Infectious Disease
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Duane R. Hospenthal
Michael G. Rinaldi *Editors*

Diagnosis and Treatment of Fungal Infections

Second Edition

Springer

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Editors

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Preface

Since publication of *Diagnosis and Treatment of Human Mycoses* in 2008 fungi have continued to emerge as important agents of human infection. Fungal infections (mycoses) continue to plague humankind as the at-risk population continues to expand with more immunosuppressive therapies, enlarging populations receiving cancer therapy, and continued support of our most ill in intensive care units and with broad-spectrum antibacterial agents. *Diagnosis and Treatment of Fungal Infections, 2nd Edition* again brings together globally recognized experts to guide readers in the use of our current knowledge to diagnose and treat patients with fungal infections.

In addition to basic and directed culturing techniques, histopathology, serological methods, and radiological studies, molecular biology techniques continue to improve our ability to diagnose fungal infection and identify the offending fungus. Genotypic identification has led to an expansion of our understanding of the fungal pathogens and has led to many new fungi being identified as the cause of human infection. This, and recent changes in taxonomy, can lead to confusion in keeping up with the most proper name for any recovered fungus and difficulty in identifying the appropriate medical literature to review.

We currently have three major classes of antifungal agents to choose from for systemic treatment of fungal infections. These include amphotericin B and the echinocandin and triazole antifungals. Selecting which drug to use can be difficult in the empirical setting and targeted therapy typically requires identification of the pathogen to species level. Antifungal susceptibility testing can assist in selecting the best antifungal drug to use, but clinical correlation of this testing with treatment success remains limited to the *Candida* species.

Diagnosis and Treatment of Fungal Infection, 2nd Edition is meant to be a concise text that will provide the busy infectious disease, hematology-oncology, pulmonology, or critical care specialist a practical tool to diagnose and manage fungal infections. In addition, the depth of the material in the text will provide these and other medical specialists and trainees an excellent reference and learning resource.

The text is divided into four parts to guide the reader. Part I provides a general introduction to the epidemiology of fungal infections and presents practical approaches for using patient risk factors, exposures, and site of infection to direct diagnostic evaluations. Part II introduces the science of mycology and the current tools available to diagnose fungal infections using basic clinical mycology laboratory techniques, with molecular biology, histopathology and immunology, and with radiological technologies. Part III provides a review of the available antifungal drugs, their use, and discussion of resistance and antifungal susceptibility testing. Part IV reviews fungal infections (mycoses) in 15 uniform, easy to read chapters, with accompanying tables and figures.

Duane R. Hospenthal, MD, PhD
Michael G. Rinaldi, PhD

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Part I
Approach to Patients

Approach to Patients with Suspected Fungal Infections

1

Duane R. Hospenthal

Introduction

Fungal infections (mycoses) are increasing in incidence throughout the world as a result of modern medical practice and rise in the population of those at risk. Supporting this increase is the expanding use of immunosuppressive therapies, broad-spectrum antibiotics, and central venous access devices. Technology has led to the improved survival of persons with malignancies, transplanted organs, HIV infection, following trauma, and at the extremes of age. The medical community has met this challenge with the introduction of new antifungal agents, often with less toxicity and improved spectrums of activity. Additionally, newer, more sensitive and specific diagnostic strategies such as improved radiographic imaging and serological tests, have provided clinicians with better tools to detect fungal infections earlier, potentially influencing disease outcomes. Molecular techniques have been introduced in the last decade which can produce a more exact identification of recovered fungal pathogens and have the potential to improve diagnosis of fungal infection. Despite these advances, the approach to the diagnosis and management of fungal infections still relies on recognizing the interaction of the pathogen and the host. Although some fungal diseases have classic presentations, many of these occur so rarely that clinicians may not initially include them in their differential diagnoses. In the setting of immunosuppression, mycoses may produce nonspecific signs and symptoms, making their diagnosis a challenge. Early recognition and treatment is fundamental to modifying disease outcomes in many fungal infections, especially those in immunocompromised individuals. Increased awareness of key risk factors and clinical presentations of the human mycoses may enable clinicians to develop an inclusive approach to the diagnosis of these diseases.

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Epidemiology

Deaths associated with mycoses have increased in the USA, moving from the tenth most common infectious disease cause of death in 1980 to the seventh in 1997 [1]. Sepsis due to fungal infection increased over 200 % in the USA between 1979 and 2000 [2]. Fungal sepsis is chiefly secondary to candidemia. *Candida* continues to be the fourth most common organism recovered from bloodstream infections in the USA; associated with a crude mortality of about 40 % [3, 4]. Candidemia and disseminated (also termed systemic or invasive) candidiasis continues to be the most common cause of nosocomial fungal infections, responsible for more than 80 % of these infections and up to 15 % of nosocomial infections overall. Infections with *Candida* have declined in patients with cancer and undergoing hematopoietic stem cell transplantation (HSCT), likely in association with antifungal prophylaxis. Candidemia, after surging in numbers in the 1980s appears to have declined, at least in the intensive care setting [5]. This overall decline is chiefly due to fewer infections with *C. albicans,* as *nonalbicans Candida* (NAC) candidemia has increased over this same period, 1989–1999.

Opportunistic mold infections, most commonly caused by the *Aspergillus* species, continue to expand their range of hosts from severely neutropenic cancer patients to patients with other risk factors, including prolonged immunosuppressive therapies with corticosteroids and newer agents, including those that inhibit tumor necrosis factor alpha (TNF-α) [6]. *Aspergillus* is the second most common cause of nosocomial fungal infection and the most common mold to cause invasive mycosis. Other rare opportunistic molds (e.g., the Mucorales, *Fusarium,* and *Scedosporium*) and yeasts (e.g., *Trichosporon* and *Malassezia*) have emerged as more frequently causes of disease in patients with a wide range of risks [7–13].

Outbreaks of endemic mycoses, including coccidioidomycosis in association with the growing urbanization of the US Southwest, and on a smaller scale, histoplasmosis, continue to be reported more frequently, often affecting

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greater numbers of persons. Outbreaks of endemic disease are occasionally diagnosed outside their known geographical areas, occurring in travelers to those locales. An outbreak of infection with the *nonneoformans Cryptococcus, C. gattii,* in mostly immunocompetent patients, has been going on in the US Northwest and Southwest Canada (Vancouver Island) over the past decade [14, 15].

Suspicion Based on Risk Factors

The risks for fungal infections are highly dependent on the combination of host immune competency and the specific exposures people have both within the health care system and in their communities.

Immunocompromise

Host immune status is probably the most import underlying factor determining whether people develop life-threatening, self-limiting, or no infection following exposure to fungi in their environment. Defense against invasive mycoses depends chiefly on intact mucosal barriers, the innate immunity provided by phagocytic cells, and cell-mediated immunity (CMI). The impact of humoral immunity is limited and remains poorly defined in defense against the fungi.

Neutropenia and Altered Phagocytic Function

Classically, neutropenia has been associated with candidemia and invasive candidiasis. With prolonged neutropenia, *Aspergillus* species become more common causes of infection. Infection with the Mucorales, *Fusarium, Scedosporium, Trichosporon,* and other rare species can also be seen with prolonged loss of neutrophils. The incidence of candidiasis in the highest-risk populations appears to have declined over the past decade in association with antifungal prophylaxis of these patients. This decrease has been associated with an increase in aspergillosis and other invasive mold infections. In addition to insufficient numbers of neutrophils, declination in phagocytic function also raises the risk of mycoses. The phagocytic dysfunction seen in chronic granulomatous disease (CGD) is associated with fungal infections, especially aspergillosis.

Impaired Cell-Mediated Immunity

Impaired CMI occurs in patients infected with HIV and those receiving many of the currently used immunosuppressive therapies. Impairment of CMI is associated with mucocutaneous candidiasis, *Pneumocystis* pneumonia, infection with *Cryptococcus,* and more severe and/or disseminated endemic mycoses. The specific mycoses associated with $CD4^+$ T lymphocyte decline as seen in HIV/AIDS have been carefully documented, allowing the clinician to increase their level of suspicion for particular fungal infections based on $CD4^+$ T lymphocyte counts of their patients (Table 1.1).

Table 1.1 Mycoses commonly associated with HIV infection

$CD4^+$ T lymphocyte cell count (cells/μl)	Fungal infections
>500	Candidal vaginitis
200–500	Thrush (oropharyngeal candidiasis)
<200	PCP, disseminated histoplasmosis, disseminated coccidioidomycosis
<100	Cryptococcosis, candidal esophagitis, penicilliosis

PCP Pneumocystis pneumonia

Table 1.2 Fungi associated with hematopoietic stem cell transplantation

Time period	Common fungi	Other fungi
Preengraftment (<30 days)	*Candida*	*Aspergillus*
Postengraphment (30–100 days)	*Aspergillus, Candida, Pneumocystis*	Mucorales, *Fusarium, Pseudallescheria* (*Scedosporium*)
Late (>100 days)	*Aspergillus, Pneumocystis*	

Organ Transplantation

Solid organ and HSCT recipients are at great risk for fungal infections [16–18]. In addition to immunosuppressive therapies, the mucosal damage and intensive therapy associated with these procedures place the persons who receive them at risk for the entire spectrum of fungal disease. Transplant medicine has seen substantial advancements in tailoring regimens to minimize the duration of neutropenia and to reduce immunosuppressive treatments used to control rejection. Unfortunately, most of these still place patients at a substantial risk for opportunistic infections. In solid organ transplantation, the risk of fungal infection is associated with risk surrounding the initial surgery and the use of immunosuppression to prevent rejection. This risk varies greatly based on organ transplanted and underlying condition of the recipient. As an example, in liver transplantation, the substantial risk of *Candida* infection in the first month is mostly associated with surgical manipulation of the gastrointestinal tract and the need for intensive care monitoring, as well as initial immunosuppressive agents given to control rejection (Table 1.2). Lung transplants are at high risk for invasive pulmonary aspergillosis, likely secondary to the route of inoculation and immunosuppression. Although a similar sequence of occurrence of fungal infection is seen in HSCT, the underlying factors creating risk differ from those of solid organ transplant (Table 1.3). In HSCT, initial conditioning commonly leads to neutropenia and breakdown of the mucosal surfaces. This neutropenia can be prolonged and as-

Table 1.3 Fungi associated with solid organ transplantation. (Table produced from data in reference [16])

Time period	Common fungi	Other fungi
First month	*Candida*	
1–6 months	*Aspergillus, Pneumocystis, Cryptococcus*	Endemic fungi[a]
>6 months	Endemic fungi[a]	*Cryptococcus*

[a] Chiefly, coccidioides and histoplasma

sociated with life-threatening mold infections. In allogeneic HSCT, graft-versus-host disease (GvHD) and its treatment may put the patient at risk for fungal infection for a prolonged period of time following engraftment.

Table 1.4 Risk factors commonly associated with health care-associated invasive mycoses (Table produced from data in reference [17])

Mycosis	Risk factors
Candidiasis	*Candida* colonization, surgery (especially abdominal), acute renal failure, parenteral nutrition, central venous catheters, neutropenia, broad-spectrum antibacterial antimicrobials, mucosal surface disruption
Aspergillosis	Prolonged neutropenia, corticosteroids, neutrophil dysfunction, hematologic malignancy, cytotoxic drugs, AIDS, HSCT (highest in allogeneic), solid organ transplantation (highest heart-lung), underlying lung disease, GvHD, GvHD therapies (TNF-α blockers)

HSCT hematopoietic stem cell transplantation, *GvHD* graft-versus-host disease, *TNF*-α tumor necrosis factor alpha

Health Care Exposure (Nosocomial)

A multitude of risk factors for nosocomial fungal infections have been identified (Table 1.4) [6, 19, 20]. Unfortunately, many of these health care-associated risk factors overlap with those associated with bacterial infections or are risks that are common to many or most hospitalized patients. This is especially true for those patients hospitalized in intensive care units, the majority of whom have central venous catheters and are receiving broad-spectrum antibiotics [21, 22]. In addition to the use of vascular catheters, other procedures including urinary catheterization and intubation establish portals of entry for fungal pathogens. Other risk factors include immunosuppression seen with the use of corticosteroids and chemotherapy, and with malnutrition and malignancy. Infusion of contaminated infusates, inclusion of lipids in parenteral nutrition, and construction within the hospital are additional exposures that can lead to fungal infections. A few specific risks allow the clinician to suspect certain fungi. Ketoacidosis and deferoxamine therapy has been clearly shown to be a risk for mucormycosis (zygomycosis). Unfortunately, given the overlapping nature of most of these risk factors with those associated with bacterial infections, it is often difficult to apply these risk factors to differentiate patients at higher risk of fungal versus bacterial infection.

Community Exposure

The fungi that cause community-acquired infections commonly originate in the environment and are "true pathogens" (i.e., cause disease in persons with normal immune status). Most are restricted to certain geographic environments or exposure risks (Table 1.5). The source of disease includes inhalation, ingestion, or traumatic inoculation of the fungi. Diseases most commonly afflict the lungs, paranasal sinuses, skin, and soft tissues. Rarely, disseminated, central nervous system, or osteoarticular disease occurs. The most commonly recognized community-acquired infections are the endemic mycoses, each with their limited geographical areas of exposure. With the extensive use of antibiotics, corticosteroids, and other immune modulators in the community, as well as the increased number of elderly, and population of immunocompromised persons receiving their care outside of the hospital, the boundaries between community-acquired and health care-associated infection have become blurred.

Other Risks

Other risks or probable risks associated with immune competency or genetic disposition include gender and race. The role of gender and potentially inhibitory effect of estrogen has been postulated to be important in the risk of clinical paracoccidioidomycosis. A clear risk exists for disseminated coccidioidomycosis in women when disease is acquired in pregnancy. Disseminated and severe coccidioidomycosis has also been associated with Filipino and African descent.

The use of antifungal therapy or prophylaxis in populations at risk should also be kept in mind when evaluating patients for potential fungal infections. The last decade has seen an emergence of NAC, *nonfumigatus Aspergillus* infection, and increased numbers of infections with the more rare yeasts and molds. This shift appears to reflect our greater usage of antifungals and use of the newer agents. Included in this change in epidemiology is the emergence of fluconazole-resistant *Candida* (i.e., *C. krusei*) and recent increase in *non-Aspergillus* molds (e.g., the Mucorales, *Fusarium,* and *Scedosporium*) which have decreased susceptibility or resistance to many of the currently available antifungal agents.

Suspicion Based on Organs Involved

Although the fungi may and often do cause disease in more than one organ system, many of these are associated with certain organ system infections. The presentation of disease

Table 1.5 Geographic areas in which the endemic mycoses reside

Mycosis	Region	Specific countries/areas with increased prevalence	Associated exposure risks[a]
Blastomycosis	North America[b]	Southeastern and South central USA, Canada	Soil exposure near fresh water (fishing, hunting, farming, construction)
Coccidioidomycosis	Western hemisphere	Southwestern USA, Central and South America	Soil/dust exposure (construction, archeology)
Histoplasmosis	Worldwide	Mississippi and Ohio River valleys, Western Africa	Soil or organic material associated with bird or bat guano (construction, demolition, spelunking)
Paracoccidioidomycosis	Latin America	Brazil, Columbia, Venezuela, Ecuador, Argentina	Farming or other outdoor employment
Penicilliosis[c]	Southeast Asia	China, Northeast India, Taiwan, Thailand, Vietnam	Rice farming, rodent burrows
Sporotrichosis	Worldwide	North America, Japan	Gardening, sphagnum moss, hay, roses/ thorns

[a] Not all well-proven
[b] Rare reports from Africa, Central and South America, India, and the Middle East
[c] Restricted almost exclusively to persons with AIDS

Table 1.6 Mycosis by organ system chiefly affected

Focus of disease on presentation	Community-associated fungi	Health care-associated fungi
Pulmonary	*Blastomyces Coccidioides, Histoplasma, Paracoccidioides*	*Aspergillus,* Mucorales*, Pseudallescheria* (*Scedosporium*)*, Fusarium, Cryptococcus, Pneumocystis*
Superficial/cutaneous/ subcutaneous	Dermatophytes (*Trichophyton, Microsporum, Epidermophyton*), *Candida, Malassezia,* agents of mycetoma*,* agents of chromblastomycosis*, Blastomyces, Paracoccidioides, Cryptococcus, Sporothrix,* Mucorales*,* phaeohyphomycetes*, Lacazia*	*Candida, Fusarium, Trichosporon*
Bone and joint	*Blastomyces, Coccidioides, Histoplasma, Paracoccidioides, Sporothrix*	*Candida, Cryptococcus*
Central nervous system	*Cryptococcus, Coccidioides, Blastomyces, Histoplasma,* phaeohyphomycetes*, Pseudallescheria* (*Scedosporium*)	*Aspergillus, Candida*
Genitourinary	*Blastomyces, Coccidioides, Histoplasma*	*Candida, Trichosporon*
Oral	*Histoplasma, Paracoccidioides, Candida*	*Candida*
Eye	Keratitis—*Candida, Aspergillus, Fusarium,* phaeohyphomycetes, other hyalohyphomycetes	Endophthalmitis/retinitis—*Candida*
Disseminated disease	*Coccidioides, Histoplasma, Paracoccidioides, Penicillium marneffei*	*Candida, Aspergillus, Fusarium,* Mucorales, *Cryptococcus, Trichosporon* and other rare yeasts

(e.g., prolonged or chronic pneumonia with lymphadenopathy on chest radiography) can guide the clinician to the diagnosis. Disease localization and presentation can be altered based upon the host immune system, route of pathogen inoculation (e.g., inhalation, cutaneous inoculation, ingestion), and quantity of inoculum. The most common presentations are pulmonary, cutaneous/subcutaneous, and disseminated diseases (Table 1.6). Other presentations include those localized or involving the central nervous system, bones, joints, genitourinary tract, oral cavity, eyes, or gastrointestinal tract. Fungal infection can affect any organ or system, often following asymptomatic respiratory system colonization and dissemination. The fungus recovered at a specific site may portend varying diagnoses based on the combination of fungus and site, often modified by patient immune status. Oral lesions in histoplasmosis or paracoccidioidomycosis typically indicate the presence of disseminated disease. Oral lesions from *Candida* in a patient recently given a short course of corticosteroids likely only indicate mild, transient, localized disease.

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Other Key Resources

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www.doctorfungus.org is an excellent internet resource for information about current taxonomy and other quick reference material.

Part II
Laboratory and Radiological Diagnosis

Basic Mycology

2

Deanna A. Sutton

Introduction

The mycology laboratory plays a vital role in the diagnosis of fungal infections through the recovery and identification of the etiologic agent. Specimen collection from appropriate sites is critical, as is the proper transport, storage, and processing of samples. Fungal elements seen by direct microscopy often provide the first clues to a fungal infection, and are the basis upon which empiric therapy is initiated. To ensure recovery of the fungus, a sufficient number and type of media should be utilized for primary isolation based upon the clinical history and the possible organisms expected. Accurate fungal identification, in combination with antifungal susceptibility testing, provides the basis for appropriate organism-directed antifungal therapy and is essential for conducting epidemiologic investigations.

Human and/or animal pathogens historically considered to be fungal are now placed in three kingdoms: *Fungi, Straminipila* (*Stramenopila*), containing the Oomycete *Pythium* [1], and *Protoctista,* with the bulk of the human pathogens in the kingdom Fungi [2]. Organisms within this kingdom are eukaryotic (have cells containing a membrane-bound nucleus), heterotrophic (lack chlorophyll or other pigments capable of photosynthesis for making food, therefore must obtain nourishment from an external food source), may be unicellular or filamentous, and have cells surrounded by cell walls containing glucan, chitin, or both. Unlike animals, fungi possess cell walls, but unlike plants, the major cell wall component is not cellulose. In the past, medical problems attributed to these organisms, in comparison to those caused by the bacteria, viruses, and parasites, have been relatively few, and included allergic symptoms, mushroom poisoning, mycotoxicoses from ingested fungal toxins, and occasional fungal infections [2]. However, with the advent of modern medical advances utilizing immunosuppressive regimens, and with an increase in diseases/underlying conditions significantly altering the human immune system, fungal infections (mycoses) have increased significantly over the past couple decades. The recovery of these organisms from host tissue and their identification is often critical to the diagnosis and treatment of mycotic disease and is the classic method for documentation of pathogenicity. Histopathology, and other adjunctive tools, such as antigen or antibody assays and molecular techniques, addressed elsewhere in this text, may also be relied upon for empiric/preemptive therapeutic decisions, when cultures are either not available or fail to provide unequivocal information. The proper collection, transport, and processing of specimens; selection of fungal stains and preliminary direct microscopy techniques; and use of appropriate media and incubation conditions are all important to the accurate identification of fungal infection. This chapter provides a cursory review of the laboratory fundamentals as they relate to medical mycology. It also reviews basic taxonomy, classification, and nomenclature regarding the kingdom fungi and changes resulting from the Melbourne Code implemented in January 2013 [3]. Also included are descriptions of mycologic terms/features common to the most frequently recovered etiologic agents in the teleomorphic (sexual) phyla Ascomycota, Basidiomycota, and Glomeromycota (formerly Zygomycota) [4] as well as those seen in the anamorphic (asexual) fungi. Fungi without known sexual states are referred to as "mitosporic" (based upon their reproductive mitotic processes). The mitosporic fungi are the most common etiologic agents of human and animal disease.

Specimen Collection, Transport, and Processing

The likelihood of recovering a fungal etiologic agent is directly proportional to the quality of methods employed in the collection, transport, and processing of clinical

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Table 2.1 Common specimen collection sites for fungal cultures[a]

Collection site	Comments
Abscesses, subcutaneous sites	Aspirate abscess; sample base of subcutaneous lesions
Blood	Use maximum amount of blood recommended for the system being used
Bone marrow	Pediatric Isolator™ recommended[b]
CSF	Do not refrigerate
Draining sinus tracts	Search for granules of eumycotic mycetoma; wash several times with saline containing antibiotics
Ear	Rotate swab firmly in outer ear
Eye	Inoculate corneal scrapings directly onto plates in a "C" shape
Hair	Use forceps to collect several hairs with shaft intact and sample any active lesions
Intravenous catheters	Use Maki ("roll") method
Lower respiratory	Process promptly for dimorphic pathogens (BAL, brush, aspirate, wash, sputum)
Medical devices (valves, hardware, etc.)	Dislodge any biofilms before inoculation into liquid medium
Nails	For dermatophytes, agents of dermatomycoses, and *Candida* spp.; clean with 70% alcohol; collect subungual debris and clip affected nails
Nasal sinus	Surgical collection, commonly ethmoid and maxillary sinuses
Open wound	Aspirate or swab vigorously
Prostatic fluid	Primarily for blastomycosis
Skin	For dermatophytes; clean with 70% alcohol and scrape vigorously
Sterile body fluids	May be concentrated by centrifugation or syringe filtration
Tissue	Surgical collection; use punch biopsies for skin lesions
Urine	Early morning midstream collection
Vagina	Primarily for refractory vaginal candidiasis
Vitreous fluid	Needle aspiration
Upper respiratory (oral)	Swab lesions, use selective media for yeasts

CSF cerebrospinal fluid, *BAL* bronchoalveolar lavage

[a] This list is not all inclusive. Also see Appendix A in reference [5]

[b] Wampole Laboratories, Princeton, NJ

specimens, and with the 2012 publication of *Principles and Procedures for Detection of Fungi in Clinical Specimens—Direct Examination and Culture*: *Approved Guideline, M54-A,* by the Clinical and Laboratory Standards Institute (CLSI), this information is now condensed into a readily available and user-friendly document addressing all aspects of these processes for the improved recovery of fungal isolates [5]. For all disease processes, recovery is highest from an active site of infection. Common (but not all inclusive) specimen types include those from the respiratory tract [6], draining sites, aspirated abscess fluids, normally sterile body fluids, urine [7], vaginal secretions [8], corneal scrapings [9], surgical tissue specimens, intravenous catheter tips (obtained by the Maki roll method [10]), and various surgically removed medical devices [11]. Although tissue may be homogenized for the recovery of *Histoplasma capsulatum,* when the patient history suggests infection with a member of the *Mucorales* or other filamentous fungi, tissue grinding should be avoided as it may be deleterious to the growth in culture of fragile fungal hyphae [4, 11]. Specimens peripheral to the site of infection, such as blood or bone marrow, may be diagnostic in disseminated disease or when foci are not easily accessible. Several blood culture systems reliably recover yeast pathogens [12]. If manual blood cultures are used, consider a broth/agar biphasic system in which an agar paddle is attached to the bottle (Septi-Chek, BD Diagnostic Systems, Sparks, MD). Several automated, continuously monitored blood culture systems are available with instruments designed and scalable for both high- and small-volume laboratories. These include the VersaTREK (Trek Diagnostic Systems/Thermo Scientific, Cleveland, Ohio), BacT/Alert (bio Mérieux, Durham, NC), and BACTEC (BD Diagnostic Systems, Sparks, MD) systems [13–19]. Always follow manufacturer's recommendations for the specific system, using the maximum amount of blood samples recommended. The ratio of blood to broth is the most critical factor in fungal recovery, and should be near 1:5 in most systems [20]. Lysis centrifugation methods, either commercially available as the Isolator™ system (Alere, Waltham, MA) or manual methods [21, 22], are recommended for dimorphic fungal pathogens and filamentous fungi [23, 24]. Intravascular catheter tips are also frequently submitted, and should be cultured according to the semiquantitative method of Maki [10]. Blood cultures should also be drawn at the time of catheter removal to correlate catheter colony counts and organisms recovered with catheter-related septicemia. Catheter colony counts of less than 15 are less likely to be associated with infection. Specimens should be maintained at room temperature and transported to the laboratory as soon as possible, ideally within 2 h. Exceptions include storage of central nervous system specimens at 30 °C, and 4 °C extended storage for specimens likely to have bacterial contamination. Hair, skin, and nails

Table 2.2 Useful direct microscopy methods for the routine mycology laboratory[a]

Method	Comments
Calcofluor white	Requires fluorescence microscope; can be used with KOH to detect all fungi, including *Pneumocystis*
Gram stain	Detects most fungi, which are present; however, *Cryptococcus* spp. may exhibit only faint staining
Giemsa stain	Several modifications; detects intracellular *H. capsulatum* and intracystic bodies and trophozoites of *Pneumocystis*
India ink stain	Commonly used from demonstration of capsular material of *Cryptococcus neoformans* in CSF
Potassium hydroxide	Clears debris so fungi are more readily observed; stains may be added for better visualization of fungal elements
Wright stain	Useful to detect intracellular *H. capsulatum* in bone marrow and peripheral smears

CSF cerebrospinal fluid
[a] Additional fungal stains are available through the histopathology laboratory. This list is not all inclusive

Table 2.3 Media useful for primary isolation and identification[a]

Medium	Uses/Comments
Sabouraud dextrose agar (SDA)	For yeasts
	Usually adequate for aspergilli
	Poor color and conidiation for black moulds
	Classic morphologic descriptions for dermatophytes
CHROMagar Candida[b]	Contains chromogenic substrates and antimicrobial agents; for isolation and identification of yeasts
Albicans ID[c]	As above
Potato dextrose agar (PDA)	Useful for all mould recovery/identification
Potato flakes agar (PFA)	
Brain heart infusion (BHI) agar	
Inhibitory mould agar (IMA)	
Yeast extract phosphate medium	
Sabhi agar	
Mycosel agar™ [§] or Mycobiotic agar	SDA with chloramphenicol and cycloheximide
Dermatophyte test medium (DTM)	
Dermatophyte identification medium (DIM)	

[a] This list is not all inclusive. All are commercially available. Please also see Sect. 10.2 in reference [5]
[b] CHROMagar Microbiology, Paris, France
[c] bioMérieux, Marcy l'Etoile, France
[d] BD Diagnostic Systems, Sparks, MD

may be transported in clean paper envelopes. Several sources provide specific guidelines for the collection, transport, and processing of various types of specimens for fungal culture [5, 11, 25, 26]. See Table 2.1 for common collection sites and consult Appendix A in the CLSI M54-A document [5] for more detailed information/procedures.

Prior to receipt in the mycology laboratory, a portion of all tissue samples submitted for culture should also be placed in formalin for submission to the histology laboratory. Histopathologic examination together with appropriate stains are usually necessary to document fungal invasion. These may include the routine hematoxylin and eosin (H&E) stain, Gomori methenamine silver (GMS) stain, periodic acid–Schiff (PAS) stain, and others. Discussion of the use of histopathology and of mycological stains is found in Chap. 4 of this book. See also Appendix C of the CLSI M54-A document [5]. As a part of routine processing, the mycology laboratory should also examine a portion of the specimen directly by microscopy, typically with the use of a potassium hydroxide (KOH) preparation, gram stain, calcofluor white fluorescent stain, India ink stain (limited to cerebrospinal fluid examination for *Cryptococcus neoformans*), or some other method (Table 2.2). Observation of fungal structures by direct microscopy and/or histopathology is essential to corroborate organism recovery in culture (rule out contamination). Table 9.4 in Sect. 9.1.1 of the CLSI M54-A document [5] provides a useful listing/description of yeasts and yeast-like fungi seen in clinical specimens.

The media used for primary isolation may vary according to personal preferences; however, certain basic tenets apply to all media used for primary recovery. Material from nonsterile sites should be cultured on media that will support fungal growth but also inhibit bacteria. Antibacterial agents, alone or in combination, are added for this purpose.

Common choices include chloramphenicol (<16 μg/ml), gentamicin (5–100 μg/ml), penicillin (20 μg/ml), streptomycin (40 μg/ml), and ciprofloxacin (5 μg/ml). These agents should not be included, however, when actinomycetes are suspected. Media may also be made selective by the addition of the eukaryotic protein synthesis inhibitor cycloheximide at 0.5 μg/ml. This may be useful in the detection of dimorphic fungi and dermatophytes; however, many clinically significant saprobic fungi may be suppressed, leading to failure in recovering opportunistic etiologic agents in compromised hosts. Therefore, media with and without this agent should routinely be employed. Enriched media with 5–10 %, sheep erythrocytes may be incorporated into the battery for fastidious thermally dimorphic fungi such as *H. capsulatum* and *Blastomyces dermatitidis*. Peptone-based versus plant-based media may also be a consideration. Many of the opportunistic filamentous fungi prefer plant-based media, producing more typical colony morphologies and more diagnostic structures, thus increasing the potential to make identification possible from primary plates. Plant-based media may also be made selective with antibacterial agents or cycloheximide. Table 2.3 lists several commercially available media that may be used for both primary isolation and identification. Please consult Sects. 10.2 and 10.3 in the CLSI M54-A document for additional information [5]. The choice of tubed versus plated media is made based upon space constraints, personal preference, and safety. The greater surface area provided by plates is preferred by many laboratorians (and always preferred by the fungi!), as manipulation of cultures, isolation procedures, etc. are more easily performed on plates. When used, plate lids should be firmly attached with an air-permeable material or plates sealed in air-permeable bags to avoid cross-contamination or laboratory worker exposure.

Optimally, cultures should be incubated at 30 °C (± 1 °C). If this temperature is not available, room temperature near 25 °C should be used. Seven-day incubation is generally adequate when screening for yeasts from oropharyngeal or vaginal sites. Although 4-week incubation times have been traditionally recommended, studies suggest that 3 weeks are adequate to detect fungal growth from most other specimens, excluding those from skin, hair, and nails, and in cultures requested specifically to attempt to recover dimorphic pathogens [27]. The time required for development of diagnostic structures, particularly for some coelomycetes and ascomycetes, may be considerably longer, up to several weeks [28, 29].

Examining Cultures

One recommendation for culture examination is every day for the first 3 days and twice a week thereafter. Alternate reading schedules can be found in the CLSI M54-A document, Sect. 11.1 [5], depending upon the source of the isolate. Cultures of yeasts are typically creamy to waxy, while moulds appear velvety to woolly to cottony. Some safety precautions common to both yeasts and moulds include the careful handling of plates and tubes so as not to create aerosols of infectious material and the prevention of contamination of patient cultures with ubiquitous fungi from the work surroundings.

Phenotypic Yeast and Yeastlike Organism Identification

Yeast cultures, consisting of unicellular organism that replicate by budding, may be handled on the open bench, adhering to the same safety precautions as for bacteria. Yeast and yeastlike fungi should be examined for their colony color (white to cream to pink; brownish-black for the yeast synanamorph of *Exophiala* species when observed on Sabouraud dextrose agar; blue to green to pink for *Candida* species on CHROMagar Candida™ (CHROMagar Microbiology, Paris, France)), growth rate, temperature requirements (or preferences), macroscopic morphology (smooth, wrinkled, glabrous, moist, dry, etc.), and microscopic morphology (size and shape, presence of blastoconidia, capsules, germ tubes, pseudohyphae, true hyphae, chlamydoconidia, etc.). Yeast morphology is most reliably observed on a cornmeal agar plate using the Dalmau method [30]. This technique involves streaking a very small amount of yeast onto a plate in two parallel lines, streaking back and forth over these lines for better isolation, and covering the area with a flame-sterilized coverslip. The plate is incubated at room temperature for 18–24 h and then examined microscopically for diagnostic structures. Tease mounts may also provide useful information. Additional procedures that may be required for the identification of yeasts include the reduction of nitrate to nitrite, urease activity, the ability of the organism to grow on media containing cycloheximide, and assimilation and fermentation patterns. Many commercial systems, both manual and automated, are available to assist in yeast identification.

Phenotypic Mould Identification

Any filamentous organisms recovered on culture should be examined and manipulated in a biological safety cabinet. While moulds can be recovered on a variety of media, conidiation/sporulation is generally enhanced on plant-based media. If not used in primary isolation, plant-based media should be employed in the identification process. Moulds should be examined for their growth rate, temperature requirements, and macroscopic morphology to include color

(hyaline to brightly colored or phaeoid (brownish to blackish)), texture (velvety, woolly, granular, cottony, etc.), and the observation of any diagnostic features visible to the naked eye. The microscopic detail may be studied using tease mounts or temporary tape mounts (clear tape only) in lactophenol cotton blue. The preferred technique to demonstrate diagnostic structures and methods of conidiogenesis for most filamentous fungi is the slide-culture method. Additionally, this method can provide a permanent mount that can be preserved in a slide collection for future studies and is extremely useful for comparison with other similar isolates or atypical strains. Members of the order *Mucorales* (which includes *Rhizopus, Lichtheimia* (formerly *Absidia*), *Mucor,* and several other genera) may rapidly overgrow slide cultures, making this method less than optimal for studying these fungi.

Slide cultures should also not be set up on Moulds where the clinical history suggests a dimorphic pathogen such as *H. capsulatum, B. dermatitidis, Coccidioides* species, *Paracoccidioides brasiliensis* (not commonly seen in the USA), or *Talaromyces* (*Penicillium*) *marneffei* (usually restricted to HIV-infected individuals from endemic areas of Southeast Asia) [31]. Tease mounts should be prepared for these isolates in a mounting fluid known to kill the fungus, such as lactophenol cotton blue. *Sporothrix schenckii,* and other *Sporothrix* spp. [32], also dimorphic organisms, pose less of an exposure risk, and may be examined by slide culture. *H. capsulatum* and *B. dermatitidis* may be definitively identified using the DNA Gen-Probe® (AccuProbe, San Diego, CA) methodology. This method, which confirms a *Coccidioides* species, does not, however, differentiate between *Coccidioides immitis* and *Coccidioides posadasii*.

Molecular Fungal Identification

While this work contains a chapter on diagnostic molecular biology (Chap. 3), information about fungal identification would be incomplete without brief mention here of the most commonly used molecular method for the identification of clinical isolates. The method most often cited is sequencing with identification of the isolates by comparative sequence analysis [33–35]. Briefly, genomic DNA is extracted, an optimal gene target is amplified and sequenced, and the data are aligned and analyzed. The most common targets useful for a wide range of fungi are within the ribosomal DNA (rDNA) and include the internal transcribed spacer (ITS1 and ITS2) regions and the D1/D2 regions of the large ribosomal subunit. Sequence results are then compared against type strains or other credible deposits in a database using the BLASTn algorithm. GenBank is a commonly used database; however, it is non-curated, so there is no assurance that the sequence data deposited is from a correctly identified isolate, thus making it imperative that the molecular identification is compatible with the phenotypic features. While the ITS and D1/D2 regions are very useful, additional targets may be needed for some genera such as beta-tubulin (TUB), calmodulin (CAL), actin (ACT), CO1 (the mitochondrial cytochrome oxidase 1 gene), the translation elongation factor (TEF), and others [36]. Other databases that are curated such as those at the Centraalbureau voor Schimmelcultures (CBS) and the online *Fusarium* database [37] are often necessary for a definitive identification. In light of cost containment and the dwindling number of individuals trained in classical morphologic identification, more laboratories are considering adding the sequencing of isolates in conjunction with phenotypic methods or as a stand-alone method. It should be noted that sequence-alone identification of limited or non-informative targets without a comparison of the phenotypic features has the potential for misidentification of isolates that could negatively impact patient management [36].

Taxonomy, Classification, and Nomenclature

Many volumes have been dedicated to the taxonomy, classification, and nomenclature of clinically significant fungi. Herein, this work only highlights some of the basic concepts. The classification scheme accepted by most authorities will be presented for the kingdom fungi. The term classification, in the fungal sense, refers to the application of names for the categories into which the taxa (taxonomic groups) may be grouped, with some subdivisions regarding their relative order. "Taxonomy" refers to this classification in a very systematic way, and nomenclature is the assigning of names to fungi. These processes, previously regulated by the rules of the International Code of Botanical Nomenclature (ICBN), are now under the auspices of the International Code of Nomenclature for algae, fungi, and plants. The Melbourne Code, adopted in 2011, and published in 2012 [3, 38], resulted in major changes which became effective January 1, 2013. Some of the most significants are as follows: (a) the abolishment of Article 59 of the previous ICBN, permitting multiple names for the same organism (such as one name for the anamorph and one for the teleomorph, i.e., such as the use of both *Aspergillus fumigatus* and *Neosartorya fumigata*); (b) the need to determine *which* name will be used (an ongoing process); and (c) the deposition of isolate information into a recognized repository for the valid publication of a fungal name. The following is an abbreviated classification scheme for the kingdom Fungi:

Group	Group Ending
Kingdom	none
Subkingdom	none
Phylum	mycota
Subphylum	mycotina
Class	mycetes
Order	ales
Family	aceae
Genus	no specific ending
Species	no specific ending
Variety	no specific ending

The phyla in which the sexual or teleomorph forms of the majority of human/animal pathogens reside are the *Ascomycota*, *Basidiomycota* and the *Glomeromycota* (formerly the *Zygomycota*) [4]. An example of this classification scheme for the ascomycete, *Microascus cinereus,* recovered from lower respiratory sites [39], would look like this:

Kingdom	Fungi
Subkingdom	Dikarya
Phylum	Ascomycota
Subphylum	Ascomycotina
Order	Microascales
Family	Microascaceae
Genus	*Microascus*
Species	*Microascus cinereus*

M. cinereus, a sexual fungus (or the teleomorph) that produces perithecia, asci, and ascospores in culture also simultaneously produces an asexual form (the anamorph) that is microscopically quite different. Asexual fungi, previously given the prefix "form" to the classification scheme, such as form-class, form-order, etc., are now commonly known as "mitosporic" fungi, or those reproducing my mitosis rather than meiosis. The anamorphic form of *M. cinereus* is the phaeoid fungus, *Scopulariopsis cinereus* [39]. Anamorphic fungi are identified mostly on the basis of their method of conidiogenesis (how they form their reproductive structures). Asexual reproductive propagules are referred to as conidia, hence, the term conidiogenesis. Sexual fungi are mostly identified based on the method they use to form their sexual reproductive propagules (e.g., ascospores, basidiospores). Not all taxonomists have agreed that we should apply different names to the anamorph and teleomorph of the same fungus, the holomorph, or "whole fungus"; however, this has been the practice until the implementation of the Melbourne Code. As mentioned above, multiple names will no longer be permitted; however, the determinations as to which name will be used for the various genera is still ongoing. One genus that does appear to retain its anamorphic name is *Fusarium* [40]. Adding to this confusion, some multiple fungi-produced anamorph forms, such as is seen with the fungus *Pseudallescheria boydii*. *P. boydii* is the teleomorph; *Scedosporium boydii* is the anamorph (previously thought to be *S. apiospermum*) [41], and they may also produce a *Graphium* synanamorph, or another anamorphic form of the "whole fungus." Practically speaking, most etiologic agents are identified in the laboratory on the basis of structures formed by the anamorphic form of the fungus. Although many mitosporic fungi have known teleomorphs, most require two mating strains to produce the sexual form. These are referred to as heterothallic. A few clinically significant fungi require only one strain to produce the teleomorph, and these as considered homothallic. *M. cinereus* and *P. boydii,* cited above, are examples of homothallic fungi.

Fungal Identification

Yeast identification is performed in a manner similar to that for bacterial identification, and easily lends itself to various compartmentalized and automated methods that measure various physiologic characteristics. Mould identification, however, currently relies more upon the observation of macroscopic morphologies, such as color and colonial features, growth rate, temperature maximums and minimums, and microscopic structures. Some of these more common identifying characteristics are exemplified in the organisms chosen in the thumbnail sketch of the kingdom fungi as illustrated in Table 2.4.

Ascomycota

Under the phylum Ascomycota, the ascomycetous yeasts are usually identified by yeast methods, while the Mould are identified based upon the structures they produce. Some of the filamentous homothallic ascomycetes produce ascomata known as cleistothecia, perithecia, or gymnothecia in which the asci and ascospores are contained (Figs. 2.1–2.4).

Basidiomycota

Similarly, the red and white yeasts within the phylum Basidiomycota are commonly identified by yeast methodologies. The filamentous basidiomycetes pose identification dilemmas, as they frequently remain sterile in culture, producing no unique reproductive structures. *Schizophyllum commune* is one of the few that may sometimes be tentatively identified by its production of spicules along the sides of the

Table 2.4 Simplified schematic of the kingdom Fungi for some human/animal pathogens

Subkingdom: Dikarya
Phylum: Ascomycota
Class: Hemiascomycetes—yeasts
Class: Euascomycetes—mould; produce ascospores in a variety of sexual structures known as ascomata (*pl.*), ascoma (*sing.*)
Cleistothecium—round, closed ascoma
Example: *Pseudallescheria boydii*, Fig. 2.1
Perithecium—pear-shaped ascoma, with an opening or ostiole
Example: *Microascus cirrosus*, Fig. 2.2
Gymnothecium—ascoma with a loose network of hyphae
Example: *Myxotrichum deflexum*, Fig. 2.3
Asci (*pl.*), ascus (*sing.*)—within the ascoma and containing ascospores
Ascospores—various sizes, shapes, colors, ornamentation
Example: *Sporomiella* sp., Fig. 2.4
Phylum: Basidiomycota
Subphylum: **Pucciniomycotina = Urediniomycetes**
Primarily contains the rusts
Subphylum: **Ustilaginomycotina = Ustilaginomycetes**
Contains yeastlike members of smut fungi
Subphylum: Agaricomycotina = Hyenomycetes
Contains mushrooms (basidiocarps) producing yeast anamorphs (*Cryptococcus* species) and filamentous anamorphs that are frequently sterile or may produce arthroconidia
Example: *Schizophyllum commune*, a human etiologic agent, produces spicules (small protrusions) along the hyphae, Fig. 2.5
Basidiospores sometimes seen from basidiocarps of *S. commune*, Fig. 2.6
Phylum: Glomeromycota
Subphylum: ***Mucormycotina***
Order: ***Mucorales***—asexual reproduction by multi-spored or few (to one) spored sporangia (sporangiola)
Heterothallic genera (require two mating strains) include some spp. of *Rhizopus*, *Lichtheimia*, *Mucor*, and others; produce sporangiospores
Example: *Rhizopus microsporus*, Fig. 2.7
Homothallic genera/species (one mating strain required) produce zygospores
Example: *Cokeromyces recurvatus*, Fig. 2.8
Subphylum: ***Entomophthoromycotina***
Order: ***Entomophthorales***—characterized by forcibly discharged conidia. Produce asexual primary conidia and smaller secondary conidia
Example: *Conidiobolus incongruus*, Fig. 2.9
Example: *Basidiobolus ranarum*, produces zygospores
Mitosporic fungi (formerly fungi imperfecti)
Methods of conidiogenesis
Blastic—conidia blown out
Phialidic conidiogenous cell—often have discernable collarettes and produce phialoconidia
Example: *Phialophora americana,* Fig. 2.10, and *Aspergillus flavus,* Fig. 2.11
Annellidic conidiogenous cells—have rings or annellations and become longer and narrower with production of annelloconidia
Example: *Scopulariopsis cirrosus,* Fig. 2.12
Some species blow out conidia through pores on geniculate conidiophores
Example: *Curvularia hawaiiensis,* Fig. 2.13
Thallic—conidia formed from preexisting hypha
Arthroconidia produced that may or may not have intervening disjunctor cells
Example: *Coccidioides* species, Fig. 2.14, and dematiaceous arthroconidia of *Neoscytalidium dimidiatum,* Fig. 2.15
Hyphomycetes—bear their conidia free and display various colors, methods of conidiogenesis, growth rates, etc.
Example: *Aspergillus flavus,* Fig. 2.10
Coelomycetes—bear their conidia within some type of asexual structure known as a conidioma (*sing.*) (conidiomata (*pl.*)) and display various colors, methods of conidiogenesis, growth rates, etc.
Pycnidium—round conidioma with an opening (ostiole) and conidia contained within; Example: *Phoma* species, Fig. 2.16
Acervulus—flat, cup-shaped conidioma, with conidia more or less exposed; Example: *Colletotrichum* species

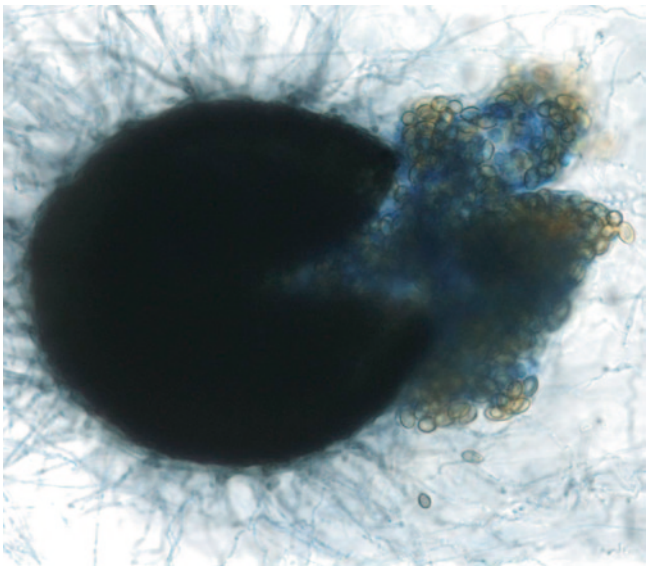

Fig. 2.1 Globose ascoma (closed cleistothecium) of *Pseudallescheria boydii*

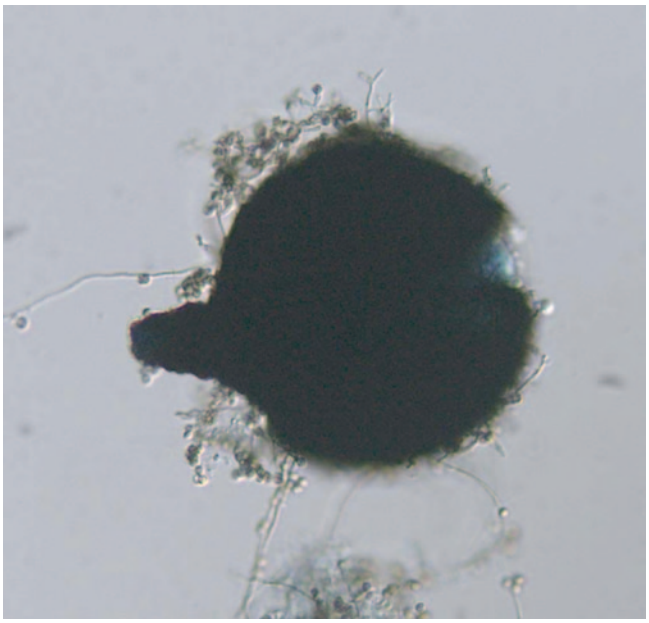

Fig. 2.2 Pear-shaped ascoma (perithecium with an opening or ostiole) of *Microascus cirrosus*

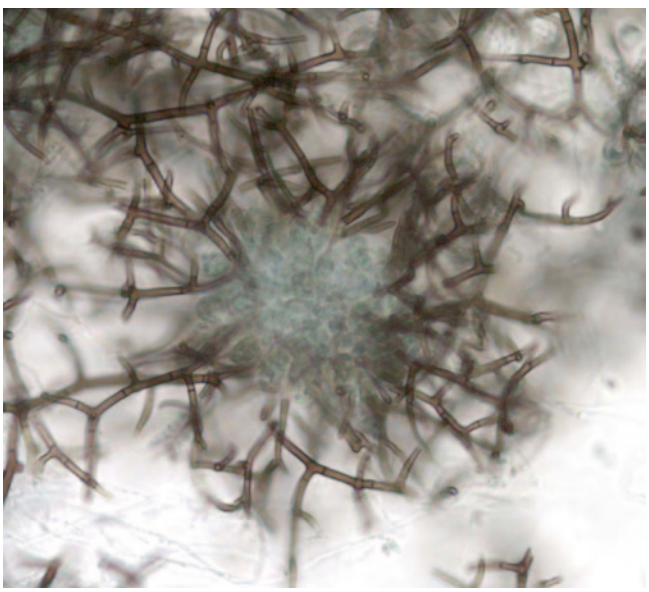

Fig. 2.3 Gymnothecium (ascoma with a loose hyphal network surrounding central ascospores) of *Myxotrichum deflexum*

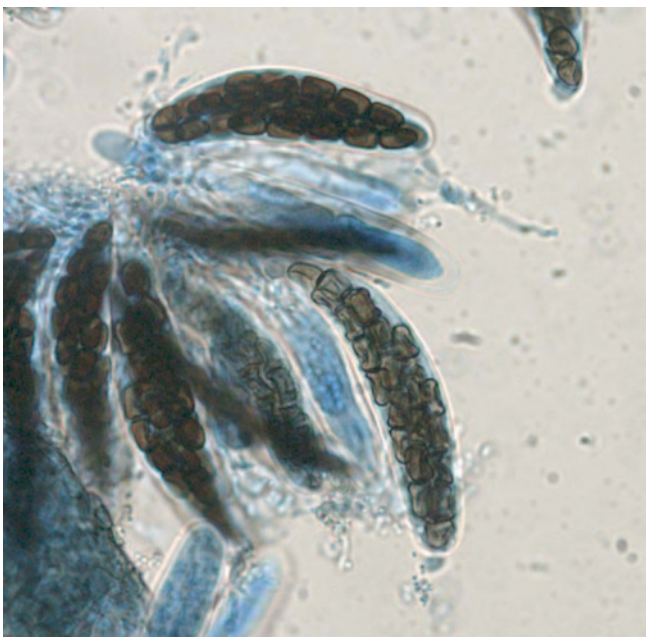

Fig. 2.4 Asci containing dark ascospores of a *Sporomiella* species

hyphae, and occasionally by clamp connections (Fig. 2.5), basidiocarps, and basidiospores (Fig. 2.6) when dikaryons (compartments of a hypha that contain two nuclei, each derived from a different parent) are present.

Glomeromycota

Human and animal pathogens in the phylum Glomeromycota (formerly Zygomycota) are contained within the subphyla *incertae sedis Mucoromycotina* containing the order *Mucorales* [42], and *Entomophthoromycotina* containing the order *Entomophthorales*. In the order, *Mucorales* are the most common mucoralean genera such as *Rhizopus* (Fig. 2.7), *Mucor, Lichtheimia* (formerly *Absidia*) [43], *Rhizomucor, Cunninghamella,* and *Cokeromyces* (Fig. 2.8), while the *Entomophthorales* encompass the less frequently seen genera

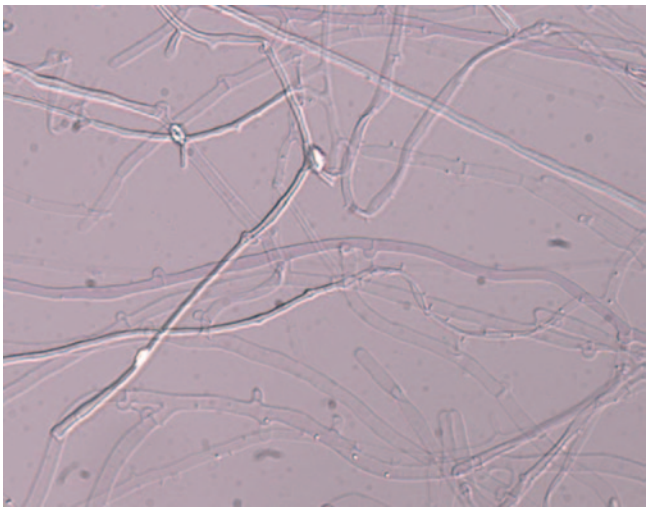

Fig. 2.5 Spicules and clamp connections on hyphae of *Schizophyllum commune*

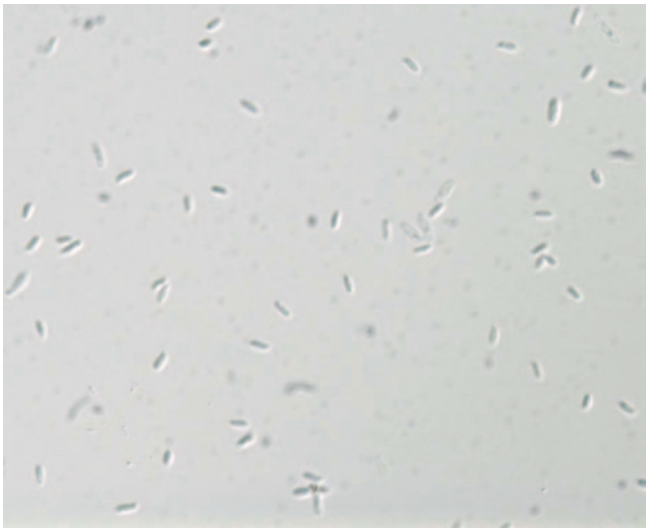

Fig. 2.6 Basidiospores produce by *Schizophyllum commune*

Fig. 2.7 Ramified rhizoids, short, dark sporangiophores, collapsed columellae, and sporangiospores of *Rhizopus microsporus*

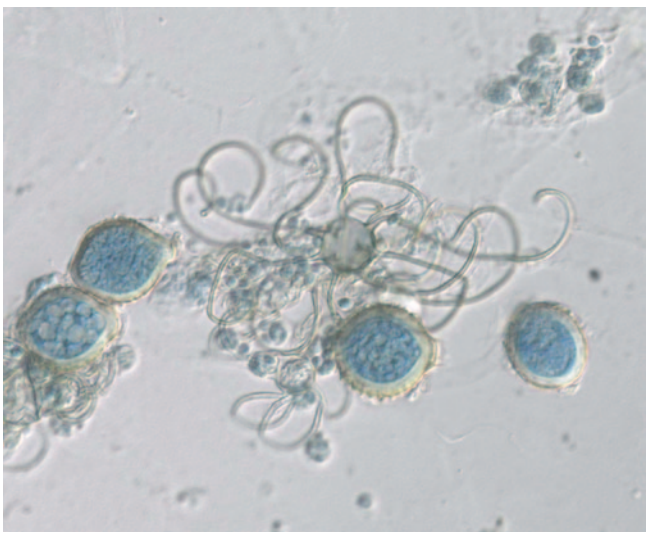

Fig. 2.8. Central vesicle, recurving stalks with terminal sporangioles containing sporangiospores, and thick-walled zygospores of *Cokeromyces recurvatus*

Conidiobolus (Fig. 2.9) and *Basidiobolus* (both characterized by forcibly discharged conidia).

Mitosporic Fungi

The group that contains the most human etiologic agents, by far, is one known as the "mitosporic fungi," or previously, the "fungi imperfecti." While these fungi may by connected to various sexual phyla, this association has not been yet demonstrated, and therefore these fungi are identified on the basis of their asexual rather than sexual reproductive propagules (method of conidial formation or conidiogenesis). Two main groups exist within the mitosporic fungi. The hyphomycetes bear their conidia free to the air, while the coelomycetes have their conidia contained within some type of enclosed to semi-enclosed structure [28, 29]. The hyphomycetes contain numerous common moniliaceous (hyaline) and phaeoid or dematiaceous (dark) genera and generally produce their conidia by either blastic or thallic methods. Blastic conidia are "blown out" of some type of conidiogenous cell. These include those produced from phialides, as in

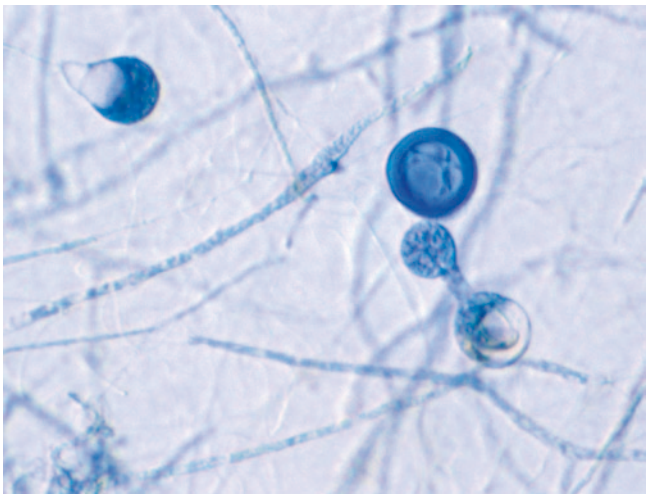

Fig. 2.9 Primary sporangiole giving rise to secondary sporangiole of *Conidiobolus coronatus*

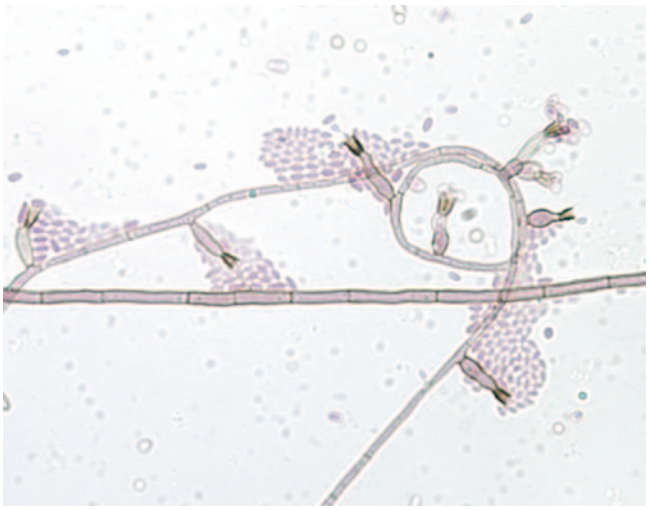

Fig. 2.10 Phialides of *Phialophora americana* with deep collarettes producing phialoconidia

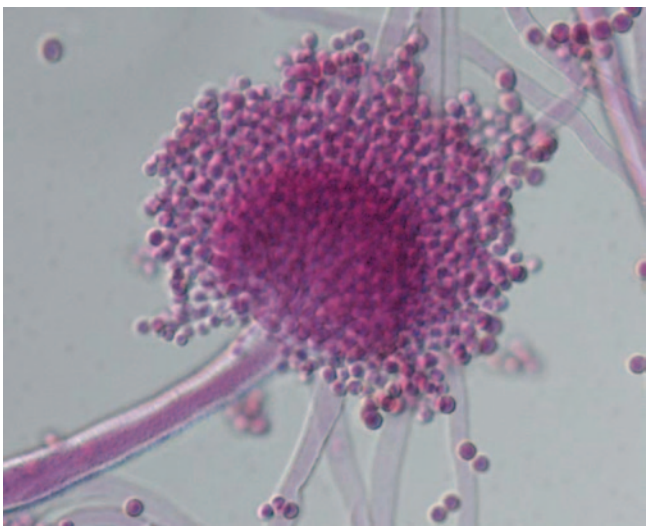

Fig. 2.11 Rough conidiophore and biseriate fruiting head of *Aspergillus flavus*

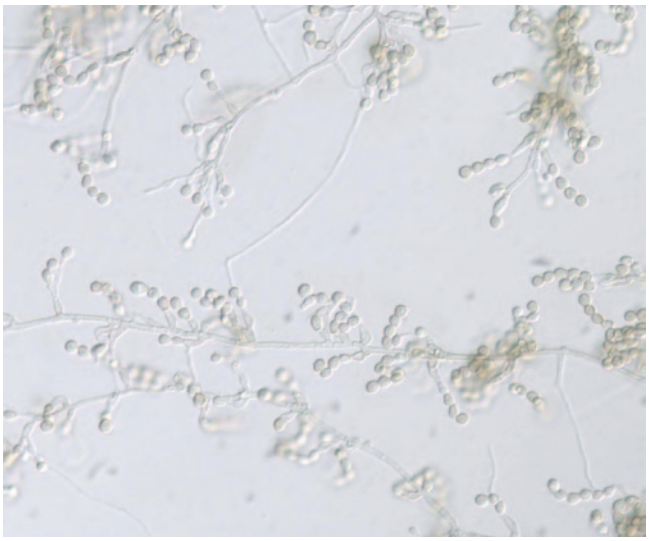

Fig. 2.12 Annellides and chains of annelloconidia produced by *Scopulariopsis cirrosus*

Phialophora species (Fig. 2.10) or *Aspergillus* species such as *Aspergillus flavus* (Fig. 2.11), or from annellides, as in *Scopulariopsis cirrosus* (Fig. 2.12). Some species blow out their conidia through pores, such as in *Curvularia* (formerly *Bipolaris*) *hawaiiensis* [44] (Fig. 2.13). Thallic conidia are formed from preexisting hyphae, as in *Coccidioides* species (Fig. 2.14), *Malbranchea* species, and *Neoscytalidium* (formerly *Scytalidium*) *dimidiatum* [45] (Fig. 2.15). The structures produced by coelomycetes to contain their conidia are known as conidioma (*sing*.) or conidiomata (*pl*.). They may be round structures with an opening or ostiole known as a pycnidium, as in *Phoma* species (Fig. 2.16), or a flat, cup-shaped, semi-enclosed structure known as an acervulus. The conidiogenous cells within both of these conidiomata may be either phialidic or annellidic. A recent review highlights coelomycete fungi seen in the clinical laboratory and salient features for their identification [29].

Fig. 2.13. Geniculate conidiophores with pores through which the conidia of *Curvularia hawaiiensis* are blown out

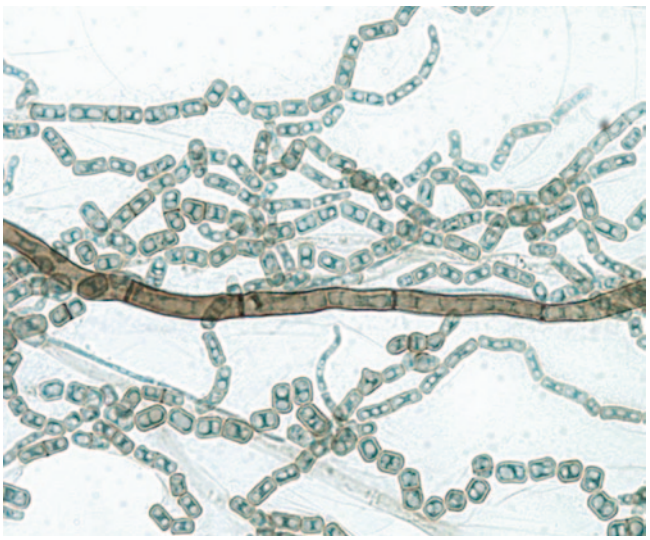

Fig. 2.15 Dematiaceous hyphae and arthroconidia of *Neoscytalidium dimidiatum* which lack disjunctor cells

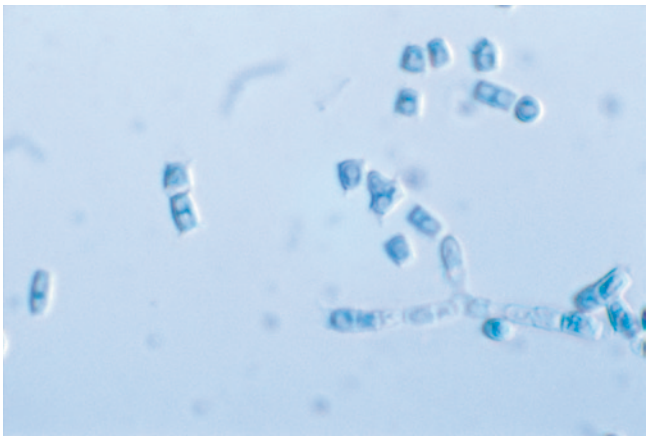

Fig. 2.14 Hyphae and arthroconidia with disjunctor cells of *Coccidioides* species

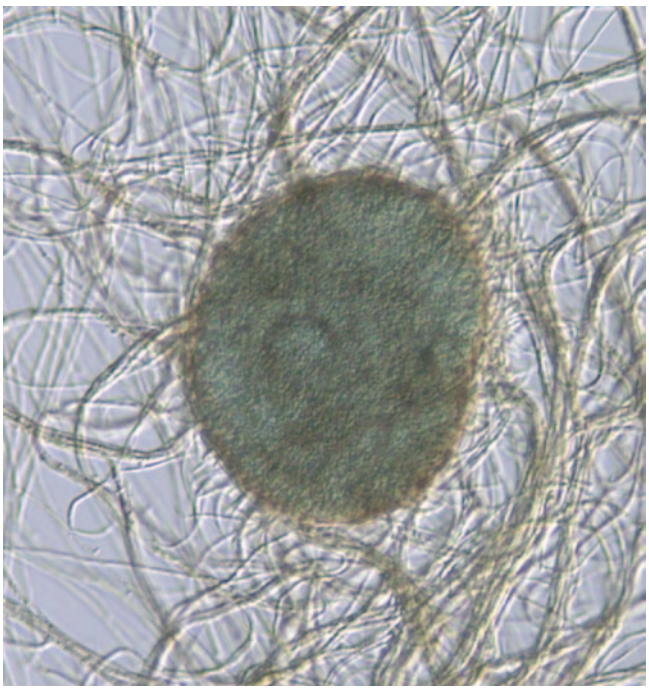

Fig. 2.16 Conidioma of a *Phoma* species containing a large central ostiole or opening.

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Suggested Reading

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Diagnostic Molecular Biology

3

Anna M. Romanelli and Brian L. Wickes

Introduction

The role of molecular biology in the clinical microbiology laboratory continues to grow at an ever-accelerating pace. Few areas of diagnostic microbiology stand to benefit more from the application of molecular diagnostics than clinical mycology. The increasing role of molecular biology in fungal diagnosis and identification is driven by two factors. First is the continually expanding spectrum of clinically significant fungi that are being recovered from patients. This list grows every year and is directly related to the growing population of immunocompromised patients at risk for the development of serious fungal infections. AIDS has been perhaps the greatest wake-up call with regard to what awaits the immunosuppressed, and organ transplantation has provided one of the strongest reminders of how desperate medical mycology is for new classes of antifungals. Second, the field of diagnostic mycology has been described as being in crisis due to the lack of experienced mycologists who can identify the increasing numbers of never before seen fungi that are recovered from patients [1]. The USA in particular, and most countries in general, abandoned education and training programs in classical mycology, which traditionally produced a steady stream of microbiologists who could identify fungi beyond the most common ones routinely encountered in a clinical microbiology laboratory. Consequently, there are few young scientists being educated and trained today to replace the retiring clinical mycologists who have expertise in classical mycology that can be translated into fungal identification using traditional phenotypic (i.e., morphology) methods. The lack of this skillset, combined with the increasing need for this expertise, is having downstream repercussions that translate into increased morbidity, mortality, and health-care costs. Furthermore, there are troubling trends in the frequency of fungal infections that place added pressure on clinical laboratories. For example, *Candida* infections, now the fourth most common nosocomial infection, carry an estimated increased cost of US$ 68,311 and an extra 23 days in the hospital [2], part of the US$ 2.6 billion expenditure on systemic fungal infections in just the USA alone [3].

Clinicians recognize that in contrast to bacterial infections, which allow multiple antibiotic treatment options, fungal infections must be treated using a very limited selection of antifungal drugs, many of which carry substantial side effects or work poorly in certain patient populations. However, the time for diagnosis of a systemic infection is a proven risk factor for increased morbidity and mortality [4], confirming the important role that mycology diagnostic expertise in the clinical microbiology laboratory plays in health care, both from a cost and patient outcome perspective. The options for strengthening this role in light of diminishing expertise are increasingly falling on technology and, more specifically, to molecular biology, as potential ways to make up lost ground by providing new tools for diagnostic mycology. There remain, however, many challenges to overcome before the full power of molecular biology can be applied in the clinical laboratory.

Current State of Fungal Detection in the Clinical Laboratory

Traditionally, fungal identification has largely been based on the subjective micro- and macroscopic examination of morphological and culture characteristics. As such, these evaluations rely on the expertise of a trained mycologist and therefore are subject to variation in identification depending on the skill and experience of the microbiologist. There are also numerous genera, species, and strains that show morphological similarities, which can also lead to fungal misidentifications

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[5]. The ability to rapidly and correctly identify positive clinical specimens has immense potential to impact the therapeutic decisions regarding empirical antifungal therapy.

Increasing numbers of immunosuppressed patients, coupled with a widening range of recognized pathogens, and the discovery of resistance of some yeasts to antifungal drugs means that the common practice of identification or exclusion of *Candida albicans* alone is no longer adequate [6]. The clinical microbiology laboratory is somewhat limited in the ability to differentiate and identify less frequently encountered yeasts. Some of the methods routinely used to identify yeasts in a clinical laboratory are Gram stain, germ tube production, culture (primarily onto chromogenic media or cornmeal agar to aid in species differentiation), and biochemical methods, including commercial platforms such as API 20 C AUX (bioMérieux, Inc., Durham, NC), use of biomarkers (Galactomannan and 1,3-β-D-glucan (BDG), and peptide nucleic acid fluorescent in situ hybridization (PNA-FISH). Culture methods, lactophenol cotton blue staining, and, more recently, polymerase chain reaction (PCR) platforms are used for the identification of molds in the clinical microbiology laboratory [6, 7]. Historically, the clinical microbiology laboratory has relied heavily on conventional methods for fungal identification, which is often not practical because they are labor-intensive and can take days to weeks to complete, depending on the isolate. Therefore, there is a constant need for new approaches, allowing for the rapid and accurate identification of pathogenic fungi.

Current Challenges in Diagnostic Molecular Biology

Presently, there are numerous molecular-based methods for the identification of fungi that are in various stages of development, but are mainly used for research purposes. They have been slow to find their way into clinical laboratories due to a number of factors that need to be overcome before molecular diagnosis becomes the predominant or even routine application in a clinical environment.

From a research perspective, many of the newer technologies have proven to be valuable and have potential for a future role in the clinical laboratory. There are, however, numerous hurdles that need to be overcome before some of the current technologies can become a major workhorse in the clinical laboratory.

Instrumentation

Many molecular assays require expensive equipment platforms that bring high capital costs, which can be in the range of US$ 100,000 or more depending on the instrument. For fungi, some of the larger platforms include DNA sequencers for sequence analysis or mass spectrometry (MS) instrumentation, such as matrix-assisted laser desorption/ionization (MALDI), which is used for analysis of biomolecules (DNA, protein, etc.). In addition to high initial capital costs, not including any supportive equipment, these instruments carry annual service contracts that are typically in the range of 10 % of the purchase price. They may also have additional requirements such as special electrical requirements that include backup power sources or separate electrical lines that are free of surges, large footprints, or space requirements that must insure they are not moved or agitated due to precise calibration requirements. For some instruments, continued development has brought down the size and cost of many platforms used in molecular biology, with many approaching benchtop size and affordability. Thermocyclers used for real-time PCR have dropped drastically in size and price over the years and can be configured to support high-throughput applications and easily interfaced with a computer for complex data management and analysis. Importantly, one of the major criteria involved in the decision-making process of whether or not to purchase an instrument is throughput. Most clinical laboratories are not going to have the fungal identification throughputs to justify large capital outlays. However, for some types of analyses, such as sequencing, cross-microbe application can justify the associated expense.

Expertise

In addition to instrumentation costs, expertise to operate the equipment often imposes additional salary pressure on microbiology laboratories. There is great pressure to develop assays and instruments that are operated by loading the specimen and retrieving data, with little or no presample or postassay analysis or manipulation required. However, in many cases, this simplicity is not possible. The instruments themselves can be complicated to run and, most importantly, downstream analysis and manipulation of data can require expertise not found in traditional clinical microbiology or medical technology training. Furthermore, unless analysis is performed in a reference laboratory, specific expertise almost always would need to be translatable between subdisciplines since workloads may not be enough to justify investment in instrumentation or personnel dedicated to a single group of microbes (e.g., fungi, parasites).

Throughput and Turnaround Time

The importance of rapid diagnosis of an infecting fungal pathogen cannot be overstated given the significance that this risk factor plays in morbidity and mortality. For some

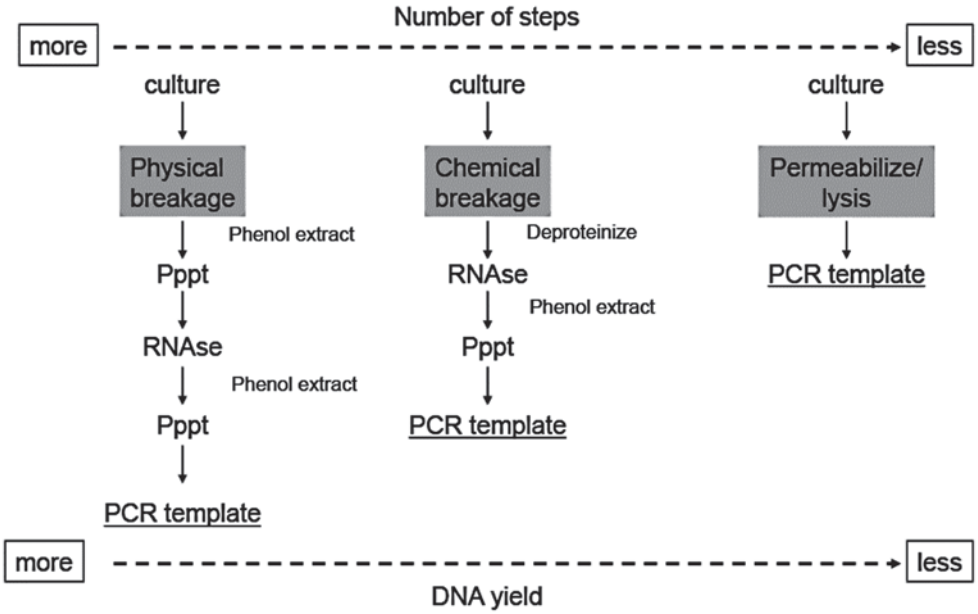

Fig. 3.1 The relationship between yield, number of steps, and type of fungal template preparation method. The greatest yield comes from laborious methods that require numerous steps, including organic extractions. In contrast, the easiest methods yield the least DNA, which is typically lower in quality and more likely to be contaminated with cellular material

patient types, it may be the most important criterion that affects survival. For example, Von Eiff et al. showed that starting antifungal therapy for pulmonary aspergillosis after 10 days increased mortality rates from 40 to 90% [8]. Unfortunately, due to potential cost, throughput becomes an issue in molecular diagnostics because there must be enough savings per isolate, through speed of identification, accuracy, and labor-saving methodology to justify implementation of a given molecular assay. For smaller platforms, such as PCR, thermocyclers can be used on non-fungal microbes (i.e., bacteria, viruses), and/or have additional applications beyond microbial diagnostics. These instruments are generally inexpensive to moderately expensive and may be within the operating budget of the clinical laboratory. Certainly, larger reference laboratories are better positioned to support this equipment. However, an important factor to consider for any platform is per assay cost. Many instruments will have prepackaged reagents dedicated to a given assay, which will be much more expensive than homemade recipes. Furthermore, in order to meet certification requirements in a clinical laboratory, specific assays will be more expensive to run than they would be in a research laboratory. For example, a research laboratory may run sequencing reagents at 1/8, 1/16, or even 1/32 of the concentration, depending on laboratory expertise, while a certified laboratory may need to run full-strength reactions to insure inter-laboratory consistency as mandated by the appropriate accrediting agency (i.e., College of American Pathologists, Clinical and Laboratory Standards Institute).

Template Preparation

There is probably no greater challenge to molecular diagnostics than template preparation because this issue impacts every other component of molecular diagnostics. Fungi are unique from virtually all other medically important microbes in that lysing the cells can be problematic and varying in difficulty depending on the genus. Additionally, once lysed, fungal cellular material, such as melanin or polysaccharides, can inhibit downstream reactions and lead to erroneous or negative results unless it is removed. Because almost all fungi have a tough cell wall, extractions can require harsh, laborious, or prolonged manipulations. For any diagnostic assay requiring an amplification step, such as PCR, multiple-sample manipulations that contain excess tube transfers should be avoided because each transfer introduces a potential opportunity for external DNA contamination. Unfortunately, this problem is ever present as even some commercial material used for DNA purification can be contaminated with exogenous fungal nucleic acid that can be amplified during PCR [9]. Using alternative methods, such as physical breakage or enzymatic spheroplasting and lysis, are suboptimal because they typically require multiple time-consuming, laborious, organic extractions and precipitations that eventually yield a DNA amount much larger than what is needed for the assay (Fig. 3.1). Automation may offer a suitable alternative because it can be standardized using specific fungal nucleic acid extraction programs and/or reagents, which give excellent yields that enable multiple downstream assays. However, this approach requires instrumentation that may be beyond a laboratory budget or which may not be used enough to justify the purchase.

An unfortunate characteristic regarding fungal template preparation is that templates can be from a variety of sources, not just pure culture. The advantage of having a live culture is that there is a limitless supply of starting material for template preparation because any problems can be addressed by simply subculturing and starting over. However, the absence

Table 3.1 Comparison of different diagnostic platforms

Method	Capital cost	Technical expertise	Assay cost	Turnaround time	Downstream analysis	Labor	Sensitivity	Specificity
PCR	Low	Lowest	Low	Low	Low	Low	High	High
qPCR	Medium	Low	Low	Low	Low	Low	Highest	Highest
NASBA	Lowest	Lowest	Low	Low	Low	Low	High	High
LAMP	Lowest	Lowest	Low	Low	Low	Low	High	High
RCA	Lowest	Lowest	Low	Low	Low	Low	Highest	Highest
REP-PCR	Low	Low	Low	Medium	Medium	Low	High	Medium
RAPD	Low	Lowest	Low	Medium	Low	Medium	Lowest	Lowest
Sequencing	Highest	Highest	Low	Medium	Medium	Medium	High	High
Array	High	Low	High	Medium	Low	Low	High	High
PNA-FISH	Medium	Low	Low	Low	Lowest	Lowest	High	Highest
MALDI-TOF	Highest	High	Lowest	Lowest	Lowest	Lowest	Medium	High

Capital costs are generally defined as high (~US$ 100,000 or more) to low (<US$ 1000). Technical expertise is defined as high (specialized training or background) to low (basic laboratory skills with no special training). Assay cost is defined as high (>US$ 50) to low (<US$ 5). Turnaround time is defined as high (>5 h) to low (<3 h). Downstream analysis is defined as high (extensive data manipulation or transformation) to low (immediate readout). Labor is defined as high (>3-h preparation) to low (<1-h preparation). Sensitivity is defined as high (detection of <100 CFU) to low (>10^4). Specificity is defined as high (species level discrimination) to low (genus or lower order)

PCR conventional polymerase chain reaction, *qPCR* quantitative real-time PCR, *NASBA* nucleic acid sequence-based amplification, *LAMP* loop-mediated isothermal amplification, *RCA* rolling circle amplification, *REP-PCR* repetitive sequence PCR, *RAPD* random amplification of polymorphic DNA, *PNA-FISH* peptide nucleic acid fluorescence in situ hybridization, MALDI-TOF matrix-associated laser desorption/ionization-time of flight

of a pure culture can be quite common in fungal infections, which, in fact, can increase the value of any molecular assay if pure cultures are optional. Instead, starting material for template preparation can be any type of patient specimen, including blood, body fluids, whole tissue, scrapings, washes, etc. Each specimen type can bring its own problems regarding template preparation. Components in blood specimens, such as hemoglobin and lactoferrin, as well as the preservatives or anticoagulatives in specimen collection, such as ethylenediaminetetraacetic acid (EDTA) and heparin, can inhibit downstream steps such as PCR, if they are not removed during template preparation [10]. Tissue specimens that are only lightly colonized with an infecting fungus can swamp template preparations with host nucleic acids, proteins, etc. such that fungal material cannot be purified or cannot be detected against the excess background of host material. Furthermore, tissue specimens too often can be limited in amount, preventing multiple preparations. Alternatively, depending on the source, specimens from non-sterile sites such as trauma, sputum, or bronchial lavage can be contaminated with other fungi that are not invasive, but, nonetheless, could show up in assays that are pan-fungal in nature [11, 12]. Finally, there is the problem of formalin-fixed paraffin-embedded (FFPE) tissue specimens, which confer difficult challenges in spite of the wide availability of commercial kits that claim to be effective for processing these types of specimens. These specimens often make molecular detection difficult because nucleic acids can be cross-linked or fragmented during the fixation, leading to non-amplification by PCR [13]. They also can be externally contaminated [14], in addition to the ever-present problem of efficiently breaking the fungal cell walls once they have been suitably deparaffinized. Unfortunately, fixed specimens are among the most valuable fungal diagnostic specimens, so there is a substantial opportunity for template preparation improvement.

As trivial as template preparation seems, this step may be the most important area of molecular diagnosis to develop and standardize. Unfortunately, because of the diverse nature of fungal cell walls, with yeasts generally being easier to lyse than molds, it has been difficult to devise a "one size fits all" method. Until this challenge is overcome, new technologies will not be able to assume most of the diagnostic burden in a clinical laboratory.

Diagnostic Strategies

Application of molecular biology to fungal diagnosis has drawn from many areas, which has resulted in the successful use and continued development of numerous molecular platforms for fungal identification. The stringent conditions that clinical microbiology laboratories operate under combined with the multiple isolate sources (pure culture, human specimens, fixed tissue, etc.) pose difficult challenges to the widespread use of molecular biology in clinical mycology. However, the numerous working assays and the continual development of new technologies offer great promise for one or more platforms to become standard equipment in a clinical laboratory (Table 3.1).

Polymerase Chain Reaction (PCR)

Among the most basic of all molecular assays, and the most common molecular technique used by mycologists, is PCR. This technique is roughly 30 years old and is the workhorse of molecular biology, with almost limitless uses [15]. There are countless diagnostic uses and, importantly, an extensive array of variations of the basic technique that have specific applications.

Conventional PCR

The most basic PCR strategy is conventional PCR, which consists of two primers that anneal to complimentary target sequences. *Taq* polymerase, in addition to the appropriate buffer mix and template DNA, is used with a thermocycler to yield a product from a suitable starting template. This product can be the end point of the assay when visualized on an ethidium bromide gel, or it can be digested to yield specific restriction patterns that can be informative. Typically, conventional PCR assays have as an end point the presence or absence of an amplification product and almost always need to utilize specific primers that typically amplify a single or very narrow range of fungi. Size markers are used to confirm that the product is from the correct target since the amplicon size is usually known based on primer position. While degeneracy or conserved target sequences can extend the species target range, this extension can defeat the purpose if the goal is species identification. In some cases, the goal can be detecting the presence of a specific gene or allele, which could carry pathogenic or drug susceptibility consequences when it is present. Continued research has resulted in many variations that have made the technique more powerful, more sensitive, cheaper, and more creative in its applications.

Quantitative (Real-Time) PCR

The most common platform of nonconventional PCR for fungal detection is quantitative real-time PCR (qPCR), which offers a number of advantages over the older gel-based conventional PCR approach. The major advantage of qPCR is that it can be used to quantitate fungal colony-forming units, or the equivalent, and it is generally faster since the technique incorporates a target-specific, fluorescently labeled probe, or a general nonspecific dye that preferentially binds double-stranded DNA. The small amplicon size in qPCR, sometimes less than 100 bp, greatly decreases cycling time as amplicons can be one tenth the size of conventional PCR amplicons. Labeled probes confer the extreme specificity that qPCR is known for, although they add substantially to reaction costs compared to a nonspecific DNA-binding dye such as SYBR Green. However, suppliers of qPCR probes often allow web-based, specific assay design using algorithms that precisely match the two PCR primers and probe in a single reaction, which can be purchased as an individual assay, often as a ready-to-go PCR kit complete with enzyme and other reaction components. The programs work on a user-supplied target sequence that is simply pasted into the website to yield each assay with individual components tailored to the most efficient amplification of the target. These programs can be proprietary, available on the Internet, or part of desktop molecular biology programs.

Reactions are performed in a dedicated thermocycling instrument that can detect multiple dyes that fluoresce at different wavelengths, which can allow multiplexing in a single tube. There are various classes of fluorescent dye technologies for target detection, depending on the need. Although a Food and Drug Administration (FDA)-approved fungal diagnostic assay using qPCR technology has not reached clinical laboratories, qPCR is used extensively for the detection and quantitation of other microbes; consequently, it is only a matter of time before a specific fungal assay is routinely used in the clinical laboratory. However, it has been applied to virtually all of the major and more common human fungal pathogens and is frequently a part of most research laboratories.

Nucleic Acid Sequence-Based Amplification

There are a number of conventional PCR variations that have displayed clear value in the area of diagnostics. Some of them are moving away from the thermocycler platform and into isothermal amplification, which has the major advantage of greatly reducing instrumentation costs because reactions are run at a single temperature that can be reached with a heating block. Nucleic acid sequence-based amplification (NASBA) is a constant temperature (41 ºC) transcription-based amplification method that uses RNA as a template and a combination of RNase H and T7 RNA polymerase as enzymes. There are numerous advantages of this technique as it amplifies RNA templates, which can be present in great abundance for some targets (i.e., rRNA). Because RNA is the template, exogenous amplification of contaminating DNA is eliminated. The constant temperature and single-tube reaction also reduce contamination possibilities. A commercial version of this technology (AccuProbe, Hologic Gen-Probe Inc., San Diego, CA) has been available for a number of years and can be used to detect *Histoplasma capsulatum, Coccidioides immitis,* and *Blastomyces dematitidis* [16–18].

Loop-Mediated Isothermal Amplification

A second type of isothermal PCR is called loop-mediated isothermal amplification (LAMP) [19]. The key component of this reaction is a DNA polymerase derived from the bacterium, *Bacillus stearothermophilus*. Reactions can be completed within an hour and are performed isothermally at a temperature range of 60–65 ºC. In addition to the isothermal temperature and relatively fast turnaround time, LAMP is resistant to exogenous contaminants due, in part, to the

requirement for four primers. Therefore, template nucleic acids do not need to be highly purified, allowing cruder, "dirty" preparations to be used; however, the large number of primers can add complexity to primer design compared to traditional PCR reactions that utilize two primers [20]. There are multiple product detection methods, which vary from ethidium bromide-stained gels to turbidity, and even, to fluorescence. LAMP has been successfully used to detect a number of fungi, including *Fusarium* spp., *Cryptococcus* spp., and *Aspergillus* spp. [21–24].

Rolling Circle Amplification

Rolling circle amplification (RCA) is a third type of isothermal amplification technology that can result in the massive amplification of target sequences (~10^9 fold) [25]. This technology utilizes bacteriophage Phi29 DNA polymerase to amplify a DNA template. RCA is highly sensitive and specific, and can be used to discriminate single nucleotide polymorphisms. The technology utilizes a circular template to generate linear amplification products, which can be detected fluorescently using a variety of different probe chemistries. This sensitivity is particularly important for fungal genera that can be difficult to distinguish at the species level, even with molecular methods, due to highly conserved target sites [26]. The method is generally refractory to inhibitory compounds present in a sample that would normally inhibit other types of PCR reactions. RCA can also be easily multiplexed in contrast to conventional PCR so that multiple targets can be detected simultaneously. RCA has been used to detect a variety of fungi, including *Penicillium marneffei*, *Fonsecaea* spp., and *Exophiala* spp. [27–29].

Random Amplification of Polymorphic DNA

In addition to these technologies, there are various other PCR-based methods that have various applications in diagnostic mycology. For example, PCR can be used for some aspects of epidemiology. For fungi, this application can be extremely important because in many cases, little is known about the infecting fungus. A major PCR-based application in fungal epidemiology is random amplification of polymorphic DNA (RAPD). RAPD is a fingerprinting method that utilizes single, short oligonucleotides (~10 bp) in a PCR reaction typically at low annealing temperatures. PCR products can be produced when two oligonucleotides anneal close enough together on complementing DNA strands to produce a PCR product. Running the PCR reaction on an agarose gel followed by staining reveals an isolate-specific pattern that can be used to distinguish two isolates from one another, depending on the oligonucleotide. The discriminating power of the assay relies on multiple products being produced in a single reaction. Genetically unrelated strains are predicted to have different RAPD patterns depending on how fast their genomes evolve, geographic isolation from each other, and the frequency of the primer site in the genome. The greatest advantage of RAPD as an epidemiological tool is that nothing needs to be known about the genome sequence of the organism. The primer choice can be made empirically by testing single primers on one isolate and selecting the primer that yields the most bands for further testing on unrelated strains. For some fungi, such as *Cryptococcus neoformans,* this method has been studied extensively and has been developed into a strain typing tool based on PCR pattern [30]. The method can be highly susceptible to variation, even within laboratories, and is therefore not generally used as an identification tool. However, it has tremendous utility as a rapid way to investigate outbreaks to determine if infections are from the same or unrelated strains.

Repetitive Sequence PCR

Repetitive sequence PCR (rep-PCR) is both an epidemiologic and diagnostic tool that can, in some cases, identify as well as discriminate individual fungal strains [31]. The method is PCR based and relies on the high frequency of repetitive elements found in some microbial genomes, including bacteria and fungi. Using a large collection of stock primers in a semiautomated platform, the method can be used to fingerprint fungi as well as identify some species of fungi by searching the generated fingerprint pattern against a reference library [32, 33]. The method is well advanced and has already been commercialized (DiversiLab, **bioMérieux, Inc.,** Durham, NC).

Sequencing

While live culture is generally considered the gold standard of fungal diagnosis, arguably, the "gold ring" of molecular diagnostics should be a system that can identify any fungus, independent of any accompanying information (patient history, symptoms, imaging, morphology, biochemistry, serology results, etc.) using a single assay. While there are a number of methodologies that may be able to fulfill this possibility, the technology that is farthest along is sequence-based identification. Key factors that have enabled sequence-based identification to be as powerful as it is are the easy access to public databases, such as GenBank, and the search algorithms such as the BLAST programs [34] that enable users to query the database with an unknown sequence. Inexpensive sequencing, through advances in instrument technology and chemistry, has enabled the generation and deposit of sequences from countries all over the world, leading to both a deep and diverse database of easily searchable fungal sequences. The field of bioinformatics has made it possible to determine which sequences are the most informative and discriminatory with regard to species identification. Bioinformatics has advanced hand in hand with the completion

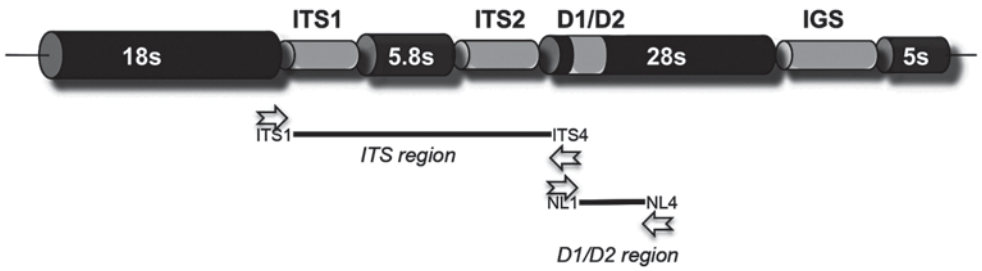

Fig. 3.2 Organization and primer location of the fungal ribosomal genes. The ITS region, generally the most informative, can be amplified by the ITS1 and ITS4 primers, and is located between the end of the 18s ribosomal subunit and the 28s ribosomal subunit. The D1/D2 region if found within the 28s ribosomal subunit towards the 5s end and can be amplified with the NL1 and NL4 primers. The IGS region is also quite variable and is typically but not always found between the 28s and 5s subunit. However, the size of this region is generally too large to amplify in a PCR reaction. Not drawn to scale. Open arrowheads indicate primer sites

of genome sequences, of which hundreds of fungal genomes are now complete. Next-generation sequencing will greatly accelerate genome sequencing and may someday be as fast and inexpensive as obtaining a single gene sequence.

Sequence-based identification of fungi evolved into a powerful diagnostic tool with the deposit of increasing numbers of ribosomally derived sequences (18s subunit, internal transcribed spacer regions 1 & 2, 5.8s subunit, 28s subunit, and the intergenic region). These sequences were important for fungal phylogeny and drove diagnostic mycology into the molecular era; however, as the deposits accumulated, both conserved and variable regions were identified. The existence of these regions allowed the design of primers to conserved regions, which enabled PCR amplification and sequencing of an unknown fungus through universal primers that could anneal to the conserved sites, and amplify the unknown intervening regions. The resultant amplicons contain the conserved priming sites in addition to the informative intervening regions, which enabled identification, often to the species level. Two of the most common pairs of primers are the ITS1 and ITS4 and the NL1 and NL4 primers (Fig. 3.2) [35, 36]. There are many other primers that are suitable for amplifying informative regions. However, these primers were used to deposit many of the fungal sequences into GenBank that are useful for diagnostic purposes. These two pairs amplify informative regions containing the internal transcribed spacer (ITS1 and ITS2) regions as well as the D1/D2 region of the 28s or large ribosomal subunit. Both regions are informative when searching GenBank, although the region amplified by the ITS1 and ITS4 primers is generally more variable than the region amplified by the NL1 and NL4 primers. Furthermore, the ITS region contains two individually informative regions, the ITS1 and ITS2 regions, which are separated by the 5.8s subunit. A third potential amplicon, in addition to the ITS and D1/D2 regions, is the intergenic sequence, sometimes referred to as the IGS region (Fig. 3.2). This region separates the 28s and 5s subunits and is extremely variable. However, it is generally too long for universal primers to be used in a PCR reaction, although it can be used to distinguish specific species, but has not been applied to general fungal molecular identification.

In addition to the conserved and near-universal PCR priming sites, a second important factor for targeting the rDNA for molecular identification is copy number. Eukaryotic ribosomal subunits are typically present in multiple copies that can exceed single copy targets by 10–100*x*. This increased copy number confers greater sensitivity for PCR reactions, which can be extremely important when template is not prepared from pure culture but, instead, from tissue specimens, body fluids, or other samples that may have a low or limited amount of organisms. In addition to some of the multiple mitochondrial targets, the multicopy nature of these targets is indispensable for limited amounts of cellular material, as is often the case when patient specimens must be tested directly.

When ribosomal targets are not specific enough and cannot distinguish isolates at the species level, additional sequencing targets are commonly used and typically are organism dependent. These targets are usually single-copy genes that are generally highly conserved, but not conserved enough to allow universal primers. In fact, degenerate primers may sometimes need to be used in order to amplify all desired species in a genus. Among the most common single-copy genes and the fungi they have used are: β-tubulin for the discrimination of *Aspergillus* spp., *Phaeoacremonium* spp., *Penicillium* spp.; elongation factor 1 alpha for discrimination of *Fusarium* spp.; cytochrome oxidase subunit 1 for discrimination of *Penicillium* spp.; calmodulin for discrimination of *Pseudallescheria* spp., *Sporothrix* spp., and *Scedosporium* spp.; glyceraldehyde-3-phosphate dehydrogenase for discrimination of *Cochliobolus* spp., *Curvularia* spp., and *Bipolaris* spp.; and actin for discrimination of *Rhizopus* spp., *Stachybotrys* spp., and *Trichophyton* spp. [37–47]. In many cases, multiple genes may need to be used to discriminate different species depending on the genus. Presently, there are no specific criteria that are used to determine what

target should be used to discriminate different species. Decisions are typically made through individual laboratory experience and publications describing phylogenetic studies that have included multiple species and multiple gene sequences. Finally, one of the great challenges to sequence-based diagnostics has been how to best standardize the methods used to arrive at an identification, and fit these guidelines within the normal function of a clinical laboratory. Presently, the most useful guidelines are provided by the Clinical Laboratory Standards Institute, which describe in detail sequence-based identification of fungi and the targets that can be used [48]. In spite of the multitude of targets and the potential to recover an informative sequence from any fungus, the most important caveat to sequence-based identification is that the value of the sequence data is completely dependent on the database it is searched against. The public databases, which are extraordinarily deep, are the best sources for rare fungi since data are collected from throughout the world for a broad spectrum of fungi, often with extensive redundancy. However, because anyone can deposit data, the error rate of data in the public databases is so high that databases such as GenBank are unlikely to meet the requirements of being a sole source for molecular identification under certified conditions. Instead, for the highest quality data, biocurated databases are needed that are closed to unauthorized deposits and contain only reference sequences derived from fungi with confirmed identity, such as culture collection isolates. There are a number of these databases with some, such as the MicroSeq Identification System (Applied Biosystems, Foster City, CA), even being commercialized. However, while the data are highly dependable, the number of unique species is going to be limited to fungi that are most commonly encountered in patients.

Arrays

In spite of the power of PCR-based technologies, even with multiplexing, it is difficult to incorporate a large number of potential identities into a single reaction. To increase the potential number of species that can be identified in a given assay, various array-based technologies have been developed that can expand the number of species that can be identified in a single assay by one to two orders of magnitude.

Arrays can be formatted in a number of different platforms and generally function by anchoring a set of known targets, in the form of a nucleic acid sequence, to some type of supportive matrix such as a bead, glass chip, or membrane. The unknown specimen is then hybridized to the array and a readout signal is generated, which can be decoded and matched to the target identity. The simplest arrays consist of spotting targets onto membranes using the Southern blotting platform developed years ago [49]. Numerous arrays of this type have been used to identify fungal species [50, 51].

Miniaturization of the arrays into a microarray format by high-density spotting enables a massive amount of spots, numbering in the thousands or tens of thousands, to be interrogated by the sample. However, the drawback is that these assays can be insensitive without an amplification step and can be slower than PCR due to the kinetics of DNA to DNA hybridization, which can take many hours. Decoding of the reaction—identifying the spot that the unknown hybridized to—can require specific instrumentation and advanced software. Furthermore, each spot carries a species-specific reagent that in turn carries a cost to be incorporated into the array. For *C. albicans,* which is frequently recovered from clinical specimens, the assay is cost effective. However, incorporating hundreds of other spots corresponding to fungi that are known to be pathogenic but are so rare that they may never be encountered in a clinical laboratory adds to cost but with questionable return.

Although the chips can be prepared robotically with extreme precision, the cost per assay can be quite expensive compared to alternatives. Scaling this process down using nanotechnology to create lab-on-a-chip assays that employ microfluidics is a newer approach to array technology that offers great promise due to the smaller reaction volumes (microliters) and user-friendly format more conducive to a clinical laboratory. Additionally, engineering cartridges containing the targets and marrying these cartridges to an instrument can greatly simplify and standardize the hybridization reactions. Large-scale application to any unknown fungal isolate has been slow to develop using arrays; however, focused use at the genome level has achieved some success. For example, Aittakorpi et al. have used microarrays to identify *Candida* spp. in cases of fungemia using the Prove-it, Sepsis microarray, which was derived from a microarray-based approach for identifying bacterial sepsis [52, 53].

Finally, bead-based arrays that combine the power of multiplexing and the simpler platform of liquid suspension have been successful in identifying a number of fungal species. An 11-plex bead array was used by Buelow et al. to identify common respiratory-associated fungi using the Luminex platform [54]. Babady et al. used the same platform to design a Luminex-based assay to identify 23 fungi commonly found to cause invasive infections in the immunocompromised while Balada-Llasat et al. used the Luminex platform to identify yeasts from blood cultures [55, 56]. These assays are targeted approaches to identify anticipated groups of fungi. The Luminex platform can multiplex almost 100 fungal analyte targets, which potentially could detect most of the commonly encountered fungi.

Peptide Nucleic Acid Fluorescent in Situ Hybridization (PNA–FISH)

PNA-FISH (AdvanDX, Woburn, MA) was adopted into some clinical microbiology laboratories to discriminate *C. albicans* from non-*C. albicans* directly from positive blood cultures [57, 58]. The assay employs PNA probes to target specific rRNA sequences in a highly sensitive and specific FISH assay that allows the technologist to view whole cells of the target pathogen [59]. The rRNA targeting brings enhanced sensitivity due to the abundance of the target. As the probe is sequence based, it can be highly discriminatory due to differences in target sequence, yet downstream manipulation of data is not required since the assay is read visually by detection of fluorescent cells. In fact, the probe is able to discriminate *C. albicans* from *Candida dubliniensis,* which is nearly impossible to do biochemically [60]. This assay requires limited sample preparation since cells do not need a prior extraction step, and quick visual results within 90 min are usually obtainable. The main drawback of the assay is based on the need for cells to be permeable to the probe since it needs to enter the cell. For *Candida* spp., permeability is not a major problem; however, filamentous fungi are much less amenable to this assay as a diagnostic tool since fungal hyphae are generally not as permeable as yeast cells.

Matrix-Associated Laser Desorption Ionization-Time of Flight (MALDI-TOF)

Culture is the "gold standard" of diagnostic mycology; however, waiting for cultures to grow and differentiate can take days, or even weeks for some fungi, such as *Histoplasma* sp. As a result, physicians may treat patients for days without knowing whether their patients are actually infected or the identity of the organism causing their patients' illness. Matrix-associated laser desorption ionization-time of flight (MALDI-TOF) is a recent development in the clinical microbiology field that is rapidly changing the routine diagnostics field because of the speed with which samples can be run through the instrument [61].

MALDI-TOF uses the unique protein fingerprint of each species to give identification in a few minutes if the unknown sample has a matching reference spectra in the library that comes linked to the instrument. After a quick sample preparation, fungal cells are mixed with an ultraviolet (UV) absorbing matrix and dried on the steel target plate. The dried preparations are loaded onto the MALDI-TOF instrument and are subsequently exposed to laser pulses, resulting in energy transfer from the matrix to the analyte, creating a "charged vapor" of the biomolecules of interest [62]. The particles in this vapor are accelerated and separated under the influence of an electric field, which requires different times to reach the detector and is referred to as the time of flight [63]. The proteins are then separated based on their mass/charge ratio, which creates a unique pattern of peaks or fingerprints at the detector, also known as the mass spectrum. The resulting spectra of biomolecules are unique and species specific. The spectrum of each organism is compared to a library of reference spectra within the instrument, leading to an identification within minutes as opposed to days or weeks for some fungi [61].

The rapid turnaround time and minimal cost for consumables per specimen compared with conventional identification methods has resulted in MALDI-TOF being increasingly used in clinical laboratories worldwide. Adoption of this technology in clinical microbiology laboratories across the USA will decrease the time to identification of fungal pathogens by an average of 1–2 days and decrease laboratory costs. The MALDI-TOF instrument has an upfront cost of US$ 213,000 (including sales tax and first year service contract) and ~US$ 35,000 to operate annually, depending on instrument manufacturer. This initial capital outlay is substantial; however, the cost per sample can be less than 5 cents, which is a significant reduction compared to the cost to run PCR, biochemical methods, or PNA-FISH.

Several platforms are available; a system from Bruker Daltonics (Billerica, MA), which includes a mass spectrometer along with the "Biotyper" software and database. Another system uses a Shimadzu Axima Assurance mass spectrometer (Columbia, MD), Launchpad software and the AnagnosTec GmbH (SARAMIS) database. This system was recently acquired by **bioMérieux,** and is being redeveloped and called "VITEK MS." These two systems exclusively use cell lysis on the target plate (without off-plate extraction).

The great advantage of MALDI-TOF is that it addresses a number of shortcomings of other molecular methods. Template extraction, while not yet completely universal, is still easy and relatively labor-free compared to other methods. However, once this step is done, the sample is ready for analysis as there are no further manipulations needed. There is no downstream analysis after the sample has been run as the instrument will call the identification based on generated spectra, which is compared to the reference library of spectra. Additionally, the instrument can analyze other microbes with little variation in sample preparation, which opens the door for standardization. Drawbacks include large capital start-up costs, and annual expenditures for service. The instrument is only as good as the reference library, and although users can add to the library, it would take years to make a spectral library as deep as the sequence databases. Furthermore, libraries are proprietary as there is no public database of spectra. Another drawback is the need to culture the organism prior to performing the test, so arguably, the analysis turnaround time benefit is mitigated by culture outgrowth delay. Validation studies have shown that some

organisms require repeat analyses and additional processing. The acceptable score cutoffs also vary between organisms [61]. Some closely related fungal organisms cannot be differentiated, and sporulation or different morphologic growth phases may present a challenge for identification since each morphology may require a different set of proteins resulting in variable spectra. Finally, these systems are not yet approved for use in the USA by the FDA. The current lack of FDA approval of any MALDI-TOF MS system for organism identification limits widespread use in the USA.

In spite of these areas, which need improvement, MALDI-TOF is an exciting new technology with many promising advantages. It is automated and does not require specific expertise in MS. It has a rapid turnaround time and high-throughput capability. It is associated with a low-exposure risk due to sample inactivation and is cost-effective with high-interlaboratory reproducibility. The broad applicability covering all types of bacteria, mycobacteria, and fungi makes the instrument more palatable to microbiology laboratory budgets.

Summary

Diagnostic mycology has a great need for molecular approaches to fungal identification. While there is no doubt that classical mycology diagnostic methods will not become obsolete anytime soon, the field needs to recognize that medical mycologists with diagnostic skills are not being trained in great numbers. Consequently, clinical microbiology laboratories are at great risk for not having the expertise to handle fungal identification. While not a substitute for classically trained mycologists, adding a molecular component to diagnostic mycology can greatly ease the loss of these skills and offer some opportunities for synergism. The increasing degree of immunosuppression, aggressive nature of modern medical treatment, and an aging population are just a few of the risk factors that contribute to infections with a broadening spectrum of fungi never seen before in the clinical laboratory. Frequent case reports of new fungal pathogens combined with regular discoveries of new species of fungi that are also capable of infecting humans clearly demonstrate that the field of clinical mycology needs to expand, not contract, with regard to the skills that can be applied to fungal identification. While there is no clear path for training a new generation of medical mycologists without the support of these programs at academic institutions, new technology, instrumentation, and strategies can offset pending losses of experienced mycologists. Therefore, from a strategic perspective, these advances should be integrated into the clinical laboratory whenever possible to enhance the possibility that one or more may emerge as a viable alternative or addition to classical identification methods.

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Diagnostic Histopathology

4

John E. Bennett

Introduction

Diagnosis of mycoses using microscopy has the advantage of rapidity and often confers the advantage of showing that the fungus is invading tissue, not colonizing bronchi, pulmonary cavities, paranasal sinuses, or surfaces of the skin or mucous membranes. Fungal contaminants that grow on culture are often too sparse to be seen microscopically so the problem of contamination is less with microscopy. Some fungi are difficult (mucormycosis, histoplasmosis) or impossible to grow (lobomycosis) and are best identified by microscopy. However, microscopic diagnosis depends on the skill of the observer and the fungal species. Yeasts are easier to identify on smear or histopathology than molds.

Specimen Preparation

Wet Mount

Wet mount is a smear of liquid specimen or, for ringworm, scrapings of skin or nail. Digestion with potassium hydroxide is the oldest method but calcofluor white staining and ultraviolet (UV) microscopy is far superior, rapid, and less likely to damage the microscope stage. Wet mounts cannot be sent for review elsewhere. The inflammatory response is generally not discernible.

Cytopathology

Needle biopsies and aspirates as well as bronchoalveolar lavage, sputum, and urine may be sent for cytopathology. The cells to be examined may be cytocentrifuged onto a glass slide or centrifuged into a pellet, fixed, mounted in paraffin, sectioned, and placed on a slide. With cytocentrifuge specimens, only one or two slides may be available, limiting the number of stains. With the primary intent of cytopathology being examination for malignancy, the stain used may be Papanicolaou, a stain not well designed to locate and identify fungi.

Histopathology

Pieces of tissue are fixed in formalin, embedded in paraffin, sections mounted on slides and stained. If the tissue is large enough, multiple sections may be stained and sent for review outside of the institution. Histopathology provides the opportunity for assessment of the associated inflammatory response.

Host Inflammatory Response

The inflammatory response varies between tissues of the same patient and also between patients. What are listed below are general characteristics that often help confirm the identity of the fungus seen on special stains. Nothing about the inflammation distinguishes mycoses from other pathogens such as tuberculosis or noninfectious diseases.

Pyogenic

Some fungi typically elicit a neutrophilic response around the fungus, provided that the patient is not granulocytopenic. Histopathology may show neutrophils around the fungus even when the surrounding response is granulomatous, forming a "pyogranuloma." Clusters of neutrophils are usually in a necrotic area, i.e., an abscess. Pus seen on clinical

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examination is an indication of a neutrophilic response and is more likely in blastomycosis and coccidioidomycosis, for example, than in histoplasmosis or cryptococcosis.

Granulomatous

This word is not clearly defined among pathologists. In cytopathology, it may mean only clumps of macrophages. In histopathology, "granuloma" usually indicates multinucleated giant cells, lymphocytes, and macrophages, particularly the long slender macrophages termed as "epithelioid cells." A "well-organized" or "tuberculoid" granuloma means a round cluster of epithelioid cells in whorls, usually with multinucleated giant cells. The most distinctive granuloma, a "caseous granuloma," is a rounded lesion with a central core devoid of cellular detail, ringed by columnar epithelioid cells, and surrounded by lymphocytes, plasma cells, macrophages, and multinucleated giant cells.

Hoeppli–Splendore Phenomenon

Hoeppli–Splendore phenomenon is an amorphous eosinophilic coating around organisms, characteristic of hyphae in basidiobolomycosis and conidiobolomycosis but occasionally seen around yeast cells in sporotrichosis. Schistosomiasis and other chronic nonmycotic infections may show this feature.

Grains

Grains are tight clusters of organisms, characteristic of actinomycosis and mycetoma, including those caused by fungi, eumycetoma, and higher bacteria, actinomycetoma. Deep dermatophyte lesions may show grains. Loose clusters of bacteria in very chronic infections are called "botryomycosis."

Stains

Hematoxylin and Eosin (H&E)

Hematoxylin and eosin (H&E) is a standard stain used in histopathology which is useful in identifying the inflammatory response and the presence of malignant cells but is not sensitive for visualizing fungal cells. Although fungi are not easily identified on H&E, sometimes the purple of hematoxylin will stain hyphae and sometimes *Blastomyces* sp. or other yeast cells. The golden color of heavily melanized hyphae or "copper penny" chromoblastomycosis cells is better seen on H&E than on gomori methenamine silver (GMS) or periodic acid Schiff (PAS).

Gomori Methenamine Silver (GMS)

GMS is the best stain for finding fungi and seeing septae in hyphae, which appear black or dark brown. GMS can stain other structures which may be misidentified as fungi. Overstaining is common and GMS stains numerous artifacts, reticulum fibers, and neutrophils. If a structure is not stained by GMS, it is not a fungus. As a rare exception, hyphae of mucormycosis may not stain with GMS. The inflammatory response is difficult to discern on GMS.

Periodic Acid Schiff (PAS)

PAS stains fungal cell wall polysaccharides red. Fungi are often more difficult to locate in many tissues with PAS when compared with GMS, but this stain typically allows the evaluation of the inflammatory response. Lipid droplets, glycogen storage vacuoles, and some artifacts also stain with PAS and may resemble yeasts.

Mayer's Mucicarmine Stain

Mucicarmine stain used in identifying mucinous adenocarcinoma can also help identify yeast cells as *Cryptococcus neoformans* or *Cryptococcus gattii*. Staining is not uniform among cryptococci in the same section and sometimes cells are not visibly stained at all. The process of tissue fixation and paraffin removal shrinks the polysaccharide capsule around the cryptococcal cell, providing a reddish rim but the wall itself is not stained.

Masson-Fontana

Agents of phaeohyphomycosis may stain brown with Masson-Fontana stain, allowing the differentiation of their hyphae from those of the hyalohyphomycosis such as *Aspergillus*, *Fusarium,* and *Scedosporium* spp. Cryptococci may also stain brown with this stain. The contrast is not sufficient to make this a useful stain for finding fungi in the section. The inflammatory response is not well seen with this stain.

Brown and Brenn Stain (Tissue Gram Stain)

Brown and Brenn is a poor stain for fungi, which may appear gram-positive or gram-negative in tissue. Candida yeast and pseudohyphae are usually gram-positive.

Histopathological Characteristics of Deep Fungal Pathogens

Yeast-like Cells in Tissue

Blastomycosis

In the skin, *Blastomyces dermatitidis* are often in small subcutaneous abscesses, surrounded by neutrophils. Differentiation between *Blastomyces, Cryptococcus,* and *Histoplasma* spp. can be difficult if only a few cells are seen. *Blastomyces* yeast cells have a thick, refractile cell wall and have initially a broad pore between mother and daughter cells (Fig. 4.1). The daughter cells remain attached until the daughter is nearly as large as the mother cells, with the pore narrowing and disappearing as the attached daughter cell enlarges. A pseudoepitheliomatous response is prominent in the dermis and may be mistaken for squamous cell carcinoma. In the lung, a granulomatous response predominates but collections of neutrophils are also present, a "pyogranulomatous response." Cavitation and fibrosis are common in chronic lung lesions but calcification in lung or lymph nodes is rare, if it occurs.

Candidiasis

Pseudohyphae and yeast cells are seen in invasive candidiasis. Pseudohyphae differ from true hyphae in having constrictions at the septae and showing branching only at points of septation. Yeast cells, also called blastospores or blastoconidia, may bud off pseudohyphae at septae (Fig. 4.2). Yeast cells are also seen separately in the tissue, along wih pseudohyphae. Yeast cells are round or elliptical, smaller than *Blastomyces,* and may show budding or even chains of budding cells. Pores between mother and daughter yeast cells are too small to be seen readily. Yeast cells are rarely seen without pseudohyphae, with the exception that *Candida glabrata* has only yeast cells (Fig. 4.3). If patients are not granulocytopenic, the predominant inflammation is neutrophilic. *Blastoschizomyces capitatus* and *Trichosporon asahii* share similar features with candida in tissue.

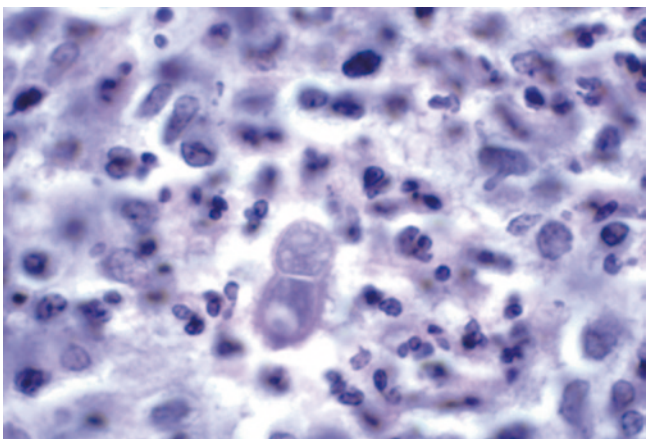

Fig. 4.1 Budding cell of *Blastomyces dermatitidis*. Note that the daughter cell is almost as large as the mother cell and still connected by a pore. *H&E* hematoxylin and eosin. (From MB Smith, MR McGinnis. Diagnostic histopathology. In Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer)

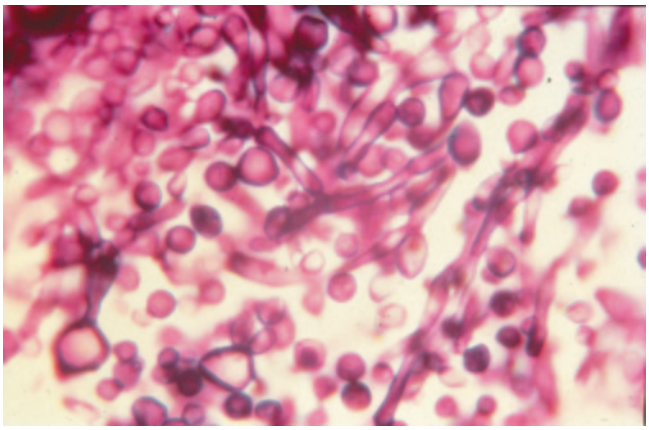

Fig. 4.2 *Candida albicans* pseudohyphae and blastospores. Note constrictions along the pseudohyphae. *GMS* Gomori methenamine silver

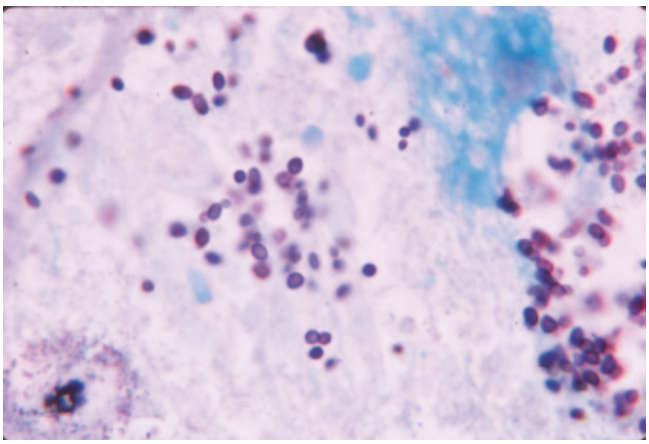

Fig. 4.3 *Candida glabrata* in a cardiac valve vegetation. Note the absence of pseudohyphae. *GMS* Gomori methenamine silver

Chromoblastomycosis

The most distinctive structures are thick-walled golden hued cells in the dermis, called "copper penny" cells, often surrounded by neutrophils and a granulomatous response (Fig. 4.4). The hyperplastic epidermis sometimes has short, wavy "fumigoid" hyphae.

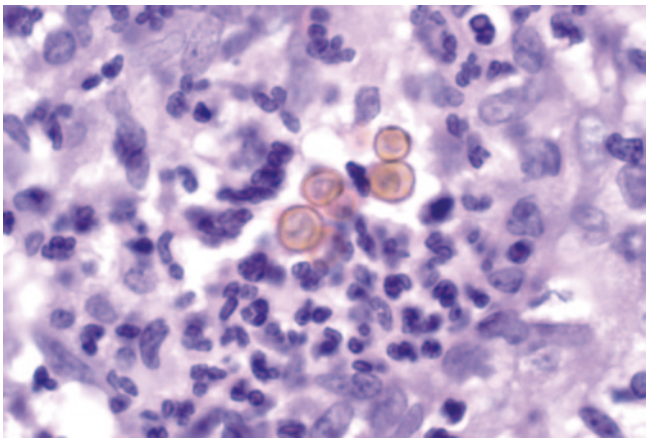

Fig. 4.4 Brown thick-walled "copper penny" cells of chromoblastomycosis in the dermis. *H&E* hematoxylin and eosin

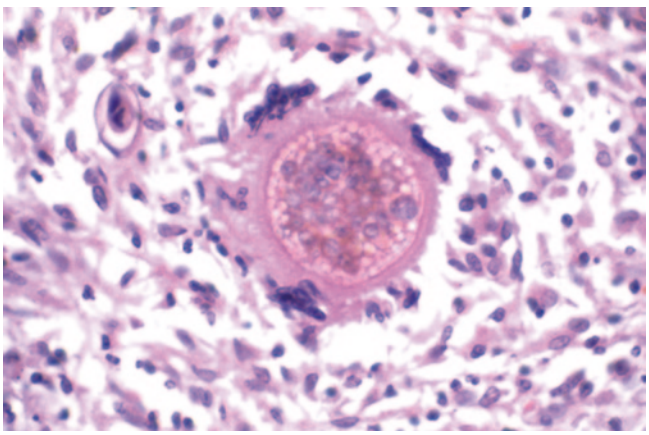

Fig. 4.5 Large spherule of *Coccidioides* species containing endospores. *H&E* hematoxylin and eosin. (From MB Smith, MR McGinnis. Diagnostic histopathology. In Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer)

Coccidioidomycosis

Spherules are all round, nonbudding, and range in size from that of *Blastomyces* or *Cryptococcus* to several times that large. Rounded endospores may be seen inside the larger spherules (Fig. 4.5). Neutrophils clusters around endospores released from ruptured spherules. Large pockets of pus may form in the tissue. Granulomatous inflammation or pyogranulomas are usual, similar to blastomycosis. Tissue eosinophilia is often present on H&E stain but easily missed. Caseous necrosis may be seen in chronic lesions, resembling tuberculosis, but calcification is not seen. Short hyphae are occasionally seen in *Coccidioides* lesions. Spherule-like structures, larger than *Coccidioides* and filled with numerous endospores, can be seen with *Rhinosporidium seeberi,* a protistan parasite of mucous membranes (Fig. 4.6), and with the *Emmonsia* species causing adiaspiromycosis of the lung.

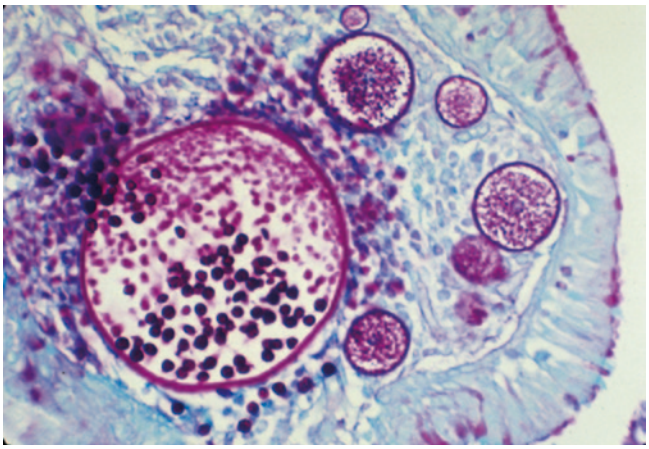

Fig. 4.6 Large cyst of *Rhinosporidium seeberi* containing spores. *PAS* Periodic acid Schiff. (From MB Smith, MR McGinnis. Diagnostic histopathology. In Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer)

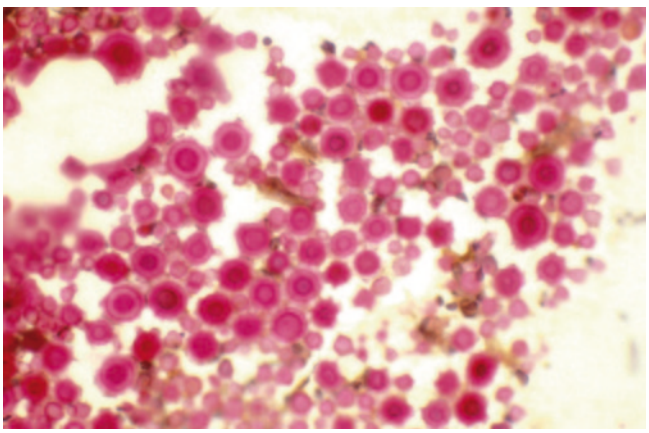

Fig. 4.7 Cryptococcal cells stained red with Mayer's mucicarmine stain. (From MB Smith, MR McGinnis. Diagnostic histopathology. In Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer)

Cryptococcosis

The yeast cells sometimes are obvious from the space around the cell, previously occupied by capsule that shrank around the cell during fixation. The capsule is easiest to detect in cells within the cytoplasm of phagocytes and in the giant "bubbles" of cryptococci in brain tissue. The yeast size overlaps *Blastomyces, Candida,* small *Coccidioides* spherules, *Paracoccidioides,* and larger *Histoplasma* cells. Definite staining with Mayer's mucicarmine can be very helpful (Fig. 4.7). If mucicarmine is negative, Masson-Fontana staining can be useful. In the lung, dense granuloma formation is usual, sometimes with clusters of cryptococci resembling caseous necrosis. Pyogranulomas, calcification and clusters of neutrophils, are not seen. In the brain, chronic inflamma-

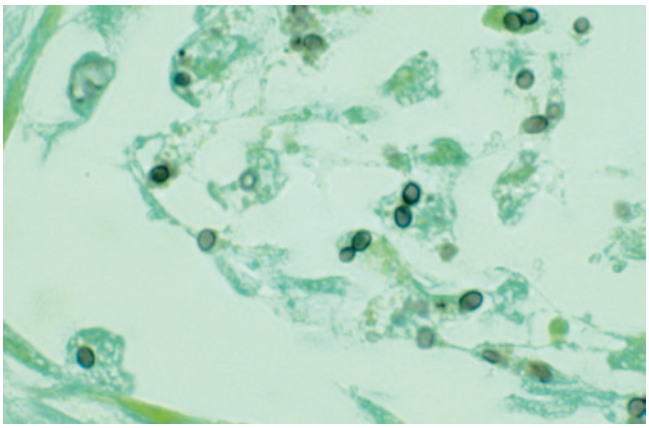

Fig. 4.8 *Histoplasma capsulatum* with an unusually large number of budding cells. *GMS* Gomori methenamine silver. (From MB Smith, MR McGinnis. Diagnostic histopathology. In Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer)

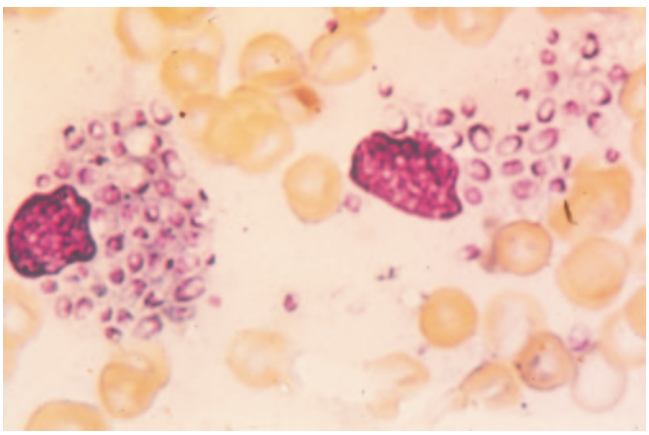

Fig. 4.9 *Histoplasma capsulatum* cells in cytoplasm of macrophages. Giemsa stain

tion with cryptococci may be visible in the subarachnoid and Virchow-Robin spaces. Large clusters of cryptococci with no surrounding inflammation are seen in the cerebral and cerebellar cortex.

Histoplasmosis

The yeast cells of *Histoplasma* are smaller than those of *Blastomyces* or *Cryptococcus,* but similar in size to *Pneumocystis jirovecii, Candida glabrata, Penicillum marneffei, Malasezzia furfur, Emmonsia pasteuriana* and the protozoa, *Leishmania* and *Trypanosoma cruzi* (Fig. 4.8 and 4.9). Even though *Histoplasma* is a budding yeast, unlike *Pneumocystis* or protozoa, pores between mother and daughter cells are rarely visible. Distinction between budding cells and cells simply touching one another may be difficult to discern in clustered cells. Location outside the lung is rare for

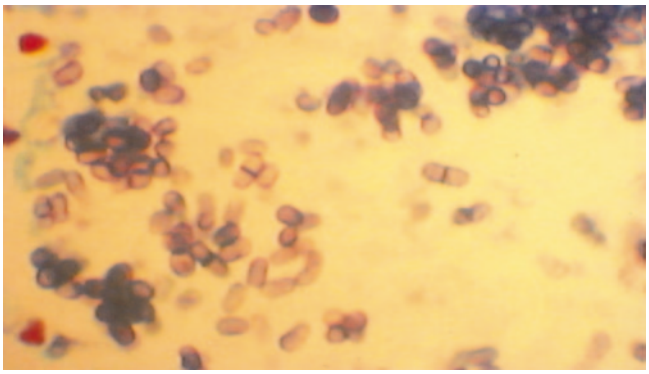

Fig. 4.10 *Penicillium marneffei*. Note cells dividing by fission with septum between mother and daughter cell. *GMS* Gomori methenamine silver

Pneumocystis, where the cells appear as clusters in alveloli. *Malasezzia* is rarely visualized outside the skin. *C. glabrata* elicits a pyogenic inflammation, not seen with histoplasmosis. *Leishmania* and the amastigotes of *T. cruzi* do not stain with GMS and contain a distinctive bar-shaped structure, a kinetoplast, that is visible on H&E, but better seen on Giemsa stain. *P. marneffei* has a distinctive geographic distribution in Southeast Asia and multiplies by fission, not by budding (Fig. 4.10). *Emmonsia pasteuriana* is a dimorphic fungus, seen in AIDS patients, that closely resembles *H. capsulatum* in tissue. Valvular vegetations in *Histoplasma* endocarditis may show only short hyphae, incorrectly suggesting a mold endocarditis. The inflammatory response in histoplasmosis resembles tuberculosis and runs the gamut from macrophages packed with yeast cells to epithelioid granulomas or caseation necrosis with rare organisms. Calcification of old granulomas in the lung, hilum, or mediastinum is common. Fibrosis can be extensive in the lung or mediastinum.

Paracoccidiodomycosis

The appearance and associated inflammation seen with *Paracoccidioides* in tissue is very similar to that observed in blastomycosis; the single difference being that a single cell may be budding from more than one site on the cell wall of the *Paracoccidioides* yeast. The pore between mother and daughter cell is tiny and the daughter cell separates while still small (Fig. 4.11). When one cell has multiple buds, the cell may resemble the "pilot wheel" of a ship, though such structures are rare in tissue.

Sporotrichosis

Budding yeast cells of *Sporothrix* species are elliptical (cigar-shaped) or spherical (Fig. 4.12). Organisms are abundant in extracutaneous sites but so scant in skin lesions that multiple GMS-stained sections may be needed to locate them. The usual inflammatory response is a pyogranuloma.

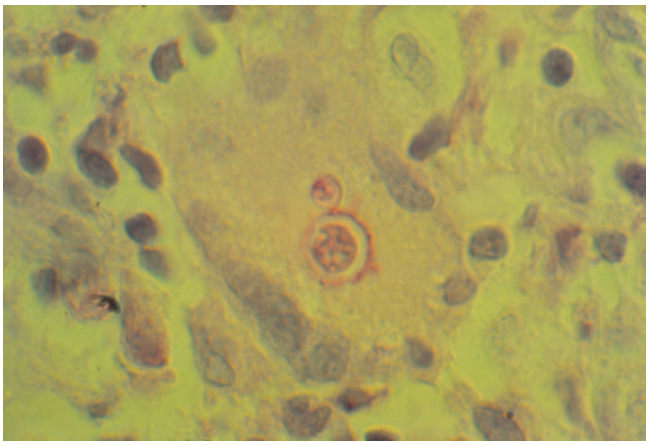

Fig. 4.11 *Paracoccidioides brasiliensis* budding cell. Note *narrow* attachment of the small daughter cell. *PAS* Periodic acid Schiff

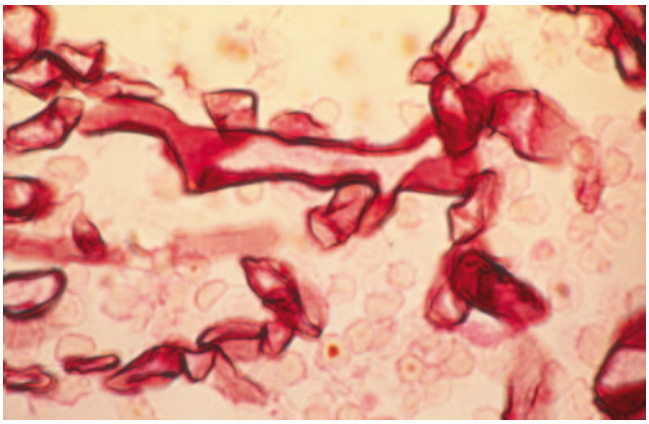

Fig. 4.13 Mucormycosis. Note variable hyphal width, absence of septae, and sharp turns. *GMS* Gomori methenamine silver

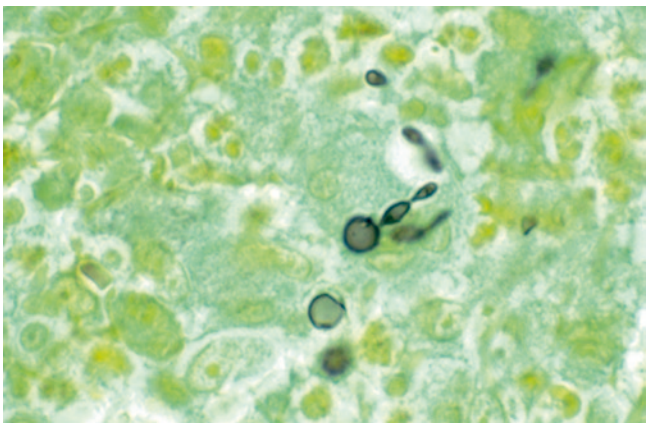

Fig. 4.12 *Sporothrix* species. Both spherical and elongated cells are seen here but either shape may occur alone. *GMS* Gomori methenamine silver. (From MB Smith, MR McGinnis. Diagnostic histopathology. *In* Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer)

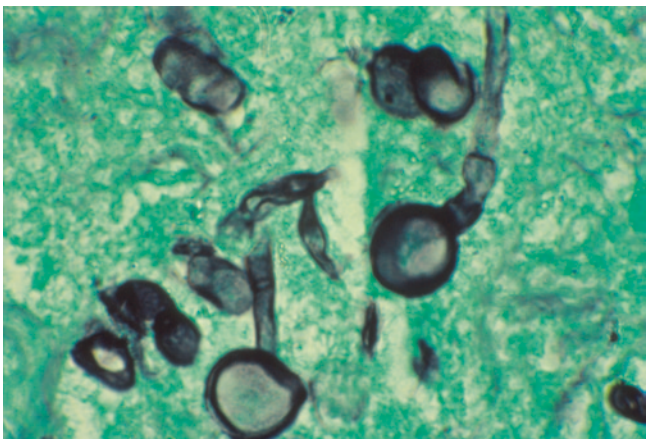

Fig. 4.14 Phaeohyphomycosis. *Curvularia lunata*. Note bulbous swellings of hyphae. *GMS* Gomori methenamine silver

Hyphae in Tissue

Identifying molds in tissue is fraught with errors. Air-containing structures, such as lung cavities, paranasal sinuses and the external auditory canal may permit sporulation, allowing identification of *Aspergillus* or *Scedosporium* hyphae by their spore-bearing structures. The broad, aseptate hyphae of basidiobolomycosis and conidiobolomycosis may be distinguished by the tissue eosinophilia and Hoeppli-Spendore phenomenon. In theory, hyphae of mucormycosis should be distinguished by being pauciseptate, broad, of variable width, lacking Y-shaped (less than 90°) branching and their ability to make sharper bends in tissue than many other molds (Fig. 4.13). In practice, mucormycosis is commonly mistaken for other molds and vice versa. Hyphae in phaeohyphomycosis often contain bulbous swelling and stain brown with Masson Fontana (Fig. 4.14). Most commonly, hyphae in tissue cannot be identified with certainty, including that of *Aspergillus* species (Fig. 4.15). Immunocytochemistry has not proven to have adequate specificity, leaving molecular technique on fresh or fixed tissue as the best option for species identification of molds in tissue.

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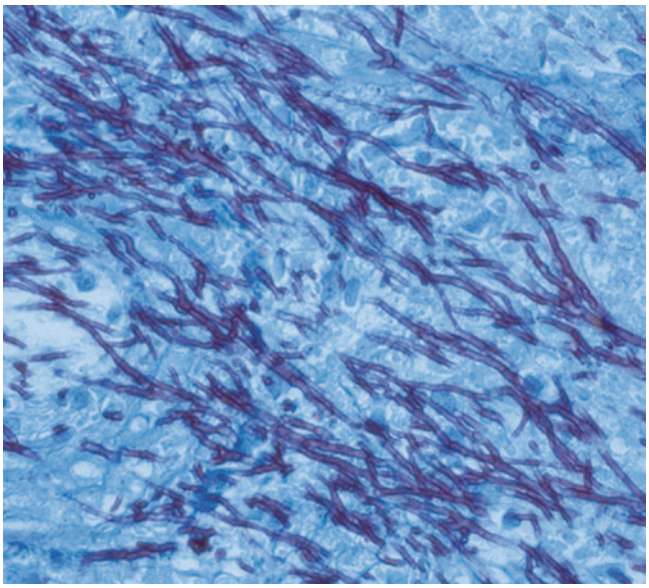

Fig. 4.15 *Aspergillus fumigatus*. Note straight hyphae of even diameter. *GMS* Gomori methenamine silver. (From MB Smith, MR McGinnis. Diagnostic histopathology. In Diagnosis and Treatment of Human Mycoses, DR Hospenthal, MG Rinaldi, eds. Humana Press. Totowa, NJ. 2008. Reprinted with permission from Springer)

Suggested Readings

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Diagnostic Immunology

5

Christopher D. Pfeiffer and Brian Wong

Introduction

The frequency of invasive fungal infections has risen dramatically in recent decades, mostly because of a larger population of at-risk patients who are immunocompromised, neutropenic, or critically ill. For clinicians evaluating these patients, it has become increasingly important to make the diagnosis early so that timely antifungal therapy can be instituted. Although culture of body fluids or tissue for the causative fungus continues to be the gold standard for definitive diagnosis, this process can sometimes take several weeks for results and often lacks sufficient sensitivity. For example, blood cultures are positive in only ~50% of the cases of invasive candidiasis (IC) and very rarely in the cases of invasive aspergillosis (IA) [1, 2]. A presumptive diagnosis can also be made on the basis of characteristic histopathology and special tissue stains. However, obtaining adequate samples from protected anatomical sites are often not feasible in the populations at highest risk for such infections. Nonculture-based diagnostic tests are classified into four groups according to what component of the invading pathogen or host immune response they target. These include detection of host antibody, fungal antigen, fungal metabolites, or fungal nucleic acid. Overall, despite these multiple potential targets and extensive efforts toward development, only a handful of nonculture-based tests have proven clinically useful, and even fewer have reached commercial availability. As current diagnostic techniques are less than ideal, development of new methods is a priority in medical mycology. This chapter outlines the available immunologic tests according to what component of the invading pathogen or host immune response they target and provides some discussion of their strengths and weaknesses. Given the growing interest in this field, there is also some introduction to newer assays that are currently being investigated. Specific recommendations for utilizing the currently available tests in conjunction with the culture and histopathology are discussed for individual fungal species and specific disease manifestations.

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Host Antibody Testing

Many tests in current use have been developed to detect host antibodies against specific fungal antigens. These require identification of one or more distinctive antigens to which host antibodies are directed, sufficient immunocompetence on the part of the host to mount a specific antibody response, and the use of a variety of techniques to detect the antibody. Tube precipitin (TP) assays, immunodiffusion (ID) assays, complement fixation (CF) assays, radioimmunoassays (RIA), and enzyme-linked immunosorbent assays (ELISA/EIA) are some such techniques. One major limitation of this general approach is that immunocompromised patients have impaired abilities to mount specific antibody responses. Moreover, these responses may be delayed and antibodies do not necessarily distinguish acute from chronic or a past history of infection. Finally, antibodies to some fungal antigens may be demonstrable in uninfected people, thereby reducing the diagnostic specificity of positive tests.

Fungal Antigen Testing

A second common method to diagnose fungal infection includes tests that use immunologic reagents to identify

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antigenic components of the fungus. These require the presence of unique antigens in a body fluid or tissue specimen that is available for testing and use of a variety of techniques to detect these antigens. Some such techniques include latex agglutination (LA) assays, dot immuno-binding assays, ELISA/EIA, RIA, and lateral flow immunoassays (LFA). Monoclonal or polyclonal antibody is often needed in these assays to help detect the antigen of interest. The major limitations of this general approach are the low level and transient nature of antigenemia in some hosts, cross-reactions between antigens derived from different fungal species, and lack of specificity for a particular antigen when polyclonal antibodies are used.

Fungal Metabolite Testing

Another methodology includes the direct detection of fungal metabolites in patient serum or other samples. These are usually by-products of a specific fungus which are detected either by gas chromatography, mass spectrometry, or an enzymatic reaction. One limitation of these tests is that the metabolic products are not unique to individual fungal species and may be present in small amounts in uninfected individuals. Furthermore, the level of metabolite may not be present in sufficient quantity outside of the local tissue being invaded, making detection difficult.

Fungal Nucleic Acid Testing

There has been a rapid expansion of diagnostic tests targeting fungal nucleic acid which is now discussed in Chap. 3 (discussed in this chapter in a previous edition of this book).

PAN-Fungal Testing: 1,3-B-D-Glucan

1,3-β-D-glucan (BG) is a major component of the cell wall of many different fungi. The ability of fungal BG to activate an enzyme in the clotting cascade of the horseshoe crab has led to the development of assays capable of detecting very small amounts of BG. Four separate assays are commercially available and each assay uses individualized cutoff values to define positivity. Available assays include Fungitell (formerly Glucatell; Associates of Cape Cod, East Falmouth, MA), Fungitec-G (Seikagaku, Tokyo, Japan), Wako turbidimetric assay (Wako Pure Chemical Industries, Tokyo, Japan), and Maruha colorimetric assay (Maruha-Nichiro Foods, Tokyo, Japan) [3]. The primary clinical scenarios where the accuracy of BG has been evaluated are discussed below and summarized in Table 5.1. In two recently performed meta-analyses, the diagnostic accuracy for invasive fungal infection (IFI) was moderate (sensitivity 77–80%; specificity 82–85%) but for *Pneumocystis jirovecii* pneumonia (PCP) was high (sensitivity 95–96%; specificity 84–86%) [4–6]. Due to the low pretest probability for IFI (5–15% in many clinical settings), the BG test has a high-negative predictive value (i.e., if the test is negative, the likelihood of IFI is quite low). A major limitation of the BG is its inability to differentiate between different species of fungi. This is because BG is a component of the cell walls of many fungi including *Candida, Aspergillus, Pneumocystis, Trichosporon, Fusarium,* and *Saccharomyces* species. Importantly, BG is not found in *Cryptococcus* or the agents of mucormycosis. False-positive results are associated with receipt of hemodialysis, recent surgery, exposure to immunoglobulin or albumin products, receipt of certain medications (e.g., amoxicillin-clavulanic acid), and bacteremia [7–14].

Diagnosis of IFI in High-Risk Hemato-Oncological Patients

The meta-analysis described above for IFI detection included a majority of studies involving the hemato-oncologic patient population. Limiting discussion to this population only, however, BG evaluation in case-control studies has demonstrated a wide range of sensitivity (50–90%) and specificity (70–100%) [15–17]. BG assessments in the cohort study setting, which more closely reflects the real world performance of the test, have been performed. These cohort studies are the subject of a systematic review and meta-analysis which evaluated the diagnostic accuracy of BG in high-risk adult hemato-oncological patients. Of the six studies meeting inclusion criteria, the diagnostic performance of two consecutive BG assays was superior to the performance of one test alone for the diagnosis of proven or probable IFI; the sensitivity and specificity of two consecutive positive tests were 49.6% (95% CI, 34.0–65.3%) and 98.9% (95% CI, 97.4–99.5%), respectively [3]. When a threshold of only one positive test was applied, the BG sensitivity and specificity were 61.5 and 90.8%, respectively. The most common IFIs detected in the six studies were invasive aspergillosis ($n=90$), invasive candidiasis ($n=80$), PCP ($n=14$), and other ($n=31$). Importantly, similar accuracy was noted across all four available BG assays. The use and interpretation of BG must be mindfully considered in context of local factors specific to a hemato-oncologic program's fungal prophylaxis and detection strategy.

Diagnosis of IFI in High-Risk Intensive Care Unit (ICU) Patients

Several reports have evaluated BG for diagnosis of IFI, primarily invasive candidiasis (IC), in the ICU setting. In a

Table 5.1 Studies of serum 1,3-β-D-glucan (BG) for invasive fungal infection (IFI) and *Pneumocystis jirovecii* pneumonia (PCP)

Population	Assay	Study design	Sensitivity (%)	Specificity (%)	Comments[b]	Ref.
All	Any	Meta-analysis of 16 studies: 2979 patients; 594 with proven/probable IFI. Excluded diagnosis of PCP	77	85	One positive test required; study population = 11 hemato-oncologic; five at risk for IC; one transplant; one miscellaneous; test performance for detection of IC and IA was similar	[4]
	Any	Systematic review and meta-analysis of 31 studies: uncertain number of patients	80	82	One positive test using the test cutoff that offered the best test performance in each individual study; diagnostic accuracy was similar for IC versus. IA; sensitivity and specificity were lower (72 and 78 %, respectively) when analysis restricted to 17 cohort studies	[5]
High-risk hemato-oncologic	Any	Systematic review and meta-analysis of six cohort studies: 1771 patients analyzed; 215 had proven or probable IFI	62	91	One positive test required	[6]
			50	99	Two consecutive positive tests required	
High-risk intensive care unit (ICU)	Fungitell[a]	Prospective cohort: 95 ICU patients with length of stay > 5 days; 16 with IFI (IC = 14)	93	94	One positive test required; analysis performed for proven IC only	[18]
	Fungitell[a]	Prospective cohort: 57 ICU patients, nine developed proven/probable IC	91	57	One positive test required; many false positives occurred on the initial sample (ICU day 3); omitting these samples and requiring two consecutive positive samples increased sensitivity and specificity to 90 and 80 %, respectively	[11]
	Fungitell[a]	Prospective, randomized: 64 ICU patients, six developed proven/probable IC	100	75	Two sequential positive tests required; those with positive tests received empiric antifungal (anidulafungin) therapy	[10]
At risk for PCP	Any	Meta-analysis of 14 studies: 1723 controls (with other medical conditions) and 357 PCP cases	95	86	One positive test using test cutoffs according to (or closest to) the manufacturers' instructions required; HIV status did not impact accuracy	[6]
	Any	Systemic review and meta-analysis of 12 studies: uncertain number of patients	96	84	One positive test using the test cutoff that offered the best test performance in each individual study; HIV status did not impact accuracy	[5]

IC invasive candidiasis, *IA* invasive aspergillosis, *IFI* invasive fungal infection, *PCP Pneumocystis jirovecii* pneumonia
[a]Associates of Cape Cod, East Falmouth, MA
[b]Positive test cutoff according to the manufacturer's instructions used unless otherwise noted

prospective study of 95 nonneutropenic ICU patients with signs of sepsis and a length of stay > 5 days, 16 (17 %) were diagnosed with IFI (14 IC, one aspergillosis, one fusariosis). In this setting, a single BG test demonstrated 94 % sensitivity and 93 % specificity; moreover, for all 13 candidemia cases, the BG assay was positive 24–72 h before a positive blood culture result [18]. Mohr et al. evaluated BG in 57 consecutively enrolled surgical ICU patients, of whom nine had proven or probable IC [11]. Overall, when only one positive sample was required, the sensitivity and specificity of the BG for IC were 91 and 57 %, respectively. Many of the false-positive tests occurred early during the ICU stay (day 3), a finding that requires further examination. BG performance was much improved (sensitivity 90 %; specificity 80 %) if the samples obtained within 72 h of ICU admission were omitted and two consecutive positive samples were required. Finally, a prospective randomized pilot study was performed and involved 64 ICU patients, six of whom developed proven or probable IC [10]. Optimal assay performance in that study was found to use two sequential positive BG tests; the

sensitivity for IC was 100% and specificity 75%. Subjects randomized to the intervention group with two positive BG tests received preemptive anidulafungin therapy, which was safe and well tolerated (the study was not powered to detect clinical outcomes). The utility of BG as an adjunctive test in the ICU setting remains to be fully evaluated.

Diagnosis of Pneumocystis jirovecii Pneumonia

BG assay has also been evaluated for the detection of PCP. Two large meta-analyses were performed to evaluate the diagnostic accuracy of BG for PCP. The first report evaluated 12 studies and found a pooled sensitivity and specificity of 96 and 84%, respectively [5]. This study found nominal differences when test performance was stratified according to HIV status, assay type (Fungitell vs. Fungitec G test), study design (cohort vs. case-control), and methodological quality. A subsequent meta-analysis of 14 studies (nine of which were included in the first meta-analysis) evaluating 357 PCP cases and 1723 controls found BG to have a sensitivity of 95% and specificity of 86% [6]. All studies in this setting relied upon only one positive BG test. Given the high sensitivity, BG testing may be sufficiently sensitive to effectively exclude a diagnosis of PCP without bronchoscopy.

Conclusions and Recommendations

See Box 5.1 for recommendations on how test use is recommended. More data regarding the optimal use of BG in various clinical scenarios is undoubtedly forthcoming.

Box 5.1 Recommendations for using the serum 1,3-β-D-glucan (BG) assay

1. A positive test generally indicates IFI but is not pathogen-specific; always interpret the results in the context of other clinical and laboratory findings.
2. Serum BG can be employed as part of IFI surveillance in a similar fashion to serum GM (e.g., perform 1–2/week on at-risk hemato-oncologic patients). The test has moderate accuracy in this setting.
3. Serum BG appears to be a sensitive test when incorporated into a screening protocol for ICU patients at high risk for invasive candidiasis (IC). However, one positive test alone, particularly early in the ICU course, may be falsely positive and should ideally be confirmed with a second test.
4. Serum BG has excellent performance characteristics for the diagnosis of PCP and may be particularly helpful in ruling out disease for patients suspected of PCP when BAL fluid cannot be readily obtained.

Candidiasis

Candida species are now the fourth most common microorganism isolated from the bloodstream of hospitalized patients in the USA and sixth most common nosocomial pathogen overall [19]. Rapid detection of IC is critical and warrants the development of nonculture diagnostic approaches. Recent attention has focused primarily on the pan-fungal BG assay (discussed above), while other *Candida*-specific investigations (discussed below) have garnered less success.

Antibody Detection

Antibody detection assays were the area of earliest interest but yielded tests with poor sensitivity and specificity. These tests have since fallen out of favor and are not recommended for routine use.

Antigen Detection

Several tests that target a variety of cell wall and cytoplasmic components have been developed to detect macromolecular *Candida* antigens (Table 5.2). Some of these are no longer available, such as an assay to detect enolase antigen. Of the available tests, the earliest was the Cand-Tec LA assay (Ramco Laboratories, Stafford, TX), which was designed to detect circulating *Candida* antigen in patients with serious, disseminated infection. Unfortunately, there are conflicting reports on its overall sensitivity and specificity, especially in patients with renal failure or rheumatoid factor positivity, making it difficult to confirm the diagnosis of candidiasis by the Cand-Tec assay alone [20, 21].

The mannan component of the *Candida* cell wall is a major antigen and the target of many serum detection assays. These assays vary in the laboratory method and type of antibody used for antigen detection. Two tests that use the same monoclonal antibody are the Pastorex *Candida* LA test (Bio-Rad, Marnes-la-Coquette, France) and the Platelia *Candida* Antigen EIA test (Bio-Rad, Marnes-la-Coquette, France). Although the EIA test is more sensitive than the LA test, they are both limited by the rapid clearance of man-

Table 5.2 Studies of serum immunological assays for *Candida* infection (excluding BG)

Target	Assay	Population tested	Sensitivity	Specificity	Comments	Ref.
Antigens as detected by						
Unknown	Cand-Tec LA[a]	Retrospective case control: 39 candidemia cases; 40 controls (20 healthy volunteers, ten patients with *Candida* colonization only, ten patients with other deep mycoses)	77%	88%	Titer of 1:4 considered positive	[20]
		Prospective cohort of patients at risk for IC: 202 patients; 23 developed IC	70%	69%	Titer of 1:4 considered positive	[21]
Mannan	Pastorex LA[b]	As above	26%	100%	–	[20]
		Retrospective case control: 43 cases of proven IC; 150 controls included ICU patients, patients with other deep mycoses, and healthy volunteers	28%	100%	–	[22]
	Platelia *Candida* Antigen EIA[b]	Per-patient analysis of 14 studies (13 retrospective) including 453 patients (proven/probable IC) and 767 controls (healthy volunteers and high-risk patients without IC). The population studied was split (seven studies mainly hemato-oncologic; seven studies mainly ICU/surgery)	58%	93%	Cutoffs used were adopted from the primary study and varied Combined testing performance was superior	[23]
	Platelia *Candida* Antibody EIA		59%	83%		
	Platelia Candida Antigen and Antibody EIA[b]		83%	86%		
	Platelia *Candida* Ag Plus EIA	Retrospective case control: 56 candidemia cases; 200 controls (100 bacteremic, 100 nonbacteremic)	59	98	Cutoffs used per manufacturer's recommendations; *C. parapsilosis* and *C. guilliermondii* were not detected by Platelia *Candida* Ag Plus; BG in comparison: sensitivity 88%, specificity 86%	[24]
	Platelia *Candida* Ab Plus EIA		63	65		
	Platelia *Candida* Ag/Ab Plus EIA		89	63		
Metabolites as detected by						
D-arabinitol	Enzymatic–chromogenic	Prospective analysis of high-risk oncology patients and control patients that included those with fever, neutropenia, and mucosal colonization with *Candida* but no culture evidence of IC, and those without these and also without culture evidence of IC	31/42 (74%)	178/206 (86%)	Candidemia patients	[25]
			25/30 (83%)	–	Persistent candidemia patients	
			4/10 (40%)	–	IC patients w/o candidemia	
			7/16 (44%)	–	Deep mucosal candidiasis patients	
	Enzymatic-fluorometric	Retrospective evaluation of patients with candidemia and healthy control patients	63/83 (76%)	89/100 (89%)	Candidemia patients	[26]
			25/30 (83%)	–	Persistent candidemia patients	

BG 1,3-β-D-glucan, *ICU* intensive care unit, *IC* invasive candidiasis, *EIA* enzyme immunoassay
[a]Ramco Laboratories, Stafford, TX
[b]Bio-Rad, Hercules, CA

nan antigenemia. In an effort to overcome this, an anti-mannan antibody EIA was developed and marketed individually (as the Platelia *Candida* Antibody test) and combined with the Platelia *Candida* Antigen (as the Platelia *Candida* Antibody and Antigen test) [22]. In a review of 14 studies that evaluated the three Platelia *Candida* assays, the pooled per-patient sensitivity of the Platelia *Candida* Antigen, Platelia *Candida* Antibody, and both tests combined was 58, 59, and 83%, respectively, while the corresponding specificities were 93, 83, and 86%, respectively [23]. Both Platelia *Candida* tests have been recently refined and are now marketed as *Candida* Ag Plus and *Candida* Ab Plus. In one initial evaluation of their performance using a case–control design, their sensitivity and specificity were, respectively,

59 and 98 % for *Candida* Ag Plus, 63 and 65 % for *Candida* Ab Plus, and 89 and 63.0 % for the tests combined (*Candida* Ag/Ab Plus) [24]. Further evaluation of the newer test is warranted before widespread clinical application.

Detection of Fungal Metabolites

D-arabinitol (DA) is a five carbon polyol metabolite that is produced by several pathogenic *Candida* species (except for *C. krusei* and *C. glabrata*). It has been shown to be present in higher serum concentrations in humans and animals with IC than in uninfected or colonized controls, making it potentially useful as a diagnostic marker for IC. There are two general methods to measure DA: gas chromatography or an enzymatic method. The former is labor intensive and not readily available in most hospital laboratories, while the latter is more suited to a commercial test kit, as is currently marketed in Japan as Arabinitec-Auto (Marukin Diagnostics, Osaka, Japan). This assay is also available for DA testing on urine samples. Several studies have shown that DA can be detected earlier than the presence of *Candida* in blood cultures and that serial measurements correlate well with clinical response to therapy [25, 26].

Conclusions and Recommendations

Immunodiagnostic tests are promising additional diagnostic strategies in the detection of IC, but they have yet to supplant traditional methods. In general, there appears to be utility for serum BG, serum mannan antigen/antibody, and serum DA as adjunctive tests in combination with cultures, histopathology, and radiology. As yet, no single test has been demonstrated to have optimal sensitivity and specificity. Their greatest value appears to be in serial testing of high-risk populations where the trend (rather than a single value) will allow an earlier, accurate diagnosis and monitor the effectiveness of empirically instituted antifungal therapy. An emerging strategy is to use these tests in combination with one another and/or molecular assays to optimize a diagnostic approach to these complex patients. Further, rigorous, prospective clinical trials are needed to determine which tests or combinations thereof will offer the greatest clinical utility.

Aspergillosis

There are a variety of clinical manifestations of aspergillosis, including aspergillomas, allergic bronchopulmonary aspergillosis (ABPA), chronic invasive aspergillosis, and IA. Different types of immunologic tests have shown different utility for this spectrum of disease.

Antibody Detection

The diagnosis of aspergilloma is made by combined radiologic and serologic testing, where IgG antibodies are usually positive. Similarly, for ABPA, a combination of routine blood tests, radiographic findings, skin testing for *Aspergillus* sensitivity, and both IgG and IgE antibody positivity are used for diagnosis. Conversely, antibody detection is less useful and not recommended for invasive disease since the immunocompromised patients most at-risk are less likely to mount a sufficient response.

Antigen Detection

Galactomannan (GM) is a polysaccharide component of the *Aspergillus* cell wall. It has been demonstrated in the serum of some patients with IA and thus has been the target of several serum detection assays. An earlier test called the Pastorex *Aspergillus* (Bio-Rad, Hercules, CA) utilized an LA method with a monoclonal antibody. This test yielded disappointing results with low sensitivity unless multiple samples were used and false-positive reactions from cross-reactivity of the antibody with several other fungal species (see Table 5.3) [27, 28]. A newer, commercially available test is the Platelia *Aspergillus* Ag (Bio-Rad, Hercules, CA). This sandwich ELISA uses the same monoclonal antibody but has the ability to detect GM at much lower limits, thereby improving the test's sensitivity and allowing earlier detection of IA. In a retrospective review of stored serum specimens on bone marrow transplant and leukemia patients, the study leading to its FDA approval, this assay had a sensitivity of 81 % and specificity of 89 % [29]. However, subsequent investigations have shown a lower sensitivity; for example, a meta-analysis of 27 studies of the Platelia *Aspergillus* assay in serum, performed in the setting of repeated surveillance in high-risk patients, demonstrated an overall sensitivity of 61 % and specificity of 93 % in proven or probable IA cases [30]. The sensitivity of the test, when limited to proven cases only, was 71 %. In subgroup analyses, the sensitivity was 79 % in those studies which reported using an optical density (OD) cutoff at the US FDA-cleared threshold of 0.5 (as opposed to a higher OD cutoff of 1 or 1.5; $n=5$). GM test performance is best in the hemato-oncologic population and is less sensitive in solid organ transplant patients [30]. An emerging role for serum GM testing is to monitor treatment response; several recent reports have demonstrated that the trend of GM during therapy predicts outcome [31–33]. False-negative results can occur due to limited angioinvasion, low fungal load, high antibody titers, or the use of prophylactic or preemptive antifungals. Alternatively, false positives occur due to cross-reactivity of the assay with other fungal species, co-administration of pipercillin-tazobactam, or several fungal-derived antibiotics.

Table 5.3 Studies of *Aspergillus* galactomannan antigen detection

Assay/body fluid	Study design and population	Sensitivity (proven/probable IA) (%)	Specificity (not IA) (%)	Comments	Ref.
Pastorex LA[a]/serum	Retrospective cohort: 61 at-risk neutropenic patients; ten developed proven or possible IA	70	86	A single positive test (only) required ELISA also performed: 90% sensitive, 83% specific	[27]
	Retrospective cohort of 215 at-risk bone marrow transplant recipients: 25 proven IA, 15 probable IA, and eight indeterminate infection cases	27	100	A single positive test (only) required ELISA also performed: 93% sensitive, 82% specific	[28]
Platelia[a] ELISA/Serum	Meta-analysis of 27 studies –21 prospective; six retrospective –23 hemato-oncologic; 3 SOT; one not specified –GM sampled in at-risk patients 1–2/week in all studies	61	93	Positive tests required: 1 (11 studies); 2 (16 studies) Proven cases only: sensitivity 71%, specificity 89% SOT subgroup: sensitivity 41% Sensitivity by OD cutoff for positivity: 79% for 0.5; 65% for 1.0; 48% for 1.5	[30]
Platelia ELISA/BAL	Meta-analysis of 30 studies –14 prospective; 16 retrospective –12 hemato-oncologic; 4 SOT; 14 other –GM typically performed as part of a diagnostic BAL	87	89	One positive BAL GM required OD≥0.5 available in 24 studies and used for primary analysis OD≥1 (21 studies) performance: sensitivity 86%; specificity 95% Serum GM: 65% sensitive; 95% specific	[37]
Platelia ELISA/BAL	Systematic review of 19 studies for PCR –7 compared PCR to GM (OD≥0.5) –75% of population was hemato-oncologic	82	96	Reference standard imperfect: positive GM or PCR	[38]

Prov proven, *Prob* probable, *Poss* possible, *ELISA* enzyme-linked immunosorbent assays, *BAL* bronchoalveolar lavage, *PCR* polymerase chain reaction, *GM* genetically modified, *IA* invasive aspergillosis, *LA* latex agglutination
[a]Bio-Rad, Hercules, CA

The Platelia *Aspergillus* assay was FDA-cleared for use on bronchoalveolar lavage (BAL) fluid in 2011, and its use has increased due to the improved sensitivity (generally >75%) as compared to serum across various patient populations [34–36]. A meta-analysis of 30 studies demonstrated a sensitivity of 87% and specificity of 89% for the BAL-GM [37]. In a systematic review of seven studies comparing BAL GM (OD cutoff 0.5) to *Aspergillus* PCR, BAL GM performed similarly; BAL GM sensitivity and specificity were reported as 82 and 97%, respectively [38]. As with serum GM, test performance depends on the selected OD cutoff required for test positivity (FDA-cleared at 0.5). While specificity is improved, sensitivity is sacrificed if higher OD cutoffs (e.g., 1.0 or 1.5) are used to indicate a positive result. Though it can be detected in other body fluids, the GM assay is validated only for serum and BAL samples at this time.

Detection of Fungal Metabolites

Mannitol, a six-carbon acyclic polyol is produced in large amounts by many different fungi, including several *Aspergillus* species in culture. Unfortunately, available data do not support the usefulness of mannitol as a diagnostic marker.

Conclusions and Recommendations

Despite the broad spectrum of disease caused by *Aspergillus* organisms, it is the invasive disease that is most important and most challenging diagnostically. The Platelia *Aspergillus* assay supports a diagnosis of IA in the appropriate clinical setting. This serum test has shown increased specificity with serial sampling and is ideally used to screen patients on a weekly or twice weekly basis during periods of severe im-

munosuppression and may be useful to monitor patients on therapy. All clinicians should keep in mind the potential for false positive and negative results and incorporate the GM results into the general clinical assessment of the patient, rather than as the sole basis on which to change management. For instance, a change in the assay from negative to positive in an immunosuppressed patient under surveillance should prompt a more thorough investigation for IA, while a change from positive to negative should lend support to other evidence that proper therapy has been instituted. Additionally, it is important to remember that the positive predictive value of this test is highest in populations with a high pretest probability; using it for routine diagnosis in lower risk populations will likely increase the chance that a positive result is a false positive. Also, BAL fluid GM testing demonstrates very good sensitivity and specificity although similar cautions regarding test interpretation should be considered. Despite its limitations, this assay is a suitable, noninvasive adjunct for diagnosing and managing IA (see Box 5.2 for recommendations).

Box 5.2 Recommendations for using the Platelia *Aspergillus* galactomannan (GM) ELISA assay

1. Serum GM is ideally used as a part of IA surveillance and performed 1–2/week on at-risk hemato-oncologic patients. The test has moderate accuracy in this setting.
2. BAL GM is a useful adjunctive diagnostic test for at-risk patients presenting with compatible clinical illness. The test has moderate-to-high accuracy in this setting.
3. Consider using serum GM testing to monitor treatment response.
4. Always interpret the results in the context of other clinical and laboratory findings.

Cryptococcosis

The worldwide burden of cryptococcosis, primarily due to *Cryptococcus neoformans,* increased in parallel with the HIV epidemic. More recently, although historically a pathogen of tropical and subtropical regions, *C. gattii* has caused outbreaks of invasive cryptococcosis in the Pacific Northwest, USA and British Columbia, Canada [39]. While culture and histopathology remain the gold standards for diagnosing cryptococcal disease, detection of cryptococcal polysaccharide antigen is a critical test which affords a more rapid diagnosis and allows for earlier disease treatment.

Antibody Detection

Tests for cryptococcal antibodies are not useful and are not widely available for clinical use because they have high false positive and false negative rates.

Antigen Detection

Some of the most important and rapid serodiagnostic tests available for any fungi are those used to detect the cryptococcal capsular polysaccharide antigen, glucuronoxylomannan (GXM) (Table 5.4). These tests utilize a variety of different laboratory techniques (e.g., LA and EIA) for antigen detection. Recently, a point-of-care LFA (IMMY, Norman, OK) was FDA-cleared in 2011. In several studies, the available assays have been directly compared; assay performance is generally excellent in serum (with some important inter-assay differences) and outstanding in CSF (with minimal inter-assay differences) [40–44]. Potential advantages of the LFA are that it is easily stored (room temperature), rapid, and simple to perform/interpret. However, the LFA requires further evaluation and particularly validation of quantitative cryptococcal antigen titers before its widespread adoption.

The performance characteristics of the cryptococcal antigen assays to diagnose cryptococcosis depend on both the disease status (localized vs. disseminated) and host status (HIV, transplant, otherwise immunosuppressed, or immune competent). Due to the multiple possible combinations, the ability to tease out these performance differences is difficult. In general, a higher fungal burden (as is typical for CNS disease) is associated with higher antigen titers. For example, the sensitivity of serum cryptococcal antigen is lower for isolated pulmonary disease than that for meningitis. Indeed, in immunosuppressed patient, a positive serum cryptococcal antigen from a patient who may only appear to have localized pulmonary disease should prompt the clinician to strongly consider checking blood cultures and CSF fluid to rule out dissemination [45]. There has been much investigation regarding the differing performing characteristics of the ability of serum and CSF antigen tests to detect cryptococcal meningitis. In a review of nine studies, the sensitivity of serum and CSF antigen titers to detect CNS disease in the following patient groups was reported: 92% (307/333) and 99% (181/183) in HIV-positive patients; 88% (38/43) and 100% (65/65) in transplant recipients; 87% (91/105) and 97% (141/154) in otherwise immunocompromised patients; and not reported and 92% (72/78) in immune competent patients, respectively [46]. Overall, the sensitivity across all reviewed patient populations for serum (94%) and CSF (94%) antigen titers was much better than that of India ink (60%) and similar (or slightly better) to that of CSF culture (92%).

Table 5.4 Studies of *Cryptococcus* antigen detection

Study design/reference	Assay	Fluid	Sensitivity (%)	Specificity (%)	Comments
Case-control: 90 serum (19 positive) and 182 CSF (30 positive) samples from 47 HIV-positive patients; unknown control population Tanner et al [42]	Crypto LA[a]	CSF	100	98	IMMY LA: used pronase on CSF and serum; CALAS: used pronase on serum
		Serum	83	98	
	Myco-Immune LA[b]	CSF	100	97	
		Serum	83	100	
	IMMY LA[c]	CSF	93	93	
		Serum	97	93	
	CALAS[d]	CSF	100	96	
		Serum	97	95	
	Premier EIA[d]	CSF	100	98	
		Serum	93	96	
Prospective cohort for serum (634 samples, nine cases) and retrospective cohort for CSF (51 samples, 18 cases); unknown patient population—studied at a reference laboratory CALAS in serum and Premier EIA in CSF were used as the "gold standard" references Binnekar et al. [44]	Premier EIA	CSF	Gold standard		Note: culture data were not incorporated in this study
		Serum	56	100	
	CALAS LA	CSF	100	100	
		Serum	Gold Standard		
	IMMY LFA	CSF	100	100	
		Serum	100	99.8	
	IMMY Alpha EIA[c]	CSF	100	100	
		Serum	100	99.7	
Prospective cohort: 106 serum, 42 CSF, and 20 urine samples obtained from 92 patients. 25 cases (four HIV+, nine other immuno-compromised, 12 immune competent) McMullan et al. [43]	IMMY LFA	CSF	100	100	–
		Serum	100	100	
		Urine	94	100 ($n=2$)	
	CALAS	CSF	100	100	
		Serum	91	93	
Prospective cohort: 100 HIV-positive patients with CNS infections; 58 cases of *Cryptococcus* meningitis Asawavichienjinda et al. [47]	Pastorex LA[e]	Serum	91	83	Note: This study used serum to screen for CNS disease

CSF cerebrospinal fluid, *LA* latex agglutination, *EIA* enzyme immunoassay, *CNS* central nervous system, *CALAS* cryptococcal antigen latex agglutination system,
[a]International Biological Labs, Cranbury, NJ
[b]American MicroScan, Mahwah, NJ
[c]IMMY, Norman, OK
[d]Meridian Diagnostics, Cincinnati, OH
[e]Sanofi Diagnostic Pasteur, France

The argument follows that for HIV-positive patients unable to undergo lumbar puncture (LP) or with vague central nervous system (CNS) symptoms not warranting an LP, a serum antigen test may be a reasonable surrogate for meningitis screening [46, 47]. On the other hand, in immune competent patients with meningitis symptoms, the utility of a serum antigen is less clear (i.e., CSF antigen is required) [48, 49].

The prognostic value of antigen titers is controversial given the above mentioned issues of disease and host factors. In the majority of studies, higher initial titers are associated with increased mortality and relapsed disease; however, other recent studies have not confirmed this association [39, 47, 50–54]. Also controversial is the use of sequential antigen titers obtained during therapy. This practice is best studied in the HIV patient population, in which setting the use of titers to monitor treatment response is probably unreliable and therefore not recommended [45, 55]. In certain situations, sites other than serum and CSF may be useful in detection of cryptococcal disease including the pleural fluid and urine [41, 56].

All cryptococcal antigen assays appear to have excellent accuracy for *C. neoformans*. However, because of different binding affinity to different GXM serotypes, the tests may have differing abilities to detect *C. gattii* (which has Serotype C GXM). In vitro data and a recent clinical report indicate that the Meridian EIA and CALAS kits may be less sensitive than IMMY LFA and EIA for *C. gattii* detection [40].

The only limitations of these assays are the occasional false-negative results in patients with extremely low or high *Cryptococcus* organism burden and infrequent false-positive results, generally resulting in low titers, in patients with other infections including disseminated trichosporonosis, *Capnocytophaga canimorsus* sepsis, and *Stomatococcus* infection [57–59].

Detection of Fungal Metabolites

Cryptococcus species, like *Aspergillus* species, also produce large amounts of mannitol, but it has not proven useful as a diagnostic marker for this disease either.

Conclusions and Recommendations

Cryptococcal infection is the rare condition where a serodiagnostic test has extremely high accuracy. A positive cryptococcal antigen result is highly suggestive of infection and can be the sole basis for initiating targeted therapy. However, definitive proof of disease still requires culture or histopathology and efforts to prove the diagnosis by these means are always warranted. The clinical utility of the antigen test depends on the extent of disease and host immune status (see Box 5.3 for recommendations). Care must be taken not to compare titers derived from different kits given the lack of standardization among manufacturers.

Box 5.3 Recommendations for using cryptococcal antigen tests in different host populations

HIV-positive patients

1. For meningeal symptoms, check both a serum and CSF antigen. If unable to do LP or the neurologic symptoms are vague, one can use the serum antigen (which has a high sensitivity for meningitis) as a surrogate screening test.
2. For nonmeningeal symptoms, check a serum antigen. If applicable/available, consider also checking a site-specific antigen (i.e., pleural fluid).
3. A positive serum antigen is associated with disseminated disease and warrants blood cultures and CSF evaluation (cultures and antigen testing).
4. Initial antigen titers correlate with disease burden and likely provide prognostic information; serial antigen titers probably do not provide prognostic information during therapy (and therefore are not recommended).

Solid organ transplant patients

1. For meningeal symptoms, check both a serum and CSF antigen. The sensitivity of a serum test to screen for CNS disease also appears to be high in this population, however an LP is mandatory to evaluate for *Cryptococcus* and other disease.
2. For nonmeningeal symptoms, check a serum antigen. If applicable/available, consider also checking a site-specific antigen (i.e., pleural fluid).
3. A positive serum antigen is associated with disseminated disease and warrants blood cultures and CSF evaluation (cultures and antigen testing).
4. Initial antigen titers correlate with disease burden and likely provide prognostic information; it is unknown whether serial titers provide prognostic information during therapy (and therefore are not specifically recommended).

Immune competent patients

1. For meningeal symptoms, check both CSF and serum antigen; serum antigen alone may be insufficient to rule out meningitis.
2. For nonmeningeal symptoms, check a serum antigen. If applicable/available, consider also checking a site-specific antigen (i.e., pleural fluid).
3. Initial antigen titers correlate with disease burden and likely provide prognostic information; some experienced clinicians monitor serial titers during therapy to document therapeutic response and predict relapse, but others do not.

Histoplasmosis

The standard method for the diagnosis of histoplasmosis remains isolation and specific identification of the causative organism. Unfortunately, this process can take 2–4 weeks and the necessary specimens can be difficult to obtain. Immunologic tests offer a more rapid alternative and in some manifestations of the disease are the preferred means of establishing a diagnosis.

Antibody Detection

The two *Histoplasma capsulatum* species-specific antigens against which host antibodies are made are the H and M antigens, which are both components of histoplasmin. Antibodies against H antigen, a β-glucosidase, form during acute histoplasmosis, while antibodies against M antigen, a catalase, may be formed in active or chronic histoplasmosis and are usually the first to arise upon seroconversion (Table 5.5). These antibodies can be detected by either ID or CF assays; the CF assay uses two antigens including histoplasmin and a suspension of killed yeast phase cells, the latter of which renders CF more sensitive but less specific than ID. Antibodies against M antigen are detected 6–8 weeks after exposure in 50–80 % of patients, but can persist for years in patients who have recovered from infection; therefore their presence does not distinguish remote infection from current disease. On the other hand, antibodies against H antigen are detected in only 10–20 % of exposed patients, but their presence signifies an active infection. In general, asymptomatic patients are less likely to have detectable antibody levels,

Table 5.5 Studies of *Histoplasma* antibody and antigen detection

Assay	PDH in patients with AIDS	PDH in patients without AIDS	Limited disease (type)[b]	Controls[b]	Specimen type[b]	Ref.[b]
Antibodies as detected by						
ID	32/52 (62)	14/21 (67)	65/81 (80)	–	Serum	[89]
	–	17/21 (81)	210/255 (82)	4/767 (1)	Serum	[90]
	–	–	5/29 (17); acute	–	Serum	[91]
ID plus CF	9/13 (69)	37/53 (71); other IC 8/9 (89); non-IC	4/6 (67); acute 39/41 (95); subacute 5/6 (83); chronic	–	Serum	[88]
CF	29/46 (63)	14/22 (64)	75/83 (90)	–	Serum	[89]
	–	–	18/28 (64)	–	Serum	[91]
	0/3 (0)	7/10 (70)	–	–	CSF	[92]
	0/3 (0)	8/9 (89)			Serum	
	–	12/21 (57)	212/255 (83)	15/357 (4)	Serum[c]	[93]
	–	17/21 (81)	197/255 (77)	8/357 (2)	Serum[d]	
Antigens as detected by						
RIA[a]	3/4 (75)	2/10 (20)	–	1/28 (4)	CSF	[92]
	3/4 (75)	4/10 (40)		–	Urine	
	2/4 (50)	2/10 (20)			Serum	
	75/79 (95)	22/27 (82)	24/82 (29)	–	Urine	[89]
	54/63 (86)	7/11 (64)	6/26 (23)		Serum	
	38/40 (95)	12/16 (75)	11/30 (37)	1/96 (1)	Urine	[60]
EIA[a]	38/40 (95)	12/16 (75)	11/30 (37)	1/96 (1)	Urine	[60]
	–	–	84/130 (65); acute	n/a	Urine	[91]
			24/35 (68); acute	n/a	Serum	
	53/56 (95)	81/87 (93); other IC 11/15 (73); non-IC	5/6 (83); acute 14/46 (30); subacute 7/8 (88); chronic	n/a	Urine	[88]

PDH progressive disseminated histoplasmosis, *RIA* radioimmunoassay, *EIA* enzyme immunoassay, *ID* immunodiffusion, *CF* complement fixation, *CSF* cerebrospinal fluid
[a]Mira Vista Diagnostics, Indianapolis, IN
[b]Number of positive tests/total number of subjects (%)
[c]Antibody to yeast phase antigen
[d]Antibody to mycelial phase antigen

and if present, they are usually in lower titers. This is evidenced by the low levels of antibody detected in ~10% of healthy patients residing in an endemic area. Antibody titers generally decline over several months following exposure, but may remain positive for years in some chronic forms of the disease. False-negative tests occur during the early stages of infection and are more common in immunocompromised patients. False-positive results occur in ~15% of patients mainly due to cross-reaction with the agents of coccidioidomycosis or blastomycosis.

Antigen Detection

One of the major developments in diagnostic strategies for histoplasmosis was the introduction of antigen detection assays that could recognize a histoplasmosis polysaccharide antigen (Table 5.5). Depending on the disease manifestation, this antigen can be present in urine, serum, plasma, CSF, or bronchoalveolar lavage (BAL) fluid. The original assay was a RIA that was costly and posed a risk to laboratory personnel because of its radioactivity. A newer assay, now in its third generation, is the MVista *Histoplasma* antigen EIA (MiraVista Diagnostics, Indianapolis, IN). This assay is a quantitative sandwich EIA that uses polyclonal rabbit *anti-Histoplasma* antibodies to bind antigen and has demonstrated favorable results compared to its earlier generations and to the original RIA test [60, 61]. Two other polyclonal antibody EIAs which have performed well in initial reports include a test developed at the Centers for Disease Control (intended for resource poor countries) and an FDA-cleared in vitro diagnostic assay, the *Histoplasma* Antigen EIA (IMMY, Norman, OK) [62, 63]. Antigen detection assays are especially useful for establishing a diagnosis in immunosuppressed patients and patients with progressive disseminated disease (i.e., those with higher fungal burden), while they are less sensitive in detection of isolated pulmonary histoplasmosis. Furthermore, these tests are useful for monitoring antigen levels during treatment, where levels decrease with appropriate therapy and increase with disease

Table 5.6 Recommendations for immunologic test selection stratified by clinical syndrome for histoplasmosis, blastomycosis, and coccidioidomycosis

Disease/clinical syndrome	Antigen sensitivity	Antibody sensitivity	Recommendations
Histoplasmosis			
Progressive disseminated disease	95% in urine: AIDS ~75–95% in urine: other hosts	Poor	Antigen testing of serum and urine is recommended
Acute pulmonary disease	35–70% in urine	>80% (at 4–6 weeks)[a]	Antibody (ID and CF) testing is recommended. Also consider antigen testing of serum and urine, particularly early in disease
Subacute pulmonary disease	~20% in urine	90%	Antibody (ID plus CF) testing is recommended
Chronic pulmonary histoplasmosis	~20% in urine (note 88% in one study)	80–100%	Sputum or BAL fluid for culture and antigen testing are preferred. Send antibody (ID and CF) for remaining cases
Fibrosing mediastinitis Broncholithiasis Asymptomatic lung granuloma Chronic mediastinal lymphadenopathy	Antigen test usually negative	50–65%	Antibody (ID and CF) testing is recommended
Meningitis	~20–70% in CSF ~40–70% in urine ~20–50% in serum	0–70% in CSF 0–80% in serum	Antigen testing in CSF, serum, and urine plus antibody testing in serum and CSF (use CF assay) is recommended
Blastomycosis			
All forms of disease	85–93% in urine 57% in serum	Poor	Obtain culture and histopathology; perform urine antigen testing in cases where immunologic diagnosis is needed
Coccidioidomycosis			
Acute pulmonary disease: immune competent	Unknown	80–95% (ID+CF)	Send serum antibodies (ID or EIA); if positive, obtain CF
Acute disease: immunosuppressed	50–70% in urine ~70% in serum	50–70% (ID+CF)	Send serum antibodies (ID+CF+/− EIA) and consider sending serum and urine antigen (not well studied)
Meningitis	Unknown	~40–80% in CSF ~75–195% in serum	Send serum antibodies (ID+CF+/− EIA). Culture CSF (alert the laboratory) and send for EIA and/or CF

EIA enzyme immunoassay, *CF* complement fixation, *CSF* cerebrospinal fluid *ID* immunodiffusion, *BAL* bronchoalveolar lavage
[a]Data and recommendations from references [60, 67, 70, 72, 74, 79, 80, 87–98]

relapse. Though urinary and serum antigen detection are the most sensitive for these patients as a whole, CSF and BAL fluid testing may prove more valuable in patients with disease at those specific sites. Cross-reactivity of the assay occurs commonly with penicilliosis, paracoccidioidomycosis, and blastomycosis; less frequently in coccidioidomycosis; rarely in aspergillosis; and possibly in sporotrichosis [64]. False-negative results can also occur depending on the population tested and the severity of illness.

Detection of Fungal Metabolites

No tests of this nature are currently available.

Skin Testing

Skin testing with histoplasmin antigen is a useful epidemiologic tool to document past exposure and to investigate histoplasmosis outbreaks. It is of little use in the diagnosis of individual cases. Prior skin test positivity can be lost with disseminated disease or immunosuppression.

Conclusions and Recommendations

Immunodiagnostic tests for histoplasmosis are a proven adjunct to the usual diagnostic methods of culture and histopathology. Due to the wide spectrum of disease with histoplasmosis, there are different recommendations to help guide the appropriate use of immunologic tests (see Table 5.6 for recommendations). Patients with acute localized disease and a low burden of organisms or patients with chronic sequelae of a prior histoplasmosis infection should predominantly be diagnosed via antibody testing. Conversely, patients with a high burden of organisms (e.g., progressive disseminated disease) are diagnosed primarily via antigen testing. Also, combining antigen with antibody testing likely adds sensitivity in certain clinical situations.

Finally, measuring antigen levels is recommended during and after completion of therapy for progressive disseminated disease to monitor response to therapy and for relapse, respectively [65].

Blastomycosis

A high level of suspicion for *Blastomyces dermatitidis* infection is important to its successful diagnosis since no clinical syndrome is characteristic for infection with this organism. While definitive diagnosis requires the growth of the organism from clinical specimens, a presumptive diagnosis can be made by histological characteristics and further supportive evidence can be gained from immunologic tests.

Antibody Detection

Early serologic tests for blastomycosis were directed toward detecting host antibodies against *B. dermatitidis* A antigen. These tests utilized different laboratory techniques including ID, CF, and ELISA. The ID test has the most specificity (while sacrificing sensitivity); the ELISA test has the most sensitivity (and sacrifices specificity); and the CF test has poor sensitivity and specificity [66, 67]. However, all the tests have limited sensitivity for diagnosing acute disease because the mean peak seroprevalence of antibody occurs 50–70 days after the onset of symptoms [68]. An additional limitation is the presence of detectable antibodies for 1 year or more even after successful treatment. Antibody detection assays directed against the WI-1 antigen of the outer cell wall of *B. dermatitidis* have also been explored with promising results, but are not currently available commercially [69].

Antigen Detection

A quantitative antigen detection assay for use on urine, serum, plasma, CSF, and BAL fluid specimens of patients with suspected blastomycosis is available (MVista *Blastomyces* Antigen EIA; MiraVista Diagnostics, Indianapolis, IN). This assay targets a glycoprotein antigen that unfortunately is not genus-specific. The test has high sensitivity in the urine, ranging from 85 to 93% across three studies [70–72]. However, the sensitivity is much lower in serum (in contrast to histoplasmosis). While the specificity is high for patients without fungal disease, the cross-reactivity is similar to the MVista *Histoplasma* Antigen EIA (common cross-reaction with penicilliosis, paracoccidioidomycosis, and blastomycosis; less frequent with coccidioidomycosis; rarely in aspergillosis; and possibly in sporotrichosis) [64].

The correlation between longitudinal measurements of antigen levels with response to therapy and/or relapse is not well established and hence, at this time, monitoring levels during treatment is not recommended.

Detection of Fungal Metabolites

No tests of this nature are currently available.

Conclusions and Recommendations

Several clinical features of blastomycosis make serodiagnosis relatively less important. Unlike histoplasmosis or coccidioidomycosis, where many of the recognized cases are acute pulmonary infections with negative sputum smears and cultures, identified blastomycosis cases are usually chronic pulmonary infections or disseminated infections of the skin and bones. In both of these conditions, histopathology and cultures are usually positive and easy to acquire. Blastomycosis urinary antigen testing should be used for the subset of cases when histopathology is unavailable or negative; however, caution is advised given the high test cross-reactivity with other fungal diseases. On the other hand, the poor accuracy of *Blastomyces* antibodies renders these tests generally unhelpful for diagnosing blastomycosis. Therefore, a negative antibody test should never be used to rule out disease, nor should a positive test be an indication to start treatment (see Table 5.6 for recommendations).

Coccidioidomycosis

Culture and histopathology are the gold standards for diagnosing coccidioidomycosis, but have several limitations. First, although *Coccidioides* species are readily cultured, growth is relatively slow and the cultures need to be performed under Biosafety Level 2 conditions as they pose a certain degree of risk to laboratory personnel. Second, since *Coccidioides* species are listed by the CDC as potential bioterrorism threats, laboratories working with these fungi must follow extensive security practices (and work under Biosafety Level 3 practices). Third, direct examination is insensitive because of the small number of *Coccidioides* organisms present in most clinical specimens. And finally, the mycelial form of growth rarely allows microscopic identification of *Coccidioides* species, requiring further testing to detect coccidioidal antigen in the fungal extract or a specific ribosomal RNA sequence using a DNA probe. This often has to be carried out by a reference laboratory. Given these limitations, immunologic tests are an important adjunct in helping to establish a diagnosis of coccidioidomycosis.

Antibody Detection

The cornerstone of serologic diagnosis is based on the detection of anti-coccidioidal antibodies via several different laboratory methods (Table 5.7). Assays initially developed included the TP and CF tests. Subsequently, two ID tests, the IDTP and IDCF, were developed which detect the same antibodies as the aforementioned TP and CF assays, respectively. The TP test (which is no longer used) and IDTP assay detect primarily IgM antibodies directed against the TP antigen, a heat-stable carbohydrate antigen of the fungal cell wall. These antibodies form early during infection with ~90% of patients developing them in the first 3 weeks of symptomatic disease. For patients with a self-limited illness, these antibodies decline to less than five percent within 7 months. In contrast, the CF assay detects primarily IgG antibodies directed against the chitinase antigen, an enzyme of the fungal cell wall, which is often detectable while the disease is active. In the 1990s the Premier EIA (Meridian Diagnostics Inc., Cincinnati, OH) became available which measures IgM and IgG antibodies directed against the TP and CF antigens. Subsequently, other EIAs have been developed. An LA assay was also developed; unfortunately, while the LA is simple and provides rapid results, its use is limited by a higher number of false-positive reactions compared to the other assays.

Three tests are widely employed today: EIA, ID (both IDTP and IDCF), and CF. The EIAs are relatively simple to perform, can be performed on serum and CSF, and are generally quite accurate. However, an isolated positive IgM (when IgG is negative) may be falsely positive. This relatively common finding (~10% of positive tests) has been the subject of several recent publications with quite discrepant conclusions (false-positive rate range 0–82%) [73]. In particular, one should question the validity of an isolated positive IgM test in an asymptomatic patient or one without classic symptoms [74]. In general, EIAs should be confirmed with ID and/or CF.

Although several ID tests are commercially available (e.g., Meridian Diagnostics, Cincinnati, OH and IMMY Norman, OK), their performance is typically limited to reference laboratories. ID assays are sensitive (although perhaps not as sensitive as EIA or the CF test) and highly specific; they are often used to confirm the results of other serologic tests. They are limited to testing serum.

The CF assay, also limited to reference laboratories, is quantitative and can be performed on body fluids other than serum. Importantly, the antibody concentrations measured by CF are expressed as titers and generally reflect the extent of infection. As variation between testing results from facility to facility exists, it is suggested that serial measurements be conducted using the same laboratory to allow comparison. Use of serial testing can help the clinician gauge disease progression, remission, or cure. Early treatment of suspected coccidioidomycosis with fluconazole may abrogate the IgG response (i.e., the CF assay does not become positive) [75].

Notably, these assays are less reliable in the immunosuppressed population. In a recent publication evaluating 27 solid organ transplant recipients with newly acquired coccidioidomycosis, the sensitivity of all assays (EIA, ID, and CF) used in conjunction was only 77% (20/26), whereas the positivity of any single assay was limited [76].

The EIA and CF may be used to detect antibody in the CSF; the tests are generally less sensitive than serum testing but, when positive, are highly specific for meningitis [77].

Antigen Detection

Research laboratories have demonstrated the ability to detect coccidioidomycosis antigen in both acute and chronic disease [78]. This sparked the development of the MVista *Coccidioides* antigen EIA (MiraVista Diagnostics, Indianapolis, IN). In the initial report of patients who were mostly immunosuppressed and who had moderate to severe disease, the test in urine demonstrated a 71% (17/24) sensitivity and 99% (159/160) specificity compared to healthy volunteers; however cross-reactivity was seen in 11% (3/28) with other endemic mycoses (two histoplasmosis, one paracoccidioidomycosis) [79]. A second study evaluated the same test in serum (pretreated with EDTA at 100°C) and urine in 28 patients with milder disease; in this limited dataset, the sensitivity in serum and urine were 73 and 50%, respectively [80].

Detection of Fungal Metabolites

No tests of this nature are currently available.

Skin Testing

Skin testing with coccidioidin antigen or spherulin antigen is a useful epidemiologic tool to document past exposure. It may also be useful in patients in whom pulmonary coccidioidomycosis has already been proven by other means. A negative skin test in such a patient may be a bad prognostic sign, suggesting current or impending dissemination. In 2011, the FDA cleared a re-formulated test, the *Coccidioides immitis* Spherule Derived Skin Test Antigen (Spherusol; Allermed Laboratories, San Diego, CA). In a published report, this test demonstrated good safety, no histoplasmosis cross-reactivity, sensitivity 98% in patients with a history of pulmonary coccidioidomycosis, and 98% specificity in adult volunteers living outside an endemic area [81]. The niche for this test in clinical practice remains unclear.

Table 5.7 Studies of *Coccidioides* antibody and antigen detection

Assay	Population tested	Sensitivity	Specificity	Comments	Ref.
Antibodies as detected by					
TP	Retrospective analysis of antibody detection *in serum* of a large group of patients with various forms of coccidioidomycosis	2524/3219 (78%)	–	Pulmonary disease	[95]
		89/226 (39%)	–	Disseminated disease	
		33/73 (45%)	–	Meningeal disease	
CF	As above	1790/3219 (56%)	–	Pulmonary disease	[95]
		222/226 (98%)	–	Disseminated disease	
		69/73 (95%)	–	Meningeal disease	
	Retrospective analysis of patients with coccidioidal *meningitis*	29/30 (97%)	–	Serum samples	[96]
		25/30 (83%)	–	CSF samples	
	Retrospective analysis of HIV patients with disseminated coccidioidomycosis	6/8 (75%)	–	Serum samples	[97]
		4/6 (67%)	–	CSF samples	
	Retrospective analysis of antibody detection *in CSF* of a group of patients with various forms of coccidioidomycosis and control patients without coccidioidomycosis	0/9 (0%)	13/13 (100%)	Pulmonary disease (CSF)	[98]
		0/2 (0%)	–	Disseminated disease (CSF)	
		14/33 (42%)	–	Meningeal disease (CSF)	
	Retrospective review of all patients diagnosed with coccidioidomycosis at a tertiary medical center	35/52 (67); IC 188/252 (75); Non-IC	–	–	[87]
IDCF + IDTP	As above	21/40 (53); IC 180/248 (73); Non-IC	–	–	[87]
IDCF[b]	As above	0/9 (0%)	13/13 (100%)	Pulmonary disease (CSF)	[98]
		1/3 (33%)	–	Disseminated disease (CSF)	
		10/19 (53%)	–	Meningeal disease (CSF)	
	Retrospective analysis: patients with proven coccidioidomycosis; control patients with noncoccidioidal pulmonary illness, other fungal illness, HIV disease and no illness	47/47 (100%)	362/362 (100%)	–	[99]
EIA[c]	As above	43/47 (92%)	352/362 (97%)	IgG alone	[99]
		36/47 (77%)	354/362 (98%)	IgM alone	
		47/47 (100%)	347/362 (96%)	IgG and IgM together	
	As above	38/57 (67); IC 212/244 (87); Non-IC	n/a	IgG and IgM together	[87]
LA	As above	8/9 (89%)	0/13 (0%)	Pulmonary disease (CSF)	[98]
		3/3 (100%)	–	Disseminated disease (CSF)	
		31/33 (94%)	–	Meningeal disease (CSF)	
Antigens as detected by					
EIA[a]	Retrospective analysis of mostly immunosuppressed/HIV-positive patients with coccidioidomycosis	17/24 (70) in urine	159/160 (99%)	More severe disease	[79]
	Retrospective analysis of mostly immunosuppressed patients with coccidioidomycosis; serology positive in 19/19 cases	12/24 (50) in urine 19/26 (73) in serum	–	Moderately severe disease	[80]

TP tube precipitin, *CF* complement fixation, *IDCF* immunodiffusion using complement fixation antigen, *EIA* enzyme immunoassay, *LA* latex agglutination

[a]Mira Vista Diagnostics, Indianapolis, IN

[b]IMMY, Norman, OK

[c]Meridian Diagnostics, Cincinnati, OH

Conclusions and Recommendations

The manifestations of most early coccidioidal infections overlap with those of other respiratory infections; therefore, specific laboratory testing is required to establish a diagnosis of coccidioidomycosis (see Table 5.6 for recommendations). Serum antibodies are important in the diagnosis of this disease and develop in most immune competent patients; however, antibodies are less reliable in the immunosuppressed host, particularly transplant, for whom the addition of serum and/or urine antigen tests may help augment disease detection. For most patients who resolve their infection, the antibody concentrations decrease to undetectable levels during the course of illness, so measurable antibodies are more likely to represent a recent or active illness. In general, screening for coccidioidomycosis is performed using either an EIA or ID. If EIA is used, it should be confirmed with ID and/or CF. The CF assay is primarily used to follow titers which gauge clinical progression and response to therapy. If initial serology is negative, repeat testing, potentially using all available assays (antibody and possibly antigen) in conjunction, will improve the diagnostic yield. Finally, skin testing may emerge as an improved method to screen individuals for past exposure.

Paracoccidioidomycosis

A definitive diagnosis of paracoccidioidomycosis requires either direct visualization of the organism in body fluids or tissues or its isolation and growth in culture. Immunologic assays are useful and rapid adjuncts for diagnosing this infection and following clinical outcomes, but unfortunately they are not widely available in the USA.

Antibody Detection

Initial efforts at antibody detection caused significant cross-reactivity with other fungal pathogens, leading to the development of antibody tests (ID, LA, counterimmunoelectrophoresis, ELISA) using more specific *P. brasiliensis* antigens. The most commonly used method is ID, which is very specific and has varying sensitivity depending on the antigen preparation used [82]. For example, the sensitivity of ID using the Ag7 antigen preparation was 84% in one multinational study. More recently, a 43-kDa glycoprotein (gp43) from culture filtrates, now believed to be the dominant immunoantigen of *P. brasiliensis,* has been the target of improved serodiagnosis. A large report on 422 patients under investigation for paracoccidioidomycosis in Brazil evaluated the use of double immunodiffusion (DID) and Western blot (WB) using a crude exoantigen (primarily consisting of gp43). In this study, the sensitivity and specificity of DID were 80 and 95%, respectively, and the corresponding values for WB were 92 and 64% [83]. CF can be used to quantify antibody titers. The major limitation of these different assays is that antibodies can be detected for years after apparent successful therapy, so their presence does not help determine disease activity. Additionally, there is still some cross-reactivity with other regional diseases including histoplasmosis and leishmaniasis, and antibody responses are less robust in immunosuppressed patients.

Antigen Detection

The same cell wall and cytoplasmic components were also used as targets in early antigen detection assays. Unfortunately, these assays were also limited because of significant cross-reactivity in sera from patients with other mycoses (namely aspergillosis and histoplasmosis). To improve on this, a new target was sought and gp43 has been used (see "antibody detection" section above). An immunoblotting assay, performed on urine specimens, has demonstrated good sensitivity and excellent specificity for the detection of this antigen [84]. An ELISA technique with a monoclonal antibody has successfully detected this antigen in serum, CSF, and BAL fluid of patients with confirmed acute and chronic disease states [85]. Furthermore, these antigen levels can be followed as a marker of treatment response [86].

Detection of Fungal Metabolites

No tests of this nature are currently available.

Conclusions and Recommendations

Immunologic tests are useful for rapid diagnosis in suspected cases of paracoccidioidomycosis as ~80–90% of patients with clinical disease have specific antibodies at the time of diagnosis. Furthermore, in disseminated disease, antibody production is elevated and titers are high, providing useful prognostic information. Antibody testing is limited, though, as their presence does not differentiate disease activity and their absence does not rule out disease, especially in patients with early disease or those who are severely immunocompromised. These are the populations where antigenemia may be detectable, prior to the development of immune complexes. It is currently advisable to use more than one test for the diagnosis of paracoccidioidomycosis. Serum antibody and serum, urine or site-specific antigen tests should both be ordered and any positive results should be monitored while on treatment. There is a concern that the assays may be detecting infection with other mycoses, so the results should be evaluated in the context of the entire clinical picture.

Other Mycoses

Immunodiagnostic tests have also been investigated for several other fungal infections, namely, mycetoma, mucormycosis, penicilliosis, sporotrichosis, and dermatophytoses. While they target a variety of antigens, antibodies, and nucleic acids, they are unfortunately still limited by a lack of prospective trials and commercial availability and cannot yet be recommended for routine clinical use.

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Diagnostic Radiology

6

Maria Angela C. Hospenthal and Aimee P. Carswell

Introduction

Radiologic imaging of the structures of the central nervous system (CNS), upper and lower respiratory tract, abdomen, and musculoskeletal system is integral to the diagnosis and management of most human mycoses. Although there are no pathognomonic radiological findings associated with fungal infections, diagnostic imaging combined with clinical data (including historical data of endemic exposures, use invasive devices, coexisting disease or immunodeficiency, surgeries, and duration of illness) can be used to improve diagnostic accuracy and aid in the long-term treatment of certain conditions.

Central Nervous System Imaging

Magnetic resonance imaging (MRI) is superior to computed tomography (CT) in evaluating fungal infections of the brain and has been shown to be more sensitive than CT for detecting abnormalities. CT commonly underestimates the extent of disease in fungal infection [1]. MRI is especially helpful in the early phases of disease when the brain CT may be nondiagnostic. MRI takes advantage of the inherent properties of molecules, especially hydrogen, and manipulates their behavior in an electromagnetic field to generate an image. The composition of tissues and their differences when there is pathology present can therefore be distinguished by altering parameters of the electromagnetic field to see the effect on the molecules of the tissue being evaluated. Terminology to include longitudinal relaxation time (T1) and transverse relaxation time (T2) relates to signal intensities which offer details on specific tissue characteristics. Findings on MRI, such as edema and contrast enhancement are affected by the inflammatory response, which itself is highly dependent on the competence of the immune system. Nevertheless, noncircumscribed, ill-defined areas with little or no contrast enhancement should raise the suspicion for fungal infection [2]. CT of the brain with contrast may be normal initially and thus is more helpful in assessing later stages of infection with eventual findings of focal ring enhancing or hemorrhagic lesions. Other brain imaging modalities include proton magnetic resonance spectroscopy with MRI, which has been reported to be useful in the evaluation of infection due to mucormycosis and cryptococcosis [3]. Diffusion-weighted imaging may also be helpful, particularly in the case of *Aspergillus* infection, as it may help to diagnose early infection or differentiate fungal infection from progressive multifocal leukoencephalopathy and neoplasm [1]. A wide variety of radiologic findings may be found, although intracerebral masses and meningeal enhancement predominate in these infections (Table 6.1).

CNS Mass Lesions

Intracerebral masses are one of the more common findings in fungal brain infections. Predominantly, granulomas or solid-enhancing lesions are reported. In *Aspergillus* infections, these have sometimes been referred to as "aspergillomas." Likewise, in patients with cryptococcal infections, the term "cryptococcoma" has been used. Cryptococcomas can be single or multiple punctate (i.e., miliary) hyperintense round lesions on T2-weighted images (T2WI) usually less than 3 mm in size [4, 5]. Intraparenchymal cryptococcomas show low signal intensity on T1-weighted images (T1WI) and high intensity on T2WI [1, 6, 7]. Granulomas are preferentially seen on the ependyma of the choroid plexus [1]. Immunocompetetent patients are more likely to present with cryptococ-

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Table 6.1 Abnormalities more commonly seen in central nervous system imaging of fungal infections

Radiological finding	Fungus
Granulomas or solid enhancing lesions	*Aspergillus*, *Cryptococcus*, *Histoplasma*, *Candida*, *Paracoccidioides*
Abscesses	*Aspergillus*, *Blastomyces* (epidural), *Coccidioides*, *Cryptococcus*, *Candida*, dematiaceous fungi, *Pseudallescheria boydii* (*Scedosporium apiospermum)*
Parenchymal/leptomeningeal nodules; pseudocysts	*Cryptococcus*
Hemorrhagic/infarcted lesions	*Aspergillus*
Meningeal enhancement	*Blastomyces*, *Coccidioides* (chronic granulomatous), *Cryptococcus*, *Histoplasma*, *Paracoccidioides*, *Aspergillus*
Hydrocephalus	*Cryptococcus*, *Coccidioides*, *Paracoccidioides*

comas [1] and are more likely to demonstrate enhancement in an immunocompetent host, secondary to the ability to mount an immune response [1, 8]. Persistence of cryptococcomas over a prolonged period of time has been documented and found to be inconsistent with active disease [9]. Pseudocysts are also seen in cryptococcal infection, and are CSF-equivalent (low signal intensity on T1WI and high signal intensity on T2WI) that are predominately seen in the basal ganglia, thalami, midbrain, cerebellum, and periventricular matter (Fig. 6.1). Lesions in the basal ganglia and thalami strongly suggest cryptococcal infection [1, 8]. Single- or multiple-enhancing brain lesions have also been reported in *Histoplasma, Candida,* and *Paracoccidioides* infections.

Abscesses are frequently found in fungal brain infections [10]. These lesions can be multiple, hypodense, and may exert little mass effect. They may or may not enhance [11] (Fig. 6.2). Although abscesses occur most commonly in the cerebral hemispheres, they have also been visualized in the cerebellum and brainstem [12]. Organisms reported to cause abscess formation include *Aspergillus, Coccidioides, Cryptococcus,* and *Candida*. Candidal organisms tend to cause focal necrosis producing microabscesses [13, 14]. Less commonly, the dematiaceous moulds and *Pseudallescheria boydii* have been reported to cause one or multiple brain abscesses [15]. CNS abscesses outside the brain parenchyma are not common, although *Blastomyces* has been reported to cause epidural abscesses [16].

Other intracerebral masses associated with fungal pathogens include edematous, hemorrhagic, or infarcted lesions such as those seen in *Aspergillus* infections [11]. The hemorrhagic lesion, usually a consequence of an area of infarction, is an early radiologic sign owing to the angioinvasive nature of certain fungi [10, 17]. A peripheral ring of isointensity or low signal intensity on T2WI relates to a dense population of fungal hyphae containing paramagnetic elements and small areas of hemorrhage [1, 18]. On cross-sectional imaging,

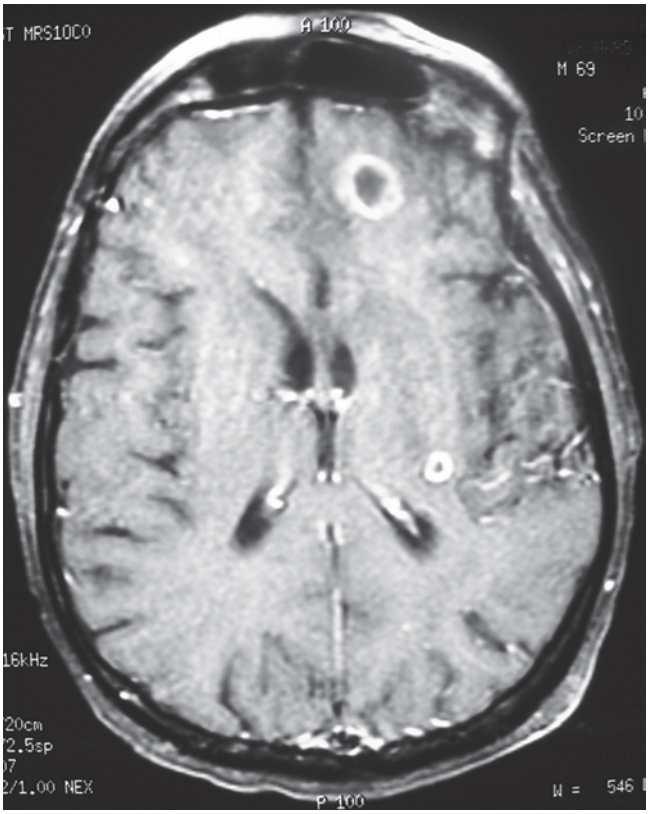

Fig. 6.1 Contrast-enhanced TI transaxial MRI image of the brain demonstrating low-signal-intensity lesions in the bilateral basal ganglia (left greater than right) associated with no significant enhancement, consistent with gelatinous pseudocysts of cryptococcosis. Also note mild meningeal enhancement. *MRI* magnetic resonance imaging

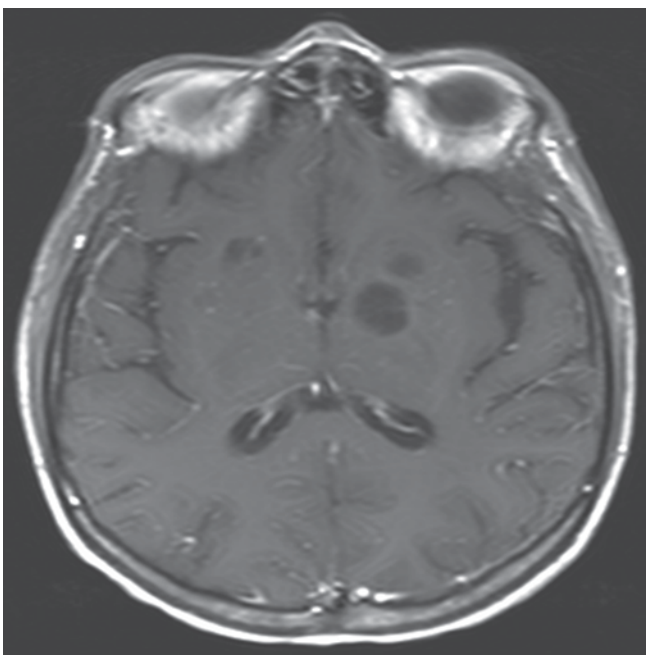

Fig. 6.2 Contrast-enhanced MRI showing multiple ring-enhancing brain abscesses in immunocompromised patient with disseminated aspergillosis. Note the lack of surrounding edema. *MRI* Magnetic resonance imaging. (Courtesy of Dr. D. R. Hospenthal)

these lesions show little or no enhancement or mass effect [11]. Similarly to pyogenic abscesses, fungal abscesses demonstrate decreased diffusion [1, 19].

Meningeal Enhancement

Diffuse enhancement of the meninges on MRI is another common radiological finding of fungal infection of the CNS, thought to be due to active inflammation (meningitis). *Histoplasma, Blastomyces, Coccidioides, Paracoccidioides, Cryptococcus,* as well as *Aspergillus* have all been observed to produce meningeal enhancement. *Coccidioides* meningitis early in its course can cause focal or nodular enhancement in the basal cisterns which represent focal organization of the fungus surrounded by inflammation [20]. Meningeal involvement may be seen secondary to direct extension from fungal infections involving the paranasal sinuses, such as blastomycosis and mucormycosis [1, 21]. Leptomeningeal enhancement in coccidiomycosis has been known to extend into the spinal canal as well [1].

Hydrocephalus

Hydrocephalus is usually a communicating hydrocephalus and is a consequence of meningeal involvement (acutely due to meningeal exudate and later because of meningeal adhesions); it is an additional finding associated with infections by *Cryptococcus, Coccidioides* and *Paracoccidioides* [1, 8, 22] (Fig. 6.3). While CT is helpful in identifying dilated ventricles, MRI appears better in determining the patency of the aqueduct of Sylvius. Other nonspecific CNS radiological findings include early vascular enhancement and diffuse cerebral edema.

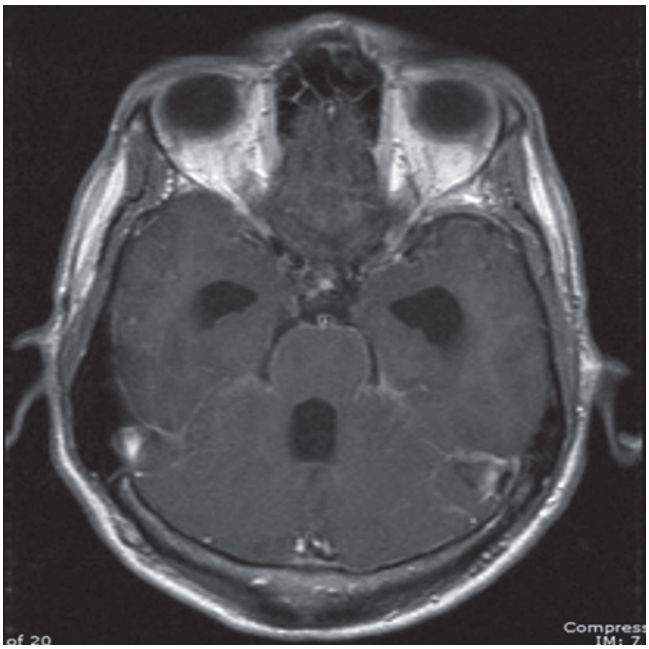

Fig. 6.3 MRI of brain revealing leptomeningeal enhancement and hydrocephalus in a patient with coccidioidomycosis. *MRI* Magnetic resonance imaging

Respiratory Tract Imaging

Sinus Imaging

CT is the imaging modality of choice in the evaluation of sinusitis [23]. It is certainly useful in evaluating the extent of fungal sinus disease [24]. The CT scan defines soft tissue invasion, necrosis, early bone erosion and cavernous sinus thrombosis [25]. When findings are suggestive of fungal sinusitis but the diagnosis is uncertain, MRI with or without gadolinium is the best radiological means to further evaluate the disease [23, 24, 26–28]. Central areas of hyperattenuation on CT correspond to hypointense signals on T1WI and signal void with T2WI MRI [29–31]. Early changes in major vessels and intracranial extension are also best seen on MRI, as is possible cavernous sinus thrombosis and embolic phenomena.

The paranasal sinuses are the most frequently affected, with the maxillary and ethmoid sinuses being the most commonly involved, followed by the sphenoid sinuses. Bilateral involvement is slightly more common than unilateral involvement [32]. Radiologic findings include opacification of multiple paranasal sinuses, with possible demonstration of sinus cavity expansion and erosion of the involved sinus wall. Bone destruction, erosion, and osteomyelitis have been reported in both the invasive and allergic form of *Aspergillus* sinusitis, as well as infections due to Mucorales (agents of mucormycosis) [26, 33, 34] (Fig. 6.4). A soft tissue mass or a sinus "aspergilloma" is reported as a major CT finding of the invasive granulomatous form of fungal sinusitis from *Aspergillus*. It can appear as sinus opacification associated with flocculent calcifications [35] (Fig. 6.5). The mass may either present as a homogenous density or have components of lower attenuation. Intraorbital and/or intracranial extension may occasionally occur [34, 36]. Air–fluid levels may be found though these are rare in either the invasive or noninvasive forms of fungal sinusitis [25]. Other findings include scattered intrasinus high-attenuation areas amid mucosal thickening on unenhanced CT scans.

Pulmonary Imaging

Definitive diagnosis of a pulmonary fungal infection by radiological imaging alone is not possible as other infectious organisms, and likewise, noninfectious pulmonary syndromes,

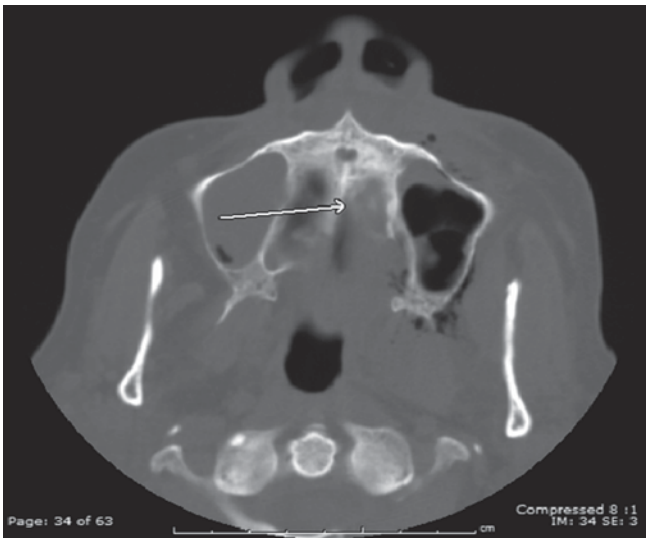

Fig. 6.4 Transaxial sinus CT of patient with mucormycosis demonstrating osteolysis of the hard palate (*arrow*) and left maxillary sinus mucosal thickening with surrounding soft tissue air. *CT* computed tomography

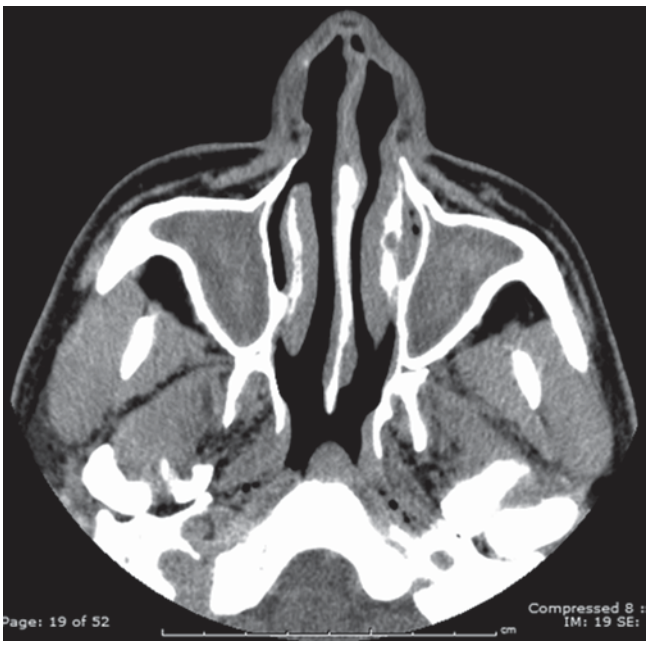

Fig. 6.5 Transaxial CT image through the sinuses demonstrating high-density opacification of the maxillary sinuses consistent with fungal sinusitis. *CT* computed tomography

can mimic radiological findings [37]. The most useful tools to assess lung infections include chest roentgenography and CT [38–40]. Chest radiography in the earlier stages of fungal disease may be normal, thus CT is the superior imaging modality as it has been shown to reveal abnormalities much earlier than chest X-rays. MRI, though reported to have been useful in the workup for *Pneumocystis* disease, has not been recognized as a significant diagnostic tool for the majority of pulmonary fungal infections [39]. Fungal infection of the lung presents generally with a wide variety of nonspecific radiographic patterns (Table 6.2).

Table 6.2 Abnormalities more commonly seen in pulmonary imaging of fungal infections

Radiological finding	Fungus
Alveolar infiltrates	*Aspergillus*, *Blastomyces*, *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Pneumocystis*, Mucorales
Interstitial infiltrates	*Aspergillus*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Paracoccidioides*, *Penicillium*, *Pneumocystis*
Nodules	*Aspergillus*/Mucorales (halo sign), *Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Paracoccidioides*, *Pneumocystis*
Masses	*Aspergillus*, *Blastomyces*, *Coccidioides*, *Cryptococcus*, Mucorales
Cavitation	*Aspergillus*/Mucorales (air crescent sign), *Blastomyces*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Paracoccidioides*, *Pneumocystis*
Abscesses	*Candida*, *Pseudallescheria* (*Scedosporium*), Mucorales
Adenopathy	*Coccidioides*, *Cryptococcus*, *Histoplasma*
Pleural effusion	*Candida*, *Coccidioides*, *Cryptococcus*, *Histoplasma*, *Pneumocystis*

Airspace and Interstitial Opacities

Nonspecific airspace opacities are the most frequent radiologic findings found with any pulmonary infectious process. Alveolar, "patchy," "air-space," or "mass-like" opacities have been identified in many fungal diseases, often progressing to areas of consolidation in the lung. Alveolar opacities have been noted in both endemic and opportunistic fungal infections. Airspace opacities have been noted as a frequent initial pattern in invasive pulmonary aspergillosis (IPA). Opacities may be unifocal or multifocal and then progress to diffuse consolidation, though segmental areas of consolidation have been noted as one of the most common CT patterns in IPA [41, 42] (Fig. 6.6). Other disease processes resulting from *Aspergillus* infection such as bronchopneumonia, hypersensitivity pneumonitis, chronic necrotizing aspergillosis, and semi-invasive aspergillosis have also presented with alveolar opacities, often progressing to consolidation [38, 41, 43]. Patchy, ill-defined opacities progressing to more diffuse, bilateral airspace consolidation are common findings in blastomycosis (seen with a prevalence of 26–76%) [21].

Interstitial, "reticular," "reticulonodular," and "linear" opacities have also been observed in many fungal infections. Diffuse bilateral interstitial opacities in a perihilar distribution [37, 44, 45] are the most common pattern seen in *Pneumocystis* infection (Fig. 6.7). Chest CT often reveals perihilar ground-glass opacity in a mosaic pattern with patchy distribution of affected lung interspersed with areas of normal lung, and noted thickening of the interlobular septa [38].

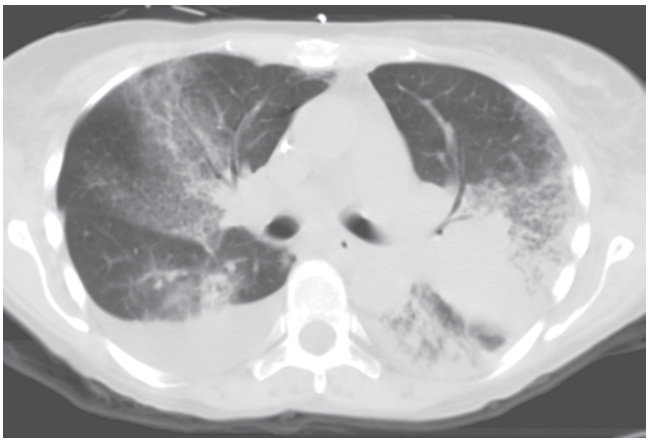

Fig. 6.6 Transaxial CT image through the chest of patient with invasive pulmonary aspergillosis demonstrating diffuse ground-glass opacity and bibasilar consolidation. *CT* computed tomography. (Courtesy of Dr. D. R. Hospenthal)

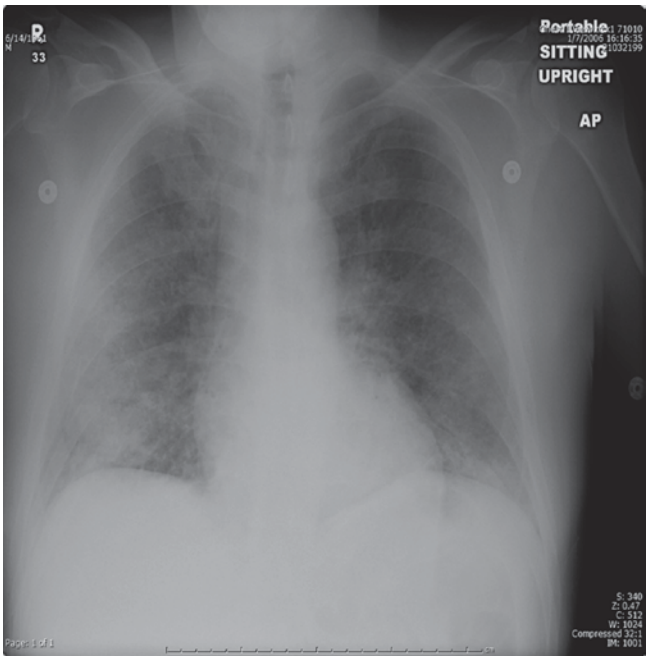

Fig. 6.7 Chest radiograph showing interstitial prominence and ground-glass opacity in a patient with *Pneumocystis* pneumonia

Interstitial opacities are also the most common pattern seen in cryptococcosis [46]. Ground-glass attenuation may be seen in AIDS patients with this infection. *Aspergillus* is able to affect the lung in a variety of ways, most of which can present in an interstitial pattern. These range from nodular opacities of invasive and semi-invasive disease, mimicking the radiologic findings of reactivation tuberculosis (TB), to coarse reticulation found in chronic hypersensitivity pneumonitis [38, 41]. A miliary or reticulonodular pattern is commonly seen in *Blastomyces* infection. *Coccidioides* pneumonia has been noted with diffuse reticulonodular lesions, especially in the setting of AIDS. Heavy exposure to *Histoplasma* can similarly present with diffuse reticulonodular opacities, such as those found in acute disseminated disease [47]. Other organisms commonly demonstrating interstitial opacities include *Penicillium marneffei* in the setting of HIV infection and paracoccidioidomycosis. In *Paracoccidioides* infection, the "reversed halo sign" of ground-glass opacity surrounded by denser airspace consolidation of crescent and ring shapes, has been reported in 10% of patients [48, 49]. The lesions predominate in the mid to lower lungs, particularly in the periphery [49]. The reversed halo sign has also be described in zygomycosis and IPA, as well as histoplasmosis and *Pneumocystis jiroveci* pneumonia. In an immunosuppressed patient with reversed halo sign, invasive fungal infection should be considered until proven otherwise [50].

Distribution and location of opacities are also nonspecific. Opacities may be confined to a lobe or they may be diffuse as seen in disseminated disease. Hematogenous candidal spread can manifest as perivascular pulmonary opacities [51]. Allergic bronchopulmonary aspergillosis often presents with fleeting or migratory upper lobe opacities [41]. Phantom opacities which resolve in one segment then reappear in another lung field have also been seen in coccidioidomycosis [51].

The shape of infiltrates can sometimes aid in the diagnosis. Wedge-shaped opacities, reflecting invasion of blood vessels with subsequent lung infarction, are suggestive of invasive *Aspergillus* or Mucorales [52].

Uncommon pathogens which can cause infections presenting with nonspecific pulmonary opacities include *Fusarium, Trichosporon, Malassezia furfur,* and the phaeohyphomycetes [53].

Nodules

A well-defined nodule, either single or multiple, has been reported as a frequent initial radiographic pattern of IPA [54]. This nodular lesion may be surrounded by a rim of hemorrhage from thrombosis of fungi within pulmonary vessels [55]. Days to weeks after treatment of neutropenia, patients infected with *Aspergillus* may present with ground-glass attenuation around the nodules recognized as the "halo sign" [41, 56, 57] (Fig. 6.8). The "halo sign" is highly suggestive of angioinvasive aspergillosis, but is nonspecific. It is thought that the surrounding ground-glass opacity may be related to hemorrhage from the vascular involvement. Other infectious processes including the mucormycosis, *Candida,* herpes simplex virus, and cytomegalovirus infections, as well as noninfectious processes such as Wegener's granulomatosis, Kaposi's sarcoma, and hemorrhagic metastatic malignancies have also been presented with "halo signs" [58–60]. Nodular lesions may also be observed with branching linear opacities recognized as the "tree-in-bud" pattern seen in *Aspergillus* bronchiolitis [38]. "Tree-in-bud" pattern suggests a small

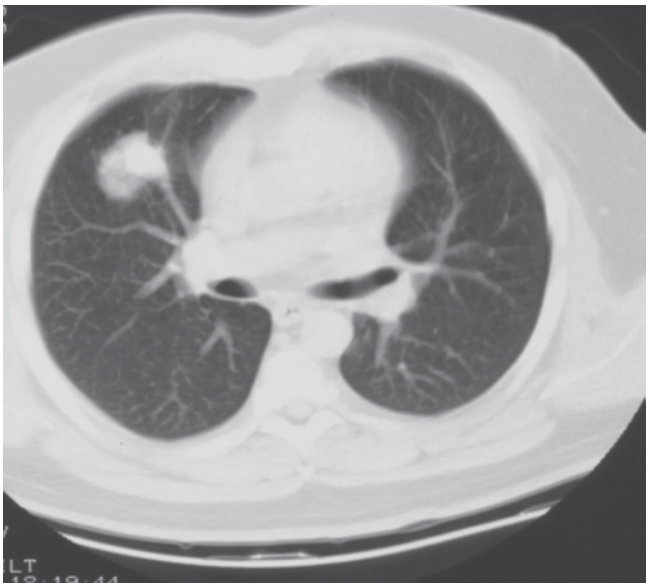

Fig. 6.8 Transaxial CT image of the chest with a right middle lobe nodule with surrounding ground-glass opacity (halo sign). This is consistent with invasive aspergillosis in an immunocompromised patient. *CT* computed tomography

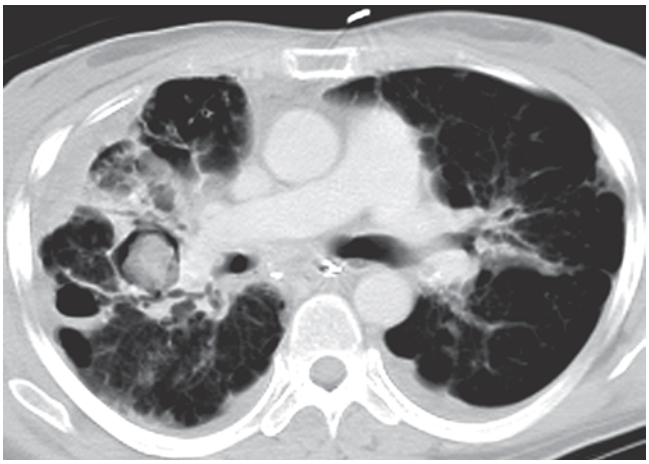

Fig. 6.9 Transaxial CT image of the chest showing bilateral upper lobe bronchiectasis, right pleural thickening, and right upper lobe ground-glass opacity and aspergilloma (soft tissue mass in preexisting cavity). *CT* computed tomography

airways process; a disease process which may be spread endobronchially. Similar lesions are found in endobronchial spread of mycobacterial, viral, and mycoplasma pneumonia. Nodules from Mucorales can be indistinguishable from IPA. The most common finding in pulmonary cryptococcosis are nodules, solitary or multiple, with or without cavitation, ranging from 5 to 20 mm in size with smooth or irregular margins, associated with other parenchymal findings such as masses and consolidation [61–63]. Miliary nodules are less commonly found in the AIDS patient with cryptococcosis [64]. Other organisms which can present with diffuse nonspecific nodular lesions include disseminated *Candida* and *Histoplasma*. Nodules can turn to "buck shot" calcifications in pulmonary histoplasmosis [52]. Approximately 5% of those who develop *Coccidioides* pneumonia may develop solitary pulmonary nodules. Infections with *Pneumocystis, Scedosporium,* and *Paracoccidioides* have also demonstrated pulmonary nodules.

Masses

Parenchymal masses may include aspergillomas, 3–5 cm mobile, round or oval masses, usually solitary, and seen in the upper lobe within a preexisting cavity (Fig. 6.9) or ectatic bronchus. These masses may be partially surrounded by a radiolucent crescent (Monod's sign) of varying thickness [41, 65, 66]. This is the pattern most often seen in immunocompetent hosts [66]. Occasionally, coccidioidal infections may leave persistent chest X-ray lesions, most common being the coccidioidoma and the peripheral cavity [51]. The occasional "fungus ball" can form inside the cavity, rupture into the pleural space and produce an air–fluid level on an upright chest X-ray. Nonspecific mass lesions have been reported in infections with *Cryptococcus, Pneumocystis,* and the Mucorales. As fungal infections may present with nodules and masses which may have irregular or speculated borders, it is not uncommon to mistake them for malignancy. Fungal infections mimicking malignancy include coccidiomycosis, histoplasmosis, aspergillosis, blastomycosis, and cryptococcus. Some have advocated for use of fluorodeoxyglucose (FDG)/positron emission tomography (PET) to differentiate between infection and malignancy; however, there is a high rate of false positives secondary to hypermetabolic nature of fungal infections. However, PET/CT does have a high negative predictive value [67].

Nonparenchymal masses may be seen in the hilar or mediastinal areas. Chronic pulmonary blastomycosis might present with a single large perihilar mass that often warrants a thoracotomy to rule out possible carcinoma. In fact, up to 31% of patients present with paramediastinal or perihilar masses, which unlike histoplasmosis, rarely contain calcification [21]. Other findings of cryptococcosis in AIDS patients include mediastinal masses.

Cavitation

Virtually any nodular lesion has the potential to cavitate. Nodular lesions seen in *Aspergillus* and Mucorales infections may progress to cavitate to what is recognized as the "air crescent sign" [41, 57, 58, 68]. The air crescent sign represents cavitation of nodules caused by resorption of necrotic tissue by returning neutrophils [55] (Fig. 6.10). It is usually unilateral and frequently in the upper lobes [38]. Other nodules, single or multiple, can cavitate and then proceed to either diffuse pulmonary consolidation or abrupt development of large wedge-shaped pleural-based lesions mimicking bland

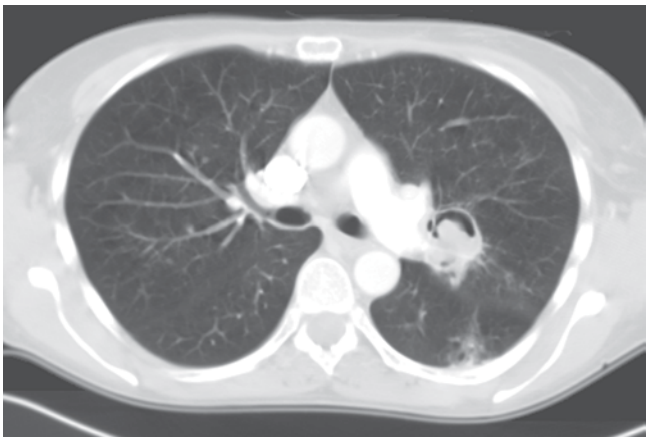

Fig. 6.10 Transaxial CT scan of the chest demonstrates a cavity in the left upper lobe with dependent soft tissue in a patient with invasive aspergillosis (air crescent sign). *CT* computed tomography. (Courtesy of Dr. D. R. Hospenthal)

infarction. Thin- or thick-walled cavities as well as cavitary infiltrates can appear in subacute invasive aspergillosis and chronic progressive coccidioidomycosis, both of which can mimic TB [52]. Approximately 5% of those who develop *Coccidioides* pneumonia develop thin-walled solitary cavities, typically near the pleura (Fig. 6.11). A chronic form of *Coccidioides* pneumonia presents as a slowly progressive fibrocavitary process of biapical fibronodular lesions with retraction and cavitation. In pulmonary histoplasmosis, upper lobe cavities are common, except in those with HIV infection. Cavitary infiltrates have also been demonstrated in disseminated disease. Fibrotic apical infiltrates with cavitation have been reported in chronic pulmonary histoplasmosis which can be confused with TB infection or co-infection on chest X-ray [52]. Other fungal infections reported to cause cavitary disease include sporotrichosis and paracoccidioidomycosis; nodular areas are sometimes confluent, often in the lower lobes; cavitation occurs in one third of cases. Cavitation from blastomycosis is unusual and not as commonly seen as in mycobacterial or *Histoplasma* infections. Cryptococcal infection may present with cavitary masses or nodules, though this is uncommonly seen in the setting of AIDS infection. In cancer patients infected with *Pneumocystis* previously given prophylactic aerosolized pentamidine, upper lobe infiltrative disease suggestive of tuberculosis may be seen, but cavitary lesions are very uncommon.

Adenopathy

Adenopathy has been observed commonly in histoplasmosis, coccidioidomycosis, and cryptococcosis. In acute histoplasmosis, a common finding in a low-level exposure includes enlarged hilar or mediastinal lymphadenopathy. In heavy exposure, the mediastinal adenopathy is usually accompanied by diffuse reticulonodular infiltrates as mentioned previously. There is potential extensive calcification of paratracheal, hilar, and subcarinal lymph nodes [69]. Occasionally, these lymph nodes combine to form granulomas which can rupture and cause chronic inflammation with subsequent diffuse mediastinal fibrosis [70], or more commonly—a localized mediastinal mass [71]. This process, known as fibrosing mediastinitis, can partially obstruct airways, vessels, and the esophagus [71]. Mediastinal lymphadenopathy is uncommon in disseminated histoplasmosis, occurring at less than 10% in one series. Coccidioidomycosis is also noted to present with bilateral hilar adenopathy. Prominent hilar adenopathy is occasionally seen in cryptococcosis. Radiologic findings vary widely in *Pneumocystis* infections but lymphadenopathy is extremely rare.

Pleural Abnormalities

The effect of fungal infections on the pleura and pleural cavity is not as common as the other previously described radiologic findings. Ultrasonography is useful in the initial detection and subsequent management of pleural effusions but the main imaging modality to visualize the pleura is CT; cross-sectional CT imaging, in particular, can be used to aid in the diagnosis and assessment of severity of disease as well guide interventional diagnostic procedures [72]. Pleural thickening with concomitant upper lobe consolidation potentially progressing to cavitation over weeks to months can be seen in semi-invasive pulmonary aspergillosis [41]. Pleural effusions have been noted in candidal pneumonia. Large parapneumonic effusions have been documented in coccidioidomycosis. Other organisms that have demonstrated pleural effusions include *Cryptococcus, Histoplasma,* and *Scedosporium*. Effusions are unusual in *Blastomyces* and *Pneumocystis* infections.

Airway Abnormalities

Tracheal or bronchial wall mucosal thickening along with airway plaques can be seen in invasive aspergillosis [38, 41]. *Cryptococcus* infection of the larynx can present on CT soft tissue images of the neck as vocal cord irregularities and asymmetric enlargement [73]. Cylindric bronchiectasis in a central distribution, as well as traction bronchiectasis, has also been noted on CT images of various forms of *Aspergillus* as well as *Paracoccidioides* infections [41, 74]. An endobronchial lesion from *Pennicillium marneffei* presented as pulmonary infiltrates due to the resultant postobstructive pneumonia [75].

Miscellaneous

Hematogenous spread of *Candida* can cause multiple abscesses in the body, including the lungs [76]. Mucormycosis and pseudallescheriasis have also been reported to cause pulmonary abscesses [77, 78]. Atelectasis, which may ap-

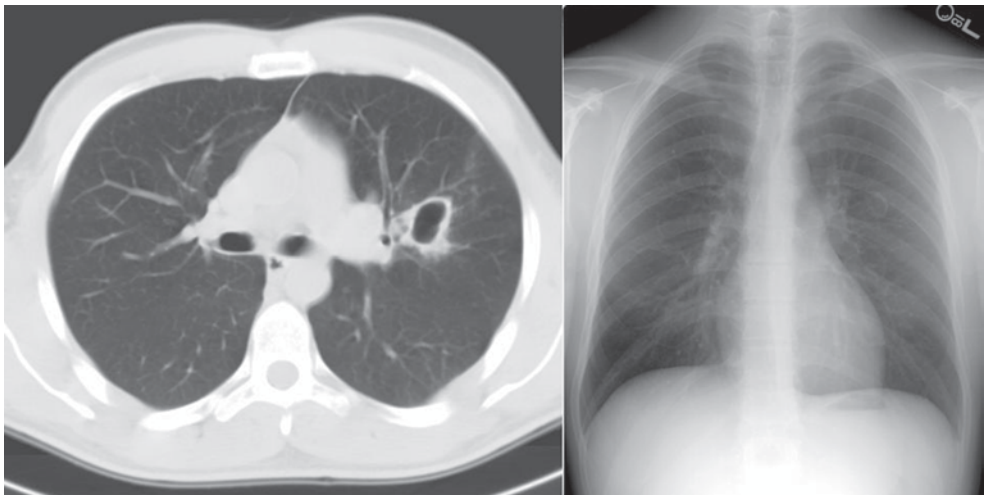

Fig. 6.11 Transaxial CT scan through the chest and chest radiograph demonstrating a thin-walled cavity in the left upper lobe in an asymptomatic patient proven to have coccidioidomycosis. *CT* computed tomography. (Courtesy of Dr. D. R. Hospenthal)

pear as bilateral lower lobe consolidation, has been noted in various pathologic processes caused by *Aspergillus* [41]. Thin-walled cysts or pneumatoceles can form in *Pneumocystis* infections, especially in those patients receiving prophylaxis with aerosolized pentamidine and TMP/SMX [44]. These upper lobe lesions increase the risk of developing pneumothoraces. End-stage honeycombing can be seen in the chronic form of hypersensitivity pneumonitis secondary to *Aspergillus* [41]. Pseudoaneurysm of the aortic arch has been noted in IPA [79].

Multidetector CT (MDCT) angiography takes advantage of the angioinvasive nature of *Aspergillus* and allows direct detection of vessel occlusion up to a peripheral lesion with high-resolution images demonstrating possibly the earliest sign of disease from *Aspergillus* [80].

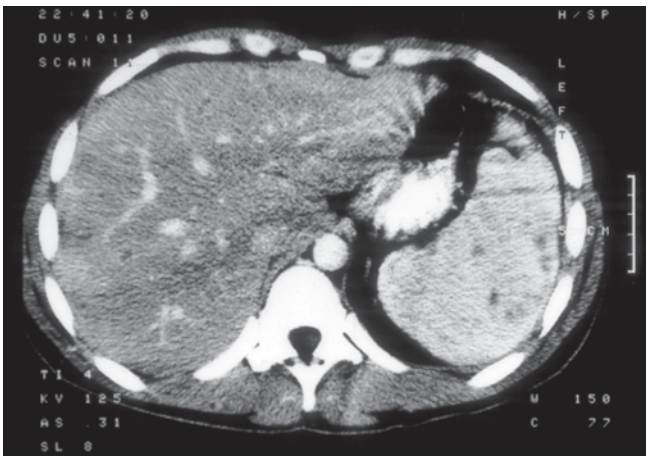

Fig. 6.12 Constrast-enhanced CT scan through the abdomen showing multiple subcentimeter low-attenuation hepatic and splenic lesions in patient with chronic disseminated candidiasis. *CT* computed tomography. (Courtesy of Dr. D. R. Hospenthal)

Abdominal Imaging

CT or MRI should be the initial imaging modality used to evaluate the abdomen for signs of fungal infection. Ultrasonography, a safer, low-cost method, may then be obtained to follow up noted disease processes. Serial ultrasounds every 3–4 weeks may be used to monitor response to therapy, typically observed as decreasing size and number of lesions or may be useful in detecting evolution of new lesions [81]. Once the ultrasound is clear, a repeat CT or MRI is suggested. Similar to other affected organs mentioned above, radiologic findings of abdominal fungal infections are varied to include nonspecific lesions, organomegaly, and lymphadenopathy.

Target Lesions

Candida is one of the main fungi to cause abdominal disease. In patients with dysphagia or odynophagia, fulminant *Candida* esophagitis can present as longitudinal plaques along the esophagus. Large filling defects from aggregated plaques can appear as "cobblestoning" in severe disease [82, 83]. Involvement of the liver, biliary tree, pancreas, and spleen has been documented in disseminated disease [84]. Target lesions seen in the spleen and liver resulting from candidal infection are most commonly detected on CT or MRI after the resolution of neutropenic episodes [81] (Fig. 6.12). On abdominal CT, chronic disseminated (formerly hepatosplenic) candidiasis is characterized by small, round, low-attenuation lesions scattered through the liver and spleen with occasional peripheral enhancement [85]. Occasionally, multiple small low-attenuation lesions in the spleen and kidneys are seen without lymph node enlargement or hepatosplenomegaly [86]. Four dominant findings on ultrasound have been described. Most commonly, uniform hypoechoic lesions are

noted and can be seen in conjunction with the other three patterns. A "wheel within a wheel" pattern can be seen representing an outer hypoechoic area of fibrosis surrounding a hyperechoic area of inflammation. A "bull's eye" measuring from 1 to 4 cm may evolve from primary lesions. "Echogenic foci," usually seen late, correlate with central fibrosis, calcifications, or both [87]. MRI has been reported to be superior to CT in characterizing chronic disseminated candidiasis. Although relatively rare, disseminated *Coccidioides* infections have been reported to affect the spleen; detected as target lesions with central areas of low attenuation on CT imaging [86, 88].

Organomegaly

Moderate to marked enlargement of the liver, spleen, and adrenals have been noted in disseminated histoplasmosis [86, 89, 90]. Cryptococcal infections have also been reported to produce marked splenomegaly and mild hepatomegaly [86, 91].

Lymphadenopathy

Enlarged lymph nodes with or without central or diffuse low attenuation are seen in the majority of patients with abdominal histoplasmosis [86, 91–93]. Cryptococcal infection has also been noted to have enlarged lymph nodes on CT imaging.

Miscellaneous

Abdominal abscesses have been reported in deeply invasive candidiasis [84, 94]. Uncommon findings in disseminated histoplasmosis include colonic wall thickening, and omental and mesenteric infiltration [86]. Adrenal masses, vascular occlusion, and extensive necrosis have also been noted. Multiple scattered low-attenuation foci can persist from focal scarring and granulomatous change which may eventually result in calcifications.

Musculoskeletal Imaging

MRI and nuclear imaging modalities are the most sensitive and specific methods for the detection of fungal infections in the skeletal system (Fig. 6.13) [95]. Osteomyelitis is the most frequent radiologic finding associated with skeletal fungal infections, though radiographic appearance is nonspecific and indistinguishable among fungi or from bacterial or neoplastic disease. Bone MRI may be sensitive for picking

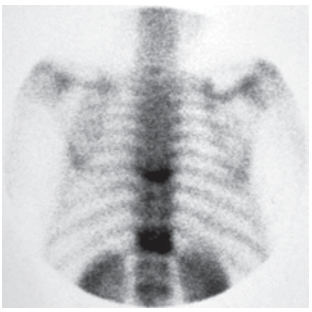

Fig. 6.13 Bone scan demonstrates increased uptake at approximately T8, T11, and T12. Although this patient had coccidioidomycosis, these findings are nonspecific and could represent another infection, inflammatory or neoplastic process.

up early lesions of osteomyelitis and is typically useful in assessing infections of the feet and vertebral column [96, 97]. Technetium uptake is dependent on blood flow, while gallium uptake is dependent on the presence of leukocytes in the area of inflammation [98]. Though positive bone scans may be seen as early as 24 h following the onset of infection, a normal scan may be the result of scanning prior to the onset of reactive hyperperfusion.

Osteomyelitis

In blastomycosis, the vertebral column, ribs, skull, and the epiphyseal ends of long bones are most commonly affected [99, 100]. In the tubular bones of the extremities, eccentric saucer-shaped erosions may be seen beneath a cutaneous abscess. Epiphyseal or metaphyseal focal or diffuse osteomyelitis has been reported as well as cystic foci or diffuse "moth-eaten" areas in the carpal or tarsal areas [101]. Histoplasmosis similarly affects the pelvis, skull, ribs, and small tubular bones.

Radiologically, osteoporosis, joint-space narrowing, and bony erosion may be seen similar to tuberculosis. *Candida* osteomyelitis usually occurs in the setting of disseminated candidiasis. The axial spine of adults and the long bones of children are primarily affected [101]. The most common abnormalities include bone destruction, soft tissue extension/swelling, intervertebral space/joint space narrowing, and epidural abscesses. Hypointense MRI images on T1WI as well as hyperintense images on T2WI were noted [102]. Irregularities of subchondral bone have also been noted [103]. Coccidioidomycosis also primarily affects the vertebral column and ribs. There is a tendency to involve multiple segments of the vertebrae, sometimes with "skip lesions" [99]. Radiographs reveal periostitis as well as multiple well-demarcated lytic foci in the metaphyses of long tubular bones and in bony prominences (Fig. 6.14). In the spine, one or more vertebral bodies may be involved, typically with paraspinal masses and contiguous rib lesions [103]. Cryptococcosis presents with nonspecific radiographic features to include osteolytic lesions with discrete margins, mild or absent

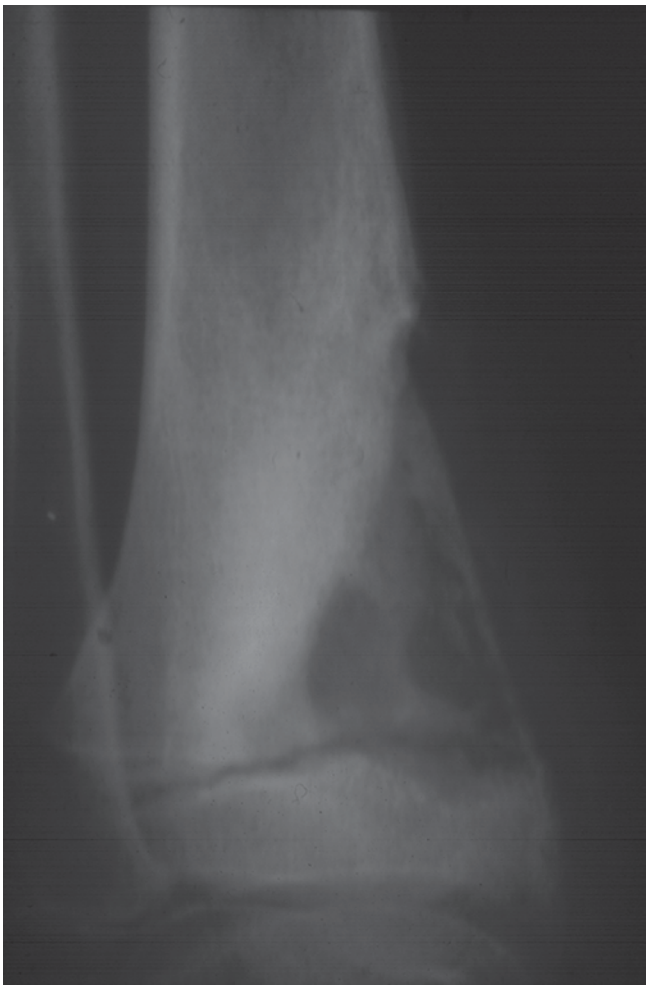

Fig. 6.14 Plain radiograph of the right ankle showing a lytic lesion in the medial aspect of the distal tibia secondary to coccidioidomycosis

surrounding sclerosis, and little or no periosteal reaction [104]. Mucormycosis generally causes osteolytic changes to the skull or face [103]; periosteal thickening and bony disruption have been noted on CT scan [105]. With Madura foot (eumycotic mycetoma), a chronic granulomatous disease of the subcutaneous tissues and bone, standard X-rays may reveal abnormalities, though CT has been reported to be more sensitive in the earlier stages of the disease. Typically, single or multiple bony defects with extensive soft tissue and bony disruption occurring with sclerosis and periostitis are seen [103]. Other organisms reported to cause radiologic abnormalities of soft tissue and bone include *Scedosporium, Paecilomyces, Pseudallescheria boydii,* and *Sporothrix schenckii* [15, 78, 103, 106, 107]. Osseous and disk-space destruction and a paraspinal mass, resembling those of TB have been reported in aspergillosis [103].

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Part III
Antifungal Agents

Antifungal Agents

7

Russell E. Lewis and Annette W. Fothergill

Introduction

Until the 1950s, relatively few drugs were available for the treatment of superficial or invasive mycoses. The era of systemic antifungal chemotherapy effectively began in 1955, with the discovery of the polyene antifungals nystatin and amphotericin B, followed closely by the discovery of the first topical azole antifungal agent, chlormidazole, in 1958 (Fig. 7.1). Although amphotericin B was to remain the mainstay of therapy for serious fungal infections for more than 40 years, infusion-related side effects and dose-limiting nephrotoxicity associated with its use-prompted continued search for equally effective but less toxic alternatives. During the 1960s, a synthetic fluorinated pyrimidine analogue, originally developed as an antineoplastic agent, flucytosine was found to have potent antifungal activity against common yeasts. Unfortunately, resistance to flucytosine developed rapidly when the drug was administered as monotherapy, thus restricting its use to combination therapy with amphotericin B. The next major milestone in antifungal therapy was not realized until 1981, when the first orally bioavailable systemic azole, ketoconazole, was introduced into clinical practice. For almost a decade, it would be regarded as the drug of choice for chronic mucocutaneous candidiasis, mild-to-moderate blastomycosis, histoplasmosis, paracoccidioidomycosis and coccidioidomycosis, and occasionally deep-seated *Candida* and *Cryptococcus* infections in patients who could not tolerate amphotericin B [1]. As ketoconazole was a highly lipophilic weak base, it had many undesirable physiochemical characteristics that increased its toxicity, and limited its usefulness in critically ill patients including:

- Limited absorption of the drug at elevated gastric pH.
- Lack of an intravenous (IV) formulation.
- Requirement for extensive cytochrome P450 biotransformation before elimination, resulting in a high propensity for drug–drug interactions.
- Dose-related gastrointestinal, hepatic, and adrenal toxicity.
- Limited penetration into anatomically privileged sites such as cerebrospinal fluid (CSF).

In an attempt to address these limitations, a new chemical group of azoles (triazoles) were developed with improved physiochemical characteristics and spectrum of activity. Fluconazole, introduced in the early 1990s, could be administered intravenously or orally, had predictable pharmacokinetics, excellent oral bioavailability, and improved penetration into anatomically restricted sites such as the vitreous humor and CSF. Importantly, fluconazole was well tolerated and was associated with few serious drug interactions in critically ill patients. As a result, fluconazole quickly became one of the most widely prescribed antifungal for superficial and life-threatening infections due to yeasts [1]. The lack of activity against opportunistic molds (e.g., *Aspergillus, Fusarium*, and the *Mucorales*) and intrinsic resistance among some non-*albicans Candida* species (i.e., *Candida glabrata* and *Candida krusei*), however, created a need among severely immunocompromised patients for broader-spectrum alternatives. The development of itraconazole and the broader-spectrum triazole derivatives, voriconazole and posaconazole and more recently isavuconazole, has largely addressed the spectrum limitations of fluconazole among these high-risk patients. Yet, these broader-spectrum triazoles still carry a potential for cross-resistance with fluconazole [2] and exhibit more complex pharmacokinetic profiles, and a higher propensity for drug interactions. Hence, less toxic alternatives to triazole antifungal therapy would be desirable in critically ill patients, especially patients at higher risk of pharmacokinetic drug–drug interactions.

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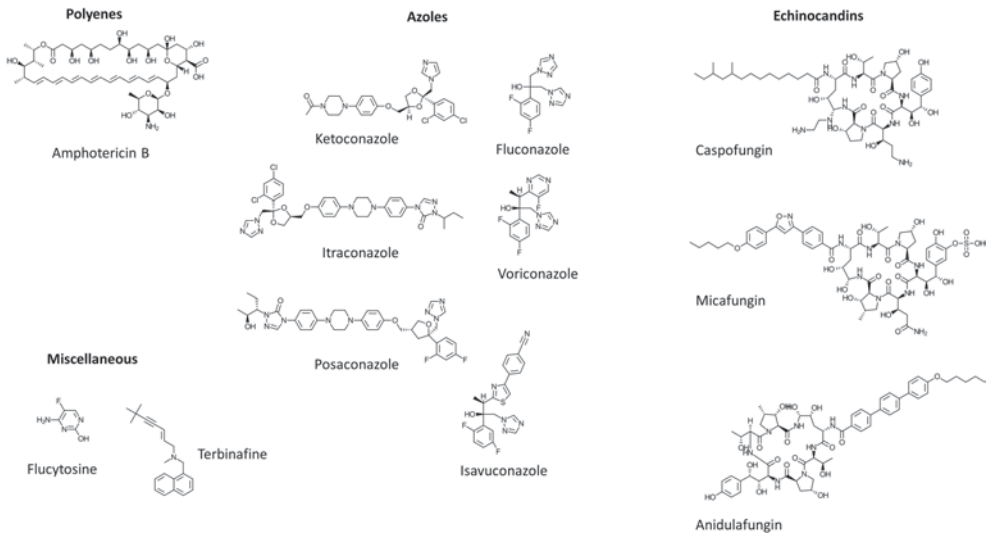

Fig. 7.1 Representative structures of systemic antifungal agents

The final milestone of antifungal drug discovery during the twentieth century was the identification and development of the echinocandins; lipopeptide molecules that inhibit glucan synthesis leading to damage of the fungal cell wall [3]. Due to the importance of the cell wall in fungal survival and the lack of this target in mammalian cells, echinocandins were predicted to be well-tolerated antifungal agents with little collateral toxicity in humans. Yet, the first echinocandin tested in humans, cilofungin, had to be abandoned prior to large-scale clinical trials due to difficulties in its preparation and the toxicity of its IV formulation [4]. Subsequent semisynthetic echinocandin derivatives demonstrated improved solubility and potency and were well tolerated even at high dosages. In 2001, caspofungin became the first echinocandin approved by the Food and Drug Administration (FDA) for the treatment of invasive fungal infections in humans. Two other echinocandin derivatives with a similar spectrum as caspofungin, anidulafungin and micafungin, have subsequently been approved for human use.

Although the arrival of new antifungal agents has clearly advanced the management of invasive fungal infections, treatment failures still occur, and some patients do not tolerate particular antifungal agents due to hypersensitivity reactions, renal or hepatic toxicity, or the potential for serious drug interactions. Therefore, no single antifungal agent is appropriate for all patients for any given mycoses. Moreover, breakthrough infections with pathogens that have acquired intrinsic resistance to multiple antifungal classes have become more common with prolonged treatment courses and improved survival of chronically immunocompromised hosts. This trend has created an urgent need for laboratory support in the treatment of invasive fungal infections including: (a) the rapid identification of fungal pathogens to the species level, and (b) in vitro susceptibility testing of clinical isolates to guide the selection of antifungal therapy. This chapter reviews key components of antifungal pharmacology with a special emphasis on systemic antifungal agents and common patterns of resistance among opportunistic mycoses in humans.

Targets of Antifungal Therapy

Despite differences in the sterol composition of the cell membrane and the presence of a cell wall, much of the cellular machinery of fungi shares remarkable homology to mammalian cells. Consequently, development of drugs that selectively target pathogenic fungi without producing collateral damage to mammalian cells is a daunting pharmacological challenge. Not surprisingly, many of toxicities and drug interactions observed with contemporary antifungal therapies can be attributed to "nonselective" interactions with homologous enzyme or cell membrane systems found in mammalian host cells [5].

With the exception of flucytosine, currently available systemic antifungals act primarily through direct or indirect interactions with the fungal cell wall or plasma membrane, and the fungal membrane sterol, ergosterol, and its biosynthetic pathways (Fig. 7.2). The fungal cell envelope has several properties that make it an ideal target for antifungal therapy [4]:

- In contrast to the cholesterol-rich cell membranes of mammalian cells, the predominant cell membrane sterol in pathogenic fungi is ergosterol. Indirect or direct targeting of ergosterol results in selective toxicity to the fungal cells.
- Mammalian cells lack a true cell wall. Drugs that target synthesis of the fungal cell wall have a low potential to cause collateral toxicity in mammalian cells.
- The cellular wall and membranes are important for ion exchange, filtration, and are a critical area for localization of enzymes involved in the metabolism and catabolism of complex nutrients [6]. Drugs that disrupt growth of the

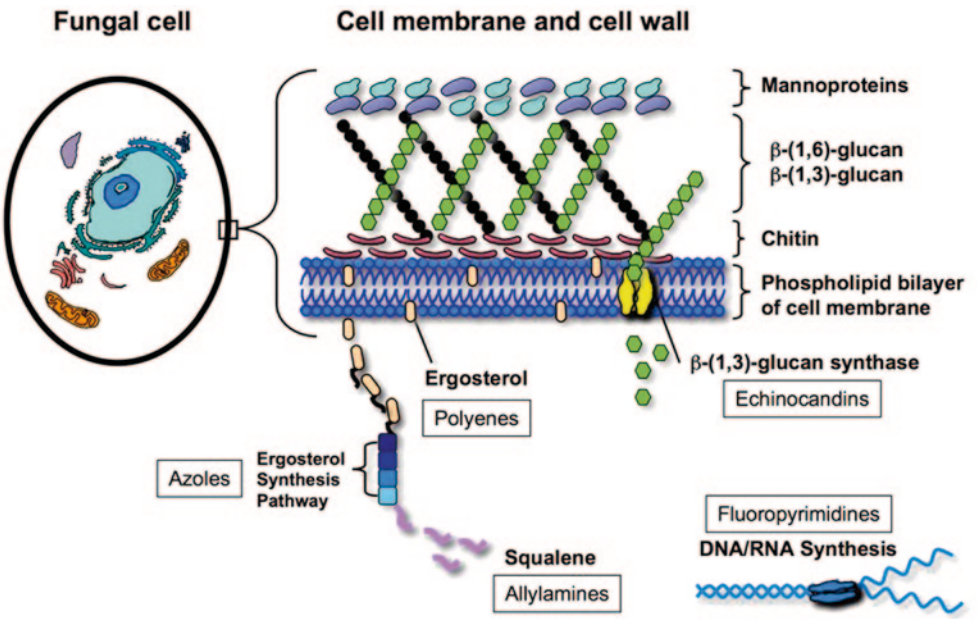

Fig. 7.2 Targets of antifungal therapy

cell membrane and wall produce a number of pleiotropic effects that can arrest fungal growth.

Polyene Mechanisms of Action

Polyene antifungals (amphotericin B) bind to ergosterol, the principal sterol in the fungal cell membrane, disrupting the structure of the fungal cell membrane to the point of causing leakage of intracellular contents. Although this binding typically results in rapid cell death, the precise mechanism of fungicidal activity remains unknown. Structurally, the fungal sterol, ergosterol, exhibits a more cylindrical three-dimensional structure than the mammalian sterol, cholesterol, which largely explains the greater affinity of amphotericin B binding to ergosterol (Fig. 7.3; [4]). However, amphotericin B undoubtedly binds to cholesterol in mammalian cell membranes; a mechanism that could account for the direct toxicity of the drug to the distal tubules of the kidneys [5].

Azole Mechanisms of Action

In contrast to the direct interactions of the polyene antifungals with ergosterol, azole antifungals indirectly affect the fungal cell membrane through inhibition of ergosterol biosynthesis. Azole antifungal compounds inhibit cytochrome P-450 sterol 14α-demethylase (Erg11p or CYP51p depending on nomenclature), an enzyme that catalyzes the oxidative removal of 14α-methyl group of lanosterol in the ergosterol biosynthetic pathway. Inhibition of 14α-demethylase by azoles results in an accumulation of 14α-methylated sterols in the cytoplasmic membrane, which disrupt phospholipid organization, impair membrane-bound enzyme systems such as ATPase and enzymes of the electron transport system; thus arresting fungal cell growth. CYP51p enzyme binding is accomplished through coordination of the triazole N3 or imidazole N4 of the azole ring with the cytochrome P-450 heme target site, while the remainder of the drug molecule binds to the apoprotein in a manner dependent on the individual structure of the azole (Fig. 7.4; [4]). Differences in the exact conformation of the active site between fungal species and drug structure largely define the spectrum of each agent. For molecules derived from the ketoconazole pharmacophore (e.g., itraconazole, posaconazole), extension of side chain enhances binding of the azole to the P-450 apoprotein, and expands the potency and spectrum against both yeast and filamentous fungi. For molecules derived from fluconazole (e.g., voriconazole) inclusion of an α-*O*-methyl group confers activity against *Aspergillus* and other filamentous fungi [7].

One drawback of targeting fungal CYP-450 enzymes involved in ergosterol biosynthesis is the homology the fungal enzyme systems share with mammalian CYP-450 enzymes involved in drug metabolism (Fig. 7.4). Indeed, all azoles inhibit to varying degrees the mammalian CYP-P450 enzymes in humans involved in drug metabolism [8]. Azole therapy can predispose patients to a number (i.e., >2000 theoretical) of pharmacokinetic drug–drug interactions when these antifungals are administered concurrently with drugs that are either substrates or inducers of CYP-P450 enzymes in humans [9]. Unfortunately, modifications of the azole pharmacophore designed to enhance binding to fungal CYP51p frequently enhances binding of mammalian CYP-P450 enzymes. Therefore, improvement in the spectrum of azole antifungals is often accompanied by an increased potential for drug interactions.

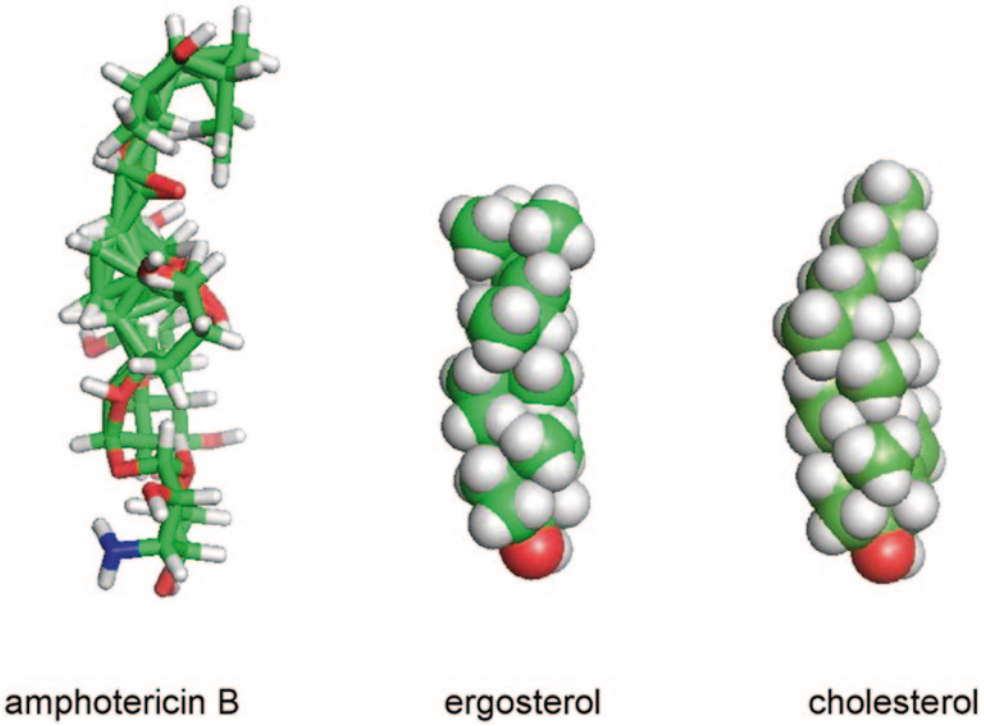

Fig. 7.3 Amphotericin B, ergosterol, and cholesterol visualized in three-dimensions

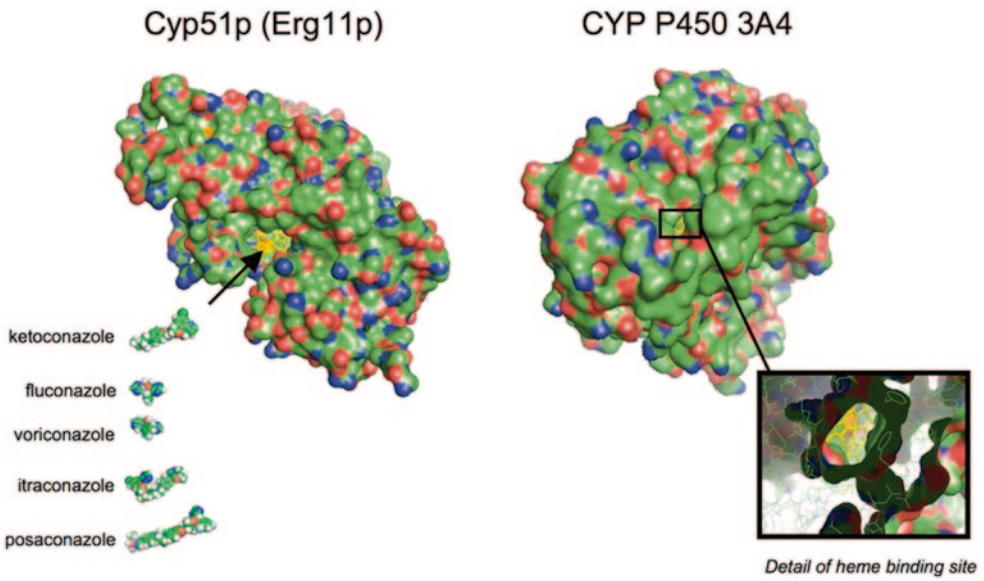

Fig. 7.4 Cyp51p and CYP-P450 3A4 visualized in three dimensions highlighting the heme-binding site for the imidazole (ketoconazole) and triazole rings

Allylamine Mechanisms of Action

Similar to azoles, allylamines inhibit ergosterol biosynthesis prior to 14 α-demethylase by inhibition of the squalene monooxygenase (formally epoxidase). This enzyme is responsible for conversion of squaline to squalene epoxide, a precursor of lanosterol in the ergosterol biosynthetic pathway. Following exposure to allylamines such as terbinafine, the fungal cell membrane accumulates squalene while becoming deficient in ergosterol resulting in arrest of cell growth. Although allylamines do not appear to produce the same degree of cross-inhibition of mammalian CYP-P450 enzymes as azole antifungals, strong inducers of mammalian CYP-P450 enzymes such as rifampin still increase the metabolism of squalene monooxygenase inhibitors such as terbinafine.

Echinocandin Mechanisms of Action

Of the currently available antifungal agents, only one class of agents, the echinocandins, are known to specifically target fungal cell wall synthesis. Echinocandins inhibit the

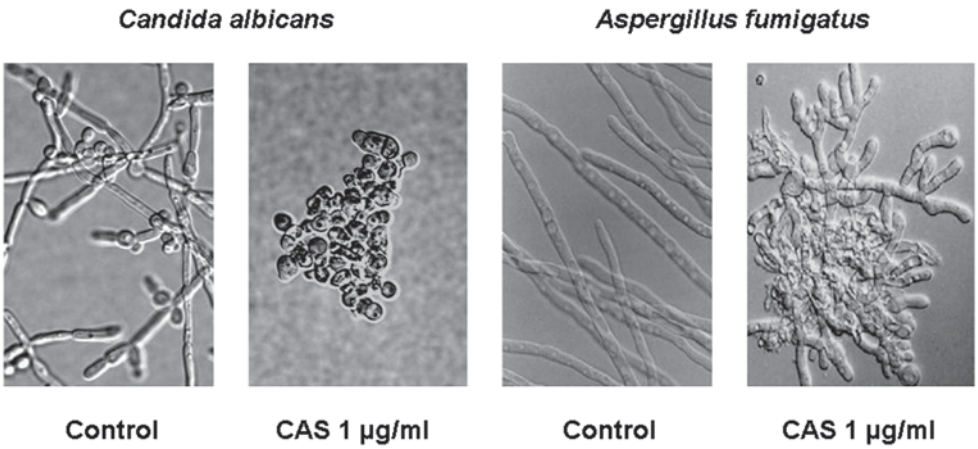

Fig. 7.5 In vitro effects of caspofungin (CAS) on fungal morphology

synthesis of 1,3-β-D-glucan polymers, which serve as essential cross-linking structural components of the cell wall. Depletion of 1,3-β-D-glucan polymers leads to a structurally impaired cell wall, osmotic instability, and lysis in rapidly growing cells. The presumed target of the echinocandins is thought to be β-1,3-D-glucan synthase, although formal proof of this target in pathogenic fungi has been complicated by technical difficulties in studying the membrane-bound protein complex. In *Saccharomyces cerevisiae*, where the enzyme complex has been best studied, the echinocandins are known to bind to the Fks1p component of the two proteins (Fks1p and Fks2p) regulated by the guanosine triphosphate (GTP)-binding peptide, Rho1p, that comprise the transmembrane β-1,3-D-glucan synthase complex [10].

The degree of β-1,3-D-glucan polymerization in the cell wall and expression of the β-1,3-D-glucan synthase target chiefly defines the spectrum and lethality of the echinocandins in pathogenic fungi. In *Candida* species, the fungal cell wall is rich in β-1,3-D-glucans, and the enzyme complex is highly expressed during rapid cell growth. Hence, echinocandins exhibit fungicidal activity against most rapidly growing *Candida* species. However, echinocandins lack clinically useful activity against *Cryptococcus neoformans* due to the limited use of β-1,3-D-glucan in the cell wall of this species [11]. Among hyaline molds, the cell wall of *Aspergillus* species contain the greatest degree of β-1,3- and β-1,6-D-glucan polymers. The β-1,3-D-glucan synthase complex, however, is expressed predominantly on the growing apical tips of the hyphae. Therefore, echinocandins kill only the growing hyphal tips of the fungus, resulting in abnormal, hyperacute branching and aberrant growth, with minimal effects on the viability of subapical components (Fig. 7.5; [12]). Other filamentous fungi, such as *Fusarium* species and *Mucorales*, utilize α-1,3-glucans in the cell wall matrix and chitosan polymers [11]. As such, echinocandins lack pronounced activity against these opportunistic fungi.

Pyrimidime Mechanisms of Action

Flucytosine (5-fluorocytosine, 5-FC) works as an antifungal agent through conversion to 5-fluorouracil within fungal cells (Fig. 7.2). Once inside cells, fluorouracil inhibits thymidylate synthase, a key enzyme in DNA synthesis and incorporates into RNA causing premature chain termination. For flucytosine to be effective, it must be internalized and converted to the active fluorouracil form through the activity of two enzymes, cytosine permease and cytosine deaminase. Mammalian cells and many filamentous fungi lack the enzymes for conversion and intracellular concentration of flucytosine, therefore the activity of the drug is most predictable for pathogenic yeast. In humans, however, resident intestinal flora may convert flucytosine to fluorouracil, resulting in nausea, vomiting, diarrhea, and bone marrow suppression [5].

Antifungal Resistance

Antifungal resistance is a broad concept describing the failure of a fungal infection to respond to antifungal therapy. Resistance has been traditionally classified as either primary (intrinsic; i.e., present before exposure to antifungal) or secondary (acquired; i.e., that which develops after antifungal exposure owing to stable or transient genotypic alterations) [13, 14]. A third type of antifungal resistance could be described as "clinical resistance," which encompasses progression or relapse of infection by a pathogenic fungus that appears, by laboratory testing, to be fully susceptible to the antifungal used to treat the infection. Clinical resistance is most commonly a result of persistent and profound immune defects (e.g., acquired immunodeficiency syndrome (AIDS), neutropenia, graft vs. host disease and its treatment) or infected prosthetic materials (i.e., central venous catheters) which become encased in protective biofilm, thus, limiting

drug activity [13, 14]. In some cases, suboptimal drug concentrations at the site of infection, resulting from poor drug absorption, drug interactions, or infrequent dosing, may contribute to clinical resistance.

Primary or secondary antifungal resistance can arise through a number of complex mechanisms and may be expressed over a wide phenotypic spectrum [13]. At one extreme, fungi may be susceptible to the effects of an antifungal agent but growth may not be completely inhibited in vitro. This so-called trailing growth may be observed for antifungals during laboratory testing (particularly azoles and flucytosine) even at high concentrations, but is generally considered an artifact of testing methods and not reflective of clinically relevant resistance. Similarly, some echinocandins may exhibit a paradoxical attenuation of activity at higher drug concentrations without clear evidence of diminished drug activity at higher dosages in animal models or patients. Heterogeneous resistance, the presence of subpopulations of fungal cells with varying degrees of resistance to an antifungal agent in a susceptible population, may indicate an increased propensity for the development of antifungal resistance. This type of resistance may not be detected unless specialized testing methods are used in the laboratory. Inducible or transiently expressed (epigenetic) antifungal resistance mechanisms have also been described in fungi, but little is known about the clinical significance of these resistance patterns in human infections [13]. The other extreme in the phenotypic expression of antifungal resistance is represented by isolates with stable and persistent growth even at high antifungal concentrations. It is important to note that most studies of antifungal resistance focus on isolates with a stable resistance phenotype. Molecular mechanisms of resistance have been best described in *Candida albicans* isolates recovered from AIDS patients with chronic, recurring fluconazole-refractory oropharyngeal candidiasis [14]. The chronic nature of these mucosal infections allow the longitudinal collection of serial, matched *Candida* isolates that exhibit progressively stable, higher degrees of resistance to antifungals. By contrast, acute bloodstream candidiasis, aspergillosis, or other less common life-threatening mycoses do not typically allow for the study of serial, matched isolates, thus complicating genotypic–phenotypic correlation of resistance development.

Laboratory Detection of Resistance

Standardization of in vitro tests used to determine the activity of antifungals has been a long process. In 1982, the Clinical Laboratory and Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standard (NCCLS)) established a subcommittee to assess the need for such testing. It was not until 1985 that the first report of this subcommittee was released. That document, NCCLS M20-CR, Antifungal Susceptibility Testing; Committee Report, was based on responses from hospitals and reference laboratories from across the nation. The committee found that approximately 20 % of the laboratories that responded were, in fact, conducting antifungal susceptibility testing. Many methods existed, but most of the respondents utilized a broth method and were testing yeast only. Comparison testing of isolates between collaborating laboratories was unacceptably low.

Based on this study, the decision was made to develop a standardized method; the goal being not to correlate between isolate and patient outcome but rather between laboratories. Methods that existed included broth, agar, and disk diffusion. The committee decided that the standard method should be a macrobroth dilution method and that only a synthetic medium should be chosen. Several centers collaborated and a preliminary method was introduced in 1992, M27-P [15]. Parameters were set to include medium, inoculum preparation and size, incubation temperature and duration, and endpoint criteria. The procedure has been refined and is now an approved method, M27-A3 [16]. Subsequent publications include M38-A2 [17], M44-A [18], and M51-A [19]. M38-A2 utilizes similar methods for mold testing, while M44-A/M51-A provides guidelines for disk diffusion testing of both yeast and molds. As a result of these approved methods, commercially available kits now allow clinical microbiology laboratories to perform testing in-house, rather than sending isolates off for reference testing. Prior to doing this, however, laboratories should have sufficient requests for this testing to ensure the volume of work needed to maintain accuracy and reproducibility.

Interpretive guidelines, last revised in 2012, have only been established for anidulafungin, caspofungin, micafungin, fluconazole, and voriconazole against specific *Candida* species. Echinocandin break points are available for *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. guilliermondii*, while fluconazole break points are available only for *C. albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis*. Voriconazole break points are given for *C. albicans*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*. Categories for the echinocandins include susceptible (S), intermediate (I), and resistant (R), while those for the azoles include susceptible (S), susceptible dose dependent (SDD), and resistant (R). The susceptible dose-dependent category relates to yeast testing only and is not interchangeable with the intermediate category used in bacterial and echinocandin break points. This category is in recognition that yeast susceptibility is dependent on achieving maximum blood levels. By maintaining blood levels with higher doses of antifungal, an isolate with an SDD endpoint may be successfully treated with a given azole ([16]; Table 7.1).

In addition to becoming species specific, break points were also lowered considerably from previous recommenda-

Table 7.1 Updated CLSI clinical break points for *Candida* sp.

Organism	Reportable reading conditions	Break points			
		Susceptible	SDD	Intermediate	Resistant
C. albicans					
Anidulafungin	24-h 50%	≤0.25	–	0.5	≥1
Caspofungin	24-h 50%	≤0.25	–	0.5	≥1
Micafungin	24-h 50%	≤0.25	–	0.5	≥1
Fluconazole	24-h 50%	≤2	4	–	≥8
Voriconazole	24-h 50%	≤0.125	0.25–0.5	–	≥1
C. parapsilosis					
Anidulafungin	24-h 50%	≤2	–	4	≥8
Caspofungin	24-h 50%	≤2	–	4	≥8
Micafungin	24-h 50%	≤2	–	4	≥8
Fluconazole	24-h 50%	≤2	4	–	≥8
Voriconazole	24-h 50%	≤0.125	0.25–0.5	–	≥1
C. tropicalis					
Anidulafungin	24-h 50%	≤0.25	–	0.5	≥1
Caspofungin	24-h 50%	≤0.25	–	0.5	≥1
Micafungin	24-h 50%	≤0.25	–	0.5	≥1
Fluconazole	24-h 50%	≤2	4	–	≥8
Voriconazole	24-h 50%	≤0.125	0.25–0.5	–	≥1
C. glabrata					
Anidulafungin	24-h 50%	≤0.125	–	0.25	≥0.5
Caspofungin	24-h 50%	≤0.125	–	0.25	≥0.5
Micafungin	24-h 50%	≤0.06	–	0.125	≥0.25
Fluconazole	24-h 50%	–	≤32	–	≥64
Voriconazole	24-h 50%	–	–	–	–
C. krusei					
Anidulafungin	24-h 50%	≤0.25	–	0.5	≥1
Caspofungin	24-h 50%	≤0.25	–	0.5	≥1
Micafungin	24-h 50%	≤0.25	–	0.5	≥1
Fluconazole[a]—Resistant	24-h 50%	–	–	–	–
Voriconazole	24-h 50%	≤0.5	1	–	≥2
C. guilliermondii					
Anidulafungin	24h-50%	≤2	–	4	≥8
Caspofungin	24h-50%	≤2	–	4	≥8
Micafungin	24h-50%	≤2	–	4	≥8

[a]considered intrinsically resistan

tions. All species of *Candida* were considered susceptible to fluconazole if the MIC was ≤8 μg/ml. This break point was decreased to ≤2 μg/ml for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. *C. krusei* is considered resistant despite the in vitro MIC and *C. glabrata* is not considered susceptible, but rather SDD at ≤32 μg/ml and resistant at ≥64 μg/ml. MIC numbers for voriconazole also dropped significantly, from a previous susceptible break point of ≤1 μg/ml for all *Candida* spp., to ≤0.125 μg/ml for *C. albicans*, *C. parapsilosis*, and *C. tropicalis*, ≤0.5 μg/ml for *C. krusei*, and a removal of all break points for *C. glabrata*. The break points for echinocandins also changed for most species. *C. parapsilosis* and *C. guilliermondii* remain susceptible at ≤2 μg/ml, but *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei* susceptibility decreased from ≤2 μg/ml to 0.06–0.25 μg/ml depending on the drug–species combination.

One important problem with any approach toward in vitro susceptibility testing is the correlation of the minimum inhibitory concentration (MIC) with patient outcome. Some assumptions can be made, however, about MIC and patient outcome. Rex and Pfaller proposed the "90–60 Rule" as a general guide for establishing clinically relevant interpretative break points for resistance [20]. This rule states that infections caused by isolates that have MICs considered susceptible respond favorably to appropriate therapy approximately 90% of the time, whereas infections caused by isolates with MICs considered resistant respond favorably in approximately 60% of cases.

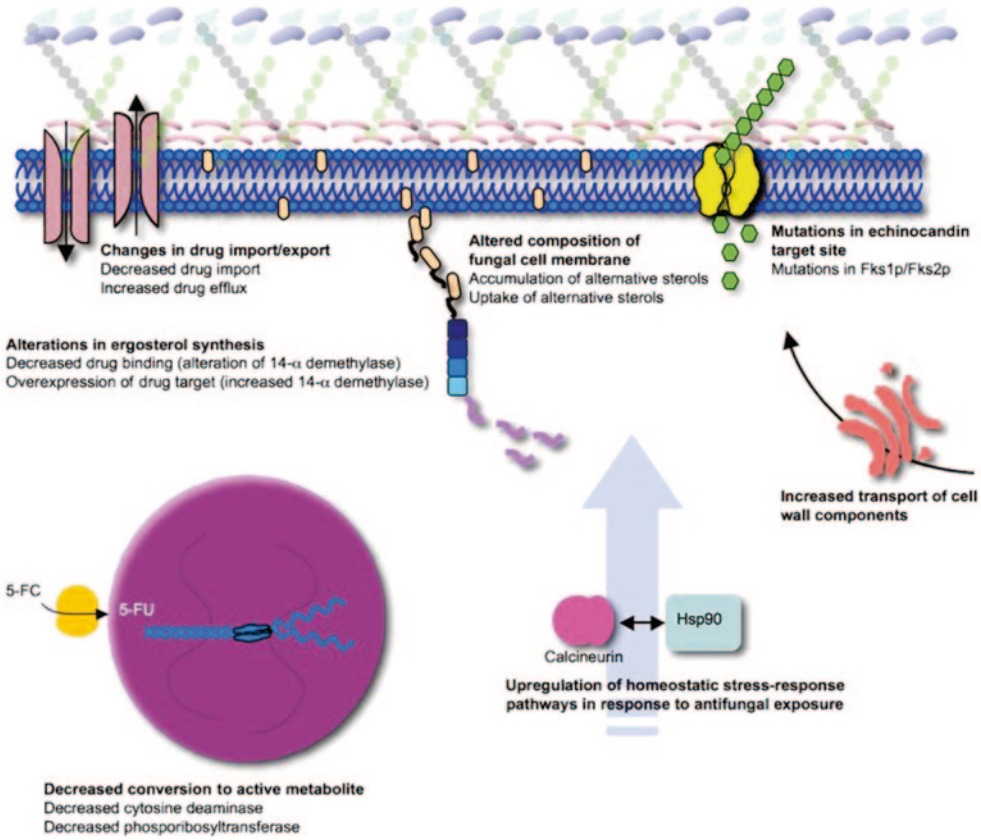

Fig. 7.6 Mechanisms of antifungal resistance

Mechanisms of Resistance

Many aspects of antifungal resistance are still poorly understood, particularly with respect to the regulation and expression of resistance mechanisms following exposure to antifungal agents (secondary resistance). Nevertheless, advances in molecular biology and genome sequencing of pathogenic fungi have yielded progress in our understanding of common mechanisms leading to antifungal resistance. These mechanisms can be grouped into five general categories (Fig. 7.6):

- Decreased drug import or increased drug exportation (efflux pumps)
- Alteration in drug target binding site
- Changes in biosynthetic pathways (particularly sterol synthesis) that circumvent or attenuate the effects of antifungal inhibition
- Alterations in intracellular drug processing
- Upregulation of homeostatic stress-response pathways to deal with antifungal-associated damage

It is important to note that multiple resistance mechanisms are often expressed simultaneously following antifungal exposure and that a single mechanism is unlikely to result in a resistant strain. Depending on the mechanisms concurrently expressed, cross-resistance may, or may not, be observed between different antifungals. Whole-genome expression profiles of *C. albicans* have revealed transient upregulation of several resistance mechanisms (e.g., ergosterol biosynthesis—*ERG 3, ERG11*; efflux pumps—*CDR1, CDR2*) following a single exposure to azole antifungals [21]. Development of resistance in longitudinally collected clinical strains in patients who fail progressively higher dosages of antifungals generally demonstrate a gradual accumulation of several alternations that result in a detectable resistance [13].

Changes in drug importation and exportation are probably the most common mechanisms associated with primary and secondary antifungal resistance [13]. Decreased drug importation is consistently associated with primary resistance to flucytosine and azoles antifungals. For example, poor uptake of flucytosine due to alterations in cytosine permease or decreased availability of this enzyme, largely account for the limited spectrum of this agent against opportunistic molds. Similarly, differences in azole susceptibility between fluconazole and itraconazole against *C. krusei* have been reported to be more closely associated with intracellular accumulation than differences in drug-binding affinity to the 14 α-demethylase target [22]. Drug importation may also be affected by the sterol composition of the plasma membrane. Several studies have demonstrated that when the ergosterol component of the membrane is altered in favor of other 14 α-methyl sterols, there is

a concomitant permeability change in the membrane to drugs, and a decrease in membrane fluidity [13].

Similar to other eukaryotic cells, fungi are known to contain two types of efflux pumps that contribute to drug resistance: ATP-binding cassette (ABC) transporters and major facilitators (MF). Overexpression of the ATP-dependent ABC transporters typically confers a multidrug resistance phenotype. In contrast, MF pumps, which expel antifungal though proton motive force (H^+ gradient across membrane), have a much narrower spectrum of substrate specificity. In *C. albicans*, overexpression of ATP-dependent efflux pumps *CDR1* and *CDR2* confer cross resistance to all azole antifungals [13]. In contrast, overexpression of MF pump *MDR1* effects only the accumulation of fluconazole and does not result in cross-resistance to itraconazole or ketoconazole. Overexpression of ATP-dependent efflux pumps is the most prevalent mechanism of efflux-mediated resistance reported in clinical isolates [13]. Recently, overexpression of ABC transporters was reported to confer a degree of cross-resistance between azoles and echinocandins in a laboratory strain of *C. albicans* [23].

Besides drug efflux, the most common mechanism associated with antifungal resistance involves changes in the binding site of the drug. Several genetic alternations in *ERG11*, the gene encoding 14 α-demethylase, have been attributed to decreases in azole activity, including point mutations that result in changes in the active pocket site or overexpression of *ERG11*. Similar alterations in other enzymes of the ergosterol biosynthetic pathway, particularly *ERG 3* (C-5-sterol desaturase), which is upregulated with inhibition of 14 α-demethylase, have also been documented in azole-resistant clinical strains. Binding site alteration is also likely to be an important mechanism of echinocandin resistance. Point mutations in the *FKS1* gene have been linked with clinical failure of echinocandins in the treatment of bloodstream infections and deep-seated candidiasis, especially among *C. glabrata* [3, 24, 25].

Changes in the target expression in the ergosterol biosynthetic pathway alter the fungal cell membrane sterol content. Substitution of alternative sterols for ergosterol, or alterations in the sterol:phospholid ratio in the cell membrane can decrease intracellular accumulation of azoles and reduce the binding of amphotericin B to the cell membrane. Indeed, most polyene-resistant yeasts recovered from patients with clear microbiological failure on amphotericin B exhibit decreased ergosterol concentrations in their fungal cell membranes. Several studies have even suggested that pathogenic fungi can scavenge free sterols for the cell membrane, including cholesterol, resulting in resistance to polyene and azole antifungals [26].

Alterations in intracellular drug processing and/or degradation and metabolism are probably the least well-studied pathways of resistance in fungi, even though these mechanisms are well characterized in other prokaryotic and eukaryotic systems. Resistance to flucytosine has been associated with alterations in cystosine deaminase, which results in decreased intracellular conversion of flucytosine to its active form.

Recent studies of antifungal resistance have begun to focus on homeostatic stress-response pathways in fungi that may be unpregulated following exposure to antifungals. Disruption of the evolutionarily conserved protein kinase C (PKC) cell-wall integrity and calcineurin pathways enhances azole and echinocandin killing in fungi [27]. Upregulation of these pathways also diminishes the lethal effects of antifungals through upregulation of ergosterol and glucan biosynthesis, increases in chitin content in the fungal cell wall, as well as increased export of cell wall components for cell-wall repair. Recently, the molecular chaperone heat shock protein 90 (Hsp90) was reported to play a critical role in regulating resistance to antifungal agents through the calcineurin pathway [28, 29]. Future efforts towards combating antifungal resistance are likely to exploit this important and evolutionarily conserved mechanism for maintaining and expressing resistance mechanisms to antifungals.

Amphotericin B

Conventional amphotericin B (Fungizone®) has long been considered to be the cornerstone of therapy for deeply invasive fungal infections. Toxicity, including infusion related fever, chills, rigors, headache, and dose-limiting nephrotoxicity, often limits the effectiveness of this agent in severely ill patients. Consequently, three lipid-based formulations (Ambisome®, Abelcet®, Amphotec®) were developed that offer several advantages over conventional amphotericin B including: (1) the ability to administer higher daily dosages of drug, (2) decreased infusion-related side effects (especially for the liposomal formulation), (3) and a reduced rate of nephrotoxicity (Table 7.2). Despite the improved therapeutic index of these formulations, there is still relatively little data from prospective clinical trials to suggest these formulations are more effective than conventional amphotericin B. Moreover, the 10–20-fold higher acquisition cost of the lipid formulations has required many institutions to restrict the use of these formulations to patients with pre-existing renal failure, or in patients who are at high risk for developing nephrotoxicity while receiving amphotericin B (e.g., patients on concomitant nephrotoxic therapies). Currently, there is no consensus opinion on the clinical or pharmacoeconomic threshold for using lipid amphotericin B formulations as first-line therapy for most invasive mycoses.

Table 7.2 Systemic antifungal therapies

Antifungals	Trade name(s)	Usual adult dose	Mechanism of action	Toxicities	Spectrum/comments
Polyenes					
Amphotericin B Lipid formulations of amphotericin B: Liposomal (L)-AMB ABLC ABCD	Fungizone Ambisome, Abelcet, Amphotec	0.25–1.5 mg/kg IV q24h 3–5 mg/kg/q24h 5 mg/kg/q24h 3–4 mg/kg/q24h	Bind to ergosterol and intercalates with the fungal cell membrane, resulting in increased membrane permeability to univalent and divalent cations	Acute fever, chills, rigor, arthralgia with infusion. Thrombophlebitis, dyspnea (rare), arrhythmias (rare) Delayed azotemia (26%), tubular acidosis, hypokalemia, hypomagnesemia, anemia	Drug of choice for severe infections caused by endemic dimorphic fungi, most *Candida* species, and common hyalohyphomycetes (including *Aspergillus*) and mucormycosis Nephrotoxicity is the dose-limiting side effect, reduced with lipid amphotericin B formulations and saline pre- and post hydration Infusion related reactions: Ambisome <Abelcet <Amphotec Ambisome considered the preferred formulation for central nervous system mycoses
Azoles					
Ketoconazole	Nizoral	200–800-mg PO q24h Divided doses recommended ≥400 mg/day)	Inhibition of cytochrome P-450 14 α-demethylase, decreased production of ergosterol, accumulation of lanosterol leading to perturbation of fungal cell membrane, fungistatic	Gastrointestinal (20–50%) including nausea and vomiting, anorexia, rash (2%), transient increases in hepatic enzymes, severe hepatotoxicity (rare), alopecia, inhibition of adrenal steroid synthesis (especially at dosages >600 mg/day)	Oral formulation only. Inconsistencies in oral absorption/poor gastrointestinal tolerance limits use for treatment of deep mycoses Potent inhibitor of mammalian cytochrome P-450 can lead to potentially severe drug interactions when administered concomitantly with other P-450-metabolized drugs
Itraconazole	Sporanox	200–400 mg PO q24h IV 200–400 mg q12h, then q24 h[a] Divided doses recommended ≥400 mg/day)	Similar to ketoconazole, but more selective for fungal P450 demethylase	Gastrointestinal (20%) including nausea and vomiting, and diarrhea rash (2%), taste disturbance (oral solution), transient increases in hepatic enzymes, severe hepatotoxicity (rare), alopecia, inhibition of adrenal steroid synthesis (especially at dosages >600 mg/day) Accumulation of hydroxypropyl-beta-cyclodextran vehicle in patients with CrCl <30 ml/min (intravenous formulation) Congestive heart failure (rare)	Spectrum similar to fluconazole with enhanced activity against *Candida krusei* and *Aspergillus*. Not active against *Fusarium* and mucormycosis. Drug of choice for mild-to-moderate infections caused by endemic dimorphic fungi Bioavailability of oral solution is improved over capsules by 30% under fed conditions and 60% in fasting conditions. Potent inhibitor of mammalian cytochrome P-450 enzymes. Serum-level monitoring is occasionally recommended, trough levels measured by HPLC should exceed 0.5 μg/ml
Fluconazole	Diflucan	100–800 mg PO/IV q24h Dosage adjustment required in renal impairment	Similar to ketoconazole, but more selective inhibitor of 14 α-demethylase	Gastrointestinal (5–10%), rash, headache, transient increases in hepatic enzymes, hepatotoxicity (rare), alopecia	Spectrum includes most *Candida* species, *Cryptococcus neoformans*, and endemic dimorphic fungi. Less active against *Candida glabrata*. *Candida krusei* are intrinsically resistant. Not clinically active for deep mycoses caused by invasive molds Best tolerated of the azoles. Higher daily dosages are recommended (e.g., 12 mg/kg/day) in critically ill patients or in institutions where *Candida glabrata* is common (>10% *Candida* species)

Table 7.2 (continued)

Voriconazole	Vfend	6 mg/kg IV q12h × 2 doses, then 4 mg/kg q12h 200 mg PO q12h if ≥40 kg, 100 mg PO q12h if 40 kg	Similar to fluconazole, but higher affinity for fungal 14 α-demethylase	Transient visual disturbances (reported up to 30%), rash, hallucinations (2%), transient increases in hepatic enzymes, severe hepatotoxicity (rare) Accumulation of sulfobutyl ester cyclodextran vehicle may occur in patients with CrCl < 50 ml/min receiving intravenous formulation Long-term therapy associated with risk of squamous cell carcinoma, periostitis (fluoride toxicity) alopecia, neuropathy	Spectrum similar to itraconazole with enhanced activity against *Aspergillus*, *Fusarium*, and *Scedosporium apiospermum* (*Pseudallescheria boydii*). Retains activity against some fluconazole-resistant *Candida glabrata* Considered by many experts as the initial drug of choice for invasive aspergillosis Inhibitor of mammalian cytochrome P-450 enzymes
Posaconazole	Noxafil	200 mg PO q6h-or q8h × 7 days, then 400 mg q12h Dose-proportional saturable oral absorption Delayed-release tablet (300 mg twice daily, day 1, then 300 mg daily) Intravenous formulation (300 mg twice daily, day 1, then 300 mg daily)	Similar to voriconazole	Gastrointestinal (5–15%), fever, headache, musculoskeletal pain (5%)	Spectrum similar to voriconazole with enhanced activity against *Fusarium*, mucormycosis, and black molds (phaeohyphomycetes) Inhibitor of mammalian cytochrome P-450 3A4 Use of delayed-release tablet improves absorption, and avoids gastric pH–drug interactions. Tablets can be given without food if necessary Intravenous formulation must be given through central line, as repeated doses through peripheral line associated with phlebitis
Echinocandins					
Caspofungin	Cancidas	70 mg IV day 1, then 50 mg q24h	Inhibition of cell wall glucan synthesis, leading to osmotic instability of fugal cell	Fever, chills, phlebitis/thrombophelibitis (peripheral line), rash. Drug concentrations decreased with P-450 3A4 inducers. Decreases tacrolimus blood levels by ~25%	Spectrum includes most *Candida* species including fluconazole–resistant *Candida krusei* and *Candida glabrata* Higher dosages may be required for *Candida parapsilosis*. Active against *Aspergillus* species Not active against *Cryptococcus neoformans*, *Trichosporon*, *Fusarium*, mucormycosis, or black molds (phaeohyphomycetes)
Micafungin	Mycamine	50–150 mg IV q24h	Similar to caspofungin	Similar to caspofungin	Similar to caspofungin
Anidulafungin	Eraxis	200 mg IV day 1 then 100 mg/day	Similar to caspofungin	Similar to caspofungin	Similar to caspofungin
Fluoropyrimidines					
Flucytosine (5-FC)	Ancobon	100 mg/kg/daily PO divided q6h Dosage adjustment required in renal impairment	Drug is transported into susceptible fungi by cytosine permease, and then deaminated to active form (5-FU) by cytosine deaminase where the drug interferes with DNA/RNA synthesis	Increase in serum transaminases (7%), nausea and vomiting (5%); diarrhea, abdominal pain, rash, enterocolitis (rare) Less common-leucopenia, thrombocytopenia, anemia	Narrow spectrum for deep mycoses: *Candida* and *Cryptococcus neoformans* only. Resistance is common when used as monotherapy Typically administered in combination with amphotericin B for cryptococcal meningitis Risk of bone marrow suppression increased with persistent flucytosine levels 100 μg/ml. Careful dosage adjustment is required in patients with renal dysfunction

PO orally, *IV* intravenously, *q6h* every 6 h, *q12h* every 12 h, *q24h* every 24 h, *P-450* cytochrome P-450
[a]Not available in the USA

Table 7.3 Comparative pharmacokinetics of the antifungal agents

	AMB	ABCD	ABLC	L-AMB	Flu	Itra[a]	Vori	Posa	Anid	Cas	Mica	*5FC*
Oral bioavailability (%)	<5	<5	<5	<5	95	50	96	60	<5	<5	<5	80
Distribution												
Total C_{max} (μg/ml)		4	0.3–1	131	0.7	11	4.6	7.8	0.83	0.27	0.24	80
AUC (mg h/L)	17	43	14	555	400	29.2	20.3	8.9	99[e]	119	158[e]	
Protein Binding (%)	>95	>95	>95	>95	10	99.8	58	99	84	97	99	4
CSF (%)	0–4				>60	<10	60		<5	<5	<5	75
Eye (%)	0–38[bc]	0–38[bc]	0–38[bc]	0–38[bc]	28–75[bc] (10–70 μg/ml)	10[b] (0.22 μg/ml)	18[b] (0.81 μg/ml)	26 (0.25 μg/ml)	NDND	ND	ND	ND
Urine (%)[d]	3–20			4.5	90	1–10	<2	<2	<2	<2	<2	90
Metabolism	Unk	Unk	Unk	Unk	+	++	+++	++	None	Hep	Hep	None
Elimination	Urine/bile	Unk	Unk	Unk	Renal	Hep	Renal	Feces	Feces	Urine	Feces	Renal
Half-life (h)	50	30	173	100–153	31	24	6	25	24	30	15	3–6

AMB amphotericin B deoxycholate, *ABCD* amphotericin B cholesterol dispersion, *ABLC* amphotericin B lipid complex, *L-AMB* liposomal amphotericin B, *Flu* fluconazole, *Itra* itraconazole, *Vori* voriconazole, *Posa* posaconazole, *Anid* anidulafungin, *Cas* caspofungin, *Mica* micafungin, *5FC* 5-fluorocytosine or flucytosine, *ND* no data available, *Unk* unknown, *Hep* hepatic

[a]Data are for oral solution; tablet and intravenous doses produce AUCs of 36.1–38.0 mg h/L

[b]Human

[c]Animal

[d]% of active drug or metabolites

[e]for doses of 100 mg/day

Spectrum and Susceptibility

Amphotericin B should be administered only to patients with progressive and possibly fatal infections. Acceptable activity can be measured in vitro against almost all fungi including *Candida*, *Cryptococcus*, *Aspergillus*, *Blastomyces*, *Histoplasma*, *Coccidioides*, *Sporothrix*, and agents of mucormycosis (including *Rhizopus*, *Mucor*, and *Lichtheimia* spp.), as well as other less frequently recovered strains.

A few species exhibit elevated MICs when tested against amphotericin (AMB) and are known to possess innate resistance to this drug. Resistant species include both *Scedosporium apiospermum* and *S. prolificans*, in addition to *Purpureocillium lilacinum*, *Aspergillus terreus*, and some *Fusarium* species. Early reports have revealed *Candida lusitaniae* resistance to AMB and have shown that this species possesses the ability to develop resistance while on treatment. The first report involved a patient whose initial isolate was susceptible but whose subsequent isolates had developed AMB resistance [30]. Later reports have shown AMB resistance may exist even prior to exposure to AMB [31]. The expected rate of resistance for *C. lusitaniae* is 8–10% of any microbiology lab stock collection.

Pharmacokinetics

Amphotericin B deoxycholate has negligible oral absorption and must be administered intravenously. Following IV administration, the drug is released from its carrier, and is highly bound by plasma proteins (91–95%) including lipoproteins, erythrocytes, and cholesterol in the plasma. Amphotericin B then redistributes from the bloodstream into tissue with an apparent volume of distribution (V_d) of 4 l/kg [5]. In adults, infusion of 0.6 mg/kg of amphotericin B deoxycholate yields peak serum concentrations of approximately 1–3 μg/ml [32]. Concentrations in other body fluids outside the serum are less than 5% of concurrent serum concentrations with poor penetration into bronchial secretions, pleura, peritoneum, synovium, and aqueous humor. Although amphotericin B poorly penetrates the CSF, fungal infections of central nervous system successfully treated with amphotericin B [5].

Tissue concentrations of amphotericin B are highest in the kidney followed by the liver, spleen, heart, skeletal, muscle, and brain. The formulation of amphotericin B into phospholipid sheets (Abelcet), cholesterol disks (Amphotec), or liposome carriers (Ambisome) alters drug distribution (particularly to the kidneys) and the elimination profile of the drug ([5]; Table 7.3).

Recent studies have suggested that amphotericin B undergoes relatively little metabolism, with a terminal elimination half-life of >11–15 days. After 168 h, approximately 60% of a single dose can be recovered from the feces (~40%) and urine (20%) [33]. Since a relatively lower fraction of the daily dosage is slowly excreted in urine and bile, dosage modification is not necessary to prevent drug accumulation in patients with renal or hepatic failure, but may be judicious in patients with organ dysfunction. Because amphotericin B behaves as a colloid in aqueous solutions and is highly protein bound, hemodialysis does not remove significant amounts of the drug unless the patients are hyperlipidemic; which enhances amphotericin B binding to the dialysis membrane [5].

Adverse Effects

The most common acute toxicity of all amphotericin B formulations is infusion-related reactions, which are characterized by fever, chills, rigors, anorexia, nausea, vomiting, myalgias, arthralgias, and headache. Hypotension, flushing, and dizziness are less common, but bronchospasm and true anaphylactic reactions have been reported with both the conventional and lipid formulations of amphotericin B [5]. Severe hypokalemia and cardiac arrhythmias have also been described in patients with central venous catheters who have received rapid infusions or excessive doses of conventional amphotericin B. Therefore, slower infusion rates (4–6 h or more) and EKG monitoring should be considered in patients with underlying cardiac conduction abnormalities. Thrombophlebitis is a common local side effect with infusion, which often necessitates the placement of a central venous line for therapy >1 week. Slower infusion rates, rotation of infusion sites, application of hot packs, low-dose heparin, and avoidance of concentrations >1 mg/ml can minimize thrombophlebitis.

Acute reactions generally subside over time and with subsequent amphotericin B infusions. In the past, a test dose of amphotericin B deoxycholate (i.e., 1–5 mg) was recommended prior to initiating therapy. This is no longer considered useful for screening patients for hypersensitivity reactions. Pre-medications such as low-dose hydrocortisone (1 mg/kg), diphenhydramine, meperidine (0.5 mg/kg), and nonsteroidal anti-inflammatory agents are often administered prior to amphotericin B infusions to blunt symptoms of acute reactions. Pre-medication can also be considered prior to infusions of the lipid amphotericin B formulations, despite the somewhat reduced rates of infusion reactions seen with these drugs, especially the liposomla formulation.

Nephrotoxicity is the most significant delayed toxicity of amphotericin B and can be classified into glomerular or tubular mechanisms. Amphotericin B directly constricts the afferent arterioles resulting in decreased renal blood flow and a drop in glomerular filtration (increased serum creatinine), eventually leading to azotemia (increased blood urea nitrogen). Amphotericin induced azotemia may be delayed ensuring patients are well hydrated prior to starting therapy and by *sodium loading*—the practice of administering intravenous normal saline (0.5–1 l) before and after amphotericin B infusion to maintain renal blood flow and adequate glomerular filtration pressure. However, many patients cannot tolerate extra fluid or salt if they have impaired cardiac function. Two small non-randomized studies have also suggested that the administration of amphotericin B deoxycholate by continuous infusion can delay glomerular toxicity [34, 35]; however, this dosing approach has not been widely adopted. Azotemia with amphotericin B is generally reversible, although 5–10% of patients may have persistent renal impairment after discontinuation of therapy.

Amphotericin B is directly toxic to the distal tubules resulting in impaired urinary acidification, impaired urinary concentrating ability, and wasting of potassium and magnesium. Hypokalemia is common in patients receiving either conventional or lipid formulations of the drug. Patients may require the administration of up to 15 mmol (15 mEq) of supplemental potassium per hour [4]. Hypokalemia and low serum magnesium frequently precede decreases in glomerular filtration (increased serum creatinine), especially in patients who are adequately hydrated or receiving lipid formulations of amphotericin B. Continued tubular damage, however, eventually results in decreases in renal blood flow and glomerular filtration through tubuloglomerular feedback mechanisms that constrict the afferent arteriole. Hence, sodium loading should still be considered for patients receiving lipid amphotericin B formulations.

Patients who receive prolonged courses of amphotericin B frequently develop normochromic, normocytic anemia due to the inhibitory effects of amphotericin B on renal erythropoietin synthesis. Patients may experience decreases in hemoglobin of 15–35% below baseline that return to normal within several months of discontinuation of the drug. Administration of recombinant erythropoietin has been suggested in patients with symptomatic anemia during amphotericin therapy.

Azoles

The availability of azole antifungals, particularly the oral triazoles itraconazole, fluconazole voriconazole, posaconazole and more recently isavuconazole, fulfill a critical need for effective and better-tolerated alternatives to amphotericin B. Miconazole was the first systemic azole approved for use in humans, but the relatively toxic IV formulation limited its use to severely ill patients. Similarly, ketoconazole was

not effective in critically ill patients due to its lack of an intravenous formulation and erratic absorption in patients with relative achlorhydria. The triazoles have proven to be much more effective in the prevention and treatment of both primary and opportunistic mycoses. All four currently approved triazoles at the time of writing (fluconazole, itraconaazole, voriconazole, and posaconazole) are available in table/capsule, oral solution, and intravenous formulations (although IV itraconazole is not currently marketed in the USA), providing clinicians with added flexibility in therapy selection. Because all azoles are potentially teratogenic, they should be avoided during pregnancy.

Spectrum and Susceptibility

The triazoles are more easily tolerated, but are primarily considered fungistatic against yeast as opposed to fungicidal (lethal) drugs. However, clear definitions of cidality are often more difficult to ascertain with antifungal agents. Although similar in mechanisms of action, each agent has a slightly different spectrum of activity.

Fluconazole is principally used for yeast infections including those caused by most *Candida* and *Cryptococcus* species. Although this drug has been used to successfully manage meningitis caused by *Coccidioides*, it is not typically a drug of choice for infections caused by other molds.

Of primary concern is acquired resistance by yeasts. *Candida krusei* is well documented to possess intrinsic resistance to fluconazole, so much so that susceptibility testing against this isolate is not recommended. Some reports place the rate of outright *C. glabrata* resistance at about 15% of any given population of isolates [36], but both *C. albicans* and *C. glabrata* are capable of developing resistance following prolonged therapy or following therapy with inappropriate dosing. Overall, by in vitro testing, about 82% of *Cryptococcus neoformans* and 91% of *C. albicans* strains appear susceptible to fluconazole.

Itraconazole possesses a wide spectrum of activity including activity against both yeasts and molds. It is useful in treating aspergillosis, blastomycosis, coccidioidomycosis, histoplasmosis, and candidiasis. In addition, itraconazole possess low MIC endpoints against the phaeoid fungi and may be considered the drug of choice for treatment of infections caused by fungi from this group. Cross-resistance is of concern between drugs within the azole class. Comparison of resistance patterns between itraconazole and fluconazole reveal similar percentages of resistance among *Candida* species.

Voriconazole is noted to have activity against *Aspergillus*, *S. apiospermum*, and *Fusarium solani*. This is remarkable due to the fact that both *S. apiospermum* and *F. solani* are notoriously resistant to other antifungal agents. In addition, voriconazole may possess lethal activity against the aspergilli as opposed to the static activity expected with the azoles. Susceptibility patterns with the yeasts are similar to both itraconazole and fluconazole. An important exception is the extremely low incidence of resistance seen with *C. krusei*, in contrast to near 100% resistance of this species to fluconazole and about 10% resistance to itraconazole.

Posaconazole and the investigational azole isavuconazole possess a similar broad spectrum of activity. Both agents show activity against aspergillosis, candidiasis, fusariosis, coccidioidomycosis, and mucormycosis. Results against the *Mucorales*, including *Rhizopus* and *Mucor*, show that this drug may provide alternative therapy to amphotericin B for infections caused by this group of fungi. Resistance patterns are similar to voriconazole with the exception of the *Mucorales* and *Pseudallescheria/Scedosporium* spp.

Fluconazole

Among the triazole antifungals, fluconazole (Diflucan®) is clearly the best-tolerated agent and has the most desirable pharmacological properties, including high bioavailability, high water solubility, low degree of protein binding, linear pharmacokinetics, and a wide volume of distribution including the CSF, eye, and urine [5, 37]. Unlike other azoles, fluconazole is eliminated primarily unchanged through the kidneys and is less susceptible to clinically significant drug interactions through mammalian cytochrome P-450 enzymes at standard dosages used to treat superficial (100–200 mg/day) or systemic (400 mg/day) infections.

Itraconazole

Itraconazole (Sporanox®) was initially introduced in the early 1990s as a capsule formulation that was effective for superficial fungal infections and mild-to-moderately severe endemic mycosis, but erratic absorption in the critically ill patient limited its effectiveness for opportunistic mycoses. The subsequent reformulation of this triazole into an oral and intravenous solution with hydroxy-beta-propyl cyclodextran significantly improved the blood levels that could be reliably obtained in critically ill and immunocompromised patients. Itraconazole is a relatively broad-spectrum triazole with activity against many common fungal pathogens including most *Candida*, *Cryptococcus*, endemic dimorphic fungi (*Histoplasma*, *Blastomyces*, and *Coccidioides*), and *Aspergillus*. The drug is lipophilic, highly protein bound and has a long half-life, nonlinear pharmacokinetics, and limited distribution into some body fluids, including the CSF and urine (Table 7.3). The drug is metabolized in the liver and to a lesser extent in the gut into more hydrophilic metabolites, one of which retains potent antifungal activity (hydroxyitraconazole). The most common adverse effects associated with itraconazole therapy are gastrointestinal (especially with the oral solution), rash, and transient increases in hepatic trans-

aminases. Prolonged therapy can be associated with metabolic disturbances (suppression of adrenal steroid synthesis) and increased risk of congestive heart failure. Itraconazole is a substrate and potent inhibitor of mammalian cytochrome P-450 enzymes and is, therefore, susceptible to a number of clinically significant drug interactions (see Azole Drug Interactions Section).

Voriconazole

Voriconazole (Vfend®) is a methylated analogue of fluconazole with enhanced activity against yeast as well as important opportunistic molds including *Aspergillus* and *Fusarium* (Table 7.2). Like fluconazole, voriconazole is well absorbed orally, has limited protein binding, and distributes widely throughout the body, including the CSF. Like itraconazole, IV voriconazole is formulated in a cyclodextran solution (sulfobutylether cyclodextran) and has nonlinear pharmacokinetics in adults. Voriconazole is metabolized to inactive metabolites through the liver and is an inhibitor of mammalian cytochrome P-450 enzymes (Table 7.3). In addition to the common adverse effects seen with other triazole antifungals (gastrointestinal, rash, increases in hepatic enzymes), voriconazole can cause transient visual disturbances in 15–30% of subjects that manifest as photophobia, perception of blinking or flashing lights (even with the eyes closed), and occasionally hazy or blurred vision. Symptoms tend to occur during the first week of therapy and disappear with continued therapy in most patients. Occasionally, visual disturbances are intensified by hallucinations—a separate side effect seen in 2–8% of patients receiving voriconazole (often with concomitant benzodiazepines and narcotic analgesic therapy). Visual disturbances are thought to be a result of temporary alterations in electrical conduction of photoreceptors in the rods and cones of the retina, which revert to normal once therapy is stopped. No permanent damage to the retina has been noted in human or animal studies of voriconazole [38].

Prolonged use of voriconazole has been associated with uncommon, but potentially severe adverse effects. Severe phototoxicity progressing to actinic keratosis and/or squamous cell carcinoma (SCC) has been reported in some patients receiving prolonged voriconazole therapy (i.e., >6 months), which may be accelerated in transplant populations [39, 40]. Although the mechanism of voriconazole-induced SCC is unknown, there are multiple retrospective studies associating the use of voriconazole with SCC possibly due to the photosensitizing effects of the voriconazole N-oxide metabolite [40]. Periostitis has also been reported in patients receiving prolonged voriconazole therapy, which may be related to fluoride toxicity [41]. Alopecia, cheilitis, and brittle nails have also been noted with prolonged therapy [42]. Similar to other triazoles, long-term therapy with voriconazole may also be associated with the development of peripheral neuropathies [43].

Posaconazole

Posaconazole (Noxafil®) is triazole analogue of itraconazole with enhanced activity against opportunistic molds including *Aspergillus*, *Fusarium*, and (notably) the agents of mucormycosis. Posaconazole is available as both an oral suspension, a pH-sensitive delayed-release tablet, and an intravenous formulation. Absorption of posaconazole suspension is dose limited at 800 mg/day and can be improved if the suspension is administered with a high-fat meal or in divided doses (twice to four times daily; Table 7.2; [44]). A more recently introduced delayed-release tablet formulation uses pH-sensitive polymers to release posaconazole at a controlled rate in the duodenum, thereby circumventing many of the problems associated with poor gastric dissolution of the drug [45]. As a result, following a loading dose (300 mg twice daily on day 1), the average serum concentration achieved with a 300-mg daily dose using delayed-release posaconazole tablets is 1400 ng/mL, which is more than double of that achieved with the oral suspension administered at 200 mg four times daily (517 ng/mL; [45, 46]). The tablet formulation also provides the opportunity to administer a loading dose on the first day of therapy to ensure therapeutic posaconazole plasma levels in the first 24–48 h. This is in contrast with the oral suspension, which typically would not approach steady-state therapeutic levels until 7–10 days of therapy [47].

Similarly, a recently introduced intravenous formulation of posaconazole solubilized in sulfobutyl ether beta cyclodextrin has been introduced. Dosing is similar to the oral delayed-release tablets (300 mg was administered intravenously twice on day 1 followed by 300 mg daily) and achieved trough concentrations greater than 1000 ng/mL within 24 h in a majority of patients [45, 46]. The intravenous formulation is preferred in critically ill patients or patients who cannot swallow the tablet formulations, which cannot be crushed.

Once absorbed, posaconazole is widely distributed into tissues throughout the body and is highly protein bound (98%). Elimination of posaconazole occurs predominantly (90%) in the feces as unchanged drug and in the urine (10%) as an inactive metabolite (Table 7.3). Despite its lack of phase I metabolism, posaconazole is a potent inhibitor of mammalian cytochrome P-450 3A4 and has a similar potential for drug interactions as itraconazole when co-administered with drugs metabolized through this pathway.

Azole Drug Interactions

As mentioned previously, an inherent limitation of azole pharmacology is that the target of antifungal activity in pathogen-

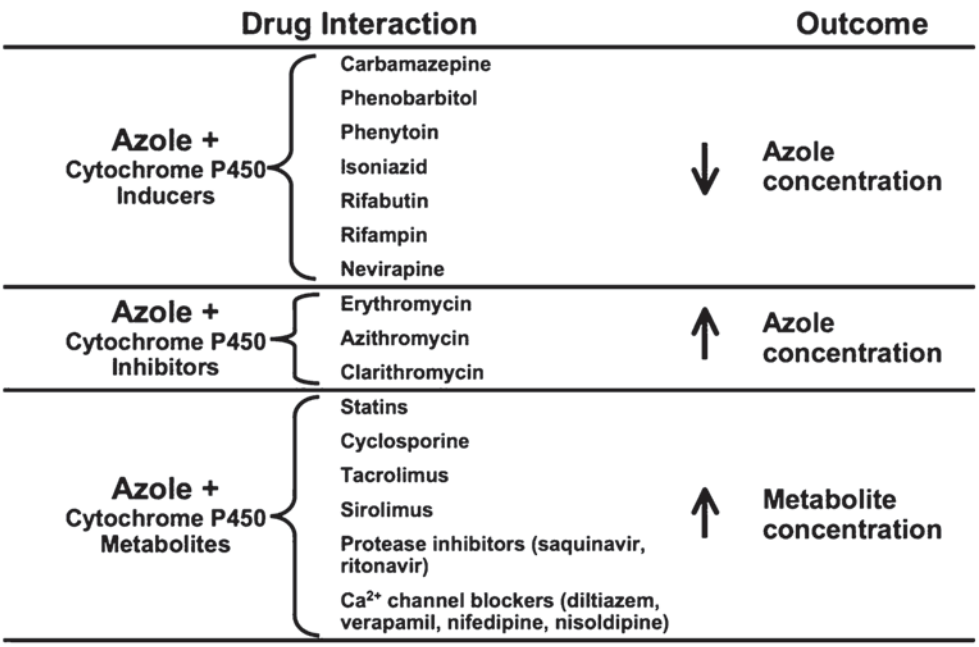

Fig. 7.7 Drug interactions of the azole antifungals

ic fungi, the cytochrome P-450 enzyme 14-α-demethylase, shares considerable homology with mammalian cytochrome P-450 enzymes involved in drug metabolism. As a result, azole antifungals can be both substrates and inhibitors of cytochrome P-450 systems in humans [8, 9]. Significant drug interactions with azole antifungals are summarized in Fig. 7.7. Many of these drug interactions are potentially severe and concomitant use should be avoided. Some azole drug interactions are less predictable and possibly dosage dependent. For example, fluconazole is a weak inhibitor of cytochrome P-450 3A4 at dosages of 50–200 mg/day and is excreted primarily (80 %) through the urine. However, as daily dosages are increased, fluconazole has a greater potential for inhibition of cytochrome P-450 3A4 and a larger percentage of the drug is metabolized via the P-450 system [8]. Cytochrome P-450 3A4 inducers increase metabolism of all azoles to varying degrees regardless of their primary excretion pathways. Co-administration of rifampin, for example, can reduce fluconazole serum concentrations by ~50 % and concentrations of itraconazole, voriconazole, and posaconazole by >90 % [8]. Azole antifungal therapy should be avoided, whenever possible, during use of high-dose conditioning chemotherapy with busulfan or cyclophosphamide due to an increased risk of acute liver toxicity and accumulation of toxic chemotherapy metabolites [47]. Although azoles themselves do not appear to exert major effects on cardiac conduction, their combined use with drugs that effect potassium channels and are metabolized through CYP-450 mechanisms (e.g., cisapride, haloperidol, certain tricyclic antidepressants) have the potential for life-threatening arrhythmias.

Echinocandins

Despite some modest differences in pharmacokinetics and potency, the echinocandins are pharmacologically similar and probably interchangeable [3]. All three currently approved agents, caspofungin, micafungin, and anidulafungin, are large semisynthetic lipopeptides that are available only as intravenous formulations. All have linear pharmacokinetics, are widely distributed (with the possible exception of the CSF and urine), and have prolonged elimination half-lives that permit once daily dosing. Slight differences in the metabolism and excretion are seen between the echinocandins, which may account for some differences in the drug interaction profile of these agents.

Spectrum and Susceptibility

Caspofungin is indicated for candidiasis and for aspergillosis in patients who are refractory to other therapies. Use for infections caused by other molds has not been as extensively studied. Activity is fungicidal against the yeasts, while static against the aspergilli. Endpoints against *Aspergillus* are determined differently than with other antifungals. Endpoints are determined as MEC or minimum effective concentration. While growth is substantial with in vitro systems, it is evident that the growth is grossly abnormal. The MEC is considered the lowest concentration of drug that causes the abnormal growth of hyphae in this species (Fig. 7.5). Endpoints for other molds would be read in like manner.

Resistance, although previously uncommon, is increasing reported for this class of antifungals with their widespread use, especially among *C. glabrata* [24]. With a few rare exceptions, resistance to one echinocandin often results in cross-resistance to other echinocandins.

Pharmacokinetics

All three echinocandins are available as intravenous formulations only, have (mostly) linear pharmacokinetics, are widely distributed (with the possible exception of the CSF and urine), have prolonged elimination half-lives, and are metabolized by chemical degradation followed by hepatic metabolism (Table 7.3). Dosage adjustment is recommended for caspofungin in patients with severe hepatic dysfunction (Child Pugh score 7–9), but is not required for micafungin or anidulafungin.

Adverse Effects

All three echinocandins were well tolerated in phase II/III clinical trials with the most common adverse effects being phlebitis/venous irritation, headache, fever, and rash. Infusion-related reactions analogous to the "red person's syndrome" seen with vancomycin have been described with caspofungin, likely due to its potential to cause histamine release from mast cells, but is seen less commonly with micafungin and anidulafungin. The most common laboratory adverse effects reported with echinocandin therapy are transient elevations in serum transaminases and alkaline phosphatase levels. The echinocandins are neither substrates nor inhibitors of cytochrome P-450 enzymes of P-glycoprotein enzymes. For reasons not completely understood, co-administration of caspofungin with inducers of P-450 3A4 (e.g., rifampin, phenytoin) results in modest (25–50%) decrease in the AUC, which can be overcome with higher dosages. P-450 inducers do not appear to have as pronounced effect on the clearance of micafungin or anidulafungin. Caspofungin modestly (~20%) decreases the AUC of concomitant tacrolimus therapy. Micafungin modestly increases the AUC of nifedipine and sirolimus. No clinically significant drug interactions have been identified thus far for anidulafungin.

Fluoropyrimidines

Flucytosine (5-fluorocytosine, 5-FC) is the only agent among the fluoropyrimidine class of antifungal agents approved for the treatment of invasive fungal infections. It is available only in oral capsule formulation in the USA. The usefulness of flucytosine for treating invasive mycoses is hampered by its relatively narrow spectrum, high rates of acquired resistance among common pathogens (e.g., *Candida* species) and significant potential for toxic effects. For these reasons, flucytosine is not used as monotherapy and has a minimal role in the treatment of most mycoses.

Spectrum and Susceptibility

5-fluorocytosine has activity against both *Candida* and *Cryptococcus* species and is not recommended for the treatment of infections caused by other fungal species. The rate of resistance against *Candida* species is expected in about 5% of isolates, while for *Cryptococcus* species resistance occurs in about 2% of isolates tested.

Pharmacokinetics

As flucytosine widely distributes throughout the body, including the CSF after oral administration, it has an adjuvant use in combination with antifungals that have slow or minimal distribution into anatomically privileged sites. Several randomized prospective studies of cryptococcal meningitis in patients with AIDS have shown that the addition of flucytosine to amphotericin B therapy results in more rapid sterilization of the CSF, decreased early mortality, and fewer relapses after completion of "induction" antifungal therapy.

Adverse Effects

Flucytosine was originally developed as an antitumor chemotherapy before it was discovered to have antifungal activity against common yeast. Not surprisingly, the most common side effects are nausea and vomiting, increases in serum transaminases, and bone marrow suppression. The risk of bone marrow suppression can be reduced if serum levels are maintained <100 μg/ml [48]. Because flucytosine is eliminated unchanged through the kidneys, serum level monitoring and dosage adjustments are required in patients receiving flucytosine in combination with amphotericin B or other nephrotoxic agents. Gastrointestinal side effects are seen in up to 6% receiving oral flucytosine including diarrhea, nausea, and vomiting. Reversible elevations in hepatic serum transmaninases and alkaline phosphatase have also been reported in 4–10% of patients receiving flucytosine. The most serious toxicity associated with flucytosine, however, is bone marrow suppression, which occurs in 6% of patients. Some evidence has accumulated in the past two decades that marrow toxicity is enhanced if serum concentrations of flucytosine exceed 100 μg/ml.

Combination Antifungal Therapy

Due to their unique mechanism of action, the introduction of the echinocandins has renewed interest in the use of combination antifungal therapy for invasive mycoses. The most common reasons for consideration of combination therapy are to: (1) broaden the spectrum of antifungal coverage of opportunistic mycoses; particularly in severely immunocompromised patients, (2) to enhance the activity of an antifungal regimen through (presumably) synergistic antifungal effects especially in severely immunocompromised patients with progressive disease, and (3) to overcome the pharmacokinetic limitations of a single antifungal agent in the treatment of life-threatening mycoses in an anatomically privileged sites such as the CNS (e.g., combined use of flucytosine and amphotericin B for cryptococcal meningitis; [49]). With the possible exception of cryptococcal meningitis, where combination therapy with amphotericin B and flucytosine is associated with improved survival [50], there are few definitive studies supporting the use of combination therapy for deep mycoses. Recently, a multicenter prospective randomized study in patients with proven or probable aspergillosis reported significantly improved survival among patients with galactomannan-diagnosed diseases who received combination anidulafungin–voriconazole therapy versus voriconazole alone [51]. Nevertheless, the benefits reported in this trial were evident only in a subgroup, post-hoc analysis, raising some lingering questions about the efficacy of combination therapy for invasive aspergillosis.

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Part IV
Mycoses

Candidiasis

8

Jack D. Sobel

Introduction

Candida species are ubiquitous fungi and the most common fungal pathogens affecting humans [1, 2]. The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the pool of patients at risk and the increased opportunity for *Candida* to invade tissues normally resistant to invasion. *Candida* are true opportunistic pathogens that exploit recent technological advances to gain access to the vascular circulation and deep tissues. *Candida*, in particular, affect high-risk patients who are either immunocompromised or critically ill.

Etiologic Agents

Candida are yeast-like fungi that can form true hyphae and pseudohyphae. These yeasts are typically confined to human and animal reservoirs; however, they are frequently recovered from the hospital environment, including food, countertops, air conditioning vents, floors, respirators, and medical personnel. They are also normal commensals of diseased skin and mucosal surfaces of the gastrointestinal (GI), genitourinary, and respiratory tracts.

More than 100 species of *Candida* exist, but only a few are recognized as causing disease in humans [1]. The medically significant *Candida* species are shown in Table 8.1. *Candida glabrata* and *Candida albicans* account for 70–80 % of yeasts isolated from patients with invasive candidiasis. *C. glabrata* has become important because of its increasing worldwide incidence and because it is intrinsically less susceptible to azoles and amphotericin B. Two uncommon *Candida* species, *Candida lusitaniae* and *Candida guilliermondii*, are important because of their innate resistance to amphotericin B. *Candida krusei*; although not as common as some *Candida* species, is clinically significant because of its intrinsic resistance to fluconazole and decreased susceptibility to all other antifungals, including amphotericin B (Table 8.1) [3]. *C. krusei* is more commonly associated with hematological malignancy and neutropenia than is *Candida parapsilosis*.

Susceptibility methods for the echinocandin antifungal agents (caspofungin, micafungin, and anidulafungin) are not standardized, and interpretive criteria are not available. The three drugs show generally similar susceptibility patterns, and, therefore, are shown as a class. Clinical responses to invasive disease have been observed with all *Candida* species for caspofungin.

Epidemiology

Candida species are the most common cause of fungal infections, primarily affecting immunocompromised patients [4–8]. Oropharyngeal colonization is found in 30–55 % of healthy young adults, and *Candida* may be detected in 40–65 % of normal fecal flora.

Clinical and autopsy studies have confirmed the marked increase in the incidence of disseminated candidiasis, reflecting a parallel increase in the frequency of candidemia. This increase is multifactorial in origin, reflecting an increased recognition as well as a growing population of patients at risk (i.e., patients undergoing complex surgical procedures and those with indwelling vascular devices). The increase in disseminated candidiasis also reflects the improved survival of patients with underlying neoplasms, collagen vascular disease, and immunosuppression. Candidiasis causes more fatalities than any other systemic mycosis.

Early studies observed that in febrile neutropenic patients who die of sepsis, there was a 20–40 % chance of finding evidence of invasive candidiasis at autopsy. Bodey described 21 % of fatal infections in leukemic patients as the result of invasive fungal disease, in contrast with 13 % and 6 % of fatal infections in patients with lymphoma and solid tumors,

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Table 8.1 General patterns of susceptibility of *Candida* species

	Fluconazole	Itraconazole	Flucytosine	Amphotericin B	Voriconazole	Posaconazole	Echinocandins
Candida species							
C. albicans	S	S	S	S	S	S	S
C. tropicalis	S	S	S	S	S	S	S
C. parapsilosis	S	S	S	S	S	S	S (to R)
C. glabrata	S-DD to R	S-DD to R	S	S to I	S to I	S to I	S
C. krusei	R	S-DD to R	I to R	S to I	S to I	S to I	S
C. lusitaniae	S	S	S	S to R	S to I	S to I	S
C. kefyr	S	S	S	S	S	S	S
C. guilliermondii	S	S	S	S to R	S	S	S
C. dubliniensis	S	S	S	S	S	S	S

S susceptible, *R* resistant, *S-DD* susceptible-dose dependent

respectively [9]. Systemic candidiasis has been described in 20–30% of patients undergoing bone marrow transplantation; however, the use of effective antifungal prophylaxis in neutropenic and other high-risk subjects has resulted in reduced occurrence of invasive candidiasis. *Candida* species remain the fourth most commonly isolated pathogens from blood cultures in hospitals [4–8]. A dramatic increase in the incidence of candidemia has occurred in the past four decades, no longer concentrated in oncology and transplantation wards, and now found mainly in non-neutropenic patients. Epidemiologic data indicate that at least 10–12% of all nosocomial infections and 8–15% of all nosocomial bloodstream infections are caused by *Candida*. Rates are now highest among adults older than 65 years.

Candidemia and disseminated candidiasis mortality rates have not improved markedly over the past few years and remain in the 30–40% range, resulting in a serious economic impact [10, 11]. Candidemia is associated with considerable prolongation of the length of hospital stay (70 days vs. 40 days in matched patients) [10–12]. Although mucocutaneous fungal infections such as oral thrush and *Candida* esophagitis are common in acquired immunodeficiency syndrome (AIDS) patients, candidemia and disseminated candidiasis are not.

Within the hospital setting, areas with the highest rates of candidemia include intensive care units (ICUs), surgical units, trauma units, and neonatal ICUs. In fact, 25–50% of all nosocomial candidemia occurs in critical care units. Neutropenic patients, formerly the highest-risk group, are no longer the most vulnerable subpopulation, likely as a result of the widespread use of fluconazole prophylaxis during neutropenia [13]. In some tertiary care centers, *C. albicans* is no longer the most frequent bloodstream isolate, having been replaced by *C. glabrata*, which has replaced *Candida tropicalis* as the most prevalent non-*albicans* species, now causing 3–50% of all candidemias. Non-*albicans Candida* have also become an increasing problem in ICUs, attributed to the more widespread use of fluconazole in this population [14]. In addition to the decline of *C. albicans*, as the dominant blood culture isolate, there is a wide global variation in the predominance of particular species, with *C. tropicalis* common in South America and *C. parapsilosis* common in Europe.[4]

Risk factors for *Candida* bloodstream infections include broad-spectrum antibiotic use, chemotherapy, corticosteroids, intravascular catheters, receipt of total parenteral nutrition (TPN), recent surgery, hospitalization in ICU, malignancy, hemodialysis, neutropenia, and fungal colonization. The most important risk factor for invasive candidiasis is a prolonged stay in the ICU [12].

Pathogenesis And Immunology

Host defects play a significant role in the development of candidal infections [1]. The intact skin constitutes a highly effective, impermeable barrier to *Candida* penetration. Disruption of the skin, from burns, wounds, and ulceration, permits invasion by colonizing opportunistic organisms. Similarly, indwelling intravascular devices provide an efficient conduit that bypasses the skin barrier. The major defense mechanisms operating at the mucosal level to maintain colonization and prevent invasion include normal protective bacterial flora and cell-mediated immunity. The importance of the latter mechanism is highlighted by chronic mucocutaneous candidiasis, a congenital *Candida* antigen-specific deficiency manifested by chronic, intractable, and severe mucocutaneous infection. However, candidemia and disseminated candidiasis are rare in the presence of an intact humoral and phagocytic system.

An effective phagocytic system is the critical defense mechanism that prevents *Candida* deep-tissue invasion, thereby limiting candidemia and preventing dissemination. Polymorphonuclear and monocytic cells are capable of ingesting and killing blastospores and hyphal phases of *Candida*, a process that is enhanced by serum complement and specific immunoglobulins. Severe leukocyte qualitative dysfunction (e.g., chronic granulomatous disease) is associated with disseminated, often life-threatening candidal infections. Myeloperoxidase deficiency also results in increased susceptibility to invasive infection.

Several *Candida* virulence factors contribute to their ability to cause infection, including surface molecules that permit adherence of the organism to other structures (human cells, extracellular matrix, prosthetic devices), acid proteases, phospholipase, and the ability to convert from yeast to hyphal form.

Candidal colonization is at the highest levels in patients at the extremes of age—neonates and adults older than 65 years. Numerous risk factors are associated with increased colonization. Once the colonized mucosal surface is disrupted by chemotherapy or trauma, organisms penetrate the injured areas and gain access to the bloodstream. Although the yeast phase of *Candida* is capable of penetrating intact mucosal cells, the more virulent hyphal phase is more often associated with tissue invasion. Indwelling central venous catheters appear to be a frequent route of bloodstream invasion, accounting for at least 20% of candidemias. Hyperalimentation (TPN) constitutes an independent risk factor. The risk of fungemia is increased with prolonged duration of catheterization, which also increases the risk of local phlebitis, occasionally progressing to suppurative thrombosis. Tunneled catheters (e.g., Hickman and Broviac) are less commonly the source of candidemia, but the intravascular portion may become colonized and infected as the result of candidemia originating from a second independent focus or portal of entry. Fungal invasion from colonized wounds occurs rarely, except in patients with extensive burns. Similarly, the respiratory tract, although frequently colonized, is not a common site for *Candida* invasion, and rarely is a source of dissemination.

Following invasion of the bloodstream, efficient phagocytic cell function rapidly clears the invading organisms, especially when the inoculum is small. More prolonged candidemia is likely in granulocytopenic patients, especially when diagnosis and treatment are delayed. This results in increased risk of hematogenous spread and metastatic seeding of multiple visceral sites, primarily the kidneys, eyes, liver, skin, and central nervous system (CNS). Manifestations of metastatic infection may be apparent immediately or may be delayed several weeks or even months, long after predisposing factors (e.g., granulocytopenia) have resolved.

A third route for bloodstream invasion is persorption via the GI wall, following massive colonization with a high titer of organisms that pass directly into the bloodstream. Candidemia and disseminated candidiasis almost invariably follow serious bacterial infections, especially bacteremia.

Clinical Manifestation

Candida infections can present in a wide spectrum of clinical syndromes, depending on the site of infection and the degree of immunosuppression of the host.

Cutaneous Candidiasis Syndromes

Generalized cutaneous candidiasis manifests as a diffuse eruption over the trunk, thorax, and extremities. Patients have a history of generalized pruritus with increased severity in the genitocrural folds, anal region, axillae, hands, and feet. Physical examination reveals a widespread rash that begins as individual vesicles and spreads into large confluent areas.

Intertrigo affects any site where skin surfaces are in close proximity, providing a warm, moist environment. A red pruritic rash develops, beginning with vesiculopustules, and enlarging to bullae, which then rupture causing maceration and fissuring. The area involved typically has a scalloped border, with a white rim, consisting of necrotic epidermis that surrounds the erythematous macerated base. Satellite lesions are frequently found. These may coalesce and extend into larger lesions. *Candida* folliculitis is predominantly found in hair follicles and rarely becomes extensive. Paronychia and onychomycosis are frequently associated with immersion of the hands in water, especially in patients with diabetes mellitus. These patients usually have a history of a painful and erythematous area around and underneath the nails and nail beds.

Chronic mucocutaneous candidiasis describes a unique group of individuals with *Candida* infections of the skin, hair, nails, and mucous membranes that tend to have a protracted and persistent course. Most infections begin in infancy or the first two decades of life; whereas onset in people older than 30 years is rare. These chronic and recurrent infections frequently result in a disfiguring form called *Candida* granuloma. Most patients survive for long periods and rarely experience disseminated fungal infections. Chronic mucocutaneous candidiasis is frequently associated with multiple endocrinopathies. Examination reveals disfiguring lesions of the face, scalp, hands, and nails occasionally associated with oral thrush and vitiligo.

Oropharyngeal Candidiasis

Oropharyngeal candidiasis (OPC) occurs in association with serious underlying conditions such as diabetes, leukemia, neoplasia, corticosteroid use, antimicrobial therapy, radiation therapy, dentures, and human immunodeficiency virus (HIV) infection. Persistent OPC in infants may be the first manifestation of childhood AIDS or chronic mucocutaneous candidiasis. Samonis et al. reported that 28% of cancer patients not receiving antifungal prophylaxis developed OPC [15]. In a similar immunocompromised, hospitalized population, Yeo et al. observed OPC in 57% of patients [16].

In the past, approximately 80–90% of patients with HIV infection developed OPC at some stage of their disease. The presence of OPC should alert the physician to the possibility of underlying HIV infection. Untreated, 60% of

HIV-infected patients develop an AIDS-related infection or Kaposi's sarcoma within 2 years of the appearance of OPC. Many AIDS patients experience recurrent episodes of OPC and esophageal candidiasis as HIV progresses, and multiple courses of antifungals administered may contribute to the development of antifungal resistance. Antifungal agents are less effective and take longer to achieve a clinical response in HIV-positive patients than in cancer patients. There has been a significant increase in the incidence of non-*albicans Candida* recovered from HIV-positive patients.

C. albicans remains the most common species responsible for OPC (80–90%). *C. albicans* adheres better in vitro to epithelial cells than non-*albicans Candida*.

The manifestations of OPC (commonly called thrush) vary significantly, from none to a sore, painful mouth, burning tongue, and dysphagia. Frequently, patients with severe objective (examination) changes are asymptomatic. Clinical signs include a diffuse erythema with white patches (pseudomembranous) that appear as discrete lesions on the surfaces of the mucosa, throat, tongue, and gums. With some difficulty, the plaques can be wiped off, revealing a raw, erythematous, and sometimes bleeding base. OPC impairs quality of life and results in a reduction in fluid or food intake. The most serious complication of untreated OPC is extension to the esophagus. Fungemia and disseminated candidiasis are uncommon.

Chronic atrophic stomatitis or denture stomatitis is a very common form of OPC, with soreness and burning of the mouth. Characteristic signs are chronic erythema and edema of the portion of the palate that comes into contact with dentures. Denture stomatitis is found in 24–60% of denture wearers and is more frequent in women than in men. Notably, *C. glabrata* has been identified in 15–30% of all cultures, a higher prevalence than generally found in the mouth. Angular cheilitis (perlèche), also called cheilosis, is characterized by soreness, erythema, and fissuring at the corners of the mouth. Chronic hyperplastic candidiasis (*Candida* leukoplakia) produces oral white patches, or leukoplakia, that are discrete, transparent-to-whitish, raised lesions of variable sizes found on the inner surface of the cheeks and, less frequently, on the tongue. Midline glossitis (median rhomboid glossitis, acute atrophic stomatitis) refers to symmetrical lesions of the center dorsum of the tongue characterized by loss of papillae and erythema.

Esophageal Candidiasis

Candida esophagitis occurs in predisposed individuals. *C. albicans* is the most common cause. The prevalence of *Candida* esophagitis increased during the first two decades of the AIDS epidemic and with increased numbers of transplant, cancer, and severely immunocompromised patients.

Esophageal candidiasis in an HIV-infected patient may be the first manifestation of AIDS. *Candida* esophagitis tends to occur later in the natural history of HIV infection and almost invariably at a much lower CD4 count. In cancer patients, factors predisposing to esophagitis include recent exposure to radiation, cytotoxic chemotherapy, antibiotic and corticosteroid therapy, and neutropenia. Clinical features include dysphagia, odynophagia, and retrosternal pain. Constitutional findings, including fever, occur only occasionally. Rarely, epigastric pain is the dominant symptom. Although esophagitis may occur as an extension of OPC, in more than two thirds of published reports, the esophagus was the only site involved; more often, infection involved the distal two thirds of the esophagus. *Candida* esophagitis in AIDS patients may occur in the absence of symptoms despite extensive objective esophageal involvement. Kodsi classified *Candida* esophagitis on the basis of its endoscopic appearance [17]. Type I cases refer to a few white or beige plaques up to 2 mm in diameter. Type II plaques are larger and more numerous. In the milder grades, plaques may be hyperemic or edematous, but there is no ulceration. Type III plaques may be confluent, linear, nodular, and elevated, with hyperemia and frank ulceration, and type IV plaques, additionally, have increased friability of the mucosa and occasional narrowing of the lumen. Uncommon complications of esophagitis include perforation, aortic-esophageal fistula formation, and rarely, candidemia or bacteremia.

A reliable diagnosis can only be made by histologic evidence of tissue invasion in biopsy material. Nevertheless, antifungal therapy is frequently initiated empirically with minimal criteria in a high-risk patient. The mere presence of *Candida* within an esophageal lesion as established by brushings, smear, or culture, does not provide sufficient evidence to distinguish *Candida* as a commensal from *Candida* as the responsible invasive pathogen.

Radiographic studies have been replaced by endoscopy, which not only provides a rapid and highly sensitive diagnosis but also is the only reliable method of differentiating among the various causes of esophagitis. The characteristic endoscopic appearance is described as yellow–white plaques on an erythematous background, with varying degrees of ulceration. Differential diagnosis includes radiation esophagitis, reflux esophagitis, cytomegalovirus or herpes simplex virus infection. In the AIDS patient, it is not uncommon to identify more than one etiologic agent causing esophagitis.

Respiratory Tract Candidiasis

Laryngeal candidiasis is seen primarily in HIV-infected patients and occasionally in those with hematologic malignancies. The patient presents with a sore throat and hoarseness, and the diagnosis is made by direct or indirect laryngoscopy.

Candida tracheobronchitis is a rare form of candidiasis seen in HIV-positive or severely immunocompromised subjects, complaining of fever, productive cough, and shortness of breath. Physical examination reveals dyspnea and scattered rhonchi. The diagnosis generally is made during bronchoscopy.

Candida pneumonia is also a rare form of candidiasis. The most common form of infection appears to be multiple lung abscesses due to the hematogenous dissemination of *Candida*. As there may be a high degree of colonization and isolation of *Candida* from the upper respiratory tract, diagnosis requires the visualization of *Candida* invasion on histopathology. Patient history usually reveals similar risk factors for disseminated candidiasis, and patients complain of shortness of breath, cough, and fever. Sputum or endotracheal secretions positive for *Candida* usually indicate upper respiratory tract colonization, have low-predictive value for pneumonia, and unfortunately are incorrectly used as a pretext for initiating antifungal therapy.

Vulvovaginal Candidiasis

In the USA, *Candida* vaginitis is the second most common vaginal infection. During the childbearing years, 75% of women experience at least one episode of vulvovaginal candidiasis (VVC), and 40–50% of these women experience a second episode. A small subpopulation of women experiences repeated, recurrent episodes of *Candida* vaginitis. *Candida* may be isolated from the genital tract of about 10–20% of asymptomatic, healthy women of childbearing age.

Candida vaginitis can be classified as complicated or uncomplicated, depending on factors such as severity and frequency of infection and the causative *Candida* species (Table 8.2). Increased rates of asymptomatic vaginal colonization with *Candida* and *Candida* vaginitis are seen in pregnancy (30–40%), with the use of oral contraceptives with a high-estrogen content, and in uncontrolled diabetes mellitus. The hormonal dependence of the infection is illustrated by the fact that *Candida* is seldom isolated from premenarchal girls, and the prevalence of *Candida* vaginitis is lower after menopause, except in women taking hormone replacement therapy. Other factors include corticosteroid and antimicrobial therapy, the use of an intrauterine device, high frequency of coitus, and refined-sugar eating binges. Recently, an increased frequency of mycotic vulvovaginal infections in type 2 diabetics receiving sodium glucose cotransporter 2 (SGLT2) inhibitors especially non-*albicans Candida* spp. has been reported.

Vulvar pruritus is the most common symptom of VVC and is present in most symptomatic patients. Vaginal discharge is often minimal and occasionally absent. Although described as being typically "cottage cheese like" in character, the discharge may vary from watery to homogeneously thick. Vaginal soreness, irritation, vulvar burning, dyspareunia, and external dysuria are common. Malodorous discharge is characteristically absent. Typically, symptoms are exacerbated during the week before menses, while the onset of menstrual flow frequently brings some relief.

Table 8.2 Classification of *Candida* vaginitis

	Uncomplicated (90%)	Complicated (10%)
Severity	Mild or moderate	Severe
Frequency	Sporadic	Recurrent
Organism	*Candida albicans*	Non-*albicans* species of *Candida*
Host	Normal	Abnormal (e.g., uncontrolled diabetes mellitus)

Examination reveals erythema and swelling of the labia and vulva, often with discrete pustulopapular peripheral lesions. The cervix is normal. Vaginal mucosal erythema with adherent whitish discharge is typically present.

In most symptomatic patients, VVC is readily diagnosed by microscopic examination of vaginal secretions. A wet mount of saline preparation has a sensitivity of only 40–60%. A 10% potassium hydroxide preparation (KOH) is more sensitive in diagnosing the presence of budding yeast. Patients with *Candida* vaginitis have a normal vaginal pH (4.0 to 4.5). A pH of more than 4.5 suggests bacterial vaginosis, trichomoniasis, or mixed infection. Routine cultures are unnecessary; but in suspicious cases with negative microscopy cases, vaginal culture should be performed. Although vaginal culture is the most sensitive method available for detecting *Candida*, a positive culture does not necessarily indicate that *Candida* is responsible for the vaginal symptoms.

Urinary Tract Candidiasis

Candiduria is rare in, otherwise, healthy people. Although epidemiologic studies have documented candiduria in approximately 10% of individuals sampled, many of these culture results reverted to negative when a clean-catch technique was used. The incidence of fungal urinary tract infections (UTIs), specifically candiduria, has dramatically increased recently, especially among patients with indwelling urinary catheters.

Platt et al. reported that 26.5% of all UTIs related to indwelling catheters were caused by fungi. *Candida* are the organisms most frequently isolated from the urine samples of patients in surgical ICUs, and 10–15% of nosocomial UTIs are caused by *Candida* [18, 19].

Diabetes mellitus may predispose patients to candiduria by enhancing *Candida* colonization of the vulvovestibular area (in women), by enhancing urinary fungal growth in the

presence of glycosuria, lowering host resistance to invasion by fungi as a consequence of impaired phagocytic activity, and promoting stasis of urine in those with neurogenic bladder.

Antibiotics also increase colonization of the GI tract by *Candida*, which are normally present in ~30% of immunocompetent adults. In patients receiving antibiotics, colonization rates approach 100%. Candiduria is almost invariably preceded by bacteriuria. Indwelling urinary catheters serve as a portal of entry for microorganisms into the urinary drainage system. Other risk factors include the extremes of age, female sex, use of immunosuppressive agents, venous catheters, interruption of urine flow, radiation therapy, and genitourinary tuberculosis.

In a large multicenter study by Kauffman et al., *C. albicans* was found in 51.8% of 861 patients with funguria. The second most common pathogen (134 patients) was *C. glabrata* [20]. Other non-*albicans Candida* are also very common and far more prevalent than in other sites (i.e., oropharynx and vagina), possibly as a function of urine composition and pH selectivity for non-*albicans* species. In approximately 10% of patients, more than one species of *Candida* are found simultaneously.

Ascending infection is, by far, the most common route for infection of the bladder. It occurs more often in women because of a shorter urethra and frequent vulvovestibular colonization with *Candida* (10–35%). Ascending infection that originates in the bladder can infrequently lead to infection of the upper urinary tract, especially if vesicoureteral reflux or obstruction of urinary flow occurs. This may eventually result in acute pyelonephritis and, rarely, candidemia. A fungus ball consisting of yeast, hyphal elements, epithelial and inflammatory cells, and, sometimes, renal medullary tissue, secondary to papillary necrosis, may complicate ascending or descending infections.

Hematogenous spread is the most common route for renal infection (i.e., renal candidiasis). *Candida* have a tropism for the kidneys; one study revealed that 90% of patients with fatal disseminated candidiasis had renal involvement at autopsy. Frequently, when renal candidiasis is suspected, blood cultures are no longer positive. Patients with renal candidiasis usually have no urinary tract symptoms.

The finding of *Candida* organisms in the urine may represent contamination, colonization of the drainage device, or infection. Contamination of a urine specimen is common, especially with suboptimal urine collection from a catheterized patient or from a woman who has heavy yeast colonization of the vulvovestibular area. Given the capacity of yeast to grow in urine, small numbers of yeast cells that migrate into the collected urine sample may multiply quickly. Therefore, high colony counts could be the result of yeast contamination or colonization. Colonization usually refers to the asymptomatic adherence and settlement of yeast, usually on drainage catheters or other foreign bodies in the urinary tract (i.e., stents and nephrostomy tubes), and it may result in a high concentration of the organisms on urine culture. Simply culturing the organism does not imply clinical significance, regardless of the concentration of organisms in the urine. In the asymptomatic patient, candiduria almost always represents colonization, and elimination of underlying risk factors, such as indwelling catheter, is frequently adequate to eradicate candiduria [20]. Diagnostic tests on urine often are not helpful in differentiating colonization from infection or identifying site of infection.

Infection is caused by superficial or deep-tissue invasion. Kozinn showed that colony counts of $>10^4$ colony-forming units (cfu)/ml of urine were associated with infection in patients without indwelling urinary catheters, although clinically significant renal candidiasis has been reported with colony counts of 10^3 cfu/ml of urine [21]. Pyuria supports the diagnosis of infection in patients with a urinary catheter, but can result from mechanical injury of the bladder mucosa by the catheter or from coexistent bacteriuria. In summary, absence of pyuria and low colony counts tend to rule out *Candida* infection, but the low specificity of pyuria and counts $>10^3$ cfu/ml require that results be interpreted in their clinical context. The number of yeast cells in urine has little value in localizing the anatomical level of infection. Rarely, a granular cast containing *Candida* hyphal elements is found in urine, allowing localization of the infection to the renal parenchyma. Declining renal function suggests urinary obstruction or renal invasion. For candiduria patients with sepsis, it is not only necessary to obtain blood cultures but also, given the frequency with which obstruction and stasis coexist, essential to perform radiographic visualization of the upper tract. Any febrile patient for whom therapy for candiduria is considered necessary should be investigated for the anatomic source of candiduria. In contrast, patients without sepsis require no additional studies unless candiduria persists after the removal of catheters.

Candiduria is most often asymptomatic, usually in hospitalized or nursing home patients with indwelling catheters. These patients usually show none of the signs or symptoms associated with UTI. Symptomatic *Candida* cystitis is uncommon. Cystoscopy, although rarely indicated, reveals soft, pearly white, elevated patches with friable mucosa underneath and hyperemia of the bladder mucosa. Emphysematous cystitis is a rare complication of lower UTI, as is prostatic abscess and epididymal orchitis.

Upper UTIs present with fever, leukocytosis, and costovertebral angle tenderness, indistinguishable from bacterial pyelonephritis and urosepsis. Ascending infection almost invariably occurs in the presence of urinary obstruction and stasis, especially in patients with diabetes or nephrolithiasis.

A major complication of upper UTI is obstruction caused by fungus balls (bezoars), which can be visualized on

ultrasonography. Renal colic may occur with the passage of fungal "stones," which are actually portions of these fungus balls.

Patients with hematogenous seeding of the kidneys caused by candidemia may present with high fever, hemodynamic instability, and variable renal insufficiency. Blood culture results are positive for *Candida* in half of these patients. Retinal or skin involvement may suggest dissemination, but candiduria and a decline in renal function are often the only clues to systemic candidiasis in a febrile, high-risk patient.

Abdominal Candidiasis, Including Peritonitis

Candida infection has been increasingly recognized as a cause of abdominal sepsis and is associated with a high mortality. Peritoneal contamination with *Candida* follows either spontaneous GI perforation or surgical opening of the gut. However, after contaminating the peritoneal cavity, *Candida* organisms do not inevitably result in peritonitis and clinical infection. Risk factors for peritonitis include recent or concomitant antimicrobial therapy, inoculum size, and acute pancreatitis. Translocation of *Candida* across the intact intestinal mucosa has been shown experimentally in animals and in a volunteer. Additional risk factors for invasive candidiasis include diabetes, malnutrition, ischemia, hyperalimentation, neoplasia, and multiple abdominal surgeries. Pancreatic transplantation, especially with enteric drainage, is associated with intra-abdominal *Candida* abscess formation. *Candida* have a unique affinity for the inflamed pancreas, resulting in intrapancreatic abscesses or infecting accompanying pseudocysts. In *Candida* peritonitis, *Candida* usually remain localized to the peritoneal cavity, with the incidence of dissemination at about 25%.

The clinical significance of *Candida* isolated from the peritoneal cavity during or after surgery has been controversial. Several earlier studies concluded that a positive culture did not require antifungal therapy. Calandra et al., in a review of *Candida* isolates from the peritoneal cavity, determined that *Candida* caused intra-abdominal infection in 19 of 49 (39%) patients [22]. In 61% of patients, *Candida* isolation occurred without signs of peritonitis. Accordingly, in each patient, clinicians should consider the clinical signs of infection and other risk factors when deciding whether to initiate antifungal therapy.

Candida peritonitis as a complication of continuous ambulatory peritoneal dialysis (CAPD) is more common, but it infrequently results in positive blood cultures or hematogenous dissemination. In a series of CAPD patients followed for 5 years, fungal peritonitis, most commonly due to *Candida*, accounted for 7% of episodes of peritonitis. Seventeen cases of fungal peritonitis were reported, with eight associated deaths. Few risk factors have emerged except for recent hospitalization, previous episodes of peritonitis, and antibacterial therapy. Clinically, fungal peritonitis cannot be differentiated from bacterial peritonitis except by Gram stain and culture of dialysate.

Yeast in the bile is not uncommon, especially after biliary surgery, and has the same significance as asymptomatic bactibilia (i.e., colonization only); however, *Candida* is an infrequent cause of cholecystitis and cholangitis. Other risk factors include diabetes, immunosuppression, abdominal malignancy, and the use of biliary stents. Biliary infection is usually polymicrobial, and *Candida* is a pathogen that should not be ignored when isolated.

Candida Osteomyelitis and Arthritis

Although previously rare, *Candida* osteomyelitis is now not uncommon, usually as the result of hematogenous dissemination, with seeding of long bones in children and the axial skeleton in adults. Sites of bone infection include the spine (vertebral and intravertebral disk), wrist, femur, humerus, and costochondral junctions.

Osteomyelitis may present weeks or months after the causal candidemic episode; therefore, at presentation, blood cultures are usually negative and radiologic findings nonspecific. Diagnosis usually requires a bone biopsy.

Occasionally, postoperative wound infections may spread to contiguous bone such as the sternum and vertebrae. Regardless of the source, manifestations resemble bacterial infection, but run a more insidious course, with a significant delay in diagnosis.

Candida arthritis generally represents a complication of hematogenous candidiasis and rarely follows local trauma, surgery, or intra-articular injections. Patients with underlying joint disease (e.g., rheumatoid arthritis, prosthetic joints) are at increased risk. *Candida* arthritis can occur in any joint, is usually monoarticular (knee), but has been reported to effect multiple joints in up to 25% of cases. Infection resembles bacterial septic arthritis, but chronic infection often develops with secondary bone involvement because of the delay in diagnosis and suboptimal treatment.

Candidemia and Disseminated Candidiasis

Clinical presentation of candidemia varies from fever alone and absence of any organ-specific manifestations to a wide spectrum of manifestations, including fulminant sepsis. Accordingly, acute candidemia is indistinguishable from bacterial sepsis and septic shock. In general, there are no specific clinical features associated with individual *Candida* species.

Candidemia may also present with manifestations of systemic and invasive metastatic candidiasis; although when these occur, blood cultures have frequently become negative. Accordingly, candidemia is a marker, although insensitive, of deep invasive candidiasis. Only 50 % of patients with disseminated candidiasis will have positive blood cultures, and an antemortem diagnosis is even lower (15–40 %). Dissemination to multiple organs may not only occur with candidemia, especially to the kidneys, eyes, brain, myocardium, liver, and spleen in leukemia patients, but infection can also involve the lungs, skin, vertebral column, and endocardium.

The possibility of asymptomatic disseminated infection drives the treatment principles of candidemia. Transient candidemia can occur from any source, but most often follows intravascular catheter infection, with prompt resolution of candidemia following catheter removal. Prolonged candidemia, especially when blood cultures remain persistently positive on appropriate antifungal treatment, suggests a persistent focus or source (e.g., intravascular catheter, abscess, suppurative thrombophlebitis, endocarditis, severe neutropenia), or antifungal resistance, which albeit rare, is more common with some of the non-*albicans Candida*. When candidemia is diagnosed, a general physical examination rarely reveals clinical signs of dissemination; but a thorough examination, including a dilated funduscopic examination, is mandatory. The crude mortality rate reported in patients with candidemia ranges from 40–60 %, with an attributable mortality of 38 %, exceeding that of most bacteremias. A 50 % reduction in national mortality rates for invasive candidiasis since 1989 was reported after a steady increase in mortality in the previous decades, reaching 0.62 death/100,000 population. The decrease in mortality, despite increased invasive disease, may be related to increased awareness, earlier diagnosis, and increased therapeutic options, primarily fluconazole and echinocandins.

Ocular Candidiasis

Candida organisms especially *C. albicans* gain access to the eye by one of two routes: Direct inoculation during eye surgery or trauma, or as the result of hematogenous spread (endogenous) causing vitritis or chorioretinitis. Once endophthalmitis occurs, therapy, especially if delayed, is often insufficient to prevent blindness. Given the recent increased incidence of nosocomial candidemia, a parallel increase in endophthalmitis has occurred. Endophthalmitis should raise the suspicion of concomitant, widely disseminated candidiasis. Estimates of the incidence of eye involvement during candidemia have been as high as 37 %, but recent studies indicate a reduced rate of less than 10 %. Only half of patients diagnosed with endophthalmitis have a history of recent candidemia.

Symptoms of chorioretinitis vary, may be absent in patients too ill to complain, and include visual blurring, floaters, scotomata, and blindness. Funduscopic examination reveals white, cotton ball-like lesions situated in the chorioretinal layer that may rapidly progress to extend into the posterior vitreous. Indirect ophthalmoscopy with pupillary dilation is necessary to achieve complete visualization. For the lesions to be visible, they require the presence of leukocytes; thus, in the presence of neutropenia, ocular lesions may be absent.

Cardiac and Endovascular Candidiasis

Candida myocarditis is the result of hematogenous dissemination with formation of microabscesses within the myocardium usually detected only on autopsy. Studies in the pre-antifungal drug era reported that 62 % of 50 patients with disseminated candidiasis had myocardial involvement at autopsy.

Candida may reach the pericardium from adjacent endocarditis or myocarditis, but pericardial involvement is most often the result of hematogenous seeding or direct inoculation during cardiac surgery. Pericarditis is purulent in nature, resembles bacterial infection, and may be complicated by constrictive pericarditis.

The advent of prosthetic cardiac valve replacement surgery and the increase in intravenous (IV) drug abuse have resulted in a dramatic increase in the incidence of *Candida* endocarditis, which previously had been rare. Fungal endocarditis is responsible for < 10 % of all cases of infective endocarditis.

Prosthetic valve endocarditis (PVE), following prosthetic valve surgery, remains the most common form of *Candida* endocarditis (> 50 %). Most episodes occur within 2 months of surgery, though endocarditis can also occur much later (> 12 months). Specific risk factors for PVE include complicated surgery, antibiotics, prolonged postoperative use of catheters, and candidemia, even if transient. Non-*albicans Candida* species are increasingly responsible for PVE, especially *C. parapsilosis*. Damaged endocardium and prosthetic material, especially suture lines, serve as foci for *Candida* adherence. Pacemaker-associated endocarditis from *Candida* has also been reported.

Clinical findings and complications in *Candida* endocarditis are similar to those seen in bacterial endocarditis, with the exceptions of increased frequency of large vegetations and large emboli to major vessels. Aortic and mitral valve involvement is the most common. The higher incidence of embolization is frequently manifested as focal and global neurologic deficits. Some studies have found a reduced incidence of cardiac failure, changing heart murmurs, and splenomegaly. PVE may recur several years after a putative cure with medical therapy, so long-term follow up is necessary.

Most patients with *Candida* endocarditis have positive blood cultures. Improved diagnosis is the result of greater awareness of the significance of candidemia, newer blood culture techniques, and echocardiography. Visualizing large vegetations via echocardiogram in patients with negative blood culture is strong circumstantial evidence of *Candida* endocarditis. Mycologic examination including culture and histopathology should be performed on all surgically removed emboli.

Candida endocarditis mortality remains high. Before cardiac surgery was available, mortality exceeded 90 %. With combined treatment, using surgery and aggressive antifungal therapy, mortality rates of ~45 % are now typical.

Phlebitis due to *Candida* is common and often associated with subcutaneous catheters. Delay in treatment often results in extensive vascular thrombosis and suppuration. Prolonged candidemia, despite adequate antifungal treatment, is not uncommon. Venous thrombi, even after removal of responsible catheters, impair drug penetration and contain persistent microabscesses, with resultant prolonged candidemia. Surgical excision of thrombi is often required in addition to prolonged antifungal therapy. Complications include superior vena cava obstruction, tricuspid valve endocarditis, right-sided mural endocarditis, and pulmonary vein thrombosis.

Central Nervous Systemic Candidiasis

CNS candidal infections usually occur as a manifestation of acute disseminated candidiasis or as a complication of a neurosurgical procedure especially involving an intracranial device. The most common presentation is that of meningitis or multiple small abscesses throughout the brain; however, large solitary brain and epidural abscesses have been described. CNS involvement is more commonly seen at the extremes of life, low-birth-weight neonates are particularly vulnerable to metastatic CNS involvement secondary to candidemia.

Chronic Disseminated Candidiasis

Hepatosplenic candidiasis (HSC) is a chronic form of disseminated candidiasis that develops as a complication of invasive candidiasis during granulocytopenia. Many now prefer the term chronic systemic or disseminated candidiasis (CDC) because other organs (eyes, skin, soft tissue) may also be involved [23]. In the past two decades, reports of HSC have increased, probably as a result of improved diagnostic imaging and increased rates of candidemia. Candidemia, although frequently secondary to intravascular catheter infection, generally follows *Candida* colonization of the gut, together with disruption of the GI mucosa, the organism reaches the submucosal blood vessels that drain into the portal venous system and into the liver, where focal lesions are established. Thus, many patients with CDC have no history of documented candidemia. As patients recover from neutropenia, the lesions that were established during the neutropenic phase become more prominent, especially in the liver, spleen, and kidneys.

Clinically, most patients have a history of a hematologic malignancy, cytotoxic chemotherapy, or recent recovery from neutropenia, during which time they were febrile and received antibacterial therapy. Upon recovery from neutropenia, symptoms of antibiotic-resistant fever and abdominal pain begin and worsen as the neutrophils infiltrate foci of *Candida* in the liver and spleen. Serum alkaline phosphatase increases, paralleling the increase in leukocytes, although hepatic transaminases are not invariably elevated.

Diagnosis

In superficial candidiasis, wet-mount smears use scrapings or smears from skin, nails, oral or vaginal mucosa examined under the microscope to identify hyphae, pseudohyphae, or budding yeast cells. Potassium hydroxide smear, Gram stain, or methylene blue stain may help directly demonstrate fungal cells. For diagnosis of invasive candidiasis, laboratory studies are nonspecific and lack sensitivity. Clinicians are required to act definitively based on a high index of suspicion. In the past, many patients with life-threatening candidiasis died without receiving antifungal therapy. For therapy to be effective, clinicians must act early, often empirically in patients who are febrile and at risk.

In candidemia and disseminated candidiasis, blood cultures are helpful, but they are positive in only 40–60 % of cases of disseminated disease. Urinalysis may be helpful and may be indicative of colonization or renal candidiasis. Nonculture, antigen-based diagnostic assays are not available in the USA. Occasionally, blood cultures obtained via central catheters may indicate contamination. Nevertheless, febrile patients with a single positive blood culture for *Candida* should always initially be considered to have a proven infection. Given the low sensitivity of blood cultures, as well as the lack of an adequate test for the diagnosis of invasive candidiasis, detection of hematogenous dissemination remains poor.

Cultures of nonsterile sites, although not useful in establishing a diagnosis, may demonstrate high degrees of candidal colonization. This may be useful in deciding whether to initiate antifungal therapy in patients with fever unresponsive to broad-spectrum antimicrobials. Positive blood cultures and cultures from sterile sites, on the other hand, are indicative of definite infection using established international definitions of European Organization for Research

Table 8.3 In vitro susceptibility of *Candida* species to azole antifungal agents[a]

	Fluconazole	Voriconazole	Itraconazole	Posaconazole
Candida species	(MIC_{50})	(MIC_{50})	(MIC_{50})	(MIC_{50})
C. albicans	1	0.06	0.5	0.13
C. tropicalis	1	2	1	1
C. glabrata	16	0.5	0.25	1
C. parapsilosis	1	0.06	0.13	0.13
C. krusei	64	1	0.5	0.5
C. lusitaniae	2	0.06	0.25	0.13

*MIC*50 median minimum inhibitory concentration, (μg/ml)

[a]Based on 2047 blood culture isolates collected from January 1997 through December 2000. Susceptibilities were calculated on the basis of National Committee for Clinical Laboratory Standards (NCCLS) methodology. Pfaller et al. [25].

and Treatment—Mycoses Study Group EORTC/MSG organization. Lesions of CDC may be detected by imaging techniques such as computerized tomography (CT) scan, ultrasonography, and magnetic resonance imaging (MRI). The characteristic "bull's-eye" lesions seen on ultrasound and/or CT are not detectable until neutrophil recovery. However, the lesions are not specific for CDC as they resolve during therapy, they may either disappear completely or calcify. Ultrasonography appears to be less sensitive but possibly more specific than CT scanning in demonstrating these lesions; however, MRI appears to be the most sensitive imaging tool. Diagnosis may be confirmed by histopathologic examination and culture of hepatic tissue obtained by either percutaneous biopsy or laparoscopy. The appearance of hyphae in a granulomatous lesion is itself not specific for *Candida* and may be caused by other fungi such as *Trichosporon*, *Fusarium*, and *Aspergillus*. Additionally, metastatic tumors may simulate the appearance of CDC. Often biopsy-visible organisms do not grow in culture, leading some investigators to propose that CDC might reflect immune reconstitution only. Diagnosis of *Candida* endophthalmitis is usually made on the basis of clinical context and characteristic funduscopic picture. Aspiration of the anterior chamber is justified, but often culture-negative; vitrectomy is often helpful. Polymerase chain reaction (PCR) studies on the aspirate may prove the presence of *Candida*.

Species identification of *Candida* is critically important because of the increase in non-*albicans Candida* infections. CHROMagar *Candida* media allows for the presumptive identification of several *Candida* species by using enzymatic reactions in specialized media that produce differing colony colors. Several biochemical assays, usually based upon fermentation reactions, can be used to identify the different *Candida* species with more accuracy. Assays that evaluate the assimilation of a number of carbon substrates and generate profiles are used in the identification of different fungal species. Once colonies are present on an agar plate or blood culture growth is recognized, significantly more rapid identification of *Candida* species, taking only 2 h, is now possible using peptide nucleic acid fluorescence in situ hybridization (PNA Fish) allowing more rapid acid fluorescence in situ hybridization (PNA Fish) allowing more rapid administration of appropriate antifungals.

Serum levels of beta-1,3-glucan are useful as an aid to the diagnosis of invasive candidiasis but is not widely used or always timely available. Sensitivity for *Candida* infections of greater than 80% has been reported. The test often provides a positive test days before clinical signs and symptoms appear, allowing earlier initiation of therapy [24]. A negative test seems to have good negative predictive value. False positive results may occur with antibiotics, surgical gauze, hemodialysis or use of IV immunoglobulins. Although not yet commercially available, a new PCR test may be useful in febrile patients, culture negative with disseminated or invasive candidiasis [25].

The Clinical and Laboratory Standards Institute CLSI (formerly NCCLS) microbroth dilution methodology has standardized antifungal susceptibility testing for *Candida* species. Although not used as a standard of care, it may be helpful in guiding difficult therapeutic decisions. Most of these difficult decisions involve antifungal therapy of refractory oral or esophageal candidiasis in patients with AIDS or of patients with persistent candidemia, including infective endocarditis.

Management

Treatment of *Candida* infections varies considerably and is based on the anatomic location of the infection, the patient's underlying disease and immune status, the patient's risk factors for infection as well as the species of *Candida* responsible for infection, and, in some cases, the susceptibility of the strain to antifungal drugs (Tables 8.1, 8.3, 8.4, and 8.5). In 2009, the Infectious Diseases Society of America published updated practice guidelines for the treatment of candidiasis [26].

Azoles have become the mainstay of therapy, including many topical and systemic agents. Polyenes include amphotericin B, lipid-based amphotericin B formulations, and topical nystatin. The echinocandin class of antifungals has excellent fungicidal activity against *Candida* species.

Table 8.4 In vitro susceptibility of *Candida* species to other antifungal agents[a]

	Amphotericin B[b]	Flucytosine[b]	Caspofungin[c]	Anidulafungin[c]	Micafungin[c]
Candida species	(MIC_{50})	(MIC_{50})	(MIC_{50})	(MIC_{50})	(MIC_{50})
C. albicans	0.5	<0.25	0.5	0.03	0.03
C. tropicalis	0.25	<0.25	1	0.06	0.06
C. glabrata	0.5	<0.25	2	0.06	0.06
C. parapsilosis	0.25	<0.25	2	2	2
C. krusei	0.25	16	2	0.25	0.13
C. lusitaniae	>1	<0.25	1	1	0.25

*MIC*50 median minimum inhibitory concentration, (μg/mL)
[a]Susceptibilities were calculated on the basis of National Committee for Clinical Laboratory Standards (NCCLS) methodology
[b]Pfaller et al. [31]
[c]Ostrosky-Zeichner L et al. [3]

Table 8.5 Clinical break points for common *Candida* species (Data from Pfaller MA et al. [35])

Organism	Clinical Break points (in mcg/mL)			
	Susceptible	Susceptible dose dependent	Intermediate	Resistant
Candida albicans				
Caspofungin	<0.25	–	0.5	>1
Anidulafungin	<0.25	–	0.5	>1
Micafungin	<0.25	–	0.5	>1
Fluconazole	<2.0	4.0	–	>8
Itraconazole	<0.12	0.25–0.5	–	>1
Voriconazole	<0.12	–	0.25–0.5	>1
Candida parapsilosis				
Caspofungin	<2	–	4	>8
Anidulafungin	<2	–	4	>8
Micafungin	<2	–	4	>8
Fluconazole	<2	4.0	–	>8
Voriconazole	<0.12	–	0.25–0.5	>1
Candida tropicalis				
Caspofungin	<0.25	–	0.5	>1
Anidulafungin	<0.25	–	0.5	>1
Micafungin	<0.25	–	0.5	>1
Fluconazole	<2	4.0	–	>8
Voriconazole	<0.12	–	0.25–0.5	>1
Candida glabrata				
Caspofungin	<0.12	–	0.25	>0.5
Anidulafungin	<0.12	–	0.25	>0.5
Micafungin	<0.06	–	0.12	>0.25
Fluconazole		<32	–	>64
Candida krusei				
Caspofungin	<0.25	–	0.5	>1
Anidulafungin	<0.25	–	0.5	>1
Micafungin	<0.25	–	0.5	>1
Fluconazole[a]	–	–	–	–
Voriconazole	<0.5	–	1	>2
Candida guilliermondii				
Caspofungin	<2	–	4	>8
Anidulafungin	<2	–	4	>8
Micafungin	<2	–	4	>8

24 h 100%, MIC endpoints read as 100% inhibition at 24-h incubation; 24 hr 50%, MIC endpoints read as 50% inhibition at 24-h incubation
[a]Fluconazole break points are not available for *C. krusei* since this species is considered intrinsically resistant to this compound. All strains should be reported as resistant

Table 8.6 Oropharyngeal and esophageal candidiasis treatment options

Drug/formulation	Dose	Comments
Nystatin		
Pastilles or lozenge	200,000 U qid[a]	Unpleasant taste; may cause nausea and gastrointestinal disturbances
Suspension	500,000 U by swish and swallow qid	
Vaginal tablet	100,000 U dissolve 1 tablet tid	Vaginal tablets in combination with unsweetened mints or chewing gum better tolerated; not recommended for esophagitis
Clotrimazole		
Troches	Dissolve in mouth five times each day	More palatable than nystatin but contains dextrose, which may promote dental caries; not recommended for esophagitis
Miconazole		
Mucoadhesive buccal tablet	50 mg, apply to gum once daily	Adheres to gum above incisor tooth; convenient once daily but expensive
Fluconazole		
Oral suspension or tablet	100 mg/day; loading dose of 200 mg for severe disease	Superior to nystatin, clotrimazole, ketoconazole. High doses (up to 800 mg/day) can be used in difficult cases. Success has been obtained even in cases with in vitro resistance
Itraconazole		
Solution	200 mg (20 ml) by swish and swallow daily	Solution has been tested only among HIV patients, but is much better absorbed and has shown efficacy equivalent to that of fluconazole
Capsule	200 mg/d (with food) x 14–28 days	Limited bioavailability; absorption improved if taken with fatty meal
Posaconazole		
Tablet	400-mg bid, then 400-mg daily. Esophagitis 400-mg bid	Oral solution availability but tablet preferred
Amphotericin B		
Suspension	1 ml (1 mg/ml) swish and swallow qid	Agent considered second-line option; reserved for severe cases and documented failures to azoles; parenteral dosing necessary for esophagitis
Lozenge	100 mg qid	
Tablet	10 mg qid	
Parenteral	0.4–0.6 mg/kg/day IV	

U units, *qid* four times daily, *tid* three times daily, *bid* two times daily, *IV* intravenously

[a]All given for 7–14 days for oropharyngeal candidiasis and up to 21 days for esophageal candidiasis orally, unless otherwise stated

In 2007, the CLSI proposed Clinical interpretive break points (CBPs) for minimal inhibitory concentration testing (MIC) for echinocandins but with time the designated CBP of <2 μg/mL were deemed too high (a) failing to reliably identify isolates with resistance mechanisms associated with treatment failure.

Cutaneous Candidiasis

Most localized, cutaneous candidiasis infections can be treated with topical antifungal agents, such as clotrimazole, econazole, ciclopirox, miconazole, ketoconazole, and nystatin. If the infection is a paronychium, the most important aspect of the therapy is drainage of the abscess, followed by oral antifungal therapy with either fluconazole or itraconazole. In cases of extensive cutaneous infections, infections in immunocompromised patients, folliculitis, or onychomycosis, systemic antifungal therapy is recommended. For *Candida* onychomycosis, oral itraconazole appears to be the most efficacious of azoles. Two treatment regimens are available: a single daily dose of itraconazole taken for 3–6 months or a pulsed-dose regimen that requires a slightly higher dose daily for 7 days, followed by 3 weeks off therapy. The cycle is repeated every month for 3–6 months. Also effective and well tolerated is terbinafine 250 mg daily for 6 weeks.

Gastrointestinal Candidiasis

OPC may be treated with topical antifungal agents (nystatin, clotrimazole, miconazole, amphotericin B oral suspension) or with systemic oral azoles (fluconazole, posaconazole, itraconazole; Table 8.6).

Candida esophagitis requires systemic therapy, usually with fluconazole or itraconazole for at least 14–21 days. Parenteral therapy with fluconazole may be required initially if the patient is unable to take oral medications. Daily suppressive antifungal therapy with fluconazole 100–200 mg/d is effective in preventing recurrent episodes, but it should only be used if the recurrences become frequent or are associated with malnutrition from poor oral intake and wasting syndrome. In patients with advanced AIDS and severe immunodeficiency, recurrent candidal esophagitis due to fluco-

Table 8.7 Azole therapy for vaginal candidiasis

Drug	Formulation	Dosage
Butoconazole	2% cream 2% vaginal suppository	5 g x 3 d (single dose) 1 suppository (5 g) once daily x 7–14 d
Clotrimazole	1% cream 10% cream 100-mg vaginal tablet 100-mg vaginal tablet 500-mg vaginal tablet	5 g x 7–14 d 5-g single application 1 tablet x 7 d 2 tablets x 3 d 1 tablet once
Econazole	150-mg vaginal tablet	1 tablet x 3 d
Fenticonazole	2% cream	5 g x 7 d
Miconazole	2% cream 100-mg vaginal suppository 200-mg vaginal suppository 1200-mg vaginal suppository	5 g x7 d 1 suppository x 7 d 1 suppository x 3 d 1 suppository once
Tioconazole	2% cream 6.5% cream	5 g x 3 d 5 g single dose
Terconazole	0.4% cream 0.8% cream 80-mg vaginal suppository	5 g x 7 d 5 g x 3 d 80 mg x 3 d
Fluconazole	Oral tablet	150-mg single dose
Ketoconazole	200-mg tablet	400 mg x 5 d
Itraconazole	100-mg tablet	200 mg x 3 d

nazole-resistant *C. albicans* or *C. glabrata* can be effectively treated with voriconazole, posaconazole, any of the echinocandins, or AmB [26].

Genital Tract Candidiasis

VVC can be managed with either topical antifungal agents or single-dose oral fluconazole in uncomplicated infections (Table 8.7). Single-dose (150 mg) oral fluconazole is the preferred method of treatment and typically preferred by women. This therapy has been shown to have clinical and microbiologic efficacy as good as that of topical antifungal agents.

A small percentage of women (5–7%) suffer from recurrent VVC infections, which often require chronic or prophylactic oral azole therapy for control. In women who suffer from recurrent attacks, the recommended regimen is fluconazole at a dose of 150 mg every third day for three doses, followed by weekly fluconazole at a dose of 150 mg for 6 months. This regimen prevents recurrent infections in more than 90% of women although symptomatic recurrence is common following cessation of maintenance suppressive prophylaxis [27].

Urinary Tract Candidiasis (Candiduria)

Asymptomatic candiduria in urinary catheterized patients is extremely common and most commonly reflects yeast colonization of the catheter and lower urinary tract and hence no antifungal therapy is not indicated. Treatment for asymptomatic candiduria should only be considered in a high risk for dissemination subgroup including infants with very-low-birth weight, neutropenic subjects and patients due to undergo urologic manipulation. Symptomatic candiduria reflects deep-tissue or parenchymal invasion and results in organ-specific as well as constitutional symptoms (e.g., fever, frequency, dysuria (lower urinary tract) or fever, renal angle pain, nausea, vomiting, and even sepsis (pyelonephritis). While amphotericin B IV has been the mainstay of indicated therapy, accompanying drug nephrotoxicity limits its use. Fluconazole 200–400 mg, daily for 2 weeks, achieves high urinary concentrations, and has emerged as the drug of first choice with small dose adjustments required for coexistent renal insufficiency. None of the other azoles, including voriconazole, are excreted in urine. Similarly, the echinocandins achieve subtherapeutic urine concentrations. A useful agent for eradicating non-*albicans* candiduria, especially *C. glabrata* is oral flucytosine in the absence of renal failure. Irrigation of the bladder with amphotericin B resolves candiduria in 80–90% of patients with disease confined to bladder especially in infections caused by non-*albicans Candida* spp. Deep-tissue invasion of kidneys or bladder can be treated by all the systemically active antifungals [28].

Candidemia and Acute Disseminated Candidiasis

Candidemia requires treatment in all patients, (Tables 8.8 and 8.9) and is related to the presence of an intravascular catheter in up to 80% of non-neutropenic patients. Removal of intravascular catheters shortens the duration of candidemia and has been associated with reduced mortality. Although some patients have been cured by catheter removal alone, even transient episodes of candidemia can be associated with hematogenous spread and subsequent diagnosis of endophthalmitis or osteomyelitis. Thus, all episodes of candidemia mandate antifungal therapy. A dilated retinal examination is important in all candidemic patients.

Table 8.8 First line therapy for candidemia and invasive candidiasis

Polyenes
Amphotericin B deoxycholate 0.5–0.7 mg/kg IV daily
Liposomal amphotericin B 3–5 mg/kg IV daily
Lipid complex amphotericin B 3–5 mg/kg IV daily
Azoles
Fluconazole 400–800 mg IV daily
Itraconazole 200 mg IV q12h x 4 doses, followed by 200-mg IV daily
Voriconazole 6 mg/kg IV q12h x 2 doses, followed by 3 mg/kg IV q12h
Posaconazole PO (not yet approved)
Echinocandins
Caspofungin 70-mg IV x 1 dose, followed by 50-mg IV daily
Micafungin 100-mg IV daily[a]
Anidulafungin 200-mg IV x 1 dose, followed by 100-mg IV daily
IV, intravenously; PO, by mouth; q12h, every 12 h

Table 8.9 Management of candidemia and disseminated candidiasis

For *Candida albicans*, *C. parapsilosis*, *C. tropicalis*, *C. lusitaniae*, *C. dubliniensis*
Fluconazole 800-mg IV x 1 dose, followed by 400-mg
Caspofungin 70-mg IV x 1 dose, followed by 50-mg IV daily
Micafungin 200-mg IV
For *C. glabrata*— (select therapy on the basis of MICs)
Caspofungin (as above)
Micafungin 200-mg IV
Anidulafungin 100-mg IC
Voriconazole 6 mg/kg IV q12h x 2 doses, followed by 3 mg/kg IV q12h
Amphotericin B 0.7–1.0 mg/kg IV daily
Fluconazole 800-mg IV daily
For *C. krusei*
Echinocandin (as above)
Voriconazole (as above)
IV intravenously, PO by mouth, q12h every 12 h

Antifungal therapy has progressed dramatically from the period when only desoxycholate amphotericin B (AmB) was available and clinicians now have a wide choice of antifungals, none of which has been shown to be superior to amphotericin B, but possess a superior safety profile. AmB was previously the standard drug for candidemia or invasive candidiasis. However, in spite of broad-spectrum activity against most *Candida* species except *C. lusitaniae*, its significant nephrotoxicity dramatically reduces its use. Lipid formulations of AmB have almost entirely replaced the desoxycholate formulation. However, these agents are expensive and although less toxic, are not therapeutically superior.

Fluconazole, a triazole and antifungal, has been widely used for all forms of candidiasis for almost two decades and has an excellent safety profile. Several large studies have shown equivalent activity and success when compared with polyene agents and echinocandins in non-neutropenic hosts [29]. Voriconazole has superior in vitro activity than fluconazole and is active against fluconazole-resistant *C. krusei*, and some, but not all (<50%), *C. glabrata* strains. IV followed by convenient oral voriconazole was shown to be equivalent or noninferior to AmB followed by fluconazole in the treatment of candidemia and invasive candidiasis [26, 29]. Nevertheless, evidence of superiority of voriconazole over fluconazole clinically has not been forthcoming. Its additional costs and less favorable toxicity profile, has resulted in voriconazole being reserved for selected patients with fluconazole-resistant but voriconazole-sensitive *Candida* species. Posaconazole, although extremely active, is still not available IV hence precluding its use in candidemia, although useful for fluconazole-resistant OPC.

Echinocandins are noncompetitive inhibitors of synthesis of beta 1, 3-glucan, a major component of fungal cell wall. The class includes caspofungin, anidulafungin and micafungin. All three drugs have excellent activity against all *Candida* species and are considered therapeutically equivalent [26]. Echinocandins are particularly useful in patients with advanced renal and hepatic disease. Only caspofungin is approved for use in pediatric patients, though all have been found to be effective. The echinocandins are preferred over azoles for infections in which *C. glabrata* is suspected or documented, particularly in critically ill, hemodynamically unstable patients regardless of species. Some consider the echinocandins class, the drug of first choice even for *C. albicans* infections [29]. Although drug concentrations of echinocandins (MICs) in vitro required to inhibit *C. parapsilosis* are slightly higher than for other *Candida* species (1–2 μg/mL), clinical efficacy is comparable and resistant organisms are extremely rare. On the other hand, recent studies identified acquired resistance to all agents in this class to be increasing among *C. glabrata* isolates especially those with fluconazole resistance although this phenomenon is still uncommon [30]. Prevalence of fluconazole resistance (7% of all *Candida* spp. remains unchanged) [31, 32].

Antifungal therapy should be started in patients who are critically ill and have risk factors for systemic candidiasis after other causes of fever have been excluded. First-line choices are fluconazole or an echinocandin; however, if *C. krusei* or *C. glabrata* are suspected or documented, an echinocandin is recommended. Similarly, this class is advised in critically ill unstable patients and in patients who have had recent exposure to an azole. Recommended alternatives are AmB or a lipid formulation [26].

In the presence of neutropenia, a slightly different order of preference is recommended. This is because febrile neutropenic patients are usually already receiving oral prophylactic azole drugs, and because of the higher risk of molds, less susceptible to azoles. Accordingly, initial antifungal recommended includes either AmB/lipid formulation AmB or an echinocandin [26, 29]. In general, IV fluconazole 400 mg/d (after a loading dose of 800 mg/d) still has an important role, in patients in whom azole resistance is not

expected and those not critically ill. Some guidelines suggest that fluconazole is the drug of choice for infections caused by *C. parapsilosis*. Finally, apart from its limited role as a diagnostic marker, beta glucan kinetics have been shown to be useful in predicting and correlating with clinical outcome in invasive candidiasis including candidemia.

Combinations of either fluconazole or amphotericin B with flucytosine at 100–150 mg/kg/d may be useful in some patients, but the precise role of this combination is unclear. The required duration of antifungal therapy is undetermined, but therapy is usually continued for about 2 weeks after the last positive blood culture [26]. With this approach, the rate of subsequent recurrent infection at a hematogenously seeded site is about 1%. For *Candida* endophthalmitis or complicated candidemia with metastatic *Candida* infection, duration of treatment should be at least 4–6 weeks.

It appears likely that the GI tract is the most common source of candidemia in neutropenic patients. In these patients, removal of intravenous catheters is still important. This applies particularly to *C. parapsilosis* fungemia, which is highly associated with intravascular catheters in cancer patients. Recovery of marrow function is critical, and no therapeutic approach is consistently successful in the face of persistent leukopenia. In this setting, most available experience is with the use of amphotericin B at 0.6–1.0 mg/kg/d, given until recovery of marrow function. The optimal dose of amphotericin B is not certain, but non-*albicans Candida* require higher doses (0.8–1.0 mg/kg/d) of amphotericin B. This appears to be especially true of *C. krusei* and *C. glabrata*. Use of flucytosine in neutropenic patients is generally limited because of its potential for marrow suppression and the lack of a readily available IV formulation. On the other hand, in non-neutropenic patients, given the penetration advantage, flucytosine 25 mg/kg four times daily should be combined with lipid formulation AmB 3–5 mg/kg/daily for CNS infection. This also applies to *Candida* endocarditis where vegetation penetration is critical to infection cure [26].

Patients may develop candidemia while receiving antifungal therapy, including prophylactic antifungals. Such breakthrough candidemia may be the result of an infected unremoved intravascular catheter. In cancer patients, breakthrough candidemia has been associated with a higher mortality and has occurred more often during an ICU stay, during prolonged neutropenia, and with the use of corticosteroids. In this setting, immunosuppression should be reduced and factors that might alter antifungal drug delivery or clearance excluded. Intravenous catheters should be changed and the possibility of drug resistance considered, especially since non-*albicans Candida* are frequently responsible. Antifungal drug susceptibility tests should be performed and therapy should be changed to an antifungal of a different class.

Crude (30–40%) and attributable mortality rates in patients with candidemia and invasive candidiasis remains unacceptable high. *C. parapsilosis* has lowest mortality rate (20–25%) and *C. krusei*, followed by *C. glabrata*, has highest crude mortality rate. Risk factors for increased mortality include neutropenia, high acute physiology and chronic health evaluation (APACHE) scores, delay in institution of appropriate antifungal therapy, failure to remove IV catheters in ICU units.

Central tunneled catheters in febrile neutropenic patients do not require mandatory removal because alternate vascular access sites are less available, removal is more difficult, and, most importantly, such catheters are less likely to be the source of candidemia, although they may become infected secondarily to bloodstream infection. Occasionally, these valuable access sites can be salvaged using the controversial antibiotic lock method using amphotericin B, but results are unpredictable.

Infections involving chorioretinal layer are more easily treated because they are highly vascular and systemic antifungal agents reach adequate concentrations with these structures, however, sight-threatening lesions near the macula and invasion into the vitreous usually necessitate intravitreal injection of antifungal agents, with or without vitrectomy in addition to systemic antifungal agents [33, 34].

Chronic Disseminated Candidiasis

Therapy of CDC traditionally consists of prolonged therapy with amphotericin B alone, especially in acutely ill patients, but this approach has not been uniformly successful. Amphotericin B (0.5–1.0 g), followed by a prolonged course of fluconazole (200–400 mg/d) for 2–14 months, is associated with cure rates of greater than 90%. Use of oral fluconazole is also recommended 400 mg (6 mg/kg) daily for clinically stable patients. Lipid-based amphotericin B has also been used successfully. For azole-resistant *Candida* species, an echinocandin or lipid formulation AmB is recommended. If the lesions have stabilized and the patient is clinically improved, antineoplastic therapies (including those that induce neutropenia) may be restarted, while antifungal therapy is continued. The duration of antifungal therapy is determined by imaging studies of the liver and spleen. Use of corticosteroids is controversial.

Prevention

Prophylaxis of Candidiasis in Transplant Patients

Invasive candidal infections are a concern in these high-risk groups. Institutions with recipients of solid organ and bone marrow transplants usually consider prophylaxis with fluconazole for the prevention of candidiasis in selected patients

only. Fluconazole is generally started 1 day before neutropenia, and although controversial, some investigators support its use for 75–100 days after bone marrow transplantation. In liver transplants, short-term fluconazole prophylaxis is indicated in selected high-risk patients.

Prophylaxis of Superficial Candidiasis in HIV-Positive Patients

There is little support for primary or secondary prevention of OPC, esophageal candidiasis, or vaginal candidiasis in HIV-positive patients. Concern about potential development of resistance or colonization by resistant species or strains of *Candida* exists. Prophylaxis may be indicated in a select group of patients with recurrent episodes of symptomatic candidiasis only.

Empirical Anti-Candida Treatment

Empirical use of antifungal agents in febrile patients in ICUs is widely used without data to support its use. Given the existent difficulties in diagnosing invasive candidiasis, it appears reasonable to recommend empirical antifungal therapy in selected febrile, high-risk patients with persistent antibiotic-resistant fever. Although caspofungin with its broad spectrum may be preferable, less expensive empirical fluconazole is recommended. The use of empirical antifungals in low-risk patients is not justified [26].

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Infection Due to Non-Candidal Yeasts

9

Jose A. Vazquez

Introduction

Yeasts exist throughout nature in association with soil, plants, mammals, fish, and insects. For that reason, humans are constantly exposed to many yeast genera through varying routes. Depending on the interaction between host defense mechanisms and fungal virulence factors, the association between yeast and humans can be either transient or persistent, and can associated with either local infection or systemic disease. Most yeast organisms are of low virulence and generally require significant alterations or reductions in host defenses prior to tissue invasion. Recently, however, because of the increased population of immunocompromised patients, the frequency of invasive infections due to yeasts as well as the number of organisms causing disease continues to grow [1–6] (Table 9.1).

Trichosporon

Trichosporon asahii was first described in 1865 by Beigel, who identified it as the causative agent of hair infections [7]. Infections due to *Trichosporon* may be classified as superficial or deep. Disseminated infections are increasingly recognized in the compromised host over the past decade and are frequently fatal [1–6]. One of the first reported cases of disseminated disease was described in a 39 year-old female with lung cancer who subsequently developed a brain abscess [8].

Etiologic Agents

The genus *Trichosporon* was first reported by Behrend [9]. Gueho and colleagues have suggested that the species known as *T. asahii* may include several different *Trichosporon* species with epidemiological and pathogenic differences [10]. Kemker et al. using isoenzyme delineation and polymerase chain reaction (PCR) DNA fingerprinting suspect that strains that produce superficial infections are distinctly different from those strains that produce invasive infection [11]. There are currently seven species of *Trichosporon*. These include *T. asahii* (formerly, *T. beigelii*), the most frequently recovered species from invasive infections, and *T. mucoides* and *T. inkin,* also known to cause systemic infections [1, 2, 12–14]. *T. asteroides* and *T. cutaneum* generally produce superficial skin infections, while *T. ovoides* generally causes white piedra of the scalp and *T. inkin,* white piedra of the pubic hair. *Trichosporon capitatum* is now known as *Blastoschizomyces capitatus* [10, 12, 13].

Trichosporon species are characterized by true hyphae, pseudohyphae, arthroconidia, and blastoconidia [10, 15] (Fig. 9.1). *T. asahii* grows readily on Sabouraud dextrose agar, producing smooth, shiny gray- to cream-colored yeast-like colonies with cerebriform radiating furrows that become dry and membranous with age. All *Trichosporon* species are easily identified using commercially available carbohydrate assimilation assays.

Epidemiology

T. asahii is generally found in the soil, but may also be recovered from air, rivers and lakes, sewage, and bird droppings [1, 2, 12]. It rarely colonizes the inanimate environment, but can colonize the mucosal surfaces of the oropharynx, the lower gastrointestinal (GI) tract, and the skin of humans [12, 13].

More than 100 documented cases of disseminated infection due to *Trichosporon* species have been reported, most due to *T. asahii* [1, 2, 12, 13, 15]. The major risk factors

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Table 9.1 Yeasts other than *Candida* and *Cryptococcus* that occasionally cause human infection

Trichosporon
T. asahii (T. beigelii)
T. inkin
T. mucoides Saccharomyces
S. cerevisiae
S. boulardii Rhodotorula
R. mucilaginosa (rubra)
R. glutinis Malassezia
M. furfur
M. pachydermati Blastoschizomyces capitatus (Geotrichum capitatum, Trichosporon capitatum) Sporobolomyces
S. salmonicolor
S. holsaticus

Table 9.2 Risk factors associated with *Trichosporon* infection

• Hematologic malignancy
• Solid organ transplantation (kidney, heart, liver)
• Neutropenia
• Broad-spectrum antibiotics
• Corticosteroids
• Use of intravenous lipids
• Bone marrow transplant
• Chronic active hepatitis
• IVDU
• Central venous catheters
• CAPD
• Burns

IVDU intravenous drug user, *CAPD* central auditory processing disorder

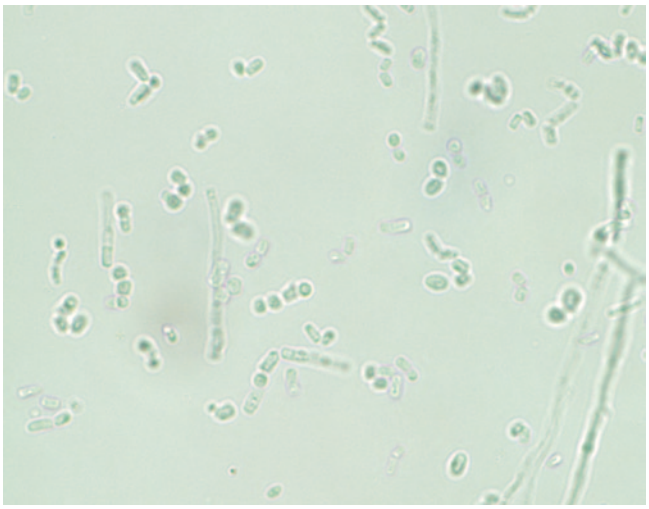

Fig. 9.1 *Trichosporon* species produce yeast-like colonies in culture and are unique in their production of hyphae, pseudohyphae, arthroconidia, and blastoconidia (budding) both in culture and in disease. (Courtesy of D. R. Hospenthal)

associated with infection include hematologic malignancies (acute leukemia, chronic leukemia, multiple myeloma), solid tumors, and neutropenia [1, 2, 12–15]. In non-neoplastic, non-neutropenic cases, the major risk factors include corticosteroids, prosthetic valve replacement, solid organ transplantation, chronic active hepatitis, and occasionally intravenous drug use (Table 9.2). The most common portal of entry appears to be either the respiratory or GI tracts. Infrequently, central venous catheters and other vascular devices have also been implicated [12–16].

Clinical Manifestations

Trichosporonosis is classified into superficial infections (white piedra (hair shaft infection), onychomycosis, and otomycosis) and invasive infections.

Deep tissue infections may involve either a single organ or multiple organs. The most commonly infected tissue is the lungs, which accounts for approximately 33 % of all localized deep tissue infections [1, 2, 12–19]. Other sites of infection may include the peritoneum, heart valves (natural and prosthetic), retina, liver, spleen, kidneys, gallbladder, and central nervous system (brain abscess and chronic fungal meningitis) [1,2, 10–19].

The signs and symptoms of disseminated infection resemble those of systemic candidiasis and include fungemia with associated organ infection. Moreover, disseminated infections may present as either acute or chronic disease. Acute disseminated trichosporonosis often has a sudden onset and progresses rapidly, especially in neutropenic patients. Patients may develop skin lesions (~33 %), pulmonary infiltrates (~30–60 %), renal, and ocular involvement.

The metastatic cutaneous lesions generally begin as an erythematous rash with raised papules on the trunk and the extremities. The rash eventually evolves into macronodular lesion, followed by central necrosis of the nodules and occasionally the formation of hemorrhagic bullae. The pulmonary infiltrates may present as lobar consolidations, bronchopneumonia, or reticulonodular patterns.

Renal involvement occurs in >75 % of the disseminated infection cases. Renal disease may manifest as proteinuria, hematuria, red blood cell (RBC) casts, with either acute renal failure or acute glomerulonephritis [12–16]. Urine cultures are frequently positive for *Trichosporon* and suggest disseminated disease, especially in immunocompromised patients [19].

Chorioretinitis is not uncommon in disseminated infection and may be a cause of visual alterations due to retinal vein occlusion or retinal detachment [12–14]. For unexplained reasons, *Trichosporon* has been found to have tropism for the choroid and retina. However, unlike candidal endophthalmitis, *Trichosporon* infects uveal tissues including the iris, but spares the vitreous [20].

Table 9.3 In vitro antifungal activity against emerging yeast infections

	Minimum inhibitory concentration (MIC; μg/ml) range[a]									
Organism	Flu	Itra	Vori	Posa	Isuv	Mica	Cas	Anid	AMB	5FC
Trichosporon	1–16	0.06–0.25	0.03–>16	0.06->16	0.015–0.5	16–64	4–>16	16–>16	1–8	16–>512
Saccharomyces	0.5–64	0.03–4	0.016–2	0.12–1.0	0.03–1	NA	0.25–1	0.25–1	0.032–4	<0.125–1
Rhodotorula	0.5–>64	0.25->16	0.25–>8	0.25–>8.0	0.125–2	>64	8–>64	>64	0.12–1.0	0.6–0.25
Malassezia	1.0–16	0.03–0.25	0.03–0.125	0.03–32	0.03–0.5	NA	NA	NA	0.3–2.5	>100
Blastoschizomyces	1–32	0.03–0.50	0.03–0.50	0.12–0.25	NA	NA	NA	1–4	0.6–0.25	0.12–16
Sporobolomyces	1.25–>64	1.0–2.0	0.25–4.0	NA	NA	>64	NA	NA	0.14–1.0	NA

Flu fluconazole, *Itra* itraconazole, *Vori* voriconazole, *Posa* posaconazole, *Isuv* isavuconazole, *Mica* micafungin, *Cas* caspofungin, *Anid* anidulafungin, *AMB* amphotericin B, *5FC* flucytosine, *NA* data not available

[a] Clinical Laboratory Standards Institute (CLSI) testing criteria and break points have not been established for any of these fungi

During disseminated infection, any tissue in the body may become infected. The organs most frequently include the liver, spleen, GI tract, lymph nodes, myocardium, bone marrow, pleura, brain, adrenal gland, and thyroid gland [1, 2, 12–20].

In chronic disseminated infection, subtle manifestations may be present for several weeks and frequently include persistent fever of unknown etiology [1, 2, 12, 13]. The infection is similar to the entity known as chronic disseminated (hepatosplenic) candidiasis. It is generally a chronic infection of the liver, spleen, and other tissues after recovery from neutropenia. Laboratory studies frequently reveal an elevated alkaline phosphatase. Computed tomography (CT) scan or magnetic resonance imaging (MRI) frequently reveals hepatic or splenic lesions compatible with abscesses. A tissue biopsy is needed to confirm the diagnosis.

Diagnosis

The diagnosis is made with a biopsy of the skin or involved organs. Blood cultures may occasionally be useful in deep tissue infection, but are positive only late in the course of infection. *Trichosporon* grows readily in conventional blood culture and on standard fungal media including Sabouraud dextrose agar [15]. The presence of *Trichosporon* in the urine of a high-risk patient should increase the suspicion of disseminated infection.

Although there are no standardized serologic assays, the serum latex agglutination test for *C. neoformans* may be positive. A potential usefulness of this assay has been postulated based on the report of positive serum latex agglutination test for *C. neoformans* in several patients with disseminated *Trichosporon* infection [21, 22].

Treatment

Disseminated trichosporonosis has a mortality rate of approximately 60–70% [2–6, 12, 13]. In most cases, however, the underlying disease contributes greatly to the overall mortality. First-line, optimal antifungal therapy has not been established. The initial step in the management of disseminated *Trichosporon* infection should be to decrease or reverse immunosuppression.

Table 9.4 Suggested antifungal agents for use in the treatment of emerging yeast infections

Yeast	Antifungal therapy[a]
Trichosporon	Fluconazole 400 mg/day
	Voriconazole 200 mg bid
	Itraconazole 400–600 mg/day
Saccharomyces	Amphotericin B
	5-Flucytosine
	Azoles (ketoconazole, clotrimazole, miconazole)
Rhodotorula	Amphotericin B + 5-flucytosine
Malassezia	Fluconazole 400 mg/day
	Voriconazole 200 mg bid
	Amphotericin B 0.7 mg/kg/day
Blastoschizomyces	Amphotericin B 1–1.5 mg/kg/day
Sporobolomyces	Amphotericin B
	Azoles (ketoconazole, itraconazole, fluconazole)

[a] No antifungal agents have specific FDA approval for therapy of any of these infections. No randomized clinical trials have been performed. Selections are based on in vitro data, limited animal studies, and/or individual case reports

In vitro susceptibility studies of *Trichosporon* species are limited (Table 9.3). In vitro susceptibility assays of *T. asahii* reveal fluconazole MIC_{90} of 4.0 μg/ml, itraconazole MIC_{90} of 0.25 μg/ml, and amphotericin B MIC_{90} of 4.0 μg/ml. In general, most strains have relatively high MICs for polyenes, flucytosine, and echinocandins, with relatively low MICs for the azoles. Among the newer triazoles, voriconazole and posaconazole have demonstrated excellent in vitro activity [22–26]. Although not yet approved, isavuconazole also shows significant in vitro activity against most strains of *Trichosporon* (Table 9.4) [25]. In fact, it appears to have better activity than fluconazole [25]. In vitro and animal models suggest that azoles and not polyenes are more effective in the eradication of *Trichosporon* species [1, 2, 20]. Suggested therapy for the treatment of disseminated disease includes the use of either voriconazole 3 mg/kg IV or 200 mg orally

twice daily, fluconazole 400–800 mg/day, or itraconazole 400–600 mg/day (Table 9.4). A potential option in patients failing azole therapy may also include a combination of an azole with an echinocandin. Serena et al. demonstrated in vitro synergy and improved outcomes in an animal model of trichosporonosis with either the combination of amphotericin B/micafungin or fluconazole/micafungin [12, 14, 24, 25]. However, because of the high MICs to polyenes and echinocandins, these antifungals should not be used alone or as first-line therapy. In a patient with disseminated infection and poor response to therapy, in vitro susceptibility testing of recovered isolates may be a helpful adjunct.

Saccharomyces

Saccharomyces is an ascomycetous yeast found throughout nature. *Saccharomyces* is commonly known as "brewer's yeast" or "baker's yeast." It is best known for its commercial use in beer and wine production, in health food supplements, and more recently, its use in DNA recombinant technology. Occasionally, these yeasts have been reported to cause severe infection in immunocompromised hosts [15]. Species include *S. cerevisiae, S. boulardii* (a subtype of *S. cerevisiae*), *S. fragilis,* and *S. carlsbergensis*. *Saccharomyces* may occasionally be part of the normal flora of the GI and genitourinary tracts [1, 2, 27]. Recently, *S. cerevisiae* has been found to cause mucosal and disseminated infection in humans, primarily in immunocompromised hosts [27–29].

Etiologic Agents

Cells are oval to spherical and exist as either haploids or diploids. When present, ascospores, one to four in number, are in either tetrahedral or linear arrangement and stain Gram negative; vegetative cells stain Gram positive. Colonies are smooth, moist, and either white or cream colored. *Saccharomyces* are generally nonpathogenic due to innate low virulence [15, 30–32]. Investigators evaluating more than 3300 yeast cultures obtained from cancer patients found only 19 isolates of *S. cerevisiae*. Recent studies by Clemons et al. using an animal model have been able to show that some strains of *S. cerevisiae,* when introduced into CD-1 mice, can proliferate and resist clearance in vivo, supporting the role of *S. cerevisiae* as a cause of clinical infection in humans [32].

Epidemiology

Isolation of *Saccharomyces* species from human surfaces is rarely of any clinical significance. It has been recovered from the bloodstream, lungs, peritoneal cavity, esophagus, urinary tract, and vagina [2–6, 27, 30, 33]. Genotyping studies evaluating the relatedness between clinical strains and commercial strains of *S. cerevisiae* have demonstrated that commercial products may occasionally be a contributing factor in human colonization and infection [34]. Nyirjesy et al. reported that four women suffering from recurrent *S. cerevisiae* vaginitis had also experienced exposure to bread dough that contained identical strains *S. cerevisiae* [29].

The risk factors associated with *Saccharomyces* infections are similar to the risk factors associated with candidemia and systemic candidiasis; including central venous catheters, neutropenia, use of antimicrobials, GI tract surgery, and occasionally HIV [28, 35–37]. The portal of entry for invasive disease is most likely the oropharynx or GI tract [36].

Clinical Manifestations

Manifestations are generally nonspecific and indistinguishable from those associated with candidemia and invasive candidiasis. In addition, *Saccharomyces* has been associated with bloodstream infections, endocarditis, peritonitis, disseminated disease, and vaginitis [1–6, 29, 33, 35–39].

Fungemia is the most common form of infection, occurring in approximately 70 % of reported cases. As in invasive candidiasis, it is seen primarily in the immunocompromised host and tends to be associated with the use of intravascular catheters, chemotherapy, and/or antimicrobials [27, 28, 36]. Manifestations are similar to those of systemic candidiasis and candidemia. Overall, fever unresponsive to broad-spectrum antimicrobials is the most frequent manifestation. Unlike infections due to *Candida* species, most patients survive.

In addition, it is not uncommon for other organ systems to become infected, including the respiratory tract, with several documented episodes of pneumonia and empyema. Diagnosis is generally established by histopathology, since *Saccharomyces* can colonize the respiratory tract without producing invasive disease [27, 36, 40]. *Saccharomyces* has also been reported to produce peritonitis, cholecystitis, and endocarditis [27, 36]. All cases of endocarditis were associated with prosthetic valves and intravenous heroin use. Furthermore, two out of the three patients were cured with antifungal therapy alone; only one patient had their valve replaced. There have also been several documented cases of urinary tract infections due to *S. cerevisiae* [29]. All patients had underlying urologic abnormalities or were associated with fungemia [36].

Mucosal infections due to *S. cerevisiae* have also been reported. Sobel et al. reported on 17 women with difficult to manage vaginitis due to *S. cerevisiae* [26, 29]. In fact, the women with symptomatic vaginitis had manifestations indistinguishable from those caused by *C. albicans*. All patients had a history of chronic vaginitis unresponsive to

conventional antifungals and all but two had systemic or local predisposing factors.

Diagnosis

Because of the fact that *Saccharomyces* species have a tendency to be nonpathogenic, the decision to attribute a causal role to *S. cerevisiae* is difficult [2–6]. Diagnostic difficulty occurs when the organism is recovered from body sites that may be colonized by *Saccharomyces,* especially in the absence of symptoms of infection. Unless the organism is found in the bloodstream, it is frequently necessary to determine whether these yeasts are causing true infection versus colonization. This is generally done via a histopathologic examination. *S. cerevisiae* readily grows from blood culture bottles and on Sabouraud dextrose media [2–6, 34].

Treatment

It is often difficult to assess the role of antifungal therapy in patients with infection due to *Saccharomyces*. There are several reports that document the resolution of fungemia and infection just by removing the intravascular catheter without providing antifungal therapy [35, 36]. Most experts advocate removing the focus of infection, whether it is an indwelling or tunneled intravenous catheter and the concurrent use of antifungal agents [36]. In vitro susceptibility studies reveal that *S. cerevisiae,* when compared to *C. albicans* isolates, are less susceptible to most antifungals, including azoles [1–5, 26] (Table 9.3). Although clinical trials have not been conducted and in vitro susceptibility assays are not standardized, *Saccharomyces* species appear to be susceptible to most antifungals including amphotericin B, 5-flucytosine, ketoconazole, clotrimazole, miconazole, and terconazole [1–5, 26] (Table 9.4).

Rhodotorula

Yeasts of the genus *Rhodotorula* are found worldwide from a variety of sources and is generally considered a contaminant when identified. Infections are occasionally seen primarily in immunocompromised hosts [2–6].

Etiologic Agents

Yeasts of the genus *Rhodotorula* are imperfect basidiomycetous yeasts belonging to the family Cryptococcaceae. Currently, eight species in the genus *Rhodotorula* are recognized [2–6, 41]. *Rhodotorula mucilaginosa* (formerly *R. rubra*) is the species most frequently associated with human infections. The other species include *R. glutinis, R. pilimanae, R. pallida*, *R. aurantiaca, R. minuta.* Most *Rhodotorula* species produce red-to-orange colonies due to the presence of carotenoid pigments [15, 42]. The yeast is mucoid, encapsulated, and readily grows on many types of culture media.

Table 9.5 Clinical manifestations of *Rhodotorula* infections

• Fungemia
• Endocarditis
• Meningitis
• Peritonitis
• Disseminated disease

Epidemiology

Rhodotorula can be isolated from a variety of sources including seawater, plants, air, food, fruit juices, and occasionally from humans [1–6, 15, 41, 42]. It is not unusual to recover it as an airborne laboratory contaminant. *Rhodotorula* can also be recovered from shower curtains, bathtub–wall junctions, and toothbrushes. In humans, *Rhodotorula* can be recovered from skin, nails, respiratory tract, urinary tract, GI tract, and bloodstream [1–6, 15, 41–46].

R. mucilaginosa and *R. glutinis* account for approximately 0.5 % of yeast isolated from the oral cavity and more than 12 % of yeast isolates recovered from stool and rectal swabs [47]. The recovery of *Rhodotorula* from non-sterile human sources such as mucosal sites has been of questionable clinical significance. Although a number of invasive infections have been documented, risk factors include underlying immunosuppression (malignancy, neutropenia, corticosteroids, collagen vascular disease, and uncontrolled diabetes mellitus), use of broad-spectrum antimicrobials, and central venous catheters.

Clinical Manifestations

Manifestations are generally nonspecific, and may vary from subtle and mild to severe, including septic shock. *Rhodotorula* have been incriminated in a wide spectrum of infections including bloodstream infections, endocarditis, peritonitis, meningitis, and disseminated disease [2–6, 41, 43, 46–57] (Table 9.5).

Fungemia is the most common form of infection and is generally due to intravascular catheter infection [1–6, 41, 43, 46–57]. Fever of unknown etiology that is unresponsive to broad-spectrum antimicrobials is the most frequent manifestation associated with fungemia.

Meningitis has also been described in patients with acute leukemia, HIV infection, and postoperatively [52, 53]. The organisms are generally recovered from the cerebrospinal fluid (CSF) on culture, and frequently seen on an India ink stain. In addition, several cases of *R. rubra* peritonitis have been described in patients undergoing continuous ambulatory

peritoneal dialysis. In these patients, environmental cultures revealed a possible common source outbreak. In all patients, the symptoms were subtle and intermittent at first, consisting of abdominal pain, anorexia, nausea, and occasional diarrhea [2, 54, 57].

Diagnosis

In the most proven infections, *Rhodotorula* is recovered from a sterile site of infection. In these cases, the decision to attribute a causal role to *Rhodotorula* is relatively simple, and the patient should be treated appropriately for an invasive fungal infection. The more difficult decision is when the organism is recovered from nonsterile body sites that may normally harbor *Rhodotorula* species, especially in the absence of signs or symptoms of infection [41, 46, 57]. In this setting, it is essential to establish the presence of infection and not just colonization.

Treatment

As with many of the uncommon yeast isolates, it is difficult to assess the role of antifungal therapy in patients infected with *Rhodotorula*. Optimal management of patients with indwelling catheters and infection due to *Rhodotorula* has not been well defined. There are several case reports that document the clearance of fungemia and the resolution of infection by removing the intravascular catheter without providing antifungal therapy [2–6, 26, 54–58]. On the other hand, there are also several documented case reports that have suggested that antifungal treatment alone may suffice without having to remove the central venous catheter. Since infections due to *Rhodotorula* have been severe and life threatening, it is probably best to manage these infections aggressively with catheter removal and antifungal therapy.

In vitro susceptibility studies reveal that *Rhodotorula* are susceptible to amphotericin B and flucytosine, but less susceptible to azoles and resistant to echinocandins [2–6, 26, 54–58] (Table 9.3). Although clinical trials have not been conducted, it appears that amphotericin B with or without flucytosine is the best recommendation for antifungal therapy at this time [1–6]. In view of the intrinsic resistance of *Rhodotorula* to the azoles and echinocandins, these agents should not be used as monotherapy unless in vitro susceptibility activity has been assessed.

Malassezia

Malassezia furfur is a yeast commonly found on human skin. It has been well documented to cause superficial skin infections such as pityriasis (tinea) versicolor and folliculitis. In addition, in immunocompromised host, it may occasionally cause invasive infection.

Etiologic Agents

The genus *Malassezia* consists of several species, the two most frequently isolated species include *M. furfur* and *M. pachydermatis* [1–6, 15]. The other less commonly isolated species includes *M. sympodialis, M. slooffiae, M. globosa, M. obtuse,* and *M. restricta*. *M. furfur* is the dominant species recovered in humans as a fungal pathogen. *M. furfur* is a dimorphic, lipophilic yeast that is unable to synthesize medium- or long-chain fatty acids and thus has a strict in vitro requirement for exogenous fatty acids of the C_{12} and C_{14} series [15, 59, 60]. Although it exists primarily in the yeast form, it may also form filamentous structures on the skin when the organism is associated with superficial infections [59, 60]. Because of its nutritional requirements, *M. furfur* is difficult to recover from clinical specimens unless its presence is suspected and special preparations are made by the microbiology laboratory. The second most common species is *M. pachydermatis,* which is generally associated with infections in dogs producing otitis externa [59, 60]. Occasionally, however, it has been implicated in human infections [59, 60]. Both *Malassezia* species, when grown under favorable conditions, produce clusters of oval-to-round, thick-walled yeast cells, with unipolar buds that form repeatedly from the same pole of the parent cell. This gives rise to the characteristic "collarette" at the bud site. Media such as Sabouraud dextrose agar, chocolate agar, trypticase soy agar with 5% sheep blood all require the addition of supplements such as olive oil, in order to permit the growth of this organism [15]. *M. pachydermatis,* however, does not require exogenous lipids for growth, can be recovered on conventional fungal media, and its colonies tend to be dry, and white to creamy in color.

Epidemiology

Malassezia is frequently found on normal individuals colonizing the skin. Distribution of this colonization tends to correlate with the more oily areas, most likely because of the organisms' requirement of exogenous fatty acids found in those areas. Thus, it is found primarily on the scalp, shoulders, chest, and back [60, 61]. The highest colonization rates are found in teenagers (>90%).

The isolation of *M. furfur* from newborns is reported to be less than 10% in non-intensive care settings. However, it has been reported to be greater than 80% in neonatal intensive care units [59–61]. Risk factors associated with increased colonization rates in neonates include prematurity, duration of hospitalization in the intensive care unit (ICU), use of

Table 9.6 Risk factors associated with *Malassezia* infections

• Prematurity
• Longer duration of hospitalization
• Use of occlusive dressings
• Administration of antibiotics
• Use of central venous catheters
• Use of intravenous lipids

occlusive dressings, and prolonged antimicrobial use [59–62]. Although the epidemiology of disseminated infection in adults has not been well studied, there appear to be several risk factors that are frequently associated with deep-seated infections (Table 9.6). These include prematurity, central venous catheters, total parenteral nutrition, parenteral lipid preparations, and immunocompromised state [60, 62–65]. Molecular epidemiologic studies using DNA fingerprinting have concluded that within the neonatal ICU there is longitudinal persistence of both *M. furfur* and *M. pachydermatis* strains [60, 62].

Clinical Manifestations

Malassezia generally produces superficial skin infections, such as pityriasis (tinea) versicolor, or folliculitis. From time to time, *Malassezia* may produce a deep-seated or hematogenous infections [60, 62–65]. The first reported case of systemic infection was described in 1981 in a premature neonate who developed fungemia and vasculitis while on lipid therapy [64]. Since then, numerous reports describing disseminated infection have been published [59–62]. The manifestations of disseminated infection vary from subclinical and mild symptomatology, such as fever, to sepsis with associated multi-organ dysfunction [59–62]. The majority of the *Malassezia* infections are diagnosed in premature infants. Occasionally, they may be seen in adults. The most commonly reported manifestations of systemic infection include fever unresponsive to broad-spectrum antimicrobials, bradycardia, respiratory distress, hepatosplenomegaly, and lethargy.

Diagnosis

Laboratory findings include leukocytosis and thrombocytopenia. Chest X-ray frequently reveals bilateral pulmonary infiltrates (>50%) [1–6, 60, 62]. Occasionally, the diagnosis of disseminated infection can be made by a Gram stain of the buffy coat of blood. The budding yeast cells may be observed using different stains such as Giemsa, periodic acid–Schiff (PAS), Gomori's methenamine silver (GMS) or Calcofluor white. Blood cultures are usually negative unless the infection is initially suspected and the laboratory uses a lipid-enriched media. The recovery of the organisms is enhanced by using the lysis centrifugation blood culture technique [60, 66]. Palmitic acid (3%) supplementation may also improve the recovery of *Malassezia* [60, 66].

Treatment

Management of *M. furfur* fungemia and disseminated infection are controversial. Most authorities recommend prompt removal of the central venous catheter and discontinuation of intravenous lipids [60, 62–64]. In most cases without a deep-seated infection, removal of the central venous catheter and discontinuation of lipids is all that is needed to clear the infection. This treatment modality accomplishes two objectives: it eradicates the nidus of infection and removes the nutritional requirements of the organism. If fungemia persists or there is evidence of deep-seated infection, it is prudent to initiate antifungal therapy. Fortunately, *Malassezia* species are susceptible to azoles and polyenes [1–6, 67] (Table 9.3). In vitro susceptibility assays of *M. furfur* strains demonstrate that most of the isolates are susceptible to amphotericin B, ketoconazole (MIC range <0.05–0.4 μg/ml), miconazole (MIC range 0.4–>50 μg/ml), and fluconazole. Most of the isolates are intrinsically resistant to flucytosine (MIC_{90} >100 μg/ml) [1–6, 26, 60]. Although randomized clinical trials have not been conducted, in most situations, either fluconazole 400 mg/day or amphotericin B 0.7 mg/kg/day is sufficient to eradicate the infection (Table 9.4). Based on excellent in vitro activity, itraconazole and voriconazole are also alternate choices.

Other Yeast

In addition to the yeasts discussed thus far, fewer reports have been published of infection due to other yeasts. These include *Blastoschizomyces, Sporobolomyces, Pichia* (formerly *Hansenula*), and *Exophiala*.

Blastoschizomyces

Blastoschizomyces capitatus (formerly *Geotrichum capitatum* or *Trichosporon capitatum*) infections, although less common than those due to *T. asahii,* have been well described in the literature [2–6, 67]. *B. capitatus* is found in wood and poultry, but has also been recovered from sputum and normal intact skin [15]. Geographically, it appears to be the opposite of *T. asahii,* with *B. capitatus* infections found primarily in Europe and *T. asahii* found in North America [2–6, 68]. In most cases, the major risk factors include neutropenia and underlying hematologic malignancies. Although the portal of entry is unknown, it is suspected to be either the respiratory tract, GI tract, or central venous catheter [2–6, 68].

Infection may involve a single organ or multiple organs and may be associated with fungemia. The clinical spectrum of disseminated infection is similar to that of systemic candidiasis and includes fungemia with or without organ infection [2–6, 68]. By and large, the manifestations begin with fever

of unknown etiology and unresponsive to antimicrobials. Diagnosis can be made with blood cultures or on biopsy of the skin or affected organs. Blood cultures are reportedly seen in >80% of cases [68]. *B. capitatus* easily grows in blood culture bottles and on fungal specific media [15]. Although skin lesions are commonly seen, fungal stains and cultures from biopsied skin lesions are frequently negative [15, 68].

Mortality rates between 60 and 80% are generally described [68]. However, underlying disease, persistent neutropenia, and concurrent infections are significant contributing factors to this overall mortality rate. Optimal therapy has not yet been established. Until recently, however, most patients have received amphotericin B [2]. As with all fungal infections, the initial step is to decrease or reverse the immunocompromised state. In vitro susceptibility studies demonstrate that the organism is susceptible to amphotericin B (MIC_{90} 0.12 μg/ml), and less susceptible to azoles such as fluconazole and ketoconazole (0.04–32 μg/ml), but appears to be susceptible to itraconazole and voriconazole. Most isolates are resistant to flucytosine [2, 15] (Table 9.3). The current recommendation is to use amphotericin B at a dose of 1–1.5 mg/kg/day [1–6]. However, since the newer azoles, voriconazole and posaconazole have demonstrated good in vitro activity that they may also be suitable alternatives [69].

Sporobolomyces

Sporobolomyces are yeast-like organisms that belong to the family Sporobolomycetaceae. These yeast are found throughout the world in soil, bark, and decaying organic material. They have occasionally been associated with infections in humans. There are seven known species of *Sporobolomyces,* but only three have been documented to cause disease, *S. salmonicolor, S. holsaticus,* and *S. roseus*. To date, there have been only six documented cases of *Sporobolomyces* infections; a nasal polyp, one case of dermatitis, one case of infected skin blisters, one case of mycetoma, and two cases of disseminated infection in patients with AIDS (lymph node and bone marrow) [2, 70]. In vitro susceptibility studies show that *S. salmonicolor* is susceptible to amphotericin B and the imidazoles [1, 2, 15, 69, 70] (Table 9.3). Despite the fact that these organisms are saprophytic, the case reports indicate their potential ability to produce invasive infection in humans, especially, in a compromised host.

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Aspergillosis

10

Helen W. Boucher and Thomas F. Patterson

Introduction

Aspergillosis is caused by *Aspergillus,* a hyaline mold responsible not only for invasive aspergillosis but also for a variety of noninvasive or semi-invasive conditions. These syndromes range from colonization to allergic responses to *Aspergillus,* including allergic bronchopulmonary aspergillosis (ABPA), to semi-invasive or invasive infections, spanning a spectrum from chronic necrotizing pneumonia to invasive pulmonary aspergillosis.

The genus *Aspergillus* was first recognized in 1729 by Micheli, in Florence. He described the resemblance between the sporulating head of an *Aspergillus* species and an aspergillum used to sprinkle holy water. In 1856, Virchow published the first complete microscopic descriptions of the organism [1].

The frequency and severity of invasive fungal infections in immunocompromised patients have increased steadily over the past three decades with the growing population of patients undergoing transplantation and the persistent challenges in preventing, diagnosing, and treating these infections [2]. Mortality due to documented invasive aspergillosis approaches 80–100% in high-risk patients, including those with underlying hematologic malignancy, bone marrow, or solid organ transplantation, and may be related to several factors, including diagnostic and therapeutic inadequacies [2–5]. Apart from organ transplant recipients, individuals with AIDS and patients hospitalized with severe illnesses, major increases in invasive fungal infections have been observed in patients with hematologic malignancies who receive induction or consolidation chemotherapy and those who undergo hematopoietic stem cell transplantation (HSCT) [5].

Successful therapy depends not only on an early diagnosis—which is often difficult to establish—but, even more importantly, on reversal of underlying host immune defects, such as neutropenia or high-dose immunosuppressive therapy [2]. Nonculture-based tests and radiological approaches can be used to establish an early diagnosis of infection and may result in improved outcomes of infection [2, 6, 7]. Even when a therapy begins promptly, efficacy of the therapy is poor, particularly in patients with disseminated or central nervous system disease [2, 3, 5]. Recent developments include more widespread use of newer diagnostic approaches and improved understanding of how best to use available antifungal agents [8].

Etiologic Agents

Aspergillus fumigatus is one of the most ubiquitous of the airborne saprophytic fungi [9]. *A. fumigatus* has emerged worldwide as a frequent cause of nosocomial infection and may be regarded as the most important airborne pathogenic fungus [9]. As *Aspergillus* species can be readily found in the environment, invasive aspergillosis is widely believed to occur as a consequence of exogenous acquisition of the conidia (spores) of the species [9]. The most common route of transmission of *Aspergillus* infection is the airborne route. *Aspergillus* conidia are resilient and may survive for long periods in fomites (any substance that can absorb, retain, and transport infectious species, e.g., woolen clothes or bedding) [10]. *Aspergillus* infection occurs less frequently through damaged mucocutaneous surfaces (e.g., following surgery or through contaminated dressings). However, the sources of *Aspergillus* may be broader than have traditionally been thought, as waterborne transmission of *Aspergillus* conidia through contaminated aerosols has been suggested [11].

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The most common species causing invasive aspergillosis include: *A. fumigatus* (Fig. 10.1), by far the most common, *A. flavus, A. terreus,* and, less commonly for invasive infection, *A. niger* [5] (Table 10.1). Recent studies have shown emergence of less common species, including *A. terreus* (which is frequently resistant to polyenes) and other unusual less pathogenic species as the etiologic agents of invasive infection [12].

Epidemiology

The incidence of invasive aspergillosis has increased substantially during the past few decades because of the use of more intensive cytotoxic anticancer chemotherapy and the introduction of novel immunosuppressive therapies for organ transplant recipients, both of which have prolonged the period of risk for many individuals. The increasing number of patients undergoing solid organ, bone marrow, and hematopoietic stem cell transplantation, and the implementation of aggressive surgical interventions has also contributed to the increased incidence [9]. The changes in epidemiology of invasive aspergillosis may also be the result of growing awareness of aspergillosis among clinicians, the introduction of noninvasive diagnostic tools and improved microbiological laboratory techniques.

Invasive fungal infections are an important cause of morbidity and mortality among patients with severely compromised immune systems. Although there have been significant advances in the management of immunosuppressed patients, invasive aspergillosis remains an important life-threatening complication, and is the leading cause of infection-related mortality in many immunocompromised individuals [13].

Immunosuppression and breakdown of anatomical barriers, such as the skin, are the major risk factors for fungal infections [7]. Individuals at risk for invasive aspergillosis include those with severely comprised immune systems as a result of anticancer chemotherapy, solid organ or bone marrow transplantation, AIDS, or use of high-dose corticosteroids. Patients with hematological disorders, such as prolonged and severe neutropenia, those undergoing transplantations, and those treated with corticosteroids and newer immunosuppressive therapies such as the tumor necrosis factor-α antagonists (e.g., inflixamab) are considered to be at highest risk for invasive aspergillosis [7, 14].

Pathogenesis and Immunity

Invasive aspergillosis most frequently originates via inhalation of *Aspergillus* conidia into the lungs, although other routes of exposure, such as inhalation of water aerosols contaminated with *Aspergillus* conidia have been suggested [11].

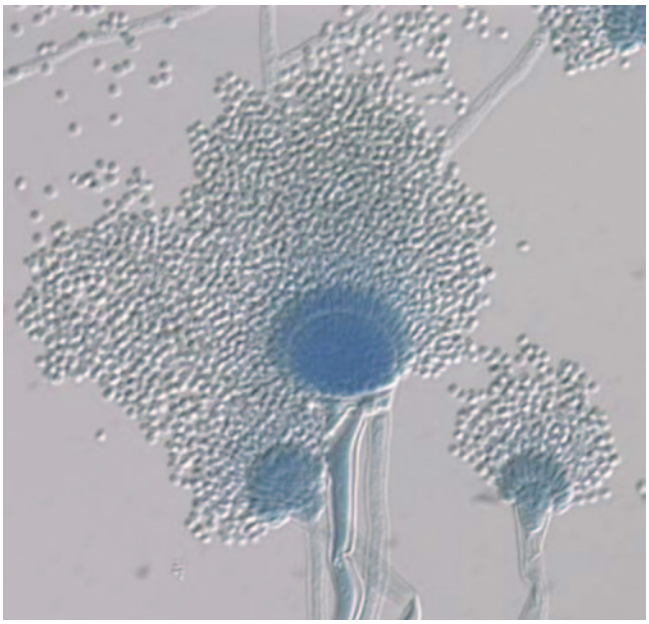

Fig. 10.1 Microscopic morphology of *Aspergillus fumigatus* showing a single role of phialides (uniseriate) bearing smooth conidia in a columnar fashion. (Courtesy of www.doctorfungus.org)

In the absence of effective pulmonary host defenses, the inhaled small resting conidia enlarge and germinate, then transform into hyphae with subsequent vascular invasion and eventual disseminated infection. The incubation period for conidial germination in pulmonary tissue is variable, ranging from 2 days to months [15]. Hydrocortisone significantly increases the growth rates of *Aspergillus*; likely one of the reasons corticosteroids pose a risk factor for invasive disease [16].

Although infection in apparently normal hosts can occur, invasive aspergillosis is extremely uncommon in immunocompetent hosts [5]. Normal pulmonary defense mechanisms usually contain the organism in a host with intact pulmonary defenses. The first line of defense against *Aspergillus* is ciliary clearance of the organism from the airways and limited access to the alveoli due to conidia size. This feature is one reason for the increased pathogenicity of *A. fumigatus* as compared with other species of *Aspergillus* [16]. Once conidia reach the alveoli, pulmonary macrophages are generally capable of ingesting and killing *Aspergillus* conidia [17]. When macrophages fail to kill the conidia (e.g., high-fungal inoculum, decreased number or function of macrophages), conidia germinate and begin to form hyphae. Polymorphonuclear leukocytes are recruited via complement activation and production of neutrophil chemotactic factors and extracellularly kill both swollen conidia and hyphae [18]. Antibodies against *Aspergillus* are common due to the ubiquitous nature of the organism, although they are not protective nor

Table 10.1 Characteristics of common *Aspergillus* species

Aspergillus species	Mycological characteristics	Clinical significance	Mycoses
A. flavus	Olive to lime green colonies	Second most common species, produces aflatoxin, may be less susceptible to polyenes	Sinusitis, cutaneous infection, pulmonary, and disseminated disease
A. fumigatus	Smoky, blue- or gray-green, small, smooth conidia (2–2.5 μm)	Most common species causing invasive infection	Invasive pulmonary aspergillosis, disseminated infection, CNS, others
A. niger	Typically black colonies, radiate conidial head, large rough conidia	Common cause of otomycosis, produces oxalate crystals which may be seen in host	Otomycosis, cutaneous, endophthalmitis, aspergilloma, invasive pulmonary, or disseminated disease less common
A. terreus	Beige to buff colonies, globose accessory conidia along hyphae	Increasing frequency, associated with soil, usually resistant to polyenes	Pulmonary, disseminated, cutaneous, keratitis, CNS
A. lentulus	Poorly sporulating variant of A. fumigatus	May be multidrug resistant, recently described variant, may be underdiagnosed	Invasive pulmonary, disseminated, other sites

CNS central nervous system

are they useful in the diagnosis of infection in high-risk patients due to the lack of consistent seroconversion following exposure or infection [19].

Corticosteroids play a major role in increasing susceptibility to *Aspergillus* by decreasing oxidative killing of the organism by pulmonary macrophages and by increasing the linear growth rate by as much as 30–40% and cell synthesis by more than 150% [16].

Many *Aspergillus* species produce toxins including aflatoxins, ochratoxin A, fumagillin, and gliotoxin. Gliotoxin works in several ways to help evade host defenses:

- Inhibition of phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation (key in host defense (versus filamentous fungi)
- Inhibition of macrophage ingestion of *Aspergillus*
- Suppression of functional T cell responses [20, 21]

In tissues, invasive aspergillosis causes extensive destruction across tissue planes via vascular invasion with resulting infarction and necrosis of distal tissues.

Clinical Manifestations

The clinical syndromes associated with aspergillosis are diverse, ranging from allergic responses to the organism including allergic bronchopulmonary aspergillosis (ABPA), asymptomatic colonization, superficial infection, and acute or subacute, and chronic invasive disease. The clinical presentation generally corresponds to the underlying immune defects and risk factors associated with each patient group, with greater immune suppression correlating with the increased risk for invasive disease. Although this chapter focuses on invasive aspergillosis, a brief description of other presentations follows. The reader is encouraged to refer to other sources for more in-depth discussion of those conditions [1].

Allergic Bronchopulmonary Aspergillosis

ABPA is a chronic allergic response to *Aspergillus* characterized by transient pulmonary infiltrates due to atelectasis. The incidence of ABPA is estimated to range from 1 to 2% in patients with persistent asthma and approximately 7% (with a range from 2 to 15%) of patients with cystic fibrosis [22]. Specific criteria are used to establish the diagnosis of ABPA as no single finding is diagnostic for the condition, although some presentations, like central bronchiectasis in patients with asthma highly suggest the diagnosis [22–24]. ABPA typically progresses through a series of remissions and exacerbations but can eventually lead to pulmonary fibrosis, which is associated with a poor long-term prognosis [24]. Management of ABPA is directed at reducing acute asthmatic symptoms and avoiding end-stage fibrosis. Corticosteroid therapy is commonly used for treating exacerbations although few randomized trials have been conducted for their use [25]. The role for antifungal therapy was evaluated with a randomized, double-blind, placebo-controlled trial that showed itraconazole at 200 mg per day for 16 weeks significantly reduced daily corticosteroid use, reduced levels of immunoglobulin E (IgE), and improved exercise tolerance and pulmonary function [23, 26].

Aspergilloma

A pulmonary fungus ball, due to *Aspergillus* or "aspergilloma," is a solid mass of hyphae growing in a previously existing pulmonary cavity, typically in patients with chronic lung disease, such as bullous emphysema, sarcoidosis, tuberculosis, histoplasmosis, congenital cyst, bacterial lung abscess, or, very rarely, in a pulmonary bleb from *Pneumocystis* pneumonia in AIDS [27, 28]. On chest radiograph,

a pulmonary aspergilloma appears as a solid round mass in a cavity. In many patients, the fungus ball due to *Aspergillus* remains asymptomatic, but in a significant number, hemoptysis occurs and can be fatal [29]. Surgical resection is considered as the definitive therapy, but the dense pleural adhesions adjacent to the fungus ball and the poor pulmonary reserve of most patients with this condition, makes surgery hazardous. Contamination of the pleural space with *Aspergillus* and the common complication of bronchopleural fistula in the postoperative period can lead to chronic *Aspergillus* empyema. Dense adhesions make pleural drainage difficult, often requiring pleural stripping or an Eloesser procedure, further compromising lung function [29].

Aspergillus can also be associated with fungal balls of the sinuses without tissue invasion [28]. The maxillary sinus is the most common site for a sinus aspergilloma to occur [28]. Clinical presentation is similar to that for any chronic sinusitis. Management is usually directed at surgical removal and a generous maxillary antrostomy for sinus drainage, along with confirmation that invasive disease has not occurred.

Other Superficial or Colonizing Syndromes

Other superficial or colonizing syndromes of aspergillosis include otomycosis, a condition of superficial colonization typically due to *A. niger* [30]; onychomycosis which, although rare, can become chronic and respond poorly to antifungal agents [31]; and keratitis, particularly following trauma or corneal surgery [32].

Chronic Pulmonary Aspergillosis

Denning et al. have described three distinct syndromes of chronic pulmonary aspergillosis in order to better characterize those patients who develop chronic pulmonary disease related to *Aspergillus* [33]. These conditions include chronic cavitary pulmonary aspergillosis, which is characterized by the formation and expansion of multiple cavities, which may contain fungus balls; chronic fibrosing aspergillosis, which as its name suggests involves extensive fibrosis; and chronic necrotizing aspergillosis or subacute aspergillosis, in which slowly progressive infection occurs usually in a single thin-walled cavity. In all of these conditions, the diagnosis is suggested by radiological and clinical features and the role for therapy remains speculative, although it appears that long-term antifungal therapy may be beneficial in a subset of patients, perhaps even with the extended spectrum triazole antifungals [33, 34].

Invasive Pulmonary Aspergillosis

Invasive pulmonary aspergillosis is the most common form of invasive aspergillosis in immunocompromised patients. This infection occurs following approximately 2 weeks of neutropenia [35] or during the course of graft versus host disease, now the most common risk factor in hematopoietic stem cell transplant recipients [36]. Symptoms include fever (may be absent in the presence of high-dose corticosteroid therapy), dry cough, shortness of breath, pleuritic chest pain, hemoptysis, as well as pulmonary infiltrates all of which lag behind disease progression. In lung transplant patients and those with AIDS, *Aspergillus* tracheobronchitis can present with cough, wheezing, and shortness of breath and chest radiographs show normal lungs with or without atelectasis [37].

Disseminated Aspergillosis

A variety of signs and symptoms are seen with disseminated invasive aspergillosis, based on the organs involved. The organs involved include kidneys, liver, spleen, and central nervous system (CNS; signs and symptoms of stroke or meningitis) most frequently, followed by the heart, bone, skin, and other organs [8]. Aspergillosis of the skin can occur either as a manifestation of disseminated disease or by direct extension from a local inoculation, for example, from an intravenous catheter [38].

Sinusitis

Aspergillosis of the sinuses presents clinically like rhinocerebral mucormycosis, but is more common in neutropenic patients than in those with diabetic ketoacidosis, and inflammatory signs may thus be less frequent. Fever, nasal congestion, facial pain can progress to visual changes, proptosis, and chemosis if the infection spreads to the orbit. Posterior extension to the brain can lead to cranial nerve palsies, other focal neurologic deficits, as well as a depressed level of consciousness [39].

Endocarditis

Aspergillus endocarditis is the second most common form of fungal endocarditis after that caused by *Candida* species and occurs in prosthetic valve recipients and in native cardiac valves in intravenous drug users and patients with indwelling central venous catheters [40]. Clinically, these patients present with fever and embolic complications. Blood cultures are rarely positive even with extensive disease [41].

Table 10.2 Diagnosis of invasive aspergillosis

Diagnostic method	Comment
Respiratory culture	Not frequently positive early in course of infection; positive result in high-risk patient (bone marrow transplant, neutropenia) highly correlates with infection; may indicate colonization in other populations (chronic pulmonary diseases, lung transplant)
Galactomannan	*Aspergillus* Platelia system (BioRad, Redmond, WA) with variable sensitivity—low (~40 %) with single samples or prior antifungal therapy, or prophylaxis; better yield with reduced threshold for positivity (>0.5), serial samples, testing on BAL samples. False positives historically with pipercillin–tazobactam, certain foods, neonates
1,3-β-D-glucan	Nonspecific detection of cell wall glucan. Commercially available Fungitell™ assay (Associates of Cape Cod, Falmouth, MA), limited validation and availability
PCR	Remains investigational due to lack of standardized reagents and methods, both false positives and negatives may occur, some recent studies have suggested less sensitive than other assays
Computed tomography	In high-risk patient, "halo" sign and/or pulmonary nodules without other documented cause may be a frequent and early sign of invasive pulmonary aspergillosis

BAL bronchoalveolar lavage, *PCR* polymerase chain reaction

Diagnosis

Current diagnostic modalities are limited and the clinician must rely on the combination of knowledge of risk factors, a high index of suspicion, clinical judgment, and the finding of fungi in tissue specimens and/or cultures from the presumed site of infection (Table 10.2). The diagnosis of proven invasive aspergillosis requires both tissue biopsy demonstrating invasion with hyphae and a culture positive for *Aspergillus* species [42]. *Aspergillus* produce hyaline, 3–6 μm wide septate hyphae that typically branch at acute angles [43] (Fig. 10.2). In tissue, these features can often distinguish *Aspergillus* from agents of mucormycosis, but they cannot distinguish *Aspergillus* from a large number of other opportunistic molds, including *Fusarium* and *Scedosporium* (*Pseudallescheria*). Thus, culture is needed to confirm the diagnosis [43]. Unfortunately, invasive, or even less invasive procedures like bronchoscopy, are often contraindicated in immunosuppressed patients, many of whom have low platelets due to chemotherapy and other complications. In this setting, positive culture can support the diagnosis of invasive aspergillosis.

Plain chest radiography is of limited utility in invasive aspergillosis as it has low sensitivity and specificity in this disease [6]. In contrast, chest CT scans have proven useful in early diagnosis of invasive pulmonary aspergillosis as the "halo sign" of low attenuation surrounding a pulmonary nodule, has successfully been used as a marker for early initiation of therapy in high-risk patients with neutropenia or who have undergone HSCT [44–46]. Of note, these radiographic findings are also consistent with other infections such as *Nocardia* species, and may increase over the first week of therapy even when the patient in improving; follow-up scans should be ordered and interpreted cautiously with full attention to the clinical progress of the patient [44].

Nonculture diagnostic tests have also been used to diagnose aspergillosis and in attempts to preempt difficult-to-treat proven disease. A sandwich enzyme immunoassay (EIA) that utilizes a monoclonal antibody to *Aspergillus* galactomannan (Platelia *Aspergillus*, BioRad, Redmond, WA) is approved for serum and bronchoalveolar lavage (BAL) fluid and is being used with varying success around the world [47–49]. Questions remain regarding the value of routine surveillance testing, frequency of testing, role of false-positive results (seen in solid organ transplant recipients, patients treated with piperacillin–tazobactam and other medications, and neonates), importance of prior antifungal therapy, and correlation of serum galactomannan results with clinical outcome [50].

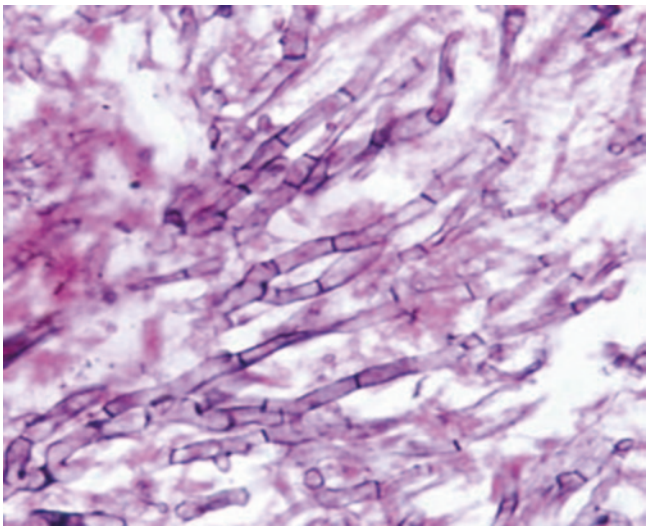

Fig. 10.2 Periodic acid–Schiff (PAS) stained tissue section of lung showing dichotomously branched, septate hyphae of *Aspergillus fumigatus*. (Courtesy of www.doctorfungus.org)

Several reports demonstrate the potential for using polymerase chain reaction (PCR) as an early diagnostic marker, which appears more sensitive than other methods including galactomannan [51, 52]. These assays may be associated with false-positive results due to the ubiquitous nature of *Aspergillus* conidia, are not standardized, and remain investigational at the present time [53–56].

Other nonculture-based methods for the diagnosis of invasive aspergillosis include detection of the nonspecific fungal marker 1,3-β-D-glucan using a variation of the *Limulus* amebocyte assay. This assay (Fungitell™, Associates of Cape Cod, Falmouth, MA) has been approved for diagnostic purposes by the Food and Drug Administration (FDA) and is a colorimetric assay that can indirectly determine the concentration of 1–3, β-D-glucan in serum samples [57]. The test appears promising as an indicator of infection due to many fungi, including *Aspergillus* and *Candida* but not *Cryptococcus* or Mucorales (which contain little or no β-D-glucan). One study suggested the utility of the assay in early diagnosis of invasive fungal infection in a leukemic population, but validation remains limited [58].

Interpretation of results is complicated with frequent false-positive β-D-glucan results, as well as reports of "interfering substances," hemodialysis with cellulose membranes, intravenous immunoglobulin, albumin, gauze packing of serosal surfaces, intravenous amoxicillin–clavulanic acid (not available in the USA) [59], and bloodstream infections with certain bacteria, such as *Pseudomonas aeruginosa* [60, 61]

Treatment

The goals of treatment of patients with invasive aspergillosis are to control infection and to reverse any correctable immunosuppression. Patients at high risk of developing invasive aspergillosis should be treated based on clinical or radiological criteria alone if microbiological or histological diagnosis would significantly delay treatment [2].

Treatment of *Aspergillus* infection is challenging due to difficulty in diagnosis, the presence of advanced disease in many by the time of diagnosis, and the presence of severe, often irreversible, immunosuppression. Mortality rates are high in patients with invasive aspergillosis and the efficacy of currently available treatments is limited by spectrum of activity, extensive drug–drug interactions, and serious toxicity. Treatment failure with currently available antifungal medication in patients with invasive aspergillosis has been reported to be 40% or higher in some series [3, 4]. Antifungal therapies with activity against *Aspergillus* include broad-spectrum triazoles (voriconazole, posaconazole, and isavuconazole), lipid formulations of amphotericin B, and the echinocandins (caspofungin, micafungin and anidulafungin), all of which offer options for therapy of this disease [62, 63] (Table 10.3). Guidelines developed by the Infectious Diseases Society of America and the American Thoracic Society provide summaries of existing data as well as recommendations. Of note, relatively few randomized trials of therapy for invasive aspergillosis have been completed so many recommendations stem from nonrandomized and noncomparative studies, as well as expert consensus [2].

Azoles

Voriconazole is a potent, broad-spectrum triazole that has fungicidal activity against many *Aspergillus* species, including *A. terreus*, is approved for therapy of invasive aspergillosis, and has replaced amphotericin as the recommended primary therapy for patients with invasive aspergillosis [2, 62, 64]. This recommendation is based on data from a randomized trial that compared voriconazole to conventional amphotericin B for the primary treatment of invasive aspergillosis, with each agent followed by other licensed antifungal therapy if needed for intolerance or progression of disease, in severely immunocompromised patients with invasive aspergillosis [45]. In this trial, voriconazole was superior to amphotericin B with successful outcomes in 52% of patients as compared to only 31% in those receiving amphotericin B. In addition, voriconazole demonstrated a survival advantage to amphotericin B with an absolute 13% difference in mortality between treatment groups.

In clinical trials, voriconazole has been adequately tolerated and the drug exhibits a favorable pharmacokinetic profile. There are a number of issues to consider, including important drug interactions, especially those with immunosuppressive agents, such as cyclosporine, tacrolimus, and sirolimus, the latter of which is contraindicated for use with voriconazole, and intolerance to the drug. The most common adverse event has been a transient and reversible visual disturbance described as an altered perception of light which has been reported in approximately 30% of the treated patients, but has not been associated with pathological changes [45]. Other adverse events include liver function test abnormalities in 10–15%, and skin rash in 6% (sometimes associated with sun exposure). Long-term voriconazole therapy has been associated with skin cancer and periostitis related to high fluoride levels [65–67].

Both toxicity (e.g., liver function abnormalities and CNS side effects increase with higher levels) and efficacy (i.e., poorer outcomes with lower levels) have been associated with voriconazole concentrations. As voriconazole metabolism varies between patients and is affected by so many relevant drug–drug interactions, many advocate the use of therapeutic drug monitoring. In the pivotal aspergillosis trial, serum concentrations between 2 and 5.5 mcg/mL were associated with successful outcomes. A more recent study suggests achieving serum trough concentrations of >1 mcg/mL and <5.5 mcg/mL [68, 69]. In patients with documented invasive aspergillosis, we recommend concentrations between 2 and 5.5 mcg/mL.

Itraconazole is approved for use as salvage therapy of aspergillosis. Its utility has been limited due to the fact that the only reliably absorbed formulation is an oral solution as its intravenous formulation is no longer marketed in the USA [70]. For these reasons, itraconazole is more frequently used

Table 10.3 Antifungal agents for treating invasive aspergillosis

Agent	Typical dose/route of administration	Comments
Azole		
Voriconazole	6 mg/kg IV q12 h x 2 doses, then 4 mg/kg IV q12 h; 200 mg PO bid (weight-based dosing should be considered)	Better efficacy and improved survival compared with amphotericin B deoxycholate; current recommended primary therapy for invasive aspergillosis; drug interactions common, hepatic toxicity (10–15 %) may be dose limiting; visual effects common (~30 %) but not usually dose limited and no long-term toxicity reported [98]
Itraconazole	200 mg tid for 3 days, then 200 mg PO bid (oral solution)	Second-line agent for invasive aspergillosis; erratic bioavailability, improved with oral solution; drug interactions including chemotherapeutic agents; intravenous formulation no longer available [2]
Posaconazole	Oral solution—200 mg PO qid loading, 400 PO bid maintenance; extended release tablets—300 mg bid x 2 doses, then 300 mg daily; intravenous—300 mg bid x 2 doses, then 300 mg daily	Recommended for salvage therapy; FDA approved for prophylaxis; P450 drug interactions; limited metabolism with favorable tolerance in clinical studies [2, 99]
Isavuconazole	Investigational	Full clinical development underway
Ravuconazole	Investigational	In vitro activity, but limited clinical development at present [63]
Polyene		
Amphotericin B deoxycholate	1.0–1.5 mg/kg IV daily	Prior "gold standard"; associated with significant toxicity and limited efficacy in severely immunosuppressed patients [100]
Liposomal amphotericin B	3–6 mg/kg IV daily	Alternative primary therapy; well tolerated; limited nephrotoxicity or infusion-related reactions; anecdotal reports of efficacy with higher doses (7.5 mg/kg/d or more)
Amphotericin B lipid complex	5 mg/kg IV daily	Indicated for salvage therapy or intolerance to standard agents, generally well tolerated [101]
Amphotericin B colloidal dispersion	3–6 mg/kg IV daily	Less nephrotoxic than amphotericin B deoxycholate, but associated with more infusion-related and pulmonary toxicity than other lipid formulations [81]
Echinocandin		
Caspofungin	70 mg x 1 dose, then 50 mg IV daily	Indicated for salvage therapy of aspergillosis, experimental and clinical data for use in combination therapy; well tolerated [84]
Micafungin	Investigational for aspergillosis (IV)	Used in doses of 100 mg/d in salvage studies; 50 mg/d for prophylaxis; well tolerated [102]
Anidulafungin	Investigational for aspergillosis (IV)	In vitro activity; studied at doses of 100 mg/d after 200 mg loading dose in other fungi; well tolerated [103]

IV intravenous, *PO* orally, *bid* twice daily, *qid* four times daily

in less immunosuppressed patients who are able to take oral therapy and for use as sequential oral therapy [5].

Posaconazole is FDA approved for prophylaxis of fungal infections in neutropenic patients and for the treatment of mucocutaneous candidiasis. It has also been studied in patients who failed to tolerate or had fungal infections refractory to standard therapy [71]. In 2005, Posaconazole was approved in the EU for salvage therapy of invasive aspergillosis. Initially available only as an oral suspension, in 2013, the FDA approved delayed release tablets with higher absorption and less dependency on having a full stomach, and in 2014 an intravenous formulation was approved. Gastrointestinal side effects are common, including stomach upset. Currently, posaconazole is recommended as a consideration in salvage therapy.

Other second-generation triazoles, including isavuconazole and ravuconazole, were developed with an expanded spectrum of activity to include *Aspergillus* [64, 72]. Isavuconazole is in phase 3 development and studies of aspergillosis have been completed. Ravuconazole has been evaluated in early phase clinical trials and has also shown activity in animal models of invasive aspergillosis [73].

Polyenes

Amphotericin B deoxycholate was the previous "gold standard" therapy in patients with invasive aspergillosis [2]. A number of studies documented the limited efficacy and substantial toxicity with amphotericin B deoxycholate in high-risk patients [45, 74, 75]. The overall response rates of amphotericin B deoxycholate are less than 25 %, with responses of only 10–15 % in more severely immunosuppressed patients [5, 45]. Wingard et al. documented increased morbidity and mortality associated with conventional amphotericin B (amphotericin B deoxcholate) in patients receiving bone marrow transplantation and those receiving concomitant nephrotoxic agents [75]. Similar

findings were documented by Bates et al. who found that renal toxicity occurred in approximately 30% of the patients receiving conventional amphotericin B and that this toxicity was associated with sixfold increase in mortality as well as a dramatic increase in hospital costs [74]. These unacceptably high mortality rates and significant toxicities highlighted the need for new therapeutic approaches in this disease.

The lipid formulations of amphotericin B were developed to decrease toxicity and allow the administration of higher doses of drug [76, 77]. To date, few comparative studies of the efficacy of lipid formulations of amphotericin B in treating invasive aspergillosis have been conducted though studies of these drugs as salvage therapy led to the approval of three lipid formulations [78]. Clinical experience has nevertheless been favorable, which is consistent with preclinical studies in animal models [79]. One small study by Leenders et al. compared liposomal amphotericin B at 5 mg/kg/d to standard amphotericin B at 1.0 mg/kg/d for proven or suspected invasive mycoses [80]. Overall outcomes of both groups in this small study were similar but analysis of patients with proven invasive aspergillosis favored the lipid preparation of amphotericin B. Another study evaluated amphotericin B colloidal dispersion for primary therapy for invasive aspergillosis [81]. In this study of severely immunosuppressed patients with invasive aspergillosis, success rates with the lipid formulation were not better than those for conventional amphotericin B although toxicity was minimally decreased. While lipid formulations of amphotericin B are dramatically more expensive than standard amphotericin B, hidden costs of standard amphotericin B in terms of morbidity and mortality as well as resource utilization justify the use of lipid formulation of amphotericin B in most patients with invasive infection except in resource-limited settings where the lipid formulations are cost prohibitive [74].

The optimal dose of lipid formulations of amphotericin B remains controversial. A small observational study suggested that using higher doses of lipid formulations of amphotericin B results in better response rates [82]. A double-blind trial in patients with confirmed aspergillosis, most with hematologic malignancy and neutropenia, compared the efficacy of 10 mg/kg per day versus 3 mg/kg per day dosing for the first 14 days of treatment, followed by receipt of 3 mg/kg/day [83]. Patients treated with higher initial doses experienced more nephrotoxicity and success rates were similar. Based on these data, liposomal amphotericin B at 3 mg/kg/day is recommend as alternative primary therapy for those patients unable to tolerate voriconazole or in whom voriconazole is contraindicated because of drug interactions or other reasons. Amphotericin B lipid complex (usually at initial doses of 5 m/kg once daily) is also a reasonable alternative [2].

Echinocandins

Echinocandins are natural cyclic hexapeptide antifungal compounds that noncompetitively inhibit 1,3 β-D-glucan synthase, an enzyme complex that is unique to a number of fungi, that forms glucan polymers in the fungal cell wall [63]. These agents are active against *Candida* species and *Pneumocystis*. Specific modifications to the N-acyl aliphatic or aryl side chains expand the antifungal spectrum to include *Aspergillus* [63]. These agents are all poorly bioavailable and produced in intravenous formulation only.

Caspofungin is approved for treating patients refractory to or intolerant of standard therapies for invasive aspergillosis based on an open-label trial that demonstrated therapeutic efficacy in 22 of 54 (41%) patients studied [84]. Caspofungin has been very well tolerated in clinical trials; in the aspergillosis study, only approximately 5% of patients discontinued therapy. Drug interactions with cyclosporine may occur, but have not been a significant issue [84, 85]. In March 2005, micafungin was approved for the treatment of esophageal candidiasis and prevention of *Candida* infections. In the one prophylaxis study, used to support this approval, micafungin may have reduced the number of *Aspergillus* infections as compared to standard prophylaxis with fluconazole [86]. Micafungin also demonstrated efficacy when used as salvage therapy and in prevention of invasive fungal infection in patients with hematologic malignancy at high risk due to neutropenia or graft versus host disease [86–88]. Anidulafungin is another echinocandin with activity against *Aspergillus* spp. that appears to have a favorable toxicity profile similar to the other echinocandins. It was approved by the FDA in February 2006 for candidemia and other *Candida* infections (including abdominal abscess, peritonitis, and esophagitis). Notably, these agents are neither classically fungicidal nor fungistatic for *Aspergillus,* but exert their effect on the growing hyphal tips where the glucan synthase target is located [89]. For this reason, they have not frequently been used for primary therapy where outcomes have been poor, and have been more frequently used as salvage therapy or more recently in combination regimens [90–92].

Combination Therapies and Therapeutic Approaches

Outcomes for patients with invasive aspergillosis remain poor despite the advent of newer antifungal agents. This together with the availability of several antifungal drugs and drug classes against *Aspergillus* has increased interest in combination antifungal therapy for this infection [93, 94]. Marr et al. reported on a historical control study of caspofungin and voriconazole compared with voriconazole alone in patients who failed amphotericin formulations in 2004.

In this study, the use of combination salvage therapy was associated with an improved 3-month survival rate [92]. In 2012, the same investigators presented results of a randomized trial of voriconazole versus voriconazole with anidulafungin for the treatment of invasive aspergillosis in patients with hematologic malignancies and/or hematopoietic cell transplant in abstract form [95]. Among the 277 patients with proven or probable invasive aspergillosis, 6-week mortality was 19.3% for combination therapy patients and 27.5% for those treated with voriconazole monotherapy (95% CI −19.0 to 1.5). A post-hoc analysis of patients with probable invasive aspergillosis showed a significant difference in mortality (16% with combination therapy versus 27% with voriconazole monotherapy; 95% CI −22.7 to −0.4). Most current guidelines do not recommend initial combination therapy, but these results suggest that some subgroups of patients may benefit from such an approach. Based on these data, current recommendations are to consider combination therapy in patients who fail to respond to initial therapy and in select patients as primary therapy.

Preventative strategies include prophylaxis and targeted preemptive therapy in high-risk patients. Two large randomized clinical trials in patients with graft versus host disease and in acute leukemia or myelodysplastic syndromes showed the benefit of posaconazole prophylaxis in those patients, with improved survival and decreased invasive mycoses, including aspergillosis [87, 88]. Other strategies include intensive use of diagnostic tools in conjunction with early antifungal therapy in order to reduce the number of invasive fungal infections. A full discussion is beyond the scope of this chapter.

Adjuvant therapies, including surgical resection or use of granulocyte transfusions and growth factors, in invasive aspergillosis can augment antifungal therapy, although their utility has not been established in randomized trials. In older studies, surgical resection of isolated pulmonary nodules prior to additional immunosuppressive therapies was shown to improve outcome of the infection. With the use of newer, more effective therapies, like voriconazole, resection may not be necessary or indicated [6, 96]. Recent studies also suggest that the majority of patients will have bilateral infection when the diagnosis is first made, limiting the utility of this approach. Surgical resection may be most appropriate in patients with severe hemoptysis or with lesions near the hilar vessels or pericardium.

Summary

In summary, prompt diagnosis and aggressive initial therapy remain critical in improving the outcome of this infection [97]. Radiography and use of galactomannan EIA may facilitate an early detection of aspergillosis in high-risk patients, for whom outcomes are especially poor [46]. Primary therapy with voriconazole is recommended in most patients [2, 45]. In patients who are intolerant of voriconazole, have a contraindication to the drug, or have progressive infection, alternative agents include lipid formulations of amphotericin B. The echinocandins or another triazole is available for salvage therapy [76, 79, 84]. Primary use of combination therapy is not recommended at the present, but the addition of another agent in a salvage setting may be considered, due to the poor outcomes of a single agent in progressive infection [92]. Sequential therapy with oral azoles after initial intravenous therapy may be a useful option [5]. Although the optimal duration of antifungal therapy is not known, improvement in underlying host defenses is crucial to successful therapy. While substantial advances have recently been made in the management of invasive aspergillosis, newer approaches to therapy including the potential of more targeted combination therapy and newer diagnostic tools are needed to improve the outcome of this disease.

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Hyalohyphomycosis: Infection Due to Hyaline Moulds

11

Duane R. Hospenthal

Introduction

Hyalohyphomycosis is a designation used to describe fungal infection caused by moulds with hyaline (clear or light colored) septate hyphae as seen microscopically in clinical samples. This terminology is similar to the use of phaeohyphomycosis to designate infections caused by fungi with dark-colored hyphae (Chap. 12). Perhaps the best use of this terminology is to describe diseases where hyaline hyphal elements are seen on smear or histopathology, but a specific fungus is not recovered by culture. Hyalohyphomycosis commonly includes infections caused by species of *Fusarium*, *Pseudallescheria*, *Scedosporium*, *Paecilomyces*, *Purpureocillium*, *Acremonium*, *Gliomastix*, *Sarocladium*, *Penicillium*, *Scopulariopsis*, *Beauveria*, and *Trichoderma*. Although *Aspergillus* also produces hyaline septate hyphae, and is considered a hyalohyphomycetes, infections secondary to this genus are typically termed aspergillosis and discussed separately as in this text (Chap. 10). *Scedosporium* and *Scopulariopsis* are commonly included in the hyalohyphomycoses, but some species produce dark hyphae and may be included in the phaeohyphomycoses. While *Penicillium marneffei* also produces hyaline hyphae, its dimorphic nature and geographical localization make placing it in the endemic mycoses also correct.

This group of fungi may cause superficial or localized infection in immunocompetent hosts (usually as a result of direct inoculation of the fungus following trauma) and invasive or disseminated infections in immunocompromised hosts. In the latter setting, the clinical infection may be indistinguishable from that of invasive aspergillosis. A remarkable feature of some of these hyaline moulds is their ability to cause fungemia (and thus be diagnosed with blood culture) and to disseminate hematogenously causing numerous embolic skin lesions. These infections may be clinically suspected on the basis of a constellation of clinical and laboratory findings. Definitive diagnosis requires isolation of these fungi from culture (or molecular methods) or from tissue. Identification to species level is ideal as these fungi have variable susceptibility to antifungal agents (Table 11.1). An important component of therapy of localized infection is surgical excision or debridement and removal of infected prosthetic devices. Outcome is usually favorable in immunocompetent hosts, but remains poor in the setting of persistent profound immunosuppression. Guidelines for the diagnosis and management of these fungal infections have recently been published [1].

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Fusarium Species

Fusarium species have emerged as a common cause of disseminated fungal infections in neutropenic patients and those undergoing allogeneic hematopoietic stem cell transplantation (HSCT). *Fusarium* represents the second most common fungal pathogen, after *Aspergillus*, as the cause of life threatening infection in recipients of hematopoietic transplant [2]. Because of this, *Fusarium* infections are sometimes discussed separately from hyalohyphomycosis as fusariosis. *Fusarium* causes a broad spectrum of infections in humans, including superficial and local infections in immunocompetent hosts; disseminated infections are seen almost exclusively in immunosuppressed patients.

Etiologic Agents

Four species are most commonly involved in human infections: *F. solani* (most common), *F. oxysporum*, *F. verticillioides* (*moniliforme*), and *F. proliferatum* [3]. *F. solani* species complex also includes *F. falciforme* (formerly *Acremonium falciforme*) and *F. lichenicola* (formerly *Cylindrocarpon lichenicola*). *Fusarium* species are septate filamentous

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Table 11.1 Overall susceptibility of hyaline fungi to available antifungals[a]

Genus/Species	AmB	Itraconazole	Voriconazole	Posaconazole	Anidulafungin	Caspofungin	Micafungin	Ref.
Fusarium spp.	++	+	++b	++	0	0	ND	[59, 60]
F. oxysporum	++	+	++++	++	ND	ND	ND	[59, 61]
F. solani	+	+	+	+	ND	ND	ND	
Scedosporium spp.	0	0	0	0	0	ND	ND	[60]
S. prolificans *S. apiospermum*	0	++	++++	+++	0	0	ND	[60, 61]
Paecilomyces spp.	++	+++	+++	+++	++	+++	ND	[59, 60]
Acremonium spp.	++	0	++	ND	0	ND	ND	[60]
Scopulariopsis spp.	0	0	0	0	+	+	+++	[55]
Trichoderma spp.	++	0	+	0	+++	+++	+++	[58]

++++, drug of choice; +++, alternative choice; ++, some strains are susceptible (<50%); +, rarely susceptible (<10%); 0, always resistant; ND, no data available

[a] Susceptibility based on MIC_{50}

[b] In USA, the only agent indicated for invasive fusariosis

fungi that produce conidiophores, phialides, macroconidia, and microconidia. *Fusarium* species are readily and rapidly recovered in almost all fungal media. On potato dextrose agar (PDA), the colonies have a velvety or cottony surface, and are white, yellow, pink, purple salmon, or gray on the surface, with a pale red, violet, brown, or sometimes blue reverse. The characteristic sickle- or banana-shaped multiseptate macroconidia with a foot cell at the base are used in identifying the genus and species of *Fusarium* (Fig. 11.1). Molecular methods may also be used for rapid identification of *Fusarium* to the species level. In tissue, the hyphae are similar to those of *Aspergillus* species, with hyaline and septate filaments that typically dichotomize in acute and right angles. In the absence of microbial growth, distinguishing fusariosis from aspergillosis and other hyalohyphomycoses is difficult, and requires the use of in situ hybridization in paraffin-embedded tissue specimens [4]. *Fusarium* species are toxigenic, and may cause mycotoxicosis in animals and humans [3].

Fig. 11.1 *Fusarium oxysporum* macroconidia. Characteristic fusiform to sickle-shaped, multiseptate mostly with an attenuated apical cell and a foot-shaped basal cell. (Courtesy of www.doctorfungus.org © 2000)

Epidemiology

Fusarium is ubiquitous in soil and water, taking part in water biofilms, and is a human and plant pathogen [5]. *Fusarium* species are causative agents of superficial and localized infections in immunocompetent hosts, most commonly onychomycosis and cutaneous and subcutaneous infections, including mycetoma and keratitis, the latter in contact lens wearers [6]. A recent large outbreak of *Fusarium* keratitis was reported in contact lens wearers in the USA and was linked to contaminated contact lens rinse solutions. Other risk factors for keratitis are trauma and use of topical corticosteroids and antibiotics [7]. *Fusarium* endophthalmitis may arise from keratitis or by direct inoculation after cataract surgery or trauma [8]. Fusariosis may also result from skin breakdown, such as burns and wounds, or the presence of foreign bodies, such as peritonitis in patients receiving continuous ambulatory peritoneal dialysis (CAPD), catheter-associated fungemia, and thrombophlebitis [9–11]. A hospital outbreak of *F. verticillioides* fungemia in immunocompetent patients has been recently reported [12]. Other infections include sinusitis, pneumonia, cutaneous and subcutaneous infections, septic arthritis, and osteomyelitis [13–17].

Immunosuppressed patients may develop locally invasive and disseminated fusariosis [18]. Risk factors include prolonged neutropenia, such as following chemotherapy for acute leukemia, and T cell immunodeficiency, which occurs most commonly after HSCT [18–20]. In HSCT, infection may develop early during neutropenia or months after neutrophil recovery following the treatment of chronic extensive graft versus host disease (GvHD). Localized infections may also develop among solid organ transplant recipients (SOT), usually as a late infection [21].

Portals of entry are the respiratory tract and skin, the latter playing a significant role in patients with tissue breakdown such as onychomycosis. Hospital water systems are

a potential reservoir for *Fusarium*; transmission may occur from inhalation of conidia aerosolized in the shower or from direct contact of contaminated water with sites of skin breakdown [22–24].

Pathogenesis and Immunology

Similar to *Aspergillus*, this organism is highly angioinvasive and leads to tissue infarction. In contrast to *Aspergillus*, however, *Fusarium* is frequently isolated from the bloodstream, likely as a result of intravascular adventitious sporulation [25]. Phagocytes appear to be the predominant line of defense against fusarial infections [18–20].

Clinical Manifestations

Infection with *Fusarium* in immunocompetent hosts may be superficial or locally invasive, involving the skin, eyes, sinuses, lungs, and joints and bones. In immunosuppressed patients, the infection may be locally invasive, usually pneumonia and/or sinusitis, or more commonly disseminated [18–20]. The clinical picture resembles that of invasive aspergillosis. Unlike aspergillosis, however, fungemia and skin lesions are common (up to 40% of patients with disseminated disease). Skin lesions may represent the primary site of infection (onychomycosis) or secondary to disseminated infection [18–20]. Recovery of *Fusarium* from preexisting skin or nail lesions has been reported as a risk factor for invasive disease in high-risk patients [26]. Metastatic skin lesions evolve from subcutaneous painful lesions to erythematous induration followed by ecthyma gangrenosum-like necrotic center, which may be surrounded by a rim of erythema [18–20].

Diagnosis

Two characteristics suggest the diagnosis of disseminated fusariosis in the severely immunocompromised host: metastatic skin lesions and positive blood cultures for mould [18–20]. Definitive diagnosis relies on cultures (tissue and/or blood) and histopathology which show a pattern common to all hyalohyphomycosis (invasion by acute-branching, septate hyaline hyphae). The use of PCR techniques and/or in situ hybridization may be required to reach the correct diagnosis in tissues [4, 27]. The 1,3-β-D-glucan test is usually positive in invasive fusarial infections, but it cannot distinguish *Fusarium* from other fungal infections (*Candida*, *Aspergillus*, and others) which are also detected by the assay [28].

Treatment

Localized infections, particularly in immunocompetent hosts, usually respond well to treatment consisting of topical therapy for fungal keratitis or excision of involved tissue (sinuses, eye, soft tissue, bone). Removal of an infected intravascular catheter may be needed in the rare cases of catheter-related fungemia.

Outcome of invasive and disseminated fusariosis in immunosuppressed patients remains quite poor, but appears to have improved with the introduction of voriconazole and posaconazole [18–20, 29, 30]. Predictors of poor outcome are persistent neutropenia and recent therapy with corticosteroids for chronic GvHD [19]. Treatment options are limited by the lack of reliable and consistent activity of antifungal agents against *Fusarium* species. Susceptibility varies among the various species, with resistance seen to all three major classes of antifungal agents [31]. Rapid species identification may be helpful, but antifungal susceptibility testing should be considered because of this variable in vitro susceptibility among *Fusarium* species (Table 11.1). The echinocandins do not appear to be active against any *Fusarium* species

Pseudallescheria and *Scedosporium* Species

Previously, only two *Scedosporium* species, *S. apiospermum* (sexual state name, *Pseudallescheria boydii*) and *S. prolificans* (formerly *S. inflatum*), were described as human fungal pathogens [32, 33]. The fungi previously denoted *P. boydii* (asexual state name, *S. apiospermum*) have now been divided into at least four species—*P. boydii* (*S. boydii*), *P. minutispora*, *S. aurantiacum*, and *S. dehoogii* [34]. Some have called these newly described (renamed) fungi the *P.* boydii complex. In this text, we refer to this group as *P. boydii*. A spectrum of disease, ranging from respiratory tract colonization to superficial and deep infections, in both immunocompetent and immunosuppressed hosts, has been reported. Rarely, disseminated infection with high mortality is seen in the setting of severe immunosuppression. *S. prolificans* belongs to the group of fungi which causes phaeohyphomycosis (dematiaceous fungi), but will be briefly discussed because of its relation to *P. boydii*.

Etiologic Agents

Scedosporium species are identified by their characteristic macroscopic (wooly to cottony, dark gray to dark brown) and microscopic (characteristic conidia, conidiophores, and hyphae) appearance. *S. prolificans* is distinguished from *P. boydii* by the production of terminal annelloconidia with

inflated bases (cylindrical in *S. apiospermum*) and growth inhibition by cycloheximide or actidione. In tissue sections, *Pseudallescheria*/*Scedosporium* appear as septate hyaline hyphae that cannot be reliably distinguished from *Aspergillus* or *Fusarium* unless conidia are present.

Epidemiology

Pseudallescheria/*Scedosporium* have been isolated from soil, potting mix, compost, animal manure, and stagnant or polluted water. Infections occur worldwide, though a large number of reports come from Northern Spain [32]. Patients at risk for invasive and/or disseminated infection include those with HIV infection, acute leukemia, and recipients of allogeneic HSCT or SOT [35, 36].

Infection is thought to be secondary to direct inoculation (such as after trauma) or inhalation of airborne conidia. In normal hosts, *P. boydii* causes infection after penetrating trauma, including keratitis, endophthalmitis, cutaneous and subcutaneous infections, bursitis, arthritis, and osteomyelitis. Following near-drowning accidents, sinusitis, pneumonia, meningoencephalitis, and brain abscesses may develop. Allergic bronchopulmonary disease due to *P. boydii* may also occur. Like *P. boydii*, *S. prolificans* causes localized infections (usually of bone or soft tissue) in immunocompetent patients following trauma, and deeply invasive infections in immunocompromised hosts, sometimes as a nosocomial outbreak [32, 35, 36].

Clinical Manifestations

Mycetoma is the most common *P. boydii* infection in normal hosts (see Chap. 22), usually occurring after penetrating injury and presenting as lower extremity swelling with draining sinuses. Other infections include non-mycetoma cutaneous and subcutaneous infections, keratitis, and endophthalmitis [36]. Invasive *P. boydii* infection is usually seen in immunocompromised patients, most commonly as pneumonia. Disseminated infection is mainly associated with *S. prolificans*, and is characterized by refractory fever, pulmonary infiltrates (diffuse or nodular), central nervous system involvement (present in one third of patients), fungemia, renal failure, erythematous, and nodular skin lesions with central necrosis.

Diagnosis

The diagnosis relies on the combination of clinical signs and symptoms and recovery of *Pseudallescheria*/*Scedosporium* from blood and/or infected tissue, with or without demonstration of colorless septate hyphae.

Treatment

Localized infections, particularly in immunocompetent hosts, usually respond well to surgical debridement. *Pseudallescheria boydii* is resistant to fluconazole and flucytosine, but susceptible to the newer azoles—voriconazole, posaconazole, and ravuconazole (Table 11.1). Voriconazole is approved for use in *P. boydii* infections [37, 38]. Caspofungin appears to be more active than itraconazole or amphotericin B. Variable strain-to-strain susceptibility to amphotericin B can be seen [39]. Surgical resection remains the only definite therapy for *S. prolificans* infections, as this organism is resistant to all available antifungal agents in vitro. In vitro synergism between terbinafine and either voriconazole or itraconazole has been reported [40].

Paecilomyces and *Purpureocillium* Species

Paecilomyces and *Purpureocillium* species are frequent airborne contaminants in clinical microbiology laboratories but have been increasingly reported as cause of human infection.

Etiologic Agents

Two species, *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) and *Paecilomyces varioti*, account for most human infections. *Paecilomyces marquandii* and *P. javanicus* have also been reported to cause human disease. These fungi grow rapidly on various agar media including blood, chocolate, Sabouraud dextrose (SDA), and PDA. *P. varioti* is thermophilic and grows well at temperatures as high as 50 °C. The color of the colony and certain microscopic features help differentiate these fungal species from each other. The colonies are flat and velvety. The color is initially white and becomes yellow-green, yellow-brown, pink, or violet according to species.

Epidemiology

Paecilomyces and *Purpureocillium* are found worldwide in soil, food products, and water and causes infection in both immunocompetent and immunosuppressed patients. A strong association of these fungi with prosthetic implants may be due to their inherent resistance to most sterilizing techniques. Prosthetic implant-related infections include keratitis in contact lens wearers and after corneal implants (rarely endophthalmitis), and in recipients of CAPD, cardiac valves, and ventriculoperitoneal shunts. Other infections involve the nails, skin, subcutaneous tissues, bones and joints, sinuses, and lungs, while disseminated infections only occur in immunosuppressed patients [41].

Clinical Manifestations

The most common infections due to *Paecilomyces* and *Purpureocillium* involve the eye and eye structures (keratitis and endophthalmitis), followed by the nails (onychomycosis) and skin and soft tissues. Skin infections are characterized by erythematous macules, nodules, pustules, vesicular lesions, or necrotic crusts [41]. Sporotrichosis-like skin infection has also been described. Other reported infections in the competent host include peritonitis in CAPD, prosthetic-valve endocarditis, catheter-related fungemia, and arthritis/osteomyelitis. In immunocompromised patients, pneumonia and disseminated disease are most commonly observed.

Diagnosis

These fungi grow readily on routine fungal media, including SDA. Like all hyalohyphomycetes, they produce hyaline septate hyphae in tissue and can be seen with periodic acid–Schiff (PAS) staining in histopathology. Both may exist in various forms in tissue (conidia and phialides) and can therefore be misdiagnosed as candidiasis.

Treatment

Treatment of invasive *Paecilomyces* and *Purpureocillium* infections relies on surgical debridement and removal of infected prosthetic materials. Because of different susceptibilities to antifungal agents, these fungi should be identified to the species level (Table 11.1). *P. varioti* is susceptible to amphotericin B, flucytosine, itraconazole, voriconazole, and posaconazole, whereas *P. lilacinum* is only susceptible to the latter two triazoles [42].

Acremonium (and *Gliomastix* and *Sarocladium*) Species

Acremonium species are filamentous fungi of low pathogenicity commonly isolated from the environment (soil, insects, sewage, plants, and water) [43].

Etiologic Agents

Many species of *Acremonium* have been reported to cause human infection. Several of these have been transferred to the genera *Gliomastix* and *Sarocladium*. *A. strictum* is the most common of these species to cause of human infection, but infection has been attributed to *A. alabamensis*, *A. potronii*, *A. recifei*, *G. roseogrisea*, *S. kiliense*, and *S. strictum* [1]. One of the other more common species, *A. falciforme*, has been reclassified *Fusarium falciforme*. *Acremonium* species grow on SDA, forming white, salmon, or yellowish-green colonies that are usually velvety or cottony with slightly raised centers. This genus is distinguished by formation of narrow hyphae bearing solitary, unbranched needle-shaped phialides. Like other hyaline moulds, septate colorless hyphae are found in tissue.

Epidemiology

Most infections occur in immunocompetent hosts and include mycetoma following trauma, keratitis in contact lenses wearers, and endophthalmitis [43–45]. Fungal colonization of humidifier water in a ventilator system was thought to be the source of infections in an outbreak of endophthalmitis. Invasive disease is almost exclusively seen in immunocompromised patients.

Clinical Manifestations

Mycetoma is the most common infection due to *Acremonium* and presents in a manner similar to that of mycetoma caused by *S. apiospermum*. Keratitis and endophthalmitis constitute the second most common infections. Colonization of soft contact lenses may proceed to corneal invasion. Other reported infections include onychomycosis, peritonitis, dialysis fistulae infection, pneumonia, empyema, septic arthritis, osteomyelitis, meningitis (following spinal anesthesia in an otherwise healthy individual), cerebritis in an intravenous drug abuser, and prosthetic valve endocarditis. Disseminated infection occurs exclusively in immunosuppressed hosts and has been characterized by endocarditis, meningitis, and bloodstream infection. In vivo sporulation can occur, facilitating dissemination and perhaps explaining the high rate of metastatic skin lesions and positive blood cultures with *Acremonium*.

Diagnosis

Acremonium species grow slowly on SDA. Hence, cultures must be kept at least 2 weeks. Blood cultures may isolate *Acremonium* in cases of disseminated disease. Like other hyaline moulds, septate colorless hyphae that stain with PAS are found on histopathologic examination [45]. *Acremonium* may be difficult to identify in tissue because of morphologic similarities with other moulds, such as *Fusarium*.

Treatment

Acremonium species have a variable susceptibility to antifungal agents [43] (Table 11.1). In vitro activity of amphotericin

B and itraconazole against *Acremonium* is variable, while resistance to fluconazole and 5-fluorocytosine is uniform. The newer azoles, voriconazole and posaconazole, appear promising.

Penicillium (*Talaromyces*) *marneffei*

Although most *Penicillium* species are recovered only as laboratory contaminants, *P. marneffei* has emerged as a significant pathogen, most commonly as an opportunistic infection in HIV-infected patients residing or traveling to Southeast Asia [46].

Etiologic Agents

Penicillium marneffei is a facultative intracellular pathogen that is the only known thermally dimorphic fungus of the genus *Penicillium*. With the change in rules governing fungal taxonomy, it has been proposed that *P. marneffei* be renamed *Talaromyces marneffei*. Throughout this text, we will continue to use the name *P. marneffei*. At room temperature, *P. marneffei* exhibits the characteristic morphology of the genus, in contrast, it grows as a yeast when found in infected tissue or at 37 °C.

Epidemiology

Infection due to *Penicillium marneffei* constitutes the third most common opportunistic infection in HIV-infected patients in certain parts of Southeast Asia. Infection is most commonly reported from Thailand and Vietnam, but it is also endemic to the Guangxi province of China, Hong Kong, and Taiwan [47]. The incidence of penicilliosis has increased significantly, paralleling the incidence of HIV infection. Although penicilliosis is most commonly seen in adults infected with HIV, the disease has also been detected in children and adults without immunodeficiency [48]. The mode of transmission is thought to be due to ingestion or inhalation of the fungus. Soil exposure, especially during rainy season, has been suggested to be a critical factor.

Clinical Manifestations

P. marneffei can clinically resemble tuberculosis, molluscum contagiosum, cryptococcosis, and histoplasmosis. The most common clinical manifestation in penicilliosis includes low-grade fever, anemia, weight loss, cough, lymphadenopathy, and hepatosplenomegaly [49–51]. Skin lesions are present in up 70 % of the cases and are characterized by a central necrotic umbilication resembling molluscum contagiosum. Palatal and pharyngeal lesions can also be present. Bloodstream infection is present in approximately 50 % of the cases, in 77 % of HIV-infected, and 47 % of HIV-uninfected patients in one recent report [48]. Pulmonary involvement has been described as being diffuse or focal with either a reticulonodular or alveolar pattern. The mean number of $CD4^+$ T lymphocytes at presentation is 64 cells/μl [47].

Diagnosis

Diagnosis of *P. marneffei* infections is usually made by identification of the organism from smear, culture, or histopathologic sections. Rapid diagnosis of suspected infection could be obtained by direct examination of bone marrow aspirate, lymph node, or skin biopsy. Microscopic examination of Wright-stained smears reveals yeast forms both within phagocytes and extracellularly. The intracellular forms resemble those seen with *Histoplasma capsulatum* infection. The demonstration of characteristic central septation and elongated "sausage-shaped" forms by methenamine silver stain, clearly distinguishes *P. marneffei* from *H. capsulatum* (see Fig. 4.10, Chap. 4).

Treatment

Penicillium marneffei is usually susceptible to both amphotericin B and the azole antifungals. Amphotericin B is effective in the majority of the cases, whereas the azoles are preferred for mild-to-moderate infections. In a nonrandomized trial of 74 HIV-infected patients with disseminated penicilliosis [52], high response rate (97 %) was demonstrated with a regimen of amphotericin B (0.6 mg/k/d) for 2 weeks, followed by itraconazole (400 mg/d) for 10 weeks. Relapse is common 6 months after discontinuation of therapy in as high as 50 % of patients who do not receive maintenance antifungal therapy. Long-term suppressive therapy with itraconazole has been recommended in patients with HIV infection and penicilliosis [53]. Recent noncontrolled trials, however, demonstrated safe discontinuation of secondary prophylaxis for penicilliosis in HIV-infected patients who were responding to highly active antiretroviral therapy (HAART) [49, 54].

Other Agents of *Hyalohyphomycosis*

Scopulariopsis species are common soil saprophytes and have been isolated worldwide (see Fig. 2.12, Chap. 2). Of the many *Scopulariopsis* (sexual state *Microascus*) species, *S. brevicaulis* is the most common cause of human infection [55]. Disease in immunocompetent hosts includes onychomycosis

(most common), keratitis, and rarely, post-traumatic endophthalmitis or subcutaneous infection. Rare cases of endocarditis associated with valvuloplasty or prosthetic valves have been described. Invasive and disseminated infections, particularly with *S. brevicaulis*, may occur in immunosuppressed patients, manifesting as pneumonia or disseminated infection with skin lesions and fungemia. Patients at risk include those with acute leukemia and HSCT [56]. Typically resistant to amphotericin and the azole antifungal, prognosis is often related to immune reconstitution and the ability to perform surgical debridement on localized infections. In vitro, echinocandins appear to have some activity [55].

Beauveria are ubiquitous fungi commonly found in soil. Because of their pathogenicity to many insect species, the organisms are incorporated into pesticides worldwide. Rarely, *Beauveria* may cause infections in humans, including keratitis and subcutaneous mycosis. Disseminated infections have occurred in patients with leukemia and HSCT [57]. The organism appears to be susceptible to itraconazole and amphotericin B.

Trichoderma species are also common environmental fungi. They are rarely reported as the cause of fungal infection, including disseminated or localized disease (e.g., keratitis, sinusitis, peritonitis, pulmonary infections, endocarditis, and brain abscess). Reported risk factors for these infections include hematologic malignancy, solid organ transplantation, and peritoneal dialysis. *T. longibrachiatum* is the most common species to cause human disease. Based on in vitro susceptibilities, the echinocandin antifungal drugs or amphotericin are most likely to be effective against these fungi [58].

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Phaeohyphomycosis: Infection Due to Dark (Dematiaceous) Molds

12

Sanjay G. Revankar

Introduction

Dematiaceous, or darkly pigmented fungi are a large, heterogeneous group of organisms that have been associated with a wide variety of clinical syndromes. These are uncommon causes of human disease, but can be responsible for life-threatening infections in both immunocompromised and immunocompetent individuals. In recent years, these fungi have been increasingly recognized as important pathogens and the spectrum of diseases they are associated with has also broadened.

The clinical syndromes caused by the dark-walled fungi are typically distinguished based on characteristic histologic findings into chromoblastomycosis, mycetoma, and phaeohyphomycosis. Chromoblastomycosis and mycetoma are caused by a small group of fungi that are associated with characteristic structures in tissues and are usually seen in tropical areas [1]. These are discussed in Chap. 22 (Fungal Infections of Implantation). Phaeohyphomycosis is a term introduced by Ajello et al. in 1974, which literally means "infection caused by dark-walled fungi" [2]. It is a catch-all term generally reserved for the remainder of clinical syndromes caused by dematiaceous fungi that range from superficial infections and allergic disease to brain abscess and widely disseminated disease [3]. These fungi are alternately called phaeoid, dematiaceous, dark, or black molds. While typically, phaeohyphomycosisis a term limited to infections caused by the dark molds, there are dark yeasts that rarely cause infection, and these are also included under this grouping by many experts.

Etiologic Agents

More than 150 species and 75 genera of dematiaceous fungi have been implicated in human disease [4]. The common characteristic among these fungi is the presence of melanin in their cell walls, which imparts the dark color to their conidia or spores and hyphae. Their colonies are typically brown to black in color as well. As the number of patients immunocompromised from diseases and medical therapy increases, additional species are being reported as causes of human disease, expanding an already long list of potential pathogens. Common genera associated with specific clinical syndromes are listed in Table 12.1.

Guidelines are available for the handling of potentially infectious fungi in the laboratory setting. Cultures of certain well-known pathogenic fungi, such as *Coccidioides immitis* and *Histoplasma capsulatum,* are suggested to be worked with in a Biosafety Level 3 facility, which requires a separate negative pressure room. Recently, certain agents of phaeohyphomycosis, in particular *Cladophialophora bantiana,* have been included in the list of fungi that should be kept under Biosafety Level 2 containment [5]. This seems reasonable given their propensity, albeit rarely, for causing life-threatening infection in normal individuals.

Epidemiology

These fungi are typically soil organisms and generally distributed worldwide [6]. However, there are species that do appear to be geographically restricted, such as *Ramichloridium mackenzei,* which has only been seen in patients from the Middle East [7]. Exposure is thought to be from inhalation or minor trauma, which may not even be noticed by the patient. Anecdotal reports suggest that smoking may be a risk factor in patients who are immunodeficient [8]. Surveys of outdoor air for fungal spores routinely observe dematiaceous fungi [9]. This suggests that most if not all individuals are exposed to them, though they remain uncommon causes of

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Table 12.1 Clinical spectrum and treatment of phaeohyphomycosis

Clinical syndrome	Commonly associated fungi	Therapy
Onychomycosis	*Onychocola*, *Alternaria*	Itraconazole or terbinafine
Subcutaneous nodules	*Exophiala*, *Alternaria*, *Phialophora*	Surgery ± itraconazole or voriconazole
Keratitis	*Curvularia*, *Bipolaris*, *Exserohilum*, *Lasiodiplodia*	Topical natamycin ± itraconazole or voriconazole
Allergic fungal sinusitis/Allergic broncho-pulmonary mycosis (disease)	*Curvularia*, *Bipolaris*	Corticosteroids± itraconazole or voriconazole
Pneumonia	*Ochroconis*, *Exophiala*, *Chaetomium*	Itraconazole or voriconazole (amphotericin B if severe)
Brain abscess	*Cladophialophora* (*C. bantiana*), *Ramichloridium* (*R. mackenzei*), *Ochroconis*	See text
Disseminated disease	*Scedosporium* (*S. prolificans*), *Bipolaris*, *Wangiella*	See text

disease. These fungi may also be found to be contaminants in cultures, making the determination of clinical significance problematic. At one institution, only 10% of positive cultures were associated with clinical disease [10]. A high degree of clinical suspicion as well as correlation with appropriate clinical findings and histopathology is required when interpreting culture results.

Pathogenesis and Immunology

Little is known regarding the pathogenic mechanisms by which these fungi cause disease. One of the likely virulence factors is the presence of melanin in the cell wall, which is common to all dematiaceous fungi. It may confer a protective advantage by scavenging free radicals that are produced by phagocytic cells in their oxidative burst that normally kill most organisms [11]. In addition, melanin may bind to hydrolytic enzymes, thereby preventing their action on the plasma membrane [11]. In the yeasts *Cryptococcus neoformans* and *Wangiella dermatitidis,* disruption of melanin production leads to markedly reduced virulence in animal models [12, 13]. Melanin has also been associated with decreased susceptibility of fungi to certain antifungals, possibly by binding these drugs [14, 15]. It is interesting to note that almost all allergic diseases and eosinophilia are caused by two genera, *Bipolaris* and *Curvularia,* though the virulence factors responsible for eliciting allergic reactions are unclear at present [16].

Clinical Manifestations

Superficial Infections

Superficial infections are the most common form of disease associated with phaeohyphomycosis. These may be divided into tinea nigra, onychomycosis, subcutaneous lesions, and keratitis and are generally associated with minor trauma or other environmental exposure. Although many pathogens have been reported, relatively few are responsible for the majority of infections.

Tinea nigra is primarily seen in tropical areas, and involves only the stratum corneum of the skin. Patients are generally asymptomatic, presenting with brownish-black macular lesions, almost exclusively on the palms and soles. *Hortaea werneckii* is the most commonly isolated species, though *Stenella araguata* has also been cultured from lesions [17]. Tinea nigra may be confused with a variety of other diseases, including dysplastic nevi, melanoma, syphilis, or Addison's disease. Diagnosis is made by scrapings of lesions and culture. As it is a very superficial infection, simple scraping or abrasion can be curative, though topical treatments such as keratolytics or imidazole creams are also highly effective [17].

Dematiaceous fungi are rare causes of onychomycosis, and the term fungal melanonychia has been used to describe this entity, which is seen predominantly in tropical regions [18]. Clinical features may include a history of trauma, the involvement of only one or two toenails, and the lack of response to standard systemic therapy [19]. Twenty-one species have been implicated as causes, including *Alternaria, Curvularia,* and *Scytalidium* have been reported, with the latter being highly resistant to therapy [18].

There are numerous case reports of subcutaneous infection due to a wide variety of species [20, 21]. Minor trauma is the usual inciting factor, though it may be unrecognized by the patient. Lesions typically occur on exposed areas of the body and often appear cystic or papular. Immunocompromised patients are at increased risk of subsequent dissemination. Occasionally, these infections may involve joints or bone.

Fungal keratitis is an important ophthalmologic problem, particularly in tropical areas of the world. In one large series, 40% of all infectious keratitis was caused by fungi, almost exclusively molds [22]. The most common fungi are *Fusarium* and *Aspergillus,* followed by dematiaceous fungi (up to 8–17% of cases) [23]. Approximately, half of the cases are associated with trauma; prior eye surgery, diabetes, and contact lens use have also been noted as important risk factors

[23]. In a study from the USA of 43 cases of *Curvularia* keratitis, almost all were associated with trauma [24]. Plants were the most common source, though several cases involving metal injuries were seen as well.

Allergic Disease

Relatively few species have been associated with allergic disease. *Alternaria alternata* is thought to be involved in some cases of asthma [25]. Whether dematiaceous fungi may be responsible for symptoms of allergic rhinitis is unclear, as it is difficult to quantitate exposure and to distinguish them from other causes [26].

Bipolaris and *Curvularia* are responsible for most cases of allergic fungal sinusitis (AFS) and allergic bronchopulmonary mycosis (ABPM). Patients with AFS usually present with chronic sinus symptoms that are not responsive to antibiotics. Previously, *Aspergillus* was thought to be the most common fungus responsible for allergic sinusitis, but it is now appreciated that disease due to dematiaceous fungi actually comprises the majority of cases [27]. Criteria suggested for this disease include (1) nasal polyps, (2) the presence of allergic mucin, containing Charcot–Leyden crystals and eosinophils, (3) hyphal elements in the mucosa without the evidence of tissue invasion, (4) positive skin test to fungal allergens, and (5) on computed tomography (CT) scans, characteristic areas of central hyperattenuation within the sinus cavity [28]. Diagnosis generally depends on the demonstration of allergic mucin, with or without actual culture of the organism.

Allergic bronchopulmonary mycosis (ABPM) (or disease (ABPD)) is similar in presentation to allergic bronchopulmonary aspergillosis (ABPA), which is typically seen in patients with asthma or cystic fibrosis [29]. Criteria for the diagnosis of ABPA in patients with asthma include: (1) asthma, (2) positive skin test for fungal allergens, (3) elevated IgE levels, (4) *Aspergillus*-specific IgE, and (5) proximal bronchiectasis [30]. Similar criteria for ABPM are not established, but finding allergic mucin (Charcot–Leyden crystals and eosinophils) without tissue invasion, as in AFS, makes this diagnosis highly likely [31].

Pneumonia

Nonallergic pulmonary disease is usually seen in immunocompromised patients, and may be due to a wide variety of species, in contrast to allergic disease [16, 31–34]. Clinical manifestations include pneumonia, asymptomatic solitary pulmonary nodules, and endobronchial lesions which may cause hemoptysis.

Brain Abscess

This is a rare, but frequently fatal manifestation of phaeohyphomycosis [35]. Interestingly, over half of the reported cases have occurred in patients with no risk factors or known immunodeficiency. Lesions are usually solitary. Symptoms may include headache, neurologic deficits, and seizures, though the classic triad seen in bacterial brain abscess (fever, headache, and focal neurologic deficit) is not usually present. The most commonly isolated organism is *Cladophialophora bantiana*, particularly in immunocompetent patients. The pathogenesis may be hematogenous spread from an initial, presumably subclinical pulmonary focus. However, other risk factors such as chronic sinusitis or smoking have been implicated in case reports [8, 36]. It remains unclear why these fungi preferentially cause central nervous disease (CNS) disease.

Disseminated Infection

This is the most uncommon manifestation of infection seen with dematiaceous fungi. Most patients are immunocompromised, though occasional patients without known immunodeficiency or risk factors have developed disseminated disease as well [37]. In contrast to most invasive mold infections, blood cultures are often positive. The most commonly isolated fungus, *Scedosporium prolificans,* may also be associated with septic shock. Peripheral eosinophilia, seen in 11 % of cases, is more commonly associated with *Bipolaris* or *Curvularia*.

Diagnosis

In contrast to other common mycoses that cause human disease, there are no specific serologic or antigen tests available to detect these fungi in blood or tissue. However, the nonspecific serum 1,3-β-D-glucan test may be positive in certain cases of invasive disease [38], and certain species have been demonstrated to contain the *FKS* gene responsible for its production [39]. The diagnosis of phaeohyphomycosis currently rests on pathologic examination of clinical specimens and careful gross and microscopic examination of cultures (Fig. 12.1). Hospital laboratories can generally identify the most common genera associated with human disease (Fig. 12.2), though referral to a reference laboratory is often needed to identify unusual species. As many of these are rarely seen in practice, a high degree of clinical suspicion is required when interpreting culture results. Increasingly, molecular techniques such as internal transcribed sequence (ITS) sequencing are being used to definitively identify

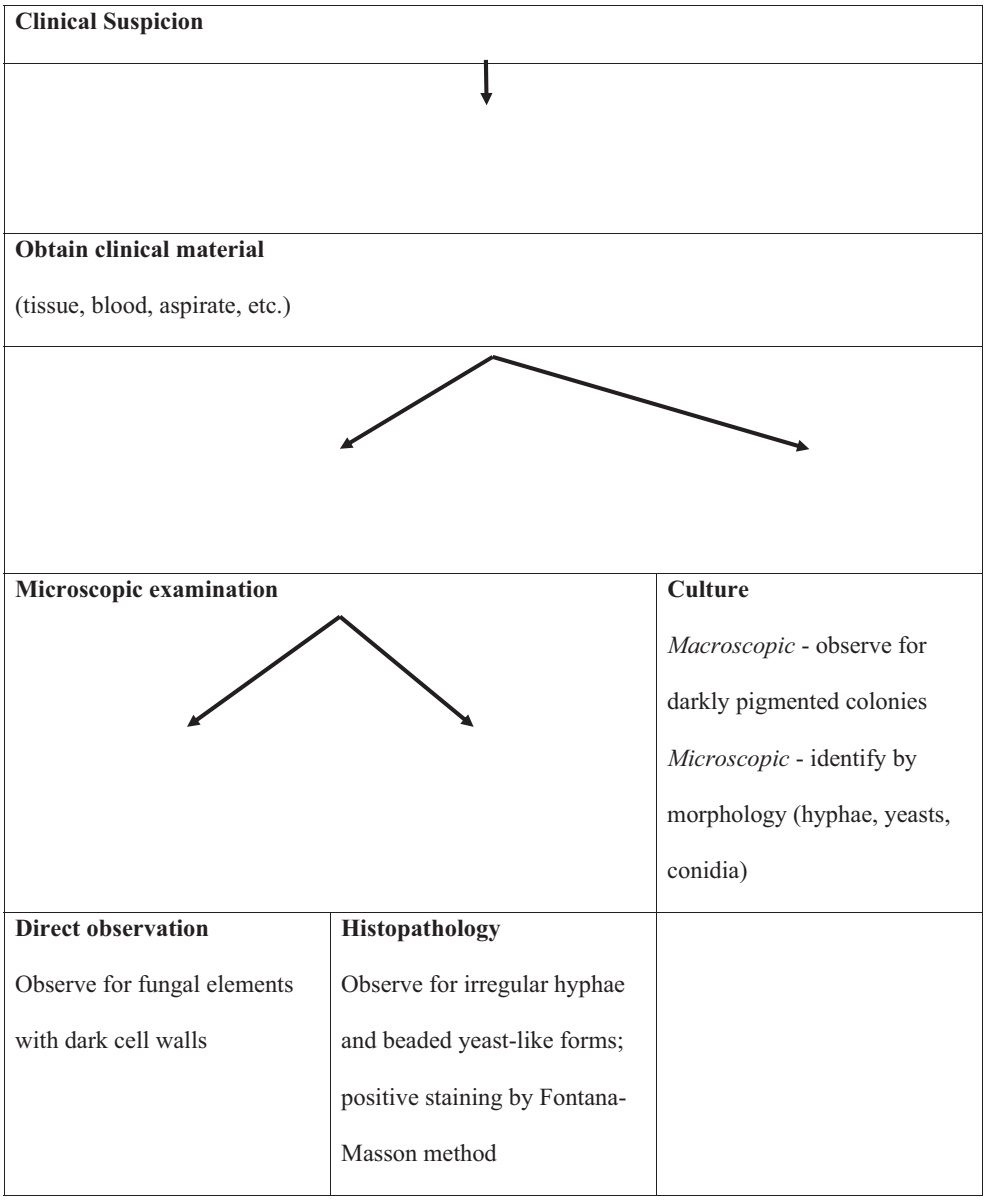

Fig. 12.1 Diagnostic approach for phaeohyphomycosis

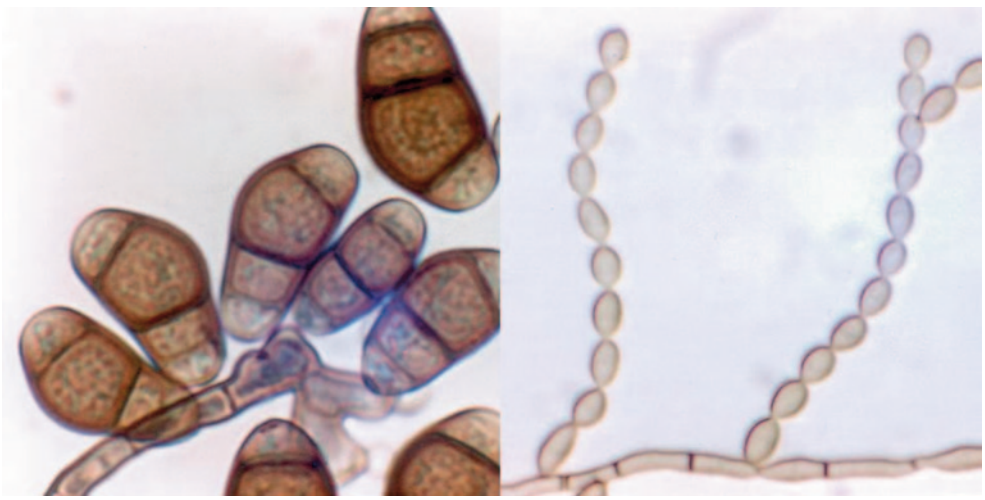

Fig. 12.2 Commonly seen fungi causing phaeohyphomycosis. *Left panel*, *Curvularia lunata*; *right panel*, *Cladophialophora bantiana*

isolates to the species level and are becoming the standard to distinguish between closely related strains and establish novel species [4].

In tissues, these fungi stain strongly with the Fontana-Masson stain, which is specific for melanin (Fig. 12.3) [3]. This can be helpful in distinguishing these fungi from other species, particularly *Aspergillus*. In addition, hyphae typically appear more fragmented in tissue than seen with *Aspergillus,* with irregular septate hyphae and beaded, yeast-like forms [3].

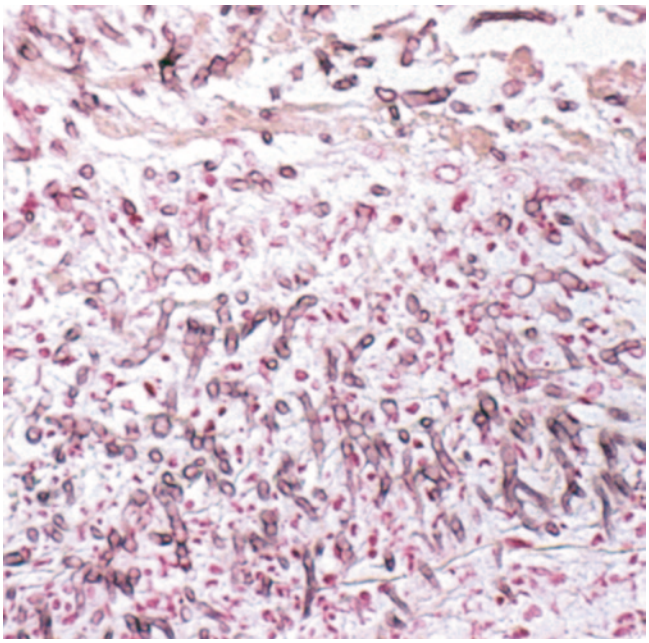

Fig. 12.3 Fontana-Masson stain of *Bipolaris* infection in the lung, demonstrating irregular hyphae and beaded yeast-like forms

Treatment

Therapy is not standardized for any of these clinical syndromes, and randomized trials are unlikely given the sporadic nature of cases. Itraconazole, voriconazole, and posaconazole demonstrate the most consistent in vitro activity against this group of fungi, though far more clinical experience has accumulated with itraconazole [40]. Isavuconazole is a novel triazole with good in vitro activity, though it is not yet approved for use [41, 42]. Amphotericin B may be used for severe infections in unstable patients; high doses of lipid formulations may have a role in the treatment of refractory cases or in patients intolerant of standard amphotericin B. However, some species of dematiaceous fungi are resistant to this agent. Once the infection is under control, longer-term therapy with a broad-spectrum oral azole is often reasonable until complete response is achieved, which may require several weeks to months.

Other agents have limited roles in treating these fungi. Ketoconazole is not well tolerated, and fluconazole has poor activity against these fungi in general. Terbinafine and flucytosine have occasionally been used for subcutaneous infections in patients refractory to other therapy. Echinocandins do not appear to be very useful as single agents. Combination therapy is a potentially useful therapeutic strategy for refractory infections, particularly brain abscess and disseminated disease, though it has not been well studied. Suggested therapies for specific infections are summarized in Table 12.1.

Superficial Infections

Itraconazole and terbinafine are the most commonly used systemic agents for onychomycosis, and may be combined with topical therapy for refractory cases [43]. There is no published experience with voriconazole.

Subcutaneous lesions will often respond to surgical excision alone [44]. Oral systemic therapy with a broad-spectrum azole antifungal agent in conjunction with surgery is frequently employed and has been used successfully, particularly in immunocompromised patients [45, 46].

For keratitis, topical 5% natamycin is used almost exclusively, with only a few severe cases requiring adjunctive therapy, usually with an azole [22, 47]. Itraconazole has the best in vitro activity. The majority of isolates are resistant to flucytosine. Surgery, including penetrating keratoplasty, is often needed. Enucleation is occasionally required due to poor clinical response. Many patients do not recover complete visual acuity despite aggressive therapy.

Allergic Disease

Steroids are the mainstay of treatment for allergic disease caused by these fungi, especially in asthma, though other modalities may have a role in specific clinical situations. For example, therapy for AFS consists of systemic corticosteroids and surgery to remove the mucin, which is often tenacious. Antifungal therapy, usually in the form of itraconazole, may play a role in reducing the requirement for corticosteroids, but this is not routinely recommended [48]. Other azoles have only rarely been used for this disease.

ABPM can be treated with systemic corticosteroids as in ABPA; prednisone at a dose of 0.5 mg/kg/day for 2 weeks, followed by a slow taper over 2–3 months or longer [29]. Itraconazole has been used as a steroid sparing agent in APBA, but its efficacy is not clear and routine use of itraconazole is not generally recommended [29].

Pneumonia

Therapy consists of systemic antifungal agents, usually amphotericin B or itraconazole initially, followed by itraconazole for a more prolonged period [16]. Mortality rates are high in immunocompromised patients. Experience with voriconazole is currently only anecdotal [49].

Brain Abscess

Therapy published in the literature has varied greatly depending on the case report, and there is no standard treatment. A retrospective analysis of 101 reported cases suggested that the combination of amphotericin B (high-dose lipid formulation), flucytosine, and itraconazole may be associated with improved survival, though it was not frequently used [35]. Voriconazole may also prove useful. High doses of azoles have been suggested as an option, though there are no studies confirming this approach. Based on animal models and anecdotal reports, some form of combination therapy may be optimal, though specific regimens have not been established [4]. Complete excision of brain abscesses may lead to better outcomes than aspiration or partial excision. Overall mortality is greater than 70 %.

Disseminated Infection

A literature review suggested the mortality rate is greater than 70 %, despite aggressive antifungal therapy [37]. There were no antifungal regimens associated with improved survival in disseminated infection. High-dose lipid amphotericin B may be reasonable for initial therapy, given its fungicidal activity for many fungi. The addition of a broad-spectrum azole or echinocandin could be considered in those failing therapies. Infection with *S. prolificans* has been associated with a nearly 100 % mortality in the absence of recovery from neutropenia, as it is generally resistant to all available antifungal agents. Recent reports have suggested that the combination of itraconazole or voriconazole with terbinafine may be synergistic against this species, though the clinical relevance of this finding is unclear [50, 51].

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Mucormycosis (Zygomycosis)

13

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Introduction

The class Mucormycetes (formerly Zygomycetes) includes a variety of filamentous fungi that may cause life-threatening human diseases and, over the past two decades, have emerged as increasingly important causes of morbidity and mortality among immunocompromised patients [1, 2]. The first case of mucormycosis (zygomycosis) in humans was reported in 1885 by Platauf as *Mycosis Mucorina*. In many of the cases reported, thereafter, the infection was identified as "mucormycosis" or *Mucor* infection based solely on histological findings of wide, rarely septate hyphae, without culture confirmation. The use of the term "mucormycosis" was further promoted by the original classification of most of the pathogenic species of Zygomycetes as members of the genus *Mucor* [3]. Consequently, the term "zygomycosis," instead of "mucormycosis," was widely used during the past three decades for infections caused by any of the species within the class of Zygomycetes.

Many experts preferred the use of "mucormycosis" and emphasized that the opportunistic disease due to those fungi in the order Mucorales differed substantially from those caused by members of the order Entomophthorales, which was also included within the class Zygomycetes [3]. Diseases caused by members of the order Mucorales, such as *Rhizopus oryzae,* are typically opportunistic infections of the lungs, sinuses, and brain with angioinvasion leading to ischemia, infarction, and necrosis. Diseases caused by members of order Entomophthorales, such as *Conidiobolus* spp. and *Basidiobolus* spp., are typically subcutaneous infections in immunocompetent hosts. With advances in molecular taxonomy, these clinical and pathophysiological distinctions correlated with the observation that the class of Zygomycetes is not monophyletic. Thus, the Zygomycota was eliminated. The taxa, conventionally classified in Zygomycota, are now distributed among the new phylum Glomeromycota and four subphyla, including the Mucoromycotina and Entomophthoromycotina [4]. As the orders Mucorales and Entomophthorales are preserved, the preferred names for the respective diseases should now be mucormycosis and entomophthoromycosis. The focus of this chapter is mucormycosis. See chapt. 22 in this text for discussion of entomophthoromycosis.

Etiologic Agents

The medically important Glomeromycota encompass two orders of filamentous fungi with distinct morphologic, epidemiologic, and pathogenic characteristics, the Mucorales and the Entomophthorales [3, 5–8] (Table 13.1). The majority of cases of mucormycosis in humans are caused by members of the order Mucorales. Organisms of the genus *Rhizopus* are, by far, the most common clinical isolates, with *R. oryzae* being the most frequently recovered species. Members of the genus *Mucor* are second to *Rhizopus* in order of frequency, while *Cunninghamella, Apophysomyces, Lichtheimia* (formerly, *Absidia*), *Saksenaea, Rhizomucor,* and other genera, each represent a significantly smaller percentage of clinical isolates [1, 3].

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Table 13.1 Taxonomic classification of the order Mucorales

Order	Family	Genus	Species causing human disease
Mucorales	Mucoraceae	*Lichtheimia*	*L. corymbifera*
		Apophysomyces	*A. elegans*
		Mucor	*M. circinelloides, M. ramosissimus, M. racemosus, M. hiemalis, M. rouxianus*
		Rhizomucor	*R. pusillus*
		Rhizopus	*R. oryzae (R. arrhizus), R. microsporus* var *rhizopodiformis*
	Cunninghamellaceae	*Cunninghamella*	*C. bertholletiae, C. echinulata*
	Mortierellaceae	*Mortierella*	(animal pathogens)
	Saksenaceae	*Saksenaea*	*S. vasiformis*
	Syncephalastraceae	*Syncephalastrum*	*S. racemosum*
	Thamnidaceae	*Cokeromyces*	*C. recurvatus*

The Mucorales are characterized in culture by broad nonseptate, or sparsely septate, hyphae and by the presence of sporangiophores supporting sporangia, which contain sporangiospores. *Cunninghamella bertholletiae* is characterized by sporangiola rather than sporangiospores. During sexual reproduction in culture, zygospores may be produced. The Mucorales are characterized, in tissue by the formation of wide, ribbon-like, hyaline, aseptate (coenocytic) or sparsely septate hyphae with wide-angle (approximately 90°) branching. The substantial differences among these and other structures allow organisms to be diagnosed by genus and species in the mycology laboratory [3].

Epidemiology

The Mucorales are ubiquitous in soil and can be isolated from decaying organic matter including hay, decaying vegetation, and a variety of food items. Human infection is usually acquired through inhalation of sporangiospores from environmental sources. Acquisition via the cutaneous or percutaneous route is also common, either through traumatic disruption of skin barriers or with the use of catheters and injections. Less commonly, infection through the gastrointestinal route may occur [1, 3, 6, 7]; however, gastrointestinal mucormycosis is relatively common (54% of total cases reported) in premature neonates [7].

Mucormycosis is approximately 10–50-fold less common than invasive *Candida* or *Aspergillus* infections, with a prevalence of 1–5 cases per 10,000 autopsies and an estimated incidence of 1.7 cases per million per year in the USA [9]. A clear male predisposition has been observed, as demonstrated by an approximate 2:1 male to female ratio among cases [1]. Unlike other filamentous fungi, targeting mainly immunocompromised patients, the Mucorales cause disease in a wider and more heterogeneous population.

The most common underlying condition for development of mucormycosis is diabetes—both type I and type II. A significant proportion of these patients will present with ketoacidosis, while, in others, mucormycosis may even present as the diabetes-defining illness. Other significant underlying conditions include the presence of hematological malignancy, solid organ or hematopoietic stem cell transplantation (HSCT), deferoxamine therapy, and injection drug use [1, 6]. During the past three decades, the percentage of pediatric and adult patients with hematological malignancy, solid organ transplantation or HSCT, and injection drug use among all cases of mucormycosis has significantly increased [1, 10, 11]. In the aforementioned groups of hematological patients and transplant recipients, factors associated with this infection have been reported to include prolonged neutropenia, corticosteroid use, and graft-versus-host disease (GvHD) [6, 11]. Less commonly, the Mucorales may cause invasive disease, in the presence of renal failure, diarrhea, and malnutrition, in low-birth-weight infants and human immunodeficiency virus (HIV) patients. Occasionally, mucormycosis has developed in patients with persistent metabolic acidosis secondary to causes other than diabetes [1, 6].

A significant proportion of mucormycosis cases have, as well, been observed in persons with no primary underlying disease at the time of infection. In many of these cases, there was a history of penetrating trauma, surgery, or burn prior to the development of infection [1, 6].

Pathogenesis and Immunology

The epidemiologic profile of mucormycosis cases (patients with diabetes, hematological malignancies or on deferoxamine therapy, transplant recipients) may, in part, be explained by our current understanding of the pathogenesis of these infections. As with other filamentous fungi, an effective immune response, following inoculation of sporangiospores, requires the presence of adequate phagocytic activity of the host effector cells, including tissue macrophages and neutrophils. Pulmonary alveolar macrophages ingest the sporangiospores to inhibit germination, while the neutrophils are involved in hyphal damage [12]. Consequently, the

host immune response against the Mucorales may be compromised if phagocytic cells are insufficient in number as in the case of chemotherapy-induced neutropenia, or dysfunctional, as in the case of corticosteroid treatment or diabetes mellitus [3, 12].

Experimental evidence also suggests an important role of iron in the pathogenesis of infections caused by *Rhizopus* species, whose growth is promoted in the presence of increased iron uptake. Deferoxamine, an iron chelator, has siderophore activity for these fungi, allowing significant increase in iron uptake. Furthermore, the availability of serum iron is increased in the presence of acidic pH, suggesting an additional mechanism for the development of mucormycosis, in patients with diabetic ketoacidosis [4, 12].

An almost universal feature in infections, caused by the Mucorales, is the presence of extensive angioinvasion associated with thrombosis and ischemic necrosis [3, 13]. This is likely an important mechanism by which these organisms survive antifungal therapy since adequate blood supply is necessary for the delivery of antifungal agents. Recent data also have demonstrated the ability of *R. oryzae* sporangiospores or hyphae to adhere to subendothelial matrix proteins and human endothelial cells [4, 13]. Pregerminated sporangiospores of *R. oryzae* are able to damage endothelial cells in vitro, following adherence to and phagocytosis by these cells. *R. oryzae* viability is not required for endothelial cell damage, suggesting that in the setting of established infection even fungicidal therapy may not prevent subsequent tissue injury [13].

Clinical Manifestations

The clinical manifestations of human infection caused by the Mucorales can be classified as sinus disease, localized or extended to the orbit and/or brain, pulmonary, cutaneous, gastrointestinal, disseminated, and miscellaneous infection [1, 14]. Some of these manifestations may occur with increased frequency in patients with certain underlying conditions (Table 13.2) [1, 6]. However, this is not always the case and mucormycosis, in these patient groups, may still present with any of the above patterns.

Paranasal Sinus Infection

Paranasal sinus disease may be confined to the sinuses or may infiltrate the orbit (sino-orbital) and/or the brain parenchyma (rhinocerebral). This form represents approximately two thirds of all cases of mucormycosis in diabetic patients [1]. The infection originates in the paranasal sinuses following inhalation of sporangiospores. Initial symptoms may suggest sinusitis and include sinus pain, discharge, soft tissue swelling, and perinasal cellulitis/paresthesia. Fever is variable and may be absent in up to half of the cases [4, 14, 15]. The tissues involved become red, violaceous, and finally black, as vascular thrombosis leads to tissue necrosis. A blood-tinged nasal discharge may be present. In sinus disease, nasal endoscopy may show black necrotic crusts on the nasal septum and turbinates; in the early phases, the mucosa may still look pink and viable [4]. We refer to these necrotic ulcers along the nasal mucosa or turbinates as "sentinel eschars," as they may represent an early phase of infection or may be more amenable to biopsy than a deep maxillary sinus infection.

Table 13.2 Predominant site of Mucorales infection according to the patient's underlying condition

Underlying condition	Predominant site of infection
Diabetes	Sinuses
Hematological malignancy	Pulmonary and sinuses
Solid organ transplantation	Pulmonary
Bone marrow transplantation	Pulmonary and sinuses
Deferoxamine therapy	Pulmonary, sinuses, disseminated[a]
Injection drug use	Cerebral
No underlying condition	Cutaneous

[a] No clear predominance among the three sites

Extension of the infection to the mouth may produce painful necrotic ulcerations in the hard palate. Extension into the periorbital area, and ultimately the orbit, may be manifested by periorbital edema, lacrimation, chemosis, and proptosis. Subsequent ocular or optic nerve involvement may be suggested by pain, diplopia, blurring, or loss of vision. Alteration of mental status and cranial nerve palsies may signify invasion of the central nervous system. Occasionally, thrombosis of the cavernous sinus or the internal carotid artery may follow, with resultant neurological deficits, while dissemination of the infection also may occur [4, 6, 15, 16].

Pulmonary Infection

Pulmonary disease is most commonly observed in patients with hematological malignancies, solid organ, or HSCT recipients, and those receiving deferoxamine treatment [1]. Not infrequently it may occur with concomitant sinus disease (sinopulmonary infection) [17]. Lung involvement may be manifested as infiltrates, consolidation and solitary, nodular, or cavitary lesions (Fig. 13.1) [18, 19]. Fungal invasion of the pulmonary vessels may result in thrombosis and subsequent infarcts in the lung parenchyma (Fig. 13.2). Angioinvasion may also lead to intraparenchymal bleeding or even hemoptysis, which can be fatal if major vessels, such as the pulmonary artery, are involved. Extension of the infection to the chest wall, pericardium, myocardium, mediastinum, and diaphragm has been described [4, 6, 18]. A predilection for

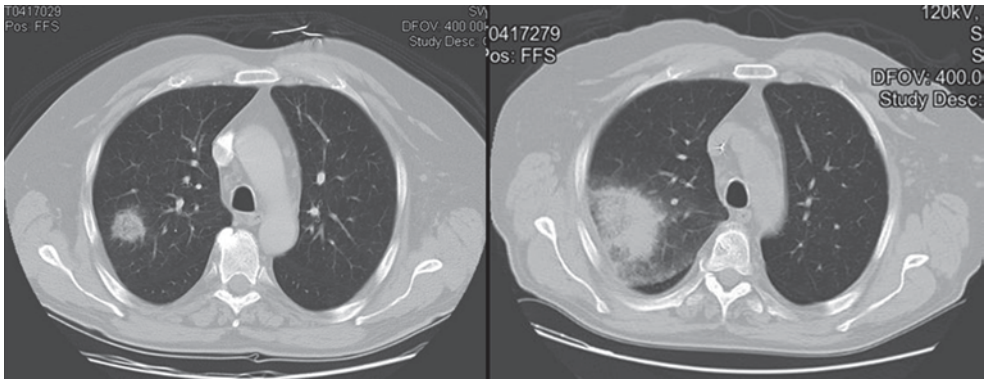

Fig. 13.1 Thoracic CT scan of profoundly neutropenic patient with pulmonary mucormycosis demonstrates rapid evolution of pulmonary nodule to involve the pleural surface and to manifest a halo sign at the interface with radiologically normal lung. The two scans are separated by 5 days. CT computerized tomography

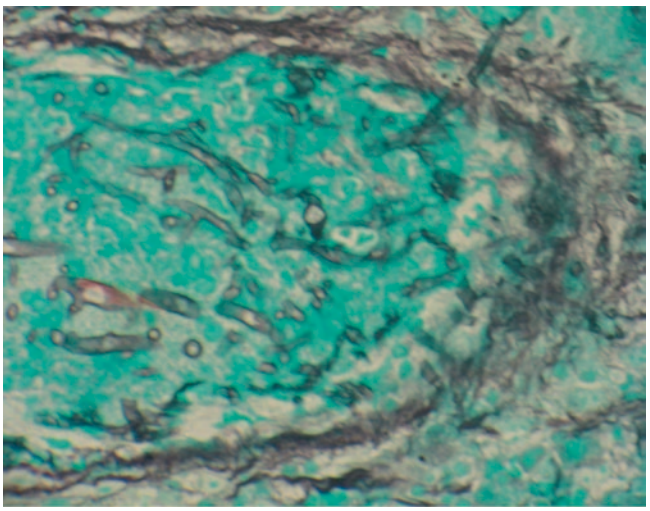

Fig. 13.2 Histopathology of pulmonary mucormycosis in this figure is characterized by broad nonseptate ribbon-like hyphae with non-dichotomous branching invading a pulmonary blood vessel. The specimen was obtained from the lung lesion seen on CT scan in Fig. 13.1. CT computerized tomography

the upper lobes has been reported; however, any part of the lung may be involved, and bilateral disease is not uncommon [18]. Recent studies of the presenting signs and symptoms are nonspecific and include fever, cough, chest pain, dyspnea, hemoptysis, tachypnea, crackles, decreased breath sounds, and wheezing [4, 18, 19]. Endobronchial findings include stenosis or airway obstruction, erythematous mucosa, fungating or polypoid mass and, less often, granulation tissue, or mucosal ulceration [18].

Cutaneous Infection

Cutaneous mucormycosis is often observed in individuals with no underlying condition as a result of infection of a preexisting lesion, such as skin trauma or burn [20]. Alternatively, it may occur in the context of disseminated disease or extensive local infection in immunocompromised hosts [1, 3, 21]. In the case of primary cutaneous inoculation, the lesion appears acutely inflamed with redness, swelling, induration, and frequent progression to necrosis. Extensive local invasion may occur involving the adjacent subcutaneous fat, muscle, bone tissues, and facial layers (Fig. 13.3). When cutaneous disease is the result of disseminated infection, it usually presents as nodular subcutaneous lesions that may ulcerate [3, 6, 21].

Gastrointestinal Infection

Gastrointestinal disease is rare, occurring mainly in malnourished patients and premature neonates, where it can present as necrotizing enterocolitis [1, 7, 22]. After ingestion of the sporangiospores, fungal invasion of the mucosa, submucosa, and vascular structures of the gastrointestinal tract may occur, often resulting in necrotic ulcers, rupture of the intestinal wall, and peritonitis. Symptoms are nonspecific, including fever, abdominal pain, distention, vomiting, and gastrointestinal hemorrhage [3, 4].

Disseminated Infection

Disseminated infection refers to involvement of at least two non-contiguous sites and is commonly observed in patients receiving deferoxamine therapy [1]. Dissemination occurs through the hematogenous route and may originate from any of the above sites of primary infection; although, it seems to be more frequently associated with lung disease. The most common site of dissemination is the brain but other organs may also be involved [4, 23].

Other Infection

Isolated cerebral mucormycosis is usually observed in injection drug users [1]. Endocarditis is a potential complication of cardiac surgery. Isolated peritonitis is often associated with peritoneal dialysis. Renal infection and external otitis also have been reported [4, 6].

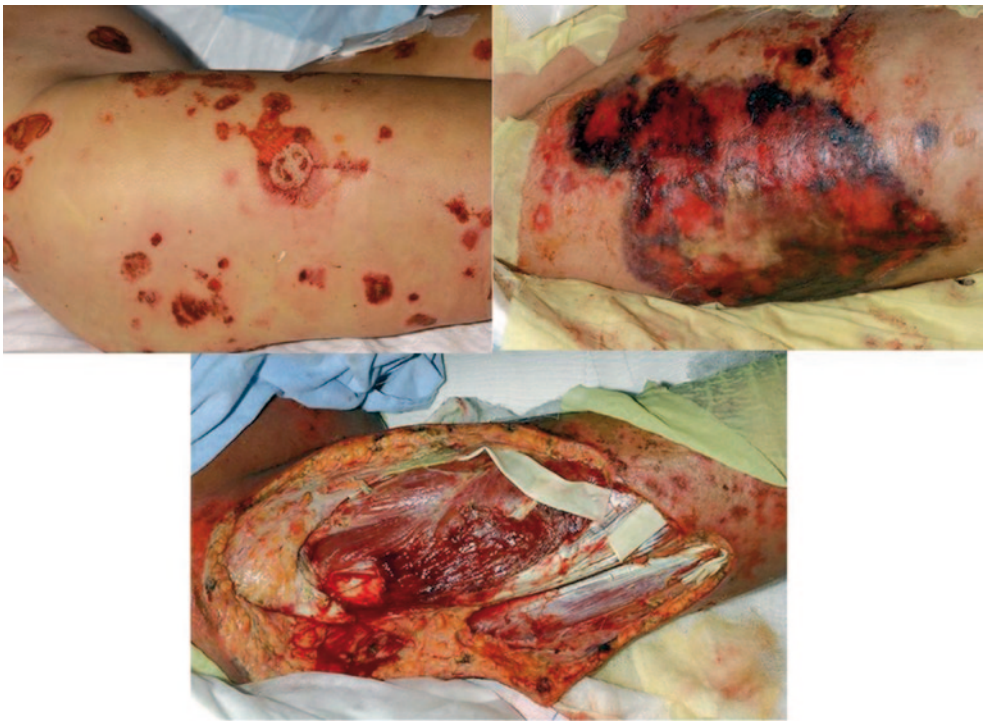

Fig. 13.3 Development of mucormycosis in the skin and subcutaneous tissues of the right lower extremity in a patient with cutaneous T cell lymphoma. The *top-left* panel depicts the lesions of cutaneous T cell lymphoma, which were possibly infected by direct inoculation. The *top-right* panel reveals the extensive necrosis and destruction of soft tissue caused by the rapidly invading hyphae. The entire region was anesthetic to any tactile or pressure stimuli. The *bottom* panel demonstrates the soft tissues following extensive surgical debridement to resect infected skin, fascia, and muscle

Diagnosis

As infections caused by the Mucorales, in humans, may be rapidly fatal, timely diagnosis is crucial to avoid treatment delay. While confirmation of the diagnosis and species identification of the causative organism should be pursued, treatment should be initiated as soon as the diagnosis is suspected, due to the severity of these infections.

Currently, the diagnosis of mucormycosis relies on a constellation of the following: high index of suspicion, assessment of presenting signs and symptoms, imaging studies, cultures of clinical specimens, and histopathology (Fig. 13.4).

Clinical Assessment

The high index of suspicion should be based on the knowledge of the underlying conditions that predispose to mucormycosis and the usual presentation of the infection in each of these conditions (Table 13.2). Nevertheless, less common manifestations of the disease should not be excluded. A common scenario is the development of mucormycosis in oncological patients or transplant recipients who are receiving antifungal therapy for prophylaxis or treatment of other opportunistic fungal infections, such as invasive aspergillosis. If antifungal agents, being administered to the patient, are not active against the Mucorales (including, fluconazole, voriconazole, and the echinocandins), then clinical deterioration or appearance of new signs and symptoms in these patients should alert the clinician to the possibility of mucormycosis [17].

Most of the signs and symptoms that are associated with the clinical manifestations of mucormycosis are nonspecific. However, their diagnostic significance may increase if they are interpreted in relation to the patient's underlying condition. For example, the development of sinusitis in a leukemic or diabetic patient should raise the suspicion of mucormycosis. Other findings have probably greater specificity for this infection, such as the presence of blood-tinged nasal discharge or necrotic eschars in the hard palate. In addition, the presence of hemoptysis in a susceptible host is consistent with angioinvasion and should raise the possibility of mucormycosis [18]. An alarming sign should also be the rapid spread of the infection. Finally, even after the diagnosis has been made, careful periodic clinical assessment should be performed in order to detect progression of the disease. For example, in a patient with pulmonary mucormycosis, palpation of the skin for subcutaneous nodules and neurological evaluation for changes in mental status and focal neurological signs should be repeatedly performed in order to detect dissemination to the skin and brain, respectively.

Diagnostic Imaging

Imaging studies are helpful in assessing the burden of the disease, involvement of adjacent tissues, and response to treatment. They are also helpful in guiding more invasive procedures to obtain biopsy specimens for histopathology and culture [24]. Although imaging findings may be suggestive of mucormycosis in the appropriate clinical setting, they are not

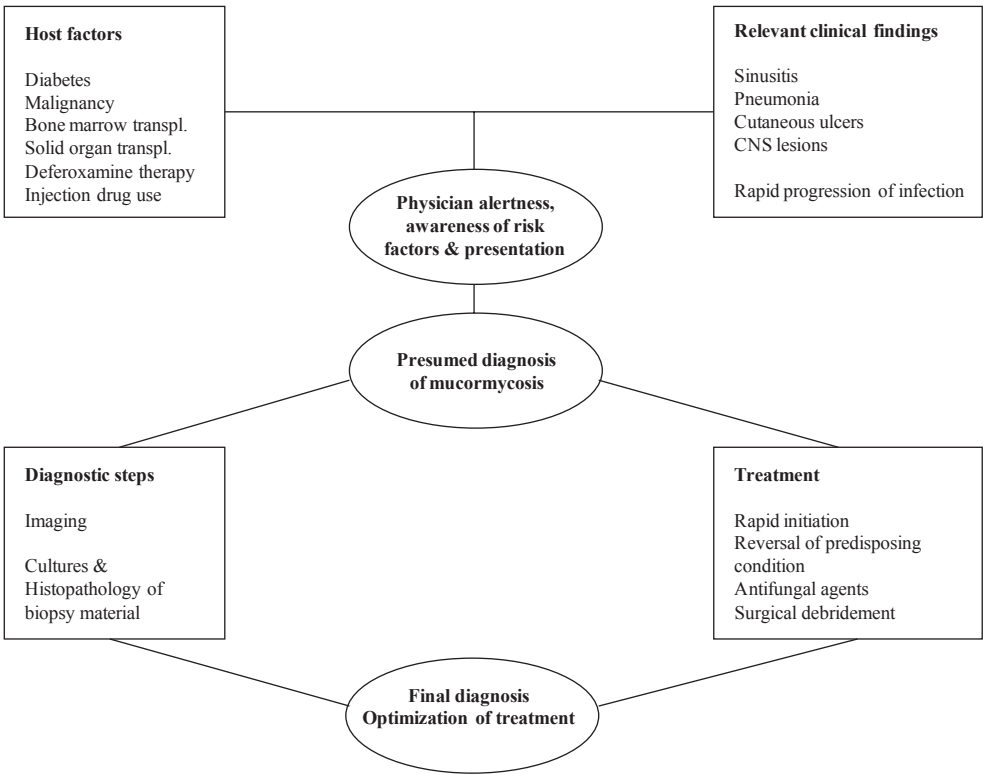

Fig. 13.4 Diagnosis and management of mucormycosis

sufficiently specific to establish the diagnosis. In sinus disease, computerized tomography (CT) detects subtle mucosal thickening or bony erosions of the sinuses, but it is less sensitive than magnetic resonance imaging (MRI) for the detection of extension of the infection to the soft tissues of the orbit [14, 25]. In the case of pulmonary disease, high-resolution CT is more sensitive than chest radiograph for early diagnosis of the infection, and can, more accurately, determine the extent of pulmonary involvement. Radiographic features consistent with pulmonary mucormycosis include nodular infiltrates, pleural effusions, cavity, consolidation, and reverse halo sign (Fig. 13.1). The air crescent and halo signs, which are recognized radiologic features of invasive aspergillosis, have been reported as well for mucormycosis, while the reverse halo sign in neutropenic patients may have more specificity for pulmonary mucormycosis [18, 19, 26]. In patients with pulmonary mucormycosis, the presence of an air crescent sign seems to be associated with increased risk for massive hemoptysis [18]. Another suggestive finding could be the expansion of a mass or consolidation across tissue planes, in particular, towards the great vessels in the mediastinum [4, 27]. In the case of cutaneous disease, MRI is superior to CT scan for assessment of extension of the infection to the adjacent soft or bone tissues.

Culture

Recovery of Mucorales from cultures of clinical specimens would allow not only establishment of diagnosis but also identification of the causative organism to the species level. Although the Mucorales may contaminate laboratory material, their isolation from clinical specimens of susceptible hosts should not be disregarded as contamination. Despite the ability of these organisms to invade tissues, they are rarely isolated from cultures of blood, urine, cerebrospinal fluid, feces, sputum, paranasal sinuses secretions, bronchoalveolar lavage, or swabs from infected areas [3, 14, 18]. The recovery of Mucorales from biopsy material may be compromised if processing of the specimens involves tissue grinding, a procedure that kills the nonseptate hyphae of these fungi. The recovery rate is, however, enhanced if thin slices of minimally manipulated tissue are placed onto the culture medium. Consequently, for proper handling of the specimens, the laboratory should be notified of the possibility of mucormycosis. In any case, negative cultures do not rule out the infection [3, 6].

Histopathology

Given the above limitations of cultures or imaging studies, diagnosis of mucormycosis is almost always based on histopathologic examination of appropriately collected samples (Fig. 13.2). The latter should be pursued in the presence of strong suspicion for mucormycosis if the cultures or imaging studies are negative or nonspecific. Depending on the presentation of the disease, the samples may be collected by fiberoptic bronchoscopy, radiographically guided transthoracic

needle aspiration, open lung biopsy, nasal endoscopy, paranasal sinus biopsy or debridement, and biopsies of skin or other infected tissues [4, 6, 18, 24].

Because the hyphae of Mucorales in tissue specimens may stain poorly with hematoxylin and eosin (H and E), a second more fungus-specific tissue stain should also be used, such as Gomori methenamine silver (GMS) or periodic acid Schiff (PAS) [3]. As already mentioned, the hallmark of mucormycosis is the demonstration of wide, ribbon-like, aseptate (coenocytic) or sparsely septate hyphae with wide-angle branching in biopsy specimens (Fig. 13.2). For Mucorales infections, the hyphae are seen to invade the adjacent blood vessels. Mycotic emboli may thrombose small vessels in which they are lodged. Extensive tissue necrosis or hemorrhage may be observed [3]. Although histopathology is sensitive and reliable for diagnosing mucormycosis, obtaining biopsy material from hematological patients may not always be feasible due to concomitant thrombocytopenia. Finally, a new approach to rapid intraoperative diagnosis of diagnosis and staging of mucormycosis utilizes fluorescent microscopy and cell-wall stain in lieu of frozen sections with special stains on tissue specimens [28].

Non-culture Diagnostic Methods

Although there are no standardized molecular or antigen detection methods available to date for primary diagnosis of mucormycosis, there are important advances being achieved. These advances are summarized elsewhere [29]. A number of molecular techniques are currently employed by research laboratories for species identification of Mucorales isolates, epidemiologic studies, or determination of taxonomic assignments [3, 17, 29].

Treatment

There are four cornerstones of successful management of mucormycosis: (1) rapid initiation of therapy, (2) reversal of the patient's underlying predisposing condition, (3) administration of appropriate antifungal agents, and (4) surgical debridement of infected tissues (Fig. 13.4) [4, 6]. If not treated or diagnosed, with delay, infections caused by the Mucorales in humans are typically fatal [1, 10, 11]. Even if a timely diagnosis is made, treatment is challenging due to a number of reasons such as the underlying condition of the patient, the rapid progression of the disease, and the high degree of angioinvasion and thrombosis that compromises the delivery of antifungal agents active against the causative organisms. As previously mentioned, in the presence of certain conditions, such as diabetes, immunosuppression, and others (Table 13.2), treatment for mucormycosis should be initiated empirically as soon as a strong suspicion for this infection is raised without awaiting formal confirmation of the diagnosis, which may take time. Meanwhile, of course, all the required actions to establish the diagnosis should be undertaken, as already outlined (Fig. 13.4).

Reversal of the underlying condition can be fairly quickly achieved in certain circumstances, such as diabetic ketoacidosis, which should be promptly corrected, or deferoxamine therapy, which should be discontinued. However, timely reversal of the disease- or treatment-related immunosuppression in patients with hematological malignancies or transplant recipients is challenging. In these patients, temporary discontinuation of corticosteroid treatment or myelotoxic chemotherapy should be strongly considered until the infection is brought under control. However, even with these measures, spontaneous restoration of phagocytic activity or recovery from neutropenia is likely to occur after several days, during which time the infection may progress. In vitro and in vivo studies, as well as case reports, have suggested that the administration of cytokines, such as granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF) and interferon-γ, may accelerate immune recovery [30]. In support of these general recommendations for cytokine augmentation of host defense are recent in vitro studies demonstrating enhancement of neutrophil activity against *R. oryzae* and other species of Mucorales in the presence of interferon-γ and GM-CSF [31]. An alternative approach has been the administration of granulocyte transfusions in neutropenic patients with invasive fungal infections [29, 30]. These immunomodulatory interventions may be considered on an individual patient basis as adjunctive therapy for mucormycosis in immunocompromised hosts. Nonetheless, as a caveat, there is a lack of adequately powered clinical trials to evaluate their clinical efficacy and potential complications [6, 29, 30].

Amphotericin B

Amphotericin B is the drug of choice for the treatment of mucormycosis. This polyene agent exerts good in vitro and in vivo activity against the Mucorales. However, apparent in vitro resistance, with elevated minimal inhibitory concentrations (MICs) of amphotericin B, may be observed among clinical isolates, and is relatively common among *Cunninghamella* species [32–34]. The efficacy of amphotericin B in the treatment of mucormycosis was demonstrated in a recent review of 929 cases, where survival was 61% for patients treated with amphotericin B deoxycholate versus 3% for those who received no treatment [1]. The lipid formulations of amphotericin B (mainly amphotericin B lipid complex and the liposomal formulation) also have been used in the treatment of mucormycosis. These formulations are associated

with significantly less toxicity than amphotericin B deoxycholate and demonstrate at least equivalent clinical efficacy [1, 35, 36]. However, no randomized controlled trials have been conducted to compare the efficacy of deoxycholate versus lipid formulations of amphotericin B in the treatment of mucormycosis.

When treatment with amphotericin B is initiated for documented mucormycosis, full doses should be given from the onset, foregoing the past practice of dose escalation. The optimal dosage of amphotericin B formulations for the treatment of mucormycosis has not been systematically evaluated in clinical studies. A study of the safety, tolerance, and plasma pharmacokinetics of liposomal amphotericin B, in patients with invasive fungal infections, found no demonstrable dose-limiting nephrotoxicity or infusion-related toxicity over a dose range of 7.5–15 mg/kg/day [37]. Plasma concentrations of liposomal amphotericin B achieved an upper limit at 10 mg/kg/day and were not increased by further dosage increases. Nevertheless, the efficacy of higher dosages of liposomal amphotericin B compared to the Food and Drug Administration (FDA)-approved dosage of 3–5 mg/kg/day for aspergillosis has not been investigated through clinical trials in mucormycosis. In the absence of such studies, an increase of dosage of liposomal amphotericin B to 7.5 or 10 mg/kg/day could be considered on an individual basis for patients with mucormycosis progressing through liposomal amphotericin B at 5 mg/kg/day [37]. Amphotericin B lipid complex has been used in a dosage of 5 mg/kg/day in salvage treatment of mucormycosis with complete or partial responses in 17 (71%) of 24 cases [36]. Further discussion of the management of mucormycosis in comparison to that of aspergillosis is discussed in detail by Lewis and colleagues [38]. A pilot study of the efficacy of 10 mg/kg liposomal amphotericin B efficacy, in initial mucormycosis treatment, has been completed but not yet reported (http://clinicaltrials.gov/ct2/search).

Triazoles

Of the triazole agents, fluconazole and voriconazole have little or no activity against the Mucorales [38]. Itraconazole is active in vitro against some of these organisms, but has demonstrated poor efficacy in animal models [39, 40]. Posaconazole is active in vitro and in vivo against many of the Mucorales [32, 33, 38–40]. There are several reports of salvage therapy with posaconazole of patients with mucormycosis refractory to amphotericin B [41–44]. The use of posaconazole, however, as monotherapy or in combination with amphotericin B, for the treatment of mucormycosis awaits further evaluation in randomized clinical trials versus deoxycholate amphotericin B or a lipid formulation of amphotericin B [44]. Isavuconazole, a new extended-spectrum triazole with in vitro and in vivo activity against the Mucorales has been used in treatment of two reported cases [45–48]. The data from a recently completed nonrandomized trial of isavuconazole as primary therapy of mucormycosis are currently being analyzed.

Combination Antifungal Therapy

Combination therapy with a lipid formation of amphotericin B and an echinocandin is another possible treatment option [44]. Although echinocandins have no intrinsic in vitro activity against the Mucorales, the combination of echinocandin and lipid formulation of amphotericin B improves outcome in laboratory animal studies and possibly in patients [44, 49].

Surgery

Appropriate and early surgical debridement is a critical intervention for the successful management of mucormycosis for a number of reasons: The infection progresses rapidly, vascular thrombosis compromises the delivery of antifungal agents to the site of infection, and there is massive tissue necrosis. Several retrospective studies have demonstrated that the survival of patients treated with antifungal therapy, combined with surgical debridement, was significantly higher than that of patients treated with antifungal therapy alone [1, 4, 6, 18]. Surgical treatment should aim in removing all necrotic tissues and should be considered for any of the clinical presentations of mucormycosis (sinus disease, pulmonary, or cutaneous). It should be performed early in the course of treatment and repeated if necessary. It may include excision of the infected sinuses, debridement of retro-orbital space, or even enucleation in the case of sinus/sino-orbital disease, and wedge resection, lobectomy or pneumonectomy, in the case of pulmonary disease [14, 15, 18, 21, 27]. If the patient survives the infection, plastic surgery is likely to be needed in order to correct disfiguring resulting from debridement [4].

Hyperbaric Oxygen

Besides the above important aspects of management of mucormycosis, hyperbaric oxygen is a therapeutic modality that has been occasionally used as adjunctive treatment. Hyperbaric oxygen has a theoretical potential for being beneficial in the treatment of mucormycosis since it is known to inhibit fungal growth at high pressures, correct tissue hypoxia and lactic acidosis, promote healing, and enhance phagocytosis [50, 51]. In a number of case reports and small case series of mucormycosis, administration of hyperbaric oxygen was associated with a favorable outcome [50]. Currently, however,

the absence of randomized controlled clinical trials on the efficacy of hyperbaric oxygen, in this setting, does not allow firm recommendations regarding its use as adjunctive treatment of mucormycosis.

Prognosis

The prognosis of mucormycosis largely depends on the patient's underlying condition, the clinical presentation of the infection, the time of initiation of therapy, and the type of treatment provided. Mortality may range from less than 10% for localized sinus disease to approximately 100% for disseminated infection, with an overall percentage of 47% for cases of mucormycosis [1, 4].

Prevention

Prevention may be feasible for a proportion of cases through adequate control of diabetes and judicious use of deferoxamine and corticosteroids. For severely immunocompromised hosts, measures to reduce the risk of exposure to airborne sporangiospores should be undertaken, including high-efficiency particulate air HEPA filtration of air supply, positive room air pressures, exclusion of plants from the wards, and wearing of masks when leaving the room. Due to the relatively low incidence of mucormycosis, the cost-effectiveness of prophylactic treatment is questionable; the development of a preemptive therapy approach, however, based on validated early indicators of the disease and risk assumption, should be a target for research in the near future. In the meantime, physicians caring for susceptible patients should maintain a high level of suspicion, and be alert to the early signs and symptoms of mucormycosis, in order to achieve early diagnosis and timely initiation of treatment.

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Suggested Reading

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Pneumocystosis

14

Francis Gigliotti and Terry W. Wright

Introduction

Pneumocystis is the classic opportunistic pathogen in that it does not produce any recognizable disease in an immunologically intact host, yet infection of the at-risk immunocompromised host results in a pneumonitis that is universally fatal if untreated. The organism was first identified in the early 1900s but was not appreciated to be a significant human pathogen until after World War II when outbreaks of *Pneumocystis* pneumonia (PCP) occurred in orphanages in Europe. These young infants who developed what was termed "interstitial plasma cell pneumonitis" were suspected to be immunosuppressed secondary to severe malnutrition. Two subsequent events firmly established *Pneumocystis* as a major opportunistic pathogen; the development of successful cancer chemotherapy in the late 1950s and 1960s and the start of the AIDS epidemic in the early 1980s. In fact, it was the recognition of a cluster of this "rare" pneumonia, PCP, in apparently healthy young gay men over a short period of time that led to the recognition that a new syndrome (AIDS) and infection (HIV) had emerged [1, 2].

Presently, the population of patients at risk to develop PCP is growing steadily as we develop new modalities of therapy and potent immunosuppressive drugs to treat malignancies, organ failure, autoimmune and inflammatory diseases. For example, in solid organ transplant recipients, as survival improves so does the recognition that these patients are at risk of developing PCP if not on specific prophylaxis. Most recently, the addition of antitumor necrosis factor (TNF) therapy to the management of patients with Crohn's disease, rheumatoid arthritis, and other inflammatory conditions has resulted in the occurrence of PCP in populations that previously had not been considered to be at risk for the development of PCP. The importance of PCP as an opportunistic pneumonia is likely to increase as the use of immunosuppressive biologic response modifiers increases.

Etiologic Agent

All strains of *Pneumocystis* are extracellular organisms found in the lungs of mammals. The taxonomic placement of these organisms has not been unequivocally established, largely due to the inability to adequately culture the organism. However, nucleic acid homologies indicate it is most closely related to the fungi, despite its morphologic features and susceptibility to drugs that are similar to those of protozoa. Both phenotypic and genotypic analysis demonstrates that each mammalian species is infected by a unique strain of *Pneumocystis* [3–5]. A biological correlate for these differences is evidenced by animal experiments that have shown organisms are not transmissible from one mammalian species to another [6]. This restricted host range is the one biologic characteristic of *Pneumocystis* that might achieve the level of uniqueness sufficient to define species of *Pneumocystis*.

Two forms of *Pneumocystis* are found in the alveolar spaces, thick-walled cysts (Fig. 14.1) that are 5–8 μm in diameter and may contain up to eight pleomorphic intracystic sporozoites, and trophozoites, which are 2–5 μm diameter cells with a more typical cell membrane, thought to be derived from excysted sporozoites. The terminologies sporozoites and trophozoites are based on the morphological similarities to protozoa, since there are not exact correlates for these forms of the organism among the fungi. Sporozoites are also called intracystic bodies and trophozoites are referred to as trophic forms.

As noted above, the host-species specificity of *Pneumocystis* has led some to propose the division of *Pneumocystis carinii* into multiple unique species, with the nomenclature *Pneumocystis jirovecii* being used to refer to human *P. carinii* [7]. The proposal for a change in nomenclature has been questioned because it also calls for species distinction

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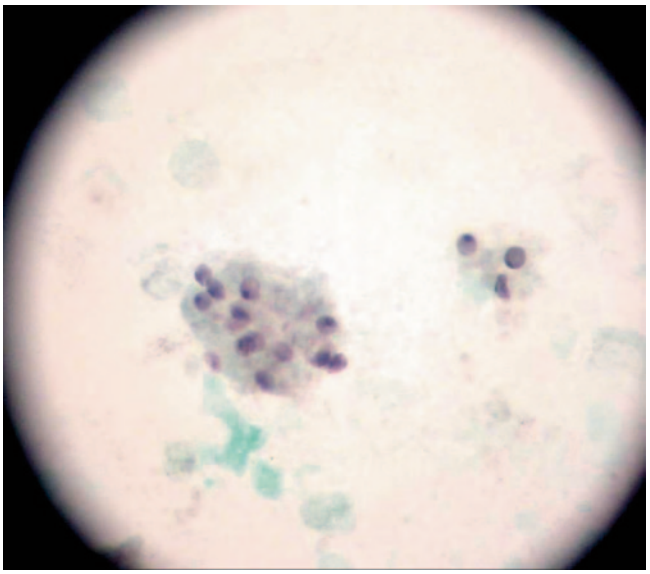

Fig. 14.1 Silver-stained bronchoalveolar lavage specimen showing characteristic clusters of *Pneumocystis* cysts

based on variation in gene sequences not known to result in a unique phenotype, so-called molecular phylogeny [8]. *Pneumocystis* that has not had a new name submitted for consideration can still be clearly defined using "special form" nomenclature (e.g., *P. carinii* f. sp. mustela for ferret *P. carinii*).

Epidemiology

PCP occurs only in patients who are significantly immunosuppressed, typically with abnormalities in $CD4^+$ T lymphocytes or B cells. Serologic studies have demonstrated that a high proportion of the population has evidence of infection and that seroconversion typically occurs during childhood. A recent prospective longitudinal study demonstrated that seroconversion began in the first few months of life and by 20 months of age, 85% of the infants in the study had seroconverted [9].

Aside from the serologic data, *Pneumocystis* was not known to actually infect the immunologically normal host. However, animal studies have proved that *Pneumocystis* produces a typical pattern of infection, transmission, and resolution in the normal host [10]. The other important biological feature of *Pneumocystis* infection is that the strain (or species) of *Pneumocystis* from any given mammalian host is transmissible only to members of the same host species. Cross-species transmission has never been convincingly demonstrated. Because of the finding of early seroconversion followed by disease later in life, PCP was postulated to be the result of reactivation of latent infection. However, no evidence for latency has ever been demonstrated, and mouse and rat models of PCP have shown that latency does not develop after infection. Considering all of these features, it would seem most likely that PCP is the result of new infection rather than reactivation of a latent infection. Person-to-person transmission is likely, based on the cumulative experience in animal models, but difficult to prove.

Without prophylaxis, PCP develops in approximately 70% of adults and 40% of infants and children with AIDS, and 10% of patients with organ transplants. It is often the sentinel event identifying infants with severe congenital immunodeficiencies such as severe combined immunodeficiency (SCID) syndrome. PCP also is a frequent occurrence in patients being treated for malignancies, occurring with an overall frequency of 10–15%. The actual incidence for any given malignancy depends on the treatment regimen and is positively correlated with the number of chemotherapeutic agents and the intensity of treatment.

Pathogenesis and Immunology

Control of infection is dependent on normally functioning $CD4^+$ T lymphocytes. Studies in patients with AIDS show an increase in the occurrence of *Pneumocystis* pneumonia as $CD4^+$ T lymphocytes drop. For adults and children over 6 years of age, a $CD4^+$ T cell count of 200 cells/μl or lower is a marker of very high risk for the development of PCP. Based on the occurrence of PCP in some patients and mouse strains with various immunologic defects which result in defective antibody production, a possible role for $CD4^+$ T lymphocytes could be to provide help for the production of specific antibody. Passively administered antibody has been shown to aid in the clearance of *Pneumocystis* in mouse models. Thus, antibody could be involved in the clearance of organisms through interaction with complement, phagocytes, and/or T lymphocytes.

The mechanism by which *Pneumocystis* damages the lung is not yet fully defined. Animal models have been valuable in helping us understand the immunopathogenesis of PCP [11]. Infection of SCID mice with *Pneumocystis* produces very little alteration in lung histology or function until very late in the course of the disease. However, if *Pneumocystis*-infected SCID mice are immunologically reconstituted with normal splenocytes, there is a rapid onset of an inflammatory response that results in an intense cellular infiltrate, markedly reduced lung compliance and significant hypoxia, all changes seen in humans with PCP. These inflammatory changes are associated with marked disruption of surfactant function. T cell subset analysis has shown that $CD4^+$ T lymphocytes produce an inflammatory response that not only clears the organisms but also results in lung injury. In contrast, $CD8^+$ T lymphocytes are ineffective in the eradication of *Pneumocystis,* but do produce a marked injurious inflammatory response, especially in the absence of $CD4^+$ T lymphocytes.

Immune reconstitution inflammatory syndrome (IRIS), also called immune restitution disease or immune reconstitu-

tion syndrome, is a recently described manifestation of pulmonary infection in AIDS patients with *Pneumocystis, Mycobacterium tuberculosis,* and other pulmonary pathogens who are experiencing rapid reconstitution of their immune system due to the administration of effective antiretroviral therapy [12]. In general, the severity of IRIS is directly related to the degree and rapidity of T cell recovery. Mouse models of PCP suggest that $CD8^+$ T lymphocytes help modulate the inflammation produced by $CD4^+$ T lymphocytes, but as mentioned above, their ineffectual inflammatory response can also contribute significantly to lung injury. These various T cell effects may be responsible for the variations in presentation and outcome of *Pneumocystis* pneumonia observed in different patient populations.

The inflammatory processes taking place during PCP do not appear to result in major long-term damage to the lung in those who recover. A long-term follow-up of 23 children with cancer and PCP showed a return to normal lung function by 6 months in all 18 survivors. Similar studies in adults are complicated by the fact that adult patients, especially those with AIDS, might have multiple pulmonary insults. While some studies, primarily of adult AIDS patients, suggest long-term pulmonary damage following PCP, other studies of renal transplant recipients have shown pulmonary function returned to nearly normal after recovery from PCP.

Clinical Manifestations

Pneumocystis Pneumonia

There are at least three distinct clinical presentations of PCP. In patients with profound immunodeficiency, such as young infants with congenital immunodeficiency, severe malnutrition, or in AIDS patients with very few $CD4^+$ T lymphocytes, the onset of hypoxia and symptoms is subtle with cough, dyspnea on exertion, or tachypnea, often without fever. Infants may show progression to nasal flaring, intercostal, suprasternal, and infrasternal retractions. As the disease progresses patients develop hypoxia, with cyanosis in severe cases. In the sporadic form of PCP, occurring in children and adults with underlying immunodeficiency, the onset of hypoxia and symptoms is usually more abrupt with fever, tachypnea, dyspnea, and cough, progressing to severe respiratory compromise. This latter type accounts for the majority of cases, although the severity of clinical expression may vary. Rales are usually not detected on physical examination. The third pattern of disease is that associated with rapid restoration of immune function referred to as IRIS. It has been best described in newly diagnosed AIDS patients who are severely immunocompromised and present with PCP as their initial manifestation of AIDS [12]. These patients appear to respond well to therapy for PCP but 3–6 weeks after beginning treatment they experience an unexpected recurrence of pulmonary symptoms and chest X-ray (CXR) abnormalities that coincide with return of immune function. IRIS may also occur in bone marrow transplant patients who engraft while infected with *Pneumocystis*.

Extrapulmonary Infections

Extrapulmonary infection with *Pneumocystis* is rare. The incidence is not well defined, but is estimated to be a 1000-fold less likely than PCP itself [13]. The most commonly reported sites of infection include the ear and eye. Why these two sites seem to predominate is unclear but may reflect the fact that infection at these sites may quickly produce readily apparent signs and symptoms. Other sites of involvement are the thyroid gland, liver, kidney, bone marrow, lymph nodes, spleen, muscles, and gastrointestinal (GI) tract. How the organism arrives at these sites is unknown. Response to treatment is usually good when extrapulmonary infections occur in the absence of pulmonary infection.

Diagnosis

Pulmonary symptoms in at-risk patients should always raise the suspicion of PCP. The classic chest radiograph reveals bilateral diffuse alveolar disease with a granular pattern (see Fig. 6.7, Chap. 6). The earliest densities are perihilar, and progression proceeds peripherally, typically sparing the apical areas until last. Less common chest radiograph appearances in PCP include cystic lesions, pneumothorax, or isolated focal infiltrates. In patients receiving aerosolized pentamidine for prophylaxis, there may be a predisposition for upper lobe infiltrates. The arterial oxygen tension (PaO_2) is invariably decreased.

A clinical pearl is that an elevated lactate dehydrogenase (LDH) may be a hint that one is dealing with PCP. This is due to the fact that LDH is a useful marker of alveolar and inflammatory cell damage. Because *Pneumocystis* is a diffuse alveolar infection, it tends to result in higher and more often elevated levels of LDH than some other more focal opportunistic pulmonary infections. For example, a recent analysis of LDH and pulmonary opportunistic infections in AIDS patients showed that about 90 % of those with definite PCP had elevated serum LDH [14]. Thus while not specific for PCP, very high LDH levels should raise one's suspicion for PCP and normal levels make the diagnosis of PCP much less likely.

PCP can only be definitively diagnosed by demonstrating *Pneumocystis* in the lungs of a patient with compatible pulmonary signs and symptoms. Appropriate specimens for analysis include bronchoalveolar lavage, tracheal aspirate, transbronchial lung biopsy, bronchial brushings,

Table 14.1 Recommended treatment for *Pneumocystis* pneumonia

Drug	Adults	Children
Treatment of first choice		
Trimethoprim–sulfamethoxazole (TMP–SMX)	TMP 15–20 mg/kg/d with SMX 75–100 mg/kg/d IV divided into three or four doses; PO for mild disease	TMP 15–20 mg/kg/d with SMX 75–100 mg/kg/d IV divided into four doses; PO for mild disease
Alternate treatment regimens		
Pentamidine	4 mg/kg/d IV as single dose	4 mg/kg/d as single dose
Atovaquone	750 mg PO bid	3–24 mo of age: 45 mg/kg/d PO divided into two doses; 1–3 mo and more than 24 mo: 30 mg/kg/d in two divided doses (max. daily dose 1500 mg)
Dapsone plus trimethoprim	Dapsone 100 mg, PO once daily; TMP 15 mg/kg/d PO in three divided doses	Dapsone 2 mg/kg/d (100 mg max.) PO once daily; TMP 15 mg/kg/d PO in three divided doses
Primaquine plus clindamycin	Primaquine 15–30 mg, PO once daily; clindamycin 600 mg IV every 8 h	Primaquine 0.3 mg/kg (max. 30 mg) PO once daily; clindamycin 40 mg/kg/d IV in four divided doses (no pediatric data)
Trimetrexate plus leucovorin	Trimetrexate	
	50 kg: 1.5 mg/kg/d IV once daily 50–80 kg: 1.2 mg/kg/d IV once daily 80 kg: 1.0 mg/kg/d IV once daily	45 mg/m^2 IV once daily
	Leucovorin (continue 3 days beyond trimetrexate)	
	50 kg: 0.8 mg/kg/d IV or PO every 6 h 50 kg: 0.5 mg/kg/d IV or PO every 6 h	20 mg/m^2 IV or PO every 6 h

Duration of therapy is typically 3 weeks in patients with AIDS and 2 weeks in other immunosuppressed patients
IV intravenous, *PO* orally, *mg/kg* milligrams/kilogram, *mg/kg/d* milligrams/kilogram/day, *mo* months of age, *bid* twice daily

percutaneous transthoracic needle aspiration, and open lung biopsy. Induced sputum samples are gaining popularity, but are helpful only if positive; the absence of *Pneumocystis* in an induced sputum sample does not exclude infection. The open lung biopsy is the most reliable method, although bronchoalveolar lavage is generally more practical. Estimates of the diagnostic yield of the various specimens are as follows: induced sputum 20–40 %, tracheal aspirate 50–60 %, bronchoalveolar lavage 75–95 %, transbronchial biopsy 75–95 %, and open lung biopsy 90–100 %. Once obtained, the specimens are typically stained with one of the four commonly used stains: Gomori methenamine silver (GMS) and toluidine blue stains only stain cyst forms; polychrome stains, such as Giemsa, stain both trophozoites and sporozoites; and the fluorescein-labeled monoclonal antibody also stains both trophozoites and cysts. *Pneumocystis* can also be visualized by Papanicolaou stain. Polymerase chain reaction analysis of respiratory specimens offers promise as a rapid diagnostic method, but a standardized system for clinical use has not been established.

Treatment

The clear drug of choice for the treatment of PCP is trimethoprim–sulfamethoxazole (TMP–SMX; Table 14.1). Generally, TMP–SMX is administered intravenously, but it may be given orally if disease is mild and no malabsorption or diarrhea is present. The duration of treatment is generally 3 weeks for patients with AIDS and 2 weeks for other patients. Adverse reactions occur frequently, more so in adults than children, with TMP–SMX. These include rash, fever, and neutropenia in patients with AIDS. These side effects are less common in non-AIDS patients. For patients who cannot tolerate or fail to respond to trimethoprim–sulfamethoxazole after 5–7 days, pentamidine isethionate may be used. Adverse reactions are frequent with pentamidine and include renal and hepatic dysfunction, hyperglycemia or hypoglycemia, rash, and thrombocytopenia. Atovaquone is an alternative treatment that has been used primarily in adults with mild-to-moderate disease. For adults and adolescents atovaquone is given twice a day with food. Less information is available for the treatment of younger children with this agent. Other effective therapies include trimetrexate glucuronate or combinations of trimethoprim plus dapsone and of clindamycin plus primaquine.

Administration of corticosteroids in addition to anti-*Pneumocystis* drugs increases the chances for survival in moderate and severe cases of PCP [15]. The recommended regimen of corticosteroids for adolescents older than 13 years of age and for adults is oral prednisone, 80 mg/day divided in two doses on days 1–5, 40 mg/day once daily on days 6–10, and 20 mg/day once daily on days 11–21. While specific studies of adjunctive corticosteroid therapy in young children are not available, a reasonable regimen for children is oral prednisone, 2 mg/kg/day for the first 7–10 days, followed by a tapering regimen for the next 10–14 days.

Table 14.2 Recommended antibiotic prophylaxis for *Pneumocystis* pneumonia

Drug	Adults	Children
Trimethoprim–sulfamethoxazole (TMP–SMX)	One single or double strength tablet daily or 3 days/week	TMP 5 mg/kg/d with SMX 25 mg/kg/d given once daily or divided into two doses
Dapsone	100 mg daily or twice weekly	2 mg/kg/d as single dose (max. 100 mg/dose)
Atovaquone	1500 mg once daily	30 mg/kg/d as single dose for children aged 1–3 months and older than 24 mo; 45 mg/kg/d as single dose for children 4–23 mo
Aerosolized pentamidine	300 mg monthly given by Respirgard II nebulizer	For children ≥ 5 years—same as for adults

IV intravenous, *PO* orally, *mg/kg/d* milligrams/kilogram/day, *mo* months of age

Prevention

PCP is effectively prevented by the use of antimicrobial prophylaxis, thus all patients at high risk for PCP should be placed on chemoprophylaxis. As noted above, $CD4^+$ T cells are the key cells in determining susceptibility to PCP. However, defining the risk for PCP is not always clear. In AIDS patients, there is a clear-cut correlation between cell number and function so that firm cutoffs can be given. In adults with AIDS, prophylaxis is indicated at $CD4^+$ T cell counts of below 200 cells/μl. Because of the rapid changes in $CD4^+$ T cell counts in young infants, prophylaxis is recommended for all HIV-infected children during their first year of life. Thereafter, prophylaxis is started at $CD4^+$ T cell counts drop below 750 cells/μl for infants 12–23 months of age, 500 cells/μl for children from 2 to 6 years of age, and 200 cells/μl for those 6 and older. Prophylaxis is also recommended for all ages if $CD4^+$ T cell percentages drop below 15%. In other disease states where patients are placed at risk of PCP from being on immunosuppressive drugs, both lymphocyte number and function will be affected. Thus while a patient may have a lymphocyte count above the threshold for susceptibility to develop PCP, suppressed function of remaining lymphocytes may place them at risk for PCP. Because of the demonstrated increased risk of PCP with increasing intensity of chemotherapy in patients with cancer it would seem prudent, in our opinion, to consider prophylaxis for patients receiving prolonged (more than 6–8 weeks) therapy with two immunosuppressive agents and to give prophylaxis to all patients receiving three or more immunosuppressive agents.

TMP–SMX is the drug of choice for *Pneumocystis* prophylaxis and may be given for 3 days each week, or, alternatively, each day (Table 14.2). The original study testing less than daily administration of TMP–SMX used a schedule of three consecutive days on TMP–SMX and 4 days off with the idea of reducing potential bone marrow suppression from the TMP–SMX. Subsequent studies have used alternate day schedules such as dosing on Monday, Wednesday, and Friday. The double strength tablet is preferred for adults receiving 3 days a week dosing. Alternatives for prophylaxis, all of which are inferior to TMP–SMX, include dapsone, atovaquone, and aerosolized pentamidine. Prophylaxis must be continued as long as the patient remains immunocompromised. Studies in adult AIDS patients who reconstitute adequate immune response during antiretroviral therapy show that prophylaxis may be withdrawn without risk of developing PCP. Small studies in children have provided similar results. Patients who maintain their $CD4^+$ T cell count at or above the at-risk threshold for age, e.g., 200 cells/μl for older children and adults, for at least 3 months are candidates for discontinuation of both primary and secondary prophylaxis. Guidelines for the management of PCP in adults and children can be found at the National Institutes of Health (NIH) AIDS information web site [16, 17].

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Cryptococcosis

15

Eileen K. Maziarz and John R. Perfect

Introduction

Cryptococcosis is an infectious disease with a wide range of clinical presentations caused by pathogenic encapsulated yeasts in the genus *Cryptococcus*. Currently, there are two species of these fungi that commonly cause disease in humans: *Cryptococcus neoformans,* which causes cryptococcosis in both immunocompetent and immunocompromised hosts, and *Cryptococcus gattii*, which is primarily a pathogen in apparently immunocompetent patients but can also cause disease in the immunocompromised. *C. neoformans* was first identified as a human pathogen in 1894 by two German physicians, Otto Busse and Abraham Buschke, when they described a circular yeast-like microorganism in a lesion on the tibia of a woman; the microorganism was initially named *Saccharomyces hominis* [1]. The name *C. neoformans* has been consistently adopted in both the mycology and medical literature since 1950 [2]. In the mid-1970s, when Kwon-Chung discovered two mating types of *C. neoformans* that produced fertile basidiospores, the organisms were subsequently separated into two varieties, var. *neoformans* (serotypes A and D) and var. *gattii* (serotypes B and C). These two varieties were recently separated into two species, *C. neoformans* and *C. gattii*, based on their genetic background and phylogenetic diversity, as proposed by Kwon-Chung in 2002 [3]. It is possible, as more molecular information is gathered from genome sequencing, that *C. neoformans* var. *neoformans* (serotype D) and *C. neoformans* var. *grubii* (serotype A) will be divided into separate species as well as other cryptic species.

The incidence of cryptococcosis began to rise in the late 1970s. Early case reports of cryptococcal infections were primarily associated with cancer, autoimmune diseases, organ transplantation, and receipt of corticosteroids as these immunocompromised populations expanded [4]. A major surge in new cases of cryptococcosis occurred during the first two decades of the HIV pandemic, when cryptococcal infection was an important opportunistic infection (OI) in all parts of the world. Furthermore, around 2000, *C. gattii* strains (previously geographically restricted to tropical and subtropical regions) caused a localized outbreak of cryptococcosis in apparently immunocompetent individuals on Vancouver Island [5]. This has increased recognition that these fungi can exploit new geographical environments and cause disease in both immunocompromised and apparently immunocompetent hosts. Despite the development of highly active antiretroviral therapy (HAART), which has decreased the rate of HIV-related cryptococcosis in developed countries, the burden of cryptococcal infection is still very high in developing countries and in those individuals without access to health care. It has been estimated that there are a million cases per year with more than 600,000 deaths due to cryptococcosis worldwide [6].

Etiologic Agents

Cryptococcus is a genus of heterobasidiomycetous fungi containing more than 30 species. However, the common pathogenic organisms of cryptococcosis currently consist of two species, which can further be classified into three varieties, five serotypes (based on structural differences in the polysaccharide capsule), and eight molecular subtypes (Table 15.1). *C. neoformans* is classified into serotype A and D and the hybrid strain AD, whereas serotype B and C strains are classified as *C. gattii*. Serotype A strains have been further classified as *C. neoformans* var. *grubii* and serotype D strains are named *C. neoformans* var. *neoformans*. Recently, both *C. neoformans* and *C. gattii* have been further divided into molecular subtypes for each species, VN I–IV, VN B and VG I–IV, respectively.

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Table 15.1 Classification of *Cryptococcus neoformans* and *Cryptococcus gattii*

Serotype	Species and varieties	Molecular types
A	*C. neoformans* var. *grubii*	VN I, VN II, VN B
B	*C. gattii*	VG I, VG II, VG III, VG IV
C	*C. gattii*	VG I, VG II, VG III, VG IV
D	*C. neoformans* var. *neoformans*	VN IV
AD	*C. neoformans*	VN III

The life cycle of *C. neoformans* and *C. gattii* involve asexual (yeast) and sexual (basidiospores/hyphae) forms. The asexual form is the encapsulated yeast that reproduces by narrow-based budding and is found most commonly in clinical specimens, whereas the sexual stage, which exists in one of two mating types, "alpha" or "a," is observed only under certain conditions, resulting in meiosis to form basidiospores. The vast majority of clinical infections and environmental isolates are caused by "alpha" mating-type locus strains. Since the sexual stage of *C. neoformans* and *C. gattii* has been described, their teleomorphs were named *Filobasidiella neoformans* and *Filobasidiella bacillospora,* respectively.

C. neoformans and *C. gattii* usually appear as white-to-cream, opaque, and mucoid colonies that grow to several millimeters in diameter on the most routine agar within 48–72 h. With some strains, a few colonies occasionally develop sectors with different pigmentation or different morphologies (i.e., wrinkled, smooth, mucoid). Both cryptococcal species will grow readily on most fungal culture media without cycloheximide at 30–37 °C in aerobic conditions. However, *C. neoformans* is generally more thermotolerant than *C. gattii,* and, within this species, serotype A is generally more tolerant than serotype D strains. In addition to their ability to grow at 37 °C, the yeast produce a thick shedding polysaccharide capsule, melanin pigments, and the enzymes urease and phospholipase, which allow *Cryptococcus* to be readily identified from other yeasts. These are also considered to be yeast virulence factors.

Epidemiology

Cryptococcosis was considered an uncommon infection prior to the AIDS epidemic, associated with malignancies, organ transplantation, and certain immunosuppressive treatments. Beginning in the early 1980s, the incidence of cryptococcosis increased significantly and between 6 and 10% of persons with AIDS developed cryptococcosis [7, 8]. In fact, HIV/AIDS was found to be associated with 80% of cryptococcosis cases worldwide. Cryptococcal infection is a major OI in HIV-infected patients as the $CD4^+$ cell count falls below 100 cells/μl. Following widespread implementation of HAART, the incidence of cryptococcosis among patients with HIV/AIDS has fallen significantly in most developed nations. The incidence of cryptococcal infection in persons not infected with HIV has remained stable during this time. Moreover, in developing nations with limited access to HAART, the prevalence of and morbidity and mortality associated with cryptococcosis remains unacceptably high, accounting for up to 600,000 deaths per year. Besides HIV infection, other risk factors for acquiring cryptococcal infections include many conditions that result in an immunocompromised status (Table 15.2). Although both *C. neoformans* and *C. gattii* can cause cryptococcosis in apparently normal hosts, the percentage of *C. gattii* infections causing disease in such patients is significantly higher than for *C. neoformans*.

Table 15.2 Predisposing factors of cryptococcosis

HIV infection
Malignancies[a] (e.g., Hodgkin's disease, other lymphomas, and chronic lymphocytic leukemia)
Lymphoproliferative disorders[a]
Idiopathic $CD4^+$ T cell lymphopenia
Rheumatologic or immunologic diseases[a]
Sarcoidosis
Systemic lupus erythematosus
Rheumatoid arthritis
Hyper-IgM syndrome or hyper-IgE syndrome
Monoclonal antibodies (etanercept, infliximab, alemtuzumab)
Corticosteroid and/or immunosuppressive therapies
Diabetes mellitus
Solid organ transplantation[a]
Chronic pulmonary diseases
Renal failure and/or peritoneal dialysis
Chronic liver diseases[b]

IgE immunoglobulin E, *IgG* immunoglobulin G
[a] Immunosuppressive therapies add to the risk
[b] Poor prognosis

C. neoformans is found throughout the world in association with excreta from certain birds such as pigeons and in tree hollows. *C. gattii* is commonly associated with several species of eucalyptus and other trees [9]. While the link between the environmental source of infection and cryptococcosis cases is not precise, there is evidence to suggest an increased risk of cryptococcosis and asymptomatic cryptococcal antigenemia following intense bird exposures. Recently, there has been a strong link between the *C. gattii* outbreak in humans on Vancouver Island and common environmental yeast exposures. Although these fungi can be detected in endobronchial specimens from humans without disease (colonization), clinicians should be alert for subclinical disease or potential for disease when *Cryptococcus* is isolated from any clinical specimen.

Approximately 95% of cryptococcal infections are caused by serotype A strains (*C. neoformans* var. *grubii*) with the remaining 4–5% of infections caused by serotype D (*C. neoformans* var. *neoformans*) or serotype B and C strains (*C. gattii*). Whereas *C. neoformans* serotype A is found worldwide, serotypes B and C are found primarily in

tropical and subtropical regions such as southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa (and more recently identified in temperate climates such as Vancouver Island and the Pacific Northwest region of the USA), and serotype D is predominantly found in European countries (Table 15.3) [10]. In Australia and New Zealand, serotypes B and C caused up to 15% of all cases of cryptococcosis cases in one study, but serotype A remains the predominant serotype even in these endemic areas [11]. To date, only *C. gattii* strains have been reported to cause a widespread defined outbreak of disease [5].

Table 15.3 Distribution of *C. neoformans* and *C. gatti*

Cryptococcus species	Primary areas of distribution
C. neoformans var. *grubii* serotype A	Worldwide; pigeon guano, tree hollows
C. gattii	Tropical and subtropical regions: southern California, Hawaii, Brazil, Australia, Southeast Asia, and central Africa; eucalyptus trees, firs, and oak trees
C. neoformans var. *neoformans* serotype D	Europe: Denmark, Germany, Italy, France, and Switzerland; less common in the environment than serotype A

Pathogenesis and Immunology

Cryptococcosis occurs primarily by inhalation of the infectious propagules, either dehydrated (poorly encapsulated) yeasts or basidiospores, into pulmonary alveoli. Direct inoculation into tissue due to trauma can be a portal of entry in occasional cases and, potentially, yeast may enter through the gastrointestinal tract. After the yeasts are inhaled into the lungs of a susceptible host, they encounter alveolar macrophages, and other inflammatory cells are recruited through release of cytokines and chemokines such as interleukin (IL)-12, IL-18, monocyte chemotactic protein (MCP)-1, and macrophage inflammatory protein (MIP)-1α. Cryptococcal infection primarily involves granulomatous inflammation, which is a result of a helper T cell (Th1) response with cytokines including tumor necrosis factor, interferon-γ, and IL-2 [12]. In many circumstances, the yeasts remain dormant (yet viable) in hilar lymph nodes or pulmonary foci of an asymptomatic individual for years and then disseminate outside those complexes when local immunity is suppressed, similar to that which is observed in cases of reactivation tuberculosis or histoplasmosis [10]. In a patient with severely compromised cellular immunity, the yeasts reactivate and proliferate at the site of infection and then disseminate to other sites causing progressive clinical symptoms.

Recent advances in the molecular biology of *Cryptococcus* have confirmed several virulence factors. The three classical virulence factors of *C. neoformans* include: capsule formation, melanin pigment production, and the ability to grow well at 37 °C [9, 12]. The prominent antiphagocytic polysaccharide capsule, which is composed of glucuronoxylomannan (GXM), is unique to *Cryptococcus* species and is considered an essential virulence factor that has multiple effects on host immunity. In addition, *C. neoformans* possesses an enzyme that catalyzes the conversion of diphenolic compounds to form melanin, which may have a biological role to protect the yeasts from host oxidative stresses and which may partially explain the organism's neurotropism. Finally, its ability to grow at 37 °C is a basic part of the virulence composite for most of the human pathogenic fungi including *Cryptococcus,* as molecular studies have linked high-temperature growth with certain signaling pathways and enzymes that this yeast has acquired or adapted over time in order to enhance its pathogenicity. Other virulence factors include phospholipase and urease production and multiple enzymes associated with protection against oxidative stresses.

Clinical Manifestations

C. neoformans and *C. gattii* have a predilection for establishing clinical disease in the lungs and central nervous system (CNS). Other organs that may be involved in cryptococcosis include skin, prostate, eyes, bone, and blood [2, 8, 10, 13]. In fact, this yeast may cause disease in any organ of the human body, and widely disseminated cryptococcal infection can affect multiple organs in severely immunosuppressed patients (Table 15.4).

Pulmonary Infection

The respiratory tract serves as the most important portal of entry for this yeast, and thus there are many clinical manifestations of pulmonary cryptococcosis, ranging from asymptomatic transient or chronic colonization of the airways or simply a pulmonary nodule on radiograph to life-threatening fungal pneumonia with acute respiratory distress syndrome (ARDS) [2, 8]. In a normal host with cryptococcal infection, asymptomatic pulmonary cryptococcosis can occur in about one third of patients with pulmonary infection and patients may present to care with only an abnormal chest radiograph. The most common radiologic findings of cryptococcosis include well-defined single or multiple noncalcified nodules (Fig. 15.1) and pulmonary infiltrates (Fig. 15.2), but other less frequent radiographic findings include pleural effusions, hilar lymphadenopathy, and lung cavitation. Patients with pulmonary cryptococcosis can present with symptoms of acute onset of fever, productive cough, respiratory distress, chest pain, and weight loss [14]. The outbreak of *C.*

Table 15.4 Clinical manifestations of cryptococcosis. (Adapted from Casadevall, A, Perfect, JR. *Cryptococcus neoformans*. Washington: ASM Press; 1998: 409 [2])

Organs	Common clinical manifestations
Central nervous system	Acute/subacute/chronic meningoencephalitis Cryptococcomas (abscesses) Spinal cord granuloma Chronic cognitive impairment (sequelae of hydrocephalus)
Lung	Asymptomatic airway colonization Pulmonary nodule(s) Hilar or mediastinal lymphadenopathy Lobar/interstitial infiltrates Miliary infiltrates Lung cavities Endobronchial lesions Pleural effusion/empyema Pneumothorax Acute/subacute pneumonia Acute respiratory distress syndrome
Skin	Papules with central ulceration (molluscum contagiosum-like) Subcutaneous abscesses Nodules/papules Cellulitis Draining sinuses Ulcers
Eye	Papilledema Endophthalmitis Optic nerve atrophy Chorioretinitis Keratitis Paresis of extraocular muscles
Genitourinary tract	Prostatitis Cryptococcuria Renal abscess Genital lesions
Bone and joints	Osteolytic lesion(s) Arthritis (acute/chronic)
Cardiovascular system	Cryptococcemia Endocarditis (native/prosthetic) Mycotic aneurysm Myocarditis Pericarditis
Other organs	Myositis Peritonitis Hepatitis Nodular/ulcerative GI mucosal lesions Pancreatic mass Breast abscess Adrenal mass and adrenal insufficiency Thyroiditis or thyroid mass Sinusitis Salivary gland enlargement

GI gastrointestinal

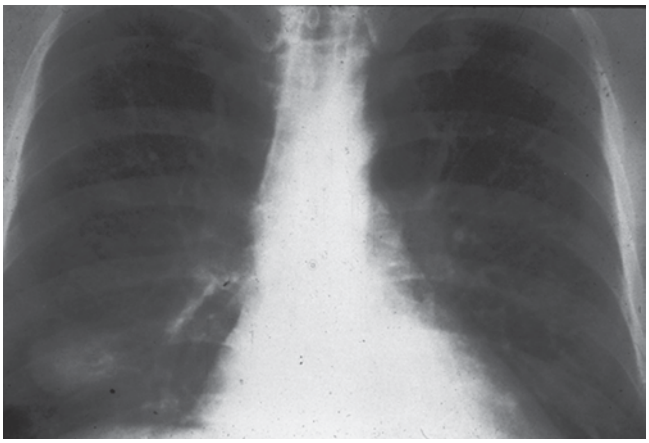

Fig. 15.1 Chest radiograph of pulmonary cryptococcosis presents as a single nodule in the lung at right lower lung field. (From A. Casadevall and J. R. Perfect, *Cryptococcus neoformans*, ASM Press, 1998. Reprinted with permission from Oxford University Press)

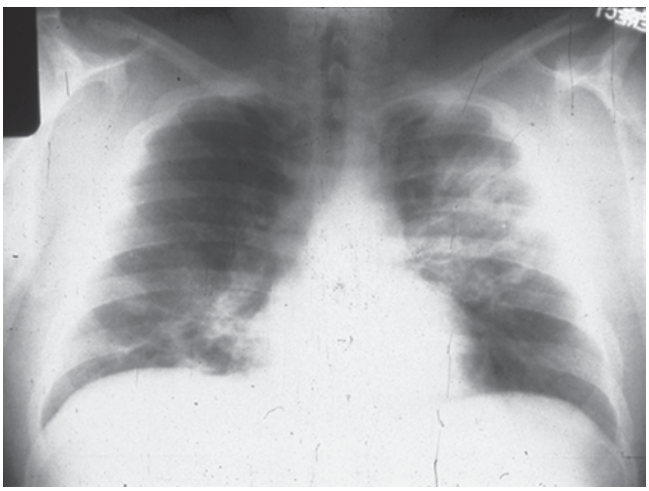

Fig. 15.2 Chest radiograph of pulmonary cryptococcosis presents as left lobar infiltrates. (From A. Casadevall and J. R. Perfect, *Cryptococcus neoformans*, ASM Press, 1998. Reprinted with permission from Oxford University Press)

gattii infections in Vancouver Island included several cases of severe symptomatic pulmonary cryptococcosis in apparently immunocompetent individuals. In an immunocompromised patient, especially those with HIV infection, cryptococcal pneumonia is usually symptomatic and can progress rapidly to ARDS, even in the absence of CNS involvement. Most immunocompromised patients with cryptococcal infection, however, present with CNS rather than pulmonary symptoms. In fact, more than 90% of HIV/AIDS patients with cryptococcal infection already have CNS cryptococcosis at the time of diagnosis, many of whom will have a paucity of respiratory complaints. The findings in chest radiographs of immunocompromised patients with pulmonary cryptococcosis are the same as those in immunocompetent patients, but alveolar and interstitial infiltrates tend to be more frequent and imaging can mimic *Pneumocystis* pneumonia. Accelerated presentations of cryptococcal pneumonia are more common among immunocompromised patients. In pulmonary cryptococcosis, if the infection is confined to the lung, serum cryptococcal polysaccharide antigen is usually negative. However, while a positive serum polysaccharide

antigen may indicate the dissemination of the yeast from the lung, it does not confirm CNS involvement. In immunocompromised individuals with pulmonary cryptococcosis, a lumbar puncture to rule out CNS disease should be considered regardless of the patient's symptoms or serum polysaccharide antigen test results. The only setting in which screening a lumbar puncture may not necessarily need to be performed in a patient with *Cryptococcus* isolated from the lung is in the asymptomatic, immunocompetent patient with disease that appears to be limited to the lungs.

CNS Infection

Clinical manifestations of CNS cryptococcosis include headache, fever, cranial neuropathy, alteration of consciousness, lethargy, memory loss, and signs of meningeal irritation [2, 8]. These findings are usually present for several weeks and therefore cause a clinical syndrome of subacute meningitis or meningoencephalitis. However, on some occasions, patients can present more acutely or lack typical features including headache. In HIV-infected patients with CNS cryptococcosis, the burden of fungal organisms in the CNS is usually high. Therefore, these patients may have a shorter onset of signs and symptoms, higher cerebrospinal fluid (CSF) polysaccharide antigen titers and intracranial pressures (ICPs), and slower CSF sterilization after starting antifungal treatment.

Different species may produce differences in clinical manifestations. For instance, one species may have a predilection to cause disease in brain parenchyma rather than the meninges. In certain areas of the world, *C. gattii* tends to cause cerebral cryptococcomas (Fig. 15.3) and/or hydrocephalus with or without large pulmonary mass lesions more frequently than *C. neoformans*. These patients with brain parenchymal involvement usually have high ICP and cranial neuropathies, and respond poorly to antifungal therapy.

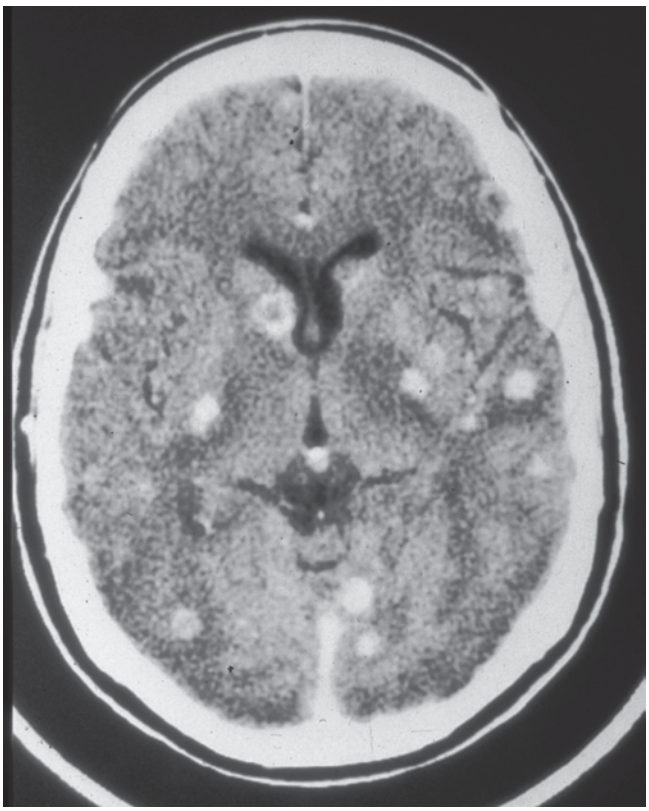

Fig. 15.3 CT scan of the brain showing multiple cryptococcomas in an apparently normal host. *CT* computed tomography. (From A. Casadevall and J. R. Perfect, *Cryptococcus neoformans*, ASM Press, 1998. Reprinted with permission from Oxford University Press)

Skin Infection

Cutaneous infections are the third most common clinical manifestations of cryptococcosis. Patients can manifest several types of skin lesions. One common skin lesion is a papule or maculopapular rash with central ulceration that may be described as "molluscum contagiosum-like." These lesions are indistinguishable from those due to other fungal infections including *Histoplasma capsulatum*, *Coccidioides immitis*, and *Penicillium marneffei*. Other cutaneous lesions of cryptococcosis include acneiform lesions, purpura, vesicles, nodules, abscesses, ulcers (Fig. 15.4), granulomas, pustules, plaques, draining sinus, and cellulitis. Because there are many skin manifestations in cryptococcosis that mimic other infectious as well as malignant conditions, skin biopsy with culture and histopathology are essential for definitive diagnosis. Skin lesions of cryptococcosis usually represent disseminated cryptococcal infection. Primary cutaneous cryptococcosis is very rare and is usually associated with skin injury and direct inoculation of the yeasts. Solid organ transplant (SOT) recipients on tacrolimus seem to be more

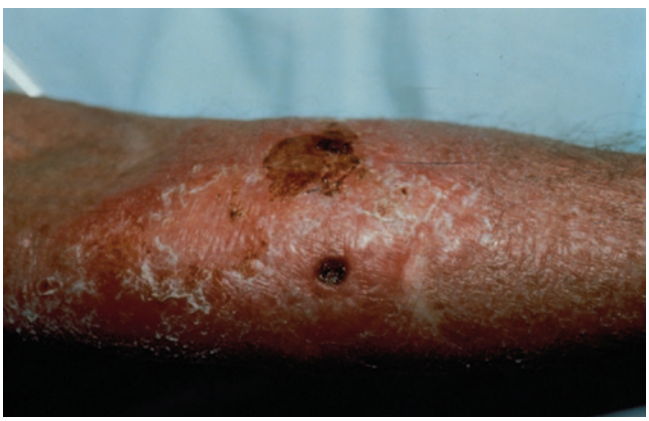

Fig. 15.4 Skin ulceration and cellulitis as cutaneous cryptococcosis

likely to develop skin, soft-tissue, and osteoarticular infections due to *Cryptococcus* [15]. Tacrolimus has anti-cryptococcal activity at high temperatures, but loses this activity as environmental temperatures decrease; this may in part explain the increased frequency of cutaneous cryptococcosis in these patients. Despite this series of patients, however, the most common site of disseminated infection in SOT recipients still remains the CNS, including patients receiving tacrolimus.

Prostate Infection

Prostatic cryptococcosis is usually asymptomatic, and the prostate gland is considered to be a sanctuary site for this yeast. The prostate may serve as an important reservoir for relapse of cryptococcosis in patients with a high fungal burden [16]. Latent *C. neoformans* infection has even been recognized to disseminate to the bloodstream during urological surgery on the prostate [17]. Cultures of urine or seminal fluid may still be positive for *Cryptococcus* after initial antifungal treatment of cryptococcal meningitis in AIDS patients [18], strongly supporting the need for prolonged antifungal treatment to clear the prostate in these severely immunocompromised patients.

Eye Infection

In the early reports of cryptococcal meningitis before the AIDS epidemic, ocular signs and symptoms were noted in approximately 45 % of cases [19]. The most common manifestations were ocular palsies and papilledema. In the present HIV era, several other manifestations of ocular cryptococcosis have been identified, including the presence of extensive retinal lesions with or without vitritis, which can lead to irreversible blindness. Furthermore, catastrophic loss of vision without evidence for endophthalmitis has also been reported [20]. Visual loss may be due to one of two pathogenic processes. The first is caused by infiltration of the optic nerve with the yeasts, producing rapid visual loss with few effective treatments. The second is due to increased ICP and compression of the ophthalmic artery. In this setting, patients have slower visual loss and treatment with serial lumbar punctures or ventricular shunts can prevent or slow down visual loss.

Infection at Other Body Sites

In addition to lung, CNS, skin, prostate, and eye, *C. neoformans* can cause disease in many other organs (Table 15.4). Cryptococcemia can occur in severely immunosuppressed patients but rarely causes endocarditis. Bone involvement of cryptococcosis typically presents as one or more circumscribed osteolytic lesions in any bone of the body, occasionally associated with "cold" soft-tissue abscesses, and has been associated with sarcoidosis. Bone marrow infiltration can be observed in severely immunocompromised hosts. Cryptococcal peritonitis [21] and cryptococcuria are also reported in several case series. Any organ of the human body can be a site of cryptococcal infections.

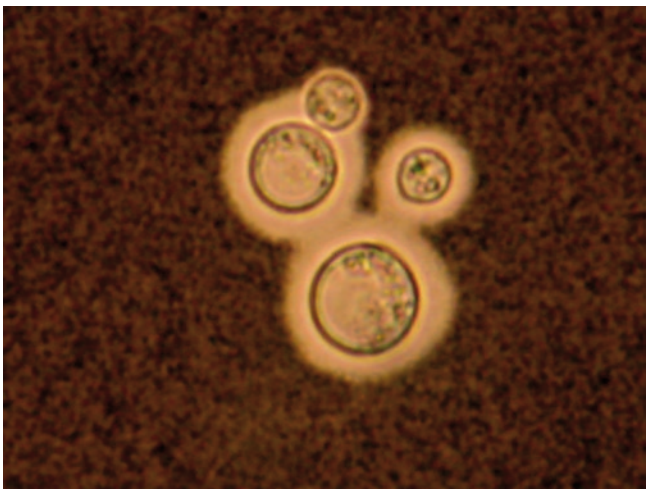

Fig. 15.5 India ink preparation showing budding encapsulated yeasts of *Cryptococcus neoformans*

Diagnosis

There are several methods used for the diagnosis of cryptococcosis. These techniques include direct examination of the fungus in body fluids, histopathology of infected tissues, serological studies, and culture of body fluids or tissues. Molecular methods, while available, are not currently used in routine clinical practice.

Direct Examination

The most rapid method for diagnosis of cryptococcal meningitis is direct microscopic examination for encapsulated yeasts by an India ink preparation of CSF. *Cryptococcus* can be visualized as a globular, encapsulated yeast cell with or without budding, ranging in size from 5 to 20 μm in diameter. It is easily distinguished in a colloidal medium of India ink when mixed with CSF (Fig. 15.5). Approximately 1–5 mL of specimen is recommended for use in the India ink preparation. India ink examination can detect encapsulated yeasts in a CSF specimen with a threshold between 10^3 and 10^4 colony-forming units of yeasts/mL of fluid. The sensitivity of the India ink preparation technique is 30–50 % in non-AIDS-related cryptococcal meningitis and

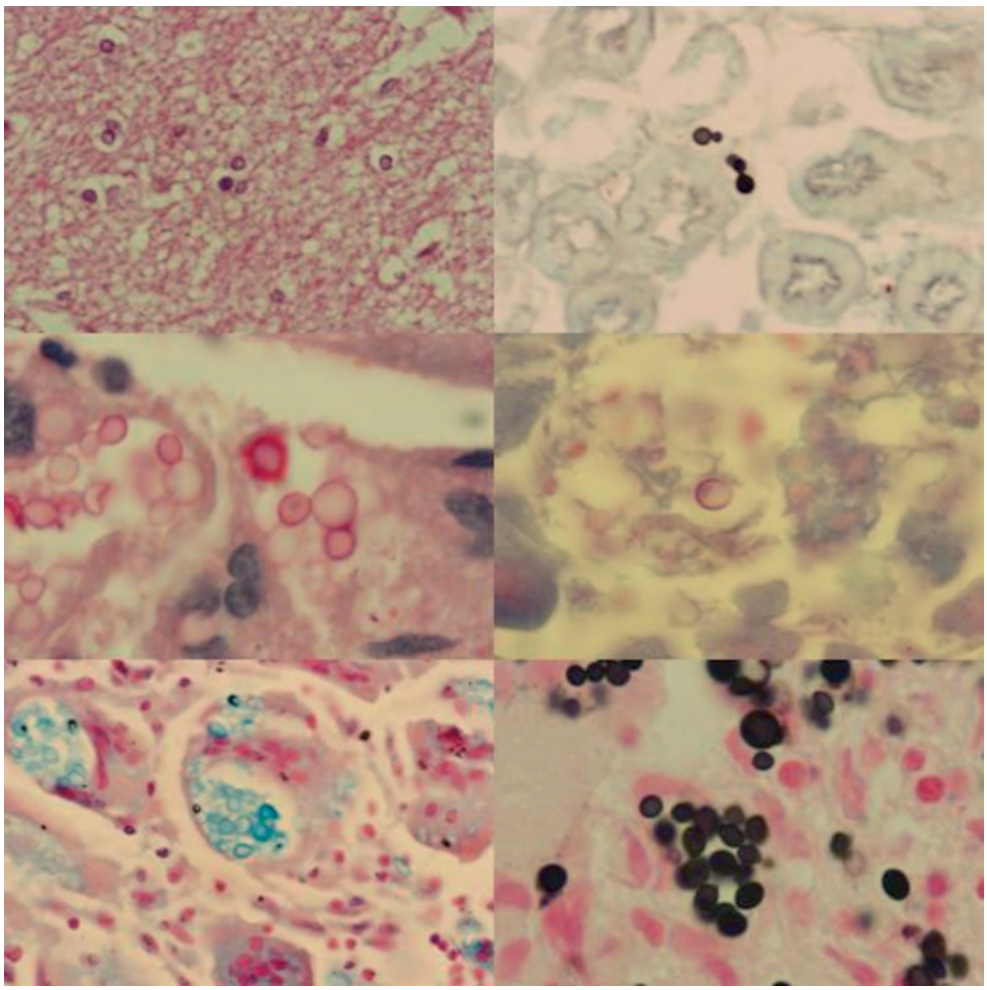

Fig. 15.6 Mouse tissues stained with various stains used to identify cryptococcal infection. *Upper left* panel is of brain stained with H&E showing meningoencephalitis with encapsulated yeast cells of *Cryptococcus neoformans*. The *upper right* panel is of kidney stained with GMS. The *middle left* panel demonstrates lung stained with Mayer's mucicarmine. Note *orange-red* staining of polysaccharide capsular material of *C. neoformans*. The *middle right* panel is liver tissue stained with PAS. Lung stained with Alcian blue stain is seen in the *bottom left* panel. Lung stained by Fontana-Masson method is seen in the *bottom right*. Melanin pigment in the cell wall of *C. neoformans* stains dark with this stain. *GMS* Gomori methenamine silver, *H&E* hematoxylin and eosin, *PAS* periodic acid-Schiff. (Courtesy of Dr. W. A. Schell)

up to 80 % in AIDS-related disease. Some false-positive results can be found from intact lymphocytes, myelin globules, fat droplets, and other tissue cells. Also, dead yeast cells can remain in the CSF and be visualized by India ink preparation for varying periods of time during and after appropriate antifungal treatment. This is a limitation of direct microscopy of CSF during the management of cryptococcal meningitis [22].

Cytology and Histopathology

Cryptococcus can be identified by histological staining of tissues from lung, skin, bone marrow, brain, or other organs [23]. Histopathological staining of centrifuged CSF sediment is more sensitive for rapid diagnosis of cryptococcal meningitis than the India ink method [24]. Peritoneal fluid from chronic ambulatory peritoneal dialysis, seminal fluid, bronchial wash, or bronchoalveolar lavage fluid can also be used for cytology preparations in the diagnosis of cryptococcal infections, whereas India ink preparations from these body fluids are difficult to interpret [25, 26]. Fine needle aspiration for cytology of peripheral lymph nodes, adrenal glands, or vitreous aspiration; percutaneous transthoracic biopsy under real-time ultrasound guidance; or video-assisted thoracoscopic lung biopsy on pulmonary nodules, masses, or infiltrative lesions can be used for obtaining tissues for cytology/histopathology [27].

A variety of positive staining methods have been described to demonstrate the yeast cells in tissue or fluids, ranging from the nonspecific Papanicolaou or hematoxylin and eosin stains to the more specific fungal stains such as Calcofluor, which binds fungal chitin, or Gomori methenamine silver (GMS), which stains the fungal cell wall [2, 25] (Fig. 15.6). Several stains can identify the polysaccharide capsular material surrounding the yeasts. These stains can be especially useful in presumptively identifying *Cryptococcus* when the organism does not grow or cultures are not obtained. They include Mayer's mucicarmine, periodic acid-Schiff (PAS), and alcian blue stains [2]. The Fontana-Masson stain appears to identify melanin in the yeast cell wall. The fungus is observed as a yeast that reproduces by formation of narrow-based budding with a prominent capsule. Gram stain is not optimal for identification of this yeast, but may show

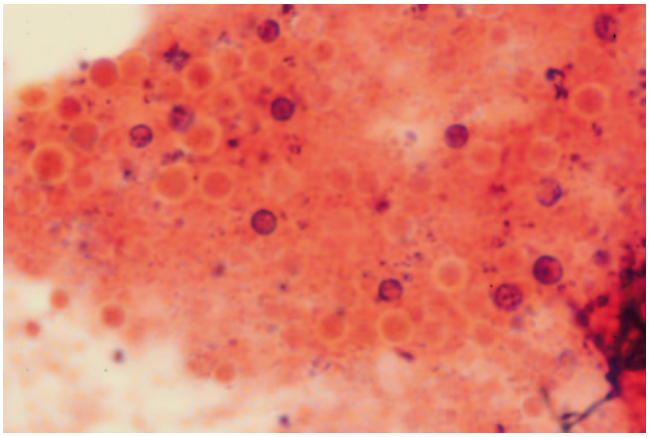

Fig. 15.7 Gram stain of sputum of a patient with pulmonary cryptococcosis. *Cryptococcus neoformans* appears as poorly stained gram-positive budding yeasts. (Courtesy of Dr. W. A. Schell, Duke University Medical Center)

C. neoformans as a poorly stained gram-positive budding yeast (Fig. 15.7) [2]. The recognition of *C. neoformans* in gram-stained smears of purulent exudates may be hampered by the presence of the large gelatinous capsule that apparently prevents definitive staining of the yeast-like cells.

Serology

Diagnosis of cryptococcosis has improved significantly over the past several decades with the development of serological tests for cryptococcal polysaccharide antigen and/or antibody. Use of serum cryptococcal antibodies for diagnosis of cryptococcosis has not been adopted. In contrast, detection of cryptococcal capsular polysaccharide antigen in serum or body fluids by a latex agglutination (LA) technique has been robust in its performance and is considered the gold standard diagnostic test for serological diagnosis of cryptococcosis. This test uses latex particles coated with polyclonal cryptococcal capsular antibodies or anti-GXM monoclonal antibodies and has overall sensitivities and specificities of 93–100% and 93–98%, respectively [28, 29]. The false-positive rate of cryptococcal capsular polysaccharide antigen testing is 0–0.4% [30]. The majority of false-positive results can be explained by technical error (improper boiling/treatment), presence of rheumatoid factor or interference proteins, and infections with *Trichosporon beigelii* [31] or some bacterial species [32]. However, most of the false-positive results of LA testing for cryptococcal polysaccharide antigen have initial reciprocal titers of 8 or less [28]. Therefore, results of such low titers must be carefully interpreted within the clinical context. False-negative results of the LA test for cryptococcal polysaccharide antigen in cryptococcal meningitis are unusual but can be seen due to a prozone effect, and, therefore, high-risk negative specimens should be diluted and retested [33]. Low fungal burden, as in chronic low-grade cryptococcal meningitis or in the very early stages of cryptococcal infection, and improper storage of patient sera can also cause false-negative results in LA cryptococcal polysaccharide antigen tests [34].

Enzyme immunoassays (EIAs) for detection and quantification of cryptococcal polysaccharide antigen of all four serotypes of *C. neoformans* in sera and CSF have been developed to detect the major component of the polysaccharide capsule, GXM, with sensitivities and specificities of 85.2–99 and 97%, respectively [28, 35]. This methodology is automated and overcomes some of the practical limitations of LA testing. Previous studies have compared EIA and LA assays and revealed no significant difference between these testing methods. EIA for cryptococcal polysaccharide antigen does not give discrepant results with rheumatoid factor or serum macroglobulins and is not affected by prozone reactions. Both LA and EIA testing have been rigorously studied and are recommended for use in both serum and CSF samples.

Recently, a lateral flow assay (LFA) was introduced in the diagnostic repertoire for cryptococcal infection and is Food and Drug Administration (FDA) approved for use in serum and CSF. The semiquantitative LFA offers many advantages over other serological methods, including rapid turnaround (approximately 15 minutes), minimal requirements for specialized laboratory infrastructure, stability at room temperature, and low cost [36]. The LFA has been evaluated against both EIA and culture, with sensitivities of 96–100% for serum and plasma and 70–94% for urine samples [36–39]. This assay has good performance across a broad range of clinical settings, including resource-limited settings and among cohorts with low burden of HIV infection and high rates of *C. gattii* infection, for which some EIA and LA tests are known to be insensitive [36–40]. The satisfactory performance of LFA combined with established cost-effectiveness and practical advantages of this approach support its use as a point-of-care testing (including preemptive screening of high-risk patients) in resource-limited settings [36, 37, 41].

Although the presence of cryptococcal polysaccharide antigen in serum is undoubtedly suggestive for dissemination of cryptococcal infection outside the lung, the precise value of cryptococcal polysaccharide antigen for diagnosis of nondisseminated pulmonary cryptococcosis remains less certain. Generally speaking, detectable cryptococcal antigen in serum should make clinicians consider that infection is now also located outside the lung. In a high-risk patient with clinical symptoms suggestive of meningitis, identification of cryptococcal antigen in CSF or serum is rapid, specific, noninvasive, and virtually diagnostic of meningoencephali-

tis or disseminated cryptococcosis even when the India ink examination or culture is negative [42, 43]. The LA test for serum cryptococcal polysaccharide antigen is widely used for detecting cryptococcal infection in patients with AIDS, as an initial screening test for those with fever of unclear etiologies or neurological symptoms. In some patients, it may represent the only means of achieving an etiologic diagnosis of invasive cryptococcosis or early diagnosis prior to CNS involvement.

Likely because of its sensitivity, the detection of cryptococcal polysaccharide antigen in the serum may precede clinically obvious disseminated cryptococcal disease ("isolated cryptococcal polysaccharidemia") in severely immunosuppressed patients [44–46]. The management of these cases, in which there is a positive serum antigen and other nonspecific clinical findings in HIV-infected patients with negative fluid or tissue cultures, is uncertain. Persons of high risk with isolated cryptococcal antigenemia probably do benefit from antifungal therapy to prevent or delay the development of overt cryptococcosis [44]. Generally, positive serum antigen tests at titers of 1:4 or more strongly suggest cryptococcal infections in these patients.

Baseline cryptococcal polysaccharide antigen titers in serum and CSF may carry prognostic significance in patients with cryptococcal meningitis [47]. A study in HIV-related acute cryptococcal meningitis indicated that a baseline titer of CSF cryptococcal polysaccharide antigen of 1:1024 or greater was a predictor of death during systemic antifungal treatment [48]. After initiation of systemic antifungal therapy, patients may respond to treatment and titers of cryptococcal polysaccharide antigen fall. Similarly, a rise in CSF cryptococcal polysaccharide antigen titers during suppressive therapy has been associated with relapse [49]. However, it is important to emphasize that the use of changing antigen titers to make therapeutic decision should be done with caution, as titers may not be equivalent across different serological modalities [39]. The kinetics of polysaccharide elimination remains unclear and, despite the accuracy of commercial kits for general diagnosis, the accuracy of specific titers can vary from kit to kit even from the same clinical specimen.

Culture and Identification

Cryptococcus can be easily grown from biologic samples such as CSF, sputum, and skin biopsy on routine fungal and bacterial culture media. Colonies can usually be observed on solid agar plates after 48–72 h of incubation at 30–35 °C in aerobic conditions. Antibacterial agents, preferably chloramphenicol, can be added to the media when bacterial contamination is considered. The yeast, however, do not grow in the presence of cycloheximide at the concentration used in selective fungal isolation media (25 μg/mL). Despite relatively rapid growth for most strains, cultures should be held for 3–4 weeks before discarding, particularly for patients already receiving antifungal treatment. Conversely, cultures may be negative despite positive microscopic examinations (India ink) due to nonviable yeast cells, which may persist for a prolonged period of time at the site of infection. Positive blood cultures are frequently reported in AIDS patients and may actually be the first positive test for cryptococcal infection in a febrile high-risk patient.

C. neoformans colonies will appear on routine fungal media as opaque, white, creamy colonies that may turn orange-tan or brown after prolonged incubation. The mucoid appearance of the colony is related to the capsule size around the yeasts. *Cryptococcus* does not routinely produce hyphae or pseudohyphae, or ferment sugars, but is able to assimilate inositol and hydrolyze urea [50]. *C. neoformans* and *C. gattii* have the ability to use galactose, maltose, galactitol, and sucrose [50]. There are special media such as canavanine-glycine-bromthymol blue (CGB) agar that can be used to differentiate *C. gattii* strains from *C. neoformans* strains [51].

Molecular Identification Methods

A number of molecular techniques have been developed for identification of cryptococcal species from biological specimens including single and multiplex polymerase chain reaction (PCR) fingerprinting, random amplified polymorphic DNA (RAPD), PCR restriction fragment length polymorphism (RFLP) analysis, multi-locus sequence typing (MLST), and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry [52–58]. These highly sensitive and specific methods have been evaluated with a variety of biologic samples [59] and can rapidly identify to the species and subspecies/genotypic level, including identification of recognized and novel strains within geographical niches [60]. While the expense and specialized techniques required of these methods preclude widespread use in clinical practice, their use in larger-scale investigations will continue to enhance our understanding of the epidemiology, pathogenesis, and nuances of antifungal management, as well as identify microevolution of different strains [61].

Treatment

The Infectious Diseases Society of America Clinical Practice Guidelines for the Management of Cryptococcal Disease (summarized in Tables 15.5, 15.6), updated in 2010,

Table 15.5 Treatment recommendations for cryptococcal meningoencephalitis. (Adapted from the 2010 IDSA Clinical Practice Guideline for the Management of Cryptococcal Disease with personal suggestions [62])

Human immunodeficiency virus-infected individuals[a]	
Induction therapy:	
Primary regimen	
AmBd (0.7–1 mg/kg/day) plus flucytosine (5-FC; 100 mg/kg/day)	2 weeks
Liposomal AmB (3–4 mg/kg/day) or AmB lipid complex (ABLC; 5 mg/kg/day)	
pls 5-FC(100 mg/kg/day) for patients predisposed to renal dysfunction	2 weeks
Alternative regimens[b]	4–6 weeks
AmBd (0.7–1 mg/kg/day) or liposomal AmB[c] (3–4 mg/kg/day) or ABLC (5 mg/kg/day) if flucytosine-intolerant	2 weeks
AmBd (0.7–1 mg/kg/day) plus fluconazole (800 mg/day)	6 weeks
Fluconazole (>800 mg/day, preferably 1200 mg/day) plus 5-FC (100 mg/kg/day)	10–12 weeks
Fluconazole (800–2000 mg/day, preferably 1200 mg/day)	10–12 weeks
Itraconazole (200 mg BID)	8 weeks
Consolidation therapy: fluconazole (400 mg/day)	>1 year[d]
Maintenance or suppressive therapy: fluconazole (200 mg/day)	>1 year[d]
Alternative regimens[b]	
Itraconazole (200 mg BID) AmBd (1 mg/kg IV per week)	>1 year[d]
Organ Transplant Recipients[e]	
Induction therapy:	2 weeks
Primary regimen	
Liposomal AmB (3–4 mg/kg/day) or ABLC (5 mg/kg/day) plus 5-FC (100 mg/kg/day)	4–6 weeks
Alternative regimen (if flucytosine-intolerant)	
Liposomal AmB[c] (up to 6 mg/kg/day) or ABLC (5 mg/kg/day) AmBd (0.7 mg/kg/day)[g]	4–6 weeks
Consolidation therapy: fluconazole (400–800 mg/day)	8 weeks
Maintenance or suppressive therapy: Fluconazole (200–400 mg/day)	6–12 months
Non-HIV-infected and nontransplant patients	
Induction therapy:	
Primary regimen	
AmBd (0.7–1 mg/kg/day) plus 5-FC (100 mg/kg/day)	4–6 weeks[f]
Alternative regimens	
Liposomal AmB (3–4 mg/kg/day) or ABLC (5 mg/kg/day) plus 5-FC (100 mg/kg/day)	4 weeks
AmBd (0.7–1 mg/kg/day) Liposomal AmB (3–4 mg/kg/day) or ABLC (5 mg/kg/day)	6 weeks 6 weeks
Consolidation therapy: fluconazole (400–800 mg/day)	8 weeks
Maintenance therapy: fluconazole (200 mg/day)	6–12 months

ABLC AmB lipid complex, *AmB* amphotericin B, *5-FC* flucytosine

[a] Initiate HAART 2–10 weeks after beginning antifungal regimen. Shorter duration (i.e., 2 weeks) of induction therapy can be considered for certain low-risk patients

[b] Can be considered as alternative regimen in circumstances in which primary regimen not available but are not encouraged as equivalent substitutes

[c] Liposomal amphotericin can be safely administered in doses as high as 6 mg/kg/day

[d] After 1 year of therapy, if successful response to ARVs (CD4 count>100 and viral load low or undetectable for>3 months), discontinuation of antifungal therapy can be considered. Consider reinstitution if CD4 count falls below 100

[e] Consider stepwise de-escalation of immunosuppressive regimen if allograft function permits

[f] If CSF culture remains positive at 2 weeks of therapy or initial presentation with neurologic complications, longer therapy preferred

[g] Caution due to concomitant calcineurin inhibitor use

provide a suitable framework for therapeutic decision making [62]. The updated guidelines provide detailed recommendations for specific "at-risk" populations and address different management strategies based on host, site of infection, and potential complications of cryptococcal infection. While subtle nuances exist based on host and site of infection, general principles for the management of cryptococcal infection can provide the cornerstone of a treatment plan in most cases.

Table 15.6 Treatment recommendations for nonmeningeal cryptococcosis

Immunosuppressed[a] and immunocompetent,[b] mild-to-moderate pulmonary disease Fluconazole (400 mg/day) *Alternatives (immunocompetent):* itraconazole 200 mg BID, Voriconazole200 mg BID or posaconazole 400 mg BID	6–12 months[c]
Immunosuppressed or immunocompetent, severe pulmonary disease Treat as CNS disease	12 months
Nonmeningeal, nonpulmonary cryptococcosis *Patients with cryptococcemia* Treat as CNS disease *No CNS disease, no fungemia, isolated focus of infection* Fluconazole 400 mg/day	12 months 6–12 months

CNS central nervous system

[a] CSF sampling should be performed to rule out CNS involvement

[b] CSF sampling can be considered but not required in the absence of neurological symptoms or high serum cryptococcal antigen

[c] If successful response to ARVs (CD4 count > 100 and viral load low or undetectable for > 3 months) and stable serum cryptococcal antigen, can consider discontinuation of antifungal therapy after 12 months of therapy

Basic Management Principles/Role of Combination Therapy

Amphotericin B deoxycholate (AmBd) remains the foundation of treatment for disseminated cryptococcosis and severe cryptococcal infection. A standard induction dose of 0.7–1 mg/kg/day is recommended. Liposomal amphotericin B (AmBisome) at 3–6 mg/kg/day has become a preferred alternative treatment with similar outcomes to that of AmBd but with less nephrotoxicity and is specifically recommended for primary induction in organ transplant patients as well as patients at risk for renal dysfunction [62–64]. Higher doses of AmBd have been shown to be more rapidly fungicidal [65, 66]. Flucytosine (5-FC) is primarily used in combination therapy with AmBd for first-line therapy in cryptococcal meningitis or severe pulmonary cryptococcosis at a dosage of 100 mg/kg/day in divided doses in patients with normal renal function [67, 68]. The combination of AmBd and 5-FC represents the most potent fungicidal regimen with more rapid sterilization of CSF cultures at 2 weeks as demonstrated by multiple studies [66, 67, 69]. Early studies from HIV infection demonstrated increased rates of CSF sterilization and fewer relapses with the combination of AmBd and 5-FC followed by itraconazole maintenance [67]. This initial combination regimen has since been compared against multiple alternatives, with the superiority of its fungicidal activity consistently confirmed [69]. Similar results have been observed among the most severe cases of cryptococcal infection [66, 70]. Early mycological failure (as defined by persistently positive CSF cultures at day 14) has for many years been associated with late treatment failure and poor outcome [71], and lack of 5-FC has been independently associated with both early [72] and late [70] mycological failure. The improved fungicidal activity of combination therapy with AmBd plus 5-FC has been shown to translate into a direct survival benefit compared with AmBd monotherapy, with improved survival at 10 weeks lasting up to 6 months [66]. 5-FC should be dose-adjusted for renal dysfunction, with therapeutic monitoring performed 3–5 days after initiation of therapy, to maintain 2-h post-dose levels under 100 μg/mL (goal 30–80), to reduce its primary side effect of bone marrow suppression [73].

Alternative Combination Regimens

Though combination induction therapy with AmBd and 5-FC remains the recommended standard of care for severe cryptococcosis including cryptococcal meningitis, limited availability of 5-FC in resource-limited settings presents significant challenges for managing patients in areas where the disease burden and mortality rates are highest. Alternative combination therapies have been investigated, the most efficacious of which has been AmBd (0.7 mg/kg/day) plus fluconazole (800 mg/day), which has demonstrated improved rates of a composite end point of CSF culture negativity, neurological improvement, and survival compared with AmBd alone or in combination with lower doses of fluconazole [74]. Fluconazole (at doses of 800–1200 mg/day) in combination with AmBd (standard dosing) has been shown to demonstrate similar rates of fungal clearance from CSF as standard AmBd plus 5-FC in a randomized study performed in HIV-infected patients in South Africa [75] and offers a potential viable option for effective initial therapy in settings where access to 5-FC is limited. Whether the survival benefit observed with AmBd plus 5-FC will be observed with this regimen remains uncertain. Additional alternative regimens for primary therapy are available in the guidelines but their use is not encouraged based on limited data on the success of these regimens [76]. Use of fluconazole in the absence of a polyene is not recommended given the fungistatic nature of this drug, poor success, higher relapse rates, and increased resistance in relapse when used as monotherapy for induction [62, 77]. However, in areas without access to AmBd, high doses (1200 mg/day) of fluconazole should be commenced.

Host Considerations

Cryptococcal Meningitis in HIV Patients

A three-stage regimen of induction/consolidation/maintenance is employed in the treatment of cryptococcal meningitis in all patients, irrespective of host risk factors [62, 67]. In HIV-infected patients, the initial induction treatment usually begins with combination therapy with AmBd plus 5-FC for at least 2 weeks as above, followed by consolidation treatment with fluconazole 400–800 mg/day for 8 weeks in patients who have demonstrated favorable response. Following consolidation, a long-term suppressive/maintenance phase is commenced with oral fluconazole, 200–400 mg given once daily. This has been demonstrated to effectively reduce rates of relapse from ~40% to less than 5% in the pre-HAART era [78]. Secondary prophylaxis can be discontinued after 1–2 years of antifungal therapy in patients who respond to HAART with rise in $CD4^+$ cell counts to greater than 100 cells/μl and decline in viral load (HIV RNA) to undetectable levels for at least 3 months [62, 79, 80].

Itraconazole can be used as an alternative consolidation treatment for cryptococcosis, but first-line therapy is with fluconazole. Despite its poor CSF penetration and inconsistent oral bioavailability, itraconazole has been successfully used in the treatment of cryptococcal meningitis [81]; however, it has been shown to be inferior to fluconazole during the suppression phase [82] and requires therapeutic drug monitoring due to its poor bioavailability. Newer triazoles including posaconazole and voriconazole are not specifically incorporated into practice guidelines but are active against cryptococcal isolates in vitro and have been shown to demonstrate moderate efficacy in patients with refractory disease [83, 84].

In patients with HIV-associated cryptococcal infection, HAART has a major impact on long-term prognosis. However, given concerns regarding immune reconstitution inflammatory syndrome (IRIS), the optimum timing for HAART initiation in the setting of OIs has been a subject of much debate. Early retrospective studies suggested an increased risk of IRIS among HIV-infected patients initiated on HAART early after the diagnosis of an OI [85, 86]. More contemporary studies have demonstrated conflicting results regarding outcomes of cryptococcal infection based on timing of HAART initiation [87–91]. The Cryptococcal Optimal ART Timing (COAT) study provides the best evidence for current recommendations regarding timing of HAART initiation in patients with cryptococcal meningitis [92]. HAART-naïve patients were randomized to receive immediate (within 48 h) or deferred (greater than four weeks) HAART following a minimum of 7 days of antifungal therapy with AmBd and high-dose fluconazole. This trial was stopped early after interim analyses suggested poorer early survival among patients receiving immediate HAART (55% vs. 70%, $p=0.03$), particularly among patients with altered mentation and low CSF white blood cell count. Although a trend toward increased rates of and earlier IRIS was observed in the immediate HAART group, this was not statistically significant.

The above data support recommendations to delay initiation of HAART in patients with cryptococcal meningitis for a minimum of 4 weeks after starting antifungal therapy (potentially longer if the primary regimen does not include AmBd) and after demonstration of a sustained clinical response to antifungal therapy [62, 93]. Interruption of HAART and/or corticosteroid treatment may be used to control symptoms if severe cryptococcal IRIS occurs.

Organ Transplant Recipients

Organ transplant recipients with CNS cryptococcal infection are managed similar to HIV-infected patients, with the exception of preferential use of lipid formulations of amphotericin B to limit nephrotoxicity [62]. The principles of induction, consolidation, and maintenance therapy remain the same. Repeat CSF sampling at 2 weeks is recommended in this population and a longer course of induction therapy should be pursued if CSF cultures remain positive at 2 weeks, as this scenario is associated with increased 6-month mortality [94]. Unlike HIV-infected patients, relapse rates among organ transplant recipients are quite low (~1.3%), such that a shorter course of maintenance therapy with fluconazole (6–12 months) can be pursued following standard consolidation [62, 94]. Drug interactions between fluconazole and immunosuppressive agents should be anticipated due to fluconazole-induced CYP3A4 inhibition, and preemptive adjustment (reduction) in calcineurin inhibitors should be made. Management of immunosuppression in the setting of cryptococcal infection requires recognition of the increased risk of IRIS associated with abrupt withdrawal or reduction of immunosuppression in organ transplant recipients with increased rates of allograft loss reported in some patients [95–97]. Stepwise reduction in immunosuppression is recommended, though the approach should be individualized for each patient.

Non-HIV-Infected, Nontransplant Patients

Screening for HIV and CD4 lymphopenia is recommended among patients who present with cryptococcosis without apparent risk factors [62]. Very little prospective data are available on the management of cryptococcal infection among this heterogeneous group of "apparently immunocompetent" patients lacking classical risk factors for cryptococcosis. What is known is based on early studies that included a heterogeneous mix of patients and were performed prior to acceptance of the standard algorithm of induction, consolidation, and maintenance therapy and higher-dose polyene

therapy [68]. Recommendations for longer induction therapy (4 weeks or more) in this population are based on the recognition of poorer outcomes and higher mortality rates in this group of patients in both early [68, 98] and contemporary [99] studies. An additional 2 weeks of therapy should be considered if 5-FC is not included in the induction regimen [62]. Recommendations for consolidation and maintenance parallel those for HIV-infected and transplant patients, and reflect early reports of relapse rates approaching 30 % within the 1st year prior to introduction of consolidation and maintenance antifungal therapy [62, 68]. Criteria for stopping treatment in these patients include resolution of symptoms, generally following at least 1 year of suppressive therapy. Patients may have prolonged positive cryptococcal polysaccharide antigen tests and/or slightly abnormal CSF findings for months during successful therapy, and if there are concerns about cure, follow-up CSF culture should be considered.

Nonmeningeal Cryptococcosis

Just as host factors influence management approaches for cryptococcal infection, site of infection also matters. Airway colonization in a nonimmunosuppressed individual poses a low risk for invasive pulmonary infection (and dissemination) and treatment can be deferred. Some experts would still favor treatment with fluconazole in this scenario, given the relative benign nature of this therapy. However, among immunocompromised patients with isolated pulmonary cryptococcosis, treatment is recommended to prevent dissemination [62]. It should be emphasized that a thorough evaluation to rule out systemic disease/dissemination is warranted in this group of patients to provide optimal treatment. This includes blood and CSF cultures as well as serum and CSF cryptococcal antigen testing. If the results of the above evaluation are negative, symptoms are mild, and there is no evidence of diffuse pulmonary infiltrates or ARDS, oral fluconazole (400 mg/day) is recommended for 6–12 months. However, in any patient in whom cryptococcemia is identified, symptoms are severe, ARDS is present, or CSF examination reveals asymptomatic CNS involvement, treatment for cryptococcal meningitis is recommended [62]. Cerebral cryptococcomas often can be managed with prolonged antifungal therapy without need for surgical removal unless mass effect or other evidence of obstruction is identified. At least 6 weeks of induction therapy with AmBd plus 5-FC, followed by 6–18 months of consolidation therapy with fluconazole (400–800 mg/day), is recommended for management. Surgery should be considered for large lesions (>3 cm) or the presence of obstructive hydrocephalus [62]. Localized infection of extrapulmonary nonmeningeal sites can occasionally occur with direct inoculation, but more commonly represents disseminated infection. Suspicion for the latter must be maintained when *Cryptococcus* is identified from a sterile body site, as management strategies will differ if disseminated disease is present. Consultation with ophthalmology is indicated in cases of cryptococcal eye disease [62].

Immune Reconstitution Inflammatory Syndrome

Restoration of pathogen-specific immunity as a result of HAART or following reduction of immunosuppression in SOT recipients can result in a destructive inflammatory response known as the *immune reconstitution inflammatory syndrome* (IRIS). IRIS is best characterized in association with *C. neoformans* infection of the CNS, particularly among HIV-infected patients, and is associated with significant morbidity and mortality [85, 86, 88, 89, 100–108]. Proposed criteria for IRIS include onset of symptoms within 12 months of HAART initiation (with concomitant $CD4^+$ recovery) [109]. In addition, IRIS is estimated to occur in 5–11 % of SOT recipients with cryptococcal infection and has been associated with an increased risk of allograft failure [95, 110–114]; cryptococcal IRIS may also be observed in non-HIV-infected, nontransplant patients [115].

Clinical features of cryptococcal IRIS are similar to cryptococcal infection, most commonly presenting as CNS disease, although lymphadenitis, pneumonitis, multifocal disease, soft-tissue involvement, and mediastinitis have all been reported [109, 116]. Meningeal disease is the most frequent and most serious presentation [109]; aseptic meningitis with associated intracranial hypertension and CSF pleocytosis is most commonly observed [100, 102, 103, 105, 106, 108]. A hallmark histopathologic finding is suppurative or necrotic granulomatous inflammation with yeast seen in tissues despite negative tissue cultures [95, 112, 116, 117]. The presence of a positive CSF culture in cases of suspected cryptococcal IRIS should raise suspicion for direct antifungal failure or resistance, particularly in settings where fluconazole therapy is widely used as the standard of care [88].

Cryptococcal IRIS represents unchecked reversal of a Th2 (anti-inflammatory) to Th1 (pro-inflammatory) immune response in the setting of immune reconstitution [118]. Prospective cohort studies of HAART-naïve individuals indicate that an ineffectual host immune response to initial infection is associated with a greater likelihood of future IRIS [105]. A three-phase theory of cryptococcal immune reconstitution has been postulated, marked by: (1) failure of antigen clearance due to inappropriate Th2 response; (2) lack of effector response despite inflammatory signaling; and, ultimately, (3) vigorous pro-inflammatory responses (both Th1 and Th17) to residual antigen, recognized clinically as IRIS [100].

There are no reliable diagnostic tests for IRIS, and establishing the diagnosis presents a considerable challenge [101, 119]. The differential diagnosis includes progressive

disease due to persistent immune deficiency, failure of antimicrobial therapy (due to resistance or nonadherence), coinfection with other OIs, and drug toxicity. A high index of suspicion is necessary for recognizing atypical presentations or manifestations at distant sites. Nevertheless, distinguishing between disease progression related to ongoing immune deficiency and clinical deterioration due to restoration of host immunity has important management implications. CSF analyses and biomarkers may be useful in distinguishing between relapse and IRIS. Prospective studies have demonstrated that CSF opening pressure [89] and WBC count [100, 105] at the time of an IRIS event are significantly higher than baseline values for individual patients, and higher CSF opening pressures can distinguish IRIS from relapsed infection [102].

Treatment options for cryptococcal IRIS are based largely on expert opinion [62]. Implicit in management is ensuring the efficacy of antifungal therapy, particularly in settings where access to AmBd may be limited and fluconazole resistance may account for recurrent meningitis episodes [120, 121]. In the absence of disease relapse or direct antifungal resistance, modification of antimicrobial therapy is not indicated [62]. Once the diagnosis of IRIS is suspected, consideration of disease severity is warranted. A significant proportion of minor cases will improve without specific treatment [86, 88, 108]. Corticosteroids have been shown to reduce the need for hospitalization and to improve short-term quality of life and functional status without increased risk of complications in paradoxical tuberculosis (TB)-associated IRIS [122]; the role of corticosteroids in cryptococcal IRIS, however, is not as well established and should be reserved for life-threatening cases, particularly in light of their association with increased mortality in one study [123]. Other anti-inflammatory agents have been used in cryptococcal IRIS, but the number of patients treated with any of these agents is too small to draw substantive conclusions [86, 124, 125]. Other management strategies, including therapeutic lumbar drainage in the setting of intracranial hypertension [62, 122, 126] and, at times, surgical drainage of suppurative lymph nodes [116, 117], are important adjunctive therapies that may be considered in severe disease.

Although no controlled studies have been performed, continuation of HAART in the setting of IRIS is recommended and has been performed safely without adverse effects in several studies [87, 88, 103, 119, 127]. Similarly, withdrawal or reduction of immunosuppressive agents is standard practice in managing infectious complications in SOT recipients [111]. Given the putative risk of IRIS with abrupt withdrawal or discontinuation of immunosuppressive agents in these patients, gradual de-escalation during the initiation of antifungal therapy is advised to reduce the risk of future IRIS [95, 111, 112].

Persistent and Relapsed Infection

Persistent and relapsed infection must be distinguished from IRIS, as management strategies will differ significantly. Persistent disease can be defined as persistently positive CSF cultures after 1 month of antifungal therapy, whereas relapse requires new clinical signs and symptoms and repeat positive cultures (at same or distant sites) after initial improvement and fungal sterilization [62]. Surrogate markers, including biochemical parameters, India ink staining, and cryptococcal antigen titers, are insufficient to define relapse or alter antifungal therapy. General recommendations for management in these cases include resumption of induction therapy, often for a longer duration and at increased dosages, if tolerable, and pursuance of antifungal susceptibility testing [62].

Antifungal Susceptibility Testing

While routine in vitro susceptibility testing of cryptococcal isolates at the time of initial therapy is not recommended, there is a role for testing in cases of suspected relapse or persistent infection [62]. It is generally recognized that primary antifungal resistance to most agents is rare, although reduced susceptibility to 5-FC has been observed in untreated patients [128] and echinocandins have no reliable activity against this yeast. Reduced susceptibility to fluconazole has been described in cases of culture-positive relapsed cryptococcal meningitis associated with prior fluconazole therapy [77, 129, 130] (Table 15.6).

Management of Elevated CSF Pressure

Along with the optimization of antifungal therapy, management of increased ICP is critically important. Elevated ICP is correlated with overall fungal burden, and is thought to be due to CSF outflow obstruction by clumped yeast forms [131]. An ICP of 250 mm H_2O or greater is considered elevated and is associated with increased morbidity and mortality [123]. Persistently elevated ICP after 2 weeks of treatment is associated with poorer clinical responses among patients with HIV-associated cryptococcal meningitis [62]. Intracranial imaging should be performed prior to lumbar puncture if impaired mentation or focal neurologic deficits are present. A baseline measurement of CSF pressure should be obtained in all patients with suspected cryptococcal meningitis. Aggressive attempts to control increased ICP should occur, if elevated and if there are signs/symptoms to suggest increased ICP (headache, mental status changes, and new focal neurological findings). Treatment options for managing acutely elevated ICP include repeated lumbar punctures

Table 15.7 Management of elevated intracranial pressure in HIV-infected patients with cryptococcosis. (Based on the IDSA Practice Guideline for the Management of Cryptococcal Diseases [62])

Initial lumbar puncture
Positive focal neurological signs or altered conscious status
Radiographic imaging before lumbar puncture is indicated
Normal opening pressure
Initiate medical therapy, with follow-up lumbar puncture at 2 weeks
Opening pressure ≥250 mm H_2O with signs or symptoms
Lumbar drainage sufficient to achieve closing pressure
<200 mm H_2O
or 50% of initial opening pressure[a]
Follow-up for elevated pressure
Repeated drainage daily until opening pressure
<200 mm H_2O and symptoms/signs
are stable
If elevated pressure persists, consider
Lumbar drain
Ventriculoperitoneal shunt

[a] Recommendations are not evidence based and are provided as a guide only

(daily until pressure and symptoms are stable for >2 days), lumbar drain insertion, ventriculostomy, or ventriculoperitoneal (VP) shunt (Table 15.7) [123]. Medical treatments such as corticosteroids (unless there is a component of IRIS), mannitol, and acetazolamide have been used in some cases, but are generally not recommended for use in management of increased ICP in cryptococcal meningitis [132]. Some patients may develop symptoms of obstructive hydrocephalus necessitating the placement of a permanent VP shunt during the first 1–2 years of treatment, and occasionally at initial presentation. Sterilization of CSF is not required prior to placement of a VP shunt, which can be inserted once a patient is receiving the appropriate antifungal therapy [133].

Prevention

Prevention of cryptococcal disease can be achieved by use of HAART in HIV-infected patients. Fluconazole prophylaxis has been shown to be effective for preventing cryptococcosis in AIDS patients with persistently low $CD4^+$ cell counts (below 100 cells/μl) [134, 135], but due to concerns regarding antifungal resistance, this approach is not currently recommended, and HAART remains the best strategy for the prevention of cryptococcal disease in this population. Routine screening for cryptococcal infection and/or prophylaxis are not recommended in SOT recipients, even when immunosuppression is augmented in patients with previously (appropriately) treated infection [136]. Although cryptococcal GXM–tetanus toxoid conjugate vaccine and specific monoclonal antibodies to cryptococci have been developed, clinical trials have not been initiated to determine their usefulness in human subjects [137, 138].

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Blastomycosis

16

Donna C. Sullivan and Rathel L. Nolan III

Introduction

Blastomycosis is a systemic mycosis, primarily involving the lungs, caused by the thermally dimorphic fungus *Blastomyces dermatitidis*. First described by Gilchrist as a cutaneous disease [1], later analysis showed that the lung was the primary route of infection [2] and that skin disease or other organ involvement occurred secondary to hematogenous dissemination. Pulmonary blastomycosis may be asymptomatic or may manifest as acute or chronic pneumonia. Hematogenous spread of the organism frequently results in extrapulmonary disease. Blastomycosis has been reported in North America, Africa, India, and parts of Europe, but the majority of cases are from the endemic region around the Mississippi and Ohio Rivers and in areas of southern Canada near the Great Lakes [3, 4].

Etiologic Agent

Phenotypic Characteristics

B. dermatitidis is the imperfect (asexual) stage of *Ajellomyces dermatitidis,* which exhibits thermal dimorphism growing as a mould (mycelial) form at 25–30 °C and as a yeast form at 37 °C (Fig. 16.1). The mycelia produce terminal conidia which, when disturbed in the environment, easily become airborne. Human and animal infections typically occur following the inhalation of conidia, which convert to large budding yeast cells inside the lungs associated with the temperature shift to 37 °C [3].

Primary isolation of *B. dermatitidis* from clinical specimens is most reliable when grown as the mycelial form at 30 °C. Mycelial colonies, which are white to brown in color, grow on agar in 1–3 weeks. Positive identification of *B. dermatitidis* requires conversion to the yeast form at 37 °C or nucleic acid amplification methods which allow early identification of mycelial phase growth. Yeast-like colonies are wrinkled and cream-to-tan in color. Asexual reproduction is by budding of single, broad-based, thick walled, multinucleated daughter cells (Fig. 16.1). The same morphologic characteristics are observed in tissue samples from infected individuals. In the appropriate clinical situation, this allows a presumptive diagnosis of blastomycosis [6].

Genotypic Characteristics

A. dermatitidis, the telomorphic form of *B. dermatitidis,* has been shown to have heterothallic mating-type cultures [5, 6]. Mating is controlled by a highly conserved domain that determines plus (MAT1) and minus (MAT2) strains. The complete sexual cycle has been defined by the DNA structure of the *MAT* locus (alpha or high mobility group, HMG, domain), mating assays, and genetic recombination [7]. The *B. dermatitidis MAT* locus is similar to those of other dimorphic fungi in which the mating-type gene is linked to *APN2, SLA2*, and *COX13* genes [7].

A variety of nucleic acid techniques have been employed to examine the genetic diversity and geographic distribution of *B. dermatitidis* isolates. Polymerase chain reaction (PCR)-based assays targeting the promoter region of the *BAD-1* virulence gene identified two major genotypic groups A and B, representing 47.2 and 48.1 %, respectively, and three minor groups (C, D, and E) in an analysis of 106 clinical and environmental isolates of *B. dermatitidis* from Wisconsin, Georgia, and Africa [8]. Recently, the technique of microsatellite analysis has been applied to a number of fungal species as well as *B. dermatitidis* [9–13]. Microsatellite analysis offers several advantages over both PCR-restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA [14, 15] (RAPD) methods. A panel of 27 microsatellite markers distinguished 112

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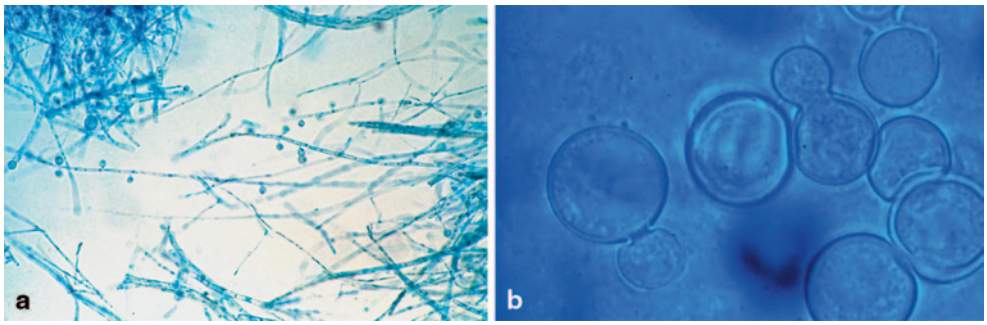

Fig. 16.1 The mycelial phase of *B. dermatitidis* (*left*) produces no unique characteristics which allow organism identification. As a yeast (*right*), *B. dermatitidis* has characteristic thick walled, multinucleated cells with single broad-based daughter cells

geographically diverse isolates into two genetically distinct *B. dermatitidis* groups [13]. Group 1 isolates were virtually monomorphic (1.8 alleles/locus) compared to Group 2 which were highly diverse (8.2 alleles/locus). The α and HMG mating types were almost evenly divided between the two groups [13].

The identification of species by sequencing a genetic marker has become common practice among mycologists [14, 16]. Genealogical concordance phylogenetic species recognition (GCPSR) techniques have been used to identify populations, delineate cryptic species, and document genetic recombination and gene flow in a number of fungal pathogens [9, 11, 17–20]. GCPSR employing seven nuclear genes to evaluate 78 human, canine, and environmental *B. dermatitidis* isolates from diverse geographic regions also revealed two distinct monophyletic clades within *B. dermatitidis,* phylogenetic species 1 (PS1, clade 1) and phylogenetic species 2 (PS2, clade 2), which may represent a genetically divergent novel cryptic species [21]. Although only a few isolates were subjected to both multilocus satellite analysis and GCPSR, GCPSR-defined PS1 and PS2 correspond to the highly diverse Group 2 and monomorphic Group 1 as described by Meece et al. [13].

Epidemiology

The ecological niche of *B. dermatitidis* has not been conclusively established. Environmental isolations of the organism associated with disease outbreaks indicate that the organism grows as microfoci in warm, moist soil in wooded areas that are rich in organic material [22–24]. Analysis of sporadic cases in humans and dogs, point source outbreaks, and infrequent environmental isolations has provided the major basis for the definition of endemic regions in North America [3, 4]. *B. dermatitidis* is endemic to the eastern USA, the Mississippi, and Ohio River valleys, extending northward to the Great Lakes and southern Canada. While most cases have been reported in Mississippi, Arkansas, Kentucky, Tennessee, and Wisconsin, up to 14% of blastomycosis cases identified from Medicare claims data occurred outside the endemic region [25, 26]. Within these known endemic regions there exist hyperendemic areas with exceptionally high attack rates [3, 4].

Other mammals, especially dogs, may become infected [27]. Early studies of endemic cases suggested middle-aged men with outdoor occupations were at greatest risk for blastomycosis. Subsequent reviews of reported outbreaks indicate no predilection for sex, age, race, occupation, or seasonal exposure [3, 4]. Vocational or avocational exposure to soil appears to be a common factor associated with both endemic and epidemic disease.

Pathogenesis and Immunology

Blastomycosis is initiated by the inhalation of the conidia of *B. dermatitidis*. Following inhalation, the infectious conidia are nonspecifically phagocytosed and killed by polymorphonuclear leukocytes (PMN), monocytes, and alveolar macrophages. This phagocytic response represents natural or innate immunity and may in part explain asymptomatic cases observed in outbreaks. Conidia which escape the initial phagocytic response rapidly convert to a yeast form that is more resistant to phagocytosis and killing. In in vitro studies, *B. dermatitidis* yeast evades macrophage defenses [28] and suppresses nitric oxide production by inhibition of inducible nitric oxide synthase [29]. Several virulence factors have been associated with the pathogenicity of *B. dermatitidis*. The thick cell wall of the yeast has been proposed to have antiphagocytic properties. Higher concentrations of lipids and phospholipids in cell walls in some strains have been associated with increased virulence.

Conversion of *B. dermatitidis* to the yeast form induces the expression of a yeast phase-specific gene designated BAD-1 (formerly WI-1). BAD-1 (WI-1) is a 120-kDa glycoprotein adhesion and immune modulator with a number of essential properties, including CR3 and $CD14^{+}$ binding, and an epidermal growth factor (EGF)-like domain [30–33]. The protein contains a repetitive domain that mimics thrombospondin type-1 (TSP-1) and suppresses T lymphocyte activation and effector function by binding heparin sulfate [34]. BAD-1 is a major virulence factor and its deletion results in attenuated pathogenicity [35, 36].

Cellular immunity in humans, as determined by antigen-induced lymphocyte proliferation, has been documented using whole yeast phase organisms, an alkali-soluble, water-soluble yeast extract, and BAD-1 [3, 4]. As with other endemic fungi, *B. dermatitidis* seems to require type 1-dependent cell-mediated immunity (CMI) [37]. Recent vaccine studies in animal models have shown that CMI is mediated by both vaccine-induced $CD4^+$ [37] and $CD8^+$ T cells [38] which produce type-1 cytokines such as gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α). In addition, the $CD28^+$ T cell receptor has been shown to be required for the induction of protective T cell responses to *B. dermatitidis* infection [39]. $CD4^+$ cells require interleukin-12 (IL-12) for the development of CMI while $CD8^+$ cells were less dependent on IL-12 for this process [40].

Development of a vaccine to prevent blastomycosis is a major goal but is hampered by an incomplete understanding of the immune response. Bronchoalveolar macrophage (BAM) activity is limited for fungal pathogens such as *B. dermatitidis* [41]. Binding of the yeast cells to surfactant protein D results in interference with BAM TNF-α production, blunting the host defense [42]. This may be compensated by the stimulation of BAM TNF-α by the abundant 1,3-β-glycan on the *B. dermatitidis* yeast cell. Intradermal administration of an attenuated *B. dermatitidis* lacking BAD-1 protects mice against lethal pulmonary challenge but intranasal vaccine delivery fails to do so [37]. Mucosal vaccination leads to poor T cell activation by induction of matrix metalloproteinase two, which impairs the chemokine response [35]. These studies in mice [37] and dogs [35] are promising and indicate that the development of a vaccine to prevent disease in humans is possible.

Clinical Manifestations

The clinical manifestations of blastomycosis are varied and include asymptomatic infection, acute or chronic pneumonia, and extrapulmonary disease. A number of factors are known to influence clinical presentation of disease. Patient characteristics and comorbidities as well as genetic variations of the infecting organism affect disease outcome. Univariate analysis of 16 clinical disease and patient demographic characteristics showed significant associations with different groups of *B. dermatitidis* [13, 43]. The monomorphic group 1 *B. dermatitidis* isolates identified by microsatellite analysis were more likely to be associated with pulmonary-only infections, while the more genetically diverse group 2 isolates were more likely to disseminate [43]. Group 2 isolates were more frequently seen in older patients who were smokers and had a comorbid condition [43].

Extrapulmonary disease results from the hematogenous spread of the fungus from a primary pulmonary infection.

Table 16.1 Organ involvement in blastomycosis

Organ system involved	No. involved/total patients (%)
Pulmonary	369/534 (69)
Cutaneous	306/534 (57)
Osseous	116/534 (22)
Genitourinary	92/534 (17)
Central nervous system	29/534 (5)

Although extrapulmonary *B. dermatitidis* infection has been reported to involve almost every organ of the human body, the skin, bones, and genital urinary system are most common (Table 16.1). It is important to note that blastomycosis mimics many other disease processes, whether acute or chronic [3]. For example, acute pulmonary blastomycosis is often mistaken for bacterial community-acquired pneumonia or influenza. Chronic pulmonary blastomycosis commonly mimics a malignancy or tuberculosis. Skin lesions are often misdiagnosed as pyoderma gangrenosa, or keratoacanthoma. Blastomycosis of the larynx is frequently misdiagnosed as carcinoma. Thus, a high index of suspicion and a careful histologic evaluation of secretions or biopsy material is needed.

Pulmonary Blastomycosis

Acute Infection

Initial infection occurs after inhalation of conidia into the lungs. Unless associated with an outbreak or group exposure, acute infection is frequently unrecognized. Clinical studies involving point-source outbreaks of infection indicate that symptomatic acute pulmonary disease occurs in only 50% of individuals, usually after an incubation period of 30–45 days [22, 23]. Signs and symptoms of acute pulmonary blastomycosis are similar to those of influenza or bacterial pneumonia. Fever, chills, pleuritic chest pain, arthralgias and myalgias usually occur abruptly. At onset, cough is nonproductive but frequently becomes purulent as disease progresses. Chest radiographs commonly reveal alveolar infiltrates with consolidation (Fig. 16.2) [3, 4]. Pleural effusions are uncommon and, if present, are typically small in volume. Hilar adenopathy is uncommon and is a useful sign in distinguishing acute blastomycosis from acute histoplasmosis. Spontaneous cures of symptomatic acute infection have been documented, but the exact frequency of these cures has not been clearly established [3, 4]. Although not an opportunistic infection, immunosuppression is a risk factor for serious pulmonary complications such as acute respiratory distress syndrome (ARDS) [3, 4].

Chronic Infection

The majority of patients diagnosed with pulmonary blastomycosis have a chronic pneumonia, which is clinically

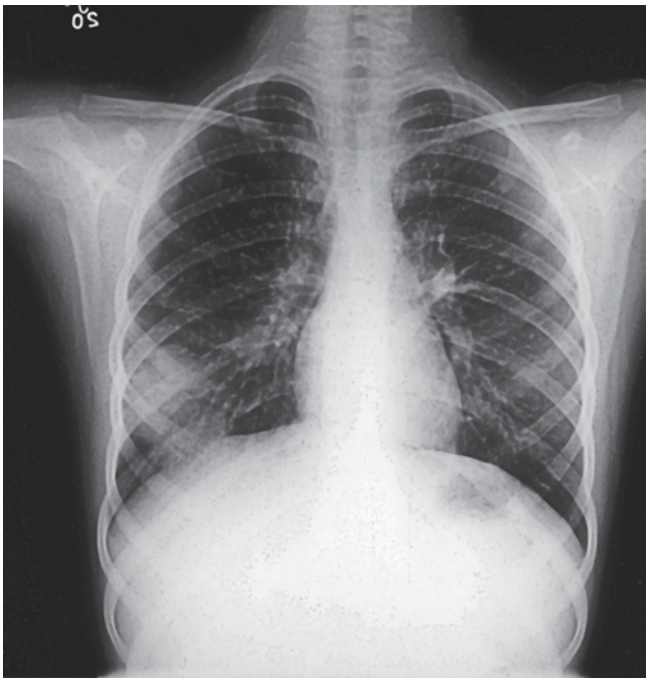

Fig. 16.2 Acute pulmonary blastomycosis. Chest radiograph reveals peripheral alveolar infiltrate which appear to be pleural based. Although this patient clinically improved with antibiotics sputum culture grew *B. dermatitidis*

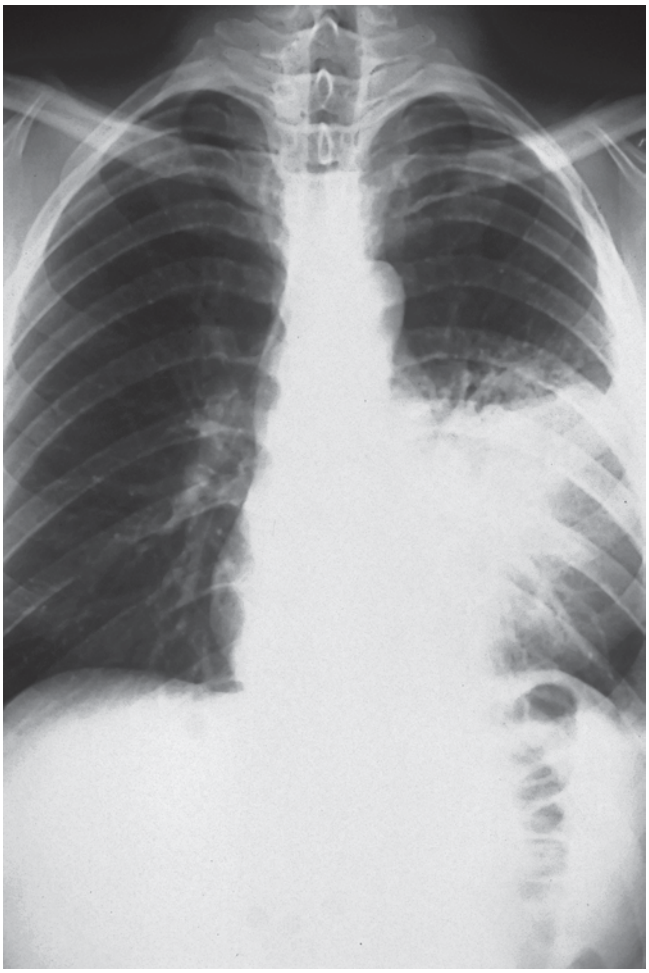

Fig. 16.3 Progressive pulmonary disease showing extensive *left* midlung alveolar infiltrate. This patient failed multiple courses of oral and intravenous antibiotics over a 2 month period prior to diagnosis of blastomycosis

similar to tuberculosis, other fungal infections, and cancer. Symptoms include fever, weight loss, chronic productive cough, and hemoptysis. The most frequent radiologic findings are alveolar infiltrates (Fig. 16.3) with or without cavitation, mass lesions that mimic bronchogenic carcinoma (Fig. 16.4), and fibronodular infiltrates [3, 4]. Although small pleural effusions have been reported, large pleural effusions (Fig. 16.5) are distinctly uncommon and, when present, have been associated with poor outcome.

Acute Respiratory Distress Syndrome

Patients may occasionally present with ARDS associated with miliary disease or diffuse pneumonitis (Fig. 16.6). Mortality exceeds 50% in these patients and most deaths occur within the first few days of therapy [44]. Diffuse pulmonary infiltrates and respiratory failure are more likely to occur in immunocompromised patients, especially those with late-stage AIDS [3, 4].

Extrapulmonary Blastomycosis

Extrapulmonary disease has been reported in as many as two thirds of patients with chronic blastomycosis. This high frequency probably reflects selection bias as these figures were reported in earlier autopsy-based studies before effective therapy was available [3, 4]. More recent studies have documented extrapulmonary disease in only 25–40% of patients with blastomycosis [44]. Extrapulmonary disease is almost always seen in conjunction with active pulmonary disease.

Skin Disease

Skin disease is the most common extrapulmonary manifestation of blastomycosis. Two types of skin lesions occur, verrucous and ulcerative (Fig. 16.7). The verrucous lesion is most common, typically with well-demarcated borders from gray to violaceous in color. These lesions may mimic squamous cell carcinoma. Microabscesses develop at the periphery of these lesions. Specimens taken from the margins usually reveal the diagnostic yeast form on wet preparation (Fig. 16.8). The second type of lesion is ulcerative. These ulcers are friable and bleed easily and usually have well-demarcated, heaped-up borders. The ulcers of blastomycosis develop from subcutaneous pustular lesions which spontaneously rupture and drain.

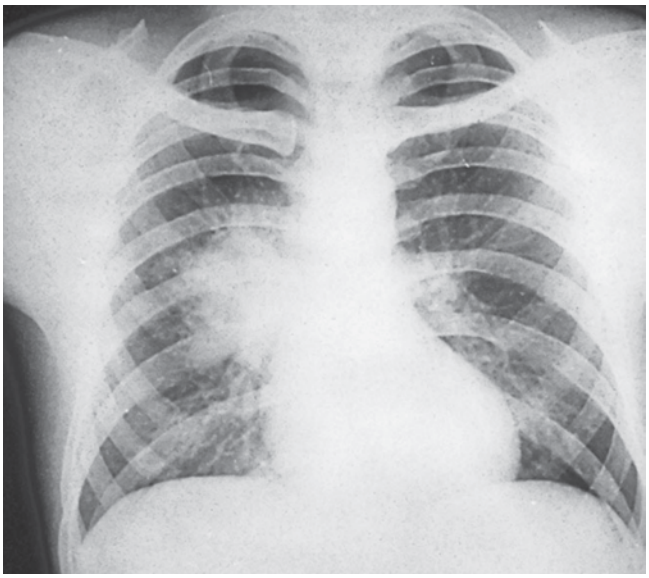

Fig. 16.4 Chest radiograph with *right* hilar infiltrate that mimics bronchogenic carcinoma. Bronchoscopy with biopsy and pulmonary cytology should be performed in these patients presenting with this radiographic finding to rule out concomitant disease

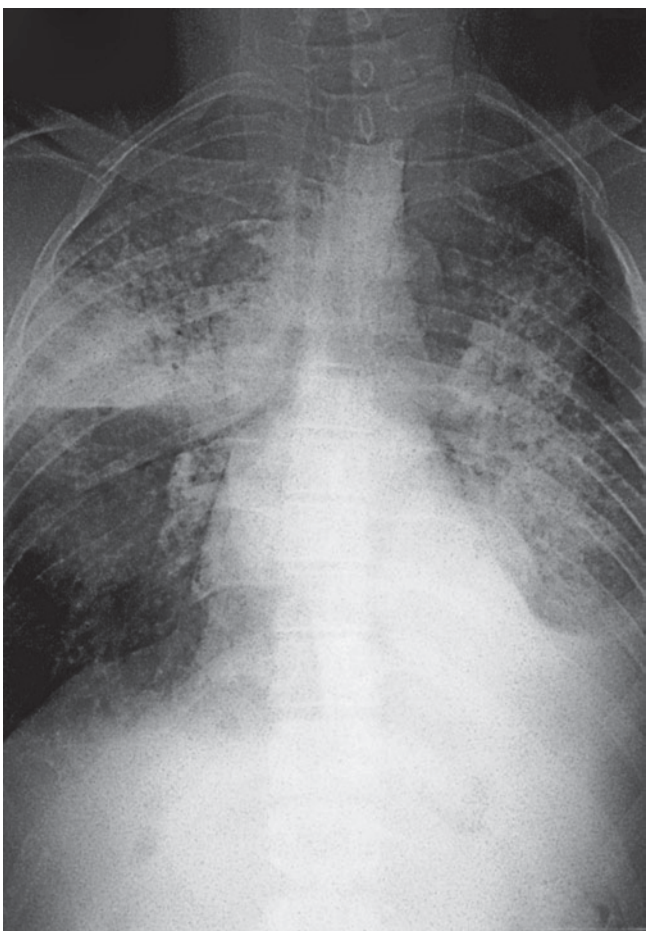

Fig. 16.5 Patient with life threatening pulmonary disease whose chest radiograph reveals bilateral alveolar infiltrates and large *left* sided pleural effusion

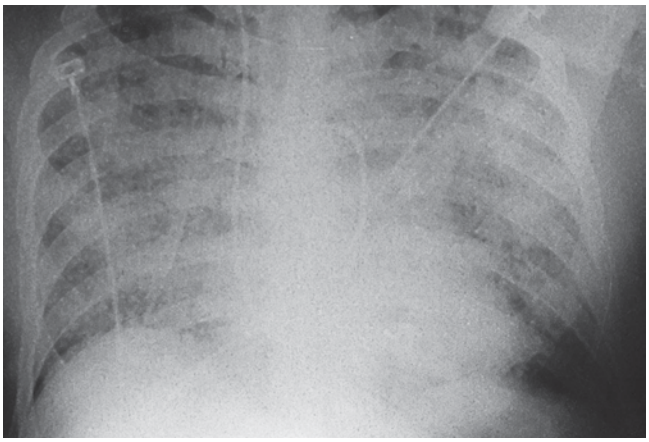

Fig. 16.6 Diffuse pulmonary infiltrates in a patient with ARDS. Patients presenting with this syndrome have a mortality rate greater than 50%

Regional lymphadenopathy is usually not present in cases of pulmonary dissemination. Inoculation blastomycosis following a dog bite or autopsy accidents often have lymphadenopathy/adenitis as a prominent feature [3, 4]. Lesions may also appear on the mucosa of the nose, mouth, and larynx. Laryngeal blastomycosis mimics well-differentiated squamous cell carcinoma both clinically and histopathologically. Subcutaneous nodules or cold abscesses may be seen in patients with multiorgan involvement.

Osseous

Osteomyelitis occurs with as many as one fourth of *B. dermatitidis* infections [3, 4]. Although any bone may be affected, the vertebrae, pelvis, sacrum, skull, ribs, or long bones are most frequently involved. Granuloma formation, suppuration, or necrosis is observed in biopsy specimens. A well-circumscribed osteolytic lesion may be observed on radiographs. Such lesions are radiographically indistinguishable from other fungal, bacterial, or neoplastic disease. Patients with *B. dermatitidis* osteomyelitis usually present with contiguous soft tissue abscesses or chronic draining sinuses. Although most bone lesions resolve with prolonged antifungal therapy, some may require surgical debridement for cure.

Genitourinary

In men, 10–30% of blastomycosis involves the genitourinary tract, primarily the prostate and epididymis [3, 4]. Prostatic involvement is frequently associated with symptoms of obstruction, and includes an enlarged, tender prostate and pyuria. Urine cultures obtained following prostate massage are frequently positive. Female genitourinary blastomycosis is rare but may include endometrial infection and tubo-ovarian abscess.

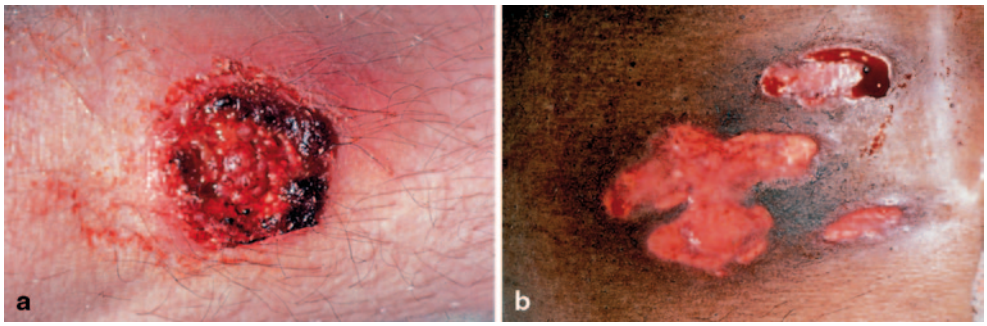

Fig. 16.7 Cutaneous blastomycosis typically produces verrucous (*left*) or ulcerative (*right*) lesions

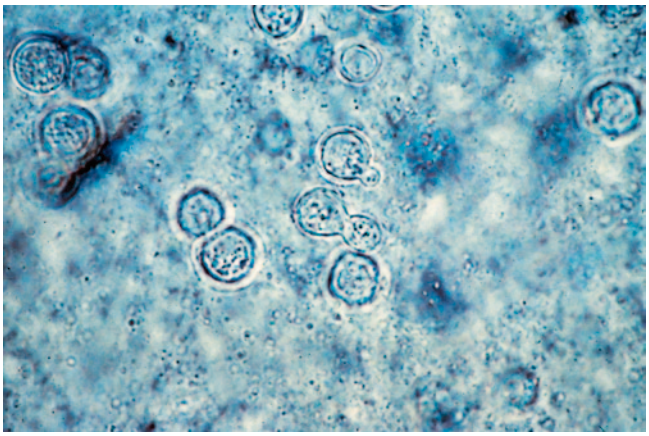

Fig. 16.8 Diagnosis of blastomycosis. This figure shows the characteristic yeast forms in a wet preparation of a skin scraping. Scraping of the edges of the verrucous and ulcerative lesions yield the best diagnostic results

Central Nervous System

CNS involvement occurs in less than 5% of cases in immunocompetent patients. Persons with AIDS have rates of CNS involvement as high as 40% [45] and other studies confirm immunosuppression as a risk factor for dissemination [46]. CNS blastomycosis may present as an abscess (epidural, cranial, or spinal) or as meningitis (Fig. 16.9) [46]. Magnetic resonance imaging (MRI), alone or in conjunction with computed tomography scans, reveals CNS abnormalities and are used to identify CNS involvement [46, 47]. Surgical intervention may be necessary both for diagnosis and to prevent neurologic deterioration [48].

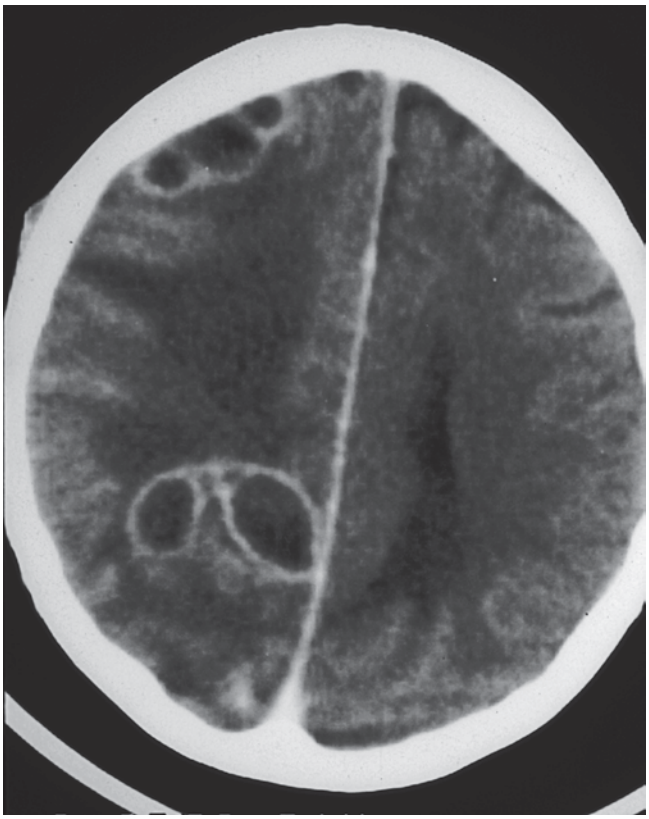

Fig. 16.9 CNS blastomycosis in an AIDS patient. Diagnosis may require aspiration of the abscesses if no active pulmonary or cutaneous disease is present

Diagnosis

Definitive diagnosis of blastomycosis requires the growth of the organism from sputum, pus, or biopsy material. The clinical laboratory should be alerted to culture specimens from suspected cases on fungal media such as Sabouraud dextrose agar. Mycelial phase cultures grown at 30 °C are the preferred method for isolation of *B. dermatitidis* from clinical specimens. These usually become positive within 1–3 weeks of incubation (Fig. 16.1). Sputum cultures in pulmonary blastomycosis have a high positive yield (75% per single sample, 86% per patient), but specimens obtained by bronchoscopy yield an even higher positive rate (92% of patients).

A presumptive diagnosis is often made by visualization of the characteristic large broad-based budding yeast in sputum, pus, or histopathologic specimens (Fig. 16.8). Because colonization with *B. dermatitidis* does not occur, observation of yeast forms in clinical specimens should prompt empiric therapy in the appropriate clinical presentation. Although direct examination of wet preparations has been reported to

have relatively low diagnostic yield [3, 4], the simplicity of the procedure, low cost, and potential for rapid diagnosis warrant its use. Cytology has been shown to have a higher diagnostic yield [4].

Serologic diagnosis of blastomycosis is of limited usefulness. Complement fixation antibodies have been used for epidemiologic purposes, but are severely limited in their specificity due to cross-reactivity to antigens of other fungi, particularly *Histoplasma capsulatum* and *Coccidioides immitis*. Immunodiffusion tests for precipitating antibodies to *B. dermatitidis* are more specific than complement fixation but lack sensitivity in early disease [4]. Commercial radioimmunoassays, enzyme immunoassays, and enzyme-linked immunosorbent assays have been developed which offer the promise of higher sensitivity but with specificities similar to complement fixation [4]. Immunoassays employing the BAD-1 yeast phase-specific protein discussed above are not currently commercially available.

A second-generation assay for *Blastomyces* that detects antigen in urine and serum has been developed by MiraVista Diagnostics (Indianapolis, IN; www.miravistalabs.com) [49]. Antigenemia is detected in 70–80% of patients with disseminated disease. Antigen detection in the urine was higher than serum, approaching 100%. Specificity is however reduced by the presence of cross-reactive antigens present in specimens obtained from patients with other fungal infections. This occurs in 96% of patients with histoplasmosis [50, 51]. Antigen levels are reported to decline with successful treatment and increase in treatment failure or relapse.

Nucleic acid detection techniques, both target and signal amplification methods, have been developed [52], including the chemiluminescent DNA probe (GEN-PROBE® AccuProbe®) for culture identification. These facilitate early identification of *B. dermatitidis* in mycelial cultures without the requirement of conversion to the yeast form. PCR amplification of the ribosomal RNA (rRNA) gene along with specific probe hybridization has been used to identify yeast phase organisms in tissue specimens. A rapid, real-time assay employing TaqMan probes to detect the *B. dermatitidis* BAD-1 promoter has been reported but is not commercially available [53]. These molecular techniques offer great promise for the rapid diagnosis of blastomycosis.

Treatment

Virtually all patients require therapy. Prior to the availability of azoles, amphotericin B was the mainstay of treatment. However, a series of clinical trials performed by the National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group have shown itraconazole and fluconazole to be effective, relatively nontoxic agents when compared to amphotericin B for treatment of patients with mild-to-moderate non-CNS disease [54]. Other than its use in diagnosis, the role of surgery is limited. Along with specific antifungal therapy, surgery may be helpful for the drainage of large abscesses, resection of cerebral blastomycomas, and debridement of devitalized bone.

Table 16.2 Treatment guidelines for Blastomyces dermatitidis infections. (Modified from [54])

Type of disease	Primary therapy	Alternate therapy
Pulmonary		
Severe	Amphotericin B 0.7–1.0 mg/kg/day; [a]total dose—1.5–2.5 g	Switch to itraconazole 200–400 mg/day once patient stabilized
Mild to moderate	Itraconazole 200–400 mg/day	Ketoconazole 400–800 mg/day, or fluconazole 400–800 mg/day
Disseminated		
CNS	Amphotericin B, 0.7–1.0 mg/kg/day; total dose—at least 2 g	If intolerant to full course of amphotericin B, fluconazole 800 mg/day
Non-CNS disease		
Serious	Amphotericin B 0.5–0.7 mg/kg/day; total dose—1.5–2.5 g	Switch to itraconazole 200–400 mg/day once patient stabilized
Mild to moderate	Itraconazole 200–400 mg/day	Ketoconazole 400–800 mg/day, or fluconazole 400–800 mg/day
Immunocompromised	Amphotericin B 0.3–0.6 mg/kg/day;[a] total—1.5–2.5 g	Selected patients with non-CNS disease may be switched to itraconazole, 200–400 mg/day, once clinically improved. Suppressive therapy with itraconazole should be considered in patients whose immunocompromised state continues. For patients with CNS disease or intolerant to itraconazole, consider fluconazole, 800 mg/day

[a] A lipid formulation of amphotericin B (3.0–5.0 mg/kg/day) may be substituted for conventional amphotericin B

Selection of an appropriate therapeutic regimen is based on three major considerations: the clinical form and severity of the disease, the immune status of the patient, and the toxicity of the antifungal agent. Specific recommendations of dose and duration of therapy in defined clinical settings are listed in Table 16.2 [54]. For more details concerning drug interactions, toxicities, adverse reactions, and pharmacokinetics about individual antifungal agents, the reader is referred to the chapter discussing individual agents in this text (Chap. 7).

All patients with progressive pulmonary infection or extrapulmonary disease and all immunocompromised patients should be treated [54]. In immunologically normal patients with mild-to-moderate pulmonary or extrapulmonary disease that does not involve the CNS, the azole antifungal agents,

itraconazole or fluconazole, administered for 6 months have proven to be effective, less toxic alternatives to amphotericin B. Although no randomized, blinded studies have been performed to compare different azoles, and only a few comparative trials for blastomycosis therapy have been reported, itraconazole appears to be the best-tolerated and most effective azole. Itraconazole is considered the drug of choice for patients with non-life-threatening, non-CNS blastomycosis [54].

Amphotericin B (including lipid-based formulations) is currently reserved for the initial treatment of patients with life-threatening disease, immunocompromised patients, and those with CNS disease. In selected patients initially presenting with life-threatening disease, itraconazole has been successfully substituted following an induction course of amphotericin B. Patients with CNS disease and severely immunocompromised patients should be treated with a full course of amphotericin B. Most experts recommend a total dose of 1.5–2.5 g. Other suggested modifications of therapy for special circumstances are reviewed in Table 16.2.

Fluconazole has been used in only a limited number of patients, but appears efficacious at doses of 400–800 mg/day. Two factors may eventually lead to more extensive use of fluconazole: It has fewer side effects and adverse drug interactions and it has excellent penetration into the CNS, suggesting a role for this drug in the treatment of CNS blastomycosis. Voriconazole also has excellent CNS penetration and has activity against *B. dermatitidis* in vitro [55]. Successful outcomes have been reported in a small number of patients treated with voriconazole for CNS blastomycosis [46, 55]. Posaconazole has been effective in the treatment of two patients with refractory pulmonary blastomycosis [56].

Amphotericin B is the drug of choice for blastomycosis occurring during pregnancy, as azoles are contraindicated [54]. The clinical spectrum of blastomycosis in pediatric patients is similar to that seen in adults. Recent reports indicate blastomycosis in children is more difficult to diagnosis and less likely to respond to oral therapy. Children with life-threatening disease should be treated with amphotericin B. Itraconazole has been used successfully at a dosage of 5–7 mg/kg/day in a small cohort of pediatric patients [54]. CNS disease occurs in approximately 40% of patients with AIDS or other diseases or therapies associated with immunosuppression. Likewise, disseminated disease and life-threatening pulmonary disease also appear more common in the clinical setting of immunosuppression. Hence, the recommendation that amphotericin B is the drug of choice for treatment of immunocompromised patients. Frequent relapses have been reported in patients whose immunosuppression persists and chronic suppressive therapy with an oral azole is recommended by some experts [54, 55].

Patients should be followed for years for evidence of relapse, especially in the CNS. Relapse rates of less than 5% are reported in patients treated with amphotericin B and itraconazole. Owing to the problems with bioavailability of oral itraconazole, serum blood levels may be clinically useful in guiding treatment of patients whose disease progresses on either formulation [54].

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Coccidioidomycosis

17

Royce H. Johnson and Arash Heidari

Introduction

Coccidioidomycosis was first described in 1892, in Buenos Aires by Posadas and Wernicke [1, 2]. They thought that the individual in their case report suffered from a malignant disease with a likely infectious cause. Organisms seen microscopically were mistakenly thought to be parasites. The disease was next described by Rixford and Gilcrest in San Francisco in 1896, whose paper was the first extensive study of coccidioidomycosis [3]. They better understood that this was an infectious illness and were the first to appreciate the importance of the parasite as the agent of a new and distinctive disease. In 1900, Williams Ophuls began his work on coccidioidal disease. Although he noted the "protozoa" of Rixford and Gilcrest in pathological sections, he discovered that culture of the organism always produced colonies of a mold, what we now know to be the mycelial (saprobic) growth of *Coccidioides*. The life cycle was roughly outlined in a preliminary report and the fungus given the name of *Coccidioides immitis* [4].

During the years 1925–1936, the early pathologic, epidemiologic, and mycologic studies were completed. Montenegro reported the first recovery of *C. immitis* from blood [5]. Coccidioidal infection in farm animals was described by Beck [6]. Meningitis was described first pathologically and subsequently clinically in the early part of the twentieth century [7, 8]. Two important observations were also made during this period; that the lung is the portal of entry and that *C. immitis* can be isolated from soil [9, 10].

Coccidioidomycosis was considered to be a rare and fatal infection until an accidental laboratory exposure of a medical student at Stanford University resulted in only a transient pulmonary infection. This led to a reassessment of the natural history of coccidioidal infection. The work in Kern County by Dr. Myrnie Gifford on a local respiratory illness in the San Joaquin Valley of California, known as valley fever, eventually elucidated the primary infection as being predominantly pulmonary [11].

During the latter part of the 1930s and 1940s, the natural history of the primary illness, the utility of the skin test, and serology were developed by Charles E. Smith and coworkers. William Winn and Hans Einstein made further contributions to disease description and therapy with amphotericin B deoxycholate, both intravenously and intrathecally for meningitis. By the 1950s, the clinical spectrum of coccidioidal infection was well described, with the publication of an excellent monograph by Fiese [12].

Etiologic Agents

Kingdom: Fungi

Phylum: Ascomycota

Class: Euascomycetes

Order: Onygenales

Family: Onygenaceae

Genus: *Coccidioides*

Coccidioides was originally described as noted above as one species, *C. immitis*. More recently, two genetically distinct populations of *Coccidioides* have been described, *C. immitis* and *Coccidioides posadasii*, correlating to separate endemic regions. Currently, the *C. immitis* is maintained as the name

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of those isolates that are predominately found in California. The new species, *C. posadasii*, is predominately found in Texas, Mexico, Central America, and South America. Both species are found in Arizona [13]. *Coccidioides* of both species, however, show few phenotypic differences and are mycologically and clinically indistinguishable. *Coccidioides* is a thermal dimorphic fungus that exists either as a mycelium or a spherule. The fungus is found as far north as the northern Central Valley in California and as far south as Argentina, the place of its original description. The fungus grows in conditions where the soil has a relatively high salinity, and in a climate that has mild winters with few freezes and hot dry summers [14]. Under these ideal conditions, the fungus grows in isolated pockets as a mycelium by apical extension. These mycelia produce specialized aerial hyphae that segment and form arthroconidia. The connecting links between arthroconidia are quite fragile and separate easily with minimal mechanical force or air turbulence. The arthroconidia become airborne in a form capable of deposition in the lungs if inhaled, and can travel substantial distances, perhaps as far as 75 miles or more. These arthroconidia, if they find an appropriate soil niche, can reestablish the saprophytic phase. However, if they are inhaled by an appropriate host, they undergo transformation from arthroconidia into spherules. Spherules reproduce by endosporulation, a process whereby the growing spherule is subdivided into numerous subcompartments, each of which becomes viable daughter cells or endospores. The spherule eventually ruptures, releasing endospores, each of which may continue to propagate in tissue or revert to mycelial growth in soil or on growth media (Fig. 17.1).

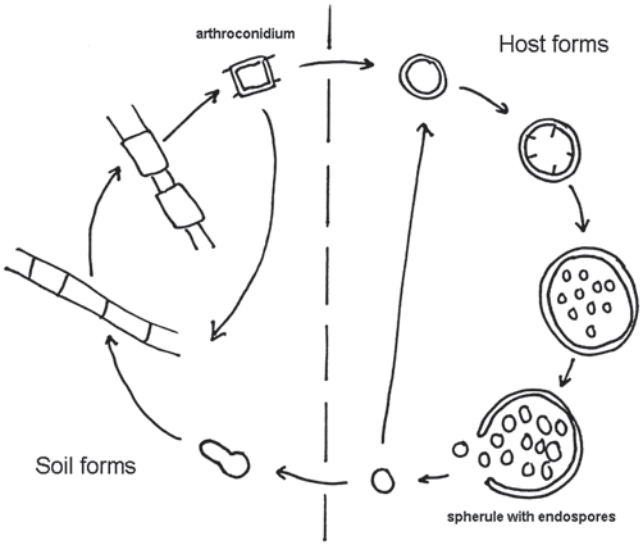

Fig. 17.1 Life cycle of *Coccidioides immitis* depicting saprophytic (soil) and parasitic (host) phases

Epidemiology

The disease was first described in Argentina, but other foci of infection in South America and Central America also exist. *Coccidioides* species are found solely in the Western Hemisphere, in the "lower Sonoran life zone" [15]. The majority of the soils which support the organism are found in North America, particularly in the southwestern USA and northwestern Mexico. The areas of greatest endemicity are in the southern San Joaquin Valley and South central Arizona. The disease extends to the northern Central Valley in California and as far as Utah in the Great Plains [16]. Recently, three isolates from clinical cases have been identified in Washington State. [17]. The total number of infections per year is not known, but prevalence surveys in the 1950s of school-age children in California's Central Valley suggested an annual risk of infection of 15%. More recent estimates from California and Arizona have indicated that the risk has declined to 3% or less [18, 19]. It has been estimated that in the USA there are approximately 100,000 infections annually [20]. Between 1998 and 2011, a total of 111,717 cases were reported. There has been a recent substantial increase in incidence of reported coccidioidomycosis from 5.3 per 100,000 population in the endemic area (Arizona, California, Nevada, New Mexico, and Utah) in 1998 to 42.6 per 100,000 in 2011 [21]. This increase is believed to be due to improved awareness, changes in demographics, and environmental factors [25]. Regions in which coccidioidomycosis is endemic have experienced a tremendous increase in their population. In 2005, it was estimated the populations of southern Arizona and Southern California increased by greater than 7 million inhabitants. Additionally, there is greater recognition of the disease in patients with compromised cellular immunity and in the elderly, who are more likely to have more severe disease. It appears, however, that the absolute incidence of disease has also increased, particularly during the epidemics described in the 1990s and in the first 2 years of the new millennium [22–25]. Despite the significant escalation in incidence, mortality associated with coccidioidomycosis has remained similar [26, 27].

Pathogenesis and Immunology

Virtually, all infections result from inhalation of arthroconidia. Inhaled arthroconidia transform into spherules, an inflammatory response ensues, and a local pulmonary lesion develops. In some infections, the *Coccidioides* species gain access to the vascular space, leaving the lungs and disseminating to other parts of the body. Control of coccidioidomycosis is predominantly cell mediated, with more severe infections seen in T-cell-deficient patients [28–30]. Additionally, in vitro observations have shown that innate cellular responses, mediated by mononuclear cells or natural killer

cells, may slow fungal proliferation after infection [31]. It is conceivable that interleukin (IL)-12, IL-23, and interferon gamma may play an important role in protective immunity in coccidioidomycosis as recently demonstrated in paracoccidioidomycosis and histoplasmosis [32, 33]. Progressive and disseminated forms of coccidioidomycosis were recently described in cases with interferon gamma receptor 1 deficiency or mutation in the beta 1 subunit of the IL-12 receptor [34, 35].

Clinical Manifestations

Pulmonary

Coccidioidomycosis most commonly presents as primary pulmonary disease. The first symptoms of primary infection usually appear 7–21 days after exposure, although infection is asymptomatic 60% of the time. In those patients presenting with symptomatic disease, the majority present with an influenza-like syndrome. Of the 40% of total infections with symptoms, only one out of four is diagnosed; the majority of these present with pneumonic or pleural disease. There are a number of pulmonary complications of primary coccidioidomycosis. The most common is severe and persistent pneumonia. This is defined as radiographic and clinical findings of pneumonic disease for greater than 6 weeks. Progressive primary coccidioidomycosis is a syndrome described in which the patient has resolution of their pulmonary parenchymal disease with persistence of hilar and mediastinal lymphadenitis. Rare cases of progressive fibrocavitary coccidioidomycosis, which often resembles pulmonary tuberculosis, are described. Solitary thin-walled pulmonary cavities are a frequent complication (see Fig. 6.11, Chap. 6). Residual nodules are often confused with a neoplasm, particularly when individuals with unrecognized primary coccidioidomycosis present with a residual nodule on routine chest radiograph long after the time of infection [36]. Modest amounts of pleural fibrosis, a residual of the primary infection, may also be seen. Cavitary disease may rupture into the pleural space causing coccidioidal empyema, not to be confused with simple pleural effusions which may occur as part of the primary disease process. Symptoms prevalent in primary coccidioidomycosis include fever (76%), cough (73%), chest pain (44%), fatigue (38%), erythema nodosum (26%), myalgias (23%), shortness of breath (22%), sputum production (22%), chills (21%), headache (21%), night sweats (21%), and other rashes (14%) [37]. Radiographic findings in primary coccidioidomycosis typically include infiltrate only (70%), infiltrate with hilar adenopathy (10%), or infiltrate with effusion (10%; Figs. 17.2, 17.3, 17.4, 17.5). Lung cavities are present in about 8% of adults, but are less common in children. Approximately, 10% of

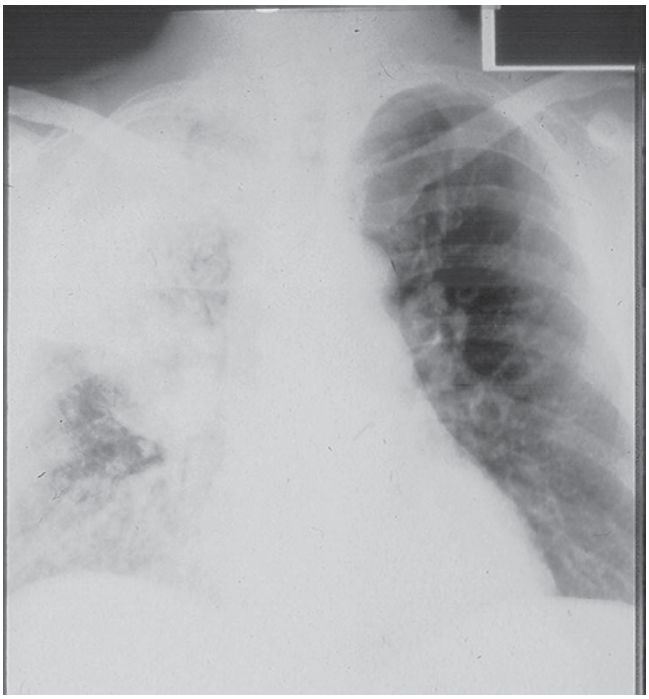

Fig. 17.2 Right upper lobe dense consolidation with associated right hilar adenopathy

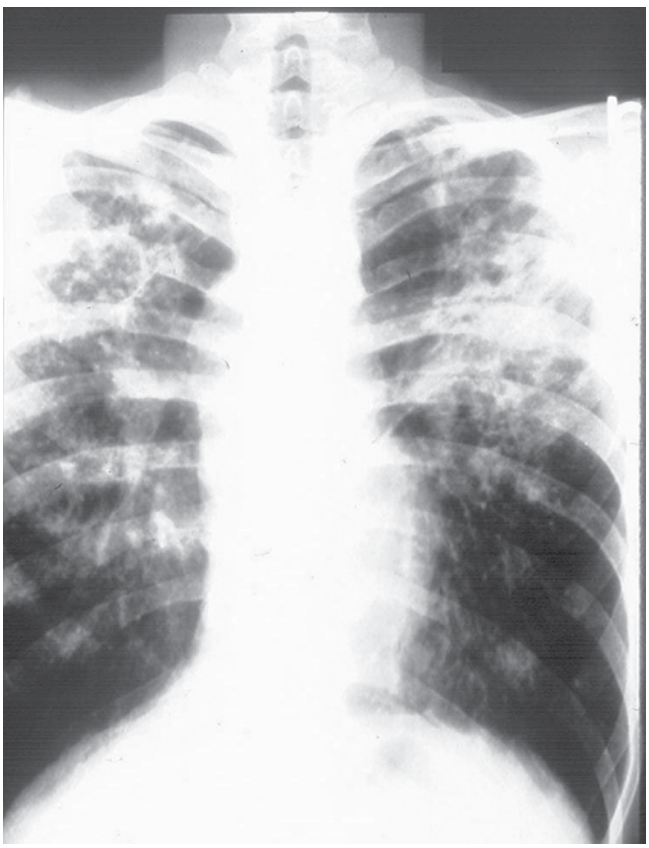

Fig. 17.3 Fibrocavitary changes involving both upper lobes. Right upper lobe cavity with air fluid level

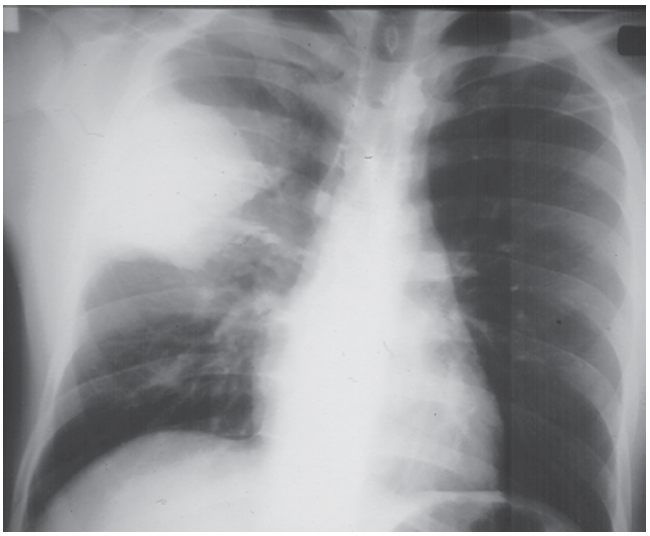

Fig. 17.4 Right upper lobe pleural-based mass with surrounding infiltrate

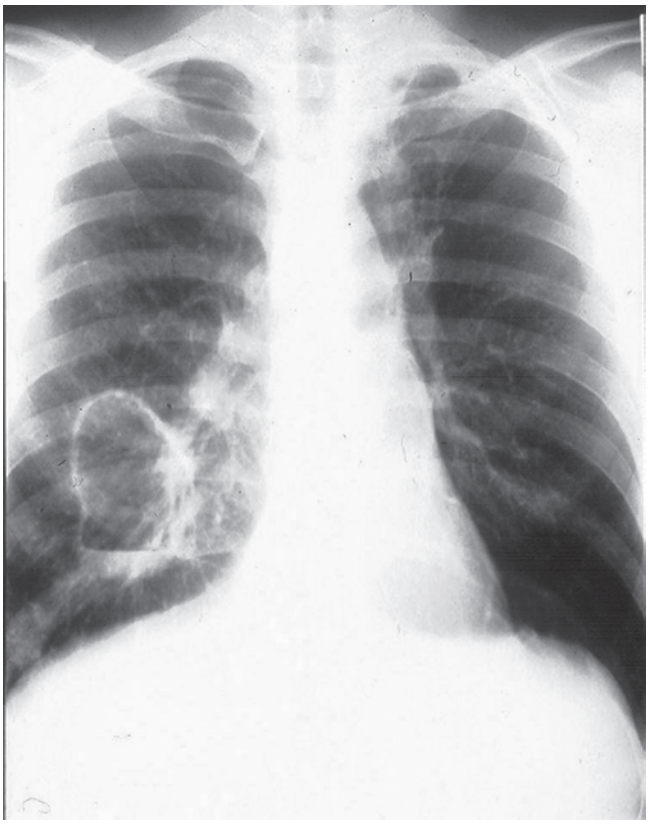

Fig. 17.5 Right middle lobe giant cavity with air fluid level and associated infiltrate

individuals will have a negative chest radiograph at diagnosis. Skin manifestations develop as part of the primary illness, most often as a transient non-pruritic fine papular rash. Erythema nodosum is fairly common in primary

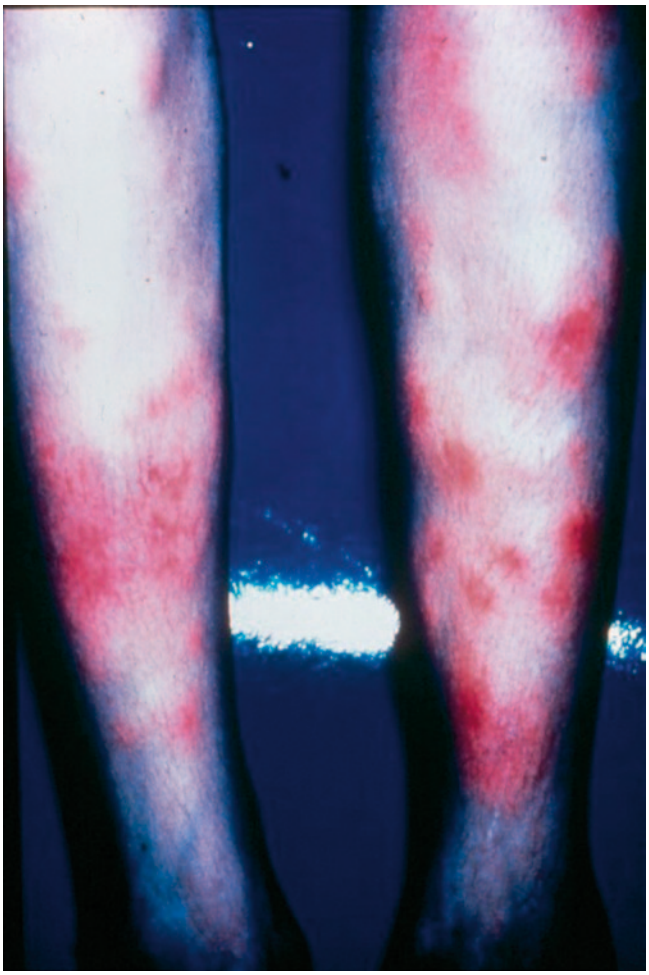

Fig. 17.6 Erythema nodosum affecting the lower extremities

coccidioidal infection, with a strong predilection for women (Fig. 17.6). Less commonly, erythema multiforme and erythema sweetobullosum are seen [38, 39]. Migratory arthralgias are common; the triad of fever, erythema nodosum, and arthralgias has been referred to as "desert rheumatism." Rarely, pulmonary coccidioidomycosis may present as a bronchial mass found on bronchoscopy. Chronic fibrocavitary pneumonia can occur, commonly in association with diabetes or preexisting pulmonary fibrosis [40]. Miliary disease with coccidioidomycosis is seen with significant frequency in the endemic area, where miliary coccidioidomycosis may be 10 times as frequent as miliary tuberculosis. Overwhelming miliary and/or alveolar coccidioidomycosis can result in respiratory failure. HIV-infected patients often have a fulminant presentation, particularly when $CD4^+$ T lymphocyte counts are less than 100 cells/μl. Probably, the most common cause of death in the endemic area from coccidioidomycosis is respiratory failure, although most coccidioidal respiratory infections resolve within several weeks to months without complications.

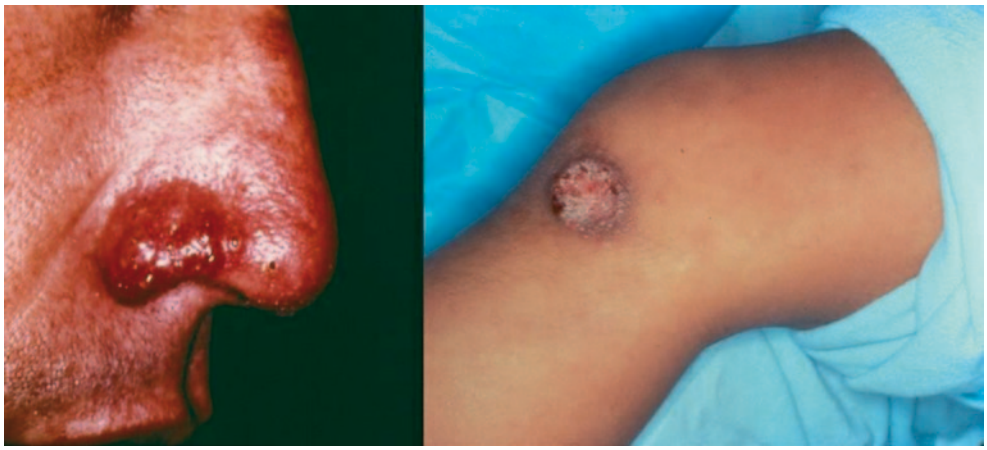

Fig. 17.7 Characteristic lesions of cutaneous coccidioidomycosis

Disseminated Disease

The rate of dissemination of coccidioidomycosis is highly dependent on the infected host. The majority of disseminated disease occurs in individuals with antecedent symptomatic pulmonary infection. However, in a minority of patients, disseminated disease presents without obvious primary pulmonary infection. Risk factors for dissemination include the extremes of age, male sex, African-American or Filipino ancestry, tobacco smoking, and low socioeconomic status [41, 42]. Persons with immunodeficiency, including those seen with advanced HIV infection, high-dose corticosteroid therapy, lymphoma, solid organ, or bone marrow transplantation, are at greater risk of dissemination [43–48]. Pregnancy also predisposes to individuals to disseminated disease [49, 50]. The majority of dissemination is to skin, subcutaneous tissue, bone, and joints. These sites taken together represent more than 50% of disseminated disease. Unfortunately, the single commonest site of dissemination is the meninges. Cutaneous dissemination has a variable clinical appearance; perhaps the most characteristic is one or more verrucous lesions, which may vary in size from a few millimeters to a few centimeters (Fig. 17.7). Subcutaneous tissue infection, which usually presents as a cold abscess, is also seen with some frequency. Infections of virtually all joints have been described. Infections of the knee, elbow, wrist, and ankle are commonly seen, with the knee most frequently involved [51–53]. Dissemination similarly has been described in almost every bone. Particularly common are infections of the axial skeleton, the pelvic bones, tibia, and femur. It is not unusual to see osteomyelitis and joint involvement in the same patient. Single bone or joint infections are most common, but multiple sites may be involved, particularly in African-American males. The most severe disseminated manifestation of coccidioidomycosis is meningitis. This is the single most common dissemination site in Caucasian and Latino males. Untreated, meningitis is fatal within a few months, although there are rare reports of survival for 2 or more years [54]. Meningitis usually develops within 6 months of the initial infection [55]. The cerebrospinal fluid has an elevated white blood cell count and protein, with depressed glucose. Eosinophils are not common, but when present, they are highly suggestive of the diagnosis of coccidioidal meningitis [56]. Finally, *Coccidioides* may disseminate to virtually any site in the body. Coccidioidal endophthalmitis, peritonitis, and prostatitis have all been described.

Diagnosis

It is essential to obtain a detailed travel history for exposure to an endemic area. Exposure does not need to be over a prolonged period of time; infection has occurred after only passing through an endemic area. The diagnosis of coccidioidomycosis is dependent on a compatible clinical illness with positive laboratory confirmation by culture, histopathology, or serology.

Culture

Suitable material for culture is sputum, tissue aspirates, or biopsy specimens. *Coccidioides* species grow well on most culture media after 5–7 days of incubation in aerobic conditions at 25, 30, or 35 °C. Typically, these fungi produce a white mold, although more pigmented strains have been observed. Laboratory cultures are highly infectious when mature arthroconidia have formed (see Fig. 2.14, Chap. 2). Typically, it takes about 10–20 days for *Coccidioides* to mature and produce arthroconidia. Because of their size, the arthroconidia are easily dispersed in the air and inhaled; therefore, *Coccidioides* is extremely hazardous when cultured in the laboratory. At a minimum, biosafety level 2 practices and facilities are recommended for handling and processing of clinical specimens. When working with known *Coccidioides*, biosafety level 3 is required [57]. Accidental percutaneous inoculation of the spherule form may result in local granuloma formation. Clinical specimens, prior to culture,

however, are not infectious to personnel. The much larger size spherules are considerably less effective as airborne pathogens. *Coccidioides* will grow in most of the media, including 5% sheep blood agar, chocolate agar, Sabouraud dextrose agar, Mycosel agar, and brain heart infusion agar with or without blood. Growth on 5% sheep blood agar and chocolate agar incubated at 35 °C can be seen in as little as 24 h. Growth on Sabouraud dextrose agar and Mycosel agar incubated at 25 °C (room temperature) can be seen after 3–4 days. Specimens from known or suspected cases should not be cultured on unsealed plated media. Use of tubed media is suggested (Fig. 17.8). Presumptive identification may be made based on colony morphology, growth rate, and the production of alternating arthroconidia. Care must be taken when attempting to identify *Coccidioides* as other mycoses may have similar macroscopic and microscopic morphologies, especially if arthroconidia are not abundant (Fig. 17.9). Laboratories that are not experienced with working with *Coccidioides* should refer these suspected isolates to qualified reference laboratories.

Coccidioides species are dimorphic fungi that have the ability to grow vegetatively at 25 °C as molds, and at 37 °C in tissue or in special medium (Converse liquid medium) in 10% CO_2 incubator as spherules. Confirmation traditionally was performed by animal inoculation with identification of endosporulating spherules on histopathology. Exoantigen tests and the production of spherules in Converse liquid medium could also be used. These methods have now been supplanted by molecular testing; a DNA probe is available commercially.

Fig. 17.8 Growth of *Coccidioides* in tubed fungal medium

Histopathology

Diagnosis may also be confirmed histopathologically with the demonstration of spherules with endosporulation usually in the setting of granulomatous inflammation (see Fig. 4.9, Chap. 4).

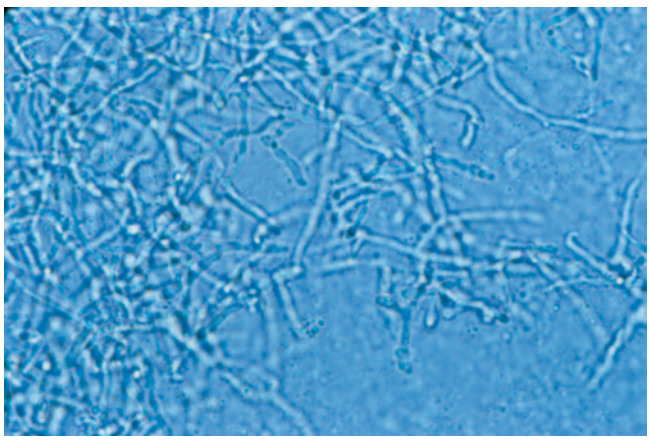

Fig. 17.9 Mycelial form of *Coccidioides immitis*

Serologic Testing

The most common method of diagnosis is serologic testing in individuals who have typical clinical features. Correctly performed serologic tests are both sensitive and specific for the disease. A negative serologic test, however, does not exclude the presence of infection, especially if recently acquired, and should be repeated over the course of several months. Serologic tests for *Coccidioides* are many. The most commonly used currently are enzyme immunoassay (EIA), immunodiffusion (ID), and complement fixation (CF) antibody tests. The EIA allows the detection of IgM antibodies for the determination of recent infection. Although this test suffers from many false positives, it is probably the most sensitive test for early infection. The ID IgM test has somewhat less sensitivity but a better specificity than the EIA test. The EIA IgG test appears to have a significant number of false negatives which limit its utility in the diagnosis of severe and advanced

disease. The ID IgG test has a high degree of specificity and fewer false negatives in individuals with significant or disseminated disease. CF tests are both sensitive and specific in the diagnosis of coccidioidal infections. The quantitative CF test is expressed as a titer and has the additional advantage of being not only diagnostic but prognostic. There is an inverse relationship of the IgG antibody titer to prognosis. Individuals with decreased amounts of IgG antibodies tend to have modest primary infections. Individuals with high amounts of IgG antibodies are more likely to have extensive primary infection or disseminated disease. It must be understood, however, that this holds true for a population of patients. In a given individual, the extent and severity of disease cannot be accurately predicted solely on the measurement of IgG antibodies.

The majority of patients with disseminated disease will eventually have a titer ≥1:16. At present, EIA results should be confirmed with the more established ID or CF tests [58, 59]. ID and CD IgG tests will have a false negative rate of approximately 1 % in disseminated disease. The majority of individuals with disseminated disease who have falsely negative *Coccidioides* serology are HIV infected. The CF titer in individuals with meningitis is higher than in individuals with primary disease but lower than those with other forms of disseminated disease [60].

Skin Testing

Skin test antigens derived from both mycelia and spherules have been marketed in the past. No skin testing reagents are currently commercially available in the USA. Skin testing detects the delayed-type hypersensitivity reaction to *Coccidioides* [61]. Since skin tests commonly remain positive for life in most people, a positive result may not be related to current illness, analogous to tuberculin skin testing. The diagnosis of coccidioidomycosis can be made by demonstrating the conversion of the skin test from negative to positive. False negative skin tests can occur in immunocompromised individuals and in the setting of overwhelming infection. Thus, a negative skin test cannot exclude a diagnosis of current or past coccidioidal infection. Skin testing therefore is limited as a screening procedure for recent infection, but may be useful in epidemiologic studies.

Other Laboratory Findings

Nonspecific laboratory tests such as the complete blood count and chemistry tests occasionally offer clues to coccidioidal infection. In an endemic area, an individual presenting with what appears to be community-acquired pneumonia who has an absolute eosinophilia (greater than 350 cells/μl) is more likely to have primary coccidioidal infection. It has also been noted that individuals who present with coccidioidomycosis and elevated alkaline phosphatase may also have liver involvement.

Radiologic Imaging

Radiographic imaging may be of great help in defining the extent and severity of both pleural pulmonary and disseminated disease. Chest radiography is mandatory in evaluation of primary disease. Computed tomography of the chest may be helpful in selected cases, especially with cavitary disease. Bone scan is the most frequently ordered test for osteomyelitis and plain radiograph is frequently utilized (see Figs. 6.13 and 6.14, Chap. 6). Contrast-enhanced magnetic resonance imaging (MRI) of bone and joint will help define problematic cases. MRI of the brain and spinal cord may reveal meningeal enhancement, hydrocephalus, or vasculitic infarction. Even though approximately 50 % of patients with meningitis will have normal neuroimaging ([62]; see Fig. 6.3, Chap. 6).

Treatment

General Approach

The treatment of coccidioidomycosis is both complex and, at times, controversial. A treatment guideline published by the Infectious Disease Society of America (IDSA) in 2005 gives a consensus framework (update is in progress) [63]. It is clear that many individuals infected with *Coccidioides* species recover without specific therapy. This is especially true when one notes that 60 % of infections are asymptomatic and, by definition, go undiagnosed and untreated. Of symptomatic individuals, large numbers also go undiagnosed and most recover uneventfully. There is some controversy as to whether those individuals who are diagnosed with symptomatic primary disease need to be treated. Some experts believe that the majority of these persons will recover without treatment and therefore treatment ought not be offered. Other authorities note that a small but significant percentage of individuals with primary disease will have either pulmonary or disseminated complications and it is difficult to predict with certainty who these individuals might be; thus, the majority should be treated. Unfortunately, there is no evidence-based study of primary disease that has examined whether improvement in the primary symptom complex, rate of pulmonary complications, or frequency of dissemination is effected by treating or not treating. What is generally agreed upon is that individuals with significant risk factors for dissemination or poor outcome

Table 17.1 Antifungal therapy of coccidioidomycosis

Disease	Primary therapy	Alternate therapy	Duration
Acute pulmonary infection			
Uncomplicated			3–6 months
Low risk	Observation	Oral azole[a]	
High risk[b]	Oral azole[c]	Amphotericin B	
Diffuse/severe	Amphotericin B[d]	High dose fluconazole[e]	≥12 months
Chronic infection			
Chronic infection			
Pneumonia	Oral azole	Surgical resection (cavitary disease only)	≥12 months
Disseminated			
Nonmeningeal	Oral azole	Amphotericin B	Years
Meningeal	Oral fluconazole[e,f]	Voriconazole, or itraconazole, or intrathecal amphotericin B	Life long
Other			
Nodule	Observation		
Cavity			
Asymptomatic	Observation		
Symptomatic	Oral azole		
Ruptured	Surgery and antifungal therapy		≥12 months

Modified from Infectious Diseases Society of America guidelines [57]
[a] Oral azoles include fluconazole and itraconazole, typically 400 mg or more daily
[b] See text for description of patients at high risk
[c] Amphotericin B should be used in pregnant patients due to the teratogenicity of azoles
[d] Typically replaced with azole therapy once patient is clinically improving and stable
[e] High-dose fluconazole (800–1200 mg daily) is often used in severe disease, at least initially
[f] Consider early shunting if hydrocephalus is present and corticosteroids if vasculitis is present

should receive treatment for primary disease (Table 17.1). Thus, advanced age; male sex; vulnerable race; presence of associated comorbid diseases such as diabetes, liver disease, and underlying lung disease; and elevated CF titers should favor treatment of primary disease. HIV infection and other conditions associated with immunodeficiency, such as lymphoma, cancer chemotherapy, and organ transplantation, mandate early and aggressive therapy of primary disease. Pregnancy represents a special circumstance. The development of primary disease during the middle trimester through the early postpartum period puts an otherwise low risk group of individuals at much higher risk. Coccidioidomycosis has been the leading cause of maternal mortality in Kern County, California, for more than 50 years [64]. Despite the risk, many women will have favorable outcomes without drug treatment, and abortions or early delivery in subjects with active infection is rare [65].

Primary Coccidioidomycosis

Most individuals with uncomplicated acute coccidioidal pneumonia, if treated, are initiated on oral azole therapy (Fig. 17.10). Fluconazole 400 mg daily has been prescribed most often. Alternatively, itraconazole 200 mg twice daily is also commonly prescribed [66]. Some institutions are initiating higher doses as primary therapy. This has especially been true with the recent availability of generic fluconazole. It should be noted that fluconazole and itraconazole are not approved for coccidioidomycosis by the Food and Drug Administration (FDA), nor are doses greater than 400 mg daily, in any disease. Despite lack of an FDA indication, these two drugs have become the mainstay in treatment of primary disease. Duration of recommended treatment ranges from 3 to 6 months, although longer courses may be prescribed in diabetics, persons of African-American or Filipino descent, seropersistant (lack of serological response to therapy), and immunocompromised patients. In individuals presenting with severe, diffuse pulmonary coccidioidomycosis or miliary disease with respiratory failure, azoles are not the initial drugs of choice. In this circumstance, amphotericin B deoxycholate, liposomal amphotericin B, or amphotericin B lipid complex are preferred. It appears that there is a more rapid response to the amphotericin than to the azole drugs. There does not seem to be a difference in efficacy between amphotericin B compounds, albeit in other diseases there has been a demonstrable difference in toxicity [67]. Several weeks of therapy with amphotericin B are often required for improvement, after which oral azole therapy is employed. A brief initial course of corticosteroids is considered beneficial by some in case of fulminant diffuse pneumonia with hypoxia [68].

Pulmonary Nodule

Antifungal therapy or resection is unnecessary for stable pulmonary nodules. If enlargement of the nodule occurs,

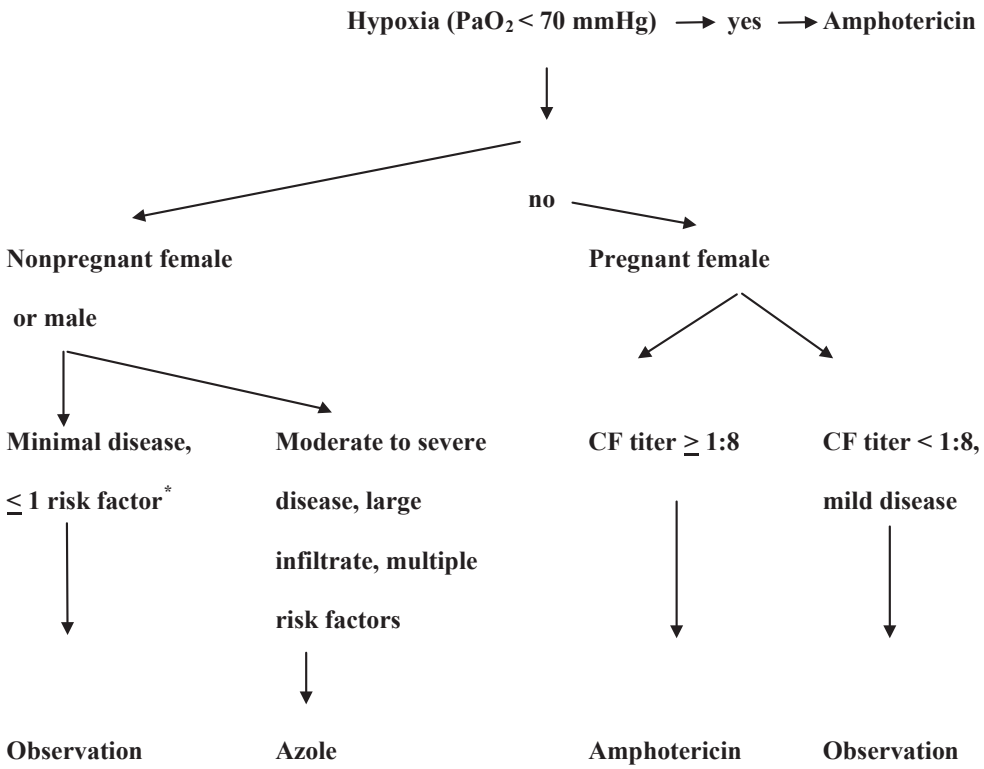

Fig. 17.10 Approach to the patient with acute pulmonary coccidioidomycosis. CF, complement fixation antibody. See text for listing of the risk factors associated with poorer prognosis. These include age >40, African or Asian ancestry, and immunodeficiency

reevaluation with sputum cultures and measurement of serum coccidioidal antibodies should be done to determine if the infection is active and warrants treatment.

Pulmonary Cavity

Asymptomatic, cavitary disease caused by *Coccidioides* often does not require intervention. Symptomatic solitary cavity coccidioidomycosis may benefit from azole therapy. A course of varying duration until symptoms are resolved is appropriate. Resolution of fever, cough and hemoptysis, improvement in appetite, and decrease in CF titers, if any, may be used to guide therapy. Approximately, one half of cavities smaller than 3 cm will resolve in 6–12 months. If the cavity persists but the symptoms abate, a trial of withdrawal of azole therapy can be undertaken. If symptoms recrudesce, reinstitution of therapy for a longer period of time is suggested. Indications for resection of the cavity include recurrent bacterial superinfections and recurrent or life-threatening hemoptysis. Rupture of the cavity into the pleural space, with development of empyema, often requires surgical as well as medical therapy.

Chronic Progressive Fibrocavitary Pneumonia

Fibrocavitary pneumonia of coccidioidomycosis in the pre-azole era often resulted in death from respiratory failure and pulmonary hypertension. Since the advent of azoles, death is less common. Fluconazole or itraconazole at 400 mg or more per day is the most common therapy. At this time, amphotericin has little role in the management of this subacute illness.

Coccidioidomycosis in Pregnancy

Because of the demonstrated concerns regarding teratogenicity with high-dose azole compounds, amphotericin is the drug of choice in pregnancy for those requiring therapy. Pregnant females with distant infection or mild disease of limited extent and with low CF titers are usually followed very closely without initiation of any therapy. Those with greater extent of disease or with high CF titers in first trimester are immediately placed on amphotericin. In second or third trimester, low-dose azoles can be used as an alternative [69]. This should be done in concert with an obstetrician who deals with high-risk pregnancies.

Disseminated Disease (Extrapulmonary)

Therapy of disseminated disease requires more expertise and judgment than does uncomplicated pulmonary disease (Fig. 17.11). It has been noted that minimal cutaneous disseminated disease may remit without specific antifungal therapy. At this time, however, no expert recommends that treatment of disseminated disease not include antifungal

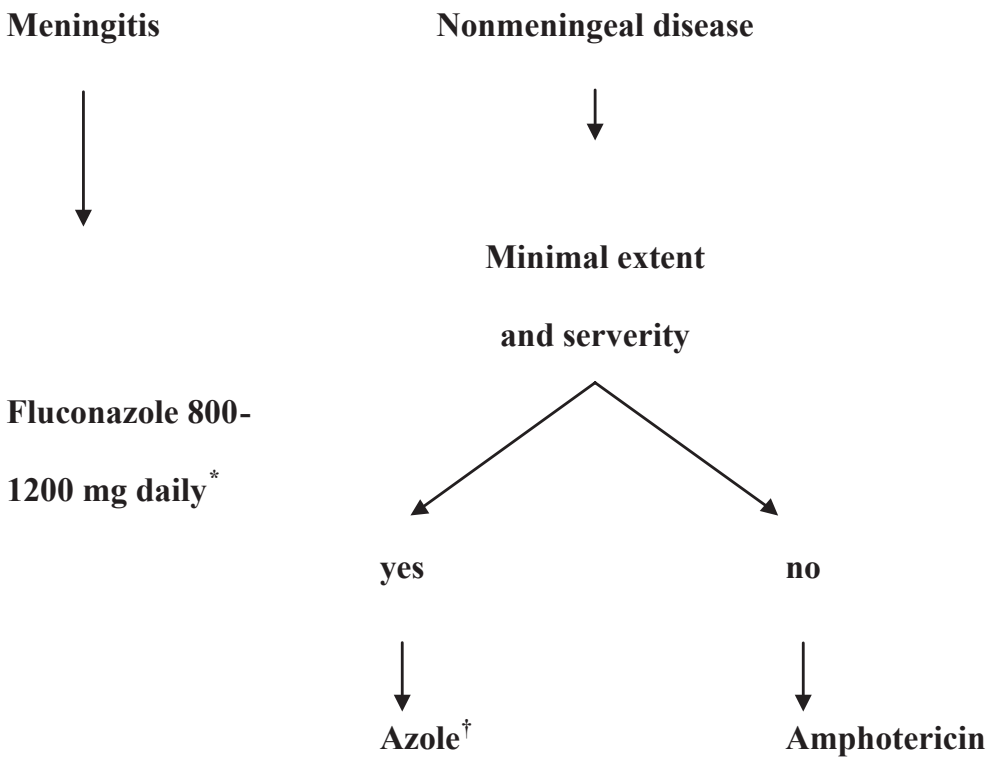

Fig. 17.11 Approach to the patient with disseminated coccidioidomycosis. Alternates for treatment failure include voriconazole, itraconazole, and intrathecal amphotericin B. Fluconazole 400–1000 mg daily (as one dose) or itraconazole 400–800 mg daily (in divided doses). Voriconazole and posaconazole may also have a role

therapy. Disseminated disease of the skin, soft tissue, joints, and bones, which is limited and not life or limb threatening is usually treated with azole therapy. Some experts prefer itraconazole for disseminated disease, particularly bony dissemination, because of a trend towards superior resolution at 1 year with itraconazole 200 mg every 12 h when compared with fluconazole 400 mg daily [66]. However, both drugs are used, albeit fluconazole is now commonly used at doses greater than 400 mg per day. Duration of therapy substantially longer than a year is frequently recommended. Some experts are recommending therapy for 3 years for significant disseminated disease. Severe multifocal osseous disease that affects the axial skeleton or a major long bone may be treated with azole therapy, though many experts prefer to use amphotericin initially in this circumstance. If the disease is amenable to surgical debridement, this may be a valuable adjunct. After individuals undergo treatment with amphotericin, secondary therapy with azoles is undertaken for protracted periods. Doses higher than 400 mg of fluconazole or itraconazole are frequently administered.

In coccidioidal meningitis, fluconazole is the preferred drug, given at doses of 800–1200 mg daily as a single dose [70, 71]. Itraconazole is not as commonly used as fluconazole but has had reported success. In patients failing high-dose fluconazole therapy, voriconazole has been used and has significant theoretical appeal as rescue therapy at a dose of 4 mg/kg every 12 h [72, 73]. Posaconazole 400 mg twice daily has been also used in refractory cases [74]. Intrathecal amphotericin B was the primary therapy of central nervous system (CNS) coccidioidomycosis until supplanted by azole therapy. This therapy can and has been given by direct cisternal injection, via ventricular or cisternal reservoir, or via intrathecal lumbar injection or reservoir. It is now used primarily in those failing other initial or secondary therapies. Coccidioidal meningitis is often complicated by hydrocephalus (Fig. 17.12), which is treated by ventriculoperitoneal shunting [75]. Therapy for coccidioidal meningitis is life long.

Monitoring Therapy

Patients with primary coccidioidomycosis should be monitored at 1–3-month intervals, both with laboratory and radiologic studies. If there is suspicion for dissemination by history or on examination, biopsy and culture of suspected sites of infection should be performed. Lumbar puncture should be performed in patients who develop headaches after the initial primary infection or who develop other neurologic signs at any time. Bone scan is indicated to evaluate bony or joint involvement.

Prevention

Developing a vaccine has been a goal for many years. A formalin-killed, whole-cell spherule vaccine was used in a human field trial but was not been found to be protective

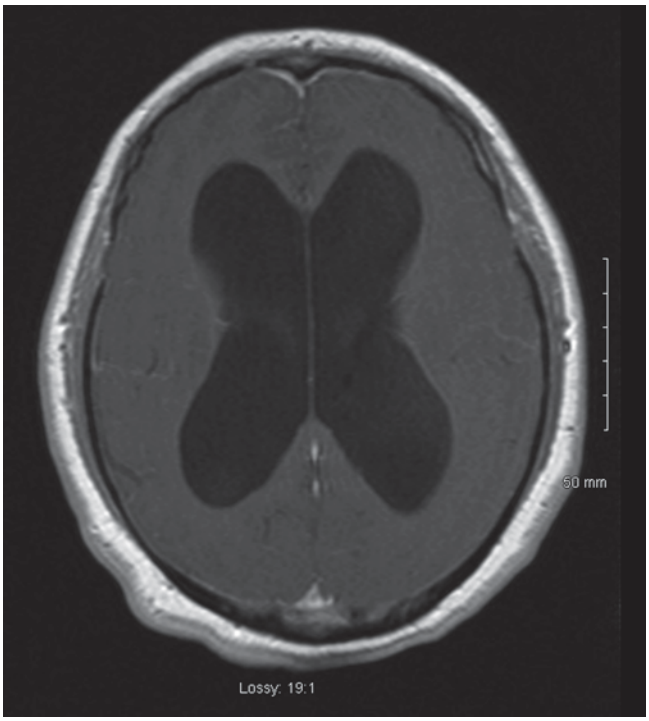

Fig. 17.12 Hydrocephalus in coccidioidal meningitis

[76]. New research on a subcellular vaccine has been initiated but has as yet not come to fruition.

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Histoplasmosis

18

L. Joseph Wheat and Chadi A. Hage

Introduction

Histoplasma capsulatum is a dimorphic fungus primarily found in the Americas, Africa, and Asia, but may be found worldwide, particularly in travelers and immigrants from the endemic areas [1]. Among the endemic mycoses, histoplasmosis is the leading cause of hospitalization and death in the USA [2]. Darling first described the organism in 1906, believing it to be *Leishmania*. First thought to cause a progressive and fatal disseminated disease, subsequently histoplasmosis was shown to be very common and usually asymptomatic or clinically self-limited.

Etiologic Agent

Histoplasma is a dimorphic fungus, defined by its ability to grow as a mold in the environment and as a yeast at 37 °C. Clinical specimens viewed via potassium hydroxide (KOH) preparation, calcofluor white, Giemsa, or hematoxylin and eosin (H&E) stains may demonstrate the 2–4-μm budding yeast cells (Fig. 18.1). *Histoplasma capsulatum* var. *capsulatum* causes the vast majority of clinical disease, while its closely related variant, *Histoplasma capsulatum* var. *duboisii*, is the etiologic agent of African histoplasmosis.

Disclaimer: Dr. Wheat is the president of MiraVista Diagnostics, the company that developed and performs histoplasma antigen testing. Dr. Hage is an assistant professor of medicine at Indiana University School of Medicine and faculty in the thoracic transplantation program at Indiana University Health.

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Epidemiology

Histoplasmosis is most commonly reported to occur in and around the Mississippi and Ohio River valleys of the USA, in South and Central America [3, 4] and less so in parts of Africa and Asia. This is thought to be due to factors such as the climate, humidity, and soil acidity. Large amounts of bird and bat excreta enrich the soil in which the fungi are found, facilitating growth, and accelerating sporulation. When disturbed, microfoci or niches harboring a large number of infective particles may lead to high infectivity rates or large outbreaks. In most cases, however, the exposure is small and infection is usually unrecognized. Cases outside the endemic area usually occur in individuals who have traveled or previously lived in endemic area(s) [5]. However, microfoci containing the organism can sometimes be found outside the endemic area and may be the source for exposure.

Pathogenesis and Immunology

Infection occurs when aerosolized microconidia (spores) are inhaled (Fig. 18.2). Infection usually is asymptomatic in healthy individuals following low-level exposure. Infection usually is self-limited except following heavy exposure or in patients with underlying diseases that impair immunity. Pulmonary infection may be progressive in patients with underlying obstructive lung disease. For unknown reasons, pulmonary infection may rarely elicit exuberant mediastinal fibrosis.

Phagocytic cells including macrophages, neutrophils, and dendritic cells are rapidly recruited to the alveolar spaces that contain conidia [6]. Within a few days, the conidia transform into yeast, which multiply within the nonactivated macrophages, and disseminate via the bloodstream to extrapulmonary organs. Neutrophils and dendritic cells inhibit proliferation of the organism, and dendritic cells present antigen to T lymphocytes as the initial step in development of specific

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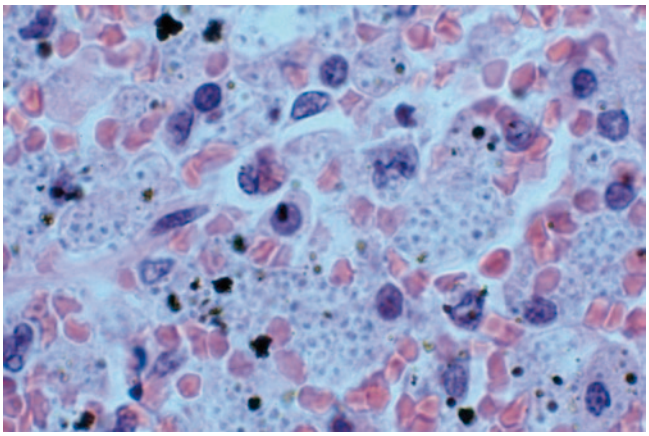

Fig. 18.1 Hematoxylin and eosin stain of yeast in macrophages

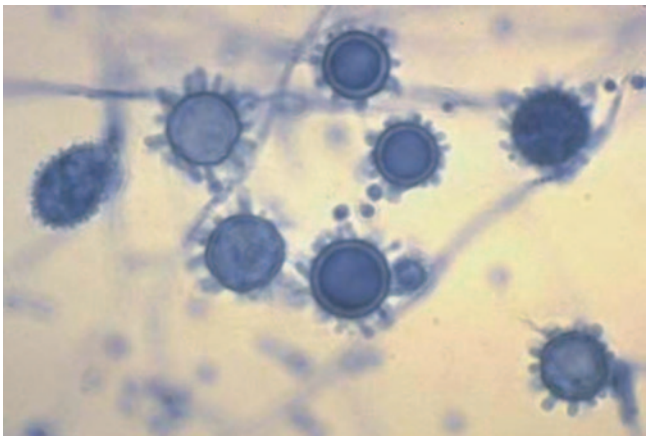

Fig. 18.2 Lactophenol cotton blue stain of the mold grown at 25°C. Note microconidia and tuberculate macroconidia

cell-mediated immunity. Consequently, tumor necrosis factor alpha and interferon gamma are induced, which activate macrophages to inhibit the growth of the organism, leading to spontaneous recovery and immunity against reinfection in most individuals [7]. Traditionally, humoral immunity is not felt to be important, but recent studies using monoclonal antibodies against specific *Histoplasma* epitopes suggest a protective role for humoral immune response.

Reactivation of latent infection has been proposed as the mechanism for progressive disseminated histoplasmosis (PDH) in immunocompromised patients. However, the rarity of PDH in immunosuppressed patients (0.1–1%) argues against reactivation as a common mechanism of pathogenesis. Histoplasmosis occurs in about 0.5% of patients undergoing solid organ transplantation in endemic areas [8]. Among 469 patients treated with TNF inhibitors for inflammatory bowel disease at a single institution, 3 (0.6%) developed histoplasmosis [9].

More likely, PDH in immunosuppressed individuals occurs because low-grade histoplasmosis was present at the time immunosuppression was initiated, or infection was acquired exogenously. In endemic areas, repeated exposure to *Histoplasma* spores probably occurs, permitting reinfection in immunosuppressed individuals whose immunity to *H. capsulatum* has waned.

Clinical Manifestations

Asymptomatic Infection

Infection is asymptomatic in most otherwise healthy individuals, who experience low inoculum exposure, as indicated by skin test positivity rates above 50–80% in endemic areas [10]. Asymptomatic infection also may be identified by radiographic findings of pulmonary nodules or mediastinal lymphadenopathy, which eventually calcify, by splenic calcifications, or by positive serologic tests performed during screening for organ or bone marrow transplantation or epidemiologic investigation. In endemic areas, about 5% of the healthy subjects have positive complement fixation tests for anti-*Histoplasma* antibodies.

Acute Pulmonary Histoplasmosis

Healthy individuals who experience a heavy exposure usually present with acute diffuse pulmonary disease one to two weeks following exposure. Fever, dyspnea, and weight loss are common, and physical examination may demonstrate hepatomegaly or splenomegaly as evidence of extrapulmonary dissemination. In many cases following heavy exposure, the illness is sufficiently severe to require hospitalization, with some individuals experiencing respiratory failure. Chest radiographs usually show diffuse infiltrates, which may be described as reticulonodular or miliary (Fig. 18.3).

Subacute Pulmonary Histoplasmosis

In symptomatic cases, the most common syndrome is a subacute pulmonary infection manifested by respiratory complaints and fever lasting for several weeks, then resolving spontaneously over 1 or 2 months. The chest radiograph or CT scan usually shows focal infiltrates with enlarged mediastinal or hilar lymphadenopathy (Fig. 18.4). Symptoms caused by a mediastinal adenopathy may prevail, and in some cases persist for months to years (granulomatous mediastinitis). In some cases, respiratory symptoms may

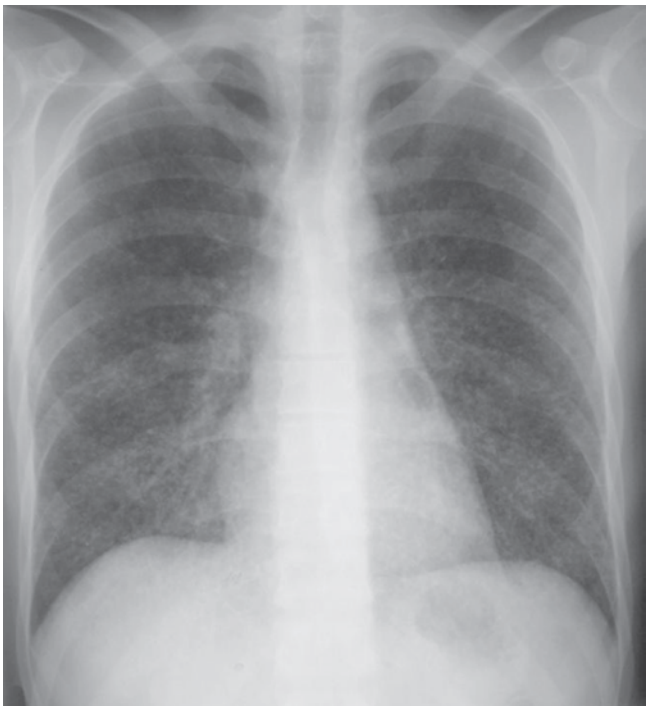

Fig. 18.3 Chest radiograph showing diffuse infiltrate seen in acute pulmonary or disseminated histoplasmosis

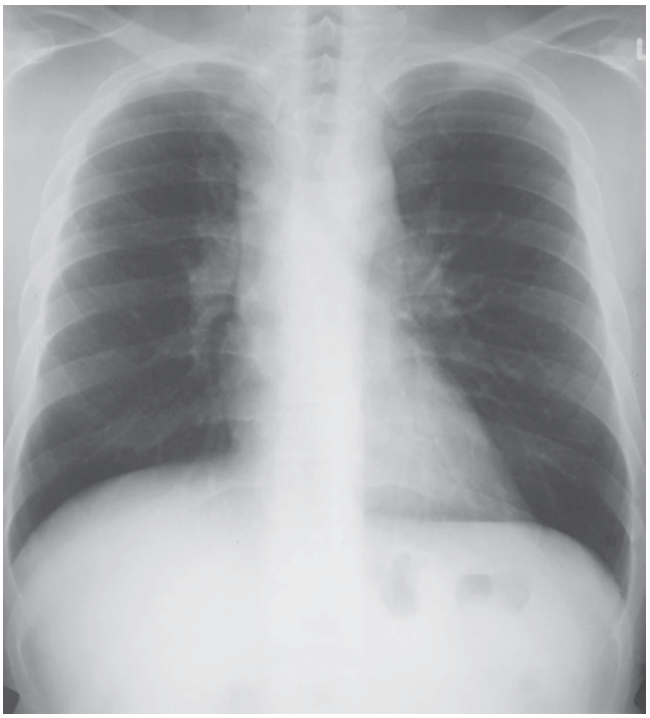

Fig. 18.4 Chest radiograph showing hilar lymphadenopathy seen in subacute pulmonary histoplasmosis

be mild or absent, and findings of pericarditis or arthritis/arthralgias may be prominent. Pericarditis and this rheumatologic syndrome represent inflammatory reactions to the acute infection, rather than infection of the pericardium or joints.

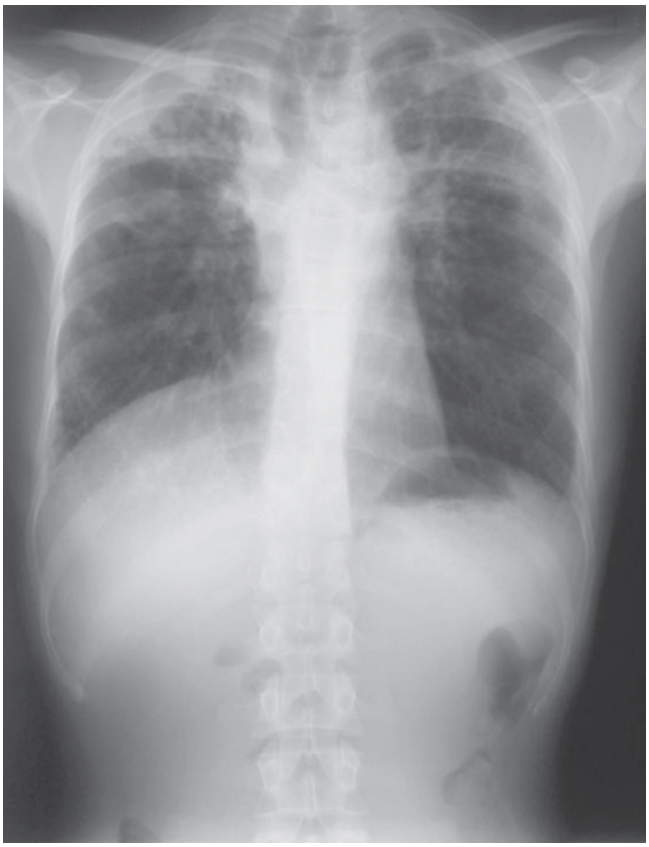

Fig. 18.5 Chest radiograph showing upper lobe infiltrates with cavitation seen in chronic pulmonary histoplasmosis

Chronic Pulmonary Histoplasmosis

Patients with underlying obstructive pulmonary disease develop chronic pulmonary disease following infection with *H. capsulatum*. The underlying lung disease prevents spontaneous resolution of the infection. Chest radiographs reveal upper lobe infiltrates with cavitation, often misdiagnosed as tuberculosis (Fig. 18.5). The course is chronic and gradually progressive, highlighted by systemic complaints of fever and sweats, associated with shortness of breath, chest pain, cough, sputum production, and occasional hemoptysis. Patients often experience repeated bacterial respiratory tract infections, and occasionally superinfection with mycobacteria or *Aspergillus*.

Progressive Disseminated Histoplasmosis (PDH)

Hematogenous dissemination is common during acute pulmonary histoplasmosis, but is nonprogressive [11]. With

the development of specific cell-mediated immunity, the infection resolves in the lung and extrapulmonary tissues. In contrast, disease is progressive in individuals with defective cell-mediated immunity. In many cases, the cause for immune deficiency is easily identified, and often includes the extremes of age, solid organ transplantation [12], treatment with immunosuppressive medications [13], acquired immunodeficiency syndrome (AIDS) [14], idiopathic $CD4^+$ leucopenia [15], deficiency in the interferon-γ/interleukin-12 pathway [16], or malignancy [17]. In other cases, the cause for immunodeficiency remains unknown, awaiting a more complete understanding of immunity in histoplasmosis and development of better tests for diagnosis of immunodeficiency.

Clinical findings in PDH include fever and progressive weight loss, often associated with hepatomegaly or splenomegaly, and laboratory abnormalities including anemia, leukopenia, thrombocytopenia, liver enzyme elevation, and ferritin elevation [11]. An elevation in serum lactic acid dehydrogenase (LDH) has been associated with disseminated histoplasmosis, particularly levels greater than 600 IU/L. Pulmonary involvement is often the prominent feature in PDH, with manifestation as severe as respiratory failure requiring mechanical ventilation and vasopressor support for shock. The classical radiographic finding in PDH is diffuse reticulonodular infiltrates on chest X-ray and CT scan. Other less frequent sites of involvement include the central nervous system, gastrointestinal tract, skin, and adrenal glands. PDH also may present as culture-negative endocarditis, accompanied by other sites of dissemination or as an isolated manifestation of histoplasmosis [18].

Fibrosing Mediastinitis and Mediastinal Granuloma

Fibrosing mediastinitis is a rare complication of pulmonary histoplasmosis [10]. The mechanism for this manifestation appears to be an excessive fibrotic response to antigens released into the mediastinal tissues rather than progressive infection. It is unclear why the immune response from subclinical histoplasmosis can lead to either mediastinal granuloma formation (with inflammation and caseating necrosis) or this fibrotic process. Genetic influences, inoculum size, and host immunity are all likely factors. The clinical findings of fibrosing mediastinitis are caused by obstruction of mediastinal structures and may include involvement of the superior vena cava, airways, or pulmonary vessels. Infiltrative inflammatory mediastinal masses that do not respect fat or fascial planes are characteristic computed tomography findings of fibrosing mediastinitis. Fibrosing mediastinitis most commonly involves the right hemithorax, although bilateral involvement may occur. In most patients, obstruction does not progress, but mild to moderate symptoms persist indefinitely. In less than one quarter of patients, the illness is progressive, highlighted by repeated bouts of pneumonia, hemoptysis, respiratory failure, or pulmonary hypertension. No proven medical therapy exists for fibrosing mediastinitis due to histoplasmosis, including antifungal or corticosteroid therapy. Since the pathogenesis involves fibrosis rather than inflammation or infection, antifungal or anti-inflammatory therapy is not effective. Because fibrosis infiltrates adjacent mediastinal structures, surgical therapy has been of little benefit and is associated with a high risk for surgical morbidity and mortality. Surgical therapy is a rarely indicated, and should be considered only after careful consideration of the risks and benefits by experts in the management of patients with fibrosing mediastinitis. Various procedures to relieve compression of vascular structures, and airway and esophageal compression are often employed with mixed results.

Distinguishing fibrosing mediastinitis from mediastinal granuloma is the key. Mediastinal granuloma represents persistent inflammation in mediastinal or hilar lymph nodes. Enlarged, inflamed notes may cause chest pain with or without impingement upon soft mediastinal structures, such as the esophagus or superior vena cava. Fistulae may develop between the lymph nodes, airways, or esophagus. Improvement may occur spontaneously or following antifungal therapy. The enlarged lymph nodes are usually encased in a discrete capsule, which can be dissected free from the adjacent tissues with a low risk for surgical morbidity or mortality. Thus, surgery may be appropriate in patients with persistent symptoms despite antifungal therapy, weighing the risk of surgery with the severity of the clinical findings. However, studies establishing the effectiveness of surgery for mediastinal granuloma are scant, and surgical therapy is rarely necessary in such cases.

Often mediastinal lymphadenopathy is asymptomatic, identified on chest radiograms or CT scans performed for other reasons. In such cases, concern often arises as to whether the mass represents malignancy. Differentiation of mediastinal lymphadenopathy caused by histoplasmosis or other granulomatous infection from that caused by a malignancy is best deferred to pulmonary disease consultants [10]. The presence of calcification strongly suggests that lymphadenopathy is caused by granulomatous infection, but cannot rule out concomitant neoplasm. Conversely, the absence of calcification does not exclude granulomatous infection, and is quite typical of histoplasmosis during the first year or two after infection. Positron emission tomography (PET) scan has been suggested as a method to distinguish malignancy from nonmalignant causes for mediastinal or pulmonary masses, but PET scan is often positive in patients with histoplasmosis. For most patients lacking risk factors for malignancy, follow-up CT scans at 3–6 month intervals

for 1 year is appropriate, lack of progressive enlargement supporting the diagnosis of histoplasmosis. In others, especially those with risk factors for malignancy, biopsy may be necessary for definitive diagnosis. Choices include surgical excision or mediastinoscopy, although many recommend avoiding the latter due to risk of excessive bleeding.

African Histoplasmosis

In addition to infection with *Histoplasma capsulatum* var. *capsulatum*, *Histoplasma capsulatum* var. *duboisii* causes disease in Africa. Bony abscesses, more commonly involving the axillary skeleton and skin lesions are much more common with African histoplasmosis. Pulmonary disease is rare, although infection with this variant also likely occurs via inhalation of spores. Disseminated African histoplasmosis resembling PDH as described above has been reported, with fever, multiorgan involvement, and a progressive course. The yeasts of *H. capsulatum* var. *duboisii* are 10–15 μm in diameter (much larger than var. *capsulatum*) and can be seen within giant cells. This can be easily confused with *Blastomyces dermatitis* or *Coccidioides immitis* on histopathological examination. DNA and antigen tests with the African variant should react similarly to *H. capsulatum* var. *capsulatum*. Likewise, therapy is similar with the exception that isolated cutaneous disease, or "cold abscesses" may heal spontaneously or with excisional surgery. Disseminated African histoplasmosis, especially with HIV coinfection, has a poor prognosis.

Diagnosis

Culture

The only test specific for histoplasmosis is culture, but the sensitivity is low, and delays up to 1 month may be required to isolate the organism [15]. Identification of *H. capsulatum* can be determined by conversion from the mold to the yeast, exoantigen detection, or through use of the commercially available DNA probe. Despite these limitations, culture should be performed unless the patient is already improving at the time diagnosis is suspected. In patients with pulmonary disease, bronchoscopy may be required if patients are unable to produce sputum. In those with suspected PDH, fungal blood cultures should be obtained, but cultures of tissues requiring invasive procedures may be deferred if tests for antigen are positive. In such cases, failure to improve within 2 weeks after initiation of therapy would raise question about the diagnosis and support additional testing.

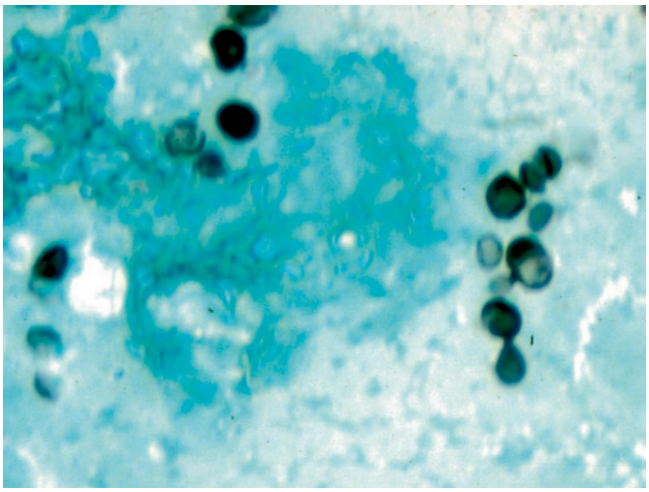

Fig. 18.6 Gomori methenamine silver (*GMS*) stain of yeast showing narrow neck budding

Antigen Detection

Antigen detection is the most useful method for rapid diagnosis of the more severe histoplasmosis syndromes, including acute diffuse pulmonary histoplasmosis and PDH [19, 20]. Antigen may be detected in any body fluid, but urine and serum testing is recommended in all cases. In some patients with pulmonary histoplasmosis, antigen may be detected in the bronchoalveolar lavage fluid but not in urine or serum [21]. Antigens may also be detected in the cerebral spinal fluid in patients with central nervous system histoplasmosis [22]; in pleural fluid, synovial fluid, peritoneal fluid, or pericardial fluid in patients with infection localized to those tissues. Antigen levels decline with effective therapy [23], persist with ineffective therapy, and rebound in patients who relapse during or following treatment [24].

Histopathology

Demonstration of yeast-like structures in body fluids or tissues may provide a rapid diagnosis in some cases [10] (Fig. 18.6; also see Fig. 4.8 and 4.8, Chap. 4). Limitations of histopathology include the need for performance of an invasive procedure and low sensitivity. Histopathology may be falsely negative or falsely positive when performed by pathologists inexperienced with recognition of fungal pathogens. If histopathology is inconsistent with clinical findings or other laboratory tests, the specimens should be reviewed by a pathologist experienced with recognition of fungal pathogens.

Table 18.1 Primary and alternate therapy for treatment for histoplasmosis

Presentation	Primary therapy	Alternate therapy
Acute pulmonary		
Mild	Itraconazole 200 mg daily or bid for 6–12 weeks	Posaconazole 400 mg bid, or voriconazole 200 mg bid, or fluconazole 800 mg daily
Moderately severe or severe	Liposomal amphotericin B 3–5 mg/kg/d for 1–2 weeks followed by itraconazole 200 mg bid for 12 weeks[a]; methyl prednisolone or prednisone 0.5–1 mg/kg/d for 1–2 weeks[b]	Amphotericin B lipid complex 3–5 mg/kg/d, or amphotericin B deoxycholate 0.7–1.0 mg/kg/d
Chronic pulmonary	Itraconazole 200 mg bid for at least 12 months	Posaconazole 400 mg bid, or voriconazole 200 mg bid, or fluconazole 800 mg daily
Disseminated	Liposomal amphotericin B 3–5 mg/kg/d for 1–2 weeks followed by itraconazole 200 mg bid at least 12 months[a]	Amphotericin B lipid complex 3–5 mg/kg/d, or amphotericin B deoxycholate 0.7–1.0 mg/kg/d

bid twice daily, *tid* three times daily, *mg/kg/d* milligram/kilogram/day

[a]Itraconazole may be given 200 mg tid x 3 days as a loading dose to achieve steady-state levels more quickly. The capsule formulation should be administered with food while the solution should be administered on an empty stomach. Measurement of drug concentrations is recommended since itraconazole drug exposure is highly variable. The intravenous formulation of itraconazole would be an alternative in a hospitalized patient with moderately severe or severe disease who is unable to be treated with any amphotericin formulation

[b]Adjunctive treatment with methyl prednisolone or prednisone may hasten recovery in otherwise healthy individuals with respiratory distress caused by acute pulmonary histoplasmosis

Serologic Tests

Serologic tests for antibodies are most useful in patients with subacute pulmonary histoplasmosis, chronic pulmonary histoplasmosis, granulomatous mediastinitis, pericarditis, or rheumatologic syndromes [24]. Immunodiffusion and complement fixation tests should be performed in all cases of suspected histoplasmosis. Serologic tests may be falsely negative during the first 2 months following acute exposure, limiting their usefulness in acute pulmonary histoplasmosis [19, 20]. Also, these tests may be falsely negative in immunosuppressed patients, limiting their role in PDH [8]. Furthermore, positive serologic tests persist for several years following recovery from histoplasmosis, and thus may provide misleading information in patients with other diseases.

Molecular Testing

Although several publications suggest that PCR is useful for diagnosis of histoplasmosis and assays are commercially available [25], their role is uncertain. The highest sensitivity has been achieved by testing tissues or respiratory secretions [25], but whether PCR is positive in specimens in which organisms are not visible by fungal stain is uncertain [26]. Sensitivity has been reported to be low in cerebrospinal fluid [27] and urine [28].

Conclusions

Accurate diagnosis of histoplasmosis requires skilled use of all of these laboratory methods. Except for culture, none of the tests are specific, and physicians must consider that the positive result may be falsely positive. Furthermore, none of the tests are positive in all cases, and the physician must consider the possibility that the test is falsely negative. In cases where the diagnosis is uncertain, or the laboratory tests are inconsistent, additional testing should be performed, including repetition of the antigen test or serology, and in some cases, invasive procedures to obtain tissues for histopathology and culture; expert advice should be sought.

Treatment

Practice guidelines published in 2007 [29] are outdated, but have been updated in a recent review [30]. Treatment is indicated in most patients with acute diffuse pulmonary histoplasmosis and all patients with chronic pulmonary histoplasmosis or PDH (Table 18.1). Adjunctive treatment with methyl prednisolone or prednisone may hasten recovery in otherwise healthy individuals with respiratory distress caused by acute pulmonary histoplasmosis, and can be administered safely if patients also receive antifungal therapy.

Treatment also should be considered in patients with subacute pulmonary histoplasmosis or granulomatous mediastinitis who are not improving within a month or two of the onset of symptoms. However, the effectiveness of therapy for these manifestations remains uncertain. Treatment for fibrosing mediastinitis is ineffective, and is not indicated except in cases where granulomatous mediastinitis cannot be reasonably excluded. Treatment is not indicated in patients with calcified or noncalcified pulmonary nodules or as prophylaxis before immunosuppression in patients without evidence of active histoplasmosis within the last 2 or 3 years.

Liposomal amphotericin B is the treatment of choice for patients with severe manifestations of histoplasmosis requiring hospitalization [31]. In some cases, however, because of

intolerance or cost, other lipid formulations may be used. In children, deoxycholate amphotericin B is well tolerated and preferred over the lipid formulations because of cost. Itraconazole is recommended for mild cases not requiring hospitalization and for continued therapy, following response to liposomal amphotericin B. Treatment should be continued for 6–12 weeks in patients with acute pulmonary histoplasmosis and 1 year or longer in patients with chronic pulmonary histoplasmosis or PDH. In patients with AIDS who achieve a good immunologic response to antiretroviral therapy, itraconazole may be stopped after 1 year if the $CD4^+$ T lymphocyte count is above 200 cells/μl and the *Histoplasma* antigen concentration in urine and serum are below 2 ng/ml [32, 33]. Similarly, lifelong maintenance therapy appears to be unnecessary in most patients with histoplasmosis complicating treatment with tumor necrosis factor inhibitors [9] or following solid organ transplantation [8]. However, in those with persistent immune deficiency, or who relapse after stopping an appropriate course of therapy, lifelong maintenance therapy may be required. Itraconazole blood levels should be monitored to assure adequate drug exposure, and the dosage should be increased or the capsule formulation replaced with the solution if random concentrations are below 1 μg/ml as determined by high-pressure liquid chromatography, or 3 μg/mL by bioassay.

If the antigen test is positive, treatment should be continued until antigen levels become undetectable or below 2 ng/ml. Furthermore, antigen levels should be monitored during the first year following discontinuation of therapy, and at the time of recurrence of symptoms suggesting relapse of histoplasmosis.

The best alternative oral therapy in patients unable to take itraconazole is posaconazole, which is highly active in vitro [34], in animal models [34], and in patients [35]. However, therapeutic concentrations of posaconazole of at least 0.5 μg/ml may be difficult to achieve using current formulations. Fluconazole is less active in histoplasmosis, and relapse associated with development of resistance has been observed in patients with AIDS [36]. Voriconazole is more active in vitro than fluconazole, but less active than itraconazole or posaconazole. Although minimum inhibitory concentrations (MICs) are lower to voriconazole than fluconazole, higher drug exposure with fluconazole offsets the lower MICs. Furthermore, prior exposure to fluconazole or voriconazole may induce resistance to voriconazole [37]. Voriconazole has not been studied in animal models or patients with histoplasmosis and offers no clear advantage over fluconazole. Furthermore, voriconazole causes several important toxicities, including photosensitivity and skin cancer, fluorosis manifesting as joint pain, and mental status changes, which must be considered before choosing it as chronic therapy in patients with mycoses that could be treated with other antifungal agents. Although CSF levels are higher with fluconazole than with itraconazole or posaconazole, the role of the former in treating *Histoplasma* meningitis is uncertain [29, 30]. Measurement of voriconazole or posaconazole blood levels is recommended because of the wide variation in drug levels. Voriconazole exhibits a short half-life (~6 h), and concentrations decline in at least twofold from the peak to the trough time after administration. Accordingly, trough concentrations of voriconazole of at least 0.5 μg/ml are recommended. Posaconazole exhibits a long half-life, similar to that of itraconazole (~24 h), supporting a similar target random concentration of 2 μg/ml. The echinocandins are not active in histoplasmosis and should not be used [38, 39].

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Paracoccidioidomycosis

19

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Introduction

Paracoccidioidomycosis (PCM) was first described in 1908 by Lutz in Brazil. Lutz and Splendore further characterized the etiologic agent, initially considered to be a strain of Coccidioides immitis. It was not until 1930 when de Almeida properly differentiated the etiologic agent, *Paracoccidioides brasiliensis*. The Brazilian disease was soon diagnosed in other Latin American countries and named South American Blastomycosis, thus stressing its peculiar geographic boundaries [1].

At present, three phylogenetically diverse *P. brasiliensis* cryptic species (S1, PS2, PS3) have been recognized as etiologic agents of this mycosis [2]. In addition, a more recently described species diverging from the former on the basis of high polygenetic diversity and exclusive morphogenetic characteristics, such as elongated conidia, has been characterized and designated *Paracoccidioides lutzii* [3–5].

These fungal pathogens are thermally dimorphic and as such exhibit two morphotypes, a mold at temperatures under 28 °C and a yeast in cultures at 35–37 °C, as well as in tissues. The yeast reproduces by multiple budding in a manner resembling a pilot's wheel [1, 6]. The genus *Paracoccidioides* is only known in its asexual (anamorph) stage, but molecular techniques have revealed the presence and expression of the mating type locus, thus allowing its classification in the phylum Ascomycota, order Onygenales, family Onygenacea, close to the phylogenetic tree encompasssing the dimorphic fungi *Histoplasma capsulatum, Blastomyces dermatitidis,* and *Emmonsia parva,* all of which present a teleomorphic or sexual stage in the genus *Ajellomyces* [7–10].

PCM is an endemic systemic mycosis that exhibits two main clinical presentations, the subclinical, or asymptomatic infection, and the clinically manifested disease. The latter is usually chronic with involvement of the primary target, the lungs, as well as of different body organs such as the mucosa, skin, adrenal glands, and lymph nodes, among others. The mycosis afflicts men more frequently than women and is common in adults [6, 11–13]. Latency is known to exist and is frequently prolonged [14].

The Etiologic Agent

The *P. brasiliensis* – *P. lutzii* complex is characterized by thermally dimorphic fungi that at temperatures between 4 and 25 °C grow as white molds, composed microscopically of thin septated hyphae, occasional chlamydospores, and rarely microconidia. The latter are infectious and capable of converting into yeast cells under the influence of temperature [15, 16]. At 35—37 ºC, colonies are soft, wrinkled, and microscopically composed of oval to round yeast cells of varying sizes (4–40 μm) that reproduce by budding. The key distinguishing feature is that of a multiple budding yeast cell with a larger mother cell surrounded by multiple daughter cells (blastoconidia), a structure resembling the pilot wheel of a ship. Additionally, a thick refractive cell wall and prominent intracytoplasmic vacuoles can also be observed (Fig. 19.1) [1, 13, 16]. The yeast morphologic characteristics are also observed in clinical specimens (Fig. 19.2) [1, 6,

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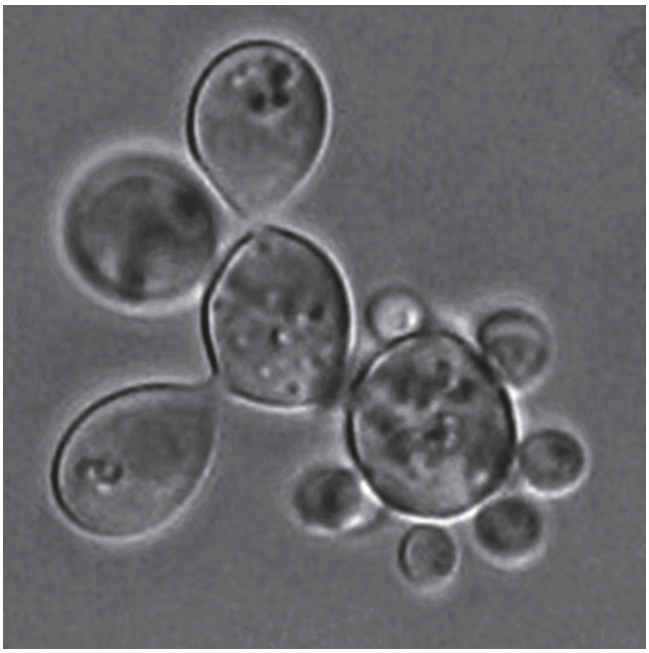

Fig. 19.1 *Paracoccidioides brasiliensis* yeast cell surrounded by multiple budding daughter blastoconidia ("pilot's wheel")

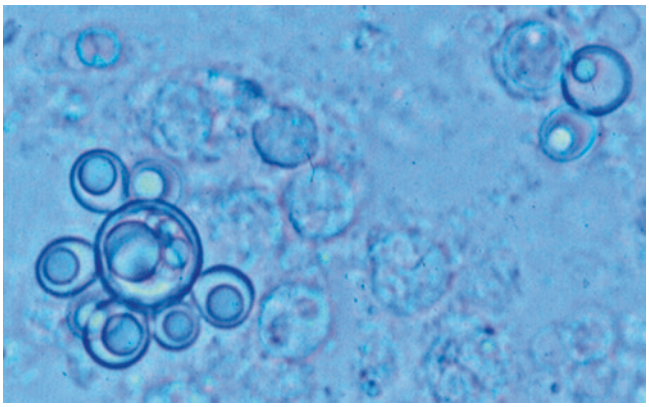

Fig. 19.2 *Paracoccidioides brasiliensis* in tissues. Note abundant round to oval yeast cells enclosed in a granuloma. One cell has multiple buds, while other cells appear empty and degenerated. Gomori Methenamine Silver stain (GMS)

11–13, 16]. As previously indicated, *P. brasiliensis* is only known in its asexual (anamorph) stage, but molecular biology techniques have revealed the presence and expression of the mating type locus, thus allowing its classification in the phylum Ascomycota, order Onygenales, family Onygenacea, close to the phylogenetic tree of *Histoplasma capsulatum, Blastomyces dermatitidis,* and *Emmonsia parva,* all of which have a teleomorphic (sexual stage) in the genus *Ajellomyces* [7–10]. Since 2009, the Broad Institute has completed the database for *P. brasiliensis* genome with the information available at: http://www.broad.mit.edu/annotation/genome/paracoccidioides brasiliensis.

Fungi in the genus *Paracoccidioides* appear to have exogenous habitats in relation to their preferred human hosts but the precise locations of such niches remain unknown [1, 12, 17]. This is why the initial stages of the infectious process can only be surmised on the basis of experimental animal studies which have shown that after inhalation of the infectious particles (conidia, short mycelial fragments) produced by the fungus saprophytic form, they settle into the lungs where they promptly transform into the tissue yeast form [18, 19]. Infection is thus coupled with a thermally regulated transition from a suspected soil-dwelling filamentous form to a yeast-like pathogenic structure [3]. Molecular epidemiology studies have shown that all *P. brasiliensis* species—including cryptic ones—are distributed preferentially in certain regions within the endemic areas. Thus, S1 polymorphism predominates in Brazil, Argentina, Paraguay, Peru, and Venezuela; PS2 prevails in Brazil and Venezuela; while PS3 is exclusive in Colombia [2, 3, 9]. *P. lutzii* occurs preferentially in the central and northern regions of Brazil [4, 9].

Epidemiology

Demographic data have consistently shown that the clinical manifestations of PCM predominate in adults (over 85 % of cases). In healthy Colombian populations, skin testing with paracoccidioidin proved reactive in 10 %, indicating a previous contact (infection) with the fungus [20]. When the same test was applied in Brazil where PCM is of greater importance, the incidence rates were higher especially in the southern, southeastern, and mid-western regions [1, 12]. Additionally, when the gp43 antigen was used for this purpose, higher (43 %) prevalence rates of infection were recorded [21, 22]. Overall, the disease prevalence rates in Latin America are low, 0.33–3 cases per 100,000 inhabitants [23, 24]. In Brazil, mortality rates attributable to the mycosis represent 3 % of all reported cases [12, 25, 26].

As a disease process, PCM is more often diagnosed in males than in females (ratio of 13:1), even though paracoccidioidin skin testing has shown similar infection rates in both sexes [1, 6, 20–22]. This mycosis is relatively uncommon in children, with approximately 5 % of all patients being less than 10 years of age. When children and adolescents (< 14 years old) are set together, approximately 15 % of all cases correspond to this age bracket, with 8 % more being less than 20 years of age [6, 12, 13, 23, 27, 28]. PCM occurs most commonly in middle-aged men (40–60 years), with the exception of immunosuppressed patients who tend to be younger [6, 11–13, 29, 30]. Most patients (73 %) have current or past agriculture-related occupations [1, 6, 11–13, 27, 28].

Risk factors include living and working in these areas, malnutrition, as well as alcoholism and smoking [1, 6, 12, 13]. A direct relation to underlying immunosuppressive conditions, including HIV disease, has not been clearly demonstrated. Sarti et al. found that in a cohort of over 1000 HIV-infected patients, PCM accounted for only 3.3 % of the deaths (in contrast with histoplasmosis which caused 38 % of fatalities) [29]. Morejon et al. estimated that in HIV patients the prevalence of *P. brasiliensis* coinfection was low, 1.4 % [30]. Isolated case reports of the mycosis in immunocompromised individuals are rarely found [31, 32].

One of the most relevant characteristics of PCM is its restricted geographic limitation to Central and South America from Mexico (23° North) to Argentina (34° South), sparing certain countries within these latitudes (Chile, Surinam, the Guyana, Nicaragua, Belize, most of the Caribbean Islands) [6, 11–13, 27, 28]. Also of note is the fact that within endemic countries the mycosis is not diagnosed everywhere, but in areas with relatively well-defined ecologic characteristics. These characteristics include the presence of tropical and subtropical forests, abundant watercourses, mild temperatures (<27ºC), high rainfall (2000–2999 mm), all of which favor crops, such as coffee and tobacco [33].

According to geospatial technologies, association between climatic factors and clinical diagnosis of this mycosis, tend to indicate that *Paracoccidioides* spp. complex would occur preferentially at sites where the environment has a high rainfall index and the soils show optimal permeability, a combination associated with high relative humidity and abundance of vegetation and watercourses [33]. Estimates indicated that during the rainy season the water volume is high, and temperatures oscillate between 18 and 28 °C, conditions that appear favorable for spore formation and aerial dispersion of the fungus [9, 12, 33, 34]. In a southern region of Brazil, the influences of soil water storage, absolute air humidity higher than normal and presence of the climatic anomaly caused by the 1982/1983 El Niño Southern Oscillation, were shown to be associated with a cluster of acute/subacute cases appearing within a lapse of 1–2 years [35].

Approximately 80 PCM cases have been reported outside the endemic areas, namely, in North America, Europe, and Asia. Of note, every patient had a record of previous residence in recognized endemic countries [36, 37]. These cases demonstrate that *P. brasiliensis* primary infections can remain dormant for long periods (mean of 13 years) from the time of infection to the moment of disease manifestations [17]. No data on imported *P. lutzii* cases have been presented. Latency/dormancy may explain why the habitat(s) corresponding to members of the *Paracoccidioides* complex had not been precisely demonstrated, as with the usual delays in diagnosis, and the mycosis slow progression, one tends to forget the site and type of activities that had led to primary infection [14]. No outbreaks have been reported and the isolation of the fungus from nature (e.g., soil) has seldom been successful [38]. By means of molecular studies (nested polymerase chain reaction, PCR), sampling by aerobiological procedures detected ITS sequences in air samples that were highly similar with the homologous *P. lutzii* sequences kept at the GenBank database, even if the fungus could not be isolated from the environment by regular culture isolation strategies [39].

Pathogenesis and Immune Response

Lack of data on two main issues, namely, the habitat of *P. brasiliensis/P. lutzii* and the manifestations of primary infection, have hindered the understanding of the initial steps in the pathogenesis of PCM. Experimental animal models have been produced by initiation of the paracoccidioidal infection via inhalation of conidia (elements small enough (<5 m) to reach the alveoli) which transform into yeast cells [18, 19]. The fungus then multiplies in the lung parenchyma and proceeds to disseminate by the venous/lymphatic routes to extrapulmonary organs (Fig. 19.3). Infection gives rise to an intense host response with alveolitis and presence of abundant neutrophils leukocytes engulfing the fungus, cells that are later on replaced by migrating mononuclears which, in turn, convert into epithelial cells thus initiating the formation of granulomas and attracting various subtypes of T lymphocytes of which the relative proportions depend on the host immune status [1, 40–42]. In mice with pulmonary infection with *P. brasiliensis,* fungal load was controlled by $CD8^+$ T cells, whereas antibody production and delayed type sensitivity reactions were regulated by $CD4^+$ T cells [43]. It has been shown that lymphocytes from PCM patients are poorly activated, express low levels of interleukin 15 receptor alpha (IL-15R alpha) and produce only basal levels of cytotoxic granules [43–46]. These findings may account for the observed defect in vitro cytotoxic activity [40, 41, 44, 46].

The characteristics of the immune responses in patients with overt disease have been the subject of numerous studies. Immune reactivity to *P. brasiliensis* antigens is characterized by depressed Th1 cellular immune responses that revert to normal or almost normal ranges with treatment and patient improvement [46, 47]. Patients with the acute type disease not only have depressed Th1 but have also polarized Th2 responses, characterized by the increased release of the cytokines IL-4, IL-5, and IL-10, high levels of circulating anti-*P. brasiliensis* antibodies of the immunoglobulin G4 (IgG4) and immunoglobulin E (IgE) subclasses, and marked eosinophilia [41, 42]. This pattern is reflected by abundant fungal multiplication and extrapulmonary dissemination with progression of disease [43, 44, 46]. This Th-2 polarization is regularly reverted with treatment. A Th1 cellular immune response to *P. brasiliensis* is detected in those individuals who

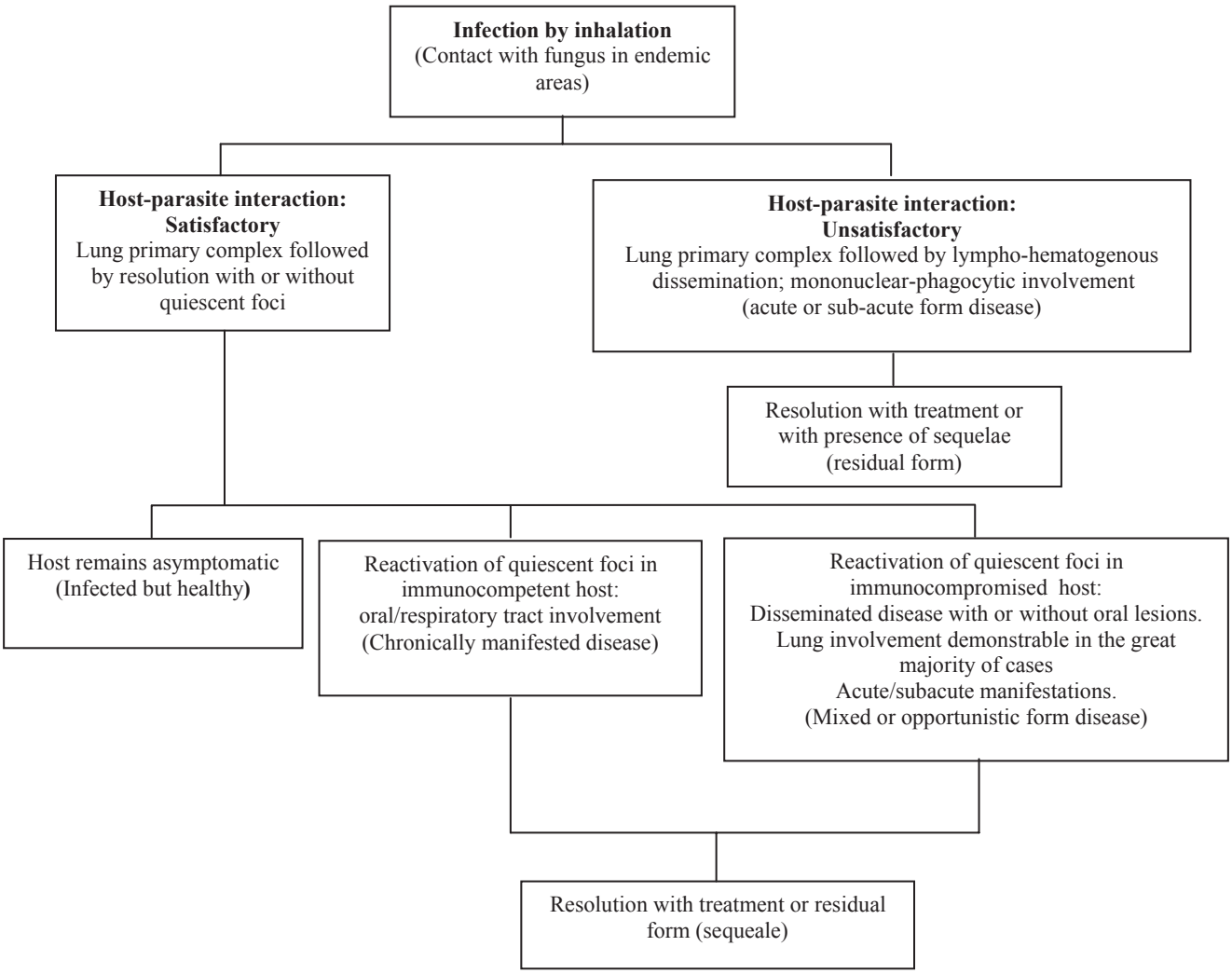

Fig. 19.3 Presumed natural history of paracoccidioidomycosis

were once exposed to the fungus but did not develop the disease [43]. More recently, a role for T-regulatory cells in cell-mediated immune suppression has been found in patients with active disease, which was also reverted with treatment [41]. The participation of the newly described Th17, Th21, and Th9 subsets in the patients' immune response has recently been documented revealing Th17 and Th21 responses in the case of the adult type disease while Th9 response was detected mostly in the juvenile type disease [46].

The factors that determine the different outcomes of the infection are not known. In most instances, the subclinical infection is resolved, possibly leaving viable yeast cells that eventually may enter into a dormant-like state for decades [14]. The chronic adult-type disease would then originate from reactivation of these dormant yeast cells and this would explain why these patients tend to develop a chronic, progressive disease, marked by pulmonary damage, and accompanied, in most cases, by dissemination to the lymphatic system, the mucosa, the adrenals, the skin, and other organs [1, 11–13, 16, 23]. Lung damage can progress to fibrosis leaving behind sequelae with variable impact on respiratory function, reinforcing the importance of early diagnosis and treatment [1, 16, 47, 48]. In the rare instances, when the primary infection is not checked by the host's immune responses, *P. brasiliensis* disseminates through the lympho-hematogenous route resulting in the acute- or juvenile-type disease [1, 42, 49, 50]. Overall, the prevalence of the disease is low even in endemic areas, while that of infected-only individuals is high, reaching up to 46% of the population in certain highly endemic settings according to skin testing studies [1, 20–22, 51].

In PCM, polymorphonuclear neutrophils (PMNs) have been implicated not only in phagocytosis and pathogen destruction but also in granuloma formation. In cases presenting only loose granulomas, a greater quantity of fungi are to be found in comparison to those corresponding to well-organized granulomas. In either case, neutrophils are present in tissues and participate in granuloma formation largely

Table 19.1 Key factors leading to consider the diagnosis of paracoccidioidomycosis

History of residence in an endemic country (even if many years previously to initiation of symptoms/lesions)
History of working in agriculture or related occupations. Mining also to be considered
Being an adult male with a chronic, progressive illness
Complaints related to external manifestations (mucous membrane, skin, and/or lymph node enlargement/draining)
General malaise, weight loss, fever
Signs and symptoms of adrenal gland dysfunction
No major pulmonary signs/symptoms contradicted by X-rays or imaging abnormalities
In children, young adults and immunocompromised individuals (mainly AIDS), hypertrophy of lymph node structures and/or involvement of liver/spleen
Multiple skin lesions or bone abnormalities in the above mentioned groups
Remember: Paracoccidioidomycosis often exhibits protean manifestations thus hindering proper diagnosis

contributing to the inflammatory response [52]. The major biological significance of granuloma is the limitation of the infection to a local area but if such formation is loose, dissemination would ensue [1, 53].

Antibodies are detected in most PCM patients although the isotypes differ according to clinical type. Thus, the reactivity of immunoglobulin G (IgG) and immunoglobulin M (IgM) to certain antigens was greater in patients with the acute form while immunoglobulin A (IgA) was more reactive in those with the chronic form [54–56]. Nonetheless, their role in the pathogenesis of human disease is still unclear.

Clinical Manifestations

PCM is a disorder characterized by protean manifestations that in most patients tend to run a chronic progressive course involving various organs and systems with mortality rates that average 0.9 per 1 million inhabitants in Brazil [46]. Most patients exhibit constitutional symptoms, such as general malaise, asthenia, adynamia, weight loss, and fever, as well as symptoms related to the infected organs. The primary infection occurs in the lungs, but often neither the patient nor the clinical examiner finds abnormalities at this site. On the basis of the clinical presentation and the host immune response to PCM, the disease is categorized as (1) subclinical infection, or (2) symptomatic infection, which is subdivided in two forms, the acute/subacute juvenile and the chronic adult type. A third residual form characterized by fibrotic sequelae is also recognized [1, 6, 11–13]. On the basis of gallium image studies, it is presently accepted that the various manifestations of PCM entail multiple organ involvement, thus negating the former division of the disease into a unifocal or a multifocal process [48]. With the increase in the number of immunocompromised individuals, particularly those with AIDS, the mycosis is being recognized more frequently [25, 26, 29, 30, 57]. In such patients, the corresponding clinical presentation does not allow to categorize the process as chronic or acute but rather as a mixed (opportunistic) form [58–62]. Table 19.1 summarizes the key factors leading to consider the diagnosis of paracoccidioidomycosis.

Subclinical Infection

The subclinical infection has no special characteristics and is detected mostly by a reactive paracoccidioidin skin testing, and sometimes by abnormal chest radiographs [11–13, 20]. Additionally, more sensitive serological or molecular techniques may also disclose healthy infected people [37, 41, 46, 47, 55]. However, *P. brasiliensis* may remain latent in the infected host giving rise to symptomatic PCM years after the initial contact, as demonstrated by the cases diagnosed outside the mycosis endemic areas, all of whom had the opportunity to become exposed to the fungus in their native countries where they had acquired the primary subclinical infection [14, 34, 35].

Symptomatic Infection

The clinically manifested disease varies with patient's age and immune status.

Juvenile-Type Disease

The juvenile-type disease is a serious disorder that afflicts children and immunocompromised individuals of either sex; it represents less than 10% of all cases. Involvement of the reticuloendothelial system organs with lymphadenopathy, hepatomegaly, and/or splenomegaly characterizes this form. Skin lesions, often multiple, are observed regularly along with bone involvement. Constitutional abnormalities such as fever, marked weight loss, and general malaise are hallmarks and become associated with anemia, eosinophilia, and hypergammaglobulinemia [12, 13, 28, 49, 50]. Abdominal and digestive tract manifestations, such as presence of abdominal masses, lymph node enlargement, diarrhea, vomiting, abdominal distention or pain, and ascites, are also more common than in the chronic adult disease. Although

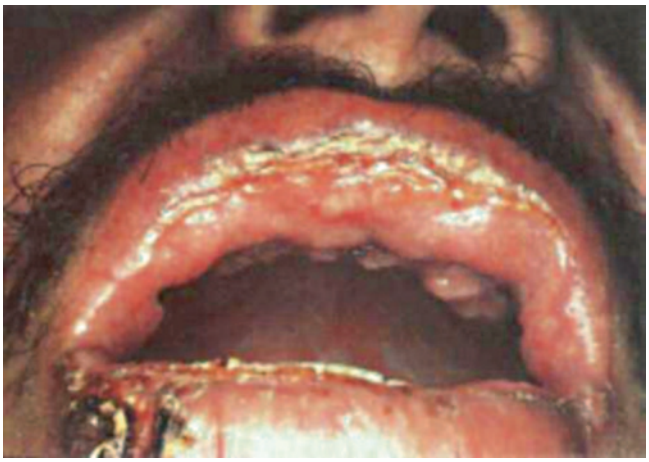

Fig. 19.4 Cutaneous and mucosal lesions in a patient with paracoccidioidomycosis. Note lip's edema, ulceration, and scarring

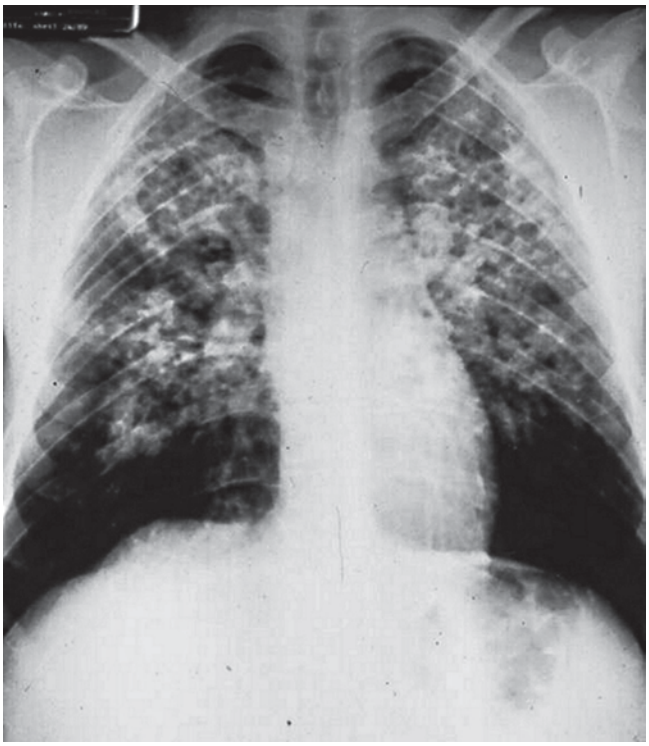

Fig. 19.5 Pulmonary paracoccidioidomycosis. Note abundant fibronodular infiltrates in central fields and basal bullae

respiratory symptoms are infrequent, in the juvenile patients, the fungus can be seen in respiratory secretions, and computed tomography studies not infrequently reveal abnormalities such as enlarged hilar lymph nodes, or, more rarely, miliary infiltrates [11–13, 48]. The juvenile-type disease may evolve rapidly, in weeks, and consequently, prompt diagnosis accompanied by instauration of antifungal therapy and supportive measures are required [11–13, 49–51, 57]. Mortality rates are higher than in the adult disease, reaching 10% in some series [57].

Chronic Adult-Type Disease

The chronic, adult-type disease predominates in all case series (80–90%) [58]. It occurs preferentially in male patients (13 men: 1 female), aged 30 years or more with agriculture-related occupations. The disease course is characterized by protracted pulmonary and extrapulmonary organ damage especially of the mucous membranes and the skin with the lesions tending to be ulcerative, granulomatous, and infiltrated (Fig. 19.4) [58, 59]. Association with AIDS is not particularly notorious [60, 61]. Sialorrhea, dysphagia, and dysphonia are common. Regional lymph nodes are hypertrophied and may spontaneously drain forming fistulae. The adrenal glands may also become involved, with associated symptoms of adrenal deficiency. CNS involvement is more frequent than thought before and its diagnosis is difficult due to the fact that its clinical manifestations, computed tomography (CT) scans, and magnetic resonance imaging (MRI) findings are not specific [62, 63]. At least 80% of these patients also present pulmonary abnormalities [12, 13, 58, 59].

High-resolution CT studies have revealed lung abnormalities in 98% of the patients with architectural distortion, reticulate and septal thickening, and centrilobular and paraseptal emphysema [64–66]. Chest radiographs reveal mixed infiltrates, mostly interstitial but at times also alveolar; these are predominantly located in the central and lower fields respecting the apices (in contrast with tuberculosis) and are bilateral [64–66]. Sequelae, represented by pulmonary fibrosis, were observed in most patients with 30% of them developing significant respiratory limitation [48, 59, 64–66]. Not infrequently, image studies show more important lesions in comparison with the patient's symptoms or even with findings at auscultation [67–69]. Fibrosis may also occur in other organs and systems and is regularly associated with functional impairments (Fig. 19.5) [1, 12, 13]. In absence of treatment, adult mortality may be as high as 30–45% based on multiple series [25–28, 51, 69].

Disease in Immunocompromised Patients

Paraccocidioidomycosis in patients with cancer, especially with hematological malignancies, frequently results in a disease with features of both the acute and chronic forms [12, 68, 70]. In patients having an impaired immune system, the pulmonary findings corresponding to those of the chronic adult-type disease which are frequently associated with signs of lymphohematogenous dissemination, such as lymph node enlargement, hepatomegaly and/or splenomegaly, and skin lesions, features of the acute type disease [49, 50]. In regard to patients with AIDS, the association between both diseases may represent 5% of all PCM cases [29, 30, 60, 61]. Although the mycosis develops mainly in patients with low $CD4^+$ lymphocyte counts (<200 cells/μl), response to

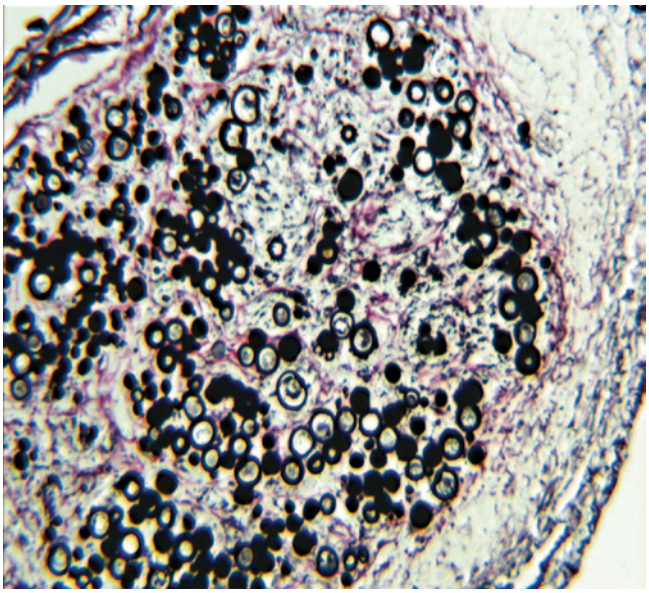

Fig. 19.6 Tissue biopsy with abundant *Paracoccidioides brasiliensis* yeasts enclosed within a granuloma. Gomori Methenamine Silver stain (GMS)

antifungal therapy is, in general, similar to that observed in non-immunocompromised patients with PCM [12, 29, 57]. Additionally, in severe cases, corticosteroids appear beneficial [71]. Despite the immunodeficiency, specific antibodies are detected in over 70 % of the cases [72]. Some clinicians discontinue antifungal therapy in patients who have responded to treatment and appear clinically cured when the CD4 lymphocyte count rises to more than 200 cells/μL.

Diagnosis

Direct Examination and Histopathology

In clinical specimens from oral, pharyngeal, and cutaneous lesions, sputum, bronchoalveolar lavage fluid, lymph nodes, adrenal glands, or biopsy materials from other tissues, *P. brasiliensis* can be identified in up to 85 % of the cases by means of fresh or wet potassium hydroxide (KOH) preparations; as well as on calcofluor and immunofluorescence preparations. *P. brasiliensis* appears as an oval to round translucent-walled yeast cell, often having multiple peripheral buds (typical "pilot wheel" configuration) (Fig. 19.1). Histopathologic preparations stained with hematoxylin and eosin (H&E), Gomori methenamine-silver (GMS), or periodic acid-Schiff are also useful as they reveal the multiple budding yeast elements, especially within granulomatous foci (Fig. 19.6). It is important to differentiate *P. brasiliensis* from *Cryptococcus neoformans, Blastomyces dermatitidis,* and even *Histoplasma capsulatum* [1, 6, 11, 16, 47, 73–75].

Culture

Isolation of *P. brasiliensis* from clinical specimens requires a battery of selective and nonselective culture media, such as Sabouraud dextrose, brain–heart infusion (BHI) plus glucose, or Mycosel^{TM}agar. The addition of antibacterial drugs (chloramphenicol or gentamicin) and mold inhibitors (cycloheximide) to the media has resulted in improved recovery rates of around 80 %, thus providing a useful differential tool in identification of the fungus [1, 6, 11, 16, 73–75]. Modified Sabouraud's (Mycosel) agar and yeast extract agars incubated at room temperature (19–24 °C) are the best media for primary isolation but the growth is slow and may take 20–30 days. Microscopically, the mold shows thin septated hyphae and chlamydospores (15–30 μm). Under conditions of nutritional deprivation, some isolates produce conidia, which vary in structure from arthroconidia to microconidia, and measure less than 5 μm. Conidia respond to temperature changes, germinating into hyphae at 20–24 °C or converting into yeasts at 36 °C on appropriate media. The mycelial form is not distinctive and dimorphism must be demonstrated for identification [1, 6, 11, 16, 73–75]. At 37 °C the *P. brasiliensis* yeast form grows in 8–10 days as a cerebriform, cream-colored colony. Microscopically, oval to spherical yeast cells (2–40 μm) are observed. As mentioned above, the large mother yeast cell bearing multiple buds (pilot's wheel) is characteristic of this fungus [1, 6, 16, 73, 75]. There is no commercial DNA probe test for identification of *P. brasiliensis*.

Immunodiagnostic Tests

Immune-based methods for antibody and antigen detection are useful not only for diagnosis but also for monitoring the patient's treatment course [74–77]. Antibody detection has been based upon antigen preparations using either mycelial or yeast cell lysates. While a series of immunoreactive antigens (27, 43, 70, and 87 kDa) are present in these lysates, the predominant antigen is the 43 kDa glycoprotein, gp43 [76–81].Because of its simplicity, the gel immunodiffusion (ID) test is typically used in endemic countries. This test demonstrates circulating antibodies in 65–95 % of the cases and is highly specific. Commercial mycelial-form culture filtrate antigen can be obtained for in-house use in ID from IMMY (*Paracoccidioides* ID Antigen; IMMY), but its sensitivity has not been widely studied [75]. Complement fixation (CF) test is performed with *P. brasiliensis* yeast-form culture-filtrate antigen but this reagent is not commercially available. CF is less specific than the ID test, and cross-reactions can occur in cases with histoplasmosis. However, CF titers of ≥1:8 are considered presumptive evidence of PCM and falling CF titers are often predictive of successful treatment [75–77].

Table 19.2 Antifungals recommended for the treatment of paracoccidioidomycosis

Antifungals	Dose (daily)	Route	Duration
Itraconazole	200 mg	PO	≥6 months
Sulfamethoxazole 400 mg-trimethoprim 80 mg	2–3 tablets bid	PO	≥1 year
Amphotericin B	1 g (cumulative dose)[a]	IV	Based on clinical response

PO oral, *IV* intravenous
[a]To be followed by an oral medication

Several laboratories have developed their own "in-house"immunodiagnostic tests based on methods such as enzyme-linked immunosorbent assay (ELISA), inhibition ELISA, competition ELISA, Western blot, or dot blot [11, 75–77]. Improvements include the detection of antibodies against chemically characterized and/or recombinant *P. brasiliensis* antigens, notably gp43, pb27, and the 87-kDa heat-shock protein [79–82]. A combination of two recombinant products has resulted in increased sensitivity (92%) and specificity (88%) [83].

Antigen detection tests have some important advantages over antibody detection in the diagnosis of PCM, particularly in immunocompromised patients and in those previously exposed to *P. brasiliensis* who may have preexisting antibody titers [79–83]. The gp43 glycoprotein and the 87-kDa heat-shock protein have been described as useful targets for serum antigen detection [83]; the 43-kDa glycoprotein has also been detected in urine, cerebrospinal fluid, and BAL samples [80]. However, these methodologies are not yet available as routine diagnostic tests. Authors have described that serum antigen levels diminished or even disappeared during successful treatment [82–85].

Skin tests play no role in diagnosis [6, 72].

Nucleic Acid Detection

No commercially available system exists for detection of *P. brasiliensis* DNA in human clinical samples. The number of clinical tests available for the molecular detection of *P. brasiliensis* in clinical samples is very limited [85–87]. The gp43 is considered the immunodominant antigen for diagnosis of PCM, and the gene coding for this glycoprotein has been used as the molecular target to detect *P. brasiliensis* [75]. A nested PCR assay to amplify the *gp43* gene was evaluated using an experimental mouse model of PCM, and the test was positive in 91% of lung homogenates and did not cross-react with other pathogens [86]. A real-time PCR that used as a target the ITS1 region of ribosomal DNA (rDNA) was developed to detect *P. brasiliensis* DNA in both cultures and clinical specimens and although the evaluation was carried out with a low number of patients ($n=10$), the authors reported 100% sensitivity and specificity [87]. A test for the gp43 antigen has also been developed with reported sensitivity and specificity of 100% [86]. An assay based on the 5′ nuclease assay gave positive results in 91% lung homogenates and did not cross react with other pathogens [87]. A loop-mediated isothermal amplification (LAMP) assay has been tested for its ability to detect the *gp43* gene of *P. brasiliensis* [88], and positive results were reported for DNA extracted from formalin-fixed paraffin-embedded tissues from PCM patients [88]. Conserved regions of the ribosomal RNA (rRNA) genes have also been used as targets in PCR-based detection assays, but the identity of the amplicons detected using these conserved genes should be verified by direct sequencing [74–77].

Treatment

Antimicrobial agents from three different classes are currently used to treat PCM. They include the sulfonamides, the polyene amphotericin B (AmB) and its lipid formulations, and certain azoles (Table 19.2) [51, 89]. Normally, the treatment of PCM is quite prolonged because of the patients' low immunity profile plus *P. brasiliensis* ability to survive in the tissues even after long-term treatment. The treatment option must consider the severity of disease; establishment of standard treatment protocols within the context of the patient clinical profile is a requisite [1, 6, 51, 89–91]. First- and second-generation azoles, such as ketoconazole, itraconazole and voriconazole, have emerged as therapeutic options for the control of the disease's moderate forms in shorter periods of time [91, 92]. To date, however, experience with voriconazole is still insufficient to permit definitive recommendations..."

Additional measures such as adequate nutrition and suspension of both alcohol intake and smoking are a must in order to accelerate recovery and diminish fibrous sequelae [1, 6, 12, 13, 26, 27, 48, 59]. In severe cases, particularly in those with the acute type disease, the antifungal agents may not have sufficient time to act and thus fatalities can occur [1, 26, 57, 59]. Use of corticosteroids has been advocated in the presence of important tissue inflammation [71].

Sulfonamides are rather effective and have low cost. In Brazil, the combination sulfamethoxazole–trimethoprim has been preferentially used for treating PCM patients with ensuing healing of most lesions (Table 19.2). In less severe forms of the disease, the latter should be administered for a period of 2 years, resulting in high-dropout rates during treatment (Table 19.2) [51, 89, 90]. Tolerability is good, with myelotoxicity (leukopenia) being the main side effect, one that can be monitored and controlled by folinic

acid administration without modification of the therapeutic regimen [1, 89]. Other side effects seen with this association include rash, gastritis, and interstitial nephritis; all reversible with interruption of treatment. This combination has the advantage of permitting parenteral administration when necessary. Duration of the acute treatment with this drug combination varies in each case, but it usually lasts for 6 months or even more in certain patients. Development of resistance to trimethoprim–sulfamethoxazole is clinically suspected occasionally, but has rarely been documented in vitro. [1,90]. Brazilian physicians most often employ trimethoprim–sulfamethoxazole (80 mg of trimethoprim and 400 mg of sulfamethoxazole per tablet) given at a dose of two tablets and administered orally at 12-h intervals for adults or 8–10 mg/kg/day (based on the trimethoprim) component in pediatric cases. This treatment can also be used in association with AmB. In severe forms of the disease, the treatment regimen involves intravenous (IV) administration of sulfamethoxazole 800 mg/trimethoprim 160 mg, every 8 h until clinical improvement of the patient, followed by administration of oral antifungal drugs [90–92].

Another treatment option for severe PCM forms is the administration of AmB at a dose of 1 mg/kg/day (maximum 50 mg/day), dissolved in dextrose 5 %, administered daily (Table 19.2). Patients may need to be hospitalized for side effects caused by the drugs [1, 89, 90]. The patient should remain in treatment and under follow-up management until achieving apparent resolution, with assessment based on clinical, radiological, and serological parameters. To avoid AmB nephrotoxicity, a liposomal AmB formulation (AmBisome) may be used in some cases [89, 90]. Three AmB lipid formulations have been developed and are now available in most countries: AmB colloidal dispersion (ABCD), AmB lipid complex (ABLC), and liposomal AmB (Ambisome). These are highly effective, but their use is now restricted to severely ill patients, to those with CNS involvement, and patients with restrictions in oral administration or gastrointestinal absorption, as they are quite costly. Based on clinical response, a typical total cumulative dose of 1–2 g should be given, followed by maintenance therapy with oral medications. Infusion-related toxicity, electrolyte abnormalities, and renal dysfunction are common adverse effects [89, 90].

With the exception of fluconazole, most of the systemically absorbed azole antifungals appear promising for PCM therapy. Currently, ketoconazole has been replaced by itraconazole, considered the best option for the treatment of PCM [89]. A comparative study showed that although both itraconazole and trimethoprim–sulfamethoxazole resulted in restoration of patients' health, itraconazole promoted a more rapid clinical and serological cure [90]. Itraconazole is typically administered at a dose of 200 mg/day for at least 6 months of therapy: Duration is based on clinical response and mycology and serology laboratory data. In severe adult cases, higher doses (400 mg/day) can be used. Itraconazole has been shown to be effective in 95 % of the patients with minimal adverse effects; relapses occur in 5 % of the cases. Despite this high response rate, itraconazole has not reduced the fibrous pulmonary sequelae [59]. Care should be taken with the administration of antacids and H2 blockers as they hinder proper absorption. An IV formulation (not available in the USA) has made it possible to treat severely ill patients at 200 mg twice daily for 4 doses, followed by 200 mg daily until favorable changes occur in the patient's clinical aspects [89, 90, 93].

Prevention

Preventive measures are difficult to place into effect as the source of infection is unknown. PCM is not transmissible from person to person. Nonetheless, precaution against aerosols is recommended when felling trees in known endemic areas or hunting armadillos in the forest [1, 33].

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Sporotrichosis

20

Carol A. Kauffman

Introduction

Sporotrichosis is a subacute to chronic mycotic infection of skin and subcutaneous tissues. The etiologic agent, *Sporothrix schenckii,* is named after Dr. Schenck who described the first case in Baltimore in 1898. Most cases of sporotrichosis arise from direct inoculation of the organism from soil, vegetation, or wood into the subcutaneous tissues. Subsequent spread along the lymphatics draining the primary lesion is common, but hematogenous spread is rare. The organism is occasionally inhaled from the environment and causes pneumonia. Zoonotic transmission has been highlighted in the last decade by a large outbreak of cat-associated sporotrichosis in Rio de Janeiro, Brazil.

Etiologic Agent

S. schenckii is a dimorphic fungus that exists as a mold in the environment and as a yeast in tissues. The dimorphism is temperature dependent. In the environment and in the laboratory, at 25–27 °C, *S. schenckii* is a mold with thin, septate, branching hyphae that have conidia that can be either dark or hyaline and that tend to arrange themselves along the hyphae in "bouquet-like" arrangements (Fig. 20.1). In the laboratory, on Sabouraud's dextrose agar, growth of a white to cream-colored mold occurs within 1–2 weeks. The colony becomes brown or black and assumes a wrinkled appearance over the ensuing weeks (Fig. 20.2).

In tissues and in vitro at 37 °C, *S. schenckii* assumes a yeast-like form. The yeasts are 4–6 μm in diameter and show budding that can be single or multiple; they are classically described as being cigar-shaped although round and oval forms are also seen (Fig. 20.3). In the laboratory, growth of the yeast phase is accomplished by incubation at 35–37 ºC using enriched media, such as brain heart infusion (BHI) agar. The colony morphology of *S. schenckii* in the yeast phase is usually off-white and wrinkled. Some strains of *S. schenckii* do not grow well at 37 ºC but do grow at 35 ºC. These strains are generally found in fixed cutaneous lesions that do not manifest lymphangitic spread [1].

In the last decade, molecular studies have made clear the fact that *S. schenckii* is not a single species but rather a complex of several phylogenetically different organisms [2–5]. Various techniques have been used to differentiate the organisms within the *S. schenckii* complex [5], creating some confusion for the nonexpert in this area. Currently, it appears that there are at least six species that previously were designated as *S. schenckii*; these six species demonstrate differences in regard to geography and virulence. *S. brasiliensis* occurs in Brazil and commonly causes disease in humans and animals, including the large ongoing zoonotic outbreak in Rio de Janeiro [5]. *S. schenckii sensu stricto* remains an important worldwide human pathogen and is the main species present in North America. *S. globosa* also is found worldwide, but is reported less frequently as a human pathogen than the above mentioned species [4]. *S. mexicana,* present in Mexico and other Latin American countries, as well as *S. luriei,* and *S. albicans* are uncommon causes of infection in humans.

Note to the reader: Throughout this chapter, the term *S. schenckii* will be used to refer to the complex of *Sporothrix* species.

Epidemiology

S. schenckii is found throughout the world. In the environment, *S. schenckii* is found in sphagnum moss, decaying wood, vegetation, hay, and soil [3]. For infection to occur, one must be exposed to an environmental source, and the organism must be inoculated through the skin. This can occur with motor vehicle accidents, hay baling, landscaping, tree

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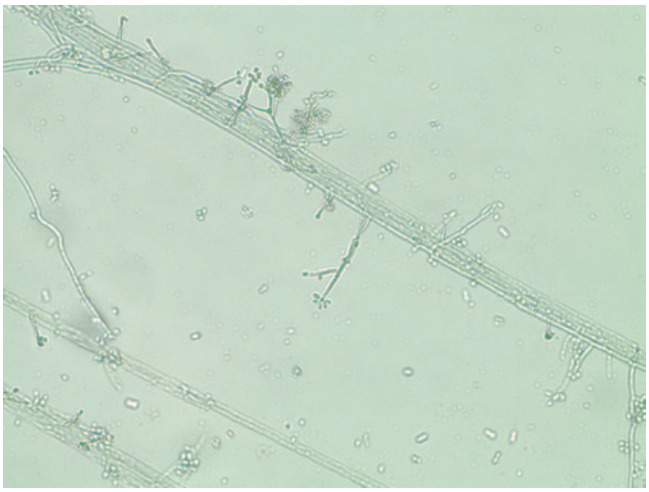

Fig. 20.1 Microscopic view of the mold form of *Sporothrix schenckii* grown at 25 °C on Sabouraud dextrose agar. Note the thin septate hyphae with conidiophores that bear oval conidia that appear "bouquet-like." (Courtesy of Dr. D. R. Hospenthal)

Fig. 20.2 Colony of *Sporothrix schenckii* grown at 25 °C on malt extract agar. Initially cream-colored, the colony darkens over time

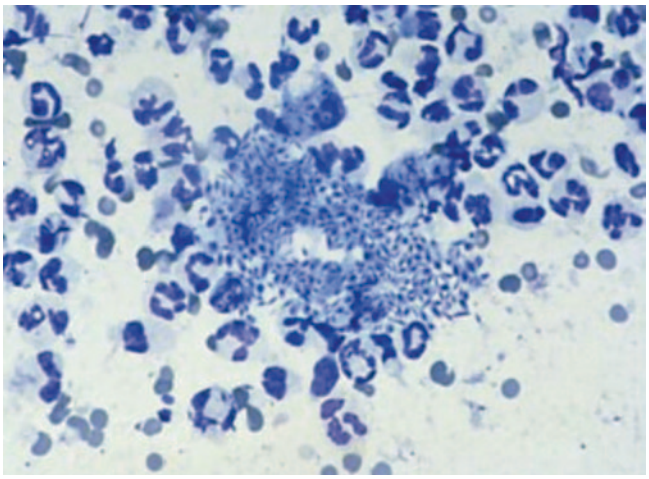

Fig. 20.3 Smear of an ulcerated lesion caused by *Sporothrix schenckii* showing a large number of oval and cigar-shaped yeasts, 4–6 μm in diameter. (Courtesy of Dr. K. Reed)

farming, and in developing countries, just the activities of daily living [6–9].

The typical person who develops sporotrichosis is a healthy person whose occupation or hobby takes him or her into the out-of-doors. Classically, landscapers and gardeners develop sporotrichosis because they are exposed to contaminated materials and their activities frequently lead to nicks and cuts on their extremities, allowing easy access for the organism.

Zoonotic transmission can occur from infected animals or from soil transferred from the nails of burrowing animals, such as armadillos [10]. Cats develop ulcerated skin lesions, often on the face, due to sporotrichosis, and many die of the infection. These ulcers are teeming with organisms and are highly infectious [11]. Sporotrichosis also has occurred in laboratory workers who, in the course of handling infected animals or culture material, have inoculated themselves or splashed material into their eye [12].

Outbreaks of sporotrichosis are not uncommon and have been traced back to a variety of point sources: contaminated timbers in a mine, sphagnum moss packed around Christmas trees, bushes, or seedlings, and hay used for Halloween parties [6, 13–15]. The outbreak in Rio de Janeiro associated with transmission from infected cats and affecting mainly housewives and children living in the poorer areas of the city has been ongoing since 1998 and has infected over 2000 persons and many thousands of cats [16–19]. Recent evidence suggests that the strains in this outbreak, which have been identified as *S. brasiliensis,* are related and likely originated from a common source [17].

Pathogenesis and Immunology

Infection with *S. schenckii* is almost always initiated when the mold that is present in the environment is inoculated into the skin, usually through minor trauma. Inhalation of the conidia of *S. schenckii* is the presumed method of transmission in the uncommon syndrome of pulmonary sporotrichosis. Defining virulence factors other than the ability to grow at 37 °C is an active area of investigation. Components of the cell wall of *S. schenckii,* especially a 70-kDa glycoprotein (GP70), mediate adhesion to extracellular matrix and endothelial cell surface proteins, initiating invasion [5]. Extracellular proteinases and melanin are the likely virulence factors [20]. The host response is comprised primarily of neutrophils, monocytes, and macrophages, cells able to ingest and kill the yeast phase of *S. schenckii* [21]. Antibody appears unimportant in immunity, but cell-mediated immunity

is crucial in containing infection with *S. schenckii* [22, 23]. The importance of cell-mediated immunity is supported by the clinical reports of disseminated sporotrichosis occurring in AIDS patients [24, 25], in patients who have hairy cell leukemia [26], in those receiving tumor necrosis factor antagonists [27], and in one patient who had a prior history of lepromatous leprosy [28].

Clinical Manifestations

The usual manifestation of sporotrichosis is localized lymphocutaneous infection. Most patients who present with typical lymphocutaneous sporotrichosis are healthy hosts. Extensive disseminated cutaneous lesions and spread to other structures, including joints, meninges, lungs, and other organs, almost always occur in those who have certain underlying illnesses. Alcoholism and diabetes mellitus are two risk factors for more severe sporotrichosis [29]. Chronic obstructive pulmonary disease is almost always present in patients who have pulmonary sporotrichosis, and disseminated sporotrichosis is rare unless cell-mediated immunity is suppressed (Table 20.1).

Lymphocutaneous Sporotrichosis

The first manifestation of infection generally occurs several days to weeks after cutaneous inoculation of the fungus when a papule appears at the site of inoculation. This primary lesion becomes nodular, and usually will eventually ulcerate. Drainage from the lesion is minimal, is not grossly purulent, and has no odor. Pain is generally mild, and most patients have no systemic symptoms. Over the next few weeks, new nodules that often ulcerate appear proximal to the initial lesion along the lymphatic distribution (Fig. 20.4).

The differential diagnosis for this form of sporotrichosis includes infection with *M. marinum* or another atypical mycobacterium, *Leishmania* species, and *Nocardia brasiliensis*

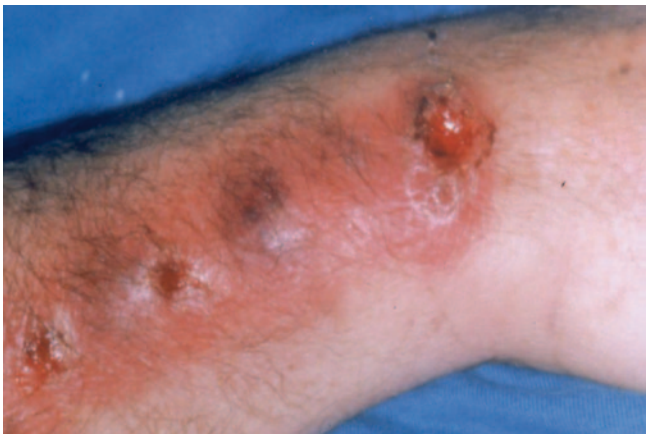

Fig. 20.4 Typical skin lesions in lymphatic distribution seen in a patient who was a horticulturist and had inoculation of *Sporothrix schenckii* in the subcutaneous tissue of the wrist. From Watanakunakorn (1996). (Reprinted with permission from Oxford University Press)

[30]. Rarely, other bacterial, fungal, and even viral infections cause a similar lymphocutaneous syndrome.

Fixed cutaneous sporotrichosis is uncommon in North America, but common in South America (Fig. 20.5). Patients with this form of sporotrichosis manifest only a single lesion, often on the face, that can be verrucous or ulcerative [8]. The lesion may regress and flare periodically, and can be present for years until it is treated. Pain and drainage are not prominent symptoms.

Pulmonary Sporotrichosis

Pulmonary sporotrichosis is a subacute to chronic illness that usually occurs in patients who have chronic obstructive pulmonary disease [31–33]. The symptoms mimic those of reactivation tuberculosis. Patients have fever, night sweats, weight loss, and fatigue; dyspnea, cough, purulent sputum, and hemoptysis also occur frequently. Chest radiography shows unilateral or bilateral fibronodular or cavitary disease;

Table 20.1 Clinical manifestations of sporotrichosis

Clinical syndrome	Known risk factors	Initiation of infection
Lymphocutaneous	None	Local inoculation
Fixed cutaneous	None	Local inoculation
Osteoarticular	Alcoholism, diabetes	Local inoculation or hematogenous spread
Pulmonary	COPD, alcoholism	Inhalation
Meningitis	AIDS	Hematogenous spread
Other focal disease (eye, breast, larynx, pericardium, epididymis, rectum, spleen, liver)	None known	Hematogenous spread or local inoculation
Disseminated	AIDS, TNF antagonist therapy, hairy cell leukemia	Hematogenous spread

COPD chronic obstructive pulmonary disease, *TNF* tumor necrosis factor

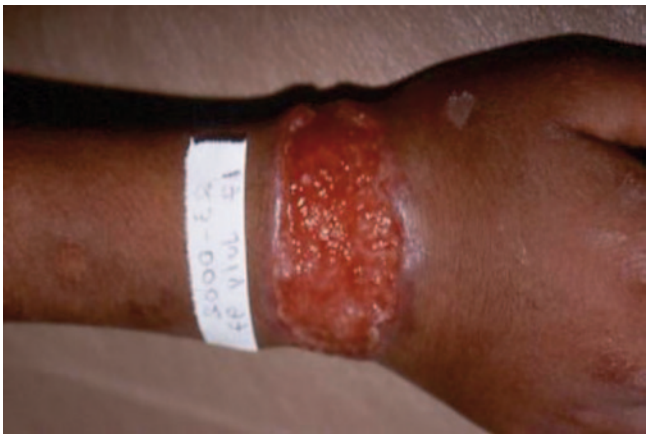

Fig. 20.5 Fixed cutaneous skin lesion of sporotrichosis. In this form of the disease, lymphatic spread does not occur, and the lesion may remain for months to years until treated. Courtesy of Dr. P. Pappas. From Kauffman (1999). (Reprinted with permission from Oxford University Press)

the upper lobes are preferentially involved (Fig. 20.6). Sporotrichosis must be differentiated from tuberculosis, chronic cavitary histoplasmosis or blastomycosis, and sarcoidosis.

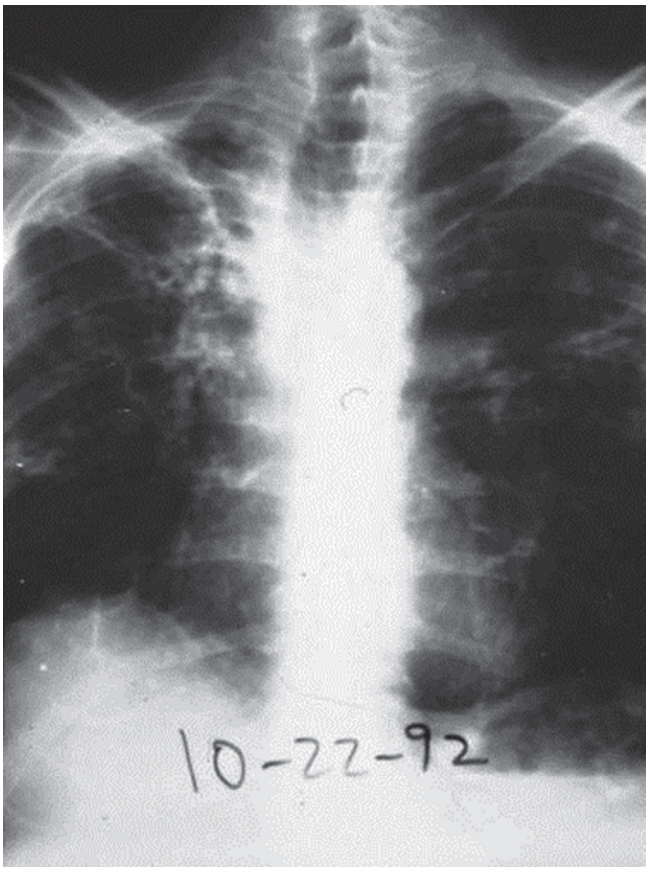

Fig. 20.6 Chest radiograph of a patient with pulmonary sporotrichosis. The patient was an alcoholic who also had diabetes mellitus and chronic obstructive pulmonary disease

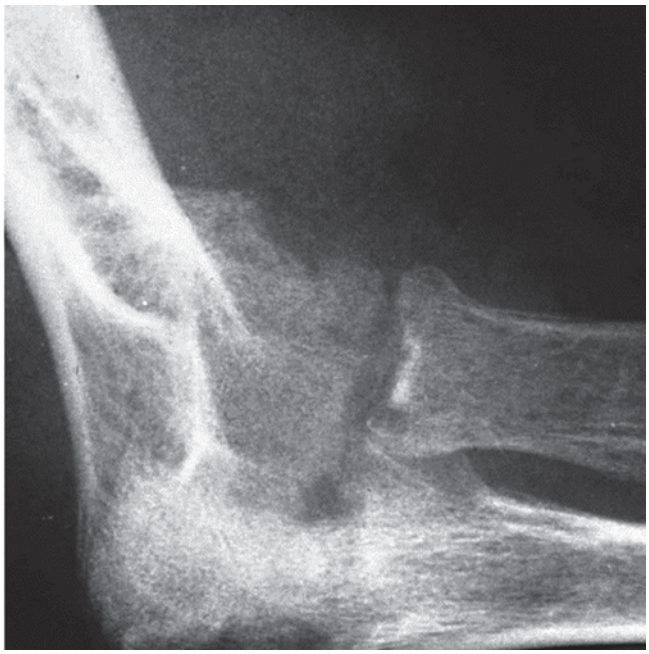

Fig. 20.7 Elbow X-ray of a patient who had osteoarticular sporotrichosis manifested by infection of both elbows and one knee. There is destruction of the joint and adjacent osteomyelitis of the radius, ulna, and humerus

Some, but not all, patients with pulmonary sporotrichosis have disease elsewhere, especially skin and osteoarticular structures.

Osteoarticular Sporotrichosis

Osteoarticular sporotrichosis is an uncommon manifestation of infection with *S. schenckii* that can occur after local inoculation, but can also arise from hematogenous spread. It is found most often in middle-aged men and appears to occur more frequently in alcoholics. Overlying cutaneous lesions may or may not be present, and one or more joints may be involved. Most commonly, the knees, elbows, wrists, and ankles are infected [34]. Bone involvement usually occurs contiguous to an infected joint (Fig. 20.7). Bursitis and tenosynovitis, the latter presenting as nerve entrapment, also have been described [35].

Meningitis and Disseminated Infection

Meningitis, a rare manifestation of sporotrichosis, occurs almost always in those with cellular immune defects and must be differentiated from tuberculosis or cryptococcosis [24]. Fever and headache are prominent symptoms, and the cerebrospinal fluid findings are those of lymphocytic meningitis with mild hypoglycorrhachia. Meningitis can be an isolated

finding, but is usually a manifestation of widespread disseminated infection.

Disseminated sporotrichosis is very uncommon, and may present with fungemia [36]. Almost all patients have had cellular immune deficiencies, and most cases have occurred in persons with AIDS [24–28, 36]. *S. schenckii* has been reported very rarely to cause infection of eye, larynx, breast, pericardium, spleen, liver, bone marrow, lymph nodes, rectum, and epididymis.

Diagnosis

Culture yielding a *Sporothrix* species is the gold standard for establishing the diagnosis. Biopsy or material aspirated from a cutaneous lesion should be sent to the laboratory. Sputum, synovial fluid, blood, and cerebrospinal fluid can also yield the organism in patients who have visceral involvement. Material obtained for culture should be inoculated onto Sabouraud's agar and incubated at room temperature to allow growth of the mold phase of *S. schenckii*. Growth usually occurs within 1–3 weeks. The characteristic arrangement of conidia on the hyphae makes the diagnosis likely, but conversion to the yeast phase at 35–37 °C, which may take several additional weeks, allows definitive identification of the organism as belonging to the *S. schenckii* complex.

The histopathology of sporotrichosis reveals a mixed granulomatous and pyogenic inflammatory process [37]. Caseous necrosis and liquefaction are frequently noted. The organism is an oval to cigar-shaped yeast, 3–5 μm in diameter, and can exhibit multiple buds. However, it is difficult to visualize the organisms within tissues, even with the use of methenamine silver or periodic acid–Schiff stains (see Fig. 4.12, Chap. 4). In some cases, an asteroid body, in which the basophilic yeast is surrounded by eosinophilic material radiating outward like spokes on a wheel, can be seen [38]. This is also known as the Splendore–Hoeppli phenomenon and is not specific for sporotrichosis, but can be seen in various parasitic, fungal, and bacterial infections. This reaction is thought to be due to deposition of antigen–antibody complexes around the organism in tissues.

Studies have been ongoing for many years to develop an enzyme immunoassay, but currently there are no commercially available serological assays to aid in the diagnosis of sporotrichosis. A nested polymerase chain reaction (PCR) assay using primers binding to specific sequences of the 18s rRNA gene of *S. schenckii* has been used to identify *Sporothrix* species in tissue samples [39], and others have used PCR to detect DNA from *Sporothrix* species in tissue samples [40]. However, PCR assays are available only through reference laboratories at the present time.

Treatment

In general, most patients who have sporotrichosis are treated with oral azole antifungal agents. Those patients who have disseminated infection, meningitis, or severe pulmonary involvement are treated initially with intravenous amphotericin B. Guidelines for the management of the various forms of sporotrichosis were published by the Infectious Diseases Society of America [41], and the suggestions that follow are modified from these guidelines (Table 20.2).

As molecular methods have defined new species in the *S. schenckii* complex, in vitro studies have shown different patterns of susceptibility for these different species [17, 42, 43]. *S. brasiliensis* appears to be more susceptible and *S. mexicana, S. albicans, and S. luriei* less susceptible to azoles and terbinafine [17, 42, 43]. However, in vitro susceptibility studies do not necessarily translate into efficacy in vivo, and very few strains of the uncommon species were available for testing.

Table 20.2 Treatment of sporotrichosis

Clinical syndrome	Primary therapy	Alternate therapy	Duration
Lymphocutaneous and cutaneous	Itraconazole 100–200 mg/d	SSKI, titrated dose Fluconazole 400–800 mg/d Terbinafine 500 mg bid Hyperthermia	Until lesions resolve; usually 3–6 months
Pulmonary[a]	Itraconazole 200 mg bid	Lipid AmB, 3–5 mg/kg/d	AmB: several weeks Itraconazole: at least 1 year
Osteoarticular	Itraconazole 200 mg bid	Lipid AmB, 3–5 mg/kg/d	AmB: several weeks Itraconazole: at least 1 year
Meningitis	Lipid AmB, 3–5 mg/kg/d, then transition to oral itraconazole, 200 mg bid	AmB deoxycholate, 0.7–1 mg/kg/d, then transition to itraconazole, 200 mg bid	AmB: 4–6 weeks Itraconazole: at least 1 year or lifelong suppression
Disseminated	Lipid AmB, 3–5 mg/kg/d, then transition to oral itraconazole, 200 mg bid	Itraconazole 200 mg bid	AmB: several weeks Itraconazole: at least 1 year or lifelong suppression

AmB amphotericin B, *bid* twice daily, *SSKI* potassium iodide

[a] For severe disease, begin with lipid amphotericin B, 3–5 mg/kg/day, treat until the patient has begun to improve (usually several weeks), and then transition to oral itraconazole for a total of at least 1 year of therapy

Lymphocutaneous Sporotrichosis

Itraconazole is the drug of choice for the treatment of this form of sporotrichosis [41, 44–46]. The dosage recommended is 200 mg daily and response rates as high as 90 % are commonly noted [41]. However, Barros et al. reported a 90 % response rate among 610 patients treated with a dosage of only 100 mg daily [44]. The oral suspension achieves higher serum levels, but is less well tolerated and is more expensive than the capsule formulation. If itraconazole capsules are used the patient cannot take acid-inhibiting drugs, such as antacids, proton pump inhibitors, or H2 blockers, and should take the capsules with food once daily to ensure adequate absorption. Treatment should continue until the lesions have resolved; this usually takes 3–6 months.

Saturated solution of potassium iodide (SSKI) has been used successfully to treat lymphocutaneous sporotrichosis for decades. It still is not clear how SSKI inhibits *S. schenckii* [47]. The initial dose is 5–10 drops three times daily in juice or milk, increasing weekly to a maximum of 40–50 drops three times daily, as tolerated. Side effects are very common and include nausea, rash, metallic taste, fever, and salivary gland swelling [48].

Several other less effective options exist if the patient is unable to tolerate itraconazole or SSKI. Terbinafine appears active in vitro [17, 42, 43], and high doses of terbinafine (500 mg twice daily) have been shown to be effective for lymphocutaneous sporotrichosis [49]. However, experience is limited, and this should be tried only in those who fail standard therapy. Fluconazole and voriconazole have little activity in vitro against *Sporothrix* species [17, 42, 43]. In spite of this, higher dosages of fluconazole (400–800 mg daily) have been used with modest success in a few patients [50]. Posaconazole has in vitro activity similar to that of itraconazole [17, 43], appeared to be efficacious in a murine model of systemic sporotrichosis [51], and has been used in a few patients [26].

Local hyperthermia can be used to treat cutaneous sporotrichosis; it is less effective for lymphocutaneous sporotrichosis [52, 53]. A variety of different warming devices are available, but each must be used faithfully for months to effect improvement in cutaneous lesions.

Pulmonary Sporotrichosis

Pulmonary sporotrichosis can be quite recalcitrant to therapy. If the patient is seriously ill, amphotericin B, preferably as a lipid formulation, 3–5 mg/kg daily, should be used initially [32, 41]. When the patient is stable, therapy can be changed to oral itraconazole, 200 mg twice daily. The duration of therapy should be at least 1 year and perhaps longer for some patients. Surgical resection should be considered, especially for those patients who have a single cavitary lesion [32]. Outcomes have improved in the last decade, but the response rates are still only about 50 %.

Osteoarticular Sporotrichosis

This form of sporotrichosis, which is almost always chronic and not life-threatening, can be treated with an oral antifungal agent [34, 41]. Itraconazole is the agent of choice, and the dosage is 200 mg twice daily [41]. Therapy should continue for at least 1 year and perhaps longer in some patients. Amphotericin B, preferably a lipid formulation at 3–5 mg/kg/day, is the only remaining option. Intra-articular, amphotericin B is not recommended [54]. Even if cure occurs, joint function rarely is recovered.

Meningitis and Disseminated Infection

A lipid formulation of amphotericin B for 4– 6 weeks is the preferred treatment for patients with life-threatening forms of sporotrichosis [41]. Itraconazole is used as step-down therapy after the patient has responded to amphotericin B. The total length of therapy is at least 1 year, and long-term suppressive therapy may be needed in markedly immunosuppressed patients. For those rare individuals who appear to have disseminated disease confined to the skin, oral itraconazole has been used as sole therapy. Outcomes in AIDS patients were dismal in cases reported prior to effective antiretroviral therapy. However, current response rates are reported to be as high as 81 % [24].

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Dermatophytosis (Tinea) and Other Superficial Fungal Infections

21

Oliverio Welsh and Gloria M. Gonzalez

Introduction

Superficial dermatophyte infection has been identified with a variety of terms through the ages although the term "tinea" has persisted as the most common. Dermatophytes are taxonomically classified into three genera: *Trichophyton, Microsporum,* and *Epidermophyton* [1]. Topical preparations have been the historic method of treatment, with the first effective oral medication being griseofulvin, developed in 1958 [1]. Current medical treatment for dermatophyte infection is based on griseofulvin, allylamines, azoles, and triazoles with other drugs becoming available as research in therapeutic efficacy of new antifungals develops.

Dermatophytes require keratin for growth. Due to this they usually affect hair, nails, and superficial skin. The clinical manifestations are named according to the affected region: tinea capitis (scalp), tinea corporis (body), tinea cruris (groin), tinea pedis (feet), tinea manuum (hands), tinea barbae (affecting the beard in men), tinea faciei (face), and tinea unguium (nails) [2]. "Ringworm" is the popular term used to identify tinea infections because lesions develop as a dermatosis characterized by a circular or oval clear area surrounded by a red, scaly, elevated border ("ring"). Tinea unguium is the term used for onychomycosis caused by dermatophytes, in contrast to nail infections caused by *Candida* or non-dermatophyte molds.

Superficial skin infections are also caused by other genera of fungi, including *Malassezia*. *Malassezia* feed on lipids in areas where sebaceous gland activity is greatest. This genus was first recognized as a pathogen in 1846. Culture was not successful until 1927 when it was found that these organisms require lipids for growth [3]. Initially, two species were described under the genus *Pityrosporum,* and by 1970 three species were recognized: *Pityrosporum ovale, Pityrosporum orbiculare,* and *Pityrosporum pachydermatis* [3]. The existence of yeast and mycelial forms made the identification difficult. This was solved by achieving conversion between forms in the laboratory in 1977. Genetic research in the 1990s confirmed at least seven species of *Malassezia*. Since then more have been identified. *Malassezia* is known to cause pityriasis (tinea) versicolor (PV) and is associated with seborrheic dermatitis (SD). These superficial conditions are included in this chapter together with dermatophyte infections.

Other rare superficial fungal infections, including candidiasis (Chap. 8), white piedra (Chap. 9), black piedra, and tinea nigra (Chap. 12), are discussed elsewhere in this text.

Etiologic Agents

Three fungal genera cause tinea: *Microsporum, Trichophyton,* and *Epidermophyton* [2]. Human infection can spread from one person to another (anthropophilic), from an animal (zoophilic), or less commonly, from soil (geophilic). The major causative species differ geographically and may change in prevalence over time due to population movements. Anthropophilic dermatophytes are the most frequent causes of onychomycosis and other superficial dermatophytoses with the most common agents being *Trichophyton rubrum* and *Trichophyton mentagrophytes*. *Trichophyton tonsurans* is currently the most frequent cause of tinea capitis in North America (northern Mexico, the USA, and Canada). *Microsporum canis* is a zoophilic organism frequently picked up by humans from contact with animals such as dogs and cats [4].

Malassezia yeast species cause the superficial skin fungal infections PV and SD. Among the most frequent species are *Malassezia furfur, Malassezia pachydermatis, Malassezia sympodialis, Malassezia globosa, Malassezia slooffiae, Malassezia restricta,* and *Malassezia obtusa* [5].

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M. pachydermatis is typically associated with animal infection [3]. Up to five new species have been described in recent literature: *Malassezia dermatis, Malassezia equi, Malassezia japonica, Malassezia yamatoensis,* and *Malassezia nana* [5]. The most common *Malassezia* species contributing to PV lesions are *M. globosa* (50–60%), *M. sympodialis* (3–59%), *M. furfur,* and *M. slooffiae* (1–10%) [6].

Epidemiology

Dermatophytosis is a common fungal infection. The risk of acquiring the disease is 10–20% in an individual's lifetime [7]. Overall rates of dermatophyte infection were measured in the USA using the National Ambulatory Medical Care Survey (NAMCS) from 1990 to 1994 [8]. This survey determined that an estimated 21.6 million physician office visits were for fungal infections during this period, breaking down the data obtained into types of infection: tinea corporis, 27.2%; tinea cruris, 16.9%; tinea pedis, 16.7%; tinea unguium, 15.6%; tinea of hair and beard, 6.9%; and tinea manuum, 1.0%.

Tinea Pedis/Manuum

Tinea pedis is estimated to affect 10% of the world population [9]. Infections are more frequent in tropical climates and may also be associated with the use of occlusive footwear [9]. Men are more often affected than women for both tinea pedis and tinea manuum with most infections occurring in the space between the fourth and fifth toes [9]. Children less frequently develop tinea pedis. Patients with atopic dermatitis or immunosuppressive disorders may develop tinea pedis. Predisposing factors for tinea manuum include manual work that results in repeated trauma to the hands, hyperhidrosis, and frequent use of soap.

Tinea Corporis/Cruris

Tinea corporis and tinea cruris are common and have a worldwide distribution [10]. Little data on prevalence in North America have been published, but tinea corporis was found to be the most common dermatophytosis for which patients sought treatment during the NAMCS (27.2% of all dermatophytoses with an estimated 2.3 million physician visits) [8]. A subset of tinea corporis affecting only the non-bearded regions of the face, tinea faciei, makes up 3–4% of tinea corporis cases, and is more frequently seen in warm, humid climates [11].

Tinea Capitis

The genus *Trichophyton,* particularly *T. tonsurans,* is the predominant cause of tinea capitis in North America. In Western Europe, *M. canis* and *Trichophyton violaceum* are the most common pathogens of tinea capitis; *T. tonsurans* and *M. canis* are dominant in the Caribbean and South America. *M. canis, T. mentagrophytes,* and *T. violaceum* dominate in the Middle East [1].

Tinea capitis is prevalent in children over the age of 6 months and before puberty. African Americans develop tinea capitis more frequently than the general US population. *Trichophyton* species affect men and women equally, although *Microsporum audouinii* and *M. canis* are more frequent in men. The spread of infection may increase in conditions of overcrowding, poor hygiene, and poverty [1].

Onychomycosis

Onychomycosis has an estimated prevalence of 6.5–12.8% in North America, accounting for up to 50% of all nail disease [12, 13]. It is more common in men and in people over the age of 60 years. Other predisposing factors include nail trauma, diabetes, peripheral artery disease, and immunodeficiency. Tinea pedis may be present in patients with toenail onychomycosis [13].

Pityriasis Versicolor

Pityriasis versicolor has a worldwide distribution. Prevalence in tropical climates has been reported at 30–40%, compared to 1–4% in temperate climates [3, 14]. PV is less frequent in prepubescent children, and more frequent in adults when sebaceous gland activity is greatest [6, 14]. Equal prevalence between the sexes has been noted [6].

Seborrheic Dermatitis

Seborrheic dermatitis is a chronic, recurrent disorder affecting between 1 and 5% of immunocompetent adults [3, 14, 15]. It is typically referred to as dandruff. Its mild form affects a large proportion of North American population, but reported numbers are likely underestimated as people tend not to seek medical advice for dandruff. Men are more frequently affected than women. The disease is more severe in winter and improves with summer sun exposure [16]. SD chiefly affects adolescents, young adults, and adults over the age of 50 years [17]. Incidence may increase in immunocompromised persons such as those infected with HIV, where estimates of incidence of SD are as high as 83% [3].

Pathogenesis and Immunology

Dermatophytes colonize keratinized tissue of the stratum corneum. Invasion by anthropophilic species usually results in less inflammation than those of zoophilic or geophilic species [18]. The epidermis functions as a barrier to microorganisms and commensal flora may help reduce infection [3]. Entry to the stratum corneum may result from trauma or some other breach of the skin. Excessive sweating and occlusive clothing or footwear aid in providing a warm, moist environment that favors tinea infection, which can be transferred from one area of the body to another. It can also be transmitted between individuals by direct or indirect contact with scales containing fungal arthroconidia from infected individuals, as occurs in contact sports including wrestling and rugby [10].

Some proteins present in the epidermis, and some skin lipids in the scalp and hair are fungicidal to certain, but not all, dermatophyte species [3]. Dermatophyte glycopeptides prompt the development of delayed hypersensitivity. Patients with inflammatory infection are more likely to demonstrate this type of reaction than patients with noninflammatory chronic disease [19]. Dermatophytes and other microorganisms can activate the alternate complement pathway of immune response, causing the production of molecules which prompt the chemotaxis of neutrophils into the skin [3]. Immunoglobulins are secreted onto the skin surface via sweat, and commensal organisms, including *Malassezia* species, have been found to be coated with these immunoglobulins [3].

Malassezia organisms are a normal part of human commensal skin flora. They are found particularly in sebaceous skin such as the chest, back, and head. They are usually found in the yeast form rather than the mycelial form [3]. *Malassezia* species vary in the antigens presented, and can alter their expressed antigens throughout their growth cycle. The ability of *Malassezia* to elicit activities of the human immune system is not well defined [3].

People and animals, though uninfected, may still be asymptomatic pathogen carriers. Fomites also play a significant role in transmission. Autoinoculation can occur; for example, tinea pedis spreading to tinea cruris, tinea capitis to tinea corporis, or onychomycosis to tinea pedis [20]. High levels of perspiration may predispose to infection, as fungal arthroconidia persist to a greater extent on the scalp with higher levels of oils [10].

Clinical Manifestations

Clinical presentations and differential diagnoses for the various superficial infections are summarized in Table 21.1.

Tinea Pedis/Manuum

Tinea pedis, also known as "athlete's foot," has three common presentations: interdigital, moccasin, and vesicobullous [9].

Interdigital is the most common and typically infects the toe webs, particularly between the fourth and fifth toes (Fig. 21.1) [9]. Interdigital infection may show fissuring, scaling, maceration, and erosion. Hyperhidrosis, pruritus, and foul odor may also be present. Dermatophytosis simplex is an uncomplicated form of interdigital tinea pedis. In contrast, dermatophytosis complex is associated with concomitant bacterial infection that can be facilitated by breakdown of the skin in preliminary infection. This form of tinea is characterized by inflammation, maceration, and odor [9].

Moccasin tinea pedis presents as fine silvery scales with underlying pink to red skin on the soles, heels, and sides of feet (Fig. 21.2) [9]. More severe cases may show cracked, inflamed skin, erythema, and odor; this type of infection is commonly produced by *T. rubrum*.

Vesicobullous tinea is the least common form of tinea pedis and appears as acute inflammatory vesicular or bullous lesions, typically at the instep; however, inflammation may spread over the sole [9]. This dermatophytosis is associated with *T. mentagrophytes* infection.

Tinea manuum is an uncommon clinical presentation that affects the palms of the hands as a chronic dermatosis with minimal erythema and dry, scaly, hyperkeratotic skin [9]. Infections are frequently caused by *T. rubrum*. Tinea manuum can be associated with tinea pedis or onychomycosis, and a two-feet–one-hand syndrome [21]. The latter consists of tinea manuum with excoriation of infected tinea pedis, and/or toenail onychomycosis.

Tinea Corporis/Cruris

Tinea corporis is a superficial dermatophyte infection of the glabrous skin, excluding the scalp, beard, face, hands, feet, and groin (Fig. 21.3), which is more common in men and children [10]. Tinea faciei is a subset of tinea corporis that affects only the face, excluding the beard region [11].

Tinea cruris, also known as "jock itch," is a dermatophyte infection of the genitalia, pubic area, perineal skin, and perianal skin (Fig. 21.4). The scrotum and labia majora are typically not affected. This form of infection is more common in men [10].

Tinea cruris and tinea corporis present as annular erythematous plaques with raised leading edges and scaling. Central clearing of the lesion may be noticed, but nodules may remain present throughout the lesion [10]. Infection is typically associated with pruritus and an erythematous papule or series of vesicles can also be present. Significant

Table 21.1 Clinical presentations and differential diagnoses of common dermatophyte and superficial fungal infections

Condition	Presentation	Differential diagnosis
Tinea pedis	*Interdigital*: scaling, fissuring, maceration, erosions, hyperhidrosis, pruritus, odor	Candidiasis, erythrasma, bacterial infection, psoriasis, contact dermatitis, dyshidrotic eczema, Reiter's syndrome
	Moccasin: fine silvery scales with underlying pink or red skin on soles, heels, sides of feet	
	Vesicobullous: inflammatory vesicular or bullous lesions, particularly at instep	
Tinea manuum	Dry, scaly, hyperkeratotic skin particularly of the palmar area, minimal erythema	Contact dermatitis, atopic dermatitis, pompholyx, psoriasis lamellar
Tinea corporis	Annular erythematous plaques with raised leading edges and scaling, over glabrous skin of trunk; may be central clearing	Impetigo, nummular dermatitis, secondary or tertiary syphilis, psoriasis, lichen planus, seborrheic dermatitis, pityriasis rosea, pityriasis rubra pilaris, candida intertrigo, atopic dermatitis, cutaneous lupus, pityriasis versicolor
Tinea cruris	Annular erythematous plaques with raised leading edges and scaling, over pubic area, perineal, and perianal skin, typically not affecting the scrotum or labia majora	Psoriasis, seborrheic dermatitis, candidiasis, erythrasma, lichen simplex chronicus, Darier's disease, pemphigus vegetans
Tinea capitis	*Noninflammatory*: erythematous papules around hair shaft spreading out with fine scaling in noticeable patches and partial or complete alopecia	Seborrheic dermatitis, psoriasis, atopic dermatitis, tinea amiantacea, alopecia areata, trichotillomania, lupus erythematosus, lichen planopilaris, traction folliculitis, bacterial pyoderma
	Black dot: noticeable black dots where hair breakage at scalp level occurs, scaling with little inflammation (particularly with *T. tonsurans* or *T. violaceum*)	
	Inflammatory: kerion with pustules, loose hair, discharge of pus	
	Favic: large yellow crusts on the scalp	
Onychomycosis	*Distal lateral subungual* (*DLSO*): infection at the distal end of nail plate; discoloration and thickening of nail plate, onycholysis, subungual debris	Psoriasis, chronic onycholysis, chronic paronychia, trachyonychia, hemorrhage, onychogryphosis, lichen planus, alopecia areata, subungual malignant melanoma, subungual squamous cell carcinoma
	Superficial white (*SWO*): white spots or patches on the surface of the nail plate	
	Proximal subungual (*PSO*): infection of the proximal nail fold, and extending distally, typically whitish in color	
	Endonyx: milky white discoloration of the nail plate without hyperkeratosis, onycholysis; may show lamellar splitting of the nail plate (typically caused by *T. soudanense* or *T. violaceum*)	
Pityriasis versicolor	Well-defined, hyperpigmented, or hypopigmented lesions of areas with high concentrations of sebaceous glands such as scalp, chest, back, upper arms, and face; showing fine scaling in most cases (caused by *Malassezia* species)	Vitiligo, chloasma, tinea corporis, pityriasis rotunda, erythrasma
Seborrheic dermatitis	Red, flaky, greasy-looking patches of skin on scalp, nasolabial folds, eyebrows, and ears: "dandruff"; may also affect groin, axillae, anterior chest; pruritus, irritation may be associated with *Malassezia* infection	Psoriasis, contact dermatitis, atopic dermatitis, tinea capitis, rosacea, lupus erythematosus

inflammation may result from infection with zoophilic organisms such as *Trichophyton verrucosum,* which produces large pustular lesions, a kerion, or the formation of frank bullae causing tinea corporis bullosa [10].

Tinea faciei has a broad range of presentations. Infection may begin as flat, scaly macules that develop a raised border that advances outward in all directions, with or without the development of papules, vesicles, and crusts (Fig. 21.5) [11]. Due to a lack of a correct diagnosis and the use of topical steroids, lesions may not be annular. The central area may become hypo- or hyperpigmented. Lesions may occur singly or in multiple patches, and can extend to other sites [11, 22].

Tinea imbricata or Tokelau is a chronic tinea of glabrous skin caused by *Trichophyton concentricum,* an anthropophilic dermatophyte. It presents as distinctive scaly, concentric, overlapping plaques that typically begin on the face and spread, involving large areas of the body [23]. This infection is endemic in Polynesia, and in Central and South America; particularly in rural areas.

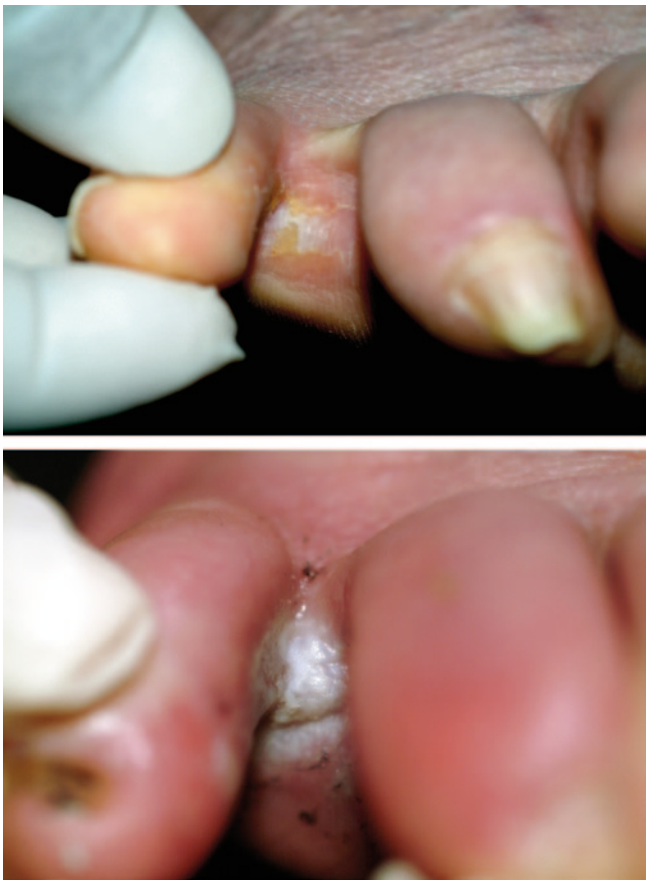

Fig. 21.1 Interdigital tinea pedis

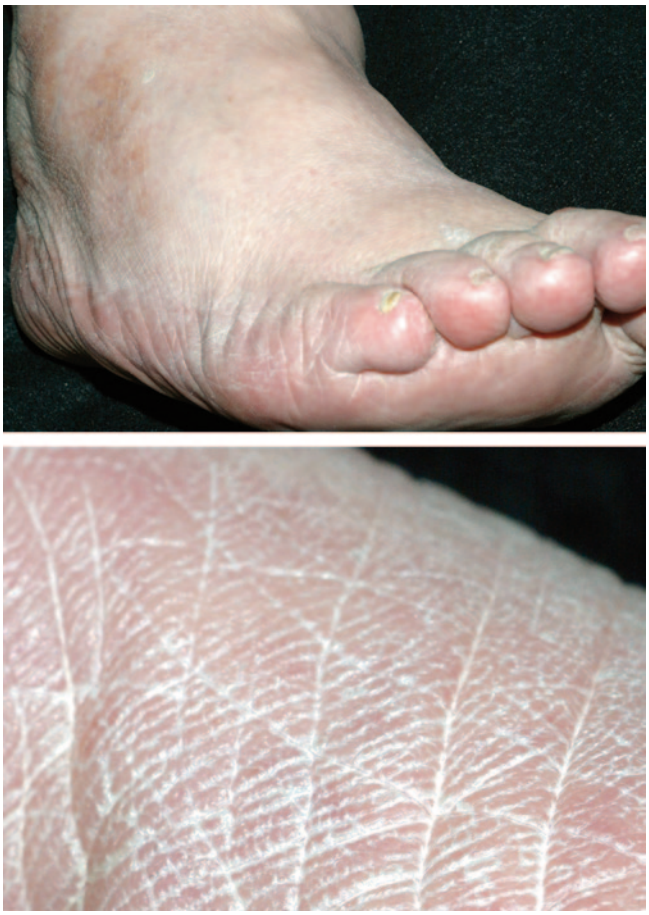

Fig. 21.2 Moccasin tinea pedis, with close-up of fine scaling

Tinea Capitis

Infection of the scalp involves hyphal proliferation in the stratum corneum that extends into the hair follicle orifice and hair shaft [1]. This clinical form is caused by the exposure of the scalp to the inoculum from an infected individual, animal, or contaminated soil [1].

Noninflammatory or epidemic tinea capitis may begin as a small erythematous papule around the hair shaft which spreads outward, developing fine scaling in noticeable patches (Fig. 21.6) [1]. Partial or complete alopecia may result because brittle hair breaks off a few millimeters from the scalp. Affected hair may appear grey due to a coating of fungi. Noninflammatory infection is associated with *M. audouinii* and *Microsporum ferrugineum*; however, *T. tonsurans* and *M. canis* may sometimes cause noninflammatory infection.

Black dot tinea capitis is most frequently associated with *T. tonsurans* or *T. violaceum* infection, and results from hair breakage at the level of the scalp, showing diseased hair in the follicle as a "black dot" [1]. Scaling is typically present with little inflammation, though inflammatory kerion, which are characterized by an oozing mass with pustules, loose hair, and discharge of pus are possible [1]. Signs of systemic illness may be present, including fever and lymphadenopathy.

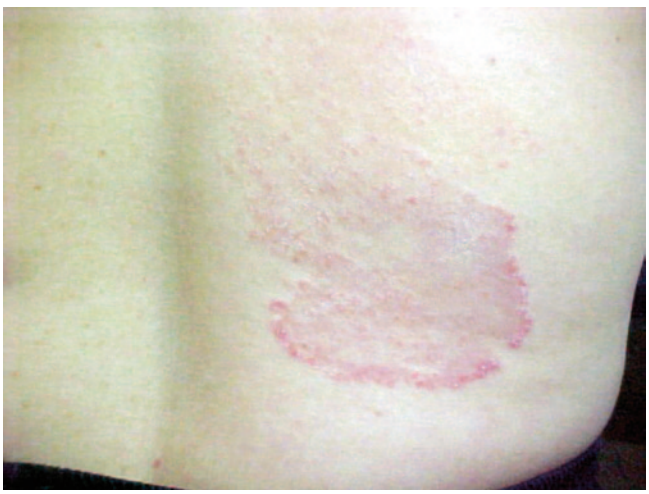

Fig. 21.3 Tinea corporis

Inflammatory tinea capitis is associated with zoophilic or geophilic species such as *M. canis* or *Microsporum gypseum,*

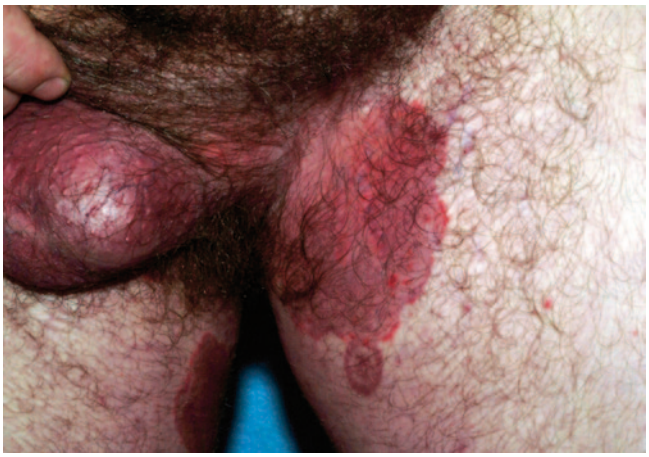

Fig. 21.4 Tinea cruris

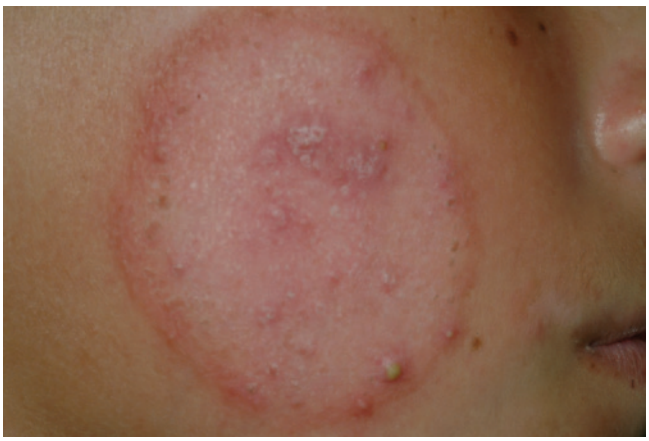

Fig. 21.5 Tinea faciei

but may also occur with *T. verrucosum, Trichophyton schoenleinii, T. tonsurans,* and *M. audouinii* [1].

Favic infection is rare in North America and it is usually caused by *T. schoenleinii,* leading to large yellow crusts [1, 24].

Onychomycosis

The most common presentation of onychomycosis is distal lateral subungual onychomycosis (DLSO), which presents as a nail with discoloration and varying degrees of hyperkeratosis, onycholysis (separation of nail from nail bed), subungual debris, and thickening [25, 26] (Fig. 21.7). DLSO begins at the distal edge of the nail (hyponychium) and travels proximally through the stratum corneum of the nail bed, involving the nail plate (Fig. 21.8). The most severe grades of DLSO may progress to total dystrophic onychomycosis (TDO) where the nail plate becomes friable and crumbles away to a varying degree, leaving an exposed thickened nail bed and subungual debris. Within the spectrum of DLSO presentations, infections may spread relatively evenly across the nail plate. Alternatively, infection may penetrate only the lateral edge or edges of the nail (lateral infection), or may penetrate longitudinally in a "spike" formation [27]. Infection may also develop as a dermatophytoma, where debris and fungi clump densely to form a thick, hyperkeratotic mass [27]. These presentations may not respond well to therapy.

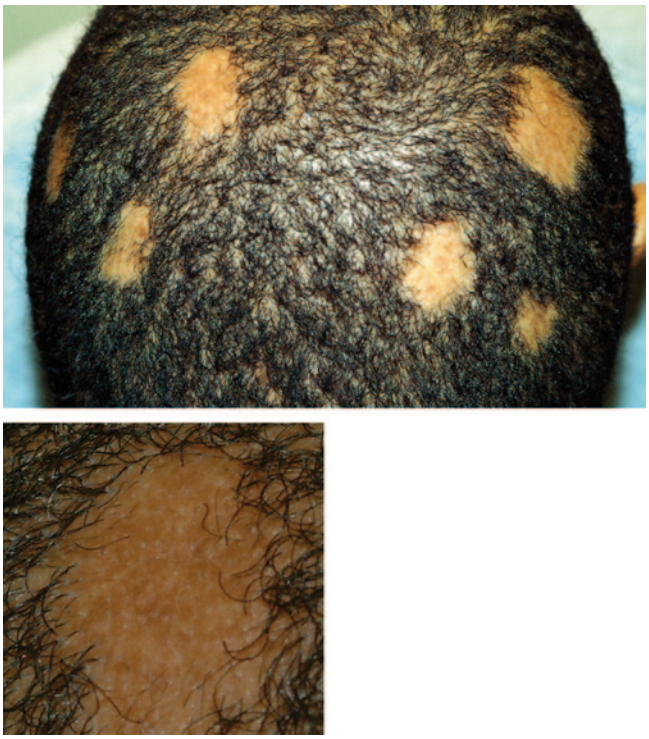

Fig. 21.6 Noninflammatory tinea capitis

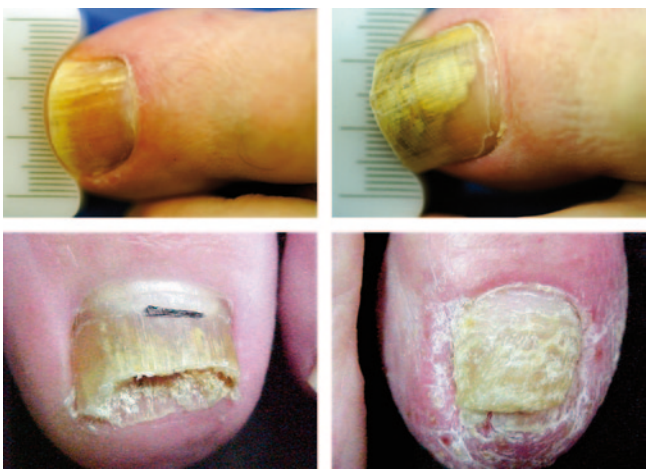

Fig. 21.7 *Top left* and *right*—onychomycosis; *bottom left*—onycholysis; *bottom right*—psoriasis

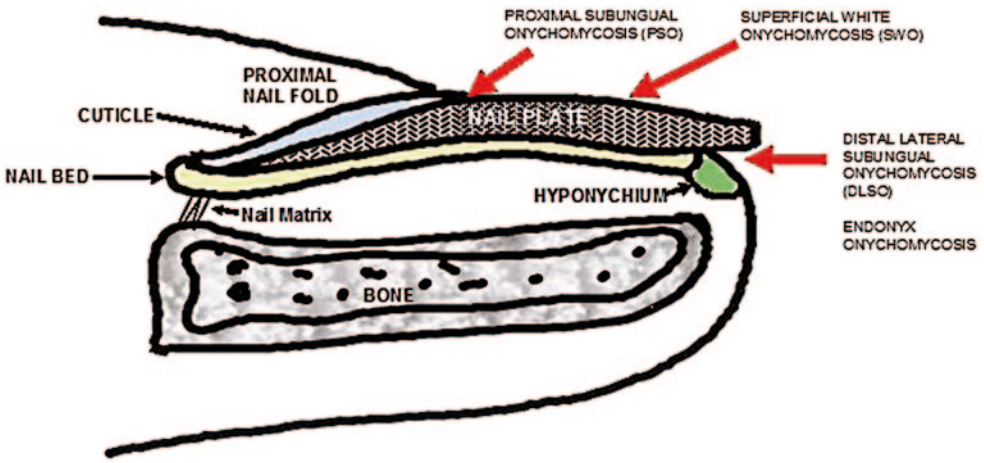

Fig. 21.8 Routes of infection causing the typical presentations of onychomycosis

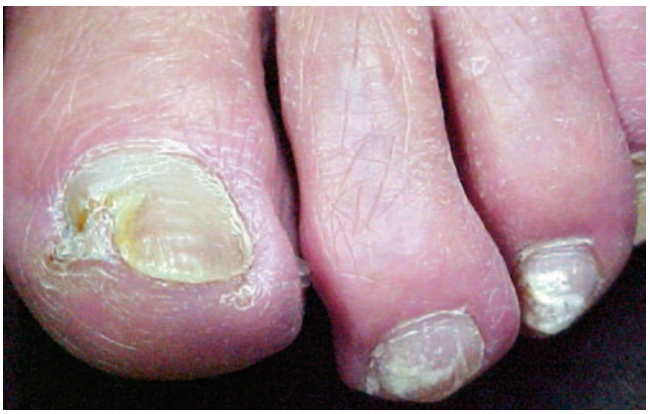

Fig. 21.9 Typical presentation of superficial white onychomycosis (SWO) on the third toenail with distal lateral subungual onychomycosis (DLSO) presented in the great toenail

Sometimes, infection may present as superficial white onychomycosis (SWO), proximal subungual onychomycosis (PSO), or endonyx onychomycosis [25, 26]. SWO involves infection of the superficial nail plate, showing patches of white discoloration on the nail surface. Multiple nails may be affected, and varying degrees of nail plate area may be covered (Fig. 21.9). The rare presentation PSO results from invasion of the proximal nail fold and extending distally along the underside of the nail plate as a white patch of infection (Fig. 21.10). PSO is more common in immunodeficient individuals, such as HIV-positive patients. It may serve as a hallmark for immunodeficiency [28]. Endonyx presents as a diffuse milky white discoloration of the nail in the absence of hyperkeratosis and onycholysis, with the nail plate surface and thickness remaining normal [25]. The nail plate may show lamellar splitting with invasion of the superficial and deep layers of the nail without excessive thickening or discoloration [26]. Endonyx infections are usually caused by *Trichophyton soudanense* or *T. violaceum* [25, 26].

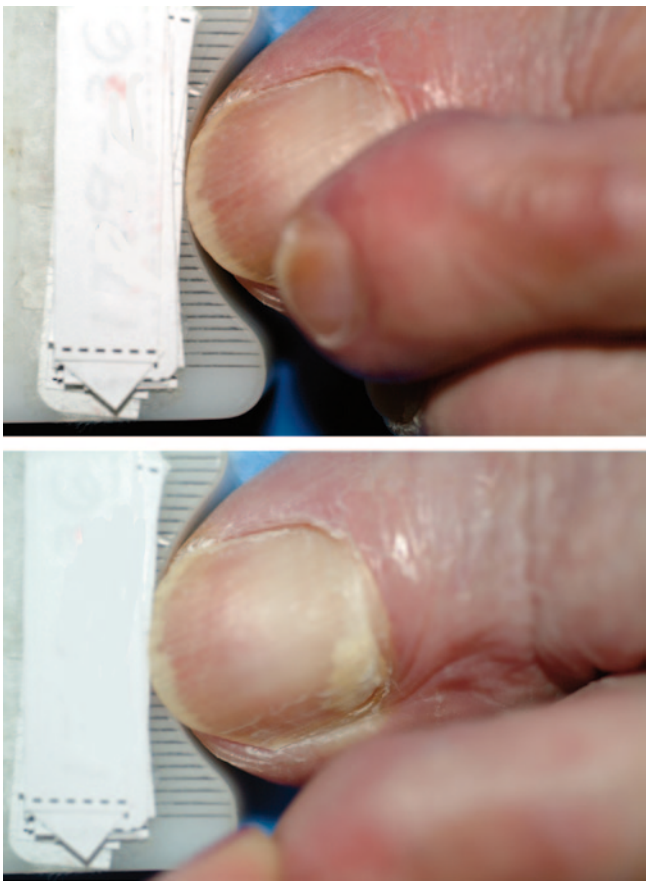

Fig. 21.10 Proximal subungual onychomycosis (PSO) developed during occlusion by the neighboring digit

Pityriasis Versicolor

Pityriasis versicolor presents as well-defined lesions with fine scales caused by desquamation. These are either hyperpigmented (pink, tan, dark brown, or black) or hypopigmented (white, or lighter than normal skin). Hypopigmentation may not always exhibit scaling (Fig. 21.11) [29]. It is a superficial

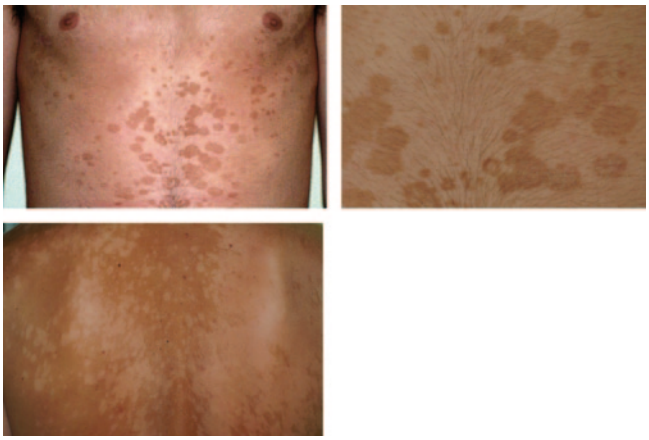

Fig. 21.11 Tinea versicolor showing hyperpigmented lesions (upper photos) and hypopigmented lesions (lower photo)

fungal infection that is largely asymptomatic, with the exception of occasional mild pruritus [6]. There is a large variation in lesion size from macules to entire trunk coverage [30]. Lesions are predominant in areas with a high number of sebaceous glands such as the scalp, chest, and back, as well as the upper arms and face [6, 29]. Facial lesions are more common in children [3]. Hypopigmentation may occur independently or following the hyperpigmented stage [29].

Seborrheic Dermatitis

Seborrheic dermatitis presents as red, flaking, greasy-looking patches of skin on the scalp and hair-bearing areas of the face such as the nasolabial folds, eyebrows, and ears (Fig. 21.12) [16]. SD is a more severe form of dandruff involving body sites of abundant sebaceous gland activity. It may occur on the groin, axillae, anterior chest, or inside/behind the ears [16, 31]. Dandruff can appear as loosely adherent white or gray flakes, while severe SD may be thick, oily, yellow–brown crusts. Pruritus, irritation, and a tight, dry feeling may be associated with the afflicted area [32]. Some cases present with little erythema, while others present as a sore scalp with occasional pustules [15].

Diagnosis

Definitive diagnosis of tineas requires confirmation of dermatophyte organisms by microscopic examination and laboratory fungal culture methods. For skin infections, scrapings or swabs can be taken from the leading edge of a lesion. Nail clippings and subungual debris can similarly be obtained. Direct examination with 15 or 20% potassium hydroxide (KOH) is undemanding, economical, and fast and its initial results allow the physician to establish treatment. KOH is added to the samples to dissociate hyphae from keratinocytes. In dermatophytosis, direct examination can demonstrate the existence of hyphae or arthroconidia. [33].

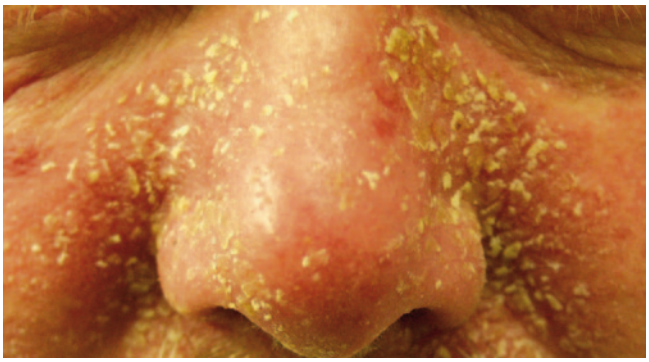

Fig. 21.12 Seborrheic dermatitis—severe presentation

In tinea versicolor, direct mycological examination can reveal the typical "spaghetti and meatball" (mixture of yeasts and short hyphae) appearance. In piedras, microscopic examination may show mycotic nodules. When clinical samples are of good quality and the observer is experienced, KOH has a high sensitivity; unfortunately, the test has a low specificity because it fails to identify the genus and species [34]. KOH examination of hairs may help differentiate types of tinea capitis infection. Ectothrix infection can be distinguished from endothrix infection because in the former arthroconidia appear as chains on the surface of the hair shaft or as a mosaic sheath around the hair [1]. Inspection under Wood's light (filtered ultraviolet light with a peak of 365 nm) may aid in diagnosis [1]. Ectothrix infections with *M. audouinii, M. canis,* and *M. ferrugineum* show bright green fluorescence under the Wood's light. *T. schoenleinii* shows dull green fluorescence. *T. tonsurans,* however, does not fluoresce, and the utility of the Wood's lamp for diagnosis is currently limited in countries where this is the major infecting agent.

Cultures are essential to establish an accurate identification of the causative microorganism. Species identification is established by the macroscopic and microscopic characteristics of the culture, but it has a low sensitivity and a high specificity. In addition, a 1–4-week turnaround time and substantial mycological expertise are needed when performing identification. Typical dermatophyte strains can be frequently recognized from primary cultures, but subcultures on specific media are usually required. Potato dextrose agar is used most frequently for enhancing sporulation and producing pigments. Biochemical and physiological tests such as urease activity, in vitro hair perforation test, and nutritional requirements tests are currently not used [35].

Molecular methods have been expanded, and are more rapid and specific for conventional identification. Young et al. developed a multiplex polymerase chain reaction (PCR) for the identification of dermatophytes. Using three

sets of primers (ITS1-2, 18S rRNA, and 28S rRNA), they effectively recognized 11 standard dermatophyte strains. This method was applied to clinical samples of 73 patients diagnosed with fungal disease. The etiological agents were *T. rubrum* (94.5%), *T. mentagrophytes* (1.4%), *T. tonsurans* (2.8%), and *M. gypseum* (1.4%) [36]. A real-time (RT) PCR based on ITS1 sequences was developed to identify 11 dermatophyte species obtained from superficial samples. It was shown to be highly sensitive and specific for direct detection of dermatophytes in clinical material. RT-PCR seems to be very appropriate as a routine diagnostic assay for dermatophytosis [37]. Sato et al. applied a simple PCR-based DNA microarray method for the detection of pathogens directly from nail material. One hundred and six onychomycosis materials were collected and corroborated for the presence of fungal elements by microscopy. Culture of the samples yielded growth in only 36. Using their PCR-based DNA microarray, they identified the species in 98 specimens [38].

Diagnostic methods in medical mycology have been changing and new techniques are an improvement over former conventional procedures. We now have optional molecular methods for rapid identification of pathogenic fungi, establishing treatment and determining epidemiological trends with more certainty.

Treatment

Treatment for superficial fungal infections varies widely. Antifungal medications that are used typically belong to the azole or allylamine drug classes (Table 21.2). Topical antifungals are effective in circumscribed areas of the skin surface. Some topical antifungals exhibit anti-inflammatory and antibacterial effects as well as antifungal activity, and are indicated for infections with inflammation and potential bacterial infection. Available oral antifungals include griseofulvin, terbinafine, itraconazole, fluconazole, albaconazole, and ketoconazole. These agents are indicated in severe or widespread infection as well as for immunocompromised patients where a prompt, thorough resolution of infection is necessary. They can also be used as an alternative to daily topical therapy.

Safety and drug interactions are a concern with oral therapy, as serum absorption tends to be minimal with topical drug use. With topical agents, most adverse effects are limited to skin reactions at the application site, which usually are mild and transient. Oral antifungals are occasionally associated with severe hepatic toxicity, rare serious skin events such as Stevens–Johnson syndrome, and possible drug–drug interactions due to metabolism through the cytochrome P-450 system. For this reason, clinicians need to evaluate the patient's medical history and be aware of potential drug interactions prior to prescribing medication.

Relapse has been noted with most dermatophyte infections. Patients must be encouraged to complete a full treatment cycle, as infection can be present without visible symptoms. Assessment must include microscopic examination and culture to confirm elimination of the pathogen. Infection transmission from symptom-free carriers such as family members and pets should be controlled.

Tinea Pedis/Manuum

Griseofulvin and topical terbinafine, butenafine, miconazole, econazole, ketoconazole, clotrimazole, and ciclopirox are Food and Drug Administration (FDA)-approved treatments (Table 21.2) [7, 18]. Studies have shown that oral terbinafine and itraconazole may be the most effective and a higher cure rate has been shown with topical allylamines [18, 39]. Topical formulations may be used for milder, limited presentations. For widespread or more severe infections, oral formulations may be required. Relapses are more common with topical agents [39].

Broad spectrum topical agents may be useful, and agents with antibacterial activity may be preferred when superimposed bacterial infection is suspected (e.g., miconazole nitrate 1%, ciclopirox olamine 1%, naftifine hydrochloride 1%, sulconazole nitrate 1%). Once daily formulations may be preferred to twice daily usage, to aid patient compliance (e.g., naftifine 1% cream, bifonazole 1%, ketoconazole cream 2%). Chronic infection may warrant the use of oral antifungals, particularly if previous topical regimens have failed. Oral itraconazole, terbinafine, and fluconazole have been successfully used in tinea pedis, though none of these agents are currently approved by the FDA for use in tinea pedis. These oral agents are preferred over ketoconazole, due to the potential for severe hepatic side effects with ketoconazole. Oral griseofulvin is less effective than newer antifungals [9]. There are no approved treatments specifically for tinea manuum; treatments for tinea pedis are effectively used to treat tinea manuum.

Tinea pedis may frequently recur. Proper foot hygiene may help prevent reinfection. Patients should avoid walking barefoot in communal areas such as bathrooms, showers, or swimming areas, and ensure that feet are dried thoroughly after bathing, showering, or swimming [40]. Additionally, patients should avoid occlusive footwear, alternate shoes every 2–3 days, and change socks often [40].

Tinea Corporis/Cruris

Griseofulvin and topical terbinafine, butenafine, econazole, miconazole, ketoconazole, clotrimazole, and ciclopirox are FDA-approved treatments (Table 21.2) [20]. Topical

Table 21.2 Treatment options available for dermatophytoses and other superficial fungal infections[a]

	Terbinafine	Itraconazole	Fluconazole	Ketoconazole	Griseofulvin	Topicals	
Tinea pedis/ manuum[a]	[b]*Cream*: apply twice daily × 1–4 weeks	*Oral*: 200 mg bid × 1 week	*Oral*: 150 mg once weekly × 2–6 weeks	[b]*2 % Cream*: apply once daily × 6 weeks	*Microsize*[b]: 1 g/day	[b]Ciclopirox 0.77% cream or gel: twice daily × 4 weeks	[b]Clotrimazole, [b]Miconazole, [b]Butenafine, [b]Econazole
	[b]1 % *Solution*: apply twice daily × 1 week			*Oral*[b]: 200–400 mg/day × >4 weeks (*seldomly used*)	*Ultramicrosize*: 660 or 750 mg/ day × 4–8 weeks	Antifungal powder for prevention	
	Oral: 250 mg/day × 2 weeks						
Tinea corporis/ cruris	[b]*Cream*: apply twice daily × 1–4 weeks	*Oral*: 200 mg/day × 1 week	*Oral*: 150–300 mg once weekly × 2–4 weeks	[b]*2 % Cream*: apply once daily × 2 weeks	[b]*Microsize*: 500 mg/day	[b]Ciclopirox 0.77% cream or gel: twice daily × 4 weeks	Clotrimazole Miconazole, Butenafine, Econazole
	[b]*1 % Solution*: apply twice daily × 1 week			[b]*Oral*: 200–400 mg/day × 4 weeks (*seldomly used*)	*Ultramicrosize*: 330–375 mg/day × 2–4 weeks		
	Oral: 250 mg/day × 2–4 weeks						
Tinea capitis	*See Table 21.3 for pediatric dosing*	*See Table 21.3 for pediatric dosing*	*See Table 21.3 for pediatric dosing*	Only effective against *Trichophyton*. 2 % shampoo used as adjunct therapy. (*seldomly used*)	*See Table 21.3 for pediatric dosing*	Selenium sulfide shampoo 1 % as adjunct therapy	Corticosteroid adjunct therapy for severe inflammatory varieties
Onychomycosis	[b]*Oral*: 250 mg/day	[b]*Oral*: [b]*Continuous therapy*: 200 mg/day ´ 12 weeks	*Oral*: 150 mg once weekly	*Oral*: 200–400 mg/day × 6 months, *not recommended due to hepatotoxicity risk*	[b]*Microsize* 1 g/day	[b]*Ciclopirox 8 % lacquer*: once daily × 48 weeks	Amorolfine 5 % lacquer—*not approved in North America*
	Toenail: *12* weeks	*Pulse therapy*: 200 mg bid for 1 week, followed by three itraconazole-free weeks *Toenails*: *three pulses* [b]*Fingernails only*: *two pulses*	*Toenail*: 9–15 months		*Ultramicrosize*: 660 or 750 mg/ day × 4–12 months		

Table 21.2 (continued)

	Terbinafine	Itraconazole	Fluconazole	Ketoconazole	Griseofulvin	Topicals	
	Fingernail: 6 weeks		*Fingernail*: 4–9 months				
Pityriasis versicolor	[b]*1% Solution*: apply twice daily × 1 week	*Oral*: 200 mg/day × 5–7 days	*2% Shampoo*: 5 days	[b]*2% Cream*: apply once daily × 2 weeks	Not effective	[b]Ciclopirox 0.77% cream; [b]Selenium disulfide	[b]Clotrimazole, [b]Miconazole, [b]Butenafine, [b]Econazole
	Oral: not effective		*Oral*: 300 mg once weekly × 2 weeks	*Oral*: 200 mg/day × 2 weeks, 10 days or 5 days; 400 mg per week × 2 weeks; 400 mg per day × 3 days; three doses of 400 mg given every 12 h.			
Seborrheic dermatitis	*1% Solution*: once daily × 4 weeks	*Oral*: 200 mg/day × 1 week	*2% Shampoo*: twice a week × 4 weeks	[b]*2% Cream*: apply twice daily × 4 weeks	Not effective	[b]Ciclopirox 0.77% cream, shampoo, or gel; [b]Selenium sulfide; [b]Coal tar [b]Hydrocortisone	[b]Zinc pyrithione Metronidazole, Bifonazole, Miconazole
	Oral: 250 mg/day × 4 weeks			[b]*Shampoo*: twice a week × 4 weeks			

bid twice daily

[a] There are no approved treatments specifically for tinea manuum; treatments shown are for tinea pedis, which are effective in the treatment of tinea manuum

[b] FDA-approved indications

Table 21.3 Pediatric tinea capitis dosing regimens

Regimen		Duration[a]	Weight (kg)				
			10–20	21–30	31–40	41–50	50+
Terbinafine (continuous)	5 mg/kg/day[b]	2–4 weeks	62.5 mg/day	125 mg/day	125 mg/day	250 mg/day	250 mg/day
Itraconazole (continuous)	5 mg/kg/day	2–4 weeks	100 mg every other day	100 mg/day	100 mg once daily alternating with twice daily	200 mg/day	200 mg/day
Itraconazole (pulse)[c]	Capsules: 5 mg/kg/day	1–3 pulses	100 mg every other day	100 mg/day	100 mg once daily alternating with twice daily	200 mg/day	200 mg bid[d]
	Oral suspension: 3 mg/kg/day	1–3 pulses					
Fluconazole (continuous)	Oral suspension: 6 mg/kg/day	20 days					
Fluconazole (pulse)[e]	Oral suspension: 6 mg/kg/day	8–12 weeks					
Griseofulvin (continuous)	Microsize: 20–25 mg/kg/day	6–12 weeks					
	Ultramicrosize: 10–15 mg/kg/day	6–12 weeks					
	Oral suspension: 15–25 mg/kg/day[f]	6–12 weeks					

bid twice daily

[a] Durations of treatment are for *Trichophyton tonsurans* infection. Longer durations are often required for *Microsporum canis* infections

[b] Drugs are given by once daily dosing unless otherwise specified

[c] Itraconazole pulses are given for 1 week, with 3 weeks "off" before starting the next pulse

[d] Itraconazole adult dose 200 mg bid (approved for pulse use in fingernail onychomycosis). No standard has been established in clinical trials for tinea capitis for children > 50 kg; use varies from once daily as with continuous regimen to twice daily 200 mg dosing

[e] Fluconazole pulses are 1 day on, 6 days off, before beginning next pulse

[f] Dosing based on Grifulvin V suspension 125 mg/5 ml

formulations may be used for infections in smaller areas (e.g., sulconazole, oxiconazole, miconazole, clotrimazole, econazole, ketoconazole) [10]. Oral therapy may be required when large areas are involved, or when infection is chronic or recurrent. Topical corticosteroid should be avoided as it may lead to suppression of signs of infection [10]. Oral itraconazole, terbinafine, and fluconazole have been used successfully for tinea corporis/cruris, although none of these agents are currently approved by the FDA for use in these indications. These oral agents are preferred over ketoconazole, due to the potential for severe hepatic side effects. Griseofulvin is not recommended as it does not adequately bind the keratin in the stratum corneum, reducing efficacy [10].

Tinea faciei is typically cleared with topical treatment. Topical ciclopirox and terbinafine may provide good anti-inflammatory effects as well as antifungal activity [11]. Miconazole or similar azoles may also be effective. Azoles should be used for 3–4 weeks, or at least 1 week after resolution of lesions. Resistant lesions, cases of extensive disease, or more severe cases may require oral therapy [11].

Tinea imbricata is best treated with oral terbinafine or griseofulvin, though a high rate of recurrence has been noted [23]. Itraconazole and fluconazole have not been effective. Adjunctive therapy with keratolytic creams such as Whitfield's ointment (benzoic and salicylic acids) may increase treatment efficacy [23].

Tinea Capitis

Oral therapy is required to adequately treat tinea capitis. Topical antifungals such as shampoos (selenium sulfide, povidone iodine, zinc pyrithione) may be used as adjunct therapy with or without oral antifungals to prevent reinfection or to treat asymptomatic carriers [1, 40]. As most infections occur in children, dosing regimens are modified from typical adult regimens provided for other indications, and are usually given on a weight-based schedule (Table 21.3). Furthermore, infections with *Microsporum* may require higher dosing than infections with *Trichophyton,* or longer regimens of therapy [1, 41]. Griseofulvin is the only FDA-approved oral treatment; however, terbinafine, itraconazole, and fluconazole have frequently been used for the successful resolution of tinea capitis. Itraconazole, terbinafine, and fluconazole have shorter treatment durations than griseofulvin [1]. Liquid formulations are available for griseofulvin, itraconazole, and fluconazole, and may aid in pediatric dosing, though dosing regimens may vary from that suggested for tablet/capsule formulations.

Infected children do not need to be kept out of school once treatment is initiated, particularly children in higher grades [40, 42]. Infection transmission from symptom-free carriers such as family members and pets may need to be controlled. Objects that may act as fomites, such as hats, combs, pillows, blankets and scissors, should be disinfected with bleach [40].

An "id" reaction has been observed with tinea capitis patients following the initiation of drug therapy, and can be confused with allergic drug reaction [1]. An "id" reaction may present as symmetrical, skin colored, or erythematous papules and plaques on the face, neck, and upper body, and sometimes be generalized. The reaction may also be present prior to initiating treatment.

Onychomycosis

Onychomycosis is difficult to cure and has a high rate of recurrence [7, 12, 43]. Typically, oral therapy is required to adequately treat this form of infection (Table 21.2). Following successful treatment, the infected nail must grow out, gradually becoming replaced by normal healthy nail. This process may take 9–18 months, depending on the nail growth rate. Fingernails may show better response rates than toenails, as they grow faster. Where the nail has been injured or shows other abnormal growth patterns, nail outgrowth may be slow, and the nail may never regain a normal appearance. Furthermore, relapse is frequent. Patient expectations should be discussed, so that the patient understands that successful treatment is unlikely to occur quickly and that long-term follow-up may be necessary to detect relapses.

Topical therapy may be effective in mild-to-moderate cases. Ciclopirox 8% lacquer is the only topical therapy currently approved for onychomycosis by the FDA. Amorolfine 5% nail lacquer has not been approved for use in North America [44–46]. Routine nail debridement may be needed to provide effective drug delivery to the infected area and to reduce the burden of fungal material needing treatment [43]. Efinaconazole 10% solution was recently evaluated in a double-blind multicenter study to define its value as topical treatment. After 52 weeks, 17.8% achieved a complete cure with efinaconazole [47].

The oral agents, terbinafine and itraconazole, are frequently used for onychomycosis. Ketoconazole is currently not used due to potential hepatic side effects. Griseofulvin is also not recommended because the required regimens are significantly longer than those of itraconazole or terbinafine, and even more important, its efficacy is low [48]. Fluconazole has shown high efficacy, low relapse rates, and usefulness with yeast coinfection; however, there have been few studies of this treatment method [20, 43, 46].

Approved oral therapy regimens for onychomycosis are: terbinafine 250 mg/day for 12 weeks (toenails) or 6 weeks (fingernails only); itraconazole 200 mg/day for 12 weeks (toenails with or without fingernail involvement); and itraconazole 200 mg twice daily as pulse therapy (one pulse: 1 week of itraconazole followed by 3 weeks without itraconazole) using two pulses (fingernails only). Though only a continuous regimen of itraconazole is FDA approved for toenail onychomycosis, the current standard of care of toenail onychomycosis used by US dermatologists is a pulse itraconazole regimen (one pulse: 1 week of itraconazole followed by 3 weeks without itraconazole; a total of three pulses is given).

Both terbinafine and itraconazole are readily taken up in the nail from the nail bed and matrix and may remain in the nail for a significant period after dosing is completed. Itraconazole tends to be fungistatic, while terbinafine is fungicidal [48]. Mycological cure rates (KOH negative and culture negative) for terbinafine use are estimated at 76% in a meta-analysis of clinical trial data [49]. By comparison, itraconazole mycological cure rates are 59% (continuous therapy) and 63% (pulse therapy). Clinical response rates (infection cleared or showing marked improvement) were: terbinafine, 66%, itraconazole continuous therapy, 70%, and itraconazole pulse therapy, 70% [49].

Itraconazole may be associated with more drug interactions than terbinafine due to its metabolism through the CYP3A4 pathway, limiting its use in some patients. Itraconazole is also prohibited in patients showing ventricular dysfunction such as current or past congestive heart failure [49]. A current country-specific product monograph should be consulted for complete listing of known drug interactions, warnings, and monitoring requirements prior to prescribing. Rare cases of hepatic injury have been reported, and monitoring of hepatic enzymes is recommended for subjects with preexisting hepatic abnormality or a history of liver toxicity with the use of other medications. Capsules must be taken with a meal or a cola beverage to ensure adequate absorption [48].

Terbinafine may interfere with the metabolism of CYP2D6 substrates, and some other drug interactions have been noted. A current country-specific product monograph should be consulted for complete listing of known drug interactions, warnings, and monitoring requirements prior to prescribing. Rare cases of hepatic injury have been reported with terbinafine. Terbinafine is not recommended for patients with existing liver disease, and all patients should be screened for hepatic enzyme abnormalities (alanine transaminase, ALT, and aspartate transaminase, AST) prior to initiating terbinafine [48]. Terbinafine may be taken in the fasted or fed state without affecting absorption.

Photodynamic therapy has been successfully used in isolated cases in which oral therapy was contraindicated [50].

Laser has recently been evaluated by several authors. Kozarev and Vizintin used variable square pulse (VSP) Nd:YAG 1064-nm laser with fluences in the range of 35–40 J/cm^2, a 4-mm spot size, a pulse duration of 35 ms, and a pulse rate of 1 Hz for a total of three passes to treat fungal onychomycosis. They used laser energy to completely cover the nail plate in 72 patients with a confirmed diagnosis of onychomycosis caused by *T. rubrum* (37 patients), *T. mentagrophytes* (22 patients), *Candida* species (ten patients), and *Aspergillus niger* (3 patients). At 3-month follow-up, 95.8 % of patients were cleared of fungal disease. At 6 and 12 months, they found 100 % clearance of fungal infection with no other treatment [51]. In contrast, Carney et al. performed a four-part in vitro, in vivo study. The first part evaluated three different nail pathogens in suspension at seven heat and time exposures. The second and third parts irradiated pure fungal colonies. A fungicidal effect for *T. rubrum* was found in vitro at 50 °C for 15 min. No growth inhibition was seen for the *T. rubrum* colony treated with direct laser irradiation with fluences of 5, 15, 20, and 50 J/cm^2 with a pulse rate of 7–10 Hz. Temperature of the media during laser treatment was 40 °C. The in vivo assessment was a 24-week pilot study of ten patients using a submillisecond (Nd:YAG) 1064-nm laser as the only treatment for onychomycosis. Fluences of 16 J/cm^2, a pulse duration of 0.3 ms with a total of five passes per session, were applied. Outcome showed no improvement in the Onychomycosis Severity Index score [52].

These results need to be further researched and validated in a well-designed multicenter, randomized, comparative study with independent evaluators using the same laser administration parameters to define the true usefulness of this therapeutic procedure.

Routine nail debridement may be used in conjunction with oral or topical therapy, particularly where the nail is thickened, or when disease presents as a dermatophytoma, spike, or lateral infection. Caution must be taken not to damage the underlying skin during debridement, particularly in subjects who are vulnerable to severe lower limb complications, such as individuals with diabetes or lower limb peripheral vascular disease [43].

As with tinea pedis, proper foot and nail hygiene may help prevent reinfection. Patients should avoid walking barefoot in public areas such as restrooms, showers, or swimming pools, and make sure that feet have been dried thoroughly after bathing, showering, or swimming [40]. Nails should be kept short and clean. Shoes should fit properly and socks should be made from absorbent material such as cotton.

Pityriasis Versicolor

A variety of topical agents may be used to treat PV (Table 21.2). Topical azoles (ketoconazole, fluconazole, bifonazole, clotrimazole, miconazole) have been effective in treating *Malassezia,* both in cream formulation or shampoos; terbinafine solution, cream, gel, or spray has also been effective [6]. Topical ciclopirox provides both antifungal and anti-inflammatory activity against *Malassezia*.

Systemic therapies may be warranted in severe cases, or cases with widespread body involvement. Patients may also prefer a short-duration oral therapy to frequent application of a topical agent. Oral therapy with ketoconazole, itraconazole, and fluconazole has been effective for PV, and the regimens reported in the literature provide similar, high efficacy rates [6]. Oral terbinafine and griseofulvin are not effective for PV [6].

Relapse of PV is common due to endogenous host factors: recurrence rates have been reported as high as 60–90 % in 2 years posttreatment [53]. Both ketoconazole (single 400 mg dose or 200 mg daily for 3 days once monthly) and itraconazole (single 400 mg dose once monthly for 6 months) have been used in prophylactic regimens for PV [6].

Individual treatments for hyper- and hypopigmented variations of PV do not exist. Although fungal organisms may be eradicated after 2 weeks of therapy, it may take significantly longer before normal skin pigmentation is restored, particularly with hypopigmented lesions [6].

Seborrheic Dermatitis

There is no definitive cure for SD; it is a recurrent disease requiring prophylactic treatment [15]. Topical corticosteroid lotions have typically been used but are being replaced by antifungals in the form of shampoos, gels, and creams (Table 21.2).

Topical ketoconazole (cream, shampoo, gel, emulsion) is the most prescribed azole for SD [54]. Bifonazole, miconazole, and fluconazole may also be effective. Low-potency corticosteroids may be useful in providing an anti-inflammatory effect, though many newer antifungal agents such as ciclopirox may also provide anti-inflammatory activity comparable to corticosteroids [54]. Ciclopirox (cream, gel or shampoo) provides effective antifungal treatment, and also has antibacterial and anti-inflammatory activities [55, 56]. Zinc pyrithione shampoos are safe and effective in controlling dandruff and SD of the scalp, and exhibit strong keratolytic and antifungal activity against *Malassezia* [16, 57]. Some patients benefit as well from non-antifungal, keratolytic agents (selenium sulfide, sulfur, salicylic acid) or anti-proliferative (coal tar) shampoos [15, 32]. Tar shampoos often cause sensitivity of the skin to sunlight and are not as favorable cosmetically [32]. Topical 1 % terbinafine solution has been effectively used for scalp SD [54].

Oral therapy should be reserved for severe inflammatory SD, widespread SD, or SD that has been refractory to topical

treatment [54]. Oral ketoconazole and oral itraconazole have been used effectively for SD. Oral itraconazole is safer than ketoconazole and is effective in severe cases which have not responded to other antifungals [16].

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Fungal Infections of Implantation (Chromoblastomycosis, Mycetoma, Entomophthoramycosis, and Lacaziosis)

22

Flavio Queiroz-Telles, Daniel Wagner de C. L. Santos and Conceição de Maria Pedrozo S. Azevedo

Introduction

Fungi may infect mammalian hosts by several manners, including inhalation and traumatic implantation through the skin. The respiratory route may result in allergic manifestations, pulmonary diseases, and/or systemic mycoses, according to the fungal pathogen and the host immune response. Among the fungal pathogens, there is a group that gain access though different kinds of transcutaneous wounds, leading to implantation mycoses. Also called the "subcutaneous mycoses," these encompassing a group of unrelated mycotic diseases whose etiologic agents are transported from their saprobiotic niche to the cutaneous tegument through wounds. The list of implantation mycoses includes sporotrichosis, eumycetoma, chromoblastomycosis (CBM; chromomycosis), phaeohyphomycosis (PHM), entomophthoramycosis (subcutaneous zygomycosis), and lacaziosis (lobomycosis) [1–7].

Chromoblastomycosis, eumycetoma, lacaziosis, and entomophthoramycosis are geographically restricted to tropical and subtropical zones. These endemic implantation mycoses are neglected diseases, affecting the low-income populations in developing regions of Africa, Asia, and Latin America. Although they rarely disseminate, their morbidity is significant due to sequelae and incapacity seen in their most severe clinical forms (Table 22.1) [2, 8].

Typically, these infections are characterized by initial lesions starting at the site of fungal implantation and with time, they be evolve according to the etiologic agent and the host immune defenses. Although the term "subcutaneous mycoses" have been used for decades, it is not strictly correct because in some of these infections the lymphatic vessels, fascia, muscles, cartilage, and bones may be affected beyond the cutaneous and subcutaneous tissues [1, 2].

Chromoblastomycosis

Chromoblastomycosis or chromomycosis is one of the most prevalent implantation mycosis in tropical and subtropical zones as well as the most frequent human mycoses caused by melanized (dark pigmented) fungi [7, 9–13]. This disease was described by Max Rudolph, a German Doctor working in Brazil, in 1914 [14, 15]. Chromoblastomycosis lesions are clinically polymorphic and if not recognized at earlier stages, they may become recalcitrant to therapy and extremely difficult to eradicate [8]. Except for the initial lesions, which should be surgically removed, moderate-to-severe clinical forms constitute a true therapeutic challenge for patients and clinicians. This disease presents with the following characteristics: primary lesion beginning at the site of implantation of the etiologic agent, chronic involvement of cutaneous and/or subcutaneous tissues, associated with a granulomatous, purulent, and fibrotic tissular reaction, and a non-protective humoral and cellular immune responses [7, 16].

Etiology

Melanized, dematiaceous, pheoid, or simply "black fungi" are ubiquitous saprobes inhabiting soil, plant fragments, and water. More than 150 species from 70 genera have been implicated in human and animal disease [17, 18]. These agents contain melanin in their cell walls, which serves a key role in virulence in black fungi as a group. The list of human infections related to the melanized fungi includes PHM, allergic and invasive sinusitis, fungemia, mycetoma, and

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Table 22.1 Endemic implantation mycoses

Disease	Affected population	Clinical manifestations	Laboratory diagnosis	Treatment
Chromoblastomycosis (also called chromomycosis, verrucous dermatitis, figueira[a])	Usually in men aged 30–50 who work as farm laborers, lumberjacks, or sellers of farm products; affected persons usually poor, without adequate protective footwear and clothing	Slowly progressive disorder usually limited to the skin and subcutaneous tissue in which initial erythematous papular lesions may gradually evolve to varying morphologies, such as nodular, tumoral (cauliflower-like), plaque, verrucous, and cicatricial lesions; affects feet and legs most frequently; may transform into squamous cell carcinoma	Microscopic finding of muriform cells (sclerotic bodies) is the hallmark of this disease. Examination of scrapings or vinyl adhesive tape preparations, wet mount, histology, culture	Surgery effective in early stages; itraconazole (200–400 mg/day), terbinafine (250–500 mg/day), terbinafine (500 mg/day) plus itraconazole (200 mg/day); combination therapy (itraconazole with terbinafine or 5-flucytosine) for severe cases, posaconazole (400 mg bid) in patients with disease refractory to itraconazole or who are intolerant of itraconazole; cryotherapy
Eumycetoma (also called mycetoma, maduromycosis, Madura foot)	Men aged 20–40 who work as herders, farmers, or other field laborers; increasingly in travelers to tropical endemic areas	Local chronic, progressive, multifistulous, suppurative, tumoral lesions discharging grains. Infection involves cutaneous and subcutaneous tissues, fascia, and eventually muscle and bone	Observation of grain color and texture; deep surgical biopsies containing grains that can be cultured or fixed for histopathology; immunodiffusion, ELISA, PCR with DNA sequencing; MRI or CT to determine bone involvement	Surgery and antifungal therapy with itraconazole (400 mg/day); posaconazole (400 mg bid) in patients with disease refractory to itraconazole or who are intolerant of itraconazole
Entomophthoramycosis (also called subcutaneous zygomicosis, subcutaneous phycomycosis, basidiobolomycosis, conidiobolomycosis)	Infections usually caused by Entomophthorales, Basidiobolus ranarum, and Conidiobolus coronatus; usually in immunocompetent persons; basidiobolomycosis usually in children, conidiobolomycosis usually in adults	*Basidiobolomycosis*: usually has chronic progressive clinical course; hard nodules that spread, often over thighs and buttocks, eventually ulcerating overlying skin *Conidiobolomycosis*: begins with swelling of inferior nasal turbinates and extends to facial and subcutaneous tissues and paranasal sinuses; eventually, subcutaneous nodules attach to underlying tissues, causing facial disfigurement	Histology—wide, sparsely septated, thin-walled hyphae with right-angle branching. Splendore-Hoeppli phenomenon present with basidiobolomycosis; culture	No standard treatment defined for entomophthoramycosis (basidiobolomycosis or conidiobolomycosis). Antifungals used to treat entomophthoramycosis include potassium iodide, miconazole, ketoconazole, itraconazole, fluconazole, terbinafine, and amphotericin B
Lacaziosis (also called lobomycosis, Lobo's disease, Paracoccidioidomycosis loboi)	Men aged 21–40 who live in tropical rain forests and work as farmers, miners, hunters, and rubber workers	Very slow evolution over many years; small papules or pustules that evolve into keloid-like lesions, which gradually increase in size; pinna of the ear most commonly affected; original lesion followed by involvement of other areas by subsequent abrasion/autoinoculation; nodule distribution follows lymphatic system	Direct microscopy of tissue smear from lesion, examination of vinyl adhesive tape preparation; cannot be cultured *Serologic tests*: have high sensitivity but lack specificity because of antigenic cross-reactivity with Paracoccidioides	Wide surgical excision, electrodessication in early stage of disease, cryosurgery; clofazimine (300 mg/day until clinical improvement, then 100 mg/day for≥2 years) Amphotericin B, 5-fluorocytosine, and azoles usually ineffective, but patient with disseminated disease undergoing treatment with itraconazole (200 mg/day)

ELISA enzyme-linked immunosorbent assay, *PCR* polymerase chain reaction, *MRI* magnetic resonance imaging, *CT* computed tomography

[a] Means "fig tree," the popular name of this disease in Brazil

CBM. The exact number of melanized (dematiaceous) fungi which may cause CBM is uncertain, especially as molecular tools continue to add to fungal taxonomy [13, 18–22]. It is believed that several species of the Herpotrichiellaceae family cause the disease. The main species, according to the taxonomic proposal, can be grouped into five genera: *Fonsecaea, Cladophialophora*, *Phialophora, Rhinocladiella,* and *Exophiala*. The majority of infections are caused by species of *Fonsecaea* and *Cladophialophora* [18–22].

Similar to the endemic fungi pathogens, the agents of CBM are dimorphic and present their mycelial form in natural habitats or in vitro culture media, but in tissues present as muriform cells (the hallmark of this disease). Muriform cells, also known as "sclerotic or fumagoid cells," "Medlar bodies," or "copper pennies," are a biological adaptation leading the agents to survive in the hostility of the host tissue environment [23, 24]. Muriform cells are characterized by thick melanized cell walls, isodiametric expansion, and unordered septum formation. They are polyhedric in shape, measuring 5–12 μm in diameter and divide by binary fission, forming two distinct planes of septation (Fig. 22.1). These are considered to be protective structures which also enhance resistance to antifungal drugs. The presence of muriform cells differentiated CBM from PHM [7, 9, 24].

In culture media, the agents of CBM grow as dark pigmented, velvety filamentous moulds. Presumed species identification can be made by conventional mycological methods, like macro- and micromorphology and physiology characteristics, but nowadays, the definitive agent identification should be based on the molecular sequence of specific genes. Although the fungal species causing CBM do not differ in clinical aspects, they may present differences in their *in vitro* susceptibility to antifungal drugs. *Cladophialophora carrionii* and *Phialophora verrucosa* are considered to be more susceptible to antifungals than *Fonsecaea pedrosoi*. Even differences in susceptibility have been reported between the species of *Fonsecaea genus*, viz. *F. pedrosoi*, *F. monophora,* and *F. nubica* [21–25].

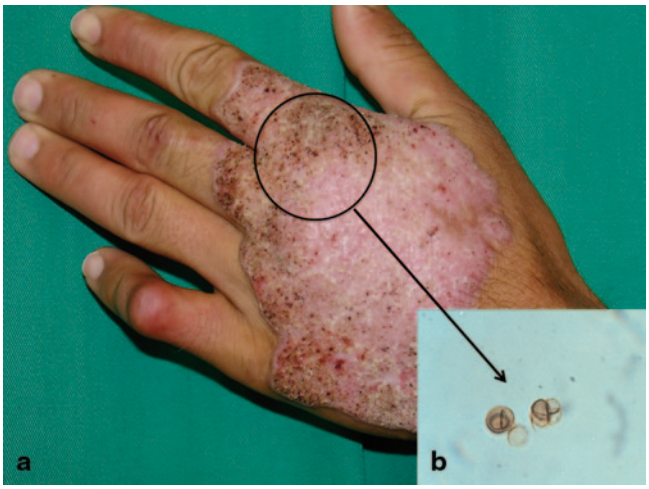

Fig. 22.1 A nodular plaque lesion of chromoblastomycosis containing several "*black dots*" (*circle*) was scraped (**a**). Muriform cells are easily observed in KOH wet mount (**b**). (*From Queiroz-Telles F, et al. Subcutaneous mycoses. Infect Dis Clin N Am 2003;17(1):59–85. Reprinted with permission from Elsevier Limited). KOH potassium hydroxide

Ecology and Epidemiology

Melanized fungi thrive in tropical and subtropical environments worldwide. It is believed that during their saprobic cycle, fungal agents of CBM live in soil and in different parts of plants such as thorns, leaves, spiny seeds, wood cortex, etc. [18, 26, 27]. Consequently, CBM lesions afflict mainly adult males involved in rural labor who do not have appropriate footwear or clothing and who are more likely to be infected by trauma. In 54–85 % of cases, CBM involves the lower extremities [13, 26]. It is considered as an occupational disease in the endemic areas of the world, but scattered cases are also reported in temperate-to-cold regions. Although CBM has no compulsory notification, gathered data from published case reports and surveys show that incidence rates may range from 1:6800 (Madagascar) to 1:8,625,000 (USA) [10, 28]. Most of the reported cases occur in Latin America, the Caribbean, Asia, Africa, and Australia. Madagascar, Brazil, Mexico, Dominican Republic, Venezuela, India, and Southern China contribute with the majority of cases. The disease is less frequent in the Northern Hemisphere, including European countries and in the USA, where it has been reported in Louisiana and Texas [2, 4, 5, 7].

Although children may present with the disease, the majority of patients are adult males. The main risk factors associated with CBM infection are adult age, male sex, rural work or outdoor activities, the lack of protective shoes, gloves or garments, and poor nutrition and hygienic habits [2, 26]. In a series of 100 patients reported from the Brazilian South Region, the majority of patients were male (4:1) of 50–59 years of age [29]. In another study of 325 cases in the Brazilian Amazon region, the main age group affected by the diseases ranged from 41 to 70 years old, 86.1 % of the patients were agricultural workers, and 93.2 % of were male [11]. In both reports, CBM lesions predominated on the lower limbs (feet and legs) and *F. pedrosoi* was the main etiologic agent [11, 29]. On the other hand, a series of cases in children and adolescents patients ranging from 2 to 19 years old have been reported in an endemic Venezuelan semiarid zone. Infection was related to *C. carrionii*, probably transmitted by cactaceae thorn implantation [30].

Table 22.2 Lesion types, severity rating, and differential diagnosis of chromoblastomycosis

Type of lesions	Severity of disease	Differential diagnosis
Nodular type Moderately elevated, fairly soft, dull-to-pink violaceous growth. Surface smooth, verrucous or scaly. With time lesions may gradually become tumorous	*Mild form* A solitary plaque or nodule measuring less than 5 cm in diameter	*Infectious diseases* *Fungi* Paracoccidioidomycosis Blastomycosis Fixed sporotrichosis Coccidioidomycosis Phaeohyphomycosis Lacaziosis Eumycetoma Granulomatous candidiasis Granulomatous trichophytosis Bacteria Cutaneous verrucous tuberculosis Leprosy Actinomycetoma, nocardiosis Botryomycosis Tertiary syphilis, yaws Ecthyma Mycobacteriosis (M. marinum, M. fortuitum) Protozoa Cutaneous leishmaniasis Rhinosporidiosis Viral Verruca, papilloma
Tumorous type Tumor-like masses, prominent, papillomatous, sometimes lobulated; "cauliflower like." Surface partly or entirely covered with epidermal debris and crusts. More exuberant on lower extremities.		
Verrucous type Hyperkeratosis is the outstanding feature Warty dry lesions. Frequently encountered along the border of the foot	*Moderate form* Solitary or multiple lesions: nodular, verrucous or plaque types, existing alone or in combination, covering one or two adjacent cutaneous regions, measuring less than 15 cm in diameter	
Cicatricial type Non-elevated lesions that enlarge by peripheral extension with atrophic scarring, while healing takes place at the centre. Usually with annular, arciform or serpiginous outline. Tends to cover extensive areas of the body		
Plaque type Slightly elevated, with variously sized and shaped areas of infiltration. Reddish to violaceous in color presenting a scaly surface, sometimes shows marked lines of cleavage. Generally found on the higher portions of the limbs	*Severe form* Any type of lesion alone or in combination, covering extensive cutaneous regions whether adjacent or nonadjacent	Helmintic Filariosis Noninfectious diseases Escamous carcinoma Bowen disease Psoriasis Sarcoidosis Lupus erythematosus Mossy foot

Immunopathogenesis

Human infection by CBM agents starts when fungal elements are transferred from their environmental saprobe life across the cutaneous barrier through transcutaneous trauma. If the parasite survives and adapts to the new hostile environment represented by the host's cutaneous/subcutaneous tissues, the infection may progress to disease [2, 16]. Because labor-related trauma is very frequent among rural workers living in tropical regions, unsuccessful infections may be the rule since melanized fungi are ubiquitous in nature, but CBM is not an everyday disease (even in the endemic areas). So other factors must play a hole in the pathogenesis of CBM, including fungus virulence, host genetic susceptibility, and continuing exposure to the agents during life. It has not been proved if hormonal factors are able to protect females like in paracoccidioidomycosis [16, 27, 31–33].

The knowledge of immunology of CBM is poorly understood because, to date, there are no reproducible animal models of this disease. Mechanisms of immunity may include humoral and cell-mediated responses. Like other chronic fungal infections, CBM patients produce specific antibodies, but not protective antibodies against the progression of the disease. It has been demonstrated that the cell-mediated immunity plays a key role in the clinical presentations of this disease. The mixed tissue response in CBM shows a granulomatous reaction associated with microabscesses, suggesting ineffective phagocytosis of muriform cells and chronic infection [34, 35].

Clinical Manifestations

Following implantation, and after an uncertain period of time, the initial lesion may be produced at the site of infection. It may start as a solitary macular lesion and latter, it may progress to a papular shape lesion with a pink smooth surface that gradually increases over a few weeks and then develop a scaly surface. The initial skin lesion may progress and evolve with diverse clinical polymorphism, eliciting differential diagnoses including many infectious and noninfectious diseases (Table 22.2). According to the modified Carrion's classification, the CBM lesions are characterized as nodular, tumoral (cauliflower-like), verrucous, cicatricial, or plaque (Fig. 22.2) [7, 13, 24]. In advanced and more severe cases, more than one type of lesion can be observed in the

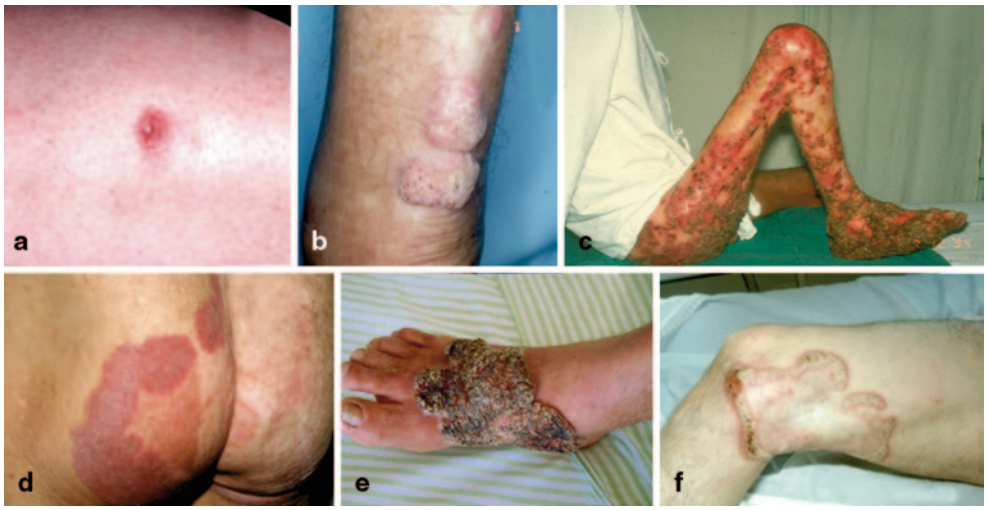

Fig. 22.2 Clinical classification of types of chromoblastomycosis lesions. Initial lesion (**a**), nodular (**b**), tumoral (**c**), plaque (**d**), verrucous (**e**) and cicatricial (**f**). (*From Queiroz-Telles F, et al. Subcutaneous mycoses. Infect Dis Clin N Am 2003;17(1):59–85. Reprinted with permission from Elsevier Limited)

same patient. The lesions may also be graded according to severity, which may help clinicians plan a patient's therapy (Table 22.2) [7, 13, 28]. Initially, the mild form lesions are asymptomatic, but with time itching becomes the predominant symptom of the disease, which in the moderate forms is intense and may be accompanied by local pain. As severity increases, edema and bacterial secondary infections may lead to limitation or incapacity to continue working. Because the CBM lesions are very pruritic, it is believed that disease dissemination usually occurs by autoinoculation and contiguous lymphatic spread [9, 13]. However, lymphatic dissemination has been reported in a few cases. In very advanced cases, chronic lymphedema, ankylosis, and malignant transformation may occur [7, 37, 38].

Diagnosis

As CBM lesions have a diverse morphology leading to an equally diverse differential diagnosis, this disease must always be confirmed by mycological exams (direct examination and culture) and/or histopathology [7].

Muriform cells may easily be observed on direct exams of crusts, skin scrapings, aspirates, debris, or tissue fragments taken from the lesions after potassium hydroxide (KOH) (10–40%) digestion. The sensitivity of the direct examination ranges from 90 to 100%. Fungal elements are easily found at the lesional surface, resembling "black dots" (which are small hematic crusts with cellular debris and fungal structures), resulting from their transepithelial elimination (Fig. 22.1). Occasionally, near the cutaneous surface, these muriform structures may germinate and undergo dimorphic transformation into filamentous fungal forms [39, 40].

In histologic sections, CBM lesions are characterized by pseudoepitheliomatous epidermal hyperplasia, hyperkeratosis, irregular acanthosis, alternating with areas of atrophy and collections of inflammatory cells forming epidermic abscesses. Granulomatous reaction with different grades of fibrosis can be found at the dermal level. Muriform cells may be observed among these structures or inside Langerhans giant cells (Fig. 22.3) [41].

In culture, all the CBM agents are slow-growing dark fungi. Initially, the colonies are deep green, developing a velvety dark aspect on their surface with time. Traditionally, their correct identification has been made through microscopic examination of the asexual reproductive structures like conidiophores, conidiogenous cells, and conidia (Fig. 22.3) [7].

However, advances in molecular taxonomy have shown that sequencing of specific genes is necessary for the correct identification at the species level [21, 22].

Therapy

Over the century that has passed since Max Rudolph first reported the disease, several therapeutic regimens have been proposed, including physical therapeutic methods and chemotherapy with antifungals (Table 22.3). As comparative trials on this disease are lacking, evidence that helps to select optimal therapy is based on a few open clinical studies and many expert opinions. Though no "gold standard" therapy for CBM has been identified, several treatment options are available. Except for its initial localized lesions, which may be treated with surgical excision, most of the CBM clinical forms require long-term continuous systemic antifungal therapy. The duration of therapy may range from months to years and even more than one decade.

Based on noncomparative trials, patients are mostly treated with itraconazole 200–400 mg per day, terbinafine 500 mg per day, or with a combination of both drugs in refractory patients. The combination of itraconazole (200–400 mg daily) with 150–200 mg/kg per day of 5-flucytosine may also be successful in recalcitrant cases [42–49]. There are several

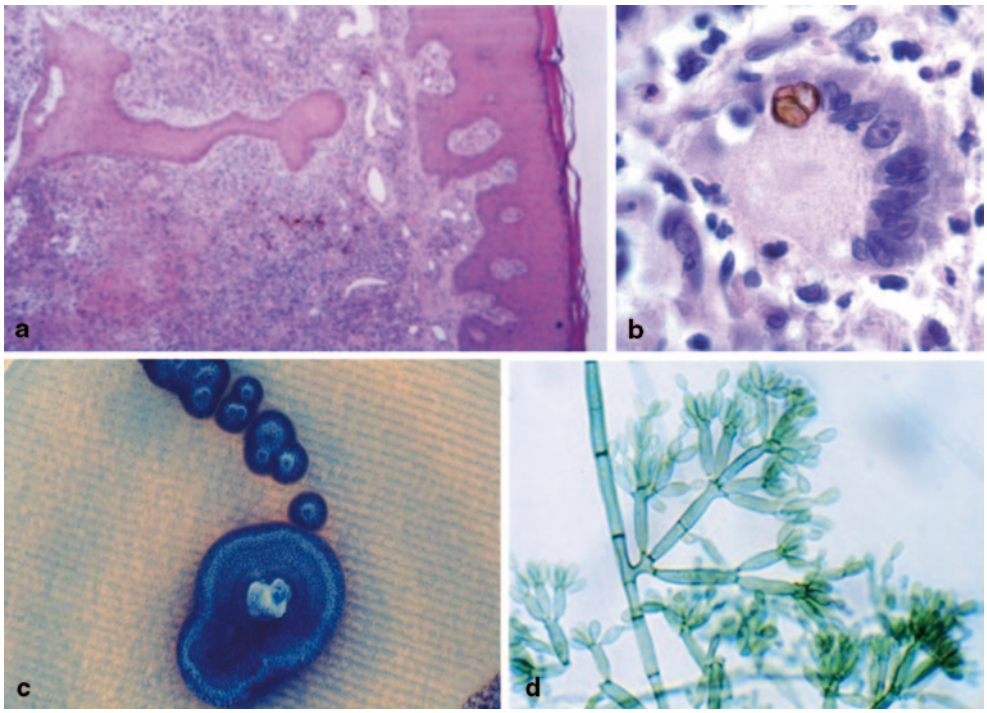

Fig. 22.3 Skin biopsy of patient with chromoblastomycosis with hyperkeratosis, acanthosis and pseudoepitheliomatous epidermal hyperplasia. The derma depicts a mixed inflammatory reaction containing granulomata and abscesses. HE × 100 (**a**). Granuloma detail showing a Langhans giant cell with a muriform cell. PAS × 1000 (**b**). Melanized colony on Sabouraud dextrose agar (**c**) and microscopic morphology of *Fonsecaea pedrosoi* conidiogenesis. Slide culture × 600 (**d**). *Courtesy of Vania Aparecida Vicente PhD, Curitiba, Brazil. HE Histology, PAS periodic acid–Schiff

reports indicating that physical methods, including photodynamic or thermo therapy (topical cold or heat), may shorten the needed duration of systemic antifungal treatment [50–55]. It is important to emphasize that physical methods must be used in combination to antifungal drugs. Among the new triazolic drug, posaconazole has achieved favorable results in severe refractory cases of CBM, while voriconazole and isavuconazole have not been effectively tested [56, 57].

Mycetoma

The term mycetoma derives from the Greek terms *mykes* (fungus) and *oma* (tumor). The disease was first mentioned in *Atharva Veda,* an ancient Indian religious book, as *pada valmikan,* which means foot anthill. The oldest known case of mycetoma most likely dates from the Byzantine period, as evidence was found in a human skeleton based on the bone morphological characteristics [58]. However, the disease was first described by John Gill in a dispensary report of the Madras Medical Service of the British Army in India in 1842, where it was named "Madura foot," as many cases originated from the city of Madurai [59]. The first description of mycetoma in the medical literature was given by Godfrey in 1846, who called it *morbus tuberculosis pedis* [60]. The fungal nature of the disease was established by Vandyke Carter, who also established several features, including its natural history, the body parts that are most commonly affected, the grain colors, the presence of bone destruction, and the higher incidence among males [61]. It was also named mycetoma

Table 22.3 Treatment options for chromoblastomycosis

Physical methods	Chemotherapy	Combination therapy
Standard surgery[a]	Itraconazole[b]	Itraconazole + terbinafin[b]
Photodynamic therapy[c]	Terbinafine[b]	Itraconazole + 5-fluorocytosine[d]
Cryotherapy[c]	Posaconazole[d]	Itraconazole + cryotherapy[d]
Iontophoresis[e]	Amphotericin B[e]	Terbinafin + cryotherapy[d]
Moh's surgery[e]	5-fluorocytosine[e]	Itraconazole + photodynamic therapy[d]
CO_2 laser[e]	5-fluorouracil[e]	Itraconazole + imiquimod[d]
Local heat (dry)[e]	Thiabendazole[e]	
	Calciferol (Vit D3)[e]	
	Ketoconazole[e]	
	Fluconazole[e]	

[a] For initial lesions only
[b] Most used therapy—References: [3–5], [7, 12, 20, 23, 73, 76, 79, 80]
[c] Used only in association to systemic antifungals
[d] Used for refractory forms—References: [6, 36–38, 83, 84, 85]
[e] Not used or not a first-line therapy or abandoned therapy

in 1860, which is the term by which it is currently known. In 1913, Pinoy subdivided the disease as a function of its bacterial or fungal etiology into two categories, actinomycetoma and eumycetoma, respectively [62].

Mycetoma is known under various names, such as fungus disease of India, Godfrey and Eyre's disease, endemic degeneration of the bones of the feet, *morbus pedis entophyticus-affection singulière,* and perforating ulcer of the foot.

Mycetoma has long been a neglected infection because of its rarity and geographical isolation in poor communities. In 2013, the World Health Organization (WHO) accepted the disease as the first fungal neglected disease, and this will promote substantial improvements in care for these stigmatized patients [63–65].

The true incidence of mycetoma is unknown due to its slow and chronic natural history and late presentation. Nevertheless, together with sporotrichosis, eumycetoma is the subcutaneous mycosis most frequently described. It exhibits global distribution, being most common in tropical and subtropical countries between the Tropic of Cancer and the Tropic of Capricorn. While actinomycetoma occurs more frequently in Central and South America, eumycetoma is most commonly found in Africa and in India [66, 67]. Approximately, 60 % of the cases of mycetoma worldwide are caused by filamentous bacteria, while the remainder of the cases are caused by fungi. However, there are regional variations, and the rate of eumycetoma in Yemen is 73 %. In fact, the prevalence of eumycetoma is high in the so-called mycetoma belt, which extends between latitudes 15° South and 30° North and includes Sudan, Mauritania, Somalia, Senegal, Egypt, Nigeria, Niger, Kenya, Ethiopia, Chad, Cameroon, Djibouti, India, and Yemen. In addition, the disease is endemic in Mexico, Venezuela, Colombia, and Argentina, and cases may also be found in temperate countries, such as Mediterranean countries, including North Africa, Greece, and Italy [2, 4, 5, 68]. Cases affecting travelers to endemic areas are frequently reported. The areas with high prevalence of mycetoma are fairly arid and have a short rainy season, lasting 4–6 months, annual precipitation of 50–1000 mm, relative humidity of 60–80 %, and a constant temperature of 30–37 °C during the night and day. The rainy season is followed by a dry one that lasts 6–8 months, with 12–18 % relative humidity, a daytime temperature of 45–60 °C and a nighttime temperature of 15–18 °C. Such extreme temperature variation might be a requisite for the survival of the etiologic organisms in their natural niches. Within the mycetoma belt, the largest number of cases occurs in Sudan, where the disease is particularly endemic and highly disabling. According to some studies, 300–400 new cases occur in Sudan every year [65, 69, 70].

Mycetoma is also highly endemic in Mexico, with an average of 70 new cases per year, most of them caused by bacteria. The ecology of the various etiological agents of mycetoma might be currently changing in the Americas, as in Brazil the ratio of actinomycetoma to eumycetoma is 1:1. In Argentina, most cases are classified as eumycetoma. Ecological factors clearly determine the geographical distributions of the etiological agents. However, the considerable displacement of large populations and the ease with which people currently move from one place to another also contribute to the changes in the pattern of disease [2, 4, 66]. A few cases of mycetoma have been reported in the USA [70].

Mycetoma more frequently affects individuals who encounter frequent and direct contact with the soil, especially those in rural areas, such as farmers and herdsmen. That fact notwithstanding, the disease is not exclusive to those individuals, as cases among urban workers, homemakers, travelers, members of humanitarian organizations, and archeologists have also been reported [63–66, 71].

The disease is known to mostly affect men, most likely as a function of their greater involvement in rural activities. Most affected women are also rural workers. These facts notwithstanding, a recent study suggested that the progesterone levels exhibited by women might inhibit the growth of some etiological agents of mycetoma, such as *Madurella mycetomatis*.

Mycetoma occurs more frequently among individuals aged 20–40 years, whereas in endemic areas, it might also affect children and older adults [2, 4, 63, 66].

Etiology

More than 30 different species of fungi have been associated with eumycetoma, some of which are able to cause a broad range of diseases, including PHM and CBM [2, 58, 66, 72].

The etiological agents of mycetoma depend on factors such as temperature, precipitation, type of soil, vegetation, and the demographic characteristics of the susceptible population. These agents are classified according to the color of the grains, which might be black, yellow, or white. The grains are also known as sclerotia and consist of aggregates of fungal hyphae embedded in a hard concrete-like matter [58, 71].

Table 22.4 summarizes the main etiological agents of eumycetoma. More than 90 % of cases of eumycetoma reported worldwide are caused by just four agents: *Madurella mycetomatis, Madurella grisea, Leptosphaeria senegalensis,* and *Pseudallescheria boydii,* the former three being melanized fungi and the latter a hyaline one [7, 58].

These organisms' distribution exhibits regional variation. *M. mycetomatis* and *L. senegalensis* are the most common pathogens in Africa. *Acremonium* spp. and *M. grisea* occur frequently in South America, but not in African dry areas. Although *M. grisea* is the most frequent of these organisms in Argentina, the prevalence of *P. boydii* and some species

Table 22.4 Main features of eumycotic and actinomycotic grains

Eumycetoma[a]	Fresh examination	Histology (HE)
Scedosporium apiospermum	<2 mm, yellowish or white, soft, oval to lobed, "fig seed like"	Compact, no cement, interwoven hyaline hyphae <5 μm and swollen cells <20 μm, eosinophilic border
Acremonium kiliense	<1.5 mm, white, soft, irregular shape	Compact, no cement, hyaline hyphae <4 μm, swollen cells <12 μm
Aspergillus nidulans Fusarium moniliforme	<2 mm, white, soft, oval to lobed	Compact, no cement, interwoven hyaline hyphae <5 μm, eosinophilic border
Neotestudina rosati	White to brownish, soft, <1 mm, fragmented angulated mass	Cement and swollen cells at periphery embedded in cement and some central vesicles
Madurella mycetomatis	<2 mm, black, firm to brittle (coal consistence), oval to lobed	*Compact type*, with brown-staining cement *Vesicular type*, with hyaline center and brown-staining cement and prominent <15 μm at edge
Madurella grisea	<1 mm, black, soft to firm, oval to lobed	Little brownish cement and polygonal cells at the periphery and central hyaline hyphae
Exophiala jeanselmei	<0.5 mm, black, soft, irregular to vermicular	No cement, hollow center, with melanin-pigmented vesicular cells <10 μm associated to short hyphae <4 μm
Leptosphaeria senegalensis	1 mm, black, soft, irregular shape	Cement in outer zone, dark periphery with hyaline vesicular center
Pyrenochaeta romeroi	<2 mm, black, firm to brittle, oval to lobed	Brownish cement at periphery, no vesicles
Actinomycetoma[b]		
Nocardia brasiliensis	<0.5 mm, white, soft, irregular	Small, basophilic stained fringe in layers, homogenous loose clumps of bacterial filaments and rare clubs. Positive Gram and Kinyoun stains
Actinomadura madurae	5 mm, yellowish to pink, oval to lobed	Anamorphous empty center with a dense basophilic or pink border associated to loose fringe and clubs. Gram positive
Actinomadura pelletieri	<1 mm, red, hard, oval to lobed	Homogenous dark staining with light periphery and no clubs. Easily fractured. Gram positive
Streptomyces somaliensis	<2 mm, yellow, hard, round to oval	Amorphous center amorphous center with basophilic layers associated to pink patches and dark bacterial filaments at the edge and no clubs. Gram positive

[a] Other eumycetoma agents: *Acremonium falciforme, A. recifei, Aspergillus flavus, Leptosphaeria tompkinsii, Pyrenochaeta mackinnonii, Curvularia geniculata, C. lunata, Fusarium solani, F. oxysporum, Pseudochaetosphaeronema larense, Exserohilum rostrata*, etc.
[b] Other actinomycetoma agents: *Nocardia asteroids, N. caviae, N. farcinica, N. dassonvillei*, etc.

of *Acremonium* are also considerable. In India, the most frequent agent is *M. grisea,* followed by *M. mycetomatis, Acremonium* spp., and *Medicocopsis* (*Pyrenochaeta*) *romeroi. M. mycetomatis* exhibits the largest global distribution and is predominant in Eastern Africa, especially Sudan. It is also the main etiological agent of eumycetoma in Yemen, Morocco, Tunisia, Saudi Arabia, and Senegal. In Western Africa, *L. senegalensis* stands out. Hyaline fungi are less frequently mentioned in the reported series; however, *P. boydii* was the most common agent found in North America and Iran, and white grains are most frequently found in the UK. Conversely, no eumycetoma with white grains has yet been reported in Chad or Djibouti [1, 2, 63–68, 73].

Pathogenesis

The factors involved in the onset of the disease are inoculum size and host immune response, in addition to the possible participation of hormones. The pathological process usually begins when the infecting agent is traumatically introduced into the host tissue through the skin, which can be caused by thorny leaves, acacia or cactus thorns, wood splinters, sharp stones, agricultural implements, knives, fish scales, or other contaminated objects [65, 74, 75].

Although trauma is the mechanism currently accepted to account for the inoculation of the infecting agent, in some cases, the skin injury might go unnoticed. The incubation period is variable and has not yet been well established; it might last from weeks to years as a function of the etiological agent involved and the host immune response. Following its introduction into the host, the fungus might exhibit local dissemination from the inoculation site to the muscles or bones, especially spongy bones, such as those in the ankles, wrists, and vertebrae, for which the fungi exhibit particular affinity. These normally weakly pathogenic organisms grow and survive through the production of grains, which are structures composed of masses of fungal mycelium and a matrix component [4, 72, 74]. The matrix material has been shown to be host-derived. The hyphae often have thickened cell walls and matrix material toward the periphery, potentially conferring protection against the host immune system. Grains are observed in histopathology within abscesses containing polymorphonuclear cells. Complement-dependent chemo-

taxis, activated by polymorphonuclear leukocytes, has been shown to be induced by fungal antigens (*M. mycetomatis* and *P. boydii*) in vitro. Cells of the innate immune system attempt to phagocytize and inactivate these organisms but fail to accomplish this goal, which is likely attributed to the failure of the neutrophil response in the individuals who develop the disease [58, 76].

The role of the immune system in the pathogenesis of mycetoma has not yet been elucidated. Several studies showed that although many residents in endemic areas develop anti-*Madurella mycetomatis* antibodies, only a low percentage of them develop the disease. For that reason, the role of innate immunity in the host resistance to fungi causing mycetoma has been quite frequently investigated in vitro and in animal models but rather rarely in human beings. The host local response, characterized by chemotaxis of neutrophils and small vessel congestion, is nonspecific. Later on, macrophages and monocytes with microbicide properties that are activated by cytokines, interferon-γ, and tumor necrosis factor-α arrive at the site of infection. Three types of immune responses have been described in response to the etiological agents of mycetoma [76]. The *type I response* is observed as neutrophils degranulate and adhere to the grain surface, leading to gradual disintegration of the grain. The *type II response* is characterized by the disappearance of neutrophils and the arrival of macrophages to clear grains and neutrophil debris. *The type III response* is marked by the formation of epithelioid granulomas to contain the infecting organism. Those host responses do not appear to be able to control infection but likely account for the partial spontaneous healing that is observed in the disease [76].

The disease might have multifactorial etiology as a function of the heterogeneity exhibited by the factors determinant of human and animal susceptibility. Only a rather small number of residents in endemic areas develop the disease, although the full population is exposed to the same risk factors, such as walking barefoot and being scratched by thorns. The fungi likely have a saprobiotic life in the soil, and repeated inoculations of small amounts of fungi might induce sensitization and increased susceptibility to infection.

Clinical Manifestations

In most cases, the disease begins as a single, small, painless, and slow-growing subcutaneous nodule, which is usually round and firm but might also be soft, lobulated, or, more rarely, cystic. The nodule slowly increases in size, while secondary nodules are formed, which become fixed to the underlying tissue and ultimately develop sterile and deep-seated draining sinus tracts beneath the lesions. These tracts open to the surface and drain purulent, serous or serosanguinous material with grains. The grains measure several millimeters in diameter, and their color and consistency vary as a function of the etiological agent involved. The grains might be found in drained pus and in the tissue around the sinus tracts [63, 66–68]. The grains are visible to the naked eye or under a microscope. The morphological characteristics and color of the grains—which might be black, brownish, white, yellow, red, or a mixture of colors—contribute to their identification. The draining sinus tracts might take weeks, months, or even years to form. The diagnosis of disease is based on the clinical triad of *tumefaction, draining sinuses,* and *grains* (Fig. 22.4).

The disease can affect the skin, subcutaneous tissue, and eventually the underlying bones, disseminating across fascial planes. The skin looks soft and shiny, is usually fixed to the underlying tissue, and can exhibit hyper- or hypopigmentation with local hyperhidrosis [1, 2, 4–6]. The affected individuals might also exhibit ulceration with honey-like crusting and scars. Swelling is usually firm and painless, while the overlying skin is not erythematous. Muscles, tendons, and nerves do not usually suffer from direct infection, but extensive local damage may cause muscle wasting, bone destruction, and deformities in the limbs secondary to bone invasion and resulting osteomyelitis. As a late and rare occurrence, lesions might affect nerves, tendons, and even organs such as

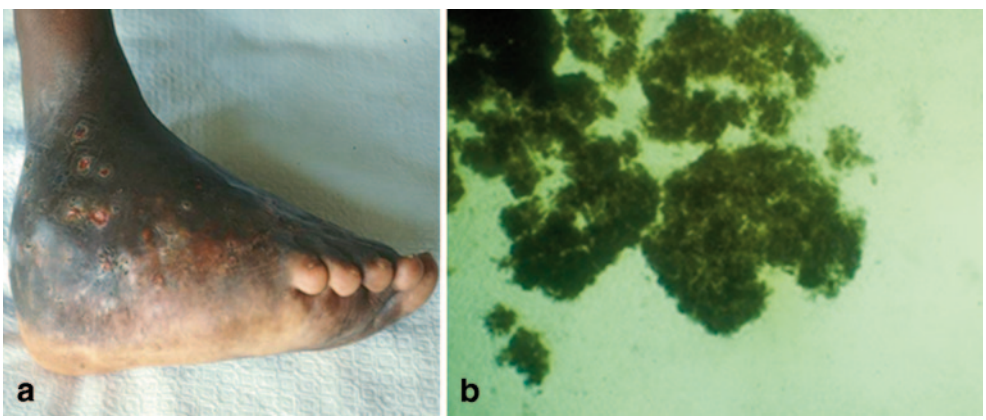

Fig. 22.4 Advanced mycetoma foot caused by *Exophiala jeanselmei* (**a**) and micromorphological aspects of dark pigmented masses of pigmented grains produced by *E. jeanselmei* (**b**). (*From Queiroz-Telles F, et al. Subcutaneous mycoses. Infect Dis Clin N Am 2003;17(1):59–85. Reprinted with permission from Elsevier Limited)

the lungs and peritoneum, as observed in cases of mycetoma affecting the trunk per contiguous dissemination. There are also reports of infection of the vertebral bodies, also due to contiguous dissemination, resulting in spinal cord compression. Although patients do not usually complain of pain, but rather report itching or burning, pain is the reason patients seek medical care in 20% of cases. It has been suggested that mycetoma may produce anesthetic substances within the lesions. In later stages, pain is usually due to nerve damage caused by intense fibrotic reaction, endarteritis obliterans, or neural hypoperfusion. Pain may also be caused by bone invasion, rupture of the abscesses formed by the sinus tracts or secondary bacterial infection [63–68].

On occasion, the disease exhibits alternative clinical patterns of progression, such as minimycetoma or the sporotrichoid pattern. Minimycetoma is characterized by a lack of tumefaction and a small number of sinus tracts, and although it has been found in children and young adults, it is a much more common finding in cases of actinomycetoma. Regional lymph nodes may be primarily involved or secondarily involved because of bacterial infection. As a consequence, chronic lymphedema may occur due to lymphatic obstruction and fibrosis. Contiguous lymphatic spread occurs quite rarely, being found in only 1–3% of cases, and it might appear after surgical treatment. No cases of hematogenous spread have been documented. Both the disease and its effects are usually localized, and thus, patients do not exhibit signs or symptoms of systemic infection, except in cases with secondary bacterial infection. Untreated mycetoma continues its progression, while bacterial superinfection might lead to local abscess formation, cellulitis, and bacterial osteomyelitis [63, 67, 70].

From the topographic point of view, the fungi tend to affect the body areas that enter in direct contact with the soil or plants, mainly the feet (70–80% of cases), followed by the legs and the hands. In Mexico, the back is affected in 20% of the cases, especially in rural workers who carry logs and hay on their backs. Other possible sites include the knees, arms, neck, thighs, and perineum, while there are a few reports of infection of the eye lids, scrotum, paranasal sinuses, orbits, vulva, external auditory meatus, old surgical wounds, skull, abdomen, and chest, which may or may not be associated with immunosuppression [66–68].

The differential diagnosis includes foreign body granulomas and benign or malignant soft tissue neoplasms, such as squamous cell carcinoma, lipoma, fibroma, fibrolipoma, sarcoma, melanoma, and cystic lesions. Minimycetoma may be confused with folliculitis and other mycoses. Mycetoma with an ulcerative pattern of proliferation might resemble epithelioma or melanoma. Lesions without draining sinus tracts may mimic PHM, hyalohyphomycosis, sporotrichosis, CBM, and basidiobolomycosis. Additionally, leishmaniasis, cutaneous and osseous tuberculosis, botryomycosis, and chronic bacterial osteomyelitis should be taken into consideration [1, 2].

Diagnosis

Several approaches might be used for the diagnosis of mycetoma, including morphological, immunological, molecular, and imaging methods. Morphological studies seek to identify the genus and species of the etiological agent and include direct examination, culture, and histopathology. The assessment of spontaneously drained grains might not be useful for diagnostic purposes, as they might contain dead organisms frequently associated with contaminating bacteria. In addition, these fungi are ubiquitous in nature and specimen contamination can occur. For that reason, a fine-needle aspiration of the draining sinus tract content is recommended for sample collection. This procedure is easy to perform and affords the material needed for culture, direct mycological examination, histopathology, and molecular techniques to identify the etiological agent [63, 72].

For the purpose of direct mycological examination, two grains should be crushed using two glass slides, and the content should be examined using 10% KOH, Gram stain, modified Kenyon stain, and lactophenol blue, which allow scientists to distinguish between thin or thick filaments and to identify other organisms such as filamentous bacteria.

Culture of the grains should be performed on Sabouraud agar or brain–heart infusion media. Ideally, 20–30 grains should be collected, washed several times with sterile saline solution with antibiotics or 70% alcohol, crushed with a sterile glass rod, and then seeded in the appropriate culture medium. Additionally, media containing cycloheximide or antibiotics (gentamycin or chloramphenicol) might be used, bearing in mind that some organisms, *P. boydii* in particular, are inhibited by them. The organisms that cause mycetoma grow slowly; thus, they should be grown for a minimum of 6–8 weeks and ultimately for 12 weeks [63, 68, 73]. Once the etiological agent is isolated, it should be subjected to the identification by microscopic morphology and gene sequencing (if available).

Histopathology reveals abscesses surrounded by inflammatory reaction, pseudoepitheliomatous hyperplasia, abundant granulation, and fibrotic tissue. The grains are observed at the center of the abscesses as masses of hyphae with filaments larger than 1 μm embedded in the intercellular cement. Special staining techniques such as Grocott's, periodic acid–Schiff (PAS) and hematoxylin and eosin (H&E) may help to distinguish the grain's etiology. Fontana-Masson stain specifically detects melanin and may be used in the assessment of black grains. When H&E is used, eumycetoma grains usually present a pale pink periphery. The grains are surrounded by a basophilic band, while its center is usually

intensely basophilic and disorganized, containing hyphae (Fig. 22.4).

Immunological methods, such as immunodiffusion, counterelectrophoresis, and immunoenzymatic assays, are only available and used in research centers.

Imaging methods are useful to assess the extent of disease. Simple radiographs might disclose early lesions, which appear as soft tissue granulomas. The findings associated with the progression of disease include variable periosteal reaction, bone deformation, osteoporosis, and multiple bone cavities. Ultrasound is useful in the case of mycetoma exhibiting cavities with thin walls without acoustic resonance, as well as to assess the extension of lesions. The so-called dot-in-circle sign is considered a characteristic radiological finding in mycetoma, and it may be identified by ultrasound or magnetic resonance imaging (MRI; Fig. 22.4).

Ultrasound is especially useful in the diagnosis of lesions lacking draining sinus tracts. The sensitivity of computed tomography for the detection of early bone changes is higher than that of MRI. The latter exhibits particular sensitivity for the detection of small lesions with the low-intensity signal on T1- and T2-weighted images, which correspond to products of the grains' metabolism. The detection of grains on MRI depends on various factors, including the size of the grains, the quality of the images, and the device parameters [77, 78].

Treatment

Amputation of the affected limb or multiple mutilating excisions were the only therapeutic approaches available in the past. No cases of spontaneous cure have been reported in the medical literature. Although pharmacological means are used, they have limitations in this disease. Eumycetoma is more resistant to pharmacological treatment than actinomycetoma, and exhibits high recurrence rates (20–90 %). In addition to the risk of recurrence, the pharmacological treatment of eumycetoma is associated with several side effects, high dropout rates, disfigurement, and disability. Factors influencing the effectiveness of pharmacological treatment include the etiological agents' susceptibility to the drug used, the drug concentration achieved at the lesion site, the localization and size of the mycetoma and patient adherence to treatment, tolerance to the prolonged therapeutic regimen, and host's immune condition [70, 79–81].

Lacaziosis

Lacaziosis, also known as lobomycosis or Lobo's disease, is a chronic infection that affects skin and subcutaneous tissues, caused by *Lacazia loboi*. *L. loboi* has previously been denominated *Paracoccidioides loboi* and *Loboa loboi*. Lacaziosis was first described in Brazil by Jorge Lobo in 1931 [82, 83]. To date, *L. loboi* has never been isolated in artificial culture media. *L. loboi* forms globular yeast-like cells, with refringent, thick cell walls, measuring 5–6 × 12–14 μm, which reproduce through simple budding. Several yeast cells may be linked assuming a catenular aspect (Fig. 22.5) [84].

Epidemiology

Lacaziosis is a disease which occurs almost exclusively in South America's Amazon area; affecting mainly men who work in forested areas or farmers. There are reports of this mycosis in dolphins off the US coast, France, south of Brazil, and the estuary of rivers in Suriname [85–89].

Lacaziosis affects, preferentially, persons between 29 and 40 years of age. In an analysis of publications up to 2006, Brito and Quaresma described 490 cases, noting 318 occurred in Brazil [84].

Clinical Manifestations

Patients with lacaziosis may present with a wide spectrum of cutaneous lesions. They may be localized or disseminated, usually slow growing. The reported clinical aspects or lesions types include nodules, papules, dyschromic macules, gummas, verruciform plaques, ulcers, scarring lesions, or keloid-like nodules. The pinna is affected frequently. Mucosal involvement has not been described (Fig. 22.5). The lesions may progress to ulceration and may present with bacterial superinfection. Usually lacaziosis lesions do not interfere with patient's general health status [2, 5, 83, 90].

Diagnosis

Diagnosis is made through direct visualization of the fungus—round, thick-walled yeast, in chains of 2–10 (Fig. 22.5). Material for diagnosis can be obtained through biopsy for direct observation and histopathological examination. Histopathological examination typically reveals a granulomatous infiltrate filled with histiocytes and with a great number of yeast cells in clusters inside the cytoplasm of multinuclear histiocytes. The granuloma may be restricted to the dermis, surrounded by delicate fibrous septa [1, 2, 91].

Treatment

Excision of lesions, particularly single lesions, may result in cure. The use of cryotherapy has also produced good results.

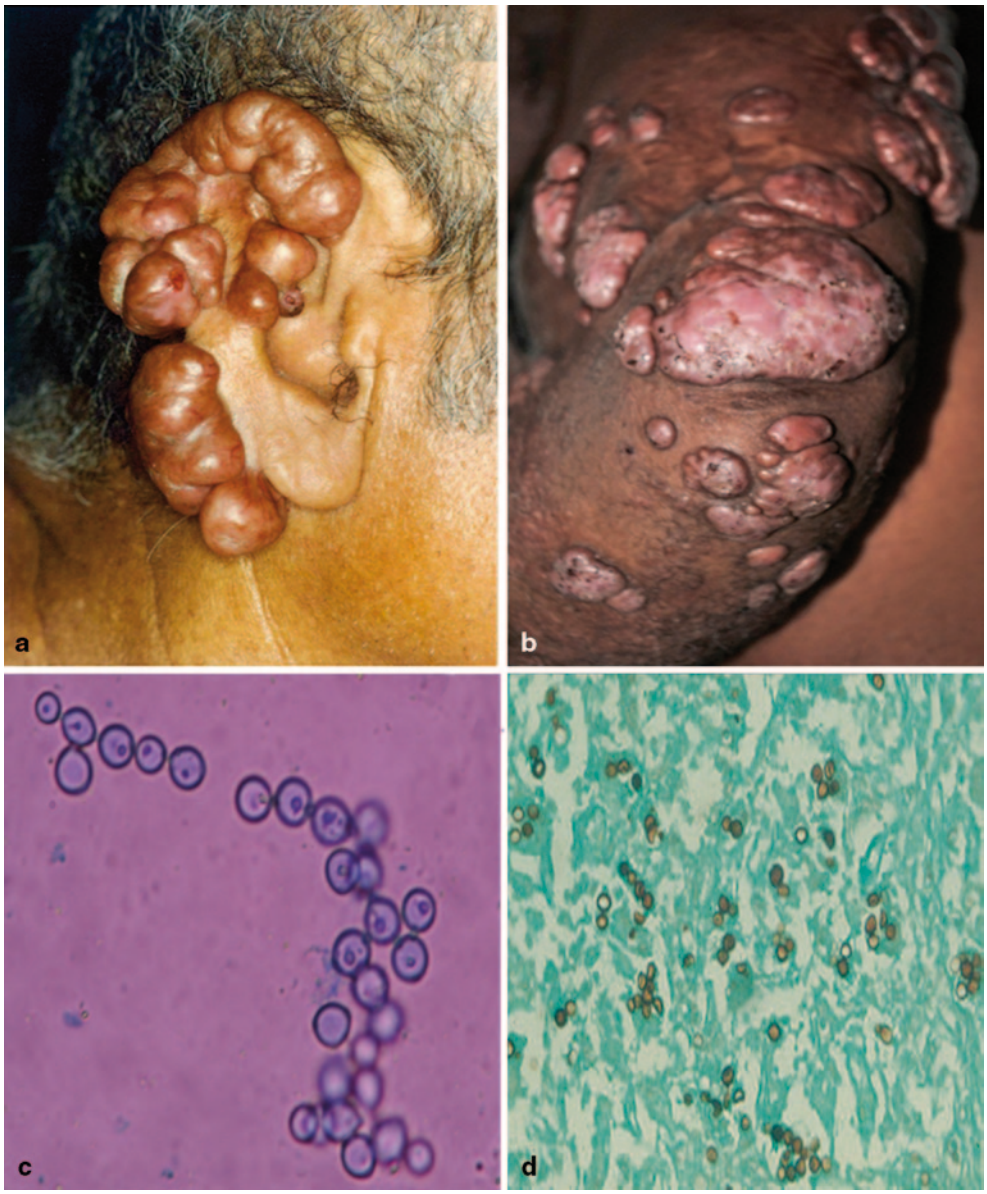

Fig. 22.5 Clinical aspects of lacaziosis (**a** and **b**). Direct examination of scrapings showing catenular budding of Lacazia loboi (**c**). Histologic section of skin biopsy of a patient with lacaziosis depicting several *L. loboi* yeast forms. GMS (Gcrocott methanamine stain) × 200 (**d**).

Lacaziosis is usually recalcitrant to antifungals and drugs used successfully in deep mycosis have not produced satisfactory results in lacaziosis [1, 2]. Clofazimine combined with itraconazole has been reported to produce clinical and histopathological remission of the disease in one treated patient after 1 year of therapy [92]. In another case report, posaconazole achieved reduction of the size of lesions in one patient treated with this drug [93].

Entomophthoramycosis

This rare mycosis, also known by several other names (Table 22.1), caused by fungi belonging to the phylum Glomeromycota (formerly Zygomycota), order Entomophthorales, was first described as a human disease by Paltauf in 1885 [94]. The Glomeromycota include two orders of fungi which cause human infection, the Mucorales and Entomophthorales. The order Mucorales contains the fungi which cause human mucormycosis (see Chap. 13), including the genera *Rhizopus* and *Mucor* [95, 96]. The order Entomophthorales includes three species which cause human infection, *Conidiobolus coronatus, C. incongruous,* and *Basidiobolus ranarum*. Infection secondary to these fungi is called entomophthoramycosis. Infections caused by *C. coronatus* and *C. incongruous* are also called conidiobolomycosis, while basidiobolomycosis is an alternative name for infection secondary to *B. ranarum* [96].

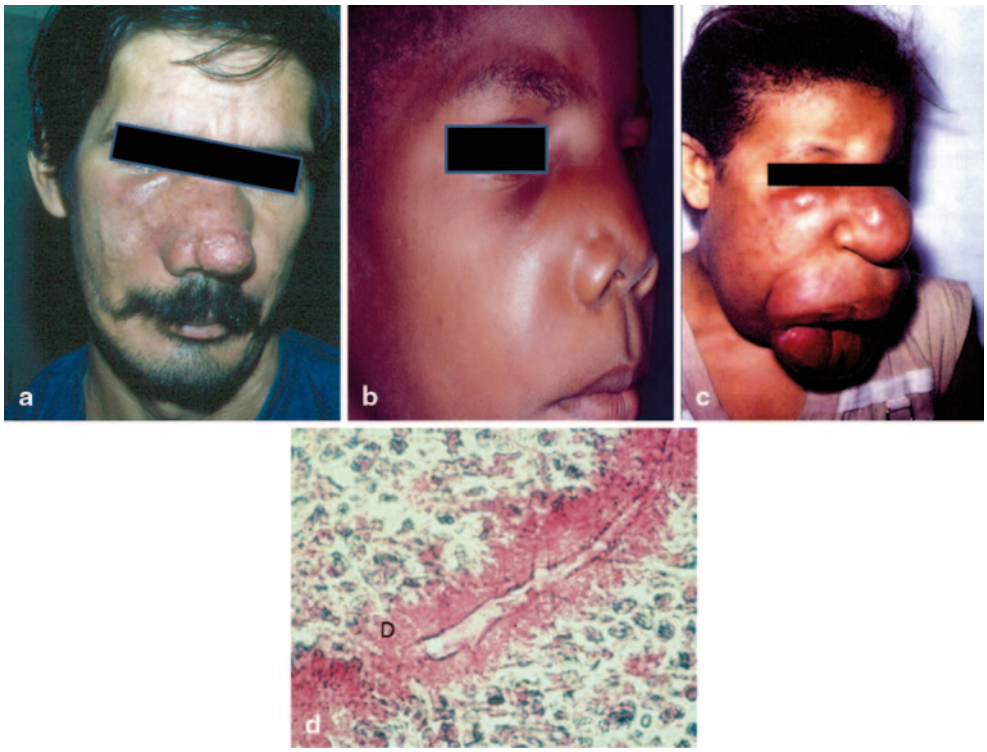

Fig. 22.6 Clinical aspects of patients with entomophthoramycosis (**a**, **b**, and **c**). Histologic section showing poorly septated hyphae of Entomophthorales. It depicts a thin nonparallel cell wall surrounded by eosinophilic material (Splendore-Hoeplli reaction) (**d**). *Courtesy of Angela Restrepo PhD. Medellin, Colombia **Courtesy of Jackson Mauricio M, D., Sao Luis, Brazil

Epidemiology

Basidiobolus spp. have been isolated from decaying plants, insects, the digestive tract of amphibians, reptiles, horses, dogs, and chimpanzees. It has a worldwide distribution and has been described in all continents, although prevalence is higher in tropical and subtropical climates. Africa has the highest number of reported cases, being identified in Uganda, Sudan, Nigeria, and among others. Disease can affect any age, but most often presents in children, and most commonly affects males [97]. *Conidiobolus* spp. are soil saprobes which are found in tropical areas, and may infect horses. These fungi have been isolated in the UK, the USA, India, and Africa [98].

Clinical Manifestations

Entomophthoramycosis is much less common than mucormycosis and is restricted to the subcutaneous and submucosal tissues. In basidiobolomycosis, infection most commonly affects the trunk and limbs. Disease starts with an isolated erythematous nodular lesion that with time may be raised and may transform in cellulitis which quickly develops a central area of necrosis with well-defined edges. These nodular lesions become confluent and in some patients there may be multiple lesions [99]. The lesions can spread to reach muscle, bone, or lymph nodes, and more rarely, involve adjacent organs. Disease is slowly progressive. Conidiobolomycosis affects the nasal mucosa or paranasal sinuses, and spreads to the skin of the nose, cheek, upper lip, and pharynx (Fig. 22.6). These lesions are nodular, indolent, associated with diffuse edema, and may affect the entire upper face, with subsequent disfigurement. Basidiobolomycosis has also been reported as a rare cause of gastrointestinal disease with clinical manifestations similar to those of inflammatory bowel disease. In adults, gastrointestinal *B. ranarum* infection may affect normal or immunocompromised hosts. Several cases of gastrointestinal basidiobolomycosis have also been reported in apparently immunocompetent children [100].

Diagnosis

Diagnosis of entomophthoramycosis is based on clinical presentation and histopathological examination of deep lesional biopsy. Culture can be used to diagnose the fungal pathogen.

Histopathological examination shows eosinophil-rich inflammation and fibrosis in the subcutaneous tissue. Aseptate hyphae 5–15 μm in diameter is usually surrounded by eosinophilic material (the Splendore-Hoeppli phenomenon; Fig. 22.6).

The etiologic agents may be recovered by inoculating small pieces of biopsy tissue on Sabouraud glucose agar with chloramphenicol and gentamicin, with or without cycloheximide and potato dextrose agar. The colonies grow rapidly with a shade of cream to gray-green color and produce a waxy, glabrous, and cerebriform surface [99].

Treatment

The treatment of choice for entomophthoramycosis caused by *B. ranarum* is potassium iodide at a dose of 30 mg/kg in single daily dose or divided into three doses daily for 6–12 months. Itraconazole has also been reported to produce good results at a dose of 100–200 mg/day. Itraconazole may also be combined with potassium iodide. Plastic reconstructive surgery may be indicated after treatment, as healing may be associated with fibrosis and deformity of the affected area [101–103].

Suggested Reading

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Entomphtoromycosis

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Instructive Cases

Instructive Case 1

A 57-year-old patient with acute myelogenous leukemia (AML) and a 3-week history of neutropenia developed fever that is unresponsive to broad-spectrum antimicrobial therapy (piperacillin/tazobactam and levofloxacin). High-resolution computed tomography (HRCT) of the lungs revealed several pleural-based nodular lesions. The patient was empirically started on amphotericin B deoxycholate 1 mg/kg every 24 h. After 5 days of therapy, the patient's serum potassium begins to drop but the serum creatinine and blood urea nitrogen (BUN) remain stable.

Questions

1. The decrease in serum potassium is most likely due to what amphotericin B toxicity?
   a. Afferent arteriole constriction in the kidney
   b. Distal tubular damage in the kidney
   c. Suppression of erythropoietin synthesis
   d. Damage of the pancreatic islet cells
2. Which of the following approaches should be used if the patient's serum creatinine doubles and he begins to develop azotemia?
   a. Change amphotericin B dosing to every other day
   b. Change amphotericin B to continuous infusion dosing
   c. Change amphotericin B to a lipid amphotericin B formulation
   d. Begin administering 500 mL of normal saline before and after infusion.

Instructive Case 2

While riding his dirt bike on a vacant lot in southeastern Michigan, a 22-year-old man abraded his right leg on the dirt while rounding a corner. He was taken to the emergency room where the large abrasion was cleaned and debrided. Because there was extensive loss of skin, a skin graft was placed several weeks later. About 2 weeks after the graft was placed, he noted that a few "bumps" had developed in the grafted area. These "bumps" became larger, broke open, and began to discharge what he described as a thin fluid with some pink discoloration. Similar lesions then developed in the thigh proximal to the original injury and graft. Several different antimicrobial agents were prescribed (including those with *Staphylococcus aureus* coverage), but the lesions did not respond, and in fact, new lesions appeared.

When seen by an infectious diseases consultant, the graft was beginning to breakdown and multiple nodules of various sizes were noted in the original abraded area that had been grafted and proximal to the graft into the upper thigh (Fig. 1). Some were ulcerated and weeping serosanguinous fluid, and others were crusted. The nodules were not tender to palpation. The patient felt well and specifically denied having chills or fever. Biopsies were taken of several nodules for culture and histopathological examination. The tissue sections showed granulomas, but no organisms were seen. Within 1 week, the cultures held at 25°C showed growth of an off-white mold that on microscopic examination showed tiny conidia arranged "bouquet-like" on thin hyphae. The diagnosis of sporotrichosis was made and the patient was begun on an experimental protocol using fluconazole (FLZ), 400 mg daily. The lesions began to resolve by 2 months (Fig. 2), and were finally all resolved by 5 months. Therapy was continued for a total of 6 months. No recurrences were noted. The patient was admonished to change hobbies from dirt bike riding to something less dangerous, but he chose to ignore that bit of medical advice.

Instructive Case 3

A 55-year-old Caucasian female was in her usual state of good health until 1 week prior to admission when she developed a nonproductive cough, fever measured at 102°F,

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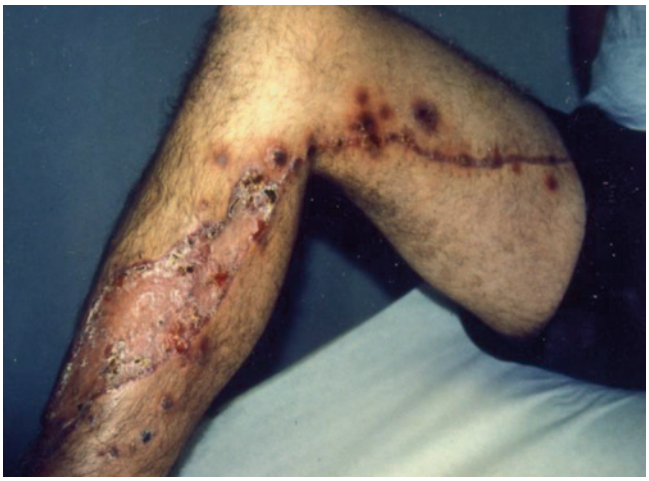

Fig. 1 Poor healing and nodular disease about lower extremity scar prior to antifungal therapy (patient later proven to have sporotrichosis)

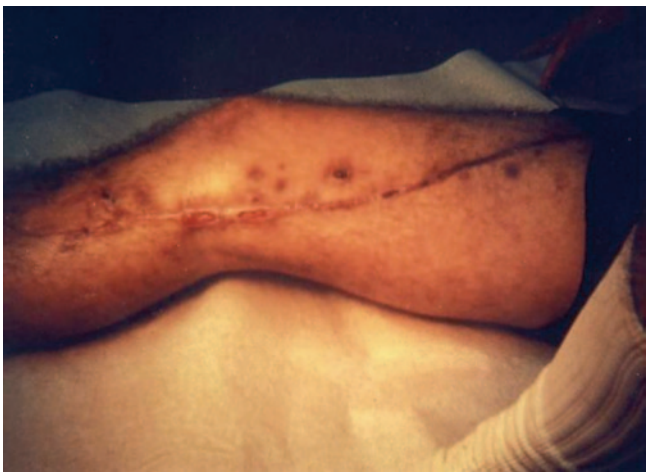

Fig. 2 Improvement of sporotrichosis after 2 months of fluconazole therapy

and a mild headache. Three days prior to admission, she developed dyspnea. She did not have chest pain or a rash. Her past medical history included coronary artery disease with stent placement. Social history included previous tobacco use and recent travel to Palm Springs, California, 3 weeks prior to admission. On physical examination, patient was in mild distress with slight intercostal retraction. Laboratory studies included hemoglobin 12.1 g/dL, white blood count $11.8 \times 10^3/\mu L$, absolute neutrophil count $9.6 \times 10^3/\mu L$, absolute lymphocyte count $0.8 \times 10^3/\mu L$, absolute monocyte count $0.9 \times 10^3/\mu L$, and absolute eosinophil count $0.5 \times 10^3/\mu L$. Chest radiograph revealed diffuse bilateral infiltrates (Fig. 3).

Evaluation led to a diagnosis of community-acquired pneumonia, and patient was admitted and treated with ceftriaxone and azithromycin without resolution of symptoms. All

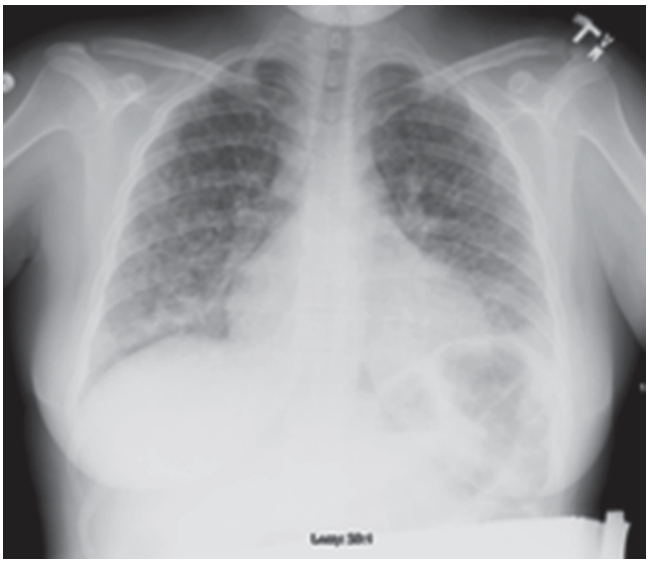

Fig. 3 Chest X-ray

cultures were negative. Pulmonary consult on day 3 was followed by a bronchoscopy on day 4. Bronchoalveolar lavage (BAL) was negative and prednisone therapy was initiated. By day 8, patient had improved clinically, antibiotics were stopped, and she was discharged on prednisone.

The patient was seen as an outpatient on day 14 with continued cough, but without fever, on prednisone taper. On day 21, patient's dyspnea had worsened and the patient presented to the emergency department and was readmitted. Two days later, she was transferred to the intensive care unit (ICU) due to hypoxia. On day 25, the patient developed severe respiratory failure requiring mechanical ventilation. Coccidioidomycosis serology was positive on day 26, and patient expired on day 28.

Instructive Case 4

A 27-year-old male agricultural worker seen in consultation to evaluate an illness of 2 months' duration characterized by the presence of rapidly enlarging lymph nodes in both cervical chains, accompanied by pain, and high fever, especially at nights. One of the nodes had drained, spontaneously producing yellow-tinged purulent material. The patient also experienced productive cough without dyspnea. On examination, he was pale, looked frail, and had enlarged cervical, axillary, and inguinal lymph nodes (Fig. 4). His spleen and liver were normal by physical examination. Lung auscultation did not reveal altered breath sounds, and chest X-rays were normal.

The HIV test was negative, hemoglobin was 8.8 g/dL, and white blood cell (WBC) count was 25,700 cells/μL. A direct

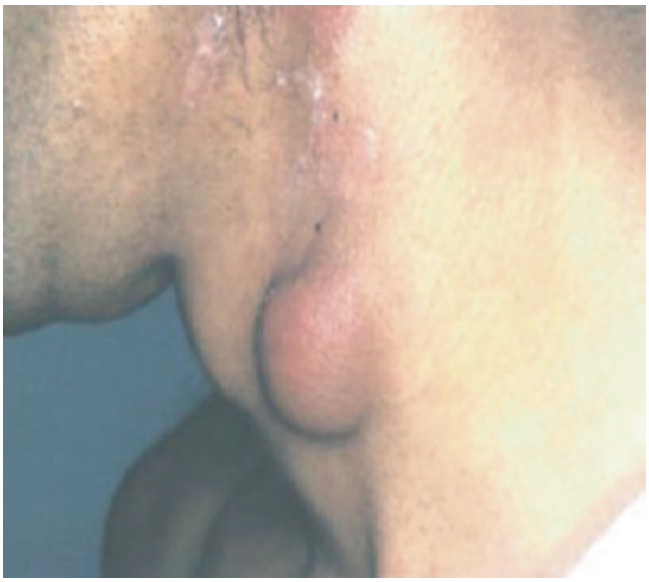

Fig. 4 Paracoccidioidomycosis. Hypertrophied cervical lymph node about to rupture. Scarring lesions of a similar process can be seen above

KOH examination of the discharge from the ruptured nodule showed abundant yeast cells, some with the characteristic multiple budding of *Paracoccidioides brasiliensis*; this fungus was also isolated in culture. Serology with paracoccidioidin proved reactive with one band of precipitate and a complement fixation (CF) titer of 1:1024.

Instructive Case 5

A 70-year-old male with hypereosinophilic syndrome, non-insulin-dependent diabetes mellitus (NIDDM), and long-term prednisone, 25 mg/day × 25 years, was admitted with fatigue, weakness, and shortness of breath. Physician examination was remarkable for a *T* 99°F, an area of erythema over the left thigh that was tender to palpation, and an effusion in the right knee. On admission, he had a hemoglobin of 5.9 g/dL, WBC 8600/mm^3, a serum creatinine 2.7 mg/dL, and a glucose 382 mg/dL. Aspiration of the right knee revealed a WBC of 40,500/mm^3 with 96 % neutrophils, and gram stain revealed gram-negative bacilli and budding yeasts. The patient was initially started on imipenem, vancomycin, tobramycin, and FLZ 400 mg/day.

Within 72 h, the blood cultures drawn on admission were found to be positive for yeast, later identified as *Trichosporon asahii* (*beigelii*). In addition, joint fluid cultures were also positive for *T. asahii* and *Pseudomonas aeruginosa*. Biopsy and culture of the left thigh area of erythema also grew *P. aeruginosa and T. asahii.*

After 5 days of FLZ, the blood cultures were still positive and caspofungin (Cancidas) 50 mg IV qd was added to the FLZ. He also underwent arthroscopic flushing of the right knee. Ten days after admission, the patient had negative blood cultures and had responded well to antimicrobial therapy. He was subsequently transferred to a rehabilitation unit in good condition.

In vitro susceptibilities (methylisocyanates (MICs)) of *T. asahii* revealed FLZ 8 µg/mg, itraconazole 0.5 µg/mL, and caspofungin 2 µg/mL.

Instructive Case 6

A 42-year-old black male presented to the University Hospital emergency department with a 1-week history of fever, chills, cough productive of white sputum, night sweats, and malaise. He denied hemoptysis. Risk factors for HIV infection included a blood transfusion in 1985 and heterosexual promiscuity. He denied intravenous drug abuse or homosexual activity. Over the past 8 months, he had lost 80 lb. Past medical history was significant for a prior appendectomy, a perirectal abscess which was surgically drained, and a chronic perirectal fistula for 5 years. There was also a history of venereal disease, including primary syphilis associated with a chancre and a positive rapid plasma reagin (RPR) of 1:32. There was no documented therapy and the patient was lost to follow up.

On admission, the patient was afebrile but cachectic. He was in no acute respiratory distress. On physical examination, he was noted to have dry crusting lesions on the tip of his nose and both cheeks of the face (Fig. 5). Similar lesions were noted over the shins, and a small draining abscess was noted over the medial aspect of the left foot. Oral hairy leukoplakia was present on the tongue, and he was noted to have prominent generalized lymphadenopathy. No rales, rhonchi, or wheezes were heard on auscultation of chest. A fistula-in-ano was also noted. Chest radiograph revealed bilateral reticulonodular infiltrates, which were more prominent in the upper lobes. The patient was lymphopenic with an absolute lymphocyte count of 410 cells/µL. Serum RPR was positive of 1:32. Skin scrapings of the lesions were performed, and wet preparations of the specimens revealed characteristic thick-walled multinucleated yeast forms compatible with *Blastomyces dermatitidis* (Fig. 6). Subsequently, the patient underwent bronchoscopy with BAL and cytology preparations of the washings also revealed *B. dermatitidis*. Cultures of skin scrapings and BAL washings grew this organism. An enzyme-linked immunosorbent assay (ELISA) and Western blot confirmed HIV infection, and total CD4$^+$ T lymphocyte count was 8 cells/µL. A computed tomography (CT)

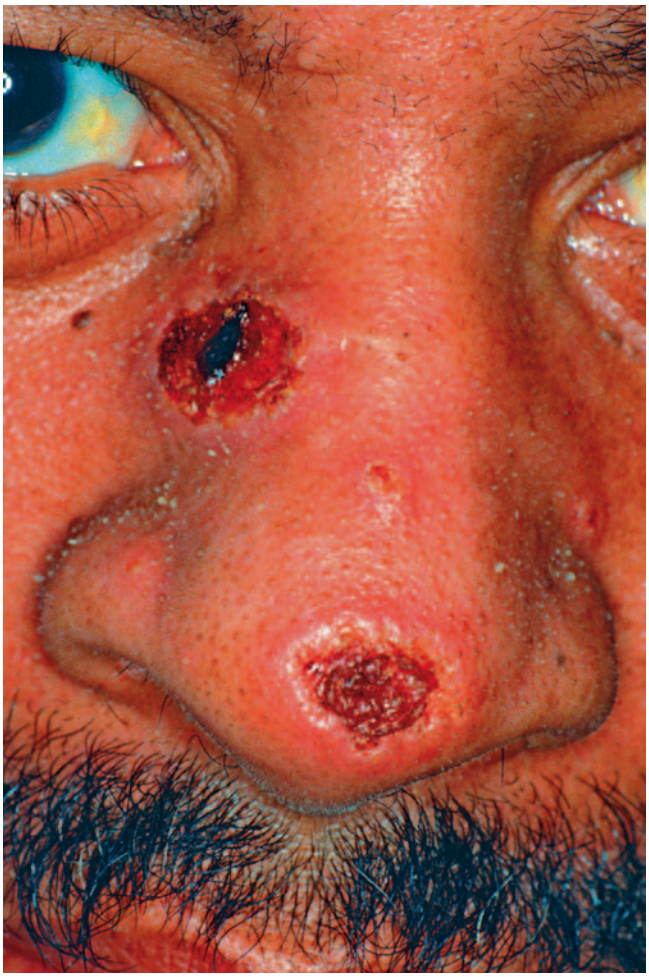

Fig. 5 Multiple cutaneous lesions in a patient with end-stage AIDS. Similar lesions were noted on both cheeks of the face and both shins

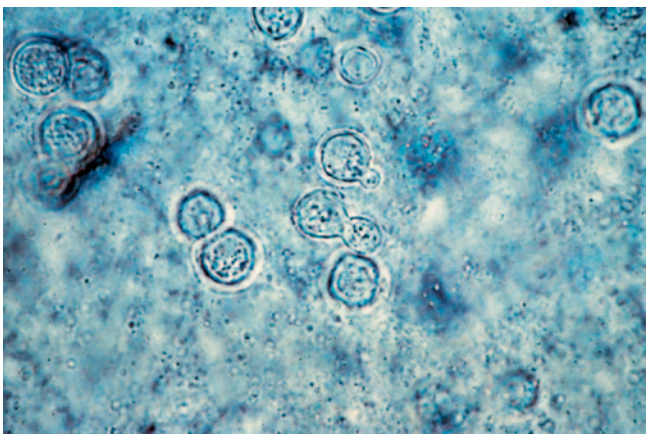

Fig. 6 Wet preparation of skin scrapings. This figure shows the characteristic yeast forms in a wet preparation of skin scrapings. Scraping of the edges of the verrucous and ulcerative lesions yields the best diagnostic results

scan of the head was performed because of complaints of headache. This study revealed multiple enhancing brain lesions (Fig. 7). Owing to concerns of a second opportunistic

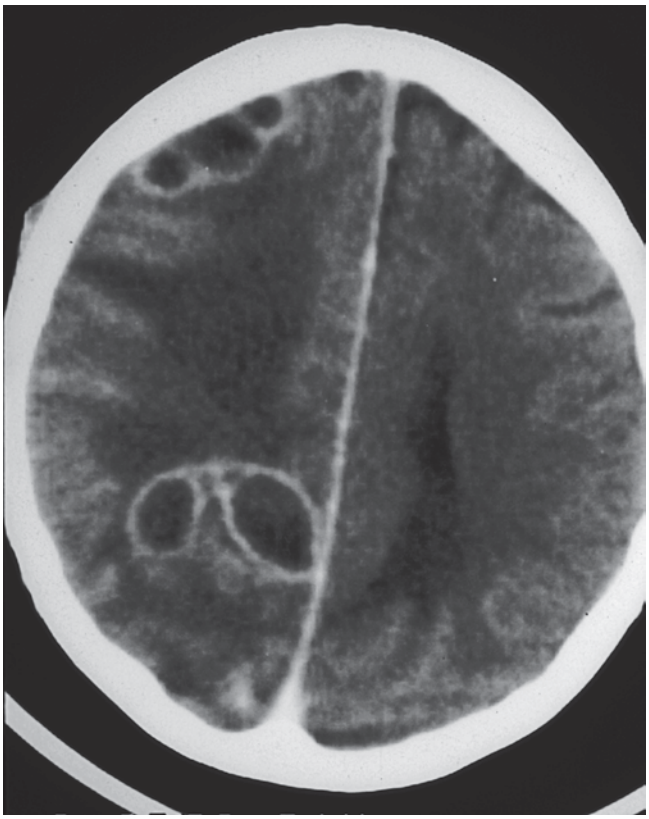

Fig. 7 CNS blastomycosis in an AIDS patient. Diagnosis may require aspiration of the abscesses if no active pulmonary or cutaneous disease is present. *CNS* central nervous system

pathogen, i.e., *Toxoplasma gondii,* the brain lesions were aspirated. Wet preparations again revealed characteristic yeast forms of *B. dermatitidis*.

The patient was begun on highly active antiretroviral therapy (HAART) and amphotericin B deoxycholate therapy at an initial dose of 1 mg/kg/day. Intravenous penicillin, 18 million units per day in divided doses, was administered for 21 days because of concerns of neurosyphilis. During the remainder of the hospitalization, the patient was carefully monitored for evidence of renal insufficiency and 500 mL of normal saline were infused prior to each amphotericin B infusion. During this hospitalization, he received a total of 1600 mL of amphotericin B. Serial CT scans documented progressive improvement of brain abscesses, and oral FLZ was substituted after the full course of amphotericin B and the patient was discharged to home. He completed eight more weeks of FLZ therapy at a dose of 800 mg/day. Although the brain abscesses had resolved on CT scan, a maintenance suppressive dose of FLZ of 400 mg/day was initiated. HAART was continued and a clinical response was documented by a falling HIV viral load and rising $CD4^+$ T lymphocyte count.

Instructive Case 7

A 45-year-old construction worker presented with a 3-week history of fever, chills, myalgias, headache, and dyspnea. Examination was unremarkable and laboratory tests revealed a WBC count of 3300 cells/μL and a platelet count of 94,000 cells/μL. Aspartate aminotransferase (AST) was 87 units/L, while alkaline phosphatase was 337 units/L. Angiotensin-converting enzyme was elevated at 213 units/L. Chest and abdomen CT showed small nodules in the lungs, enlarged mediastinal lymph nodes, small pleural effusions, and splenomegaly. Transbronchial biopsies of the subcarinal lymph node and right lower lobe and bone marrow biopsy showed noncaseating granuloma. Histopathology was negative for fungi, and cultures were negative after 1 week of incubation. Prednisone 60 mg/day was prescribed for presumed sarcoidosis, which resulted in resolution of fever and improvement of dyspnea. The prednisone dosage was tapered over 4 weeks to 10 mg/day, which was maintained.

Two months later, the patient complained of recurrent fever, 10-lb. weight loss, and worsening dyspnea. Chest CT showed more extensive diffuse interstitial infiltrates and increasing splenomegaly. Hemoglobin was 9.3 g/dL, WBC count 2500 cells/μL, and a platelet count of 89,000 cells/μL. Alkaline phosphatase was 987 units/L. Cultures from the lung tissue, lymph nodes, and bone marrow performed during the earlier admission were negative after 4 weeks of incubation. Bronchoscopy was performed, and cytology revealed small yeastlike structures resembling *Histoplasma capsulatum,* which was later confirmed by culture. Treatment was started with amphotericin B, and the patient noted progressive improvement.

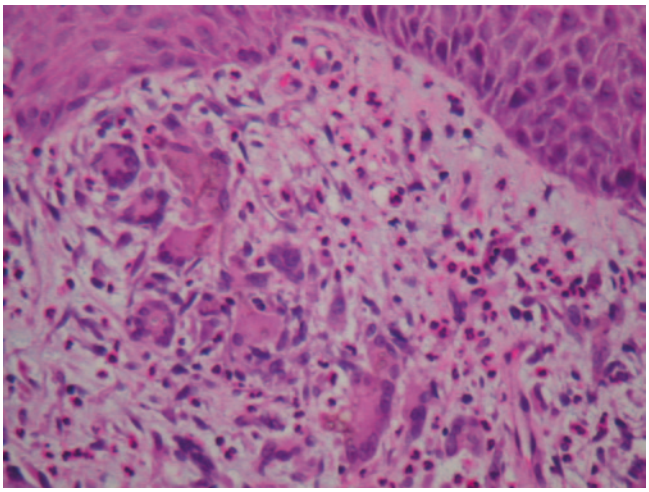

Fig. 8 Histopathological examination of subcutaneous nodule showing granulomatous changes and dark-walled fungal elements. Phaeohyphomycosis

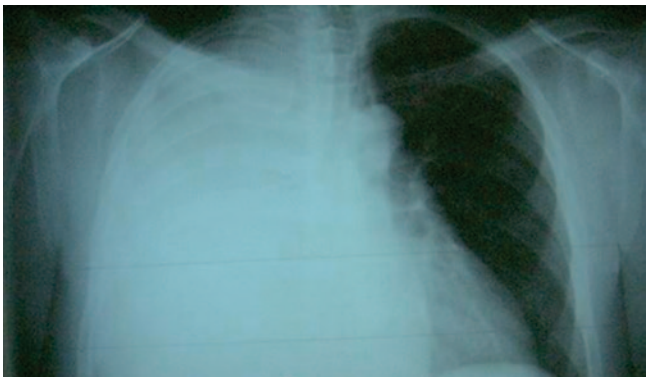

Fig. 9 Chest X-ray

Instructive Case 8

A 52-year-old male who underwent renal transplantation 2 years earlier presented with a slightly painful mass above his right knee. This mass began as a small lesion 4 weeks ago and has been slowly enlarging since first noted by the patient. He denied fever, chills, night sweats, or trauma to the area. He was taking tacrolimus and prednisone as his immunosuppressive regimen. The patient was afebrile with stable vital signs, but his physical examination was significant for a 1.5 cm firm nodule above his right knee, which was slightly tender, but without erythema or drainage. Routine laboratory studies were unremarkable.

Surgery was consulted for excision of the nodule, which was performed without complications. Pathology showed chronic inflammation and pigmented fungal elements suggestive of phaeohyphomycosis; margins were clear of infection (Fig. 8). Culture of the specimen grew *Exophiala jeanselmei*. He was given itraconazole for 3 months, and no further lesions appeared.

Instructive Case 9

A 28-year-old Caucasian male presented with a 1-day history of dyspnea and anxiety. His past medical history included major depressive disorder, and his past social history included marijuana and tobacco use. Physical exam revealed mild respiratory distress and anxiousness, with decreased breath sounds on the right thorax. Laboratory studies included hemoglobin 13.9 g/dL, WBC count 14.6×10^3/μL, absolute neutrophil count 12.5×10^3/μL, absolute lymphocyte count 2.3×10^3/μL, absolute eosinophil count 0.146×10^3/μL, and albumin 2.7 g/dL. Chest radiograph revealed complete opacity of right hemithorax (Fig. 9).

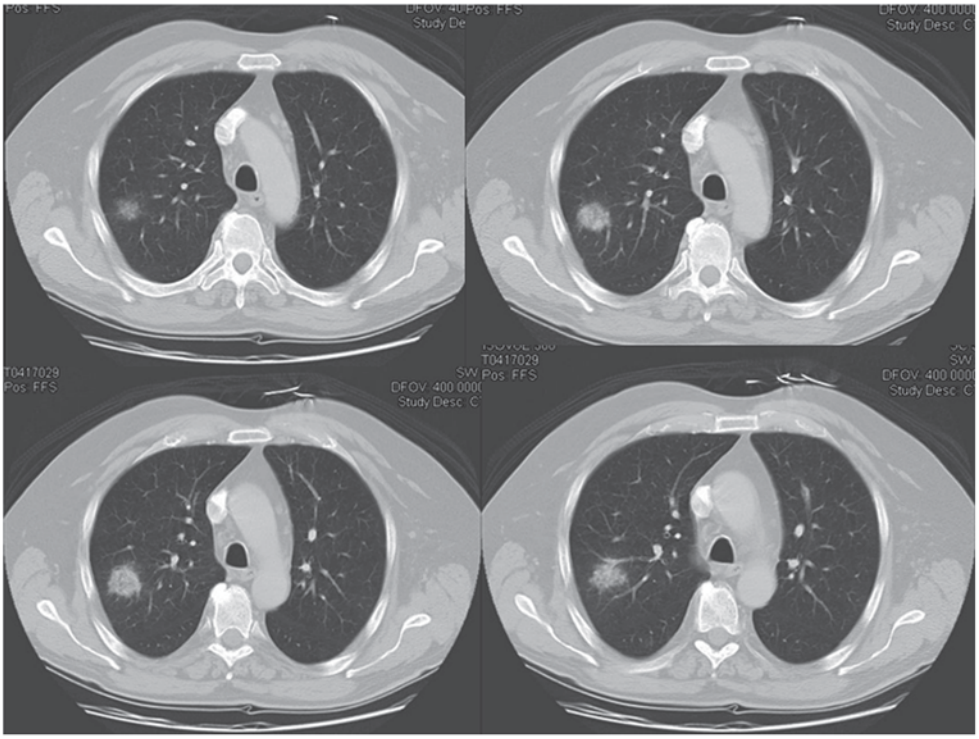

Fig. 10 CT scan of chest revealing small pulmonary nodule in the right upper lung. *CT* computed tomography

A chest tube was inserted and later video-assisted thoracic surgery (VATS) with right upper lobe (RUL) wedge resection of a cavitary lesion was performed. Coccidioidomycosis serology was positive and FLZ at 1000 mg/day was initiated. The patient's recovery was uneventful.

Instructive Case 10

A 68-year-old man with a long-standing history of hairy cell leukemia was admitted to receive treatment with the experimental immunotoxin BL-22. He had been pancytopenic for several months and had been receiving antifungal prophylaxis with itraconazole. Upon admission, antifungal prophylaxis was changed to FLZ. On day 2, the patient developed fever. At that time, his absolute neutrophil count was 76 cells/μL. Blood cultures recovered a highly susceptible *Escherichia coli,* and the patient defervesced promptly on ceftazidime. On day 7, a new fever developed and meropenem, tobramycin, and caspofungin were started. A chest CT was obtained which showed a 3-cm RUL nodule (Fig. 10). A BAL was performed, and voriconazole and levofloxacin were added. Bacterial, fungal, and viral cultures, as well as Gram, calcofluor white, acid-fast, modified acid-fast, and Gomori methenamine silver (GMS) stains were negative. Polymerase chain reaction (PCR) for *Pneumocystis, Chlamydophila, Mycoplasma,* and *Legionella* were also negative. The patient continued to have fever up to 40°C without new symptoms or hypotension. On day 11, he complained of chest pain and dry cough. A repeat CT showed marked enlargement of the nodule with development of a halo sign and abutting of the fissure (Fig. 11). A fine-needle aspirate of the mass showed broad, ribbonlike, nonseptate hyphae (Fig. 12). Voriconazole was discontinued and liposomal amphotericin B 7.5 mg/kg/day was started. The patient developed hemoptysis, and a repeat CT showed further progression of the mass, but with apparent localization in the RUL (Fig. 13). An emergent right upper lobectomy was performed on day 18 (Fig. 14). An angioinvasive mold was readily seen on the GMS stain (Fig. 15), and was later identified as *Rhizomucor pusillus*. On the day of the surgery, granulocyte transfusions were initiated. Hemoptysis and fever resolved 2 days after the surgery. Despite local control of the fungal disease, the patient never recovered his WBC counts and over the next 6 weeks developed several complications in the ICU, including herpes simplex pneumonia. At his own request, care was withdrawn.

Instructive Case 11

A 48-year-old male rural worker was referred for consultation to evaluate the presence of painful, ulcerated lesions in the external region of his right foot (Fig. 16). This process had gone on for 18 months and multiple local and systemic treatments had been given without success. The patient looked well and had no other symptoms. Physical examination also revealed the presence of an oral mucosal ulceration and of hypertrophied cervical lymph nodes. Lung auscultation found fine rales and the chest X-ray showed interstitial infiltrates in the central fields with fibrous zones and basal bullae (Fig. 17).

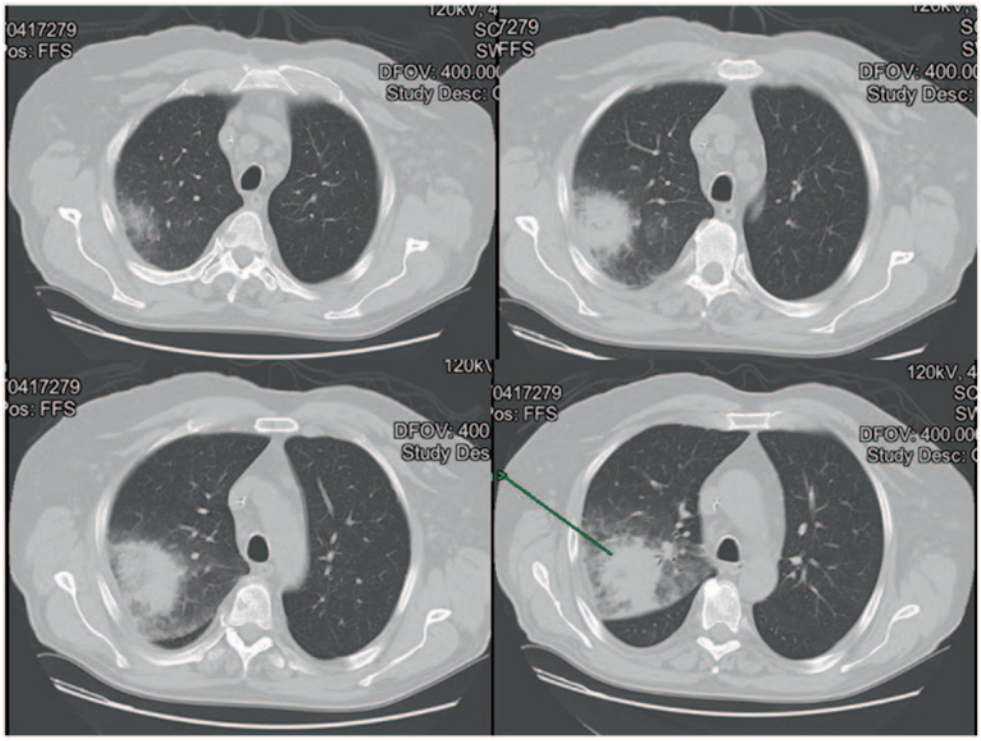

Fig. 11 CT scan of chest revealing progression of pulmonary nodule in right upper lung field to involve the right pleural surface. *CT* computed tomography

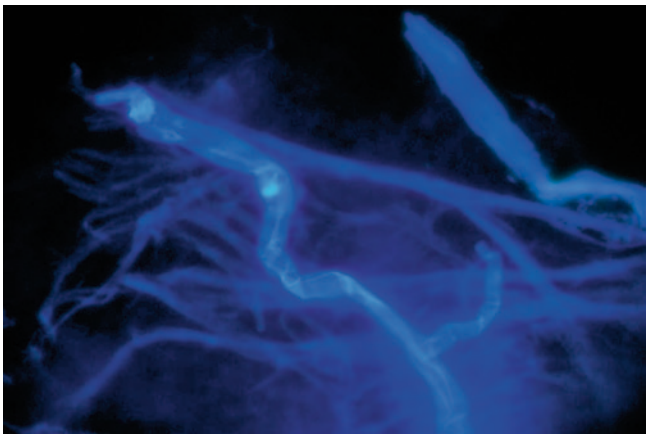

Fig. 12 Calcofluor staining of fine-needle aspiration demonstrates broad nonseptate hyphae consistent with mucormycosis

Direct KOH examination of ulcer exudate revealed multiple budding yeasts consistent with *P. brasiliensis*; cultures later grew the fungus. Patient's serum gave a band of precipitate and a CF titer of 1:32 with paracoccidioidin.

Instructive Case 12

A 40-year-old female with AML now 115 days following matched allogeneic donor hematopoietic stem cell transplantation complicated by a suspected *Aspergillus* pneumonia presented to the clinic with increasing complaints of nausea, stomach cramping, and rash on the hands spreading up her arms. By laboratory examination, she was noted to have an alanine transaminases (ALT) of 85 U/L, AST of 75 U/L, and total bilirubin of 2.1 mg/dL. Her current medications include tacrolimus 5 mg twice daily (recent level 8 ng/mL), voriconazole 200 mg twice daily, levofloxacin 500 mg daily, valacyclovir 500 mg twice daily, metoprolol 25 mg twice daily, and benzonatate (Tessalon Perles). She is admitted to the hospital for suspected graft versus host disease exacerbation. The primary service wishes to continue voriconazole therapy as this patient has a history of poorly tolerating lipid amphotericin B formulations, but they are concerned about the possibility of drug-induced hepatitis caused by voriconazole.

Question

Which of the following approaches should be recommended to the primary team to manage the suspected drug-induced hepatotoxicity?

a. Discontinue voriconazole and switch to itraconazole
b. Discontinue voriconazole and switch to lipid amphotericin B with premedications
c. Continue voriconazole and lower dose by 50%
d. Continue voriconazole and closely monitor the patient.

Instructive Case 13

A 45-year-old Hispanic male presented with chief complaint of back pain. He was in his usual state of health until he developed fever, rigors, nonproductive cough, nausea, and

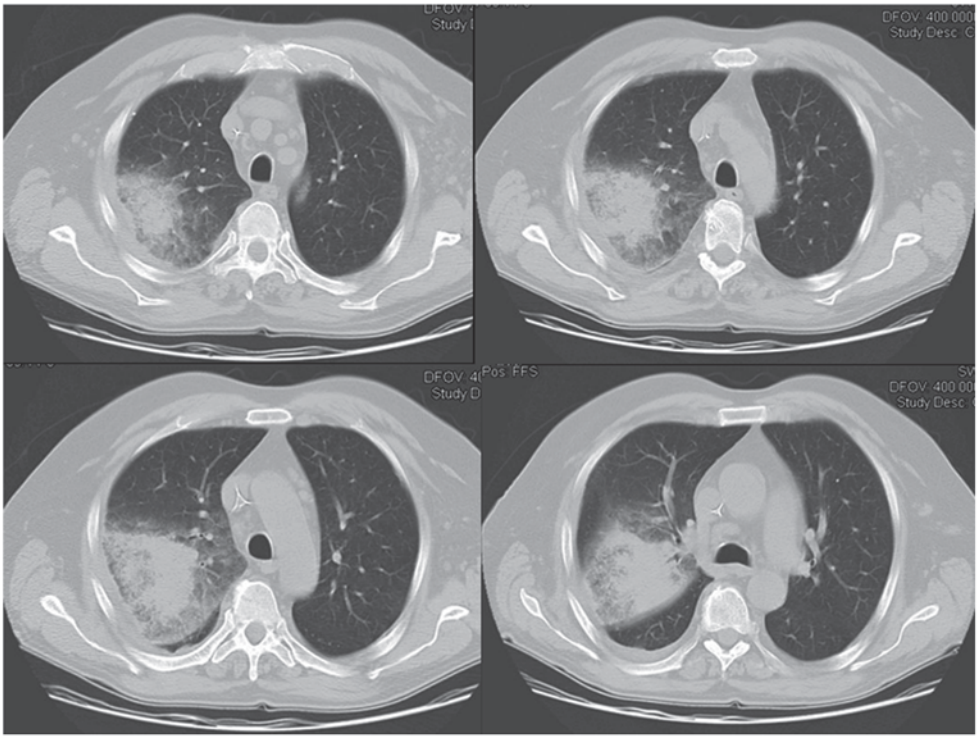

Fig. 13 CT scan of chest with severe advancement of locally progressive disease in the right upper lung fields. *CT* computed tomography

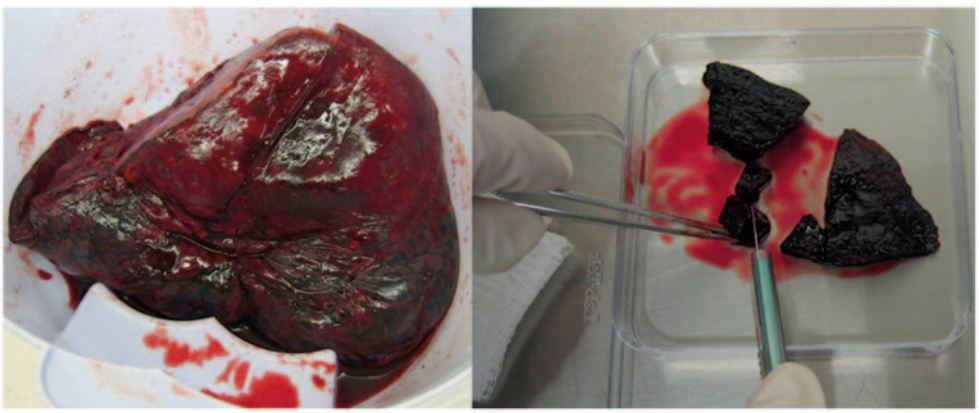

Fig. 14 Gross pathology of right upper lobectomy

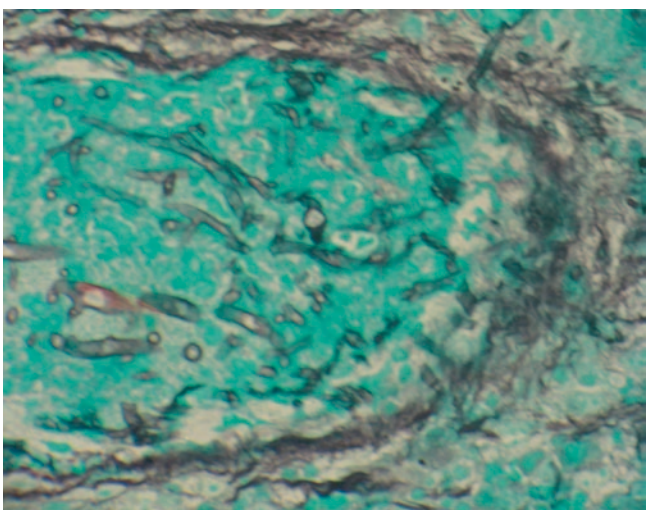

Fig. 15 Mucormycosis with hyphae invading blood vessel, infiltrating vascular wall, and causing subsequent thrombosis. GMS

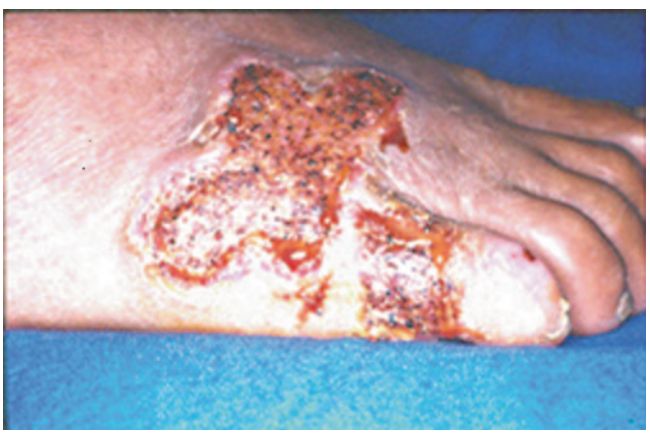

Fig. 16 Paracoccidioidomycosis. Multiple ulcerated lesions in the right foot with crusting, exudation, and hemorrhagic dots. The borders are granulomatous and show some scarring

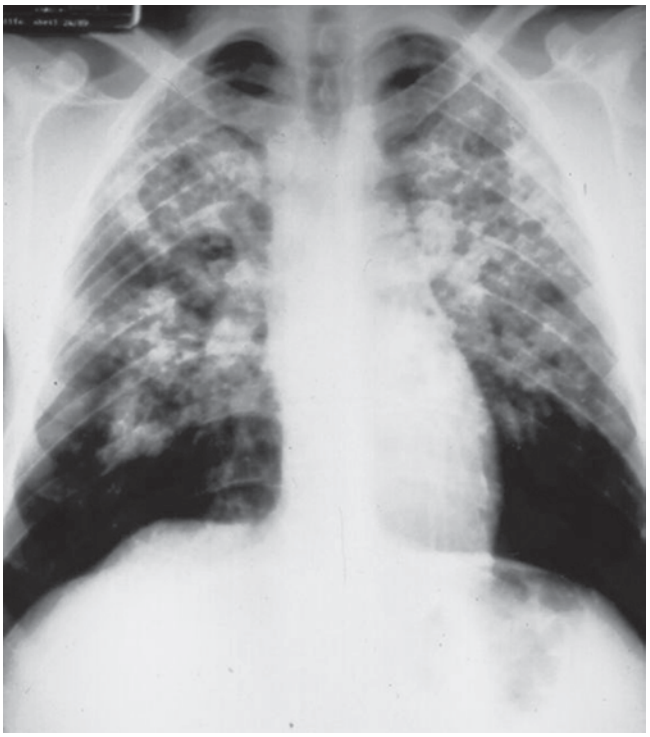

Fig. 17 Paracoccidioidomycosis. Bilateral interstitial infiltrates in central fields, basal bullae, and fibrous areas. The apices appear free of disease

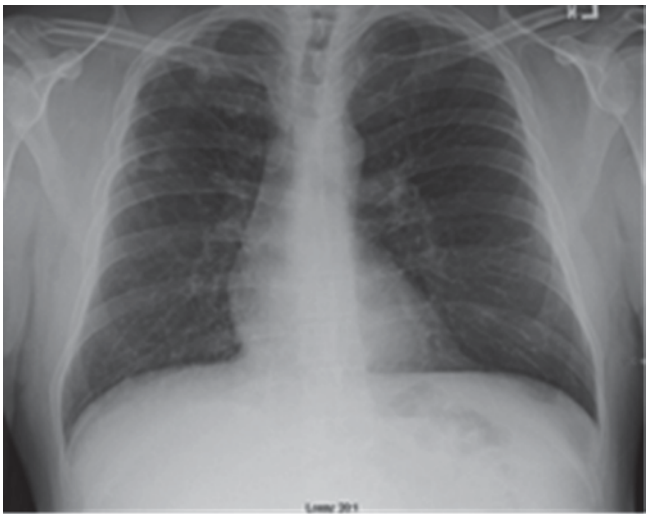

Fig. 18 Chest X-ray

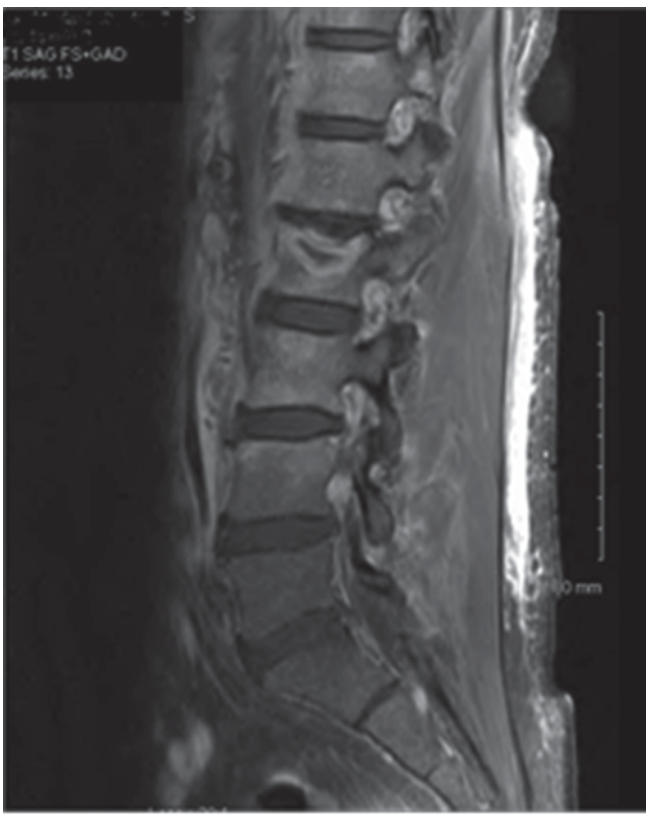

Fig. 19 MRI of thoracic and lumbar spine. *MRI* magnetic resonance imaging

vomiting. He was treated at another institution with antibiotics without improvement. Respiratory symptoms gradually abated, but he continued to have night sweats, fever, weight loss, and increasing spine pain. Abscesses on his right arm and mid back were noted. Incision and drainage was attempted with "no pus" recovered.

Seven months later, the patient was referred to surgery for evaluation of a possible "tumor." Pathology from surgery revealed coccidioidomycosis. Medical treatment with FLZ 400 mg/day was initiated (later increased to 1000 mg). Patient was referred to specialty coccidioidomycosis clinic for evaluation and patient was admitted for further evaluation and treatment.

Past medical history included chronic low back pain for 5 years, resulting in disability. Physical examination revealed temperature 97°F, pulse 86/min, blood pressure 109/68 mmHg, and respirations 18/min. He appeared chronically ill, in mild to moderate pain, with pale conjunctiva, temporal wasting, draining wounds of his right posterior upper arm and right mid back, decreased motor strength of his lower extremities, and decreased sensation to pin prick testing at T-10 level. Laboratory testing revealed hemoglobin 8.1 g/dL, WBC count $3.3 \times 10^3/\mu L$, potassium 2.8 mEq/L, creatinine 0.8 mg/dL, albumin 2.4 g/dL, and coccidioidomycosis serology (CF) 1:64. Chest radiograph showed RUL nodular infiltrate with cavity (Fig. 18), and bone scan showed evidence of osteomyelitis in distal thoracic spine. Magnetic resonance imaging (MRI) of the thoracic spine revealed osteomyelitis at T-11/12 with epidural paraspinous phlegmon (Fig. 19) and MRI of the lumbar spine revealed osteomyelitis at L-4/5 with paraspinous psoas phlegmon extending into the perinephric space (Fig. 20).

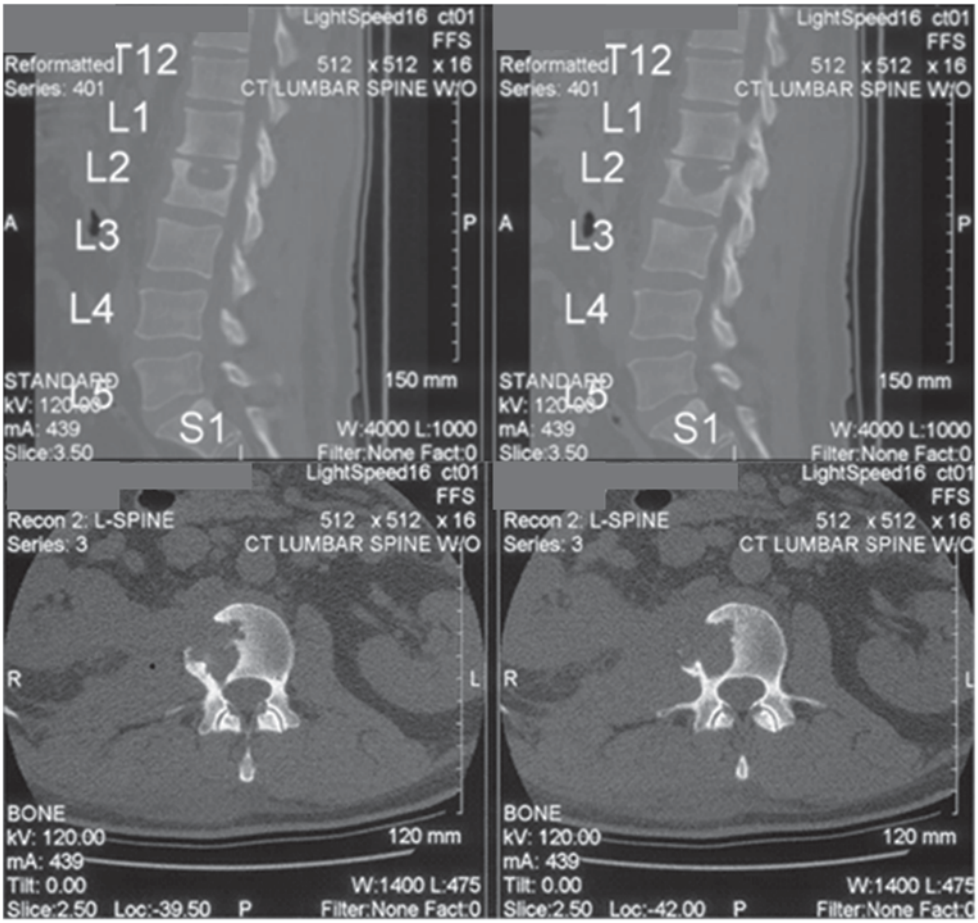

Fig. 20 MRI of lumbar spine. *MRI* magnetic resonance imaging

Instructive Case 14

A 22-year-old female with a failing 4-year-old renal allograft received several doses of Orthoclone (OKT3) and high doses of corticosteroids in an attempt to reverse the acute rejection of the transplanted kidney. Three months after this increased immunosuppressive trial and still receiving her normal immunosuppressive regimen of tacrolimus, mycophenolate, and prednisone, she presented with several weeks course of headaches, nausea, and vomiting. Her temperature was 37.2°C, and although her mental status was normal, she had bilateral clonus and papilledema on physical exam. Her laboratory results showed a normal complete blood count and a serum creatinine of 4 mg/dL. An MRI of her brain demonstrated basilar inflammation, and lumbar puncture (LP) revealed a WBC count of 100 cells/μL with 80 % lymphocytes. Cerebrospinal fluid (CSF) glucose was 43 mg/dL and protein 79 mg/dL. India ink was positive for encapsulated yeasts, CSF cryptococcal polysaccharide antigen test was ≥ 1:256, and culture grew *Cryptococcus neoformans*. Her opening pressure was 400 mmH_2O. She was started on 5 mg/kg/day of AmBisome for 20 days and flucytosine at 25 mg/kg/day for 14 days, and then placed on 200 mg/day of FLZ. Patient's symptoms did not worsen, and she was reevaluated at 2 weeks with a repeat LP. That LP found an opening pressure of 140 mmH_2O and India ink and culture were negative. CSF antigen was 1:256 and CSF WBC count was 28 cells/μL.

Instructive Case 15

A 47-year-old black male presented to the University Hospital emergency department complaining of pleuritic chest pain for 2 weeks prior to admission. He subsequently developed severe dyspnea on exertion, fever and chills, and productive cough with hemoptysis. Over the 24 h preceding admission, pleuritic chest pain, which was initially only on the left side, became bilateral and he presented to the emergency department for evaluation. Past medical history was pertinent for hyperthyroidism and cigarette smoking. He denied any risk factors for HIV infection.

In the emergency department, he was in moderate respiratory distress but was afebrile. Chest radiograph revealed diffuse bilateral miliary infiltrates with a mass-like lesion in the lung field. WBC count was 14,400 cells/μL with a left shift. Arterial blood gases on room air revealed a pH of 7.41, pO_2 of 33 mmHg, and pCO_2 of 46 mmHg.

Patient was admitted to the hospital and placed in respiratory isolation. Differential diagnosis included severe community-acquired pneumonia, atypical pneumonia, miliary

tuberculosis, and fungal disease. He was placed on supplemental oxygen, intravenous azithromycin and ceftriaxone, and a four-drug antituberculous treatment regimen. Multiple sputum samples were only remarkable for many polymorphonuclear leukocytes. Direct fluorescent antibody (DFA) and urinary antigen were negative for *Legionella*. Likewise, fungal and acid-fast stains of sputum samples were negative. An ELISA for HIV was also negative.

On day 3 in the hospital the patient became febrile; chest radiograph revealed worsening bilateral pulmonary infiltrates, and intravenous trimethoprim/sulfamethoxazole (TMP–SMX) was added to the existing antibacterial regimen as empiric therapy for possible *Pneumocystis* pneumonia. On day 5 in the hospital, he complained of increasing dyspnea and was noted as having a respiratory rate of 60 breaths/min. Arterial blood gases on 100 % oxygen by face mask revealed a pH of 7.45, pO_2 of 102 mmHg, and pCO_2 of 42 mmHg. The patient was transferred to ICU, where he was intubated and placed on mechanical ventilation. Chest radiographs revealed bilateral pulmonary infiltrates (Fig. 21) with acute lung injury. Cytology samples obtained via the endotracheal tube at the time of intubation revealed numerous broad-based budding yeast forms compatible with *Blastomyces* species (Fig. 22).

The patient received intravenous amphotericin B immediately at a dose of 0.7 mg/kg/day, but was rapidly increased to 1 mg/kg/day. During the remainder of his hospitalization, the patient became increasingly difficult to oxygenate, developed hypotension requiring pressers, progressed to multiorgan failure, and died with pulseless electrical activity.

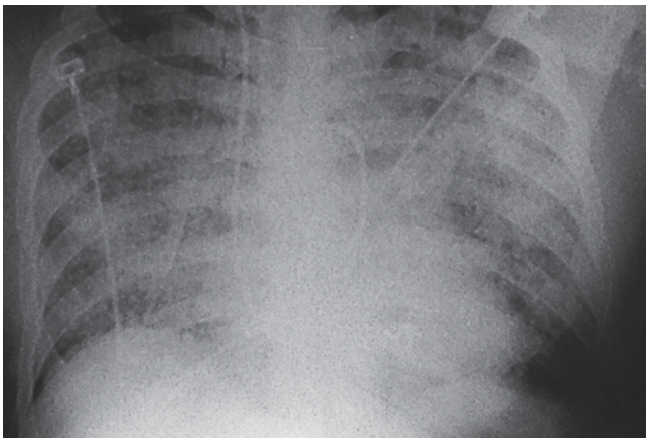

Fig. 21 Diffuse pulmonary infiltrates in a patient with ARDS. Patients presenting with this syndrome have a mortality rate greater than 50 %. *ARDS* acute respiratory distress syndrome

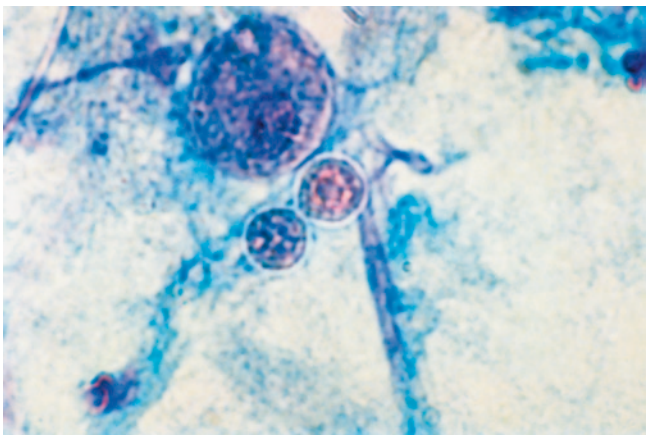

Fig. 22 Cytology preparation of endotracheal tube specimen revealing large, thick-walled yeasts consistent with *Blastomyces dermatitidis*

Instructive Case 16

An 18-year-old female presented with chest discomfort. Dilated veins were noted over the chest and upper abdomen. Chest X-ray showed right hilar enlargement, which was calcified on CT. The right pulmonary artery was narrowed and the superior vena cava was occluded. Ventilation–perfusion lung scan showed reduced blood flow to the right lung. Pulmonary function tests showed normal air flow and lung capacity. Tuberculin skin test was negative. *Histoplasma* immunodiffusion tests showed an M band, and the *Histoplasma* CF test showed titers of 1:32 to the yeast and 1:16 to the mycelial antigen. Mediastinal biopsy showed chronic inflammatory cells, granuloma, and fibrosis. Culture of the mediastinal biopsy tissue was negative for fungus.

Question

Is surgery indicated to correct the obstruction of the pulmonary artery or of the superior vena cava, and should the patient receive a course of antifungal therapy?

Instructive Case 17

A 41-year-old male rural worker and heavy smoker consulted because of 3 months of dry cough, severe progressive dyspnea, weight loss, asthenia, adynamia, and anorexia. He looked emaciated and experienced difficulties in breathing even at rest. Respiratory rate was noted to be 36 breaths/min with accessory muscles utilization. Upon auscultation, rales, rhonchi, and hypoventilation were noticed. The chest X-ray (Fig. 23) revealed the presence of a diffuse reticulonodular infiltrates predominating in both central fields with fibrous lines. Follow-up CT of chest documented widespread fibrosis (Fig. 24). Arterial blood gas analysis revealed pH 7.44, pO_2 37 mmHg, pCO_2 23 mmHg, O_2 saturation 81 %, and bicarbonate 16 mEq/l.

A BAL fluid sample was examined for acid-fast bacilli with negative results, but multiple budding cells

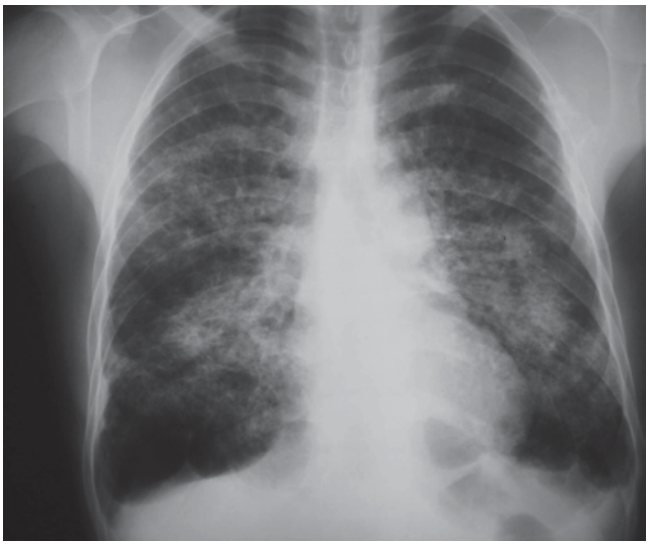

Fig. 23 Paracoccidioidomycosis. Lung fibrosis involving specially the central field with apices appearing free. Bilateral basal bullae and pleural adhesions are seen in both lower fields

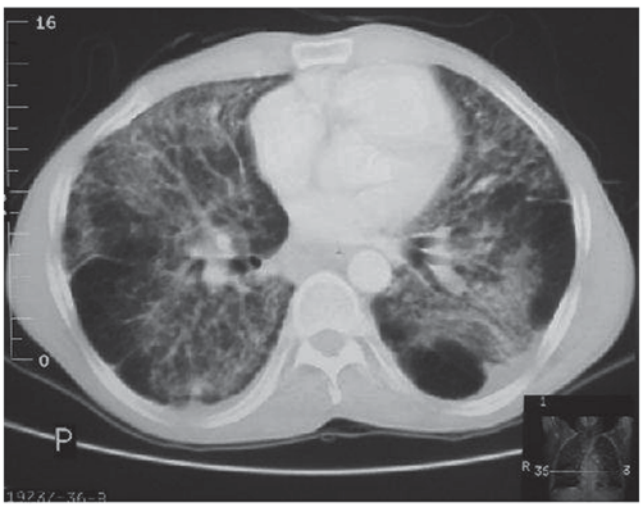

Fig. 24 Paracoccidioidomycosis. High-resolution CT showing widespread fibrosis with honeybee aspect, bullae formation, and pleural thickening in both lower lung fields

corresponding to *P. brasiliensis* were seen on direct examination and recovered in culture later on. Serologic tests with paracoccidioidin were reactive with one band of precipitate and a titer of 1:1024 in the CF test.

Instructive Case 18

A 24-year-old Caucasian male presented with a chief complaint of headache of 3 weeks' duration. He was in his usual state of health until 11 weeks prior to admission when he developed a headache. Nine weeks prior to admission, the patient was seen at another facility with headache and confusion. LP without opening pressure was performed at that time and revealed meningitis. He was treated with vancomycin, ceftriaxone, FLZ, and acyclovir. The patient was discharged home after 1 day with oral acyclovir. His headache continued to wax and wane, with decreasing ability to perform activities of daily living. His primary care physician referred the patient to the emergency room. Past social history included occasional marijuana use and lost job due to headaches. Physical examination revealed the patient to be alert, but listless and in mild distress. His neck was supple, but his tandem gait testing was abnormal. Imaging studies, including CT scan, MRI, and chest radiograph, were normal. LP found an opening pressure of 390 mm of water, with 750 WBCs/μL; 52% were neutrophils, 19% were eosinophils, and 20% were lymphocytes. The protein was 130 mg/dL and glucose was 20 mg/dL. Medical treatment was started with FLZ 1000 mg-day and dexamethasone 20 mg/day (tapered by 5 mg every other day). A plan was made to repeat LP daily if opening pressure was greater than 250 mmH_2O to reduce this reading by 50% or to less than 200 mmH_2O, whichever is greater. If this treatment fails, the plan would be to pursue lumboperitoneal shunt placement.

Instructive Case 19

A 68-year-old female was admitted to the hospital for a right frontal craniotomy to debulk a pituitary adenoma. Her past medical history was significant for poorly controlled diabetes mellitus, hypertension, and sleep apnea. Her home medications included oral prednisone. Postoperatively, she was lethargic and developed diabetes insipidus due to the surgery. One week after admission, she remained obtunded and developed a temperature of 39°C and was noted to have a brownish drainage from her craniotomy wound adjacent to a pulse-oximetry sensor. Laboratory testing was significant for a leukocytosis of 23,100 cells/μL. Underneath the sensor, a small superficial ulceration approximately 1 × 2 cm was discovered. Within 6 days, the ulceration developed into a black eschar that measured 6.5 × 7.5 cm (Fig. 25). A biopsy of the lesion revealed necrotic tissue with arterial occlusion. Fungal stains revealed broad-based, nonseptate hyphae with acute or wide-angle branching. The culture was positive for *Mucor* species. Liposomal amphotericin B at 5 mg/kg/day was initiated and the patient underwent extensive debridement of scalp tissue, underlying bone, dura, and subdural tissue, all of which were necrotic and culture positive for *Mucor*. The patient remained comatose and expired about 1 week postoperatively. On autopsy, histopathology of the affected areas revealed *Mucor* infection extending from the scalp through the craniotomy incision into the pituitary tissue (Figs. 26, 27).

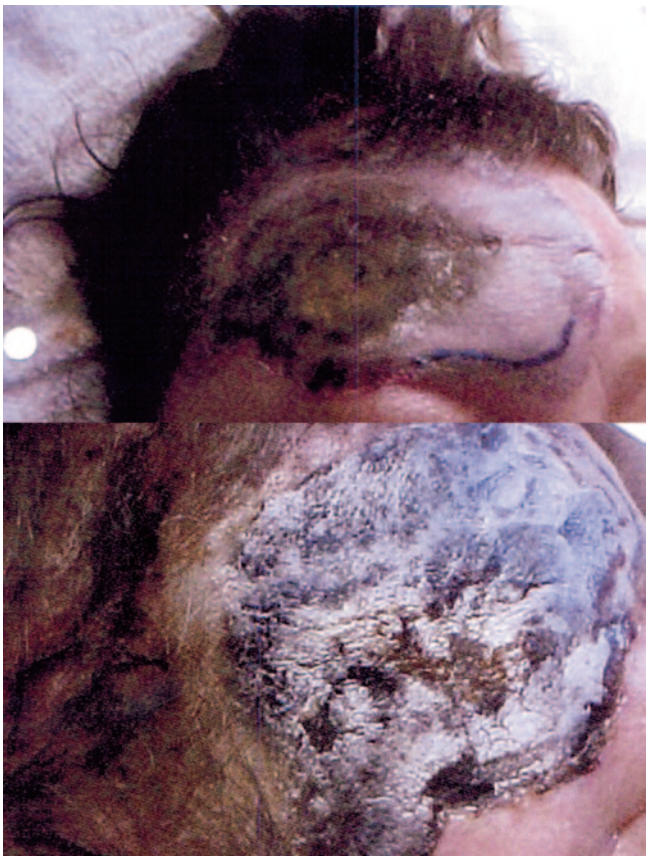

Fig. 25 Cutaneous lesion over the right frontoparietal scalp

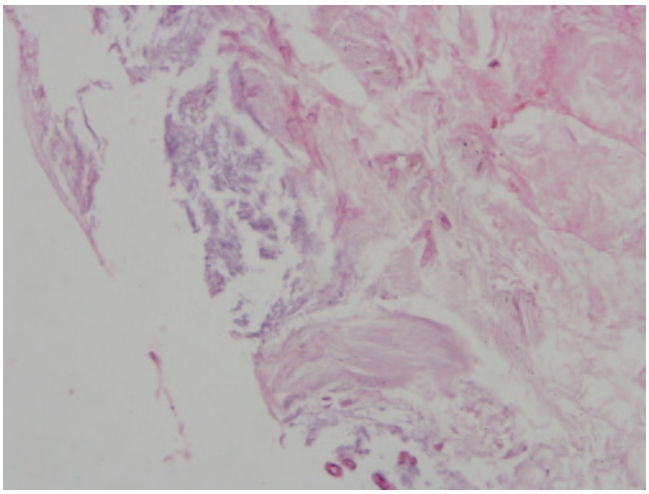

Fig. 26 Histopathologic examination with H&E stain of the skin demonstrating the broad, nonseptate hyphae with acute or wide-angle branching. *H&E* hematoxylin and eosin

Instructive Case 20

A 30-year-old man with known HIV infection presented with a 4-week history of increasing dyspnea on exertion, tachypnea, and fever. His chest X-ray revealed diffuse alveolar infiltrates and blood gas, hypoxemia. He had been noncompliant with this antiretroviral therapy and was profoundly lymphopenic. *Pneumocystis* pneumonia was confirmed by BAL.

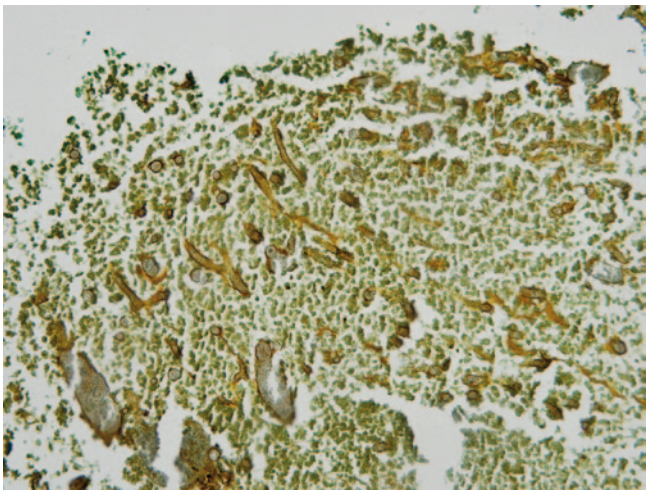

Fig. 27 Histopathologic examination with GMS stain of pituitary tissue demonstrating broad, nonseptate hyphae with acute or wide-angle branching. *GMS* Gomori methenamine silver

The patient was treated with TMP–SMX and his antiretroviral treatment restarted. On TMP–SMX treatment, he showed a gradual improvement, but at 4 weeks after having starting these therapies, his respiratory symptoms recurred and chest X-ray found new diffuse interstitial infiltrates. *Pneumocystis* organisms were not visualized on repeat BAL. A recheck of his lymphocyte subsets demonstrated his $CD4^+$ T cell count had risen to 300 cells/μL.

Question

What is the most likely diagnosis for the patient's sudden deterioration after responding to initial therapy?

a. Bacterial superinfection
b. Relapse of *Pneumocystis* pneumonia due to infection with TMP–SMX resistant *Pneumocystis*
c. Immune restitution disease (IRD)/immune reconstitution inflammatory syndrome (IRIS)
d. Stevens–Johnson Syndrome secondary to TMP–SMX

Instructive Case 21

A 68-year-old Caucasian man with polycystic renal disease presented with a chief complaint of swelling of his left lower extremity. His past history included progressive renal failure treated with renal transplantation. Patient was admitted for a diagnosis of cellulitis and placed on vancomycin and piperacillin/tazobactam. When ultrasound revealed deep venous thrombosis, antibiotics were discontinued and

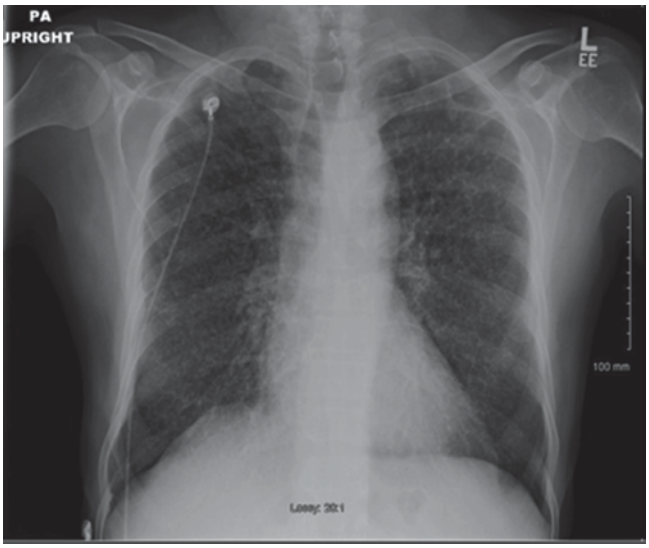

Fig. 28 Chest X-ray

anticoagulation was commenced. Chest radiograph was abnormal, but no initial respiratory symptoms were noted (Fig. 28). He subsequently developed progressive respiratory symptoms and radiographic changes and was transferred to the ICU.

Bronchoscopy revealed lymphocytic and eosinophilic cellularity and culture grew *Coccidioides immitis*. His antirejection drugs were stopped and lipid amphotericin B therapy was started. The patient gradually improved. When his dialysis graft became nonfunctional after 1 month of therapy, he was changed to oral posaconazole. He is currently on hemodialysis and FLZ therapy and doing well. No pretransplantation evaluation of the donor or recipient for coccidioidomycosis was performed.

Instructive Case 1

Answers: 1. B, 2. C

Discussion, Question 1

Amphotericin B-induced nephrotoxicity occurs primarily through two mechanisms: (1) constriction of afferent arterioles leading to direct decreases in glomerular filtration rate (GFR) (glomerular toxicity) and (2) direct damage to the distal tubules (tubular toxicity; answer B), which in turn can lead to glomerular feedback that further results in constriction of the afferent arterioles. Tubular toxicity of amphotericin B is essentially limited to the distal tubules and most commonly evident as hypokalemia (answer B). It occurs in the majority of patients receiving amphotericin B and may require up to 15 mmol of supplemental potassium per hour. Amphotericin B-induced hypokalemia is not associated with increased plasma aldosterone or renin levels and appears to result from increased permeability of the distal tubular cells due to direct toxic effects of amphotericin B. Although lipid amphotericin B formulations reduce distal tubular toxicity, they do not eliminate this side effect. Distal tubular toxicity (decreases in serum potassium and magnesium) frequently precede decreases in glomerular filtration rate (increases in serum creatinine, blood urea nitrogen) during amphotericin B therapy, particularly in patients receiving lipid amphotericin B formulations. Afferent arteriole constriction (answer A) would be more specifically associated with decreases in glomerular filtration (decrease in serum creatinine). Suppression of erythropoietin synthesis (answer C) is a more chronic effect of amphotericin B and manifests primarily as normochromic, normocytic anemia. Amphotericin B has not been shown to directly damage pancreatic islet cells (answer D).

Discussion, Question 2

Nephrotoxicity is the dose-limiting toxicity of amphotericin B therapy. Although all of the answers are potential approaches that have been applied to prevent the development of nephrotoxicity during amphotericin B therapy, switching to a lipid amphotericin B formulation (answer C) is advocated by most infectious diseases experts once glomerular toxicity has developed during amphotericin B therapy. Alternative daily dosing of amphotericin B (answer A) is no longer recommended and there is no evidence that this method is less nephrotoxic than daily dosing. Administering amphotericin B by continuous infusion (answer B) has been shown in two small prospective trials to reduce infusion-related and nephrotoxic side effects; however, this dosing method is not practical and the efficacy of amphotericin B administered by continuous infusion has not been well explored. Saline loading (answer D) can reduce tubular–glomerular feedback and delay the onset of glomerular toxicity; however, it will not reverse glomerular toxicity once it has developed.

Instructive case 1 contributed by R. E. Lewis and A. W. Fothergill.

Instructive Case 2

Discussion

This case is somewhat unusual in that the site of inoculation of *Sporothrix schenckii* had received a skin graft, and thus the lesions were atypical. They arose in the grafted area and contributed to loss of portions of the graft. The lack of response to antistaphylococcal antibiotics was a clue that this was not a typical bacterial infection. The possibility of sporotrichosis was raised by the infectious diseases consultant because of the proximal spread of nodules and the exposure to soil during the original accident. Biopsy confirmed this suspicion when the cultures yielded a mould. The fact that no organisms were seen on the biopsy is not unusual.

Fluconazole was used because an experimental protocol was available at the time, and the patient had no insurance and was unable to purchase other antifungal agents. The re-

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sponse to fluconazole was adequate, but slower than usually noted with itraconazole, which is the treatment of choice for sporotrichosis.

Instructive case 2 contributed by C. A. Kauffman.

Instructive Case 3

Beware of "steroid-responsive pneumonia" with or without eosinophils.

Discussion

This case demonstrates that coccidioidal pneumonia may present as community-acquired pneumonia. However, it is protean in its presentation and any pneumonia (with exposure in endemic areas) that fails initial antimicrobial therapy should be evaluated for the possibility of *Coccidioides*.

Instructive case 3 contributed by R. H. Johnson and A. Heidari.

Instructive Case 4

Discussion

This is an example of the juvenile-type paracoccidioidomycosis.

Instructive case 4 contributed by A. Restrepo, A. M. Tobón, and C. A. Agudelo.

Instructive Case 5

Discussion

This case is instructive for several reasons. The patients past medical history are significant for a long history of immunosuppression due to long-term corticosteroids and his diabetes mellitus. Both of these conditions predispose the patient to an increased risk of fungal infections because of alterations in cell-mediated immunity. The case demonstrates the increasing incidence and the capacity of previously nonpathogenic yeast to produce invasive infection. In addition, the presentation of the patient with nonspecific signs and symptoms is not uncommonly seen in many invasive fungal infections. In fact, the problem with the diagnosis of invasive fungal infections is that there are no "classic or pathognomonic manifestations," this makes the diagnosis difficult to establish and creates a delay in the initiation of appropriate antifungal therapy. In this case, the aspiration of the infected knee demonstrated several organisms (gram-negative bacilli and yeast), thus assisting with the diagnosis. However, *Candida,* not *Trichosporon* would have been the more common cause of infection in this patient. It is not until the laboratory identifies the organisms that the true diagnosis is established. Although there are no clinical trials establishing the best antifungal agent for *Trichosporon* species, the azoles (fluconazole, voriconazole) have been shown to have in vitro activity. In this case, in vitro susceptibility results demonstrated that both fluconazole and itraconazole had good activity. Although there are no established breakpoints for the echinocandins, the MIC of 2.0 μg/ml for caspofungin appears to be within the "standard" ranges described for clinical activity against *Candida* species. Furthermore, although there are some in vitro and animal studies demonstrating additive or synergistic activity with the combination of echinocandins and azoles, the use of combination antifungal therapy has not been demonstrated to be any better than monotherapy.

Instructive case 5 contributed by J. A. Vazquez.

Instructive Case 6

Blastomycosis in AIDS

Discussion

1. Blastomycosis in AIDS patients is more likely to be multi-organ disease with central nervous system (CNS) involvement being noted in up to 40% of patients. Routine magnetic resonance imaging (MRI) or computed tomography (CT) scans should definitely be performed in any patient with severe end-stage AIDS whether or not they have neurologic signs or symptoms.
2. Wet preparations of skin lesions allowed a presumptive clinical diagnosis and early initiation of amphotericin B therapy in this patient.
3. Central nervous system disease in end-stage AIDS patients should be treated with a full course of amphotericin B, e.g., 1.5–2.5 g.
4. Fluconazole at high doses (800 mg/day) may be a reasonable substitute in patients intolerant to amphotericin B or as step-down therapy in patients who have responded to initial treatment with amphotericin B.
5. In immunosuppressed patients, chronic suppressive therapy with fluconazole should be considered.

Instructive case 6 contributed by S. W. Chapman and D. C. Sullivan.

Instructive Case 7

Discussion

This case represents acute pulmonary histoplasmosis misdiagnosed as sarcoidosis. This case illustrates the importance of thorough testing to exclude histoplasmosis before beginning immunosuppressive treatment for sarcoidosis. While there are clinical features that help to distinguish sarcoidosis from histoplasmosis, differentiation requires laboratory testing to exclude histoplasmosis. Administration of corticosteroids resulted in transient clinical improvement, followed by progression with worsening pulmonary disease and progressive dissemination. Failure to demonstrate yeast resembling *H. capsulatum* on the initial bronchoscopy resulted in a mistaken diagnosis of sarcoidosis. Of note is that cytology and culture of respiratory secretions are often negative in acute histoplasmosis, and cannot be used to exclude the diagnosis.

Additional testing should include serology for antibodies to *H. capsulatum,* and tests for *Histoplasma* antigen in urine and respiratory secretions. Serology is often negative during the first month after exposure, but positive thereafter. Antigen may be detected in the urine or bronchoscopy specimen of 75% of cases during the acute illness and before antibodies have appeared. Corticosteroids for sarcoidosis should not be given without thorough evaluation to exclude histoplasmosis. Of note is that fungal cultures require up to 4 weeks of incubation for isolation of *H. capsulatum,* during which corticosteroids should be withheld except in severe cases. Tests for antigen and antibody, and cytology and histopathology on tissues including bronchial washing or bronchoalveolar lavage (BAL), lung and bone marrow should be performed and may provide early evidence for histoplasmosis, but do not exclude the diagnosis.

If cytology, histopathology, antigen testing, and serology are negative and corticosteroids are required for severe sarcoidosis, itraconazole may be given while waiting for culture results in selected patients in whom the diagnosis of histoplasmosis is suspected based on epidemiologic grounds.

Instructive case 7 contributed by L. J. Wheat and N. G. Conger.

Instructive Case 8

Discussion

Subcutaneous phaeohyphomycosis is among the most common manifestations of disease due to dematiaceous fungi. It is seen in both immunocompetent and immunocompromised individuals and is not usually associated with dissemination, though the risk of dissemination is higher in immunosuppressed patients. Complete excision alone has been reported as a successful therapy, particularly in immunocompetent patients. In immunocompromised patients, antifungal therapy is often given after surgical excision to reduce the risk of dissemination. However, itraconazole and voriconazole both have significant interactions with immunosuppressive agents such as tacrolimus and sirolimus, and the combined use of these drugs requires close monitoring and commonly adjustment of the immunosuppressive agents.

Instructive case 8 contributed by S. G. Revankar.

Instructive Case 9

Discussion

This case demonstrates that pleural pulmonary coccidioidomycosis may present as hydropneumothorax secondary to a ruptured coccidioidal cavity.

Instructive case 9 contributed by R. H. Johnson and A. Heidari.

Instructive Case 10

Discussion

Nodular infiltrates in the neutropenic patient are often caused by pathogenic fungi. Identification is critical for proper management. This case illustrates that pulmonary mucormycosis in a neutropenic host may be associated with fever but a paucity of other findings on initial presentation. A BAL is often negative and more invasive procedures, such as a fine-needle aspiration, may be necessary to establish a diagnosis. During neutropenia, pulmonary mucormycosis may progress rapidly, despite amphotericin B therapy. Surgery has an important role in these patients, as it may be the only way of controlling this angioinvasive infection. Granulocyte transfusions may have a role to gain time until neutropenia resolves. Ultimately, however, recovery from these infections is often contingent on recovery of bone marrow function. In the case presented, control of the pulmonary infection was achieved with combined medical and surgical intervention. Unfortunately, the patient ultimately succumbed to complications of his hairy cell leukemia.

Instructive case 10 contributed by C. Antachopoulos, J. C. Gea-Banacloche, and T. J. Walsh.

Instructive Case 11

Discussion

This patient is an example of chronic, adult multifocal paracoccidioidomycosis.

Instructive case 11 contributed by A. Restrepo, A. M. Tobón, and C. A. Agudelo.

Instructive Case 12

Answer: D

Discussion

Unpredictable, low-frequency idiosyncratic liver failure with azoles occurs on a background of a much higher frequency of mild asymptomatic liver injury. Mild liver injury is exacerbated by concomitant medications or dominated by other disease states (as in this case with the graft versus host disease that often affects the liver). Because mild liver injury is generally reversible and transient, immediate discontinuation of voriconazole is not necessary (answers A or B). In clinical trials, voriconazole was continued in the majority of patients with elevated serum transaminases until they reached greater than three times the upper limit of normal (which has not been reached in this patient). Because the patient will likely receive corticosteroids for the graft versus host disease reactivation, continuation of voriconazole and monitoring of liver function tests with the initiation of steroid therapy (answer D) would be the most reasonable approach. Although reduction of the voriconazole dose is an option (answer C), reducing antifungal intensity in an immunocompromised patient with active graft versus host disease and receiving steroids is undesirable. Many clinicians would potentially add a second antifungal agent in this patient if she had other signs of infection (i.e., pulmonary nodules in lung).

Instructive case 12 contributed by R. E. Lewis and A. W. Fothergill.

Instructive Case 13

Discussion

The most common bone disease secondary to coccidioidomycosis is osteomyelitis of the spine. Persistent spine pain should be evaluated for malignancy and infectious diseases. Coccidioidal infection most often presents as progressive pain with evidence of discitis.

Instructive case 13 contributed by R. H. Johnson and A. Heidari.

Instructive Case 14

Discussion

For induction therapy, she had received combination therapy with amphotericin B in a lipid formulation and a reduced dose of flucytosine because of kidney dysfunction. Her clinical response did not require repeated lumbar punctures to control raised intracranial pressure because symptoms did not worsen and actually improved. Her response to antifungal combination therapy was appropriate with a negative CSF culture at the end of 2 weeks of induction therapy.

Case Continued

Patient did relatively well on her suppressive fluconazole therapy at 200 mg/day for 4 months (dosed for reduced renal function) when she developed severe headaches and an MRI scan showed diffuse supra- and infratentorial leptomeningeal enhancement. At that time, CSF cryptococcal polysaccharide antigen was 1:16, white blood cell count was 100 cells/μl, and cultures negative. After 2 weeks of AmBisome at 5 mg/kg/d and no improvement, patient was continued on fluconazole and a 6-week dexamethasone taper was begun with immediate improvement in symptoms. Tacrolimus, mycophenolate were stopped and patient was started on dialysis. One week after stopping the 6-week taper of corticosteroids, her headaches returned and a 4-month steroid taper was begun. She improved and was eventually weaned off corticosteroids and now has received suppressive fluconazole for 1–2 years and is doing well awaiting a new transplant.

Discussion

In this case, the patient developed cryptococcal meningitis after receiving severe therapeutic immunosuppression in an attempt to save her renal transplant from rejection. She initially responded to potent antifungal combination therapy, which was adjusted to renal dysfunction. Initially, she did well on this suppressive therapy, but as she completely lost kidney function and immunosuppressive therapy was reduced, she again developed meningeal symptoms and signs, but the workup did not reveal evidence of an ongoing viable yeast infection. It was then decided that this may represent immune reconstitution inflammatory syndrome (IRIS) and she was started on corticosteroids which improved and eventually resolved her symptoms.

This case illustrates the dynamic relationship between the immune system and cryptococcosis. Although there are standardized, well-studied antifungal treatment regimens, there are clearly times when clinical judgment must be used regarding management. Currently, management of increased intracranial pressure and IRIS is performed without the luxury of guidance from robust evidence-based studies.

Instructive case 14 contributed by M. Chayakulkeeree and J. R. Perfect.

Instructive Case 15

Blastomycosis Presenting with Acute Respiratory Distress Syndrome

Discussion

1. Patients presenting with severe pulmonary disease, whether miliary or acute respiratory distress syndrome (ARDS), have a high rate of mortality (>50%). All patients presenting in this fashion should be initially treated with amphotericin B.
2. Life-threatening pulmonary disease may be seen in non-immunocompromised patients. Hence, blastomycosis must be considered in any patient living in or with recent travel to the endemic area and who presents with severe or overwhelming pneumonia.
3. Most patients who die do so within the first week of therapy emphasizing that amphotericin B therapy should be initiated as soon as possible after diagnosis.
4. Cytology may have a higher diagnostic yield than expectorated sputum examined under wet preparation.

Instructive case 15 contributed by S. W. Chapman and D. C. Sullivan.

Instructive Case 16

Discussion

Fibrosing mediastinitis (FM) results from excessive scarring around the hilar and mediastinal lymph nodes. This scar tissue extends from the lymph nodes to invade important nearby structures, such as the pulmonary arteries or veins, bronchial arteries, vena cava, trachea, main stem and lobar or segmental bronchi, esophagus, pericardium, and even the heart. FM represents a scarring response to a prior episode of histoplasmosis rather than an active and progressive infection.

The severity of the illness depends on the extent of the scarring, and the specific structures that are involved. In many cases, the consequences are mild and nonprogressive, causing minimal or no limitation to function, and requiring no consideration for therapy. In others, symptoms may be more severe, or even disabling, prompting consideration of the treatment options. In those with extensive involvement in both lungs, the illness is progressive and eventually fatal in nearly half of the cases.

Medical treatment with antifungal drugs that are used for treatment of other types of histoplasmosis is not effective in patients with fibrosing mediastinitis, because the manifestations of FM are caused by the scar tissue, not by active infection, and scar tissue is not affected by any medical treatments.

Minimally invasive procedures to open the blockages are useful in some cases. These procedures are relatively safe and sometimes effective, although the long-term results are not fully understood. The largest experience has been with stenting of the occluded blood vessels. Stenting of obstructed airways is not felt to be as useful, and there is little experience with this procedure. Stenting or dilatation of obstructed vessels is not always successful because the fibrotic tissue may be as hard as stone, preventing passage of a small wire past the blockage. Active bleeding may be stopped by embolization of the involved blood vessel.

Surgical correction of the obstructed blood vessel or airway also is not often possible, and carries a high operative mortality. Thus, surgery should be reserved for severe cases and only after less risky procedures are tried. The operative mortality is at least 20% overall, but can range from 50 to 75% in patients who undergo total removal of the involved lung (pneumonectomy). Of note is that the operative mortality may be even higher if the surgeon is not experienced with treatment of fibrosing mediastinitis. The reason that the mortality is so high is that the scar tissue is like cement, encasing the blood vessels and airways and obliterating tissue planes. Vital structures are often damaged while attempting to remove the scar tissue, causing bleeding or airway leaks. Death is caused by uncontrollable bleeding, respiratory failure, infection, and other postoperative complications. The indications for surgery are not well described, but at a minimum should include patient limitations severe enough to justify the risk of the known operative mortality. Surgical indications might include severe and recurrent bleeding not responsive to embolization, recurrent pneumonias that are not preventable by antibiotic prophylaxis, or respiratory failure. Surgery should only be conducted by surgeons who are experienced with fibrosing mediastinitis.

Instructive case 16 contributed by L. J. Wheat and N. G. Conger.

Instructive Case 17

Discussion

This patient is an example of the chronic unifocal pulmonary paracoccidioidomycosis.

Instructive case 17 contributed by A. Restrepo, A. M. Tobón, and C. A. Agudelo.

Instructive Case 18

Discussion

This case illustrates that chronic meningitis including coccidioidal disease may present with an increased intracranial pressure with or without radiographic changes. The increased pressure requires its own separate management plan.

Instructive case 18 contributed by R. H. Johnson and A. Heidari.

Instructive Case 19

Cutaneous/Locally Invasive Mucormycosis

Discussion

This case illustrates primary cutaneous mucormycosis with direct invasiveness of adjacent tissues. This entity is uncommon and represents less than 5% of all infections due to *Mucor* species. The case demonstrates the direct invasion via the scalp, following the craniotomy scar and subsequently leading to direct invasion of pituitary gland tissue. This case also demonstrates the difficulty in establishing an early definitive diagnosis. Although the infection was initially a superficial wound infection, the diagnosis was not made until the size of the necrotic lesion had increased by about 200% and a biopsy was performed. Furthermore, it also demonstrates the high morbidity and mortality associated with a delayed diagnosis and the resultant delays in appropriate and adequate management. Although in this case, complete excision alone would probably have resulted in a cure early on, most cases require wide and aggressive surgical removal of all necrotic tissue, along with appropriate antifungal therapy. The important reminder in this case is to always maintain a broad differential diagnosis in patients at high-risk of developing an invasive fungal infection.

Instructive case 19 contributed by J. A. Vazquez

Instructive Case 20

Answer: C

Discussion

Immune restitution disease, also called IRIS, is an acute inflammatory insult to the host, most often to the lungs. The pathophysiology is not fully understood but it is felt to represent an antigen-driven immune response initiated by a recovering immune system as a result of treatment in patients with AIDS. It is seen most often in the setting of newly diagnosed AIDS presenting with a pulmonary opportunistic infection. The role of retreatment of the opportunistic infection (OI) has not been addressed, but often there is no evidence of ongoing infection. Because the pathogenesis is felt to involve the return of pathogen-specific T cells, treatment with corticosteroids has been suggested.

Symptomatic *Pneumocystis* pneumonia coinciding with engraftment following bone marrow transplant is sometimes considered as a manifestation of IRIS but in reality this should be considered as a distinct entity, even though it may appear clinically similar. In this latter case, the inflammatory injury is an appropriate response of a newly functioning immune system to an active infection with *Pneumocystis*.

Instructive case 20 contributed by F. Gigliotti and T.W. Wright.

Instructive Case 21

Discussion

This case demonstrates that immunosuppressive therapy and transplantation may result in pulmonary, miliary, or disseminated coccidioidomycosis. The infection may be primary reactivation of prior known or unknown coccidioidal infection or may be from the allograft. In the proper endemic setting, it is probably cost effective to screen all donors and recipients for prior coccidioidal disease.

Instructive case 21 contributed by R. H. Johnson and A. Heidari.

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