Ian Feavers · Andrew J Pollard · Manish Sadarangani

Handbook of Meningococcal Disease Management

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Contents

Editor biographies

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Andrew J Pollard, FRCPCH PhD, is Professor of Paediatric Infection and Immunity at the University of Oxford, Director of the Oxford Vaccine Group, Fellow of St Cross College, and Honorary Consultant Paediatrician at the Children's Hospital, Oxford, UK. He obtained his medical degree at St Bartholomew's Hospital Medical School, University of London in 1989 and trained in Paediatrics at Birmingham Children's Hospital, UK, specializing in Paediatric Infectious Diseases at St Mary's Hospital, London, UK and at British Columbia Children's Hospital, Vancouver, Canada. He

obtained his PhD at St Mary's Hospital, London, UK in 1999 studying immunity to *Neisseria meningitidis* in children and proceeded to work on anti-bacterial innate immune responses in children in Canada before returning to his current position at the University of Oxford, UK in 2001. He chaired the UK's NICE meningitis guidelines development group, the NICE topic expert group developing quality standards for management of meningitis and meningococcal septicemia. He chairs the UK Department of Health's Joint Committee on Vaccination and Immunisation and the European Medicines Agency scientific advisory group on vaccines. His research includes the design, development and clinical evaluation of vaccines; including those for meningococcal disease and enteric fever and leads studies using a human challenge model of (para)typhoid. He also runs surveillance for invasive bacterial diseases and studies the impact of pneumococcal vaccines in children in Nepal. He has supervised 23 PhD students and his publications include over 300 manuscripts and books on various topics in pediatrics and infectious diseases.

Manish Sadarangani, MRCPCH, DPhil, is a Clinical Lecturer and Honorary Consultant in Paediatric Infectious Diseases and Immunology at The Children's Hospital, Oxford, UK. He completed his DPhil with the Oxford Vaccine Group in 2011, studying Opa proteins as a potential novel vaccine candidate for protection against capsular group B meningococcal disease, and completed a Fellowship in Paediatric Infectious Diseases in Vancouver, Canada in 2013. Current research interests include meningococcal disease, in particular the development of new meningococcal vaccines, childhood meningitis and encephalitis, the influence of the human microbiome on allergic and infectious disease, and antimicrobial stewardship.

Author biographies

Petter Brandtzaeg, MD, PhD, is Professor Emeritus and former head of the Infectious Disease Unit, Department of Paediatrics, Oslo University Hospital, Ullevål. He obtained his MD at University of Groningen, the Netherlands, and his PhD at University of Oslo. In 1973 he started training in Pediatrics and subsequently in Internal Medicine, with special focus on infectious diseases, at Ullevål University Hospital and National Institute of Public Health, Oslo. He has been particularly interested in the molecular pathophysiology of meningococcal infections and has worked for more than 30 years in this field. During these years, the Meningococcal Research Group (based at the Department of Clinical Chemistry at Oslo University Hospital) in collaboration with many other laboratories, has elucidated the crucial role of endotoxin in meningococcal disease. He has been a member of various groups appointed by the Norwegian Ministry of Health to work on guidelines related to meningococcal disease, group A streptococcal disease, antibiotic resistance, bioterrorism, and other aspects related to human infections.

Joseph Carcillo, MD, is Associate Professor of Critical Care Medicine and Pediatrics at the Children's Hospital of Pittsburgh and University Pittsburgh Medical Center. He graduated from the George Washington University School of Medicine in 1982, completed his residency training in Pediatrics, and then in Pediatric Critical Care Medicine at the National Children's Medical Center, garnering research training in sepsis at the Naval Medical Research Institute and the National Institutes of Health.

He served in the National Health Service Corps for 4 years before returning to Pediatric Intensive Care Medicine at the Children's Hospital of Pittsburgh. His research career has focused on systematic translational investigation in the pathophysiology and treatment of pediatric sepsis, septic shock, and multiple organ failure, establishing the rationale for clear differences in the management of children compared to adult patients. Presently, he is the two-time chair for the American College of

Critical Care Medicine task force on Hemodynamic Support of Newborns and Children with Septic Shock, and the Principal Investigator for the National Institute of Child Health and Development Collaborative Pediatric Critical Care Research Network's Critical Illness Stress-induced Immune Suppression (CRISIS) prevention trial.

Amanda Cohn, MD, is a medical epidemiologist at the Meningitis and Vaccine Preventable Diseases Branch at the Centers for Disease Control and Prevention (CDC), USA. She attended Emory School of Medicine in Atlanta and completed an internship and residency in pediatrics at Boston Medical Center and Boston Children's Hospital. She completed the Epidemic Intelligence Officer training program in applied epidemiology at CDC. She is the CDC lead for domestic meningococcal disease. She serves as CDC lead of the Advisory Committee on Immunization Practices meningococcal vaccine working group and she has written multiple updates to the meningococcal conjugate vaccine recommendations in the United States. She is responsible for US surveillance for *Neisseria meningitidis* and *Haemophilus influenzae*. She leads CDC efforts to evaluate vaccine effectiveness of quadrivalent meningococcal conjugate vaccine (MCV4) as well as pertussis booster vaccines (Tdap) and nH1N1 vaccine effectiveness on influenza hospitalizations. She is the recipient of the CDC 2009 Iain Hardy Memorial Award for outstanding contributions to vaccine-preventable diseases.

Mathieu Coureuil, PhD, is a Research Scientist at the Institut Necker Enfants Malades, Paris, France. He trained as a cell biologist and microbiologist. His work focuses on the pathogenicity of *Neisseria meningitidis* as a model of cell infection by extracellular pathogens. His team combines genomic techniques and cell biology approaches to understand the interactions of the *N. meningitidis* with human cells, which lead to blood and brain invasion.

Linda Glennie is the MRF Head of Research and Medical Information at the Meningitis Research Foundation, UK. She has a background in biological sciences and currently works with scientists and health professionals across a range of disciplines to develop information materials for the public and for health professionals. She has co-authored several educational resources for nurses and trainee doctors.

In collaboration with the Department of Social Medicine at the University of Bristol she manages the Impact of Meningitis project. Previously, she was a member of the Guideline Development Group for the NICE guideline on Bacterial Meningitis and Meningococcal Septicaemia, published June 2010.

Lee Harrison, MD, is an infectious diseases physician and Professor of Epidemiology and Medicine at the University of Pittsburgh, USA. He was trained in epidemiology and molecular epidemiology as an Epidemic Intelligence Service officer at the Centers for Disease Control and Prevention in Atlanta. His research focuses on the epidemiology and molecular epidemiology of *Neisseria meningitidis*, *Clostridium difficile*, and other serious bacterial pathogens. His laboratory utilizes a variety of microbial genomic techniques; including multilocus sequence typing, multilocus variable number tandem repeat analysis, and whole genome sequencing, to understand the epidemiology, transmission, and spread of pathogenic bacteria. Recently, he was appointed to serve a four-year term on the Advisory Committee on Immunization Practices (ACIP).

Bernt Christian Hellerud, PhD, is a Post doc research fellow within the Complement Research Group at Oslo University Hospital Ullevål and the University of Oslo, Norway. The main research goal for the Complement Research Group is to elucidate the role of complement as a primary inducer of the inflammatory reaction and thereby form a basis for a future therapeutic approach in complement-mediated disease processes.

Michael Levin, MD, is Professor of Paediatrics and International Child Health at Imperial College London, UK. He trained in medicine in South Africa and in pediatrics in the UK before specializing in infectious diseases. He was Consultant in Infectious Diseases at Great Ormond Street hospital before being appointed as Professor of Paediatrics at Imperial College London in 1990. His research has focused on life threatening infections of childhood, including meningococcal disease, childhood tuberculosis, malaria, Kawasaki disease, and severe respiratory infections. He currently heads an international EU-FP7 funded consortium studying the genetic basis of meningococcal and other life threatening bacterial infections of childhood. Previously, he has led an EUAid for poverty-related diseases project researching novel diagnostic methods for tuberculosis in Africa. He recently led an ESPID funded consortium studying the genetic basis of meningococcal disease, and is a co-investigator on the FEAST trial. Professor Levin's research group continues to study the pathophysiology and genetics of life threatening childhood infections by applying gene expression profiling and proteomic methods to understand pathophysiology of severe childhood illnesses, and are undertaking genome wide SNP studies linked to expression studies of meningococcal disease, Kawasaki disease, and tuberculosis.

Martin CJ Maiden, is a Wellcome Trust Senior Research Fellow and Professor of Molecular Epidemiology at the University of Oxford, UK. His research group studies the population biology and evolution of bacterial pathogens, with the objective of translating insights into benefits for human health. His work focuses mainly on two globally important pathogens, *Neisseria meningitidis* and *Campylobacter jejuni*. He uses a 'Population Genomics' approach and explores data with a range of analysis approaches including: epidemiological studies, dynamic modelling, and phylogenetic and genealogical investigations.

Simon Nadel, MD, is a Consultant in Paediatric Intensive Care at St Mary's and Imperial College London, UK. He became a consultant in 1994, having completed his training in pediatric infectious diseases at the Children's Hospital in Philadelphia, and pediatric intensive care training at Great Ormond Street Hospital and St Mary's Hospital. He holds a major clinical and research interest in life-threatening infection in children, and has been involved in many studies examining the susceptibility, treatment, and outcome of infections. He is the lead for Paediatric Intensive Care at Imperial, and was recently appointed the Adjunct Professor at Imperial College.

Xavier Nassif, MD, PhD, specializes in infectious diseases at the Institut Necker Enfants Malades, Paris, France. He is also the head of the INSERM unit in the Department of Clinical Microbiology at the Hopital Necker in Paris, where he focuses on the pathogenesis of bacterial systemic infections. In this unit he leads a group working on the pathogenesis of *Neisseria meningitidis*, specifically in the understanding of the mechanisms used to cross the blood-brain barrier and cause meningitis.

Reidun Øvstebø, PhD, is the Group Leader of the Blood Cell Research Group in the Department of Medical Biochemistry at Oslo University Hospital Ullevål, Norway. Her research group focuses on mechanisms regulating inflammation and coagulation, with the aim to disclose the roles of human monocytes and monocyte-derived microparticles in the coagulation initiation and for cytokines in inflammation. Other projects include research to validate differential leukocyte counts, identification and characterization of hemoglobin variants, and their influence on erythrocyte variables. The group is engaged in collaboration projects determining possible association between genetic variation in 'vulnerable genes' and different clinical end points. The Blood Cell Research Group is the coordinating group in the Regional Research Network on Extracellular Vesicles (RRNEV).

Stephen Pelton, MD, is Professor of Pediatrics and Epidemiology at the Boston University Schools of Medicine and Public Health and Chief of the pediatric infectious diseases program at Boston Medical Center. He is principal site investigator for the IMPAACT program evaluating new prevention strategies and therapies for children with HIV. His career goals have been to reduce the burden of middle ear disease and its sequelae, as well as respiratory tract infections in children. The current focus is evaluating new antimicrobial treatments for bacterial otitis media, the potential of immune prophylaxis for prevention of acute otitis media, understanding the impact of pneumococcal conjugate vaccines on epidemiology of pneumococcal disease, and most recently, identifying specific bacterial (pneumococcal) genes necessary for pathogenesis. His group has been leader in surveillance of the changing ecology of *Streptococcus pneumoniae* in the era of conjugate vaccines. Recent reports include the risk features for pneumococcal disease in the current era of widespread PCV immunization; identifying that comorbid disease remains an important risk feature; and a novel finding that risk stacking (the accumulation of comorbid conditions) is associated with a high incidence of disease comparable to immunocompromised hosts.

Mary Ramsay, MD, is a member of the Scientific Advisory Panel for the Meningitis Research Foundation. She obtained her medical degree at University College in London, and has since held an academic post at St Mary's Hospital Medical School in London, before becoming a Consultant Epidemiologist in 1994. She regularly produces information to the JCVI to inform policy on vaccination and for a range of groups on the prevention and control of hepatitis. She is joint Chief Editor of *Immunisation Against Infectious Diseases* (the recognized national source of advice on vaccination). She has also been involved in several national guidance documents on public health policy in her disease areas. In addition she provides expert clinical and public health advice and her work has directly contributed to several major decisions on national vaccination policy. She often acts as a temporary advisor to WHO on vaccine preventable diseases and advises the

European CDC on surveillance and epidemiology of vaccine preventable diseases. Over the past two years she has been Head of the Immunisation, Hepatitis and Blood Safety Department. Dr Ramsay's research interests involve establishing the potential role for new vaccines.

Muhamed-Kheir Taha, MD PhD, is currently Associate Professor at the Institut Pasteur, France, where he is the Head of the Unit of Invasive Bacterial Infections and the Director of the French National Reference Centre for Meningococci. He trained in medicine at Damascus University in Syria and in microbiology at the Institut Pasteur in France. He obtained his PhD from the University of Diderot in France. His main areas of research include molecular pathogenesis of *Neisseria meningitidis* and experimental animal models, as well as molecular epidemiology of meningococcal infections. He has co-authored more than 150 papers in peer reviewed international journals. He is a member of the French National Advisory Committee on Immunization.

Caroline Trotter, PhD, is a Senior Lecturer in Epidemiology at the University of Cambridge, UK. She trained as an infectious disease epidemiologist at the London School of Hygiene and Tropical Medicine. Her research focuses on the epidemiology and control of vaccine-preventable infections, particularly *Neisseria meningitidis*. She uses a range of methods including mathematical modelling, cost-effectiveness analyses, observational studies of vaccine effectiveness, carriage studies, and seroprevalence surveys to research the dynamics of infection and the role of immunization programs.

Raymond Tsang, M Med Sc, PhD is Chief of the Laboratory for Syphilis Diagnostic and Vaccine Preventable Bacterial Diseases at the National Microbiology Laboratory of the Public Health Agency of Canada. He obtained his M Med Sc and PhD degrees from the University of Hong Kong, and he has professional experience in university teaching hospitals, research institutes, government laboratories, and industry. His interests

include diagnostic bacteriology, infection and immunity, and molecular epidemiology of invasive bacterial diseases, and he has over 100 peer reviewed publications. Currently he is a member of a number of task groups on vaccine preventable bacterial diseases in Canada.

Abbreviations

ACIP	Advisory Committee on Immunization Practices
ARDS	Acute respiratory distress syndrome
AST	Antibiotic susceptibility testing
BBB	Blood-brain barrier
BE	Base excess
BEP	Base excess-platelet score
BIGSdb	Bacterial isolate genome sequence database
CFH	Complement factor H
CFHR3	Complement factor H-related protein 3
CFR	Case fatality rate
CRP	C-reactive protein
CRRT	Continuous renal replacement therapy
CSF	Cerebrospinal fluid
CT	Computed tomography
DIC	Disseminated intravascular coagulation
dUTP	Deoxyuridine-triphosphatase
EBV	Epstein-Barr virus
EMA	European Medicines Agency
ERM proteins	Ezrin, Radixin, and Moesin family of proteins
ESR	Erythrocyte sedimentation rate
EU	Endotoxin units
FDP	Fibrin degradation products
FH	Factor H
fHbp	Factor H binding protein
GGT	Gamma-glutamyl transferase
GMSPS	Glasgow meningococcal septicemia prognostic score
GP	General practitioner
ICP	Intracranial pressure
IL	Interleukin
IMD	Invasive meningococcal disease
JCVI	Joint Committee on Vaccination and Immunisation

KDO	2-keto-3-deoxy-octulosonic acid
LAL	Limulus amebocyte lysate
LOC	Level of consciousness
LOS	Lipooligosaccharide
LP	Lumbar puncture
LPS	Lipopolysaccharide
LR	Likelihood ratio
MAbs	Monoclonal antibodies
MALDI-TOF	Matrix-assisted laser desorption ionization-time of flight
MD	Myeloid differentiation factor
MH	Mueller-Hinton
MIC	Mean inhibitory concentration
MLSA	Multilocus sequence analysis
MLST	Multilocus sequence typing
MS	Mass spectrometry
NGS	Next generation sequencing
NO	Nitric oxide
OMV	Outer membrane vesicle
PAI-1	Plasminogen activator inhibitior-1
PBP	Penicillin binding protein
PCR	Polymerase chain reaction
PEEP	Positive end expiratory pressure
PFGE	Pulsed-field gel electrophoresis
PICU	Pediatric intensive care unit
PN	Platelet-neutrophil score
$rBPI_{21}$	Recombinant bactericidal permeability increasing protein
RDT	Rapid diagnostic test
rMLST	Ribosomal multilocus sequence typing
SBA	Serum bactericidal antibodies
$ScvO_2$	Central venous oxygen saturation
SIADH	Syndrome of inappropriate antidiuretic hormone

SNP	Single nucleotide polymorphism
ST	Sequence type
TAFI	Thrombin-activatable fibrinolysis inhibitor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
UNG	Uracil-N-glycosylase
VNTRs	Variable number tandem repeats
VR	Variable region
WBC	White blood cell
WGS	Whole genome sequencing
WHO	World Health Organization

Chapter 1

Introduction and epidemiology of meningococcal disease

Caroline Trotter, Mary Ramsay, Lee Harrison

Neisseria meningitidis

Neisseria meningitidis, the meningococcus, is a Gram-negative diplococcal bacterium that is only found naturally in humans. The meningococcus is part of the normal microbiota of the human nasopharynx and is commonly carried in healthy individuals (Chapter 2). In rare cases systemic invasion occurs, which can lead to meningitis and/or septicemia. Other clinical manifestations of meningococcal infection include pneumonia, urethritis, conjunctivitis, septic arthritis, and pericarditis (Chapter 5).

The meningococcus was first described as a cause of meningitis in 1887 by Weichselbaum [1], a Viennese doctor, although a striking clinical account of an outbreak of cerebrospinal fever in Geneva was provided by Vieusseux in 1805 [2]. Today, the meningococcus is an important cause of serious bacterial infections in most regions of the world.

There are twelve meningococcal capsular groups, defined on the basis of unique capsular polysaccharides, which vary in their biochemical composition [3]. Six of these groups (A, B, C, W, X, and Y) are responsible for nearly all disease. The capsule serves as a major virulence factor and capsular polysaccharides have been utilized as vaccine antigens (Chapter 7). The meningococcus can be characterized in a variety of other ways and molecular techniques are increasingly used in place of

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traditional serological methods. Subtypes are defined by variation in the two variable regions (VR1 and VR2) of the class 1 outer membrane protein (PorA) [4]. Another immunogenic outer membrane protein used as a molecular marker (though historically not characterized using serological methods) is the iron-regulated FetA protein.

Multilocus sequence typing (MLST) is used to characterize seven 'housekeeping' genes and determine the sequence type (ST), which can be grouped into clonal complexes. Housekeeping genes are used because they are generally not under selective pressure for rapid change and therefore can be used to assess the genetic lineage of meningococci. The European recommendation for molecular typing of meningococci takes the form [5]:

1. capsular group;
2. PorA type;
3. FetA type; and
4. sequence type (clonal complex), for example B: 1.19,15: F5-1: ST33 (cc32).

Antigens employed in the new generation of vaccines designed to prevent group B disease will also become important targets for typing, including factor H binding protein (fHbp) (Chapter 7). Recently, whole genome sequencing has become increasingly used for molecular characterization of *N. meningitidis*, including antigen gene typing, determination of ST, and phylogenetic analyses.

Disease surveillance

The ideal surveillance for meningococcal disease is an active, population-based system in which clinical cases are followed up for comprehensive laboratory testing and strain characterization. Few countries reach this standard and may use passive rather than active surveillance, syndromic surveillance rather than laboratory confirmation, sentinel rather than whole population coverage, or some combination of the above. As such, caution is required in interpreting data from different jurisdictions and the true global incidence of meningococcal disease is unknown [6]. The European Union case definition is shown in Box 1.1 [7].

Clinical criteria

Any person with at least one of the following five:
- Fever
- Meningeal signs
- Petechial rash
- Septic shock
- Septic arthritis

Laboratory criteria

At least one of the following four:
- Isolation of *Neisseria meningitidis* from a normally sterile site, including purpuric skin lesions
- Detection of *Neisseria meningitidis* nucleic acid from a normally sterile site, including purpuric skin lesions
- Detection of *Neisseria meningitidis* antigen in CSF
- Detection of Gram-negative stained diplococcus in CSF

Epidemiological criteria

An epidemiological link by human to human transmission

Case classification

A. Possible case
- Any person meeting the clinical criteria

B. Probable case
- Any person meeting the clinical criteria and with an epidemiological link

C. Confirmed case
- Any person meeting the laboratory criteria

Box 1.1 The European Union 2008 case definition of invasive meningococcal disease [7]. CSF, cerebrospinal fluid.

Case fatality and sequelae

The case fatality from invasive meningococcal disease (IMD) in high-income countries is usually in the range of 5 to 15% overall. In the UK, reported case fatality is approximately 5% [8], in Europe as a whole approximately 9%, and 11% in the USA [9]. Case fatality rates are variable depending on reporting practices, the age of the patient, and strain characteristics. For example, the ST-11 clonal complex circulating in Europe from the mid-1990s was associated with odds of death twice that of the most common ST-41/44 complex, even after adjusting for age [10]. In a systematic review of bacterial meningitis among African children, the median case fatality for patients hospitalized for meningococcal meningitis was around 4%, although the range from individual studies was between 1 and 13% [11] and the quality of included studies was variable.

A systematic review and meta-analysis of the risk of disabling sequelae following bacterial meningitis, based on the literature published between 1980 and 2008, found that a median of 9.5% of survivors of meningococcal meningitis experienced at least one major or minor sequela and 7.2% experienced a major impairment, including hearing loss, visual disturbance, and motor deficits [12]. The risk of sequelae was highest in low-income countries [12] and, as for mortality, may vary according to the infecting strain. For example, in Canada in the early 1990s, 3% of survivors of capsular group B disease had physical sequelae, compared to 15% of survivors of group C (ST-11) disease [13].

Epidemiology of meningococcal disease

Patterns of disease

Although our knowledge of meningococcal disease epidemiology is influenced by the type and quality of surveillance that is employed, there are three basic patterns of disease.

1. **Endemic disease.** In much of the world, including most high-income countries, meningococcal disease is endemic but rare, with annual incidence of less than 5 per 100,000 and in many areas less than 1 per 100,000 people. Cases are mainly sporadic, with occasional small clusters of epidemiologically linked cases.
2. **Hyper-endemic disease.** Some countries, or particular regions within countries, can experience prolonged periods of elevated incidence of 5 to 20 per 100,000 people. Most accurately described as hyper-endemic disease and often associated with the emergence of a new meningococcal clone.
3. **Epidemic disease.** In certain regions of the world, particularly the African meningitis belt, epidemics of meningococcal disease occur periodically but irregularly [14]. The scale of these epidemics is quite different to hyper-endemic disease; the epidemic threshold defined by WHO is a weekly incidence of 10 per 100,000 (usually at a district level) and over the course of an epidemic season, cumulative incidence can be well in excess of 100 per 100,000.

Age distribution

The burden of meningococcal disease is not equally distributed by age in any setting. In high-income countries where disease is endemic, incidence is usually highest in children aged less than 5 years, with infants under 1 year of age being at highest risk [15] (Figure 1.1). This correlates with low natural immunity; Goldschneider et al first described the inverse relationship between serum bactericidal antibodies (which increase with age) and disease incidence (which generally decreases with age) in seminal studies in the 1960s [16]. Often there is a smaller secondary peak in disease incidence in teenagers and young adults, likely due to increased exposure and transmission of meningococci in this age group (Chapter 2). This secondary peak in incidence can be more pronounced with particular strains, as seen with the emergence of capsular group C ST-11 in Canada, the US, and Europe.

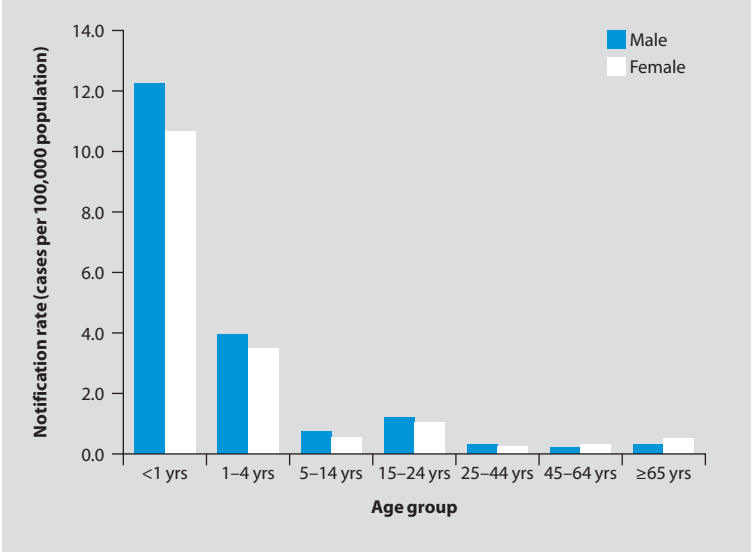

Figure 1.1 Age and sex distribution of 3439 cases of notified invasive meningococcal disease in Europe 2012. Contributing countries: Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom. Reproduced with permission from the European Centre for Disease Prevention and Control [15].

Meningococcal disease is relatively rare in older adults. In hyperendemic settings, the youngest children are often similarly at highest risk of disease. Between 1991 and 2003, New Zealand experienced hyperendemic incidence of disease due to group B/ST-41-44 clonal complex; here incidence was 15 per 100,000 overall, but 124 and 60 per 100,000 in infants and 1- to 4-year-olds, respectively [17].

In the African meningitis belt disease tends to be more evenly distributed across age groups compared to high-income countries, whereby individuals under 20 years of age experience a high incidence, but disease is uncommon in adults over the age of 30 years. In Niger, the proportion of cases in children under 5 years of age was higher in epidemic compared to non-epidemic years [18].

Seasonality

In temperate countries, the incidence of meningococcal disease is highest in the cold winter months. The most striking seasonality is observed in the African meningitis belt where epidemics occur in the dry season and end with the onset of the rains. Environmental and climatic factors, including low humidity and high levels of dust from the Harmattan winds, contribute to this pattern of disease [19], possibly by damaging the mucosal surfaces, thus making invasion more likely [14].

Other risk factors

The incidence of meningococcal disease tends to be higher in males than females. Socio-economic status is also important in high-income countries, with a higher incidence being observed in the most deprived populations [20]. Having contact with an infected person is a well-established risk factor [21]. Individuals who are deficient in components of the alternative and terminal complement pathways are highly predisposed to invasive, often recurrent, meningococcal infections [22]. Patients with HIV infection have an increased risk of disease, with a reported adjusted relative risk of 11 in a South African study [23]. Passive smoking (contact with the smoke of others) appears to be a risk factor for disease [24,25]. Concomitant or recent influenza infection

has also been shown to increase the risk of meningococcal disease in some settings [26].

Capsular groups causing disease

The capsular groups causing IMD vary substantially by region and over time (Figure 1.2). Recent epidemiology has been particularly influenced by the use of effective protein-polysaccharide conjugate vaccines against groups A, C, W, and Y (Chapter 7). The major trends by groups and region are described below.

Sub-Saharan Africa

Different patterns of disease are seen across Africa where the geography varies from desert to tropical rainforest. In Southern Africa there is a paucity of surveillance data (except in South Africa where disease is monitored by the national surveillance centre). Here disease is endemic, with incidence generally less than 2 per 100,000, with groups B and W predominating in recent years. The highest burden of meningococcal disease anywhere in the world is experienced by countries in the Sahel and sub-Sahel, in a region known as the African meningitis belt. First described by Lapeyssonnie [27], the meningitis belt stretches from Senegal in the West to Ethiopia in the East, populated by around 400 million people. The irregular but periodic nature of epidemics, of varying magnitude, is illustrated by the time series from Chad (Figure 1.3). Epidemic waves affecting many countries also occur; in 1996 there were over 250,000 recorded cases and 25,000 deaths in the African meningitis belt. Even outside of epidemics, the endemic incidence is many times higher than that experienced in most other regions of the world. Most epidemics in the African meningitis belt over the last 100 years have been due to group A, with some due to C, W, and more recently X, but never group B or Y. The predominance of group A as a cause of epidemic meningitis prompted the development of a group A specific vaccine for Africa by the Meningitis Vaccine Project, with early results demonstrating that the vaccine halts both transmission and prevents invasive group A disease (Chapter 7). Due to this success,

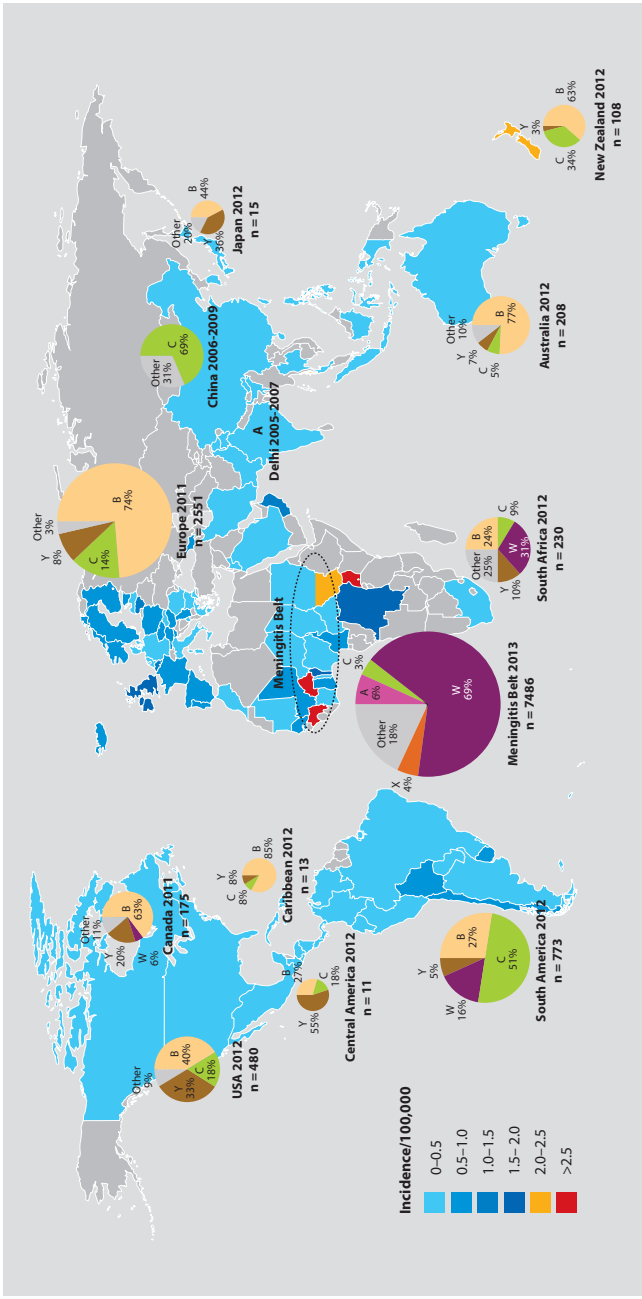

Figure 1.2 Global burden and capsular group distribution of meningococcal disease, based on most recent data available until 2013. Figure kindly provided by Siobhan Botwright.

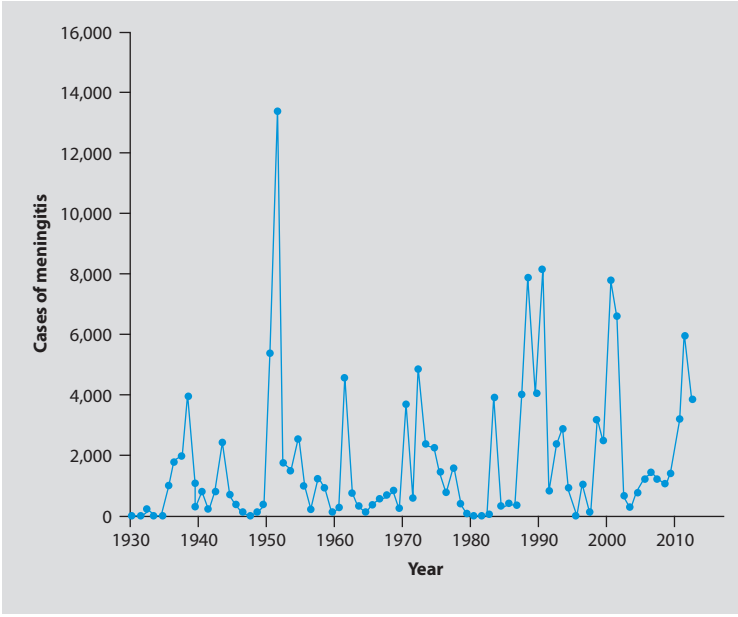

Figure 1.3 Periodic but irregular epidemics of meningitis in Chad, 1930 to 2012 [28]. Reproduced with permission from © Lancet Publishing Group.

overall meningitis incidence is much decreased and group W currently predominates in the meningitis belt.

Middle East and North Africa

The burden of meningococcal disease in the Middle East and North Africa is not well studied, but disease due to groups A, B, W, and Y has been reported in recent years [29]. The Hajj pilgrimage in Saudi Arabia has been a key driver for the global dissemination of hypervirulent meningococcal strains, in particular capsular group W; less so now that vaccination of pilgrims is compulsory.

Europe

Since at least the 1970s most meningococcal disease in Europe has been due to capsular groups B and C. In 2012, 74% of IMD in Europe was due to group B; effective group C conjugate vaccines have been

used in many countries from 1999 onwards, reducing the burden of disease due to these strains (Chapter 7). The pattern of disease is primarily endemic, with confirmed overall incidence of 0.69 cases per 100,000 in 2012, ranging from 1.76 (Lithuania) to 0.12 (Bulgaria) per 100,000 per year. The trend of declining incidence continues in many countries, despite the predominance of group B and the lack of specific control measures. In England and Wales there were only 621 cases of confirmed group B disease in 2012/13 compared to 1210 a decade earlier [30].

North America

In the US, the historical annual incidence of meningococcal disease tended to fluctuate between 0.5 and 1.5 cases per 100,000 population. However, following a peak in 1997, the incidence has fallen to historically low levels, with an incidence of 0.15 per 100,000 in 2012 [31]. The declining incidence preceded the routine use in adolescents of quadrivalent meningococcal vaccines, the first of which was introduced in 2005, and declines were also noted for group B in the absence of any immunization program. The incidence is highest in infants and there is a peak in adolescents that has been attenuated in recent years. Most disease in the US is caused by groups B, C, and Y, with a small percentage being caused by group W.

In Canada, the incidence is also highest in infants. With the widespread use of group C conjugate vaccines, the incidence of group C disease declined by 77% and was 0.03 cases per 100,000 during 2009 to 2012 [32]. Group B is by far the most common group, followed by group Y and group C.

Latin America

The pattern of disease is endemic in Latin America, with occasional hyper-endemic periods in particular areas. As in Europe and North America, group A disease no longer occurs [33]. The very low incidence rates reported by some countries, coupled with a high proportion of meningitis without microbiological confirmation, suggests that the true burden of disease is underestimated in this region. Groups B and C have

dominated in recent decades, but more recently, increases in the incidence of group W disease (mainly ST-11 clonal complex) have been observed in several countries, including Argentina, Brazil, Chile, and Uruguay [34]. In 2011 and 2012 Chile experienced a large increase in the number of cases of group W disease and a high reported case fatality of 25%, leading to the implementation of a national immunization campaign in children aged 9 months to 4 years with a quadrivalent conjugate vaccine [34]. The annual incidence of meningococcal disease in Mexico is reported to be <0.1 per 100,000, with most isolates characterized during 2000 and 2005 being group C, followed by groups B and Y [33].

Australia and New Zealand

In Australia, meningococcal disease is endemic. Current annual incidence is around 1 per 100,000, with most disease due to group B, following the successful introduction of group C conjugate vaccines in 2003. In New Zealand, the incidence of meningococcal disease is now much lower than in the previous decade when the country experienced hyper-endemic incidence due to the B:4:P1.7-2,4 strain. A tailor-made vaccine was temporarily deployed from 2004 to 2008, which contributed to the control of disease. In 2010 incidence was 2.4 cases per 100,000; typical for a high-income endemic country.

Asia

There is in general a paucity of information from Asia, which may or may not reflect the burden of disease and the relative priority afforded to IMD. In India, although the burden of disease is not reliably known, *N. meningitidis* is the third most common cause of sporadic bacterial meningitis in children aged <5 years in laboratory confirmed cases. Incidence is higher in temperate Northern versus tropical Southern India and there have been a number of outbreaks due to group A disease in the past decade [35]. Several other Asian countries have also experienced localized disease outbreaks of group A or C (eg, Philippines in 2005). China historically experienced group A epidemics until a national immunization program using a group A polysaccharide vaccine in early 1980s;

a national campaign with A+C polysaccharide took place in 2005 following the introduction of a hypervirulent ST-4281 group C clone [36].

Current issues

The epidemiology of meningococcal disease will continue to evolve over the next few years, whether through natural cyclical fluctuations or due to the expansion of meningococcal vaccination programs. Improved surveillance is required in many parts of the world so that optimal prevention strategies can be devised. A new vaccine designed to protect against group B disease is now licensed (4CMenB) and it will be important to determine its efficacy and public health impact in large scale population based programs. Vaccine-type replacement was not a public health issue for group C vaccination, but remains a potential threat to the success of the monovalent group A conjugate and 4CMenB, so must be monitored carefully (see Chapter 7).

Summary points

- The epidemiology of meningococcal disease is geographically diverse and dynamic.
- Continued, and in many areas, improved surveillance is required to monitor epidemiological trends, assess the global burden of disease and evaluate the impact of immunization programs.

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Chapter 2

Carriage and transmission of *Neisseria meningitidis*

Caroline L Trotter, Martin CJ Maiden

Introduction

Despite the fact that the meningococcus is a pathogen of global significance, causing the severe syndromes of meningitis and septicemia, it is a member of the normal microbiota of the nasopharynx of healthy humans. Indeed, this is the natural habitat of *Neisseria meningitidis*, and no other reservoir is known to exist for this bacterium. As the process of causing disease does not contribute to person-to-person transmission of meningococci, it is most usefully characterized as an 'accidental pathogen' of humans; host invasion is a dysfunctional event for both the microbe and its host, from which neither gains any benefit [1] (Figure 2.1). Although the significance of the carrier state has been long recognized, it has become increasingly apparent that an understanding of carriage is crucial to an understanding of meningococcal disease and its prevention. Here we shall outline the important features of meningococcal carriage and its study, and their implications for understanding disease.

Detecting the carrier state

The presence of meningococci in asymptomatic carriage has been appreciated from the early 20th century, when early carriage and transmission studies were conducted in UK military populations [2]. In most of

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the studies conducted since that time, carriage has been detected by conventional microbiological techniques, which remains the predominant method. Typically, a cotton-wool tipped (or similar) swab is used to sample the back of the throat and the material is transferred directly or indirectly on to a selective culture plate. There are various approaches to this type of sampling and culture, with success depending on choosing the appropriate location (uvula and/or tonsils), route of access (per-oral preferred to per-nasal) and ensuring that the meningococci remain viable until they are incubated for growth.

The density of growth in the nasopharynx can vary appreciably and after incubation culture plates can exhibit anything from very few colonies to confluent growth of putative *Neisseria,* which are identified as Gram-negative, oxidase-positive diplococci. *N. meningitidis* has to be

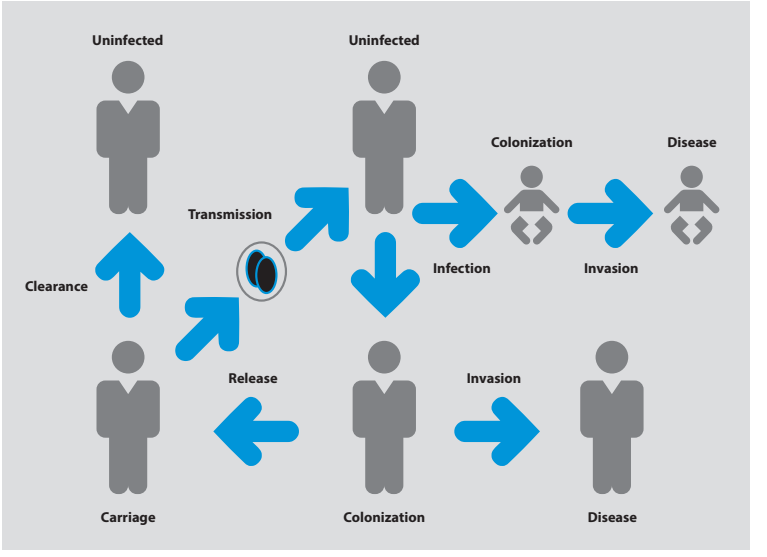

Figure 2.1 The natural history of infection with *Neisseria meningitidis* in high-income countries. Meningococcal carriage is common, at around 10% of the population, and capsulated meningococci are released from colonized individuals in aerosols. These bacteria infect human hosts and the great majority of these infections result in asymptomatic colonization. In adults carriage is common and the development of disease is very rare, whilst in small children carriage is rare but disease relatively more common. There is no onward transmission from invasive disease. In most individuals carriage is cleared, presumably as a consequence of host immune responses. Adapted from Trotter and Maiden [3].

distinguished from related organisms, especially members of the genus *Moraxella* and other *Neisseria* (studies prior to 1960 are of limited value as they did not differentiate adequately between species). Biochemical techniques have been used to do this for many years, and they can be effective, although they are time-consuming and can lack resolution. In particular, the detection and differentiation of *Neisseria* species relies on a few biochemical characteristics that are not always reliable. Molecular methods are increasingly used, although it has been necessary to develop high-resolution methods for speciation: the small subunit 16S rRNA can distinguish *Neisseria* from other genera, particularly *Moraxella*, but will not discriminate species within the genus [4]. Other sequence-based techniques such as multilocus sequence analysis (MLSA) and ribosomal multilocus sequence typing (rMLST) are effective for speciation within the genus, and the latter has been developed into a high-resolution rapid single–single locus approach, the *rplF* assay [5–7]. Developments in very high throughput 'next generation' sequencing (NGS) present the prospect of the use of metagenomic techniques to monitor the frequency and density of colonization of meningococci in the context of other organisms, but at the time of writing the potential of these approaches has not been realized.

Most carriage studies are cross-sectional, designed to measure the prevalence of carriage and provide a snapshot of the population. The prevalence of carriage is determined by the acquisition rate and the duration of carriage, which can only be determined empirically through longitudinal studies that follow-up individuals over time. Although less well described, the acquisition rate is important because disease is thought to occur soon after acquisition.

The natural history of meningococcal carriage

Exposure to meningococci through respiratory droplets can result in acquisition of carriage. For colonization to occur the bacteria must adhere to the mucosal surface, exploit locally available nutrients (including iron), and evade the human immune system. There are several important structures and molecules that facilitate colonization; pili facilitate initial attachment to the epithelial cell surfaces and opacity-associated proteins allow a more intimate engagement [8]. Phase and antigenic

variation of a number of surface components permits immune evasion during infection [8].

A period of carriage may be transient or last for many months. The duration of carriage is not well established as few longitudinal studies of carriage have been performed. Studies in Europe have suggested periods of nine or more months, whereas a study in Africa estimated a much shorter duration of three months [9].

Antibiotic chemoprophylaxis may be used to eliminate carriage from an individual and is an appropriate public health response for close contacts of a case or during an outbreak (Chapter 6). Asymptomatic carriage will otherwise resolve naturally. It is likely that carriage is an immunizing event, although the immune responses elicited by carriage, particularly in the mucosa, are not well characterized [10].

Given the extent of horizontal gene transfer observed in meningococci, it is likely that carriage of multiple meningococcal strains is relatively frequent. However, most culture-based studies have not been designed to be able to measure this, as they tend to pick a single colony, or at most very few, for characterization. As culturing many isolates from single throat swabs by conventional microbiology is very time-consuming, appropriately designed carriage studies using molecular techniques, especially metagenomic approaches, provide novel means of addressing this issue.

Epidemiology of carriage

Meningococcal carriage appears to be universal among human populations with carriage prevalence varying from a few percent to a substantial proportion of the population under study, according to time, place, host, and bacterial factors [11]. Unfortunately, there is no reliable relationship between carriage prevalence and disease incidence, and carriage studies remain uninformative in predicting disease risk.

The meningococcus is a highly diverse organism. This diversity includes surface antigens, which is perhaps to be expected in an organism that lives in close association with the human immune system, but diversity is also evident genome wide, including in 'housekeeping' genes, which are apparently not exposed to host immune responses [12]. Studies of meningococci isolated from asymptomatic carriage and disease have

demonstrated that not all variants are equally likely to cause invasive disease [13]. Typically the meningococci isolated from carriage are more diverse than collections obtained from disease. Disease isolates are likely to belong to one of a few particular genetic types referred to as 'hyper-invasive lineages'. It is important to emphasize that the spread of even the hyper-invasive meningococci is by asymptomatic carriage: while all meningococci have to be efficient at asymptomatic carriage and transmission, only a small subset of meningococci exhibit a propensity to cause disease [1].

Meningococcal carriage is highly age dependent. The great majority of studies have been conducted in industrialized societies, and in such settings meningococcal carriage is usually very rare in infancy and increases with age, carriage prevalence reaching a peak during the teenage years (Figure 2.2) [14]. This has been shown to be largely due to social behavior with carriage rates, and by extension transmission, increased by factors such as smoking, pub and club attendance, and kissing [15]. Studies in Africa have shown a less consistent age distribution, carriage prevalence being generally highest in younger children aged 5 to 14 years [16].

Seminal studies suggest that the peak in meningococcal disease incidence in infants, where carriage is rare, is due to poor immunity following the waning of maternal antibodies [17], whilst the second peak in disease incidence in young adults, which is characteristic of the meningococcus, is probably attributable to increased exposure of individuals to virulent meningococci that they have not experienced earlier in life. The conundrum that individuals acquire immunity whilst apparently not carrying meningococcus is yet to be resolved, but the carriage of closely related members of the genus *Neisseria* may be involved.

Meningococcal carriage prevalence is generally higher in males than females and particularly high rates of carriage have been observed in closed or partially closed communities, such as military training establishments and university halls of residence. This is most likely due to intense mixing of people from different geographical locations carrying a diversity of strains [18,19]. The annual Hajj pilgrimage has provided opportunities for the regional and global spread of meningococci [20].

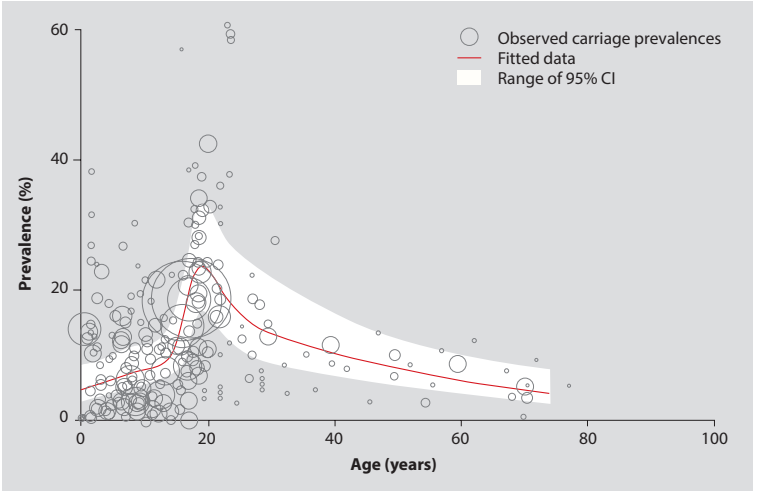

Figure 2.2 Estimates of meningococcal carriage by age in high-income countries. Swabs were plated immediately after collection, from a systematic review and meta-analysis [14]. Circles are the data-points included, with the larger circles representing a larger sample size. The largest circles represent the results of the serial cross-sectional studies in teenagers aged 15 to 19 years in the UK, before and after the introduction of the meningococcal capsular group C vaccine. Shaded area represents 95% bias-corrected confidence intervals. Reproduced with permission from © Elsevier.

Other *Neisseria* species

With the exception of *Neisseria gonorrhoeae*, other members of the genus have not been studied as extensively as the meningococcus, as they do not cause disease frequently. *Neisseria lactamica* has attracted most interest as it is closely related to the meningococcus and shares the same ecological niche. The age distribution of *N. lactamica* is very different from that of the meningococcus, with carriage in children more common than carriage in adults. Following birth, children commonly acquire *N. lactamica* and carriage persists for months and perhaps years [21]. It has been proposed that carriage of this organism leads to immunity to the meningococcus, but this has not been definitively established [22]. However, recent genomic studies have shown that at least two other *Neisseria* species, *N. polysaccharea* and *N. bergeri* [23], are more closely related to the meningococcus than *N. lactamica* and these species may be important in the development of immunity. The study of these organisms remains limited at the time of writing, but their interactions with

the human host may also be important in childhood when neither the meningococcus nor *N. lactamica* are routinely isolated from carriage or in settings other than high-income countries.

Effect of vaccination on carriage

Carriage studies have assumed more importance with the realization that herd immunity (or 'herd protection') acting on carriage is an important element in the success of meningococcal protein-polysaccharide conjugate vaccines (Chapter 7). Large-scale studies of meningococcal carriage following the introduction of capsular group C conjugate vaccines in the UK showed a marked drop in the prevalence of carriage of group C meningococci within a year, a trend that continued over subsequent years [24]. Incidence of disease declined in the unimmunized, also an indication of herd protection. As protective responses declined in those immunized under 1 year of age, herd protection was a major reason for the success of these vaccines, with the outbreak strain (the hyper-invasive ST-11 complex group C meningococcus) particularly affected by the vaccination program [10].

These observations have been highly influential in the implementation of these vaccines in other countries, the modification of the immunization schedule in the UK, and the implementation of the group A protein-polysaccharide conjugate vaccine (PsA-TT) in the African meningitis belt. Evidence gathered following mass vaccination campaigns suggests that PsA-TT can also successfully protect against carriage and generate herd protection [25]. Most evidence suggests that plain polysaccharide vaccines do not affect carriage and interrupt transmission [26]. The effect of outer membrane vesicle (OMV) and other noncapsular vaccines on carriage is less clear but they are certainly not as effective as conjugate vaccines [27].

Summary points

- An understanding of carriage is central to understanding the population and transmission dynamics of meningococci.
- It is now appreciated that the spectacular effects of the bacterial conjugate vaccines are due to the herd protection that they

confer through their effect against asymptomatic carriage, which reduces transmission.

- Carriage studies have been very important in understanding the virulence of the hyper-invasive lineages and will play a central role if we are to understand the reasons for the high levels of variability in invasiveness of these organisms and design effective interventions against them.

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Chapter 3

Pathogenesis of invasive disease

Reidun Øvstebø, Bernt Christian Hellerud, Mathieu Coureuil, Xavier Nassif, Petter Brandtzaeg

Introduction

Before 1905 (the year treatment with antimeningococcal serum started) the case fatality rate (CFR) of invasive meningococcal disease (IMD) was 70 to 90% [1]. Patients with severe sepsis or septic shock died rapidly, usually within 24 hours. Patients with meningitis and other clinical manifestations could live as long as 6 to 8 weeks before succumbing [1]. Since *Neisseria meningitidis* did not produce any recognizable exotoxin it was hypothesized that endotoxin, a yet undefined chemical component of Gram-negative bacteria, played an important role in the pathogenesis of this disease. Research conducted during the last 30 years has confirmed the crucial role of lipopolysaccharide (LPS, endotoxin) in meningococcal disease and also documented the role of non-LPS components as proinflammatory molecules [2].

Transmission, adaptation, and penetration to the circulation

N. meningitidis (Figure 3.1) is transmitted by droplets or exchange of saliva. Infection starts within 10 days, usually 2 to 4 days after a nonimmune person has been exposed to an asymptomatic carrier or an untreated patient. Before the symptoms develop, the transmitted virulent meningococci adapt locally on specific nonciliated epithelial cells

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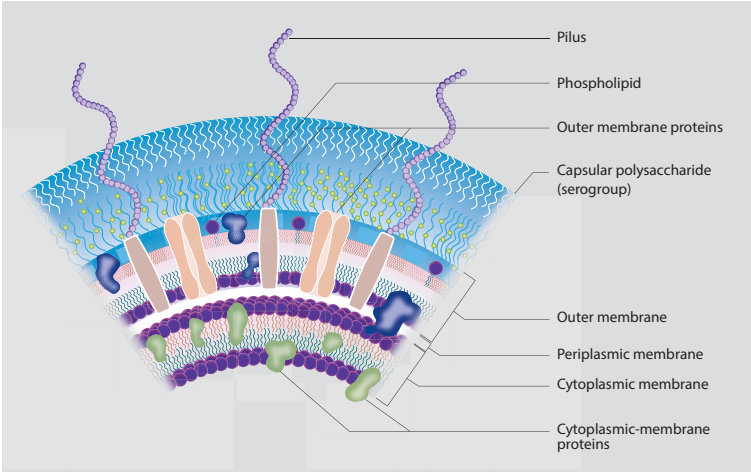

Figure 3.1 Cross-sectional view of the meningococcal cell membrane. Adapted from Rosenstein et al [7].

in the nasopharynx and tonsils (Figure 3.2) [2,3]. Bacteria evade the immune system partly via phase and antigenic variation, altering LPS and certain outer membrane proteins and down-regulating pili, before passing through the mucosal barrier and penetrating into the submucosal layer [3–5]. Subsequently they enter the circulation, most likely in the upper respiratory tract. When reaching the circulation it is assumed that they once more undergo phase variation of surface structures, thereby decreasing immune recognition through molecular mimicry [4–6]. This is accomplished by:

- up-regulating type IV pili;
- altering the terminal part of the LPS side chain to express the L3, 7, 9 epitopes; and
- adding sialic acid to LPS.

These changes result in an increased resistance to antibodies and complement. The capsule polysaccharides consist of long sugar filaments that protect meningococci from the antibacterial effect of human blood, mainly by down-regulating phagocytosis. Survival and growth in the blood are required for meningococci to develop into a systemic infection. *N. meningitidis* has the propensity to invade the meninges

and can be detected in both blood and/or the cerebrospinal fluid (CSF) during clinical disease (Figure 3.2 and Table 3.1) [6].

Neisseria meningitidis and intravascular survival

The combination of bactericidal antibodies and an intact complement system protects individuals from virulent meningococci [3,7,10–12]. When protective antibodies are absent the bacterial growth rate in the circulation

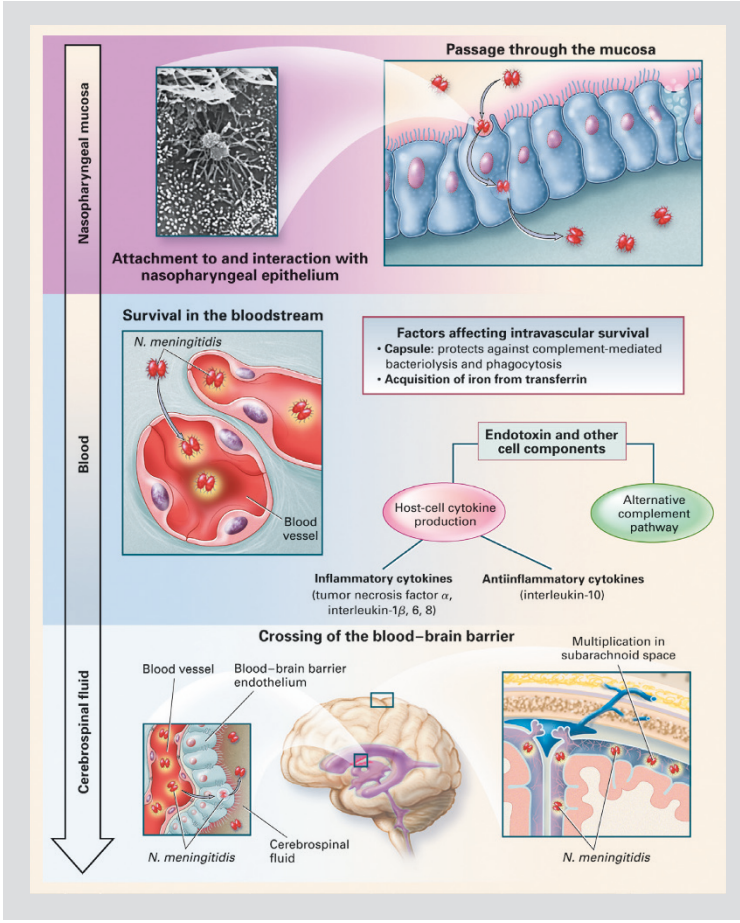

Figure 3.2 How *Neisseria meningitidis* colonizes the nasopharynx and enters into the bloodstream and cerebrospinal fluid. Reproduced with permission from © Massachusetts Medical Society [7].

varies from patient to patient infected with seemingly identical strains. This can be observed during clonal outbreaks of *N. meningitidis*. The reasons for this variation are not well understood but genetic variations in the innate immune system (including polymorphisms of complement factor-H-like protein, plasminogen activator inhibitor 1, and interleukin [IL]-1β) may play a role [3,6,13,14].

It has been known since the 1960s that patients presenting with clinical signs of meningitis without shock have a much better prognosis than patients with shock alone or shock and meningitis [6,8,15]. Subsequent studies have confirmed these observations [2]. In the 1980s a classification system for research on meningococcal disease was developed based on the presence or absence of the two critical clinical manifestations (meningitis and shock) [2,6,8,15]. This classification enabled patients to be divided into four clinical presentation groups to aid studies of pathogenesis (Table 3.2) [2,6,8,15]:

1. meningitis without shock;
2. shock without meningitis;
3. shock and meningitis; and
4. no shock and no meningitis (mild meningococcemia).

The underlying pathophysiology reflects compartmentalization of the bacterial growth to primarily the circulation in those developing shock, or to the subarachnoid space and meninges in those with marked symptoms of meningitis. The difference in magnitude of bacterial growth between

	Blood culture (% positive)	CSF culture (% positive)	Plasma Nm(DNA)/mL	Plasma LPS (EU/mL)
Meningitis	50.0	84.0	$<10^3$	<0.5
Shock	93.0	59.0	$2x10^7$	43.0
Shock + meningitis	87.0	83.0	10^6	9.4
Mild systemic meningococcal disease	77.0	47.0	$7.7x10^3$	<0.5

Table 3.1 Pathophysiological characteristics of clinical presentations. Blood and cerebrospinal fluid cultures are collected before antibiotic treatment is started. LPS (EU/mL) denotes the median biological activity given as endotoxin units and determined by the chromogenic limulus amebocyte lysate (LAL) assay [8,9]. Nm(DNA) denotes the median number of *N. meningitidis* DNA and equals the number of live and dead bacteria as determined by real-time polymerase chain reaction (PCR) [9]. CSF, cerebrospinal fluid; EU, endotoxin units; LPS, lipopolysaccharide. Adapted from Brandtzaeg [6] and Ovstebo et al [9].

these two compartments could be 1000- to 100,000-fold in the same patient [2,3,6,8,15].

Meningococcemia: a disease of the endothelial cells

As revealed by post mortem examination and histological observation, a specific feature of meningococcal infection is the ability of the bacteria to interact with the endothelium of small blood vessels. This interaction leads to the formation of microcolonies throughout the capillaries [16]. This phenomenon is very unusual during sepsis due to Gram-negative bacteria. Indeed, *N. meningitidis* has been documented to consistently adhere to endothelial cells in vivo [3,16]. It suggests that this interaction is responsible for the specific clinical characteristics of meningococcal infection, ie, the ability to cross the blood-brain barrier (BBB) to cause meningitis. Furthermore, the interaction of meningococci with endothelial cells induces the thrombotic/leakage syndrome, which in mild form is responsible for purpuric lesions, and in extensive forms for purpura fulminans, thereby characterizing the disease clinically (see Chapter 5).

In vivo *N. meningitidis* is encapsulated and, using a humanized mouse model, it has recently been shown that type IV pili are responsible for meningococcal interaction with microvessel endothelial cells [17–20]. Type IV pili are polymeric filaments found on many Gram-negative bacteria [21], the major pilin (PilE) is assembled into fibers from a platform in the inner-membrane [22]. Three other minor pilins are localized into the fiber (ComP, PilX, and PilV) [23–26]. Recent data obtained in vitro

Presenting group	Definition
Meningitis	≥100x10^6 leucocytes per liter cerebrospinal fluid.
Shock	Persistent hypoperfusion requiring fluid therapy and treatment with vasoactive drugs for at least 24 hours or until death.
Shock and meningitis	Shock as defined above combined with ≥100x10^6 leucocytes per liter cerebrospinal fluid.
No shock and no meningitis (mild meningococcemia)	Absence of shock and meningitis as defined above.

Table 3.2 Definitions used in the studies of meningococcal pathophysiology. Adapted from Brandtzaeg et al [8].

have demonstrated that pilus-mediated adhesion is a consequence of the interaction of both the major pilin (PilE) and the minor pilin (PilV) with the endothelial cell receptor CD147. This initial interaction is a prerequisite to the formation of large bacterial colonies on the apical surface of endothelial cells. As the colonies grow meningococci induce a host–cell surface reorganization, leading to the formation of filopodia-like structures that allow the bacteria to hide and resist shear stress. The signalling induced by the type IV pili is due to activation of the β_2-adrenergic/β-arrestins pathway [27,28]. PilE and PilV interact with the N-terminal domain of the β_2-adrenergic receptor, in turn activating the β_2-arrestin pathway. The β_2-arrestin pathway is responsible for the activation of Src tyrosine kinase, Rho GTPases (such as RhoA, Rac1, and Cdc42), actin polymerization, and leads to the accumulation of ERM proteins (Ezrin, Radixin, and Moesin family of proteins) and ERM binding receptors (such as ICAM-1, VCAM-1, or E-selectin) [29–31]. Since these receptors are required for optimal leucocyte adhesion to endothelial cells it was suggested that *N. meningitidis* inhibits leucocyte adhesion during infection [30,32]. In addition, Cdc42 allows the recruitment of Par3/Par6/PKCz, which leads to the accumulation of adhesins and tight junction proteins (such as VE-cadherin, ZO-1, and claudin 5) [29]. As a consequence, proteins are depleted at the intercellular junctions, thus opening the paracellular route that allows dissemination of *N. meningitidis* through the endothelium (Figure 3.3). This opening of the paracellular route is believed to be responsible for the crossing of the BBB and subsequent meningeal invasion.

Another consequence of meningococcal interaction with microvessels is the formation of thrombi. The use of the humanized animal models has clearly shown that type IV pilus-mediated interaction with endothelial cells was a prerequisite for the formation of thrombi. Despite this model, the mechanisms following this interaction that lead to an increase of the procoagulant activity remain unexplained.

The reason why some patients develop a fulminant meningococcemia while others develop meningitis may be directly linked to the level of bacteremia. During high-grade bacteremia (up to 10^8 bacteria per mL) colonization of the peripheral microvasculature occurs throughout the

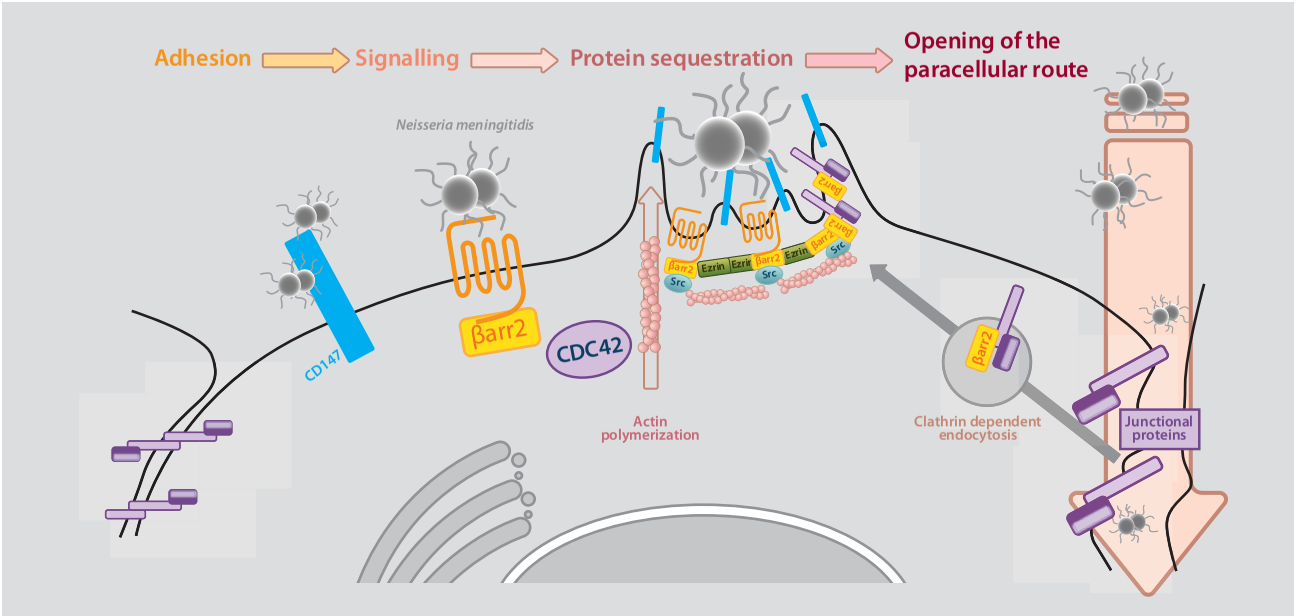

Figure 3.3 Interaction of *Neisseria meningitidis* with endothelial cells. *N. meningitidis* adheres to brain microvascular endothelial cells through an interaction between type IV pili and the CD147 receptor [18]. Following initial adhesion, type IV pili mediate the recruitment and activation of the β_2-adrenergic receptor/β-arrestins complex, thus leading to the organization of a specific cytoplasmic molecular complex referred to as the cortical plaque [27], enriched with Ezrin, Src Kinase, actin, cellular receptors, and that of proteins from junctions (VE-cadherin, ZO-1, claudins) [29,31]. As a result this promotes the opening of the cell–cell junctions and allows the transmigration of bacteria through the endothelium. Reproduced with permission from © Cold Spring Harbor Laboratory Press [33].

body, where bacteria interacting with endothelial cells induce extensive thrombosis and subsequent organ failure. By contrast, when the bacteremia remains low-grade (from $<10^3$ to 10^4 bacteria per mL), the meningococci will only sparsely interact with the peripheral microvasculature and be responsible for localized petechiae. In the latter case bacteria have time to colonize brain vessels, cross the BBB, and invade the meninges.

Pathophysiological characteristics of clinical presentations of meningococcal disease

Clinical meningitis without shock

The pathophysiology is characterized by a comparatively low grade bacterial proliferation in the blood (median $<10^3$/mL), a transition to the meninges and subarachnoid space (which may take hours), followed by a more rapid proliferation in the CSF (reaching levels as high as 10^9/mL). The median time between the onset of the disease and hospital admission (onset–admission time) varied between 23 and 29 hours, in five European studies, and the CFR was up to 1% [2,34]. Fatality is related to brain edema, herniation, and arrest of the brain circulation. Petechiae, indicating meningococcemia, are common (occurring in 50–60% of cases) but large ecchymoses are uncommon [3,34,35]. Approximately half of patients have positive blood cultures on hospital admission if no prehospital antibiotic treatment was given (Table 3.1) [6,34]. Thus, the bacteremia appears to be transient and not present on admission in half of these patients. In those with bacteremia, the bacterial load is moderate, with a median of $<10^3$ *N. meningitidis* DNA copies (= number of meningococci)/mL of plasma (when measured using real-time PCR) [35]. The median LPS (endotoxin) level in plasma is low (<0.5 endotoxin units [EU]/mL) [9]. The levels of cardinal cytokines (tumor necrosis factor [TNF], IL-1β, IL-6, and IL-10), key chemokines (IL-8, MCP-1, and MIP-1α), as well as markers of complement and coagulation activation are low in plasma [2,3,6,15].

The levels of bacteria, LPS, and key inflammatory mediators in the CSF are several 100- to 10,000-fold higher [2,3,6,9,15]. This reflects a compartmentalization of the growth of meningococci primarily to the meninges and subarachnoid space. In meningitis bacteria proliferate in the CSF and up-regulate vascular and neutrophil adhesion molecules, facilitating the

influx of neutrophils into the subarachnoid space, causing inflammation and the clinical features and biochemical evidence of meningitis [35]. Appropriate antibiotic treatment stops intra- and extracranial bacterial growth and down-regulates the intracranial inflammation and pressure. If the intracranial pressure is not too high, the CFR is low in high-income countries [2,36,37]. The long-term sequelae include educational difficulties, deafness, seizures, diffuse brain damage, altered behavior and sleep pattern, and concentration difficulties [37].

Shock without clinical meningitis

This clinical presentation is characterized by a rapid proliferation of the meningococci in the circulation, leading to a harmful initiation of the innate immune system. Few meningococci penetrate through the BBB to cause symptoms of meningitis, although 50 to 60% of cases may reveal a slight pleocytosis and a positive CSF culture (Table 3.1) [13]. Patients usually develop alarming symptoms and are often hospitalized at an early stage. Several studies reported a median onset–admission time, for patients with shock only, of 12 hours and a CFR of 16 to 52% [2,6,16,35]. Shock without meningitis paradoxically has a higher CFR than shock combined with meningitis [2,34]. This appears to be caused by a more extreme proliferation of meningococci in the circulation, leading to higher levels of LPS in patients with shock alone as compared with patients with shock and meningitis [2,6,36]. During rapid growth in the vasculature meningococci appear to seed the lungs, liver, spleen, heart, muscles, and adrenals, inducing a high grade and destructive activation of the innate immune system in these organs [38]. On hospital admission the circulation is often hyperdynamic with low peripheral vascular resistance, although infants and children may initially have an increased vascular resistance [6,15]. The vascular response is complex and related to the myriad effects of LPS and other cell wall components, including generation of nitric oxide (NO) in the endothelium and the extreme activation of the complement system, generating the anaphylatoxins C3a, C4a, and C5a [2,3,6,15]. The capillary leakage increases with a massive transcapillary flux of albumin across the endothelial barrier — owing to a reduced barrier function and opening of the intercellular

junctions. Deterioration often occurs despite treatment with antibiotics, fluids, and vasoactive drugs (Chapter 6). Gradually, the peripheral vascular resistance and myocardial contractility decrease [2,3,6,15] and there is a pronounced metabolic acidosis. Serial ultrasonographic examinations of the heart document increasing volumes of the ventricles (Starling effect) and decreasing ejection fraction [2]. Many patients die of a terminal arrhythmia [8]. The acute septic heart failure has been related to bioactive TNF, IL-1β, and IL-6 in plasma [2,3,6,15,39]. High levels of the same cytokines are produced locally in the myocardium, directly influencing the cardiomyocytes [38]. NO production induced by LPS in the myocardium and exhaustion of mitochondria are assumed to contribute to the failing myocardium [2,3,6,15,40]. Most patients have extensive hemorrhagic skin lesions (large petechiae and ecchymoses) associated with severe coagulopathy.

Tissue factor acting on human monocytes and monocyte-derived microparticles activate the extrinsic pathway of the coagulation system [2,3,15,35,41,42]. Complement activation augments the coagulopathy [2,3,6,43]. Many patients develop purpura fulminans (massive thrombosis of the skin vessels and thrombosis of peripheral vasculature) leading to gangrene. Both clinical manifestations are associated with very low levels of protein C and increasing consumption of coagulation factors augments the bleeding tendency [2,3,6,15,35].

Renal failure is present in almost all meningococcal shock patients on admission, as observed by high plasma creatinine and dwindling glomerular filtration rate [3,6,15,35]. Several factors contribute to renal failure, including [3,6,8,15,35,38]:

- thrombosis in the glomeruli capillaries;
- acute necrosis of the epithelial cells of proximal tubuli;
- circulating myoglobin caused by rhabdomyolysis; and
- high concentrations of key cytokines and chemokines in the kidney tissue.

Acute respiratory distress syndrome (ARDS) is common and often manifests during treatment with fluid to combat the shock [3,6,8,15,35]. There is a massive concentration of meningococci, as revealed by real-time PCR, inducing proinflammatory cytokines and chemokines in pulmonary

tissue [38]. Few meningococci have yet reached the subarachnoid space. Pleocytosis is absent or minimal (<100x10^6 leukocytes/L CSF) owing to the short onset–admission time [2,3,6,8,15,35]. The low number of meningococci and the minimal inflammatory response in the subarachnoid space explains the absence of clinical signs of meningitis [2,3,6,35]. Real-time PCR, as an indicator of the true bacterial load in plasma, has been compared with quantitative blood cultures. The results revealed that only 1 in 1000 to 1 in 10,000 meningococci were viable in culture [8,9]. The median number of meningococci (*N. meningitidis* DNA copies/mL) was 10,000-fold higher (median 2x10^7/mL) in the plasma of 21 patients with shock without meningitis, as compared to 28 patients with meningitis without shock (median <10^3/mL), or 14 patients with mild meningococcemia (median 7x10^3/mL) [9] (Table 3.1). The LPS levels were closely correlated to the number of meningococci (*N. meningitidis* DNA copies/mL) in plasma (r = 0.91) and CSF (r = 0.98) [9].

In 150 patients with meningococcal infections, septic shock developed in 95% of 51 patients when the LPS level in plasma passed 10 EU/mL [2,6,8,15]. Among the 51 patients with plasma LPS >10 EU/mL the CFR caused by shock was 25% for those (n = 24) with LPS levels of 10 to 50 EU/mL, 85% for 50 to 250 EU/mL (n = 20), and 100% for >250 EU/mL (n = 7) [2,3,6,8,15,35]. In a UK study, the first to document the high-grade meningococcemia, the numbers of *N. meningitidis* DNA/mL in plasma were positively correlated to the Glasgow meningococcal septicemia prognostic score [44]. A doubling time of growing meningococci of 30 to 45 minutes is associated with a rapidly escalating bacterial load and rising plasma LPS levels [6]. This was observed in a patient with an initially unrecognized meningococcal infection that developed into lethal septic shock. The LPS plasma level increased from 11 to 144 EU/mL during a period of 10.5 hours, with a parallel increase in TNF from 377 to 7627 pg/mL. After initiation of antibiotic treatment LPS in plasma was cleared with 50% reduction in 160 minutes. In parallel, TNF declined by 50% in 70 minutes [Brandtzaeg, unpublished results]. The natural clearance rate (half-life) of meningococcal LPS in plasma, as measured in patients after initiation of antibiotic treatment, is 60 to 180 (median 120) minutes [8,9,15]. Benzylpenicillin, chloramphenicol, and

third-generation cephalosporins immediately stop further proliferation of meningococci, and no increase in plasma LPS levels is observed [8,15]. Cytokines, chemokines, and many other inflammatory mediators are also down regulated and decline in parallel with LPS in plasma [6,8,15]. Clinical deterioration is commonly observed after initiation of treatment. It is related to progressive organ dysfunction caused by the inflammation and not by LPS release [8,15]. Sequelae are common and are primarily related to thrombotic complications in peripheral vessels leading to gangrene of limbs and localized skin necrosis requiring amputation and skin grafting [8,15,35,37]. Cardiac, renal, adrenal, and pulmonary failure is usually reversible, although normal function may not be regained.

Shock and clinical meningitis

This clinical presentation is explained by rapid intravascular growth, although slower than most patients with shock alone, combined with rapid transition to and proliferation in the subarachnoid space. The median bacterial load in plasma was 10^6/mL and median LPS was 9.4 EU/mL for patients with shock and meningitis, versus a median of $2x10^7$/mL and 43 EU/mL in patients with shock alone (Table 3.1) [6,9]. In a Dutch study the median onset–admission time was 16 hours for patients with shock and meningitis, versus 12 hours for those with shock alone, compatible with a slower intravascular growth of meningococci [34]. The more gradual development leads to seeding of the meninges and development of symptoms of meningitis before these patients are admitted to hospital.

Patients without shock or clinical meningitis

This is a composite group of patients characterized by a transient or persistent low grade meningococcemia (median $<10^4$/mL) and low levels of LPS (median <0.5 EU/mL) (Table 3.1) [2,3,8,9,15,35]. Patients usually have fever and rash, often with hemorrhagic elements, but not septic shock or clinical features of meningitis when admitted to hospital (although low levels of bacteria and a slight pleocytosis may be present) (Table 3.1) [6]. The median onset–admission time was 20 hours among Dutch patients [32], but some patients with short onset–admission time might have evolved to shock or meningitis if left untreated. The CFR in this setting was reported

as ≤5% [2] and fatalities were caused by brain edema or shock, which developed after hospital admission. Patients developing pericarditis, septic arthritis, uveitis, or other focal infections belong to this category, with a low grade and often transient meningococcemia. A few patients may have symptoms lasting from days to weeks.

Chronic meningococcemia is a rare condition lasting weeks to months, and is characterized by intermittent fever, rash, and joint symptoms. It has recently been associated with *N. meningitidis* mutants with abnormal lipid A (the toxic part of LPS), which is composed of five acylated fatty acids instead of the usual six seen in the most invasive wild type strains [45]. This mutated LPS is biologically less active than wild type. Similar mutants occurred in 6.5% of 448 invasive strains analyzed in the Netherlands and appeared to induce a less serious clinical picture and less coagulopathy [46]. On the opposite end of the spectrum a few patients have been identified who tolerate higher levels of LPS (up to 214 EU/mL and meningococci plasma levels of $4.2x10^7$/mL) without developing persistent shock, multiple organ failure, severe coagulopathy, or fatal sequelae [6,47]. This suggests that polymorphisms exist in the human genome that determine the response to LPS in the circulation.

The immune response

LPS, also denoted lipooligosaccharide (LOS), is the endotoxin of *N. meningitidis*. It comprises lipid A with six symmetrically acylated fatty acids attached to two glucosamine molecules and two 2-keto-3-deoxy-octulosonic acid (KDO) molecules (Figure 3.4). This structure is the primary toxic moiety of *N. meningitidis* through its ability to activate the innate immune system in a harmful manner [3,6,48]. Two short side chains comprising different sugars, with epitopes similar to those covering human cells (molecular mimicry), are linked to KDO [3,6,49].

LPS is embedded in the outer leaflet of the outer membrane of meningococci (Figure 3.1) and released as outer membrane vesicles, disintegrated meningococci, or single molecules attached to LPS binding protein and albumin [2,3,6,50,51]. LPS is the cardinal trigger of the innate immune response via activation of CD14, Toll-like receptor 4 (TLR4), and myeloid differentiation factor 2 (MD-2) [2,3,6,15,48,50]. *N. meningitidis*

LPS activates >4600 genes in human monocytes, suggesting a profound biological effect [52]. Non-LPS molecules of meningococci, such as porins, lipoproteins, peptidoglycan fragments, meningococcal DNA, and capsule polysaccharide have a much weaker pro-inflammatory effect than LPS [2,3,6,53,54]. The influence of each of these molecules in human pathophysiology is presently uncertain. However, recently a patient with acute meningitis caused by a mutant strain of *N. meningitidis,* completely lacking LPS, was described [55]. This rare patient documents the effect of non-LPS molecules on the activity of the innate immune system and the development of disease symptoms. The genetically manipulated LPS-deficient laboratory strain (H44/76lpxA-) requires 10- to 1000- fold higher concentration to induce the same degree of inflammation as the wild type parent strain (H44/76) [53,54].

Bactericidal antibodies combined with a normal functioning complement system protect from invasive meningococcal infection. Massive complement activation, which is independent of LPS, is a

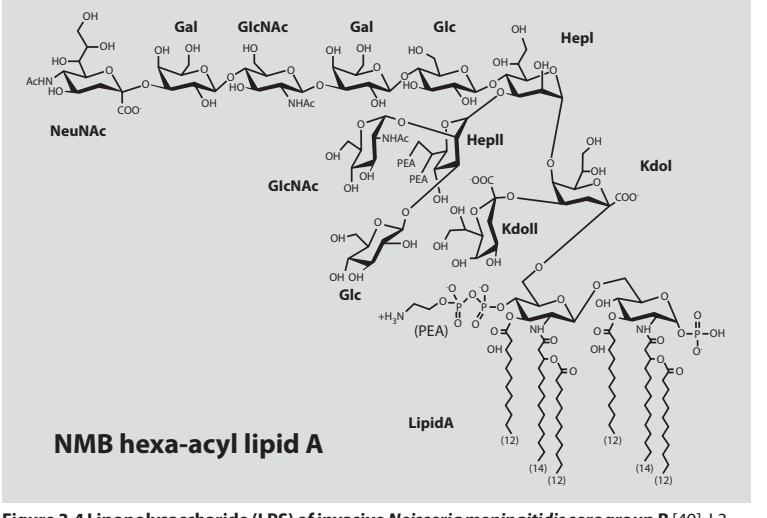

Figure 3.4 Lipopolysaccharide (LPS) of invasive *Neisseria meningitidis* serogroup B [49]. L3 comprising lipid A with six fatty acids, two 2-keto-3-deoxy-octulosonic acid (Kdo I and Kdo II), two heptoses (Hep I and Hep II) to which glucose (Glc) and glucosamine (GlcNAc) are attached. Lacto-N-neotetraose, consisting of glucose, galactose (Gal), glucoseamine and galactose, is attached to Hep I. Neuraminic acid (NeuNAc) (synonymous with sialic acid) is attached to the terminal part of the lacto-N-neotetraose. Both structures represent epitopes similar to epitopes on human cells (molecular mimicry) [6]. PEA, phosphoethanolamine.

characteristic feature among patients who do not survive meningococcal shock and is primarily caused by alternative pathway activation [2,6,15,56–58]. Massive complement activation requires [2,6,54,57]:

- 1000- to 10,000-fold higher doses of *N. meningitidis* compared to the doses required to activate the TLR-4 receptor complex; and
- a bacterial load reaching 10^7/mL plasma and LPS plasma level of ≥100 EU/mL (as found in clinical and experimental studies).

Disseminated intravascular coagulation (DIC) is triggered by up-regulated tissue factor combined with phosphatidylserine, both exposed on the surface of decaying monocytes and microparticles released from monocytes [3,6,15,39,42]. Protein C and antithrombin, both powerful natural anticoagulation factors, are subnormal owing to consumption caused by on-going DIC [2,3,6,15,35]. Low levels of protein C (<20% of healthy controls) are associated with purpura fulminans. The fibrinolytic system is strongly inhibited through increased plasminogen activator inhibitor-1 (PAI-1) in plasma [2,3,6,15,35]. Diffuse capillary thrombosis appears to be facilitated by the combination of high levels of microvesicles carrying tissue factor, high plasma levels of PAI-1 inhibiting the fibrinolysis, massive complement activation, very low plasma levels of protein C, and meningococci attached to an altered and denuded endothelium surface [3,6,15,35,41–43]. This is observed in different organs including the choroid plexus and the skin. In the skin the combination leads to purpura fulminans. The same mechanisms are associated with arterial thrombosis of the larger vessels [3,6,35].

Summary points

- Meningococci proliferate at different rates in different patients, who subsequently develop septic shock (rapid intravascular growth) or low grade meningococcemia (gradually leading to growth in the subarachnoid space and clinical meningitis).
- In 20 to 30% of patients, *N. meningitidis* causes a comparatively mild meningococcemia with little or no CSF involvement.
- Bacterial growth and accompanying inflammatory response is highly compartmentalized in most patients.

- The factors that determine whether or not patients develop septic shock owing to massive intravascular proliferations remain to be determined.
- Molecular mimicry, ie, the capacity of meningococci to produce molecules with epitopes similar to human cells, may play an important role in circumventing immune recognition and possibly the varying clinical presentations of meningococcal disease observed in patients.
- LPS (endotoxin) plays a dominant role in pathophysiology, but the picture is complex given the capacity of non-LPS molecules to trigger the innate immune system.

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Chapter 4

Diagnosis of meningococcal disease

Raymond Tsang, Muhamed-Kheir Taha

Introduction

Invasive meningococcal disease (IMD) is a reportable disease in many countries and is considered by the World Health Organization (WHO) as one of several epidemic-prone infectious diseases [1]. Tracking the spread of IMD within and between countries relies on laboratory case confirmation as well as characterization of *Neisseria meningitidis*. Laboratory detection of *N. meningitidis* is the only way to identify a confirmed case and is therefore critical to understanding the true disease burden and epidemiology.

Since IMD can be fatal and laboratory-acquired infections have been documented, those working with live cultures should be aware of the current safety protocols [2], including immunization recommendations.

Unless accompanied by typical signs of meningitis and/or purpuric/petechial rash, IMD cannot be diagnosed on clinical grounds alone. Because the disease may progress very rapidly from initially seeking medical attention to death within hours, physicians should maintain a high vigilance for IMD, and antibiotics should be given prior to laboratory confirmation. Nevertheless, appropriate clinical specimens should be collected to confirm the diagnosis.

Blood and cerebrospinal fluid (CSF) are the most common specimen types; others may include joint or pericardial fluids, depending on the

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site of infection. Rarely, skin lesions may be submitted for bacteriological culture, Gram stain, or polymerase chain reaction (PCR) detection of meningococcal DNA.

If blood or CSF cultures are negative, due to prior antibiotic treatment, throat or nasopharyngeal culture may be considered as meningococci in these sites are less likely affected by the standard antibiotic therapy for IMD [3]. However, positive findings from nonsterile body sites normally do not constitute IMD according to most national case definitions (Chapter 1). In the event that IMD is suspected several days after the death of an individual, vitreous humor may be a suitable specimen for PCR-based diagnosis [4].

Conventional diagnosis

Conventional diagnosis by culture

Bacteriological culture, if successful, provides the *N. meningitidis* isolate for subsequent testing and can be stored as a biobank archive for future reference as typing methods evolve, to enable retrospective assessment of genetic evolution of the organism.

N. meningitidis grows in various commercially available blood culture systems; 5% sheep blood or chocolate blood agar. Identification relies on its characteristics, defined in Table 4.1 and morphology shown in Figure 4.1. However, aberrant strains that fail to produce acid or are gamma-glutamyl transferase (GGT) negative may be identified by molecular signatures.

Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) has been described for the routine identification

Test	Result
Morphology	Diplococci
Gram stain	–
Oxidase	+
Glucose + maltose	Acid
Sucrose	–
Lactose	–
Catalase	+
Gamma-glutamyl transferase (GGT)	+

Table 4.1 Diagnostic characteristics of *Neisseria meningitidis*.

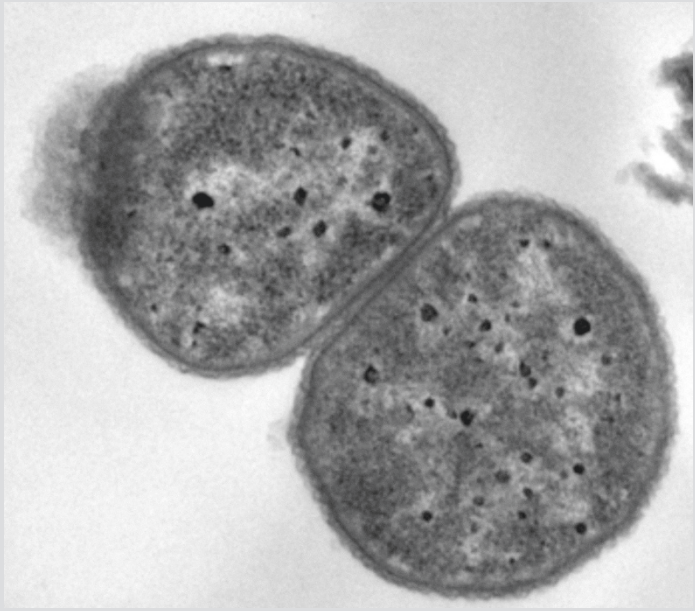

Figure 4.1 Electronic microscopy image of *Neisseria meningitidis* showing a typical diplococcus.

of clinically important microorganisms [5], including the pathogenic *Neisseria* species. However, misidentification of *Neisseria polysaccharea* as *N. meningitidis* can occur [6], suggesting further refinement of the test is required.

Temporal and geographical variations in the distribution of *N. meningitidis* capsular groups are well known. Reference laboratories should maintain antisera for common groups A, B, C, Y, and W as well as rare groups X, Z, E, H, I, K, and L. Strains expressing both groups Y and W antigenic specificities have been documented [7].

Nonculture conventional diagnosis

Although less sensitive than the molecular methods, they are still useful in resource challenging environments. The latex agglutination test uses specific antibodies to detect capsule antigens but requires heating the

CSF specimen before testing and refrigeration to prevent deterioration of the antibody latex particles.

An immunochromatography method to detect A, C, Y, and W capsule in unheated CSF specimens has been evaluated and demonstrated a sensitivity of 88% and specificity of 99% [8]. Recently, a dipstick rapid diagnostic test (RDT) for detection of group X meningococcal capsule antigens in CSF has been developed and validated using both retrospective laboratory samples and limited prospective filed samples from countries within the African meningitis belt. This RDT, with a sensitivity of 96% and a specificity of 100%, compared very favorably with PCR [9]. However, further field testing may be required to know the true value of these RDTs.

Molecular diagnosis

Culturing *N. meningitidis* remains problematic. Even without early antibiotic treatment, the rate of positive culture varies between 50 and 70% [10]. PCR is now widely used for rapid diagnosis and to help immediate management of meningococcal disease. Two-step approaches are usually used: the first round of PCR detects meningococcal DNA and the second detects the capsular group. DNA-free material and reagents are used to minimize DNA contamination. Ultra-violet irradiation, heat labile uracil-N-glycosylase (UNG), and deoxyuridine-triphosphatase (dUTP) (instead of dTTP) may prevent carryover products. Several points should be considered:

- Storing and sending samples at 4°C should reduce the risk of DNA degradation. DNA extraction is required to reduce inhibitory substances in the samples.
- PCR facilities need to be separated from other activities in the laboratory. The PCR laboratory should be compartmentalized with 'one way' workflow and a gradient of atmospheric pressure. The DNA extraction module should be separated from where PCR reagents are prepared. Protocols and equipment should be validated.
- Internal and external quality assurance should be regularly organized.

PCR-based detection and genogrouping

Several genes are used for the detection of meningococcal DNA, such as:

- *IS1106,* the insertion sequence;
- *dhps,* the gene encoding dihydropteroate synthase;
- *porA* and *porB,* the major porin genes;
- *ctrA* gene, which encodes an outer membrane protein involved in capsule transport;
- *crgA* gene, which encodes a transcriptional regulator belonging to the LysR; and
- *sodC* genes encoding the Cu-Zn superoxide dismutase.

ctrA is specific for *N. meningitidis,* however, it may be absent in nongroupable isolates. Such isolates are rare in invasive meningococcal infections but can be encountered in immunocompromised patients [11]. The *sodC* gene may offer an alternative as it is also specific for *N. meningitidis*. However, *sodC* PCR was suggested to be less sensitive than that of *ctrA* [12]. While 'in-house' PCR methods and commercial kits are now available, it is recommended that reference laboratories keep several PCR targets in routine use.

PCR-based grouping targets specific genes for groups A, B, C, Y, W, and X (respectively, *csaB*, *csb*, *csc*, *csy*, *csw,* and *csxA*). For groups E and Z, PCR-based assays that target specific regions of the *ctrA* gene have been reported [13].

Conventional PCR

End-point PCR was initially developed whereby PCR products are analyzed by electrophoresis on agarose gel to determine band sizes next to a positive control. However, low sensitivity for some groups was reported [14].

Real-time PCR

Real-time PCR relies on the use of primers/probes that are conjugated to fluorophores and the detection of fluorescence, which increases during DNA amplification. Appropriate software is required to view and analyze the results. High specificity and sensitivity are achieved by the TaqMan method. Data are listed according to values where each sample crosses the fluorescence threshold (C_t) that is higher than the negative controls and negative samples, and is within the start of the exponential phase for

positive control. The following cut-off values (Box 4.1) are used according to the WHO recommendation for the diagnosis of meningitis [15].

$C_t \leq 35$: Positive.

$C_t > 40$: Negative.

C_t 36-40: Not interpretable. Repeat the PCR after dilution.

Box 4.1 The cut-off values for detection of meningococcal DNA.

Antibiotic susceptibility testing

Conventional antibiogram

Antibiotic susceptibility testing (AST) for *N. meningitidis* is performed on Mueller-Hinton agar (MH), supplemented with sheep blood, to determine the minimal inhibitory concentration (MIC) of the tested antibiotics using agar dilution and/or Etest®. The disc diffusion method is not reliable for meningococcal AST [16].

The antibiotics tested are those that are currently used in treatment and/or prophylaxis: β-lactams (penicillin G, third generation cephalosporins), rifampicin, and ciprofloxacin.

Meningococcal suspensions should be prepared at 0.5 McFarland density and then inoculated onto the plate with a nontoxic swab to produce confluent growth. Etest® strips containing the antimicrobial agent are then placed on the inoculated plates with sterile forceps (Figure 4.2). The plates are incubated for 24 hours at 37°C under 5% CO_2.

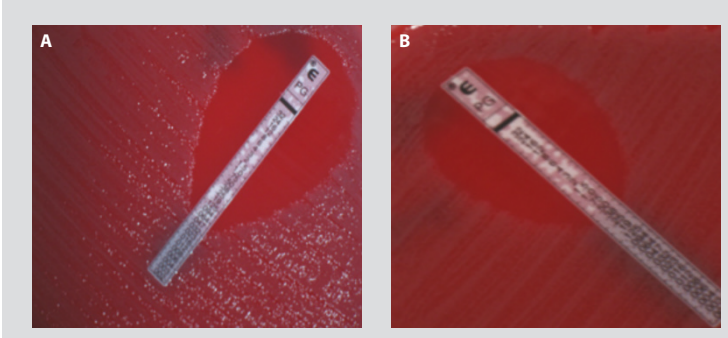

Figure 4.2 Etest® strips containing antimicrobial agent. A, meningococcal strain susceptible to penicillin G. B, meningococcal strain showing reduced susceptibility to penicillin G.

For agar dilution, bacterial suspension is prepared and spotted on the plates at 10^5 colony forming units per spot. After allowing the surface to absorb the inoculum, all the plates are incubated for 24 hours at 37°C in 5% CO_2. The plates are prepared with serial twofold dilutions with the following concentrations:

- penicillin G — 0.007 to 2 mg/L;
- cefotaxime — 0.0003 to 0.12 mg/L;
- ceftriaxone — 0.00007 to 0.12 mg/L;
- rifampicin — 0.007 to 64 mg/L; and
- ciprofloxacin and ofloxacin — 0.0007 to 0.12 mg/L.

The MIC is defined as the lowest concentration of antibiotic that prevents visible growth and is expressed using whole MIC steps (0.03, 0.06, 0.125) rather than gradient steps as in the Etest®.

Molecular prediction of meningococcal antibiotic susceptibility

Molecular detection of antibiotic resistance in meningococci has been recently developed and is based on the detection of genetic events that are directly shown to be responsible for antibiotic resistance. Reduced susceptibility for penicillin G is encountered in meningococci, while β-lactamase-positive meningococci are extremely rare, if nonexistent. This reduced susceptibility is also observed for amoxicillin, but not for third generation cephalosporins. This phenotype is directly correlated to alteration of the *penA* gene, encoding the penicillin binding protein 2 (PBP2) that can be detected by PCR amplification and sequencing of the *penA* gene [17].

For rifampicin and ciprofloxacin, rare resistant invasive meningococci have been described and found to be due to alterations/mutations in *rpoB* and *gyrA* genes [18,19]. The data on molecular typing of antibiotic susceptibility, primers, and protocols are now available through the *Neisseria* multilocus sequence typing (MLST) database at the University of Oxford, UK [20].

Defining the breakpoints

The critical points (breakpoints) that define susceptibility/resistance in meningococci have been determined according to the following scheme:

1. sequencing of large collections of isolates to define wild type alleles for *penA* (penicillin G), *rpoB* (rifampicin), and *gyrA* (ciprofloxacin);
2. correlate wild type genes to MICs to define the epidemiological cut-off values; and
3. use of animal models to explore the impact of increased MIC during experimental infections (see Table 4.2).

Typing of *Neisseria meningitidis*

Serological typing (serotyping and serosubtyping)

Serotyping and serosubtyping using monoclonal antibodies (MAbs) have significant limitations. The availability of MAbs and many nontypeable strains, as *N. meningitidis* evolves to immune selection, has made this method less useful in the DNA era. Consequently, most laboratories have adopted DNA sequencing of the *porA* (and sometimes *porB*) genes as the standard.

Molecular typing

This can be grouped into two broad categories: DNA sequence-based and nonsequence-based methods.

Multilocus sequence typing

MLST of *N. meningitidis*, based on partial nucleotide sequences of seven house-keeping enzyme genes, has successfully replaced multilocus enzyme

Antibiotic/category	Penicillin G	Ciprofloxacin	Rifampicin
Breakpoints (mg/L) according to the EUCAST			
S (Susceptible)	≤0.06	≤0.03	≤0.25
I (Intermediate)	0.12–0.25	0.06[b]	—
R (Resistant)	>0.25[a]	>0.06	>0.25[c]
Breakpoints (mg/L) according to the CLSI			
S (Susceptible)	≤0.06	≤0.03	≤0.50
I (Intermediate)	0.12–0.25	0.06	1.00
R (Resistant)	≥0.50	≥0.12	≥2.00

Table 4.2 The impact of increased minimal inhibitory concentration (MIC) during experimental infections. [a]Combining sequencing of *penA* genes from large collections of isolates to animal works are in progress to better define penicillin G breakpoints. [b]Intermediate isolates do not correspond to separate category and should be included into the resistant category (defined as R ≥0.06 mg/L) [18]. [c]Isolates with MIC >0.25 mg/L and up to 1 mg/L by gradient MIC methods (Etest®) may not correspond to true resistance. They need to be retested by agar dilution/*rpoB* sequencing. CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee for Antimicrobial Susceptibility Testing.

electrophoresis for clonal analysis. Nucleotide sequences are assigned allelic numbers and, based on their gene alleles, isolates are assigned sequence types (STs). STs are grouped together into clonal complexes depending on similarities of their allelic profiles [20].

Sequencing of other gene targets

Other targets for genetic typing include those encoding the iron-regulated OMP FetA and the vaccine component factor H binding protein. Details of the use of these typing targets can be found at the *Neisseria* MLST website [20].

Whole genome sequencing

A major challenge in using whole genome sequencing (WGS) for typing *N. meningitidis* is to decipher the roughly 2 million bases-worth of information into meaningful typing data. Building on the success of MLST, a platform called Bacterial Isolate Genome Sequence Database (BIGSdb) has been established in the PubMLST.org website [21]. Users can interrogate the BIGSdb database with WGS data to generate a gene-by-gene comparison and a report of the gene profile, with allelic numbers based on a user-defined set of loci. Using *N. meningitidis* as an example, this platform has been described to provide an automated approach to extract useful typing information from WGS data [22].

Other nonsequence-based molecular typing techniques

Based on DNA fragment sizes identified on agarose gel, pulsed-field gel electrophoresis (PFGE) and variable number tandem repeats (VNTRs) are useful for studying local short-term molecular epidemiology of *N. meningitidis,* especially for outbreak investigation [23].

Importance of meningococcal typing in the management of disease

When involving antibiotic susceptibility, gene typing will have immediate patient management benefits, including choice of antibiotics for treatment and chemoprophylaxis. Typing that identifies the capsular group may impact on the vaccination decisions to prevent further spread in

outbreak situations. VNTR or PFGE are useful in outbreak investigations, while MLST provides a long-term picture at the bacterial population level (useful for national and global surveillance). Currently, many typing methods, except PFGE, can be done directly on the clinical specimens even when culture techniques fail to yield isolates.

Summary and future outlook

With the introduction of protein-polysaccharide conjugate A, C, Y, and W meningococcal vaccines and the recently licensed protein-based vaccines against group B meningococci, the epidemiology of IMD is likely to change over the next decade. As fewer IMD cases are caused by common capsular groups, will we see more of the currently rare groups in the future? Will usage of protein-based meningococcal vaccine induce vaccine escape mutants due to mutations in the vaccine antigen genes? These theories would require surveillance expertise that can identify *N. meningitidis* strains of unusual groups or with altered vaccine targets. In order to maintain proficiency in the diagnostic and reference laboratories, quality control systems are an integral component in the detection, identification, and typing of *N. meningitidis* in the post-vaccine era [24].

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Chapter 5

Clinical aspects of meningococcal disease

Stephen Pelton, Manish Sadarangani, Linda Glennie, Michael Levin

Introduction

The spectrum of meningococcal infection ranges from asymptomatic carriage in the nasopharynx to fulminant septic shock, which can result in death within a few hours of symptom onset. Classical meningococcal septicemia is one of the most recognizable clinical syndromes, with fever and widespread purpura, often in the presence of shock. However, it is often difficult to distinguish early disease from more commonplace infections on the basis of clinical features alone. Acute meningitis is also part of the clinical spectrum, and can be difficult to identify among febrile children because it is relatively rare in countries where meningococcal vaccines are routinely used. For this reason, the possibility of meningococcal disease should be considered in any febrile or acutely unwell child. When meningococcal disease is highly suspected early consideration should be given to the need for intensive care therapy. Despite the advances in our knowledge of the pathophysiology of the disease and medical technology over the last few decades outcomes from meningococcal disease remain poor, with a case fatality rate (CFR) of 5 to 15% in resource-rich countries and high rates of long-term neurological and non-neurological sequelae among survivors. It is highly likely that the significant burden of this

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disease will only be reduced by use of effective meningococcal vaccines against all capsular groups in the future.

Clinical spectrum of disease

The majority of people infected with *Neisseria meningitidis* carry it asymptomatically in the nasopharynx, with progression to invasive disease being a relatively uncommon event. When disease does occur, this is usually manifested as sepsis (25 to 45%), acute meningitis only (15 to 40%), or a combination of both (15 to 60%). Occult bacteremia and chronic meningococcemia can also occur. A small number of individuals develop focal infections such as pneumonia, septic arthritis, osteomyelitis, myocarditis, pericarditis, peritonitis, conjunctivitis, endophthalmitis, sinusitis, and otitis media. These may progress to disseminated disease or may be a result of metastatic infection. Urethritis, cervicitis, vulvovaginitis, orchitis, and proctitis also rarely occur.

Symptoms of disease

The symptoms of both the meningitis and septicemia evolve rapidly and change with time. A study of prehospital symptoms [1] showed that most affected patients have only nonspecific symptoms in the first 4 to 6 hours. Leg pains, cold hands and feet, and abnormal skin color were noted in 72% of patients, at a median time of 8 hours after onset of symptoms. The classic features of disease, petechial/hemorrhagic rash, meningism, and impaired consciousness, developed later (median onset 13 to 22 hours).

Septicemia

Early in disease the most common nonspecific symptoms are fever, nausea and vomiting, and lethargy (Table 5.1). Other nonspecific symptoms include poor feeding, runny nose, cough, irritability, and muscle and/or joint pain. A blanching, salmon colored, maculopapular rash, which is indistinguishable from rashes seen during viral infections, can also occur early during the course of invasive infection. As disease progresses signs of shock start to become apparent, including skin pallor or cyanosis, cold extremities, a prolonged capillary refill time, and reduced urine output. A rash is

Features	% with symptom or sign		
	Meningococcal disease	Meningococcal septicemia	Bacterial meningitis
Non-specific symptoms			
Fever	58–97	98	66–97
Chills or shivering	39	—	—
Lethargy	36–65	59	13–87
Irritability or unsettled	36–67	32	21–79
Muscle ache or joint pain	7–65	30	23
Refusing food or drink	13–60	27	26–76
Thirst	8	—	—
Nausea or vomiting	44–76	64	18–70
Diarrhea	7–9	—	21–19
Abdominal pain or distension	4	—	17
Runny nose	24	31	—
Coughing	15–27	33	—
Sore throat, coryza, or throat infection	24	—	18
Ear, nose, or throat infection	—	—	18–49
Chest infection	—	—	14
Respiratory symptoms	16–23	—	25–49
Leg pain	11–37	—	—
Specific symptoms			
Rash	59–100	70	9–62
Headache	16–49	40	3–59
Seizures	7–17	—	14–38
Nonspecific signs			
Ill appearance	79	—	—
Cold hands or feet	43	—	—
Abnormal skin color	19	—	—
Capillary refill time >2 seconds	83	—	—
Hypotension	28	—	—

Table 5.1 Clinical features of meningococcal disease and meningococcal septicemia, compared to bacterial meningitis of all causes (continued overleaf).

	% with symptom or sign		
Features	**Meningococcal disease**	**Meningococcal septicemia**	**Bacterial meningitis**
Breathing difficulty	11	—	13–34
Specific signs			
Shock	27–29	—	8–16
Toxic or moribund	—	—	3–49
Altered mental state*	45–81	—	26–93
Impaired consciousness	10–72	—	60–87
Unconsciousness	—	—	4–18
Photophobia	2–31	—	5–16
Stiff neck	5–71	—	13–74
Back rigidity	—	—	46
Bulging fontanelle	—	—	13–45
Brudziński's sign	—	—	11–66
Kernig's sign	—	—	10–53
Abnormal pupils	—	—	10
Focal neurological deficit	—	—	6–47
Cranial nerve pair involvement	—	—	4
Paresis	—	—	6

Table 5.1 Clinical features of meningococcal disease and meningococcal septicemia, compared to bacterial meningitis of all causes (continued). *Includes confusion, delirium and drowsiness. Adapted from UK NICE guideline CG102 [2].

present in 70 to 80% of meningococcal bacteremia cases at presentation to hospital, and is usually nonblanching (ie, petechial or purpuric). However, in approximately 10 to 15% of cases the rash will always be blanching, and meningococcal disease should still be considered in such cases. A small proportion of children (5 to 10%) with meningococcal bacteremia will never develop a rash. There is often rapid progression to severe septic shock, characterized by increasing tachycardia, respiratory distress and hypoxia, reduced level of consciousness, hypotension (a late sign in children), and metabolic acidosis. In purpura fulminans the rash has the appearance of prominent purpura and ecchymoses (Figure 5.1). These signs are caused by hypoperfusion of vital organs (brain, kidneys, and lungs) and also indicate attempts by the body to compensate for hypovolemia and pulmonary

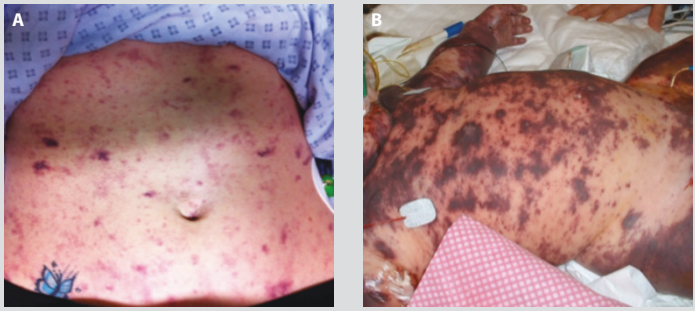

Figure 5.1 Clinical signs of invasive meningococcal disease. A, Purpuric rash on the abdomen of an affected adult. B, an infant with fulminant pupura fulminans. Reproduced with permission from © Meningitis Research Foundation UK.

edema caused largely by capillary leak. Common electrolyte disturbances include hypoglycemia, hypokalemia, hypocalcemia, hypomagnesemia, and disseminated intravascular coagulation, resulting in thrombocytopenia and coagulopathy.

Meningitis

Meningococcal meningitis is indistinguishable from other causes of bacterial meningitis, and can also be difficult to differentiate from viral meningitis and other infections. The classical manifestations present in older children are rarely present in infants and young children. The illness usually begins with fever, nausea and vomiting, photophobia, and severe headache. Occasionally, the first sign is a seizure, but this can also occur later in disease. Irritability, delirium, and altered level of consciousness develop as CNS inflammation progresses. The most specific signs are neck stiffness, associated with Kernig (inability to fully extend knee while hip is flexed due to contraction of hamstring muscles and pain) and Brudziński (automatic flexion of the hips and knees after passive neck flexion) signs, but these are often absent in children. Focal neurological abnormalities may also occur in the absence of seizures consistent with cortical necrosis, occlusive vasculitis, or venous sinus thrombosis. In infants and young children symptoms are nonspecific, and include fever or hypothermia, poor feeding, vomiting, lethargy, irritability, inconsolable crying, jaundice,

respiratory distress or apnea, and seizures. A bulging fontanelle may be present. In some cases meningitis will progress to cause clinical signs of raised intracranial pressure, such as a significantly reduced (Glasgow coma score <8) or fluctuating consciousness level, relative bradycardia and hypotension, irregular breathing pattern, abnormal posture and pupils, abnormal 'doll's eye' movements, focal neurological deficits, and seizures. Papilledema is a late sign and is unusual in the context of acute meningitis — if present other conditions should be considered, including venous sinus thrombosis, subdural empyema, brain abscess, and other intracranial space-occupying lesions. Where septicemia and meningitis co-exist, neurological symptoms and signs will be due to a combination of cerebral ischemia and meningeal inflammation.

Occult bacteremia

This syndrome of fever with no clinically evident localizing signs of infection, in a reasonably well-looking child, who subsequently has a positive blood culture, has historically been described mostly in association with pneumococcal infection. However, in rare cases it can occur with meningococcal infection. Although resolution of bacteremia may rarely occur without antibiotics, persistent bacteremia leads to meningitis in approximately 60% of cases as well as septic emboli to other tissues.

Chronic meningococcemia

This is defined as meningococcal bacteremia without meningeal symptoms in which fever has been present for at least one week before antibiotics. It is a rare manifestation of meningococcal disease, and can be associated with arthralgia or arthritis, headache, splenomegaly, and a maculopapular or petechial rash. The differential diagnosis includes acute rheumatic fever, subacute bacterial endocarditis, Epstein-Barr viral (EBV) infection, disseminated gonococcal infection, Henoch-Schönlein purpura, and immune-mediated vasculitis. Symptoms are intermittent over weeks to months (mean duration 6 to 8 weeks) and bacteremia often clears without treatment, with the same isolate usually responsible for recurrences. Untreated, it may progress to acute bacteremia and/or localized infections, most commonly meningitis. Blood culture results are

usually positive, but only if obtained during the acute episodes. A recent report indicated that almost 50% of isolates from patients with chronic meningococcemia (compared with less than 10% in acute cases) have a mutation in the *lpxL1* gene, which leads to a penta-acylated lipopolysaccharide (LPS) on the surface of the organism, in contrast to the normal form, which is hexa-acylated [3]. This modified LPS is a less potent endotoxin than the wild-type form, resulting in a reduced inflammatory response and therefore milder infection (Chapter 3). In addition, cases of chronic meningococcemia have been associated with inherited deficiencies of terminal complement pathway components, and the use of sulfonamide therapy.

Other focal infections

These are rare and can be secondary to bacteremia, or can develop as the primary infection that progresses to bacteremia. Pneumonia is difficult to confirm in the absence of bacteremia because it is almost impossible to be certain that a positive culture from the respiratory tract is not due to nasopharyngeal carriage rather than infection of the lower respiratory tract. Patients with pneumonia tend to be older (94% over 10 years old) and more likely to be infected with capsular group Y organisms. Conjunctivitis has a similar appearance to bacterial conjunctivitis caused by other organisms, but should be treated with systemic antibiotics due to poor penetration of topical agents. Arthritis occurs predominantly in adults and is usually mono- or oligo-articular. It can be due to acute infection of a joint, although joint fluid culture is usually negative (with the exception of children who have arthritis at presentation and have not received antibiotics). Arthritis can also occur as a secondary phenomenon as part of the meningococcal postinfectious inflammatory syndrome — an immune complex disease that typically occurs 3 to 7 days after onset of meningococcal disease. In addition to arthritis, features include fever, maculopapular or vasculitic rash, iritis, pericarditis, and polyserositis.

Differential diagnosis

Although meningococcal septic shock can be very characteristic in the presence of purpura, it can also be easily mistaken for other infectious

and noninfectious diseases. Other organisms that commonly result in septic shock include *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib) (much less common in countries where Hib conjugate vaccine is routinely used), and other Gram-negative bacteria. *Staphylococcus aureus* and *Streptococcus pyogenes* (Group A Streptococcus) toxic shock can also cause a similar clinical syndrome. Other infections that could be mistaken for meningococcal bacteremia are Rocky Mountain spotted fever, ehrlichiosis, disseminated gonococcal infection, typhus, secondary syphilis, and other viral exanthems, particularly if there are no petechiae or purpura. Only 2 to 11% of children with a fever and petechial rash have meningococcal disease. Viral infections are the most common cause, including enteroviruses, influenza and other respiratory viruses, measles, EBV, cytomegalovirus, and parvovirus. Other conditions that should be considered include protein C or S deficiency, other causes of thrombocytopenia (such as idiopathic thrombocytopenic purpura and hematological malignancies), Henoch-Schönlein purpura, connective tissue diseases, drug eruptions, and trauma (including nonaccidental injury). Viral and other infectious and noninfectious causes of meningoencephalitis should be considered in cases of suspected meningitis.

The poor prognosis of meningococcal disease has led to attempts to identify specific features that occur early in disease and that may be used to enable more rapid diagnosis and initiation of appropriate treatment. One observational study of children associated leg pain, cold extremities, and abnormal skin color with the early stages of disease [1]. However, the time between onset of symptoms and the child becoming moribund was short; around 24 hours for most children. These early symptoms occurred at a median time of 8 hours, with more classical features of purpura, meningism, and impaired consciousness at a median of 13 to 22 hours. A subsequent study of children presenting to their general practitioner (GP) with an acute illness suggested that confusion (likelihood ratio [LR] = +24.2), leg pain (LR = +7.6), photophobia (LR = +6.5), rash (LR = +5.5), and neck pain or stiffness (LR = +5.3) were most likely to indicate meningococcal disease compared to other infections. Cold hands and feet were a less useful discriminator (LR = +2.3) [4]. A number of studies have also tried to distinguish children with meningococcal

disease amongst the group with fever and a petechial rash, and while a number of algorithms exist, none have been adequately validated [5–8].

Disease outcomes and prognosis

Despite advances in intensive care and specific therapy with β-lactam antibiotics, the CFR in resource-rich countries has remained around 5 to 15%, with little improvement since the 1950s (Figure 5.2). Some specialist centers have recently published improved survival data (CFR of 5%) following early aggressive circulatory support [9,10]. A number of scoring systems have been proposed to identify those at highest risk of death and therefore aid prioritization of health care resources and counselling for patients and their families (Table 5.2). However, most of these have been developed in a limited subgroup of patients (mostly children with meningococcal septicemia) and data have often been collected retrospectively. The clinical utility of these scores has not been definitively established.

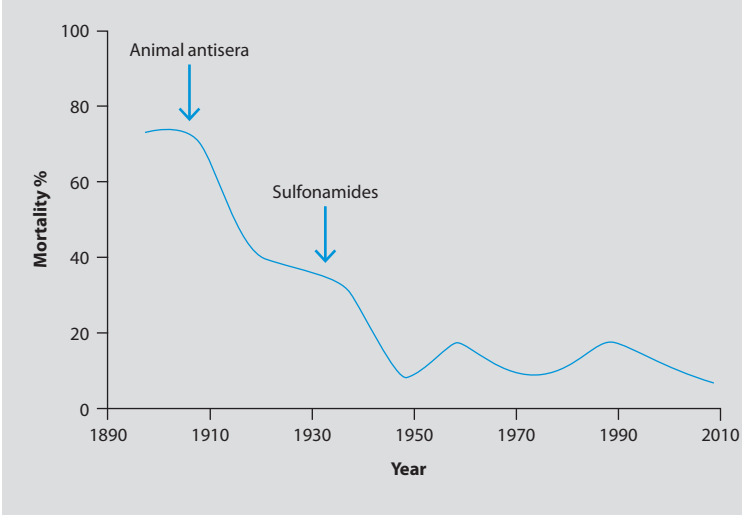

Figure 5.2 Mortality from meningococcal disease in North America and Europe. The introduction of animal antisera and sulfonamides both had a significant impact on mortality, which has remained relatively unchanged since 1950. Data from www.hpa.org.uk, www.cdc.gov, and [11–20].

Item	Stiehm and Damrosch [16]	Niklasson et al [21]	Kahn and Blum [22]	Lewis [23]	Ansari et al [24]	Leclerc et al [25]
Years	1947–1962	1959–1968	1971–1976		1972–1976	1981
Population*	63 C	80 AC	67 C	37 C	54 C	90 C
Clinical						
Rash	Petechiae within 12 hours	Petechiae within 12 hours			Purpura	Ecchymosis
Shock	✓	✓	✓	✓	✓	✓
Temperature		>40°C				<36°C
↓LOC			✓	✓		✓
DIC					✓	
Symptoms <24 hours					✓	
Hyperventilation						
Cyanosis						
Meningism						
Cold extremities						
Diarrhea						
Deterioration in last hour						
Laboratory						
CSF WBC (per µL)	<20	<100			<10	<400
Blood WBC (x10^9/L)	<10	<15	<10		<10	<10
ESR (mm/hour)	<10				<10	
Platelets (x10^9/L)		<100	<150			<100
FDP (µg/mL)			>40			
BE			✓			
Presence of group A antigen				✓		
Potassium (mmol/L)						>5
Fibrinogen						
Serum CRP						

T**able 5.2 Factors associated with worse prognosis in meningococcal disease in different prognostic scoring systems.** A, adults; BE, base excess; BEP, base excess-platelet score; C, children; CRP, C-reactive protein; CSF, cerebrospinal fluid; DIC, disseminated intravascular coagulation; ESR, erythrocyte sedimentation rate; FDP, fibrin degradation products; GMSPS, Glasgow meningococcal septicemia prognostic score; LOC, level of consciousness; PN, platelet-neutrophil score; WBC, white blood cell. *Study population represents number of subjects in the study and target groups. Adapted from Couto-Alves et al [29] and Kirsch et al [33].

	Stokland [26]	Gedde-Dahl et al [13]	Sinclair et al (GMSPS) [27]	Tesoro and Selbst [28]	Couto-Alves et al (BEP score) [29]	Peters et al (PN score) [30]	Kornelisse et al (Rotterdam score) [31]	Malley et al [32]
	1977–1984	1981–1982		1979–1987	1996–2011	1993–1997	1988–1995	1985–1994
	82 C	113 C	12 C	73 C	1207 C	227 C	75 C	245 C
	Number and size of petechiae	Ecchymosis	Extending pupura or ecchymosis	✓				
	Vasoconstriction	✓	✓	✓				↓ perfusion
	Fever	>40°C						
	✓		✓	✓				
	✓							
	✓	✓						
	Absent	Absent	Absent					
		✓	Skin–rectal temperature difference >3°C					
		✓						
			✓					
				<5000				
						Neutrophil count		Neutrophil count <3
					✓	✓	✓	<150
			< -8		✓		✓	
							✓	
								<2.5 g/L
							✓	

Early neurological complications resulting from meningitis and cerebral hypoxic-ischemic damage secondary to shock include seizures, syndrome of inappropriate antidiuretic hormone (SIADH), subdural effusions and empyema, hydrocephalus, raised intracranial pressure, focal neurological abnormalities, venous sinus thrombosis, and cerebral infarction. Effusion/empyema should be considered in all patients with persistent fever after 1 week of appropriate therapy. Venous thrombosis is more common than arterial thrombosis and is generally seen in the second week or later. Hydrocephalus is most common in young infants, particularly if diagnosis is delayed, and usually occurs at 3 to 4 weeks. It is therefore important to closely monitor head circumference. Sequelae secondary to severe shock occur due to tissue hypoperfusion, leading to necrosis. This can range from skin necrosis and subsequent scarring (which may need skin grafting) to gangrene of parts of or entire limbs, requiring amputation. Growth plate damage (thought to be more common in children with scarring) may require surgery and external fixation, usually 4 to 5 years after the acute illness, with further procedures to lengthen or straighten limbs every few years until growth has been completed. Long-term, survivors have very high rates of significant sequelae (up to 20 to 30% in most studies), leading to long-term disability. These include sensorineural hearing loss (all children should have a follow-up hearing test), epilepsy, learning difficulties, and motor/cognitive impairment [34]. Arthritis can lead to permanent joint damage in a small minority of patients. Studies with long-term follow-up of up to 15 years have found rates of sequelae up to 50 to 60%, including physical and neuropsychiatric problems [35–41]. Significant emotional problems in close family members have also been found in these studies, highlighting the societal impact of this disease.

Raising awareness of disease

There is wide-ranging evidence for the effectiveness of health and disease awareness campaigns in improving knowledge and changing behavior across diverse areas, including, for example, smoking cessation, folic acid supplementation, and use of bicycle helmets. Although the published evidence for the effectiveness of raising awareness in meningococcal disease is sparse [42,43] its value is reinforced by public health bodies.

For example, Public Health England and its antecedent organizations, Centers for Disease Control and Prevention in the US, and the Australian Department of Health formally recommend that public health action in response to cases of meningococcal disease include disseminating symptom awareness amongst the affected community, along with chemoprophylaxis and vaccination for contacts in some circumstances.

Raising public awareness of meningitis and septicemia and their symptoms has from the outset been a primary activity of meningitis charities in various countries. There are over 45 individual meningitis charities, most of which are listed as members by the Confederation of Meningitis Organizations. Nearly all of the meningitis charities were set up by parents whose children were affected by the illness for whom raising awareness was a means to prevent similar tragedies for other families. Nationwide and local public awareness programs using traditional mass and social media continue to keep meningococcal disease in the public eye. Awareness campaigns target higher-risk groups, such as children and adolescents attending GP surgeries, schools, nurseries, and universities, to provide information materials. The success of these activities in the UK, which is home to two of the longest established charities, is borne out by the fact that repeated Department of Health surveys show 'meningitis' and 'septicemia', which are relatively rare, are the illnesses parents regard as most severe [44,45].

A few of the meningitis charities also raise awareness amongst health professionals, working with expert clinicians and medical societies to provide education and educational materials. The Meningitis Research Foundation, for example, produces a management algorithm for childhood meningococcal disease in the UK, incorporating the NICE bacterial meningitis and meningococcal disease guidelines (see Appendix) [2], endorsed by the Royal College of Paediatrics and Child Health. It advocates aggressive circulatory support, which has been found to improve outcome [9,10], and the deviations from the management plan it advocates have been shown to increase the risk of fatal outcome [46]. The Spanish Fundación Irene Megías produces a widely used Clinical Practice Guideline and Meningococcal Education in Australia produces a managing meningococcal disease DVD.

Raising awareness of meningococcal disease also involves demonstrating the impact and burden of the illness and its importance. This has been a critical part of supporting the case for vaccine introduction, encouraging vaccine uptake, and highlighting the continued need for further research. Meningitis charities on at least five continents also actively campaign for the introduction of meningitis vaccines.

Genetic factors

There is clear evidence that genetic factors contribute to invasive meningococcal disease (IMD) susceptibility and outcome, with both rare Mendelian defects and polygenic variants playing a role. The sibling risk ratio for meningococcal disease is similar to other common diseases where susceptibility shows polygenic inheritance [47]. A number of host genetic factors have now been identified that affect either susceptibility to IMD or severity of disease. While early genetic studies may have produced unreliable results due to small sample size and failure to account for population admixture and multiple hypothesis testing, a number of large scale and methodologically sound studies have identified validated associations of meningococcal disease susceptibility and severity with genetic variants. The genes associated include epithelial surface receptors, the complement cascade, pattern recognition receptors, clotting factors, and inflammatory mediators. Mendelian deficiencies in several components of the complement membrane attack pathway, properdin, and factor D are well established causes of meningococcal disease. Although rare, recognition of these defects is important as other family members may be affected and recurrent disease may occur. Genome wide association studies involving over 1500 European patients with meningococcal disease and over 5000 controls have definitively identified single nucleotide polymorphisms (SNPs) within genes encoding complement factor H (CFH) and CFH-related protein 3 (CFHR3), which were associated with host susceptibility to disease [48,49]. As *N. meningitidis* possesses a factor H binding protein (fHbp), and binding of the human factor H (FH) to the meningococcal fHbp impairs complement mediated killing of the bacteria, it appears that the meningococcus uses the human FH to evade host bactericidal defenses. Recent data suggests that genetic

variation in both FH and FH-related proteins are important determinants of susceptibility.

In terms of disease severity, a recent meta-analysis performed to collate data from smaller studies found that SNPs in genes encoding plasminogen activator inhibitor 1 (SERPINE1), IL-1 receptor antagonist (IL1RN), and IL-1β (IL1B) were associated with increased mortality from IMD, which again would be predicted from the known pathophysiological changes that occur during invasive disease [50]. Given that any single specific SNP is likely to have only a small impact on disease susceptibility or severity, it is likely that multiple variants acting in key biological pathways contribute to disease outcome.

Summary points

- Meningococcal conjugate vaccines have been highly successful in reducing the incidence of meningococcal disease globally. However, meningococcal disease is still a major cause of septicemia and meningitis in children and adults throughout the world.
- Early meningococcal disease can be difficult to distinguish from other more common infectious conditions, so it is important to always consider meningococcal disease in the differential diagnosis of any febrile or unwell child or adult.
- As disease progresses more characteristic features, such as signs of meningitis and purpura, usually appear, but by this stage the disease is often very advanced and may result in death or significant long-term sequelae in a large proportion of cases.
- Until vaccination is more widely available and effective at preventing all cases of the disease, early recognition, initiation of antibiotic therapy, and effective management of septic shock will reduce the mortality from this devastating illness.

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Chapter 6

Treatment of meningococcal disease

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Introduction

Prompt recognition of meningococcal infection and aggressive early treatment are the only measures proven to be effective in treating this disease. Immediate administration of appropriate antibiotic therapy, and rapid recognition and management of the significant complications of meningococcal infection, such as shock, raised intracranial pressure (ICP), or both, have been shown in recent years to be effective in reduction of mortality [1].

Much of the improvement in mortality is the result of several factors, such as centralization of the care of seriously ill children in pediatric intensive care units (PICUs), the establishment of specialized mobile intensive care teams, the development of protocols for the treatment of meningococcal infection and sepsis in general, and the dissemination by national bodies and charities of guidance about early recognition and management of meningococcal disease.

Clinical features of disease and presentation have been discussed previously (see Chapter 5). However, meningococcal disease may progress rapidly, even after appropriate treatment has commenced. All children admitted to hospital with suspected meningococcal disease should be closely monitored for signs of deterioration. The outcome of disease may

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critically depend on prompt recognition of two important complications: shock and/or raised ICP. Although children with comparatively mild disease may have neither shock nor features of raised ICP, these two clinical problems may coexist in some cases, and present a formidable treatment challenge.

Shock

Shock in meningococcal disease is multi-factorial and results from a combination of several processes, such as endothelial cell dysfunction (including hypovolemia consequent to capillary leak syndrome), myocardial dysfunction, altered vasomotor tone, and impaired cellular metabolism [2]. The clinical features of shock arise because perfusion of vital organs (such as the brain and heart) is maintained at the expense of perfusion of less-vital organs (eg, skin, kidneys, and gut).

In the early phase of shock (compensated) these processes compensate for hypovolemia and maintain central circulating blood volume and cardiac output. The compensatory vasoconstriction that occurs in shock reduces blood flow to skin, peripheries, and certain organs (particularly the kidneys and gut). As a result, patients with meningococcal septicemia often present with cool peripheries, prolonged capillary refill time, delayed and sluggish skin blood flow, and oliguria. In the most severe cases, focal ischemia of the skin or even whole limbs may occur. In addition, many patients with septic shock will develop acute kidney injury, often leading to acute renal failure.

Despite the presence of shock, preservation of brain perfusion and function is often present until decompensation occurs; so that the child's relatively alert state may make observers underestimate the degree of cardiovascular collapse. Eventually, a decreased level of consciousness indicates a loss of cerebral vascular homeostasis and reduced brain perfusion. The onset of hypotension signifies a failure of the compensatory mechanisms. It should be remembered that diagnosis of shock in children is not dependent on the presence of arterial hypotension. Children with healthy cardiovascular systems may be able to compensate for loss of up to 40% of circulating blood volume without developing hypotension, and may have normal blood pressure until shock is advanced.

The presence of a hemorrhagic rash is pathognomonic of meningococcal infection and reflects coagulopathy. Coagulopathy is universal in severe sepsis, regardless of the etiology. Both procoagulant and anticoagulant pathways of hemostasis are dysregulated as a consequence of activation of inflammatory and coagulation cascades, in addition to endothelial dysfunction. Disseminated intravascular coagulation (DIC), as seen in meningococcal sepsis, arises from loss of anticoagulant proteins (eg, proteins C and S) from the plasma and failure of anticoagulant mechanisms on the endothelial surface. The endothelial receptors required for protein C activation (including the endothelial protein C receptor and thrombomodulin) are down regulated in patients with meningococcal septicemia [3]. In addition, levels of circulating activated protein C and antithrombin III are reduced. Fibrinolysis is suppressed due to reduced production of endothelial tissue plasminogen activator and increased production of plasminogen activator inhibitior-1 (PAI-1), and other fibrinolysis inhibitors, such as thrombin-activatable fibrinolysis inhibitor (TAFI). These abnormalities result in DIC and lead to purpura fulminans [4].

Impaired myocardial function occurs due to several pathological processes that are activated in septic shock. Hypovolemia leads to decreased ventricular filling, metabolic derangements (including hypoxia, acidosis, hypokalemia, hypocalcemia, hypophosphatemia, hypomagnesemia, hypoglycemia, and disturbed fatty acid metabolism), and myocardial contractility. Bacterial products and inflammatory cytokines directly suppress myocardial contractility, and plasma interleukin 6 (IL-6) has been identified as a specific myocardial depressant factor in meningococcal septicemia [5]. Myocardial contractility improves with volume resuscitation and correction of metabolic derangements, but patients with signs of ongoing shock (despite adequate volume resuscitation) require inotropic support to improve myocardial function.

Initial assessment and management

The use of prehospital parenteral antibiotic therapy is recommended in many countries following recognition of meningococcal disease. Observational studies that have attempted to assess the impact of such use in clinical practice, however, have reported conflicting results. In

1992, Cartwright et al [6] reported a 40% reduction in case fatality in children given parenteral penicillin before admission. However, two studies from Denmark reported a two- to threefold increase in mortality associated with antibiotics given before admission [7,8]. A more recent study from the UK confirmed the Danish data, showing that the administration of parenteral penicillin by general practitioners was associated with increased odds ratios for death (7.4, 95% CI 1.5–37.7) and complications in survivors (5.0, 95% CI, 1.7–15.0) [9]. However, as suggested in the Danish studies, the children who received penicillin had more severe disease on admission (median Glasgow meningococcal septicemia prognostic score [GMSPS] 6.5 versus 4.0, $P = 0.002$). It is therefore likely that prehospital antibiotics are given to more severely ill children and, despite the above data, theoretically should be beneficial. Brandtzaeg et al [10] have shown that antibiotic therapy reduces the plasma endotoxin level on admission. Parenteral antimicrobial therapy given within 1 hour of recognition of meningococcal disease is recommended in the most recent national and international guidelines [11,12].

The initial assessment of any patient with potentially life-threatening illness follows the standard 'ABC' algorithms (Figure 6.1) that are widely taught in acute life-support training.

Unless consciousness is impaired, the airway is usually patent in meningococcal disease, but breathing may be compromised by pulmonary edema due to capillary leak in the lungs, and hypoxia may be present. In addition, the patient may have severe metabolic acidosis, which may cause tachypnea and respiratory exhaustion. Circulation is affected as described above.

Many prognostic scoring systems have been evaluated for use in patients with acute meningococcal disease. They all lack precision in their prediction of outcome, but the clinically-based GMSPS has proven

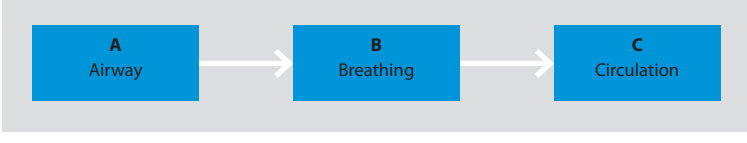

Figure 6.1 Standard 'ABC' algorithm for acute life support.

beneficial in determining whether patients are at high risk of poor outcome and should therefore be managed in an area that can offer a high level of support and monitoring (ie, a high dependency or intensive care area) [13].

Management of shock

The goal of circulatory support in shock is maintenance of oxygenation and adequate tissue perfusion. The priority in achieving these goals is fluid resuscitation to restore intravascular volume. Early and aggressive fluid resuscitation is associated with an improved survival in pediatric septic shock [14]. In addition, inotropic support is frequently necessary in order to improve cardiac output and tissue perfusion.

The establishment of central venous access is a priority in the critically ill patient. This will aid and guide fluid resuscitation and the measurement of central venous oxygen saturation ($ScvO_2$) [15]. $ScvO_2$ may be an important surrogate indicator of cardiac output and therefore a level of >70% has been targeted in studies of goal-directed resuscitation [15,16]. The most recent studies suggest that protocolized resuscitation improves outcome in children with septic shock [17]. An initial fluid bolus of 20 mL kg^{-1} should be given over 5 to 10 minutes to children with signs of shock. The expected response to this rapid volume replacement is reduction in heart rate and improvement in peripheral perfusion, with a concomitant decrease in capillary refill time. In milder cases compensated shock is rapidly reversed by this initial fluid bolus, but repeated review is mandatory as the disease may progress due to ongoing capillary leakage.

Another marker of improvement in perfusion is increased urine output, and bladder catheterization should be performed early to allow this to be accurately assessed. When signs of shock persist after an initial 20 mL kg^{-1} of fluid, further 20 mL kg^{-1} fluid boluses should be given until signs of circulatory compromise improve. If shock persists despite administration of 40 to 60 mL kg^{-1} of fluid resuscitation within 15 to 30 minutes there is a significant risk of pulmonary edema developing. Elective tracheal intubation and mechanical ventilation is recommended at this stage, even in the absence of overt signs of respiratory failure. If performed early, before signs of pulmonary edema manifest, this is associated with improvement in outcome [12]. Timely tracheal intubation and mechanical ventilation

is thought to be beneficial by consequent reduction of myocardial and respiratory muscle oxygen consumption, and by facilitating delivery of positive end expiratory pressure (PEEP) to aid oxygenation. The sedation and muscle relaxation used in these circumstances additionally facilitates placement of arterial and central venous catheters to aid monitoring. However, judicious use of sedation is required as sedative agents may cause further reduction of cardiac function.

Fluid resuscitation therapy should be monitored continuously using heart rate, blood pressure, central venous pressure, urine output, metabolic status, and peripheral perfusion as indicators. There is evidence to suggest that monitoring of mixed venous or central venous oxygen saturation may provide a surrogate indicator of cardiac output and help guide fluid and inotrope requirements [15]. However, more recent data suggest that rate of clearance of arterial lactate may give just as useful information regarding resuscitation of shock [18].

Fluid boluses should not be given to children who have rales, hepatomegaly, or other signs of fluid overload. Interestingly, some children with severe shock do not become intravascularly fluid overloaded, due to capillary leak syndrome, and are only stabilized after resuscitation with very large volumes of fluid (100 to 200 mL kg^{-1}) together with concurrent inotropic support. However, large volume fluid resuscitation is not without consequence; fluid overload has been associated with increased mortality and morbidity [19].

As myocardial depression is invariably a contributory feature of persistent shock, inotropic support with adrenaline or noradrenaline should be initiated early, via a central vein or the intraosseous route. It is usually impractical to gain central venous access in children before tracheal intubation. Dilute solutions of adrenaline, dopamine, or dobutamine can be administered as an infusion through a peripheral vein until central venous access is obtained [12].

In patients who are unresponsive to increasing amounts of catecholamines there is anecdotal data that vasopressin, or its analogues, may be valuable in raising blood pressure [20]. However, use of vasoconstrictors, while increasing blood pressure, may be associated with reduction in cardiac output, and the use of some vasoconstrictors in shock (such

as nonspecific inhibitors of nitric oxide synthase) has been associated with a worsening of outcome [21].

Respiratory support

High flow facial oxygen should be delivered routinely from the outset during initial assessment. If no major airway or breathing problem is present, priority is given to the assessment and treatment of circulatory failure. Indications for immediate tracheal intubation are hypoxia, severe respiratory distress, persistent shock not responsive to 40 to 60 mL kg^{-1} of fluid resuscitation, fluctuating or decreasing conscious level (Glasgow coma score [GCS] ≤8, or decrease of 3 points within 1 hour), or other signs of raised ICP.

Biochemical and hematological derangements

Children with meningococcal sepsis often have profound derangements in blood chemistry, including acidosis, hypoglycemia, hypocalcemia, hypokalemia, hypomagnesemia, or hypophosphatemia. These should be detected by repeated blood analysis and treated if present.

Hyperglycemia may occur following resuscitation and stabilization. Data from critically ill children and adults indicate that control of blood glucose using insulin is not associated with any major improvement in outcome, and may be associated with increased morbidity due to hypoglycemia, and is now not recommended.

DIC is common; there may be bleeding from mucosal surfaces and puncture sites. In addition, spontaneous pulmonary, gastric, or cerebral hemorrhage may occur, particularly if there is associated thrombocytopenia. If bleeding occurs, correction of coagulopathy with fresh frozen plasma, vitamin K, platelets, and cryoprecipitate, may prevent life-threatening hemorrhage. Correction of coagulopathy in the absence of overt bleeding has not been shown to reduce the risk of bleeding, unless anticoagulant treatment is being used. In this case, it is prudent to maintain the platelet count above 50,000/mm^3. The most effective rescue therapy for this severe coagulopathy syndrome may be plasma exchange, but there are no controlled trial data to confirm these findings [22].

The skin may be severely compromised in meningococcal disease through inadequate perfusion, as a result of vasoconstriction and DIC. In addition to treatment of DIC with plasma exchange, continuous renal replacement therapy (CRRT) and protein C infusion, or plasma infusion with CRRT, vasodilator therapies (such as milrinone, nitroglycerin, nitroprusside, prostacyclin, and pentoxifylline) may improve microcirculation.

Decreased skin perfusion may predispose pressure areas to ischemic damage, and tissue edema from capillary leak may cause compartment syndrome. The role of fasciotomy to treat ischemic limbs is not clearly established, but has been used in circumstances where there is evidence of an increase in compartment pressure. Multi-disciplinary input from orthopedic, vascular, and plastic surgeons may be needed for limb salvage. Amputation should not be considered until it is felt to be absolutely necessary and only performed following extensive consultation.

Raised intracranial pressure

Raised ICP occurs due to inflammation of the meninges and capillary leak leading to cerebral edema. Most patients with meningococcal meningitis have mildly raised ICP, but significantly raised ICP is uncommon. Although most critically ill children with meningococcal infection have shock as their primary clinical problem, a small proportion present primarily with meningitis and raised ICP as their predominant clinical manifestation. Signs of raised ICP include:

- a declining level of consciousness;
- focal neurological signs (including unequal, dilated or poorly responsive pupils, relative hypertension, and bradycardia); and
- papilledema (late finding in acutely raised ICP).

Patients with meningococcal septicemia, without meningeal inflammation, who have severe shock may present with impaired consciousness as a result of cerebral hypoperfusion. Conversely, patients without shock who have raised ICP may have peripheral vasoconstriction and these signs may be confused with compensated shock. In the latter case, poor peripheral perfusion is not associated with metabolic acidosis, and tachycardia, hypotension, and other signs of shock are absent. There is relative bradycardia, normal or high blood pressure, and a decrease in

level of consciousness, or the presence of other neurological signs. In these circumstances it should be assumed that the abnormal neurology is due to raised ICP, and aggressive fluid resuscitation should be avoided, as excess fluid will exacerbate cerebral edema.

If raised ICP is suspected, an intravenous infusion of mannitol (0.25 to 0.5 g/kg over 5 minutes), or 3% saline (3 mL/kg over 5 minutes), may prevent cerebral herniation and may be life-saving [23]. Urgent tracheal intubation to protect the airway and control ventilation is indicated. In children with raised ICP from meningococcal infection, initial assessment may reveal coexistent shock. In this case the priority is to correct shock before specific measures to control ICP.

Neuroprotective measures should be instituted: a relatively high blood pressure is necessary in order to maintain cerebral perfusion pressure. It is not clear that monitoring of intracranial pressure or cerebral perfusion pressure improves outcome in severe central nervous system infection [24]. In the absence of shock, cautious fluid restriction may be indicated, but fluid balance requires careful monitoring. Controlled mechanical ventilation to maintain normocapnia should be initiated.

Sedation is essential following tracheal intubation in order to prevent acute rises in ICP caused by agitation and coughing, but muscle relaxants should generally be avoided as they may mask seizures. Seizures should be aggressively managed to avoid any further increases in ICP.

Neuro-intensive care should be instituted using a head-up position, head midline, minimal suction, deep sedation, normothermia, and strict fluid balance. There is no evidence that hypothermia improves outcome from raised ICP due to meningitis, and may be associated with worse outcome. What appears to be important is avoidance of pyrexia [25].

Lumbar puncture

Lumbar puncture (LP) can yield rapid microbiological confirmation of meningococcal meningitis and can exclude other causes of meningeal infection. However, the procedure may be dangerous in the presence of raised ICP or shock, as it may cause cerebral herniation or further cardiovascular compromise. Contraindications to LP include [11]:

- cardiorespiratory insufficiency;

- raised ICP (evidence for which includes fluctuating or deteriorating levels of consciousness [GCS ≤8]; normal or high blood pressure in the presence of a slow or normal heart rate; unequal, dilated, or poorly reacting pupils; focal neurological signs or abnormal posturing; seizures; and papilledema); and
- coagulopathy.

In view of the rapid and unpredictable progression of meningococcal disease, we have previously argued that LP should be avoided or deferred in the initial assessment of patients with clinically obvious meningococcal disease. This is because the additional information provided by the LP adds little to the diagnosis. Clearly, microbiological confirmation is important, but with use of high sensitivity molecular diagnostics, which can be deployed even after treatment has begun, it is unlikely that LP at the outset will add vital information that will otherwise be lost. In a child with a hemorrhagic rash, with the most likely diagnosis of meningococcal infection, the routine use of broad-spectrum antibiotics, such as the third generation cephalosporins (which have excellent cerebrospinal fluid penetration and little reported meningococcal resistance), further reduces the absolute dependence on early microbiological diagnosis. However, where diagnosis is unclear, or in areas where resistant meningococci are emerging, important information may be obtained by carrying out an LP, but this should only be done in the absence of any of the contraindications described above.

Computed tomographic (CT) brain imaging is frequently used in patients with depressed conscious level, and is particularly recommended where there is a broader differential diagnosis. However, urgent cranial imaging is rarely justified in children with meningitis and a hemorrhagic rash, unless there is focal neurology or a suspicion of neurosurgical emergency. It is hazardous to take a critically ill patient to the radiology department before they have been adequately stabilized and monitored, and unjustifiable if it is unlikely that the scan will significantly alter clinical management.

In any case, cranial CT scanning is not a sensitive way of assessing ICP, and cannot therefore help in making the decision to perform an LP, which must be made on a basis of clinical assessment [11].

Antibiotic therapy

Cefotaxime (50 mg/kg, four times daily) or ceftriaxone (80 mg/kg, once daily) is preferred as the initial therapy in patients with a clinical diagnosis of meningococcal disease. Penicillin resistance is rare in the UK and therefore benzylpenicillin is the logical choice for urgent treatment in the community. However, until a positive bacterial identification is available, there remains the possibility of either penicillin resistance or alternative diagnoses that might not be adequately treated by penicillin therapy. Other rarer bacterial causes of purpura fulminans include *Streptococcus pneumoniae*, *Staphylococcus aureus,* and other Gram-negative bacteria.

The duration of antibiotic therapy for meningococcal disease does not need to be prolonged and most centers use a 5- to 7-day course of a third generation cephalosporin for both meningococcal meningitis and septicemia.

Adjunctive therapies

Steroids given with the first dose of antibiotics appear to reduce the incidence of neurological sequelae in *Haemophilus influenzae* type b, and may be beneficial in pneumococcal meningitis. There is also a trend of improved outcome in meningococcal meningitis. The NICE guidelines state that systemic high-dose dexamethasone should be given in cases of suspected bacterial meningitis before (ideally within 4 hours), and no longer than 12 hours following the first dose of parenteral antibiotics. A dose of 0.15 mg/kg, four times daily, for 4 days is recommended [11]. High dose corticosteroid use is contraindicated in meningococcal shock in the absence of meningitis, as this has been shown to worsen the outcome of adults with septic shock [26].

There is some evidence that refractory septic shock may be more common in children with impaired adrenal gland responsiveness in the acute phase. Death in meningococcal septic shock is more likely to occur in the first 8 hours following presentation, so adrenal replacement therapy should be given early if signs of inotrope unresponsiveness are present [27].

There have only been two properly conducted randomized controlled studies of other adjunctive therapies in meningococcal disease, and one in children with septic shock in general:

- HA-1A (anti-endotoxin antibody) — investigated in a randomized controlled trial in children with meningococcal septicemia. This study showed that there was no significant reduction in mortality in the children treated with HA-1A when compared with placebo [28]. Subsequent studies in adults with Gram-negative septicemia also showed no benefit of adjunctive therapy with HA-1A.
- Recombinant bactericidal permeability increasing protein ($rBPI_{21}$) — binds and neutralizes endotoxin and blocks the inflammatory cascade. In a large placebo-controlled randomized multicenter trial, there was evidence of improvement in a variety of parameters. Unfortunately, the study was not sufficiently powerful to be able to detect a reduction in mortality [29]. However, the patients treated with $rBPI_{21}$ suffered fewer amputations, fewer blood product transfusions, and improved functional outcome compared to those treated with placebo. In addition, fewer children died who had received a full 24 hour infusion of $rBPI_{21}$ (2% $rBPI_{21}$ vs 6% placebo, $P = 0.07$), compared to those who had not received the full infusion, suggesting that $rBPI_{21}$ used earlier may be beneficial in children with meningococcal disease. However, the only published data would not support its routine use.
- Activated Protein C — a randomized controlled trial has been carried out in children with septic shock, with its primary endpoint being reduction in time to resolve respiratory, cardiovascular, and renal organ failure, as a surrogate indicator of mortality [30]. The study was terminated early as it was felt it would be unlikely to reach its primary endpoint, with suggestions of an unfavorable risk/benefit profile.

Where to manage children with meningococcal disease?

Different countries will have differing management pathways for patients with meningococcal disease. Much of the discussion below relates to the situation in the UK. However, in many countries, children diagnosed with meningococcal disease will initially be managed in an ICU setting, partly due to the difficulties in arranging rapid transfer in the event of a later deterioration.

Most patients with meningococcal disease will not require intensive care. However, those with persistent shock or signs of raised ICP should be managed in an area where they can be closely monitored and treatment can be stepped up immediately if required.

For those who do not immediately require transfer to an intensive care unit, management on the general ward should be undertaken, with frequent careful monitoring of vital signs (pulse, blood pressure, transcutaneous oxygen saturation, respiratory rate, urine output, and conscious level) for the first 24 to 48 hours. This is likely to be better facilitated by initial placement in a high dependency unit. Pediatric early warning scores are currently being validated to determine whether they assist in identification of the deteriorating child [31].

Failure to recognize deterioration following hospital admission is associated with increased mortality. A large case-control study of health care delivery in children with meningococcal disease in the UK has demonstrated that suboptimal recognition and management significantly increased the likelihood of death [32]. In 143 children with meningococcal disease who died, there were significantly more departures from optimal (per protocol) management compared with control children (children with meningococcal disease who survived). Multivariate analysis identified three factors independently associated with an increased risk of death:

- failure of patients <16 years to be looked after by a pediatrician;
- inadequate supervision of junior medical staff; and
- failure to administer adequate inotrope doses.

In addition, failure to recognize complications of the disease was a significant risk factor for death, although not independent of the absence of pediatric care. The odds ratio for death was 8.7 (95% CI 2.3–33) with two failures in management, increasing with multiple failures in management. Ninis et al [32] concluded that suboptimal health care delivery significantly reduced likelihood of survival in children with meningococcal disease, and that improved training of medical and nursing staff, adherence to published protocols, and increased supervision of junior staff by consultants may improve outcome for these children as well as those with other life threatening illnesses.

The decision to transfer critically ill or unstable patients to a more specialized unit can be difficult. A prolonged period of resuscitation may be necessary in the emergency department before a child with severe disease is stable enough to move. Transporting children before they are adequately resuscitated is hazardous. The child should be fully stabilized, with monitoring equipment securely in place before transfer to the PICU is undertaken.

The benefits of transferring patients to specialized units for ongoing intensive care management have been confirmed by the demonstration of significant reduction in mortality of children with severe meningococcal infection [1]. It is likely that centralization of care of critically ill children with meningococcal sepsis into units experienced in the management of such patients has had a significant impact in reducing mortality.

The organization of pediatric intensive care in the UK has necessitated the development of pediatric critical care transport teams, with a network of outreach education to general hospitals. Together with the widespread implementation of protocol-based care, there is evidence that this has led to improvement in outcome of patients with severe sepsis [33].

Treatment guidelines have been developed over many years. These are regularly updated and have now incorporated NICE and RCPCH guidance. They are useful reminders of the management principles for infants, children, and young adults with meningococcal septicemia and meningitis (see Appendix) [11].

Summary points

- The outcome of meningococcal disease has improved in recent years due to enhancements in the recognition, resuscitation, stabilization, transfer, and ongoing care of individuals with the disease.
- Despite these advances, meningococcal infections remain a major cause of morbidity and mortality throughout the world.
- Introduction of serogroup C conjugated meningococcal vaccine has been an impressive success, but the challenge remains to develop effective vaccines against all the disease-causing serogroups.
- Restructuring health services to allow patients with meningococcal infection to gain access to units with a large experience in treating this fulminant disease should be made a priority.

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Chapter 7

Prevention of meningococcal disease through vaccination

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Introduction

Meningococcal disease, in its early stages, is indistinguishable from minor viral illnesses, delaying recognition and treatment. Furthermore, the infection can progress rapidly to critical illness or death, leaving little time for life-saving interventions to be instituted. For these reasons, prevention through vaccination has been a priority for public health experts for decades. This section will consider the use of purified polysaccharide vaccines, protein-polysaccharide conjugate vaccines, and capsular group B vaccines.

Polysaccharide vaccines

The first meningococcal vaccines were developed in the 1960s and were made using polysaccharides purified from the bacterial capsule. The purified polysaccharides were used to make either a bivalent vaccine containing 50 μg of each capsular group A and C, or a quadrivalent vaccine covering capsular groups A, C, Y, and W (Table 7.1). These T-independent vaccines were found to be immunogenic in older children and adults but, with the exception of capsular group A, induced little humoral response in young children [1,2]. It has been proposed that marginal zone B cells are important in the response to polysaccharide vaccines

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	Proprietary name	Manufacturer
Polysaccharide vaccines	Menomune®	Sanofi Pasteur
	ACWY Vax®	Pfizer
	NmVac4-A/C/Y/W-135®	JN-International Medical Corporation
Monovalent conjugate vaccines	Meningitec®	Nuron Biotech
	Menjugate Kit®	GlaxoSmithKline
	NeisVac-C®	Pfizer
	MenAfriVac®	Serum Institute of India
Multivalent conjugate vaccines	Menitorix®	GlaxoSmithKline
	Menhibrix®	GlaxoSmithKline
	Menactra®	Sanofi Pasteur
	Menveo®	GlaxoSmithKline
	Nimenrix®	Pfizer

Table 7.1 Meningococcal polysaccharide and conjugate vaccines.

Presentation	Active ingredients (per dose)	Adjuvant
Single dose freeze-dried vial and separate diluent	50 µg each of group A, C, W, and Y polysaccharide	None
Single dose freeze-dried vial, separate diluent in prefilled syringe	50 µg each of group A, C, W, and Y polysaccharide	None
10 and 50 dose freeze-dried vial, separate diluent	50 µg each of group A, C, W, and Y polysaccharide	None
Single dose, liquid filled vial	10 µg *O*-acetylated group C oligosaccharide conjugated to CRM_{197} (~15 µg)	$AlPO_4$
Single dose freeze-dried vial, separate diluent in prefilled syringe	10 µg *O*-acetylated group C oligosaccharide conjugated to CRM_{197} (12.5–25.0 µg)	$Al(OH)_3$
Single dose, prefilled syringe	10 µg de-*O*-acetylated group C polysaccharide conjugated to tetanus toxoid (10–20 µg)	$Al(OH)_3$
Single and 10 dose freeze-dried vial, separate diluent	10 µg group A polysaccharide conjugated to tetanus toxoid (11–33 µg)	$AlPO_4$
Single dose freeze-dried vial, separate diluent	5 µg meningococcal group C polysaccharide conjugated to 5 µg tetanus toxoid	None
	5 µg *H. influenzae* type b polysaccharide conjugated to 12.5 µg tetanus toxoid	
Single dose freeze-dried vial, separate diluent	5 µg meningococcal group C polysaccharide conjugated to ~5 µg tetanus toxoid	None
	5 µg meningococcal group Y polysaccharide conjugated to ~6.5 µg tetanus toxoid	
	2.5 µg *H. influenzae* type b polysaccharide conjugated to ~6.25 µg tetanus toxoid	
Single dose, liquid filled vial	4 µg each of group A, C, W, and Y oligosaccharides conjugated separately to diphtheria toxoid (~48 µg total)	None
Single dose freeze-dried vial, separate diluent	10 µg group A oligosaccharide conjugated to CRM_{197} (16.7–33.3 µg)	None
	5 µg group C oligosaccharide conjugated to CRM_{197} (7.1–12.5 µg)	
	5 µg group W oligosaccharide conjugated to CRM_{197} (3.3–8.3 µg)	
	5 µg group Y oligosaccharide conjugated to CRM_{197} (5.6–10.0 µg)	
Single dose freeze-dried vial, separate diluent in prefilled syringe	5 µg each of group A, C, W, and Y polysaccharides conjugated separately to tetanus toxoid (~44 µg total)	None

and that the lack of a response in children under 18 months is thought to be a result of the immaturity of the marginal zone of the spleen in humans until about 18 months of age. The repeating sugar sub-units of these T-independent vaccines are thought to induce antibody response by cross-linking the B-cell receptor and driving antigen-specific B cells to differentiate into antibody-secreting cells, without the formation of germinal centres in the draining lymph node. A consequence of the lack of T cell involvement in the immune response is that these vaccines do not induce immunological memory and are associated with immunological hyporesponsiveness (reduced responses following subsequent doses of vaccine) [3,4]. However, studies have shown that the capsular groups A and C components of these purified polysaccharide vaccines have over 90% effectiveness in the short term against disease caused by these organisms, though protection wanes over time [5,6].

Duration of the antibody response is limited, especially among children, and intervention studies in Quebec have shown that effectiveness of the C component of these vaccines is very limited in the youngest members of the population. The serogroup A component of the vaccine appears to be immunogenic from a few months of age and may therefore be unlike other polysaccharide vaccines, which do not offer relevant protection before 2 years of age. There are no protection data currently available for capsular group W or Y polysaccharides. There is no polysaccharide vaccine for capsular group B disease, since the polysialic acid capsule is recognized by the human immune system as a self-antigen (which decorates neural cell adhesion molecules in the fetus) [7]. The A, C and A, C, W, Y polysaccharide vaccines have been widely used for individuals with complement deficiency and splenic hypofunction, travellers, and laboratory workers and for decades have been the mainstay of response to outbreaks caused by epidemic serogroup A strains of meningococci. However, these vaccines have now largely been replaced by protein-polysaccharide conjugate vaccines.

Protein-polysaccharide conjugate vaccines

The development of *Haemophilus influenza* type b (Hib) vaccines during the 1980s showed that polysaccharides could be converted into T-cell-dependent antigens by chemical conjugation to a protein carrier, thus

overcoming the limitations previously associated with purified polysaccharides [8]. This in turn led to the successful development of pneumococcal and meningococcal conjugate vaccines. The protein carriers most commonly used in licensed conjugate vaccines are tetanus and diphtheria toxoids, and CRM_{197}, which is a nontoxic genetic variant of diphtheria toxin [9]. The first monovalent meningococcal group C (MenC) conjugate vaccines were licensed in the UK in 1999 and subsequently in the rest of Europe by the mutual recognition of the UK licenses.

These conjugate vaccines were introduced into the UK routine immunization program from November 1999, along with catch-up doses for older children and teenagers provided at school [10]. They had an immediate impact on group C meningococcal disease; between 1999 and 2001 the overall reduction in laboratory confirmed cases of MenC disease among the vaccinated age groups was 86.7% [11]. There was also evidence that the vaccine had an impact on the transmission of group C meningococci, thus providing a level of herd protection [12]. Following the introduction of the vaccine the number of cases among unvaccinated age groups fell by 67%, corresponding to a similar reduction in MenC carriage rates seen in vaccinated young adults [13]. This herd or indirect effect is a hallmark of conjugate vaccines and has made an important contribution to the near elimination of MenC disease where the vaccine has been implemented.

Initial analyses suggested that the vaccine was highly effective in all age groups; however, protection waned rapidly in those children vaccinated in the routine immunization program. Effectiveness during the first year after receiving the scheduled vaccination, at 93%, was similar to other age groups but by the second year there was little evidence of protection [11]. Despite this there were relatively few cases of MenC disease among infants because of the high level of herd protection. Nevertheless, a booster dose of MenC conjugate was introduced, together with a Hib booster, at 12 months of age in the form of a bivalent conjugate vaccine. More recent data suggest that vaccination later in childhood results in higher and more persistent antibody levels [14]. Therefore, conscious of the need to maintain herd immunity in teenagers and young adults, the Joint Committee on Vaccination and Immunisation (JCVI) in the UK advocated a booster dose of monovalent MenC conjugate vaccine

at the age of 13 to 14 years, reducing the number of infant doses from two to one. In 2015, in response to an outbreak of MenW, the booster was changed to MenACWY with a catch-up for young people between 14 and 18 years of age.

Although the prevalence of an ST-11 meningococcal clone expressing group C capsular polysaccharide prompted the development of monovalent MenC conjugates, disease caused by other capsular groups required different vaccine formulations. To address the recurrent and devastating group A (MenA) meningococcal epidemics occurring in sub-Saharan Africa, an affordable monovalent MenA-tetanus toxoid vaccine (PsA-TT) was produced in India as a public–private partnership involving the WHO, FDA, PATH, National Institute of Health, and the Serum Institute of India, supported by the Bill & Melinda Gates Foundation [15]. It was licensed for infants and adults up to the age of 29 years, and was rolled out across the sub-Saharan countries from the end of 2010. This vaccine has had a significant impact on both disease and carriage, with the incidence of disease reduced by 94% and a significant reduction in carriage (OR = 0.019) [16,17]. By the end of 2013, over 150 million persons in sub-Saharan Africa had been vaccinated; and there have been no reports of serogroup A meningitis among vaccinated persons, and serogroup A outbreaks have been eliminated in countries that have introduced PsA-TT. Introduction of PsA-TT into the routine immunization program in these countries will be needed to maintain herd immunity and to protect new birth cohorts.

In 2005, the first multivalent ACWY conjugate (MenACWY-D) was licensed by the FDA. This vaccine contains the four meningococcal polysaccharides conjugated to diphtheria toxoid. It has not been licensed in Europe, unlike a CRM_{197} conjugate (MenACWY-CRM), which was licensed by both the FDA and the European Medicines Agency (EMA) in 2010. In the US, where there is a higher incidence of group Y disease than in Europe, the Advisory Committee on Immunization Practices (ACIP) recommends the routine immunization of adolescents at 11 to 12 years of age, with a booster dose at age 16 years. Children and adults who have an increased risk of meningococcal disease should also be vaccinated with one of these multivalent conjugates. Since its licensure

in 2012, a three component vaccine (Hib-MenCY tetanus toxoid conjugate) has been recommended for infants from 2 months of age who are at an increased risk for meningococcal disease; MenACWY-CRM may also be used in infants from 2 months of age. The routine immunization of infants with meningococcal vaccines is not recommended in the US, given the low incidence of meningococcal disease. A third quadrivalent ACWY conjugate vaccine, in which the polysaccharides are conjugated to tetanus toxoid, was authorized by the EMA in 2012. As mentioned above, MenACWY is now used routinely for adolescents in the UK. The multivalent formulations elicit antibody responses that are at least as good as their plain polysaccharide counterparts but have the inherent advantages of conjugate vaccines, and look likely to replace them in the coming years.

Clinical studies and post-marketing surveillance have shown that meningococcal conjugate vaccines are broadly compatible with a range of other vaccines used in national immunization programs. Nevertheless, in some circumstances immune interference in the form of either carrier-specific enhancement or carrier-induced epitopic suppression has been observed [18].

Production and quality control of polysaccharide and conjugate vaccines

Purified polysaccharides used to make plain polysaccharide vaccines have to be of sufficient molecular size to be immunogenic [19]. The licensed vaccines contain high molecular weight polysaccharides purified by precipitation from broth cultures of meningococci using the cationic detergent, cetrimonium bromide. In contrast, the essential requirement of conjugate vaccines is that the saccharide is covalently linked to a carrier protein. The molecular size of the saccharide moiety itself is less critical as long as it contains enough repeating units of the capsular polysaccharide to be recognized as an epitope by the immune system [9].

The polysaccharide starting material used to make conjugate vaccines is generally the same as that used in plain polysaccharide formulations, although conjugate vaccines based on synthetic oligosaccharides are also in development. The purified polysaccharide may be used in its native form

or partially size-reduced before random activation. Alternatively, it may be size-reduced by the process used to generate active functional groups, either at one or both ends of the resulting oligosaccharide, for covalently linking it to the carrier. Thus the size and structure is characteristic of a particular conjugate, depending on a number of factors, including:

- the size of the saccharide;
- the ratio of saccharide to carrier;
- the protein carrier itself; and
- the conjugation chemistry.

Because they consist of highly purified components, both plain and conjugated polysaccharide vaccines are relatively straightforward to quality control using physicochemical assays [20]. Both types of vaccine typically contain very low levels of bacterial endotoxin and have been shown to be safe and not reactogenic in clinical use. There is no evidence of any safety issues in pregnancy, but data are currently limited. No animal tests are used in the quality control of these products and vaccine potency is assumed from the amount of saccharide per dose — as measured by physical, chemical, or serological assays. As it is important for the immunogenicity of plain polysaccharide vaccines and is a distinctive characteristic of a consistently manufactured conjugate vaccine, the molecular size of the product is determined by size exclusion chromatography. Comparison of the total and free saccharide content of conjugate vaccines is used to assess whether the vaccine is adequately conjugated.

Immunological correlates of protection

The accepted correlate of protection for polysaccharide vaccines is based on the observation that susceptibility to meningococcal disease corresponds to a lack of serum bactericidal antibodies (SBA). In a study of group C disease among US military recruits, only 5.6% of recruits who succumbed to disease had an SBA titer of greater than one in four, compared with 82.2% of healthy recruits [21]. Since these studies in the 1960s, an SBA titer of one in four, using a human complement source in the assay, has been taken as the correlate of protection in adults for all polysaccharide vaccine antigens.

The meningococcal conjugate vaccines were not evaluated in clinical protection studies but were licensed based on the SBA as the correlate of protection [10]. However, in most cases baby rabbit serum was used as the source of complement in the clinical trials of conjugate vaccines. This created a problem for comparison with the earlier studies, as meningococci have evolved a level of resistance to human but not rabbit complement, to which they are more sensitive. Following the rollout of the MenC conjugate in infants, analysis of age-specific efficacy data showed that an SBA titer of one in eight correlated best with protection when using rabbit complement in the assay [22].

Capsular group B vaccines

As discussed above, the polysaccharide capsule of group B meningococci is a self-antigen and therefore is poorly immunogenic in humans, and is not currently being pursued as a vaccine candidate. Unfortunately, subcapsular structures, which have been the focus of vaccine development for 40 years, are antigenically diverse unlike the group-defining B polysaccharide capsule, and therefore it is likely that a successful vaccine must either contain multiple antigens or multiple variants of an antigen that is expressed on all B meningococci [23]. Various recombinant surface proteins have been tested as vaccines but the clinical investigations with most of these candidates have not led to further development as a result of these issues. An additional challenge for vaccine developers is that the focus of research has been on the use of membrane-proteins, which are difficult to fold in the absence of a membrane. Consequently, to obtain the appropriate conformational structure, the most widely tested vaccines over the past 30 years have been based on outer membrane vesicles (OMVs), which are derived from the membrane of the bacteria using a detergent-extraction method. These vesicles contain multiple membrane proteins embedded in the lipid membrane, though porins are the most abundant, but have reduced levels of endotoxin and lower amounts of some proteins that are not stable in the membrane in the presence of the detergent (eg, factor H binding protein [fHbp]). OMV vaccines constructed using this approach were used to control outbreaks of meningococcal disease in Norway, Cuba, New Zealand, and France,

with effectiveness rates ranging from 50 to 70% [24]. These vaccines appear to provide protection against the outbreak strain from which they were derived, but show limited cross-protection to other strains. Whilst these vaccines appear to be an adaptable resource for clonal outbreaks, a different approach is required for endemic disease settings, such as the UK, where multiple clones of meningococci are circulating at any one time.

The main protective antigen contained in OMVs is the immunodominant protein PorA, and one promising approach was the development of a vaccine in The Netherlands containing six to nine OMVs, each with a different PorA [25]. This vaccine was immunogenic in both adults and children and had a high predicted coverage of endemic invasive strains, but is not currently being developed further. A vaccine containing two variants of the immunogenic outer membrane lipoprotein, fHbp, is now licensed in the US and recommended for those in risk groups. The bivalent vaccine was developed with adolescents as the anticipated target population, but a routine program has not yet been advised with this vaccine [26]. fHbp is expressed on the surface of almost all meningococci, with protein variants divided into two to three families. If the bactericidal antibody induced by these vaccines is sufficiently cross-protective within each of the main protein families, the vaccine could provide wide protection against circulating invasive strains.

A multicomponent vaccine, 4CMenB, was recently licensed in various jurisdictions and contains three recombinant proteins (neisserial heparin binding antigen, fHbp, and neisserial adhesin A) formulated with the OMV derived from the New Zealand outbreak strain, to provide coverage against ST-41/44 complex organisms [27]. This vaccine was immunogenic in infants, children, and adults in clinical trials, and in vitro assays have been used to predict coverage of 60 to 80% of disease-causing strains, though this can only be confirmed with effectiveness data following implementation. Studies with this vaccine found that the magnitude, duration, and breadth of the antibody response was greater among adolescents and adults than in infants. A study investigating the impact of immunization with 4CMenB on carriage of meningococci among university students in the UK found that there was some limited impact on carriage, but additional studies are necessary to further define the

potential of the vaccine to induce herd protection. 4CMenB has recently been used to control outbreaks of meningococcal disease at two US universities and is now being used for hyper-endemic disease in regions of Quebec. It has also been introduced in a new infant program in the UK that commenced in September 2015. Data on the impact of these population interventions are eagerly awaited.

Since capsular group B meningococcal disease rates are highest in infancy, this is the logical target population for immunization with new vaccines. However, immunogenicity in this population is lower than at older ages and both 4CMenB and the fHbp vaccine are reactogenic in young children [28,29]. Experience with 4CMenB indicated that it induces fever in a high proportion of infants — leading to a recommendation in the UK that it should be administered with paracetamol (acetaminophen) [30]. Most studies considered infant schedules with three early doses (at 2, 3, 4 months or 2, 4, 6 months) and a booster at 12 months of age, but the infant program in the UK will use a reduced dose 2, 4, and 12 month schedule. Two doses are recommended for older children and adolescents. In older children and adults, pain at the injection site has been prominent in studies of 4CMenB. Immunization of adolescents with a MenB vaccine has the potential advantage of inducing herd protection and providing broader protection among unimmunized populations and in those whose immunity has waned. However, data on induction of herd protection remain limited, and without substantial herd impact, cost-effectiveness thresholds are difficult to meet for routine adolescent immunization programs.

In the UK, 4CMenB is recommended for laboratory workers, individuals with asplenia, splenic dysfunction, complement disorders, or in certain outbreak situations where disease is caused by a strain that is expected to be vaccine-preventable.

Summary points

- Meningococcal vaccines are now available that cover the main disease-causing capsular groups of meningococci, with the exception of capsular group X.

- Implementation of the conjugate vaccines has produced powerful control of disease through direct protection of vaccinees and herd immunity.
- Efforts are currently underway to sustain herd immunity in the long-term by adjusting vaccine schedules to produce high levels of antibody amongst adolescents and young adults.
- The new capsular group B vaccines, while somewhat reactogenic, hold the potential to reduce the burden of MenB disease, but the scale of the impact is difficult to predict prior to implementation.

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Appendix

This appendix contains the Meningitis Research Foundation (UK) algorithms for the Management of Bacterial Meningitis and Meningococcal Disease in Children and Young People. These algorithms are based on UK NICE Guidelines, and endorsed by the Royal College of Paediatrics and Child Health (RCPCH).

It also contains the British Infection Association algorithm for Early Management of Suspected Bacterial Meningitis and Meningococcal Septicaemia in Immunocompetent Adults. A new edition of this algorithm developed by the British Infection Association, Public Health England, the Intensive Care Society, the Association of British Neurologists, the Society for Acute Medicine, and Meningitis Research Foundation will be published in the *Journal of Infection* and will be available early in 2016. Latest versions and multiple formats are available at www.meningitis.org.

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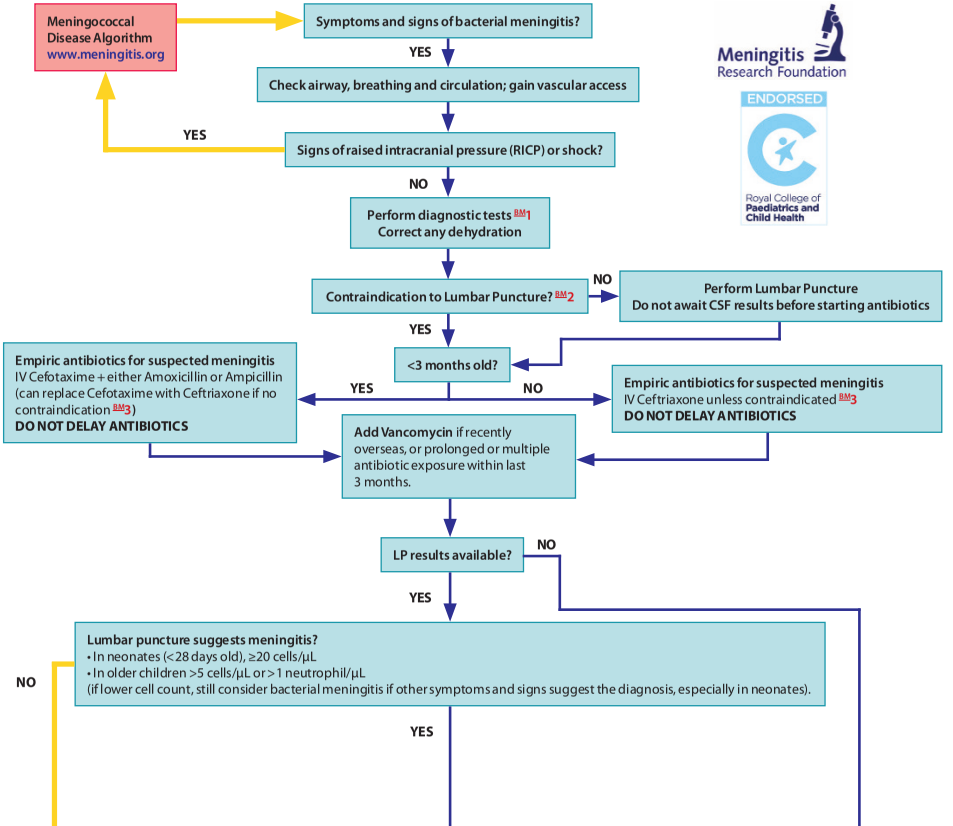
Management of Bacterial Meningitis in Children and Young People

Incorporates NICE Bacterial Meningitis and Meningococcal Septicaemia Guideline CG102. Distributed in partnership with NICE 2nd Edition



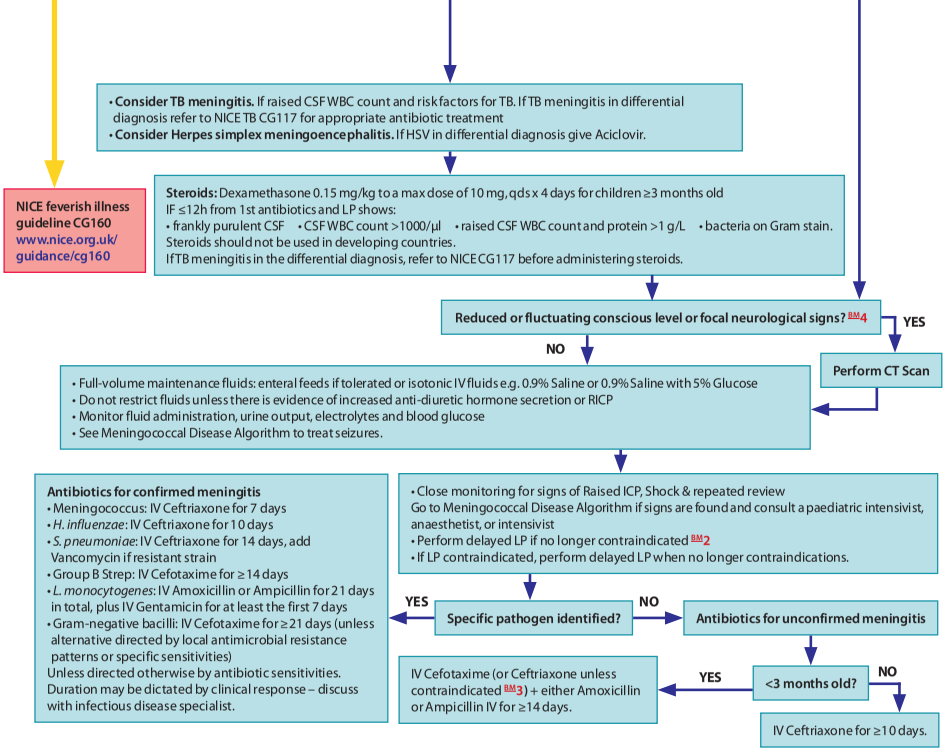
BM1 Diagnostic and other laboratory tests:
Take bloods for Blood gas (bicarb, base deficit), Lactate, Glucose, FBC, U&E, Ca++, Mg++, PO_4, Clotting, CRP, Blood cultures, Whole blood (EDTA) for PCR, X-match. Take Throat swab. If limited blood volume, prioritise blood gas, lactate, glucose, electrolytes, FBC, clotting.

BM2 Contraindications to Lumbar Puncture
Clinical or radiological signs of raised intracranial pressure
- Shock
- After convulsions until stabilised
- Coagulation abnormalities
  - Clotting study results (if obtained) outside the normal range
  - Platelet count below 100 x 10^9/L
  - On Anticoagulant therapy
- Local superficial infection at LP site
- Respiratory insufficiency

Perform delayed LP in children with suspected bacterial meningitis when contraindications no longer present

BM3 Contraindications to Ceftriaxone
Premature neonates with corrected gestational age <41 weeks and other neonates <1 month old, particularly those with jaundice, hypoalbuminaemia, or acidosis; or receiving concomitant treatment with intravenous calcium.

BM4 Indications for CT scan in children with suspected bacterial meningitis
CT scan cannot reliably detect raised intracranial pressure. This should be assessed clinically.
Perform a CT scan to detect other intracranial pathologies if GCS ≤8 or focal neurological signs in the absence of an explanation for the clinical features.
Do not delay treatment to undertake a CT scan.
Clinically stabilise the child before CT scanning.
Consult a paediatric intensivist, anaesthetist, or intensivist.



BM5 Indications for tracheal intubation and mechanical ventilation

Threatened or actual loss of airway patency (e.g. GCS ≤8, response to pain only).

- Need for any form of assisted ventilation e.g. bag–mask ventilation
- Clinical observation of increased work of breathing
- Hypoventilation or Apnoea
- Features of respiratory failure, including
  - Irregular respiration (e.g. Cheyne–Stokes breathing)
  - Hypoxia (saturation <94% in air, PaO_2 <13 kPa or 97.5 mmHg), hypercapnoea ($PaCO_2$ >6 kPa or 45 mmHg)
- Continuing shock following 40 mL/kg of resuscitation fluid
- Signs of raised intracranial pressure
- Impaired mental status
  - GCS drop of ≥3, or score ≤8, or fluctuation in conscious level
  - Moribund state
- Control of intractable seizures
- Need for Stabilisation for brain imaging or for transfer to PICU.

Should be undertaken by a health professional with expertise in paediatric airway management, Consult PICU. (See MD4)

BM6 Repeat LP in neonates after starting treatment if: persistent or re-emergent fever, new clinical findings (especially neurological findings), deteriorating clinical condition, or persistently abnormal inflammatory markers.

BM7 Long-term management: Before discharge consider need for after care, discuss potential long-term effects with parents, arrange hearing test. Refer children with severe or profound deafness for cochlear implant assessment ASAP. Use MRF discharge checklist **http://www.meningitis.org/assets/x/56050.** Provide 'Your Guide' and direct to meningitis support organisations **www.meningitis.org/recovery** or **www.meningitisnow.org/recovery**. Offer further care on discharge as needed. Paediatrician to review child with results of their hearing test 4–6 weeks after discharge from hospital considering all potential morbidities and offer referral. Inform GP, health visitor or school nurse.

Based on NICE CG102 **www.nice.org.uk/guidance/CG102**

Authors AJ Pollard (GDG chair), A Cloke, SN Faust, L Glennie, C Haines, PT Heath, JS Kroll, M Levin, I Maconochie, S McQueen, P Monk, S Nadel, N Ninis, MP Richardson, MJ Thompson, AP Thomson, D Turner. Further copies from **www.meningitis.org** or 0808 8003344.

 A charity registered in England and Wales no 1091105 and in Scotland no SC037586.

Management of Meningococcal Disease in Children and Young People

Incorporates NICE Bacterial Meningitis and Meningococcal Septicaemia Guideline CG102. Distributed in partnership with NICE 8th Edition

Meningitis Research Foundation

ENDORSED

Royal College of Paediatrics and Child Health

MD1 Estimate of child's weight (1–10 years)
Weight (kg) = 2 x (age in years + 4)

MD2 Observe HR, RR, BP, perfusion, conscious level
Cardiac monitor & pulse oximetry.
Conscious Level
Alert
Responds to **V**oice
Responds to **P**ain
Unresponsive

Normal Values

Age	Heart Rate/min	Resp Rate/min
<1	110–160	30–40
1–2	100–150	25–35
2–5	95–140	25–30
5–12	80–120	20–25
Over 12	60–100	15–20

Normal systolic blood pressure = 80 + (age in years x 2)
N.B. Low BP is a pre-terminal sign in children

MD3 Take bloods for Blood gas (bicarb, base deficit), Lactate, Glucose, FBC, U&E, Ca++, Mg++, PO_4, Clotting, CRP, Blood cultures, Whole blood (EDTA) for PCR, X-match. Take Throat swab. If limited blood volume, prioritise blood gas, lactate, glucose, electrolytes, FBC, clotting.

MD4 Intubation (call anaesthetist and consult PICU) see **BM5**
Consider using: Atropine 20 mcg/kg (max 600 mcg) AND Ketamine 1–2 mg/kg in shock or Thiopental (thiopentone) 3–5 mg/kg in RICP AND Suxamethonium 2 mg/kg (caution, high potassium). ETT size = age/4 + 4, ETT length (oral) = age/2 + 12 (use cuffed ET tube if possible). Then: Morphine (100 mcg/kg) and Midazolam (100 mcg/kg) every 30 min.

MD5 Inotropes
Dopamine at 10–20 mcg/kg/min. Make up 3 x weight (kg) mg in 50 mL 5% dextrose and run at 10 mL/hr = 10 mcg/kg/min. (These dilute solutions can be used via a peripheral vein).
Start Adrenaline via a central or IO line only at 0.1 mcg/kg/min.
Start Noradrenaline via a central or IO line only at 0.1 mcg/kg/min for 'warm shock'.
Adrenaline & Noradrenaline: Make up 300 mcg/kg in 50 mL of normal saline at 1 mL/hour = 0.1 mcg/kg/min.

RECOGNITION
May present with predominant **SEPTICAEMIA (with shock), MENINGITIS (with raised ICP)** or **both. Purpuric/petechial non-blanching rash is typical.** Some may have neither shock nor meningitis. Rash may be atypical or absent in some cases.

Call consultant in Emergency Medicine, Paediatrics, Anaesthesia or Intensive Care
Initial assessment looking for shock/raised ICP
Do not perform Lumbar Puncture yet

Give IV Ceftriaxone (80 mg/kg od) without delay
Do not use Ceftriaxone at the same time as calcium-containing solutions: in this situation use Cefotaxime (50 mg/kg qds)

Bacterial meningitis algorithm
www.meningitis.org

SIGNS OF SHOCK? MD2
- Tachycardia
- Capillary refill time > 2 seconds
- Cold hands/feet; pale or blue skin
- Respiratory distress/oxygen saturation <95% in air
- Altered mental state/decreased conscious level
- Decreased urine output (<1 mL/kg/hr)
- Hypotension (late sign)
- Hypoxia on arterial blood gas
- Base deficit (worse than -5 mmol/L)
- Increased lactate (>2 mmol/L)

NO

YES

Do not perform Lumbar Puncture; Nil by mouth

- ABC & High flow Oxygen (minimum 10 L/min) by face mask
- Insert 2 large IV cannulae (or intraosseous); Take bloods, see MD3
- Take blood gas (including lactate). Measure glucose.

VOLUME RESUSCITATION
- Immediate bolus of 20 mL/kg of 0.9% Saline over 5–10 minutes and reassess immediately
- If shock persists immediately give second bolus of 20 mL/kg of 0.9% Saline or of 4.5% Human Albumin over 5–10 minutes and reassess immediately
- Observe closely for response/deterioration
- Consider Urinary catheter to monitor output

RAISED INTRACRANIAL PRESSURE?
- Reduced (GCS ≤8) or fluctuating level of consciousness
- Relative Bradycardia and Hypertension
- Focal neurological signs
- Abnormal posture or posturing
- Seizures
- Unequal, dilated or poorly responsive pupils
- Papilloedema (late sign)
- Abnormal 'doll's eye' movements

NO

YES

Do not perform Lumbar Puncture; Nil by mouth

- ABC and Oxygen (minimum 10 L/min) by face mask
- Insert 2 large IV cannulae (or IO); Take bloods, see MD3
- Take blood gas (including lactate). Measure glucose.
- Treat shock if present

Call anaesthetist and contact PICU
- Intubate and ventilate to control $PaCO_2$ (4–4.5 kPa)
- Urinary catheter and monitor output, NG tube

Do not perform Lumbar Puncture

CLINICAL FEATURES OF MENINGITIS?

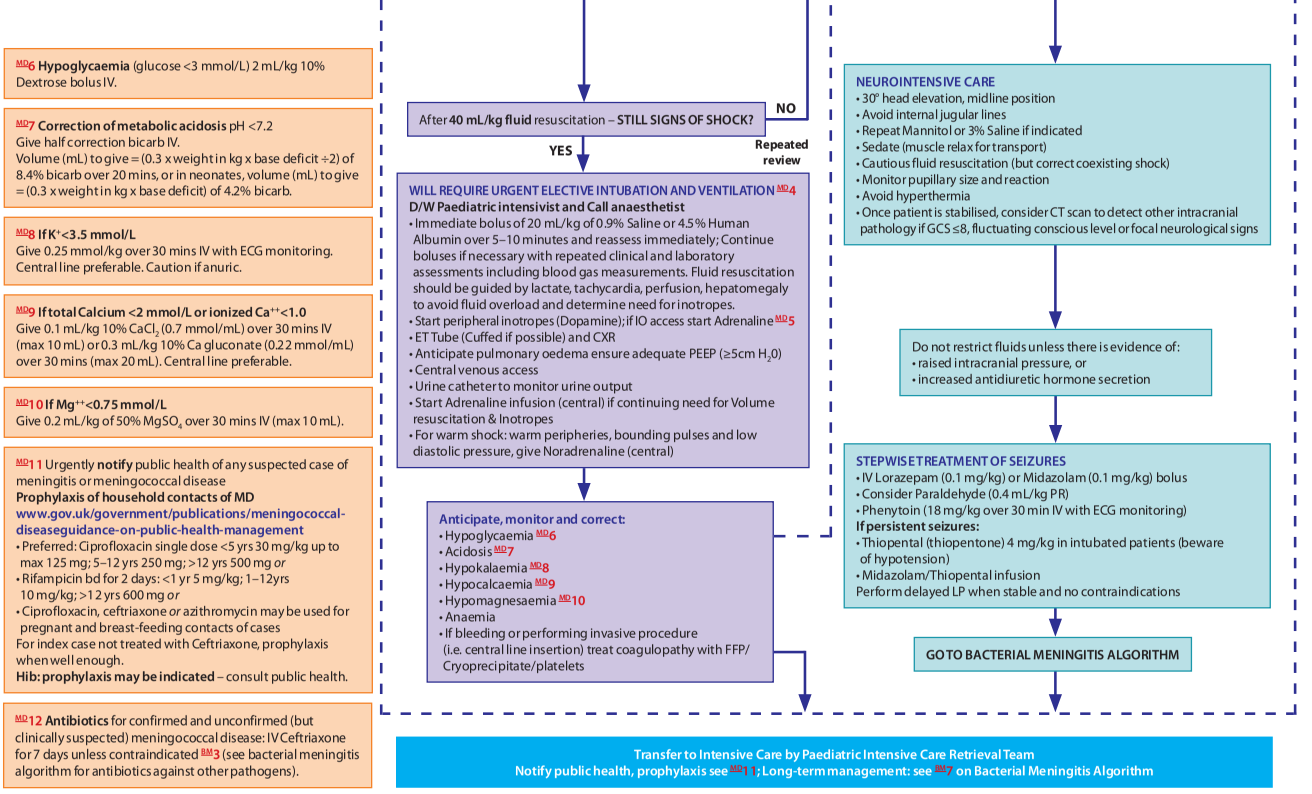
YES

NO

Take bloods, see MD3
Close monitoring for signs of:
- Raised ICP
- Shock

Perform LP if no contraindication
DO NOT DELAY ANTIBIOTICS

Repeated Review
Children with MD can deteriorate rapidly.
Deterioration detected?



Based on Early Management algorithm, Dept Paediatrics, Imperial College at St Mary's Hospital as described in Arch Dis Child 1999;80:290 & 2007;92:283 & on NICE CG102 www.nice.org.uk/guidance/cg102
Authors AJ Pollard (GDG chair), A Cloke, SN Faust, L Glennie, C Haines, PT Heath, JS Kroll, M Levin, I Maconochie, S McQueen, P Monk, S Nadel, N Ninis, MP Richardson, MJ Thompson, AP Thomson, D Turner.
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BIAMM British Infection Association

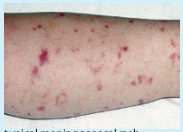
Edition 2A

Meningitis Research Foundation

Early Management of Suspected Bacterial Meningitis and Meningococcal Septicaemia in Immunocompetent Adults*

Early Recognition

- **Petechial/purpuric non-blanching rash or signs of meningitis**
- A rash may be absent or atypical at presentation
- Neck stiffness may be absent in up to 30% of cases of meningitis
- Prior antibiotics may mask the severity of the illness



Courtesy: Dr A Riordan

typical meningococcal rash

Assess Severity & Immediate Intervention[a]

- **A**irway
- **B**reathing - Respiratory Rate & O_2 Saturation
- **C**irculation - Pulse; Capillary Refill Time (hypotension late); Urine output
- Mental status (deterioration may be a sign of shock or meningitis)
- Neurology – Focal neurological signs; Persistent seizures; Papilloedema

Secure Airway
High Flow O_2
Large bore IV Cannula ± fluid resuscitation

Priority Investigations:

- FBC; U+Es; Blood sugar, LFTs; CRP
- Clotting profile
- Blood gases

Microbiology:

- Blood culture
- Throat swab
- Clotted blood
- EDTA blood for PCR

Predominantly Meningococcal Septicaemia

- Do not attempt LP
- IV 2 g Cefotaxime or Ceftriaxone
- Call critical care team for review

Predominantly Meningitis[b,c,d]

- Assess patient carefully before performing LP
- Call critical care team if any features of raised intracranial pressure, shock or respiratory failure
- If uncertain ask for senior review
- Monitor and stabilise circulation

Signs of shock[a]

YES — NO

No Raised ICP
No shock
No Respiratory Failure[a,b]

Signs of Raised ICP[a,b]

Additional Information

[a]Warning Signs (see refs)

The following warn of impending/worsening shock, respiratory failure or raised intracranial pressure and require urgent senior review and intervention (see algorithm):

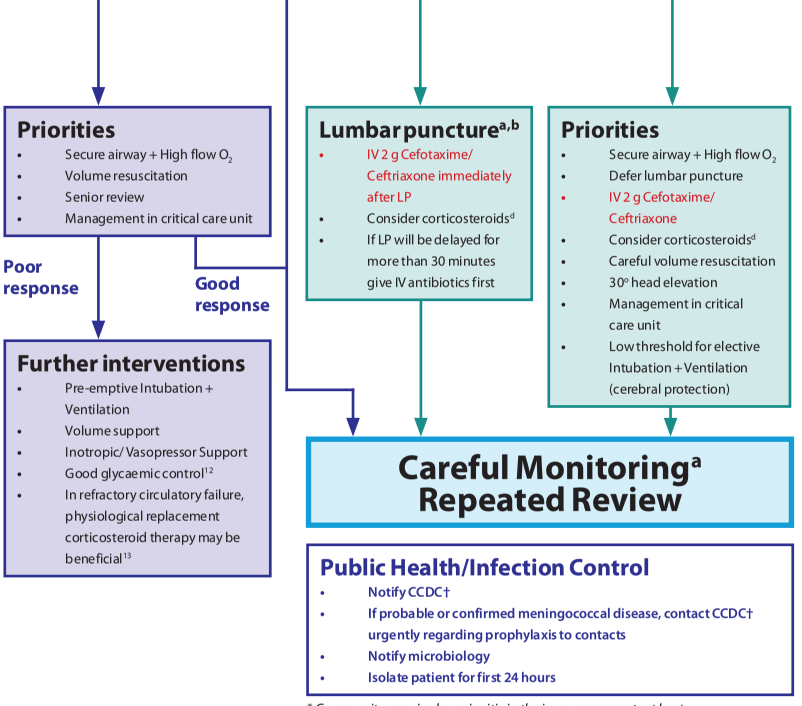
- Rapidly progressive rash
- Poor peripheral perfusion, CRT >4 secs, oliguria and systolic BP <90 (hypotension often a late sign)
- RR <8 or >30
- Pulse rate <40 or >140
- Acidosis pH <7.3 or BE worse than -5
- WBC <4

- Marked depressed conscious level (GCS <12) or a fluctuating conscious level (fall in GCS >2)
- Focal neurology
- Persistent seizures
- Bradycardia and hypertension
- Papilloedema

[b]CT scan and meningitis (see refs)

This investigation should only be used when appropriate:

- A normal CT scan does not exclude raised intracranial pressure
- If there are no clinical contraindications to LP, a CT scan is not necessary beforehand
- Subsequently a CT scan may be useful in identifying dural defects predisposing to meningitis



** Community acquired meningitis in the immunocompetent host.*
In the immunocompromised seek additional expert advice
†CPHM in Scotland

[c]Appropriate antibiotics for bacterial meningitis (see refs)

Review with microbiology:

- **Ampicillin IV 2 g qds should be added for individuals >55 years to cover Listeria**
- **Vancomycin ± rifampicin if pneumococcal penicillin resistance suspected**
- **Amend antibiotics on the basis of microbiology results**

[d]Corticosteroids in adult meningitis (see refs)

- **Dexamethasone 0.15 mg/kg qds for 4 days started with or just before the first dose of antibiotics, particularly where pneumococcal meningitis is suspected**
- **Do not give unless you are confident you are using the correct antimicrobials**
- **Stop the dexamethasone if a non-bacterial cause is identified**

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