the study suggest that selected MMP-1 inhibition might not suffice to influence remodeling. Dosage, interactions with standard medication, and timing are some of the other potential reasons for the outcome that are being considered. The latter might be of special importance, since the first dose of the medication was given immediately after PCI in the case of the TIPTOP trial and 54h after MI in the PREMIER trial. Therefore PG116800 might have been given too late to counteract infarct expansion, which is considered a critical phase in the remodeling process [327]. At the same time the treatment duration in the PREMIER trial was much longer than in the TIPTOP trial (90 days versus 7 days). Blocking MMPs in this later phase after MI was shown to cause adverse effects on the LV remodeling process by inhibiting wound healing and inflammation [328].

12.4 α-1 Antitrypsin

α-1 antitrypsin (AAT) is regarded as a serine protease inhibitor with additional broad antiinflammatory and immunomodulating properties that is found abundantly in the plasma [329]. It was shown to inhibit the endogenous production of the cytokines IL-8, IL-6, TNF-α, and IL-1β [330] and lipopolysaccharide-mediated human monocyte activation [331]. AAT is thought to have a protective role in the tissue response to ischemic injury as plasma levels are increased in patients that suffered from an MI [332,333]. Administration of human plasma-derived AAT was shown to be protective in models of acute MI in mice [334].

12.4.1 Substitution Therapy With α-1 Antitrypsin

12.4.1.1 Prolastin C

Prolastin (Grifols, Spain) represents a concentrated form of α-1 antitrypsin that is purified from human plasma. Its intravenous application was shown to increase

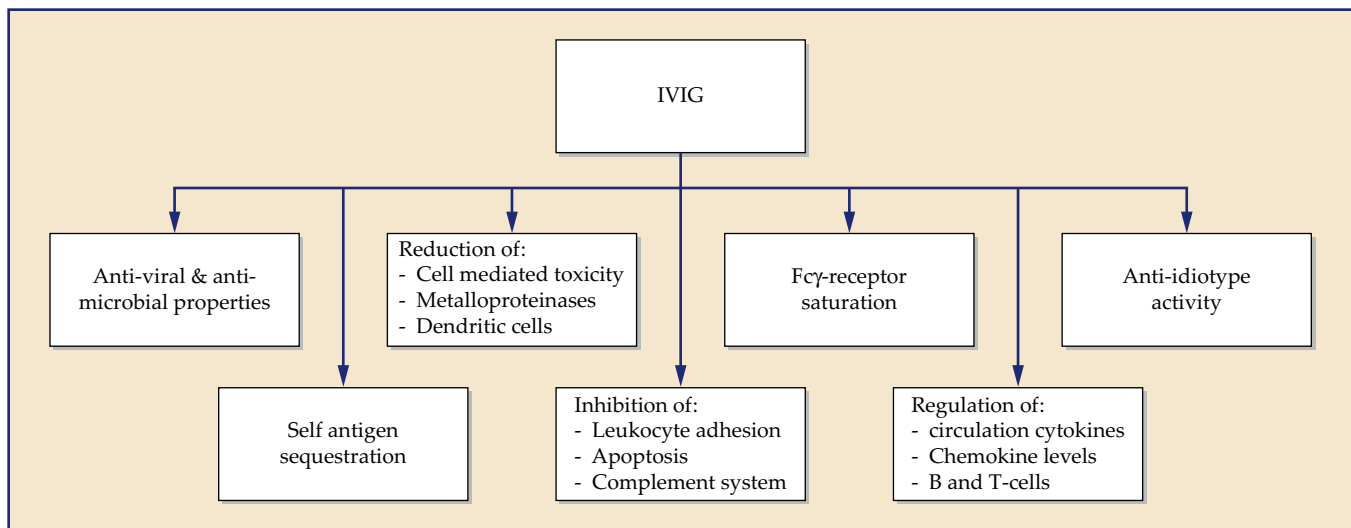


FIGURE 28.6 Therapeutic properties of IVIG. Adapted from Nussinovitch and Shoenfeld [337].

AAT levels in the blood and lung. In a mouse model of ischemia-reperfusion injury intraperitoneally administration of 60 mg/kg AAT led to significant decrease in infarct size, but this was not the case for the control group that had received albumin (Fig. 28.6) [334]. Regarding the potential mechanism of the AAT effect it was found that AAT treatment reduces caspase-1 activity, which was confirmed in vitro via examination of AAT-treated cultured adult cardiomyocytes. At the same time IL-6, IL-10, IL-17, MCP-1, TNF- α , and keratinocyte-derived cytokine levels in the heart 24h after ischemia/reperfusion turned out to be significantly lower in the AAT treatment groups than in the mice that received albumin [334]. The VCU- α 1-RT pilot study (NTC01936896) determined the safety and tolerability of human plasma-derived AAT and its effects on the acute inflammatory response after MI. In the frame of this open-label trial 10 non-AAT deficient patients with STEMI received 60 mg/kg AAT i.v. within 12h of admission followed by standard care treatment. While there was no placebo arm included, comparing results from the prolactin C group to the results of patients that had received placebo in former clinical studies (VCU-ART and VCU-ART2) with a similar setting revealed significantly lower CRP levels in the treatment group (75.9 mg/L in the prolactin C group versus 205.6 mg/L in the placebo group, $p=0.048$). There was no difference in creatine kinase-MB, regarded as a surrogate for infarct size [335], between the prolactin C and the placebo group. While such a difference was seen in the animal model of MI the authors stress that prolactin C was not given at the time of reperfusion as was the case in the mouse model [334] and that the later application time might have prevented an effect of the drug on the infarct size. None of the patients in the treatment group had recurrent MIs or new onset heart failure compared to 3 patients (15%) with recurrent acute MI and 6 patients

(30%) who developed HF in the placebo group. No infusion reactions or drug-related adverse events necessitated further evaluation of possible clinical use. Currently, there are five other clinical studies underway examining the potential of prolactin C in the therapy of new onset type-I diabetes (NTC02093221), the amelioration of organ injury after cardiac surgery (NTC02191839), and graft-versus-host-disease (NTC01183468, NTC01523821, and NTC01700036).

12.5 Intravenous γ -globulin Products

Immunoglobulins are blood product derivatives that consist of the collective plasma of between 2000 and 100,000 donors per batch. Initially, intravenous immunoglobulin (IVIG) was developed as antibody-replacement therapy to prevent infections in patients with primary humoral deficiency disorders. Since a variety of positive effects of IVIG including immunomodulation, anti-idiotypic, and anti-inflammatory effects as well as a decrease in autoantibody production were observed the use was expanded to various autoimmune disorders [336]. Other mechanisms include the mediation of various cytokines and chemokines, the reduction of apoptosis, and inhibition of leukocyte adhesion as well as complement activation and inhibition (Fig. 28.7) [337]. The Ig molecules contained in IVIG were shown to scavenge activated complement fragments like C3a, C5a, C3b, and C4b, thereby preventing the binding of complement fragments to their receptors on target cells limiting immune damage [338]. IgA may also contribute to the anti-inflammatory action of IVIG via scavenging specific receptors and/or complement fragments [339]. Inflammation is also supposed to be inhibited by the glycosylation of the Fc and Fab fragments induced by IVIG. Albert et al. showed that sugar domains attached

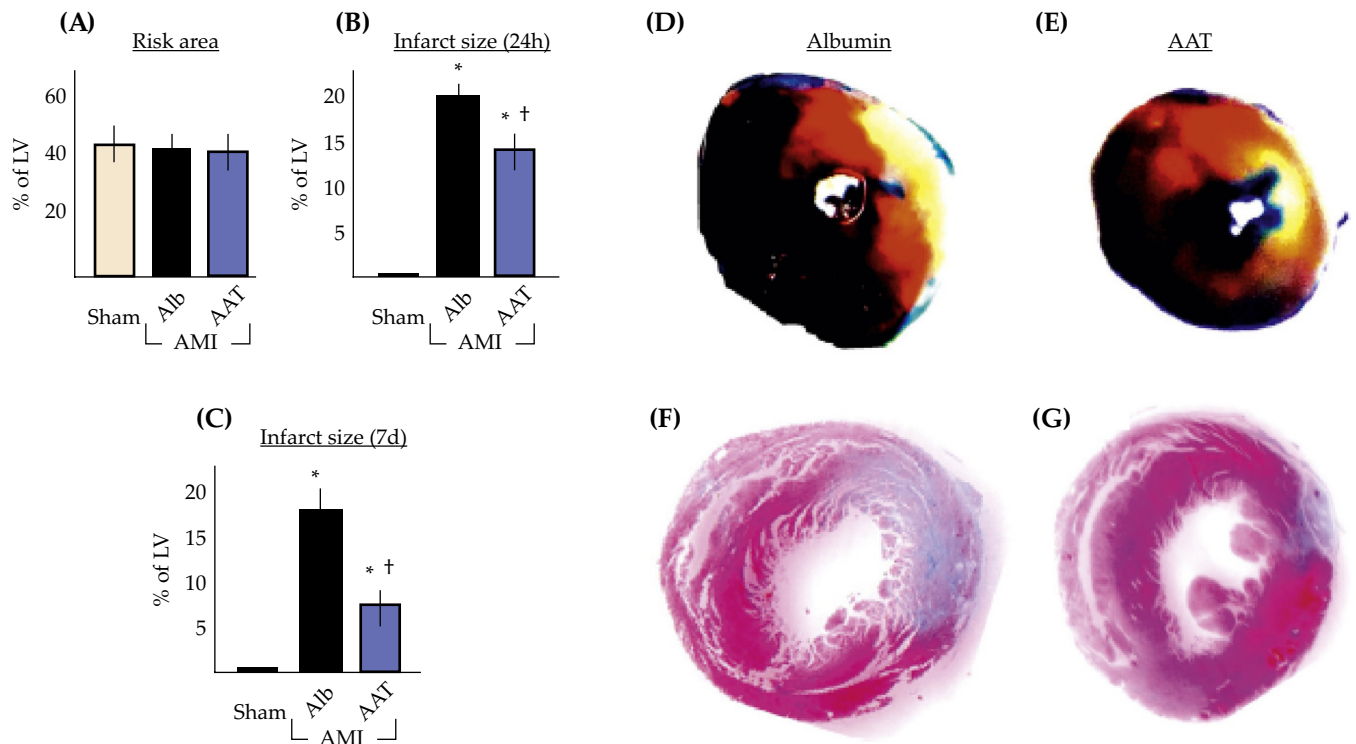


FIGURE 28.7 AAT reduces infarct size following acute myocardial infarction (AMI). (A) Mean \pm SEM percent of left ventricle at risk of infarction (risk area) following I/R event. (B) Mean \pm SEM percent of LV infarct size 24h following I/R event evaluated by triphenyl tetrazolium chloride (TTC) stain. * $p < 0.001$ vs sham operated; $\dagger p = .001$, AAT vs Alb-treated mice. (C) Mean \pm SEM percent of LV infarct size 7 days following I/R event evaluated by Masson's trichrome stain * $p < 0.001$ vs sham operated; $\dagger p = .001$, AAT vs Alb-treated mice. (D-E) Triphenyl tetrazolium chloride (TTC) stain of a midventricular heart section from a representative control mouse treated with albumin (D) or AAT (E) 24h after the I/R event. The infarct area is evident in white/gray within the risk area, which appears red/dark pink. The blue area is nonrisk area. *Alb*, albumin. $N = 6$ mice per group. (F-G) Masson's trichrome stain of a midventricular heart section from a representative control mouse treated with albumin (D) or AAT (E) 7 days after the I/R event. The infarct scar is evident in blue. *Alb*, albumin. $N = 6$ mice per group [334]. Adapted from the personal collection of the authors.

to the IgG Fc-fragment have an influence on pro- and antiinflammatory effector functions [340]. The improvement of clinical outcome in Guillain-Barré syndrome patients was shown to be associated with an increase in IgG glycosylation, especially galactosylation but also sialylation following IVIG treatment [341]. Currently six clinical applications have been approved by the FDA including the treatment of primary immunodeficiencies, the prevention of bacterial infections in patients with hypo- γ -globulinemia or B-cell chronic lymphocytic leukemia, the prevention of coronary artery aneurysms in Kawasaki disease, the prevention of infections, pneumonitis, and acute graft-versus-host disease after bone marrow transplantation, the reduction of serious bacterial infection in children with HIV, and the increase of platelet counts in idiopathic thrombocytopenic purpura to prevent or control bleeding. In addition to these applications, more than 150 off-label uses for IVIG have been reported so far. While some applications are quite controversially due to lacking evidence of effectiveness and safety partly due to low patient numbers with rare diseases [342], IVIGs are used off-label in the first-line

treatment for indications, such as Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, dermatomyositis, stiff-person syndrome, fetal alloimmune hemolytic disease, and various autoimmune diseases [337].

There are considerable differences in the composition of IVIG that seem to have a huge impact on both the treatment efficacy and related side effects. According to the Paul Ehrlich Institute, there are currently 42 different preparations on the market. All products contain IgG molecules (90–99%) but differ considerably in excipient, osmolality, pH, sugar, and sodium content [343]. There have been some reports on patients developing a MI under IVIG treatment [344–356]. These patients received 2g/kg/cycle or lower doses of IVIG and most of them developed a MI within 24h after the first infusion. None of them was treated for immunodeficiency states. In most cases patients had a history of cardiovascular risk factors or diseases including hypertension [348], prior MIs [345,350], recent coronary bypass surgery [353], and diabetes mellitus [347]. While patients without prevalent vascular disorders support the increase in viscosity

caused by the immunoglobulin products without further problems predisposed patients might experience thromboembolic events like MI or stroke as a result [357]. Therefore patients need to be screened for cardiovascular risk factors and caution in the use of IVIGs in patients with known risk factors should be used. Vo and colleagues state that patients who have a high risk of MI or thrombosis should receive aspirin and hydration (250 mL of 0.9 normal saline over 30 min) to reduce the likelihood of adverse events [343]. They observed significant differences in the side-effect pattern of IVIG products and believe that products with high concentrations of sodium (10%) are especially likely to cause MI. While substances with higher osmolality like Polygam might have pronounced prothrombotic properties a possible effect on platelet aggregation is being discussed controversially. According to the experience of Vo and colleagues products with lower osmolality or diluted products should be preferred [343]. Another IVIG preparation called Carimune was shown to induce acute renal failure. Since carimune is a sucrose-based product the osmotic load of sucrose is considered responsible for renal complications. While most patients are reported to eventually regain baseline renal function patients that are prone to develop acute renal failure like patients suffering from diabetes or heart failure should not be treated with sucrose-based preparations [343]. Gamimune-N is iso-osmolar and not associated with an increased number of myocardial infarcts or acute renal failure. A slow infusion rate equivalent to an administration time of 8 h has proven to decrease the number of side effects like headache [343].

In addition to these disadvantageous side effects, which seem to be at least partly predictable and avoidable, IVIG preparations have also proven to be useful in the therapy of cardiovascular diseases or cardiac manifestations of other diseases. The affection of the coronary arteries is a common feature of acute febrile childhood vasculitis of medium-sized vessels called Kawasaki disease [358]. Even though the underlying cause of illness is currently not established it is believed that the disease is of infectious or postinfectious origin. The development of coronary aneurysms presents a major complication of Kawasaki disease. In order to prevent this threat, diseased children receive IVIG in conjunction with aspirin the first 10 days of the syndrome but preferably as soon as the diagnosis is established. It was shown that a single dose of IVIG (2 g/kg) independent of the product used leads to significant decrease in new coronary artery abnormalities 30 days after diagnosis [359]. A meta-analysis revealed that high-dose IVIGs ideally given in combination with low doses of aspirin (≤ 80 mg/kg) have the best effect in preventing coronary artery aneurysms in children with Kawasaki disease [360]. While the exact mechanisms of this protective effect of IVIGs are still

unclear, neutralization of bacterial superantigen toxins, the antiidiotype inhibition of antiendothelial antibodies, the regulation of the cytokine milieu, the inhibition of vascular endothelial activation, and the prevention of complement-mediated tissue damage are thought to play important roles [361,362]. A reduction in fever, neutrophil counts, and acute-phase reactants typically occur within 24 h after treatment [342].

In the setting of a double-blind study, Gullestad et al. stratified 40 patients with LV-EF < 40%, heart failure (NYHA II/III) according to cause of heart failure (ie, ischemic and idiopathic dilated cardiomyopathy) and treated them either with an IVIG preparation or placebo in addition to standard medication [363]. The primary goal of the study was to examine the effect of IVIG on inflammatory and antiinflammatory mediators in heart-failure patients. In addition the influence of the potential changes in the cytokine network was correlated with variations in clinical and hemodynamic variables. IVIG treatment led to a significant increase in LV-EF from baseline $26 \pm 2\%$ to $31 \pm 3\%$ after 6 months ($p < .01$), while this was not the case for the placebo group (28 ± 2 to 29 ± 2). The verum group also showed a significant decrease in mean pulmonary artery pressure (23 ± 2 at baseline to 21 ± 2 at 6 months) and pulmonary capillary wedge pressure (13.6 ± 1.8 to 10.6 ± 1.3), an increase of exercise capacity indicated by an increase in maximum oxygen uptake (peak VO_2 at baseline 1.35 ± 0.11 and 1.43 ± 0.11 after 6 months), and maximum performance (achieved peak exercise capacity in watts 122 ± 12 at baseline and 134 ± 12 after 6 months) as well as quality of life. The improvement of LV-EF correlated with an IVIG treatment induced change in the balance between inflammatory and antiinflammatory mediators in heart failure, favoring an antiinflammatory net effect. IVIG-treated patients showed an increase in circulatory IL-1 α , IL-10, soluble p55-TNFR, and p75-TNFR with a resulting decrease of the ratios of IL-1 β /IL-1 α and TNF- α /sTNFRs [363]. In addition, mononuclear cells from some of these heart-failure patients were examined to see the gene expression of chemokines and their corresponding receptors and compared to the data of healthy blood donors. While various chemokines and their corresponding receptors were increased in the cells of heart-failure patients IVIG treatment modulated the chemokine and receptor levels toward that seen in healthy controls [364]. Another positive effect on heart failure might involve the IVIG-mediated neutralization of autoantibodies, which is thought to be involved in the development of heart failure [363].

Dilated cardiomyopathy (DCM) is another disease that is considered related to autoantibodies targeting different cardiac epitopes such as the β_1 -adrenergic receptor, which led to the notion that IVIGs may be applicable to DCM treatment. While various trials showed that IVIG treatment leads to improvement of LVEF in

patients with DCM [337] the neutralization of anti- β 1 AR autoantibodies was not shown to contribute to this beneficial effect since IVIG application led to an increase in the anti- β 1 AR autoantibodies level rather than the expected decrease [365]. Recently, it was shown that β 1-AR autoantibodies differ in their effects on the adrenergic receptor. While some induce an active receptor confirmation that leads to cAMP signaling, others cause inactive receptor confirmation and are mostly found in healthy people, while still others lead to internalization of the receptor [366]. Therefore, the positive effect of IVIG on the heart function of heart-failure patients may be due to a shift in autoantibody subgroups rather than a decrease in total antibody count, some of which trigger confirmation changes on the receptor level including attenuation of the internalization of the receptor. It might also be possible that IVIG improves LV function independent of the anti- β 1-AR ie, via its antiinflammatory properties [337,365]. In contrast to the first trials the prospective randomized placebo-controlled Intervention in Myocarditis and Acute Cardiomyopathy (IMAC) trial did not find any benefit of IVIG treatment in patients with recent-onset cardiomyopathy [367]. Within the scope of the IMAC trial adult patients with recent-onset dilated cardiomyopathy received a single dose of IVIG or placebo on top of the standard therapy. While both groups showed an unexpected high rate of spontaneous recovery the treatment group showed no increase in LVEF improvement compared to the placebo group [367]. It might be possible that IVIG treatment for DCM is limited to certain product preparation, disease stages or defined subsets of patients.

Since IVIG influences a number of mechanisms involved in the development and progression of atherosclerosis it is also under discussion as a possible therapeutic agent for the therapy of atherosclerosis. IVIG was shown to increase the levels of IL-10, an antiinflammatory cytokine known to play a role in arteriosclerosis and reduction of matrix metalloproteinase-9 secretion. IVIG also contain antibodies against oxidized low-density lipoprotein (ox-LDL). On the one hand, serum autoantibodies against MDA-modified LDL were shown to be associated with carotid artery atherosclerosis progression but on the other hand ox-LDL autoantibodies were also found to have protective effects via reduction of oxLDL uptake by macrophages. These contradictory findings have led to the hypothesis that anti-oxLDL antibodies represent a family of autoantibodies with different properties [368,369]. The antiox-LDL antibody binding to oxLDL is partly blocked by anti-anti-oxLDL also present in IVIG preparations [370]. In LDL-receptor-deficient mice the use of antibodies against CD40 ligands present in arteriosclerotic plaques was found to reduce disease progression. Since these antibodies are also found in IVIG preparations this might be an additional reason

for the beneficial effects of IVIG on atherosclerosis progression seen in animal studies [371]. Further studies are needed to test the possible application of IVIG for atherosclerosis treatment.

Various case reports and small clinical trials suggest that virus-induced myocarditis, lupus-associated myocarditis as well as myocarditis associated with dermatomyositis/polymyositis or Kawasaki syndrome, DCM, and peripartum cardiomyopathy may also be successfully treated using IVIG. In addition to the reduction of proinflammatory cytokines and the reduction of humoral immunity-induced myocyte damage its antiidiotypic properties are thought to play an important role [337,372]. While it is commonly believed that IVIG treatment of myocarditis is more efficient when started early IVIG is also supposed to be effective in the treatment of chronic pericarditis as an alternative to long-standing steroid or colchicine treatment [337]. Other case reports have successfully used IVIG to treat cardiac tamponade following SLE-related pericarditis [373].

12.6 Cytokine Profile-Modulating Therapy

Vasogen Inc., Canada recently developed an immunomodulating therapy (Celacade) for the treatment of chronic heart failure. On a monthly basis about 10 mL blood is taken from the patient, exposed to heat, ozone, and ultraviolet light and then readministered to the patient. The oxidative stress is used to induce apoptosis in the patient's white blood cells [374,375]. The dying cells are then taken up by macrophages, inducing the production of the antiinflammatory cytokines IL-10 and TGF- β [376]. The presence of these cytokines then induces the differentiation of T cells to regulatory T cells, which have been shown to inhibit inflammatory cells by cell-cell interaction and paracrine mechanisms involving the production of antiinflammatory cytokines. Via these mechanisms the immunomodulating therapy is supposed to lead to a reduction in the tissue levels of inflammatory cytokines like IL-6, INF- γ , IL-1 β , and TNF- α [377] partly restoring the balance of pro- and antiinflammatory in patients with heart failure [378]. Celacade treatment was shown to be associated with fewer hospitalizations and deaths as well as improvement in the quality of life and heart failure-related symptoms according to NYHA classification compared to placebo [379]. This pilot study included patients with moderate heart failure and its promising results led to the Advance Chronic Heart Failure Clinical Assessment of Immune Modulation (ACCLAIM, NCT00111969), a large placebo-controlled trial involving 2426 patients with NYHA class II-IV chronic heart failure [375]. The verum group was treated for at least 22 weeks or until study completion, receiving two treatments on consecutive days after randomization, then

on day 14, and subsequently every 4 weeks. While this study was not able to show a difference in time to death from any cause or first hospitalization for cardiovascular reasons between the treatment and the placebo group a posthoc analysis revealed that certain subgroups did benefit from the treatment such as patients with heart failure NYHA II and those without a history of MI [380]. While the study results show that immune modulation therapy may be regarded as a new treatment option for a subgroup of heart-failure patients [378] some question the methodology [381]. The positive effect of the therapy may be limited by a proinflammatory effect of the apoptotic red blood cells present in the treated blood whose cross-talk with immune cells is supposed to have a proatherogenic effect [382].

13. FUTURE PERSPECTIVES

13.1 Hormonal Immunomodulation

Hormones like estrogen and progesterone have been shown to be important modulators of inflammation, immunity, and autoimmunity [383]. The higher prevalence of some autoimmune diseases like SLE in women (overall ratio 9:1) is considered to be at least partly due to the gender-specific differences in hormone levels [384,385]. It also seems reasonable that the pregnancy-associated amelioration of RA is at least partly due to the hormone-mediated suppression of inflammation and the stimulation of immunological tolerance induced in the mother [386]. In order to use this effect of the hormones on autoimmunity for the therapy of autoimmune diseases it is necessary to understand the underlying mechanisms in detail. Currently it remains unclear why the clinical symptoms of RA improve during pregnancy while those of SLE worsen [387]. In this context it was already shown that estrogens amplify the type 1 IFN response and favor the survival of self-reactive B cells as well as the production of pathogenic IgG autoantibodies, thereby increasing the risk of developing SLE [386]. In contrast, progesterone seems to inhibit these effects. The favorable effects of high levels of estrogen and progesterone on the symptoms of RA during pregnancy are thought to involve the suppression of the production and signaling of proinflammatory cytokines, like TNF- α , IL-6, and IL-1 β [388,389] and the induction of proinflammatory T cells [390–392]. In addition, estrogen and progesterone seem to be able to induce the production of autoantibodies with reduced inflammatory potential via alteration of class-switch recombination of B cells. In line with these findings, the use of oral contraceptive agents and hormone replacement therapy were linked to increased risk of SLE. In 2009, a study examined the connection between

the administration of combined oral contraceptives and the risk of developing SLE [393]. The adjusted rate ratio was 1.54 (95% CI 1.15–2.07) for women who were currently using a combined oral contraceptive and even 2.52 (95% CI 1.14–5.57) for those women who had recently started the therapy. The risk of developing SLE increased with the amount of ethinyl estradiol contained in the contraceptive (RR 1.42, 1.63, and 2.92 for ≤ 30 , 31–49, and 50 μg , respectively). Since it is thought that chronic estrogen exposure increases the risk of SLE development, while progesterone might be protective [386], progesterone-only birth control may be associated with decreased risk of SLE as indicated by the Baltimore Lupus Environmental Study [394]. At the same time various studies show that combined oral contraceptives do not increase flares of disease when given to women with inactive or stable active SLE [395] but should not be used in patients suffering from antiphospholipid syndrome who have a history of thrombosis or additional risk factors [396].

13.2 Vaccinations Against Atherosclerosis

Modulation of the adaptive immune response during atherogenesis represents a potential new therapeutic option to reduce the occurrence of atherothrombotic cardiovascular events [397]. The goal would be to provoke an antigen-specific atheroprotective immune response. In order to do so, specific antigens involved in atherogenesis have to be identified. Since LDL and other apoB-100-containing lipoproteins have a strong link to the formation of atherosclerotic plaques they are currently the focus of vaccine development. Many efforts have been made to find potential antigenic epitopes within apoB-100. Fredrikson et al. screened the entire 4536 amino acid human apoB-100 protein [398] and subsequently identified several immunoreactive peptides that were shown to cause a 40–70% reduction in atherosclerosis and plaque inflammation when used in vaccine formulation in hypercholesterolemic mice [399,400]. Another group identified MHC-II restricted peptides from ApoB-100 that reduced atherosclerosis in ApoE knockout mice [401]. Likewise, a variety of vaccines have been developed including antibodies targeting epitopes in oxLDL and formulations consisting of heat-shock proteins [397]. Many substances are currently still in the state of preclinical testing and may offer an option to affirmatively influence plaque inflammation and disease progression [402]. But before they enter clinical testing many questions have to be answered involving the proper dosage, application mode, time and frequency, interactions between adjuvants used, and occurrence of possible side effects [397].

In conclusion, immunomodulating strategies used in rheumatic and autoimmune diseases are associated

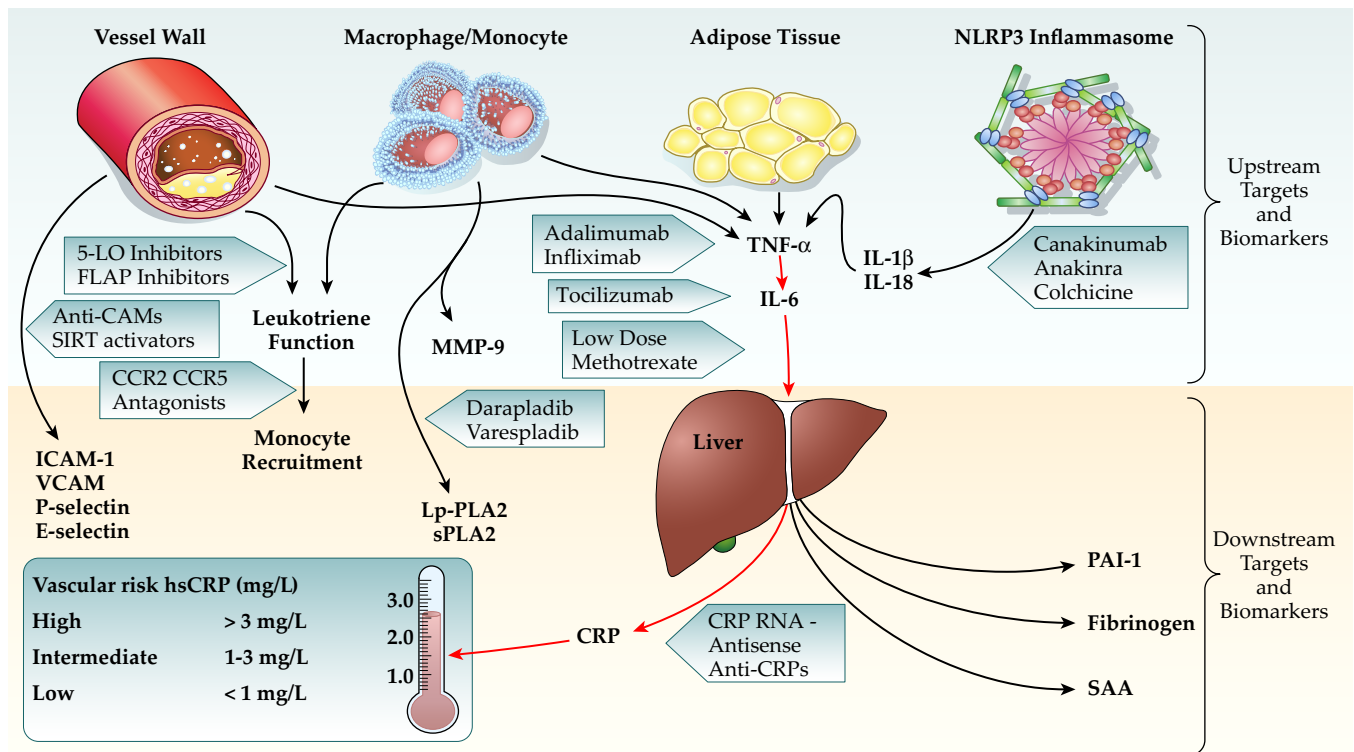


FIGURE 28.8 Targeting inflammatory pathways for the treatment of cardiovascular disease. Inhibition of the phospholipase A₂ superfamily members lipoprotein-associated phospholipase A₂ (Lp-PLA₂) by darapladib or the secretory PLA₂ (sPLA₂) by varespladib results in the reduction of potentially atherogenic lipid fractions and oxidative stress. Targeting interleukin-6 (IL-6) and (tumor necrosis factor- α (TNF- α) via low-dose methotrexate was shown to reduce the risk of future cardiovascular events by 21% [407]. Colchicine has a variety of antiinflammatory properties including the inhibition of neutrophil function, the NLRP3 inflammasome and the release of interleukin 1 β (IL-1 β), interleukin-18 (IL-18), and the C-reactive protein (CRP). Its application is supposed to be associated with a reduction in the recurrence of acute coronary syndromes [408]. CRP can also be targeted directly using CRP RNA antisense and anti-CRPs leading to a reduction in the widely used biomarker high-sensitivity (hs) CRP. The acute-phase proteins plasminogen activator inhibitor type-1 (PAI-1), fibrinogen, and serum amyloid A (SAA) represent further biomarkers that seem to be associated with the risk of atherosclerosis. Since an increase in expression of the 5-lipoxygenase (5-LO) has been shown to be associated with unstable atheroma and chronic ischaemia while increased levels of the 5-LO-activating protein (FLAP) seem to be associated with increased risk of myocardial infarction [409], 5-LO and FLAP inhibitors represent another possible new therapeutic option for cardiovascular diseases. Antagonists of chemokine receptor type 2 (CCR2) and chemokine receptor type 5 (CCR5), which are involved in monocyte chemotaxis, are able to inhibit the monocyte infiltration in inflammatory diseases. Adhesion molecules like intercellular adhesion molecule type 1 (ICAM-1), vascular cellular adhesion molecule (VCAM), E-selectin, and P-selectin play a crucial role in the recruitment and adhesion of leukocytes during inflammation and may be blocked using anti-CAMs. Activators of sirtuin-1 (SIRT1) aim to increase the protein's protective effects involving inhibition of atherothrombosis and the promotion of angiogenesis [410,411]. Abbreviation: MMP-9, matrix metalloproteinase-9; SIRT1, sirtuin-1. Adapted from Ridker and Luscher [113].

with various adverse or beneficial cardiovascular side effects. Since autoimmune diseases themselves have an influence on classical cardiac risk factors like LDL levels [125] and are known to be associated with increased cardiovascular-related mortality [2–4], pinpointing the cardiovascular risk profile of therapeutics for autoimmune diseases appears complicated [5]. Still, some substances like omalizumab and leflunomide have been associated with increased cardiovascular risk and should only be used with caution in patients and if possible not in patients with known cardiovascular risk factors. Likewise, there are reports linking the TNF- α antibodies infliximab, adalimumab, and etanercept as well as CD20

antibodies to the development of arrhythmias. Therefore in particular patients with heart failure who are more prone to develop arrhythmias should be treated with alternative substances.

At the same time some immunomodulating therapies are used for the treatment of cardiovascular diseases. The inflammatory pathway contains a variety of potential targets for therapeutic interventions not limited to those seen in Fig. 28.8. IL-1 β blockers such as anakinra and canakinumab seem especially promising in this regard. First studies show beneficial effects of anakinra in the treatment of heart failure. Currently the large multicenter trial CANTOS is underway to confirm the value

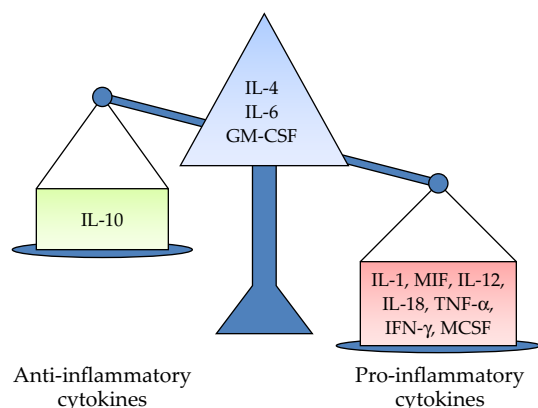


FIGURE 28.9 Atherosclerosis development is triggered by an imbalance of proinflammatory and antiinflammatory cytokines. IL-4, IL-6, and GM-CSF seem to play variable roles. Adapted from Kleemann *et al.* [403].

of anakinra in heart-failure therapy, while the D-HART2 trial aims to evaluate the potential role of canakinumab in the prevention of the recurrence of cardiovascular events. At the same time other promising therapeutic options like targeting IL-17, which has been linked to the development of various cardiovascular risk factors and diseases, have yet to be clinically tested for cardiovascular applications. Taken together, the novel strategies in the therapy of cardiovascular diseases or cardiac manifestations of inflammatory diseases are for the most part still in the trial phase and need further evaluation and improvement. Current evidence is mainly based on smaller trials or case reports. For most substances, large prospective multicenter, double-blind studies would be useful to investigate their true treatment properties. Detailed subgroup analysis may identify patient characteristics associated with an advantageous disease response or would help to improve formulations. With respect to potential cardiovascular target diseases, atherosclerosis in particular seems to be of great interest given that its pathogenesis is strongly mediated or influenced by cytokines (Fig. 28.9). An imbalance of proinflammatory and antiinflammatory cytokines was shown to promote the progression of heart failure [403]. Possibly combining approaches that suppress proatherogenic cytokines with interventions that increase antiatherogenic factors might be a reasonable path for future developments of therapeutic concepts. Since interleukins are indispensable for infection control and severe infections represent the most common side effects of interleukin inhibition it might be advantageous to target molecules that are involved in the regulation of cytokine levels rather than blocking the cytokines themselves. Proteins like galectin-1, which was shown to influence the expression and secretion of IL-17 [404–406], might be useful to tone down inflammation while keeping up essential defense mechanisms.

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