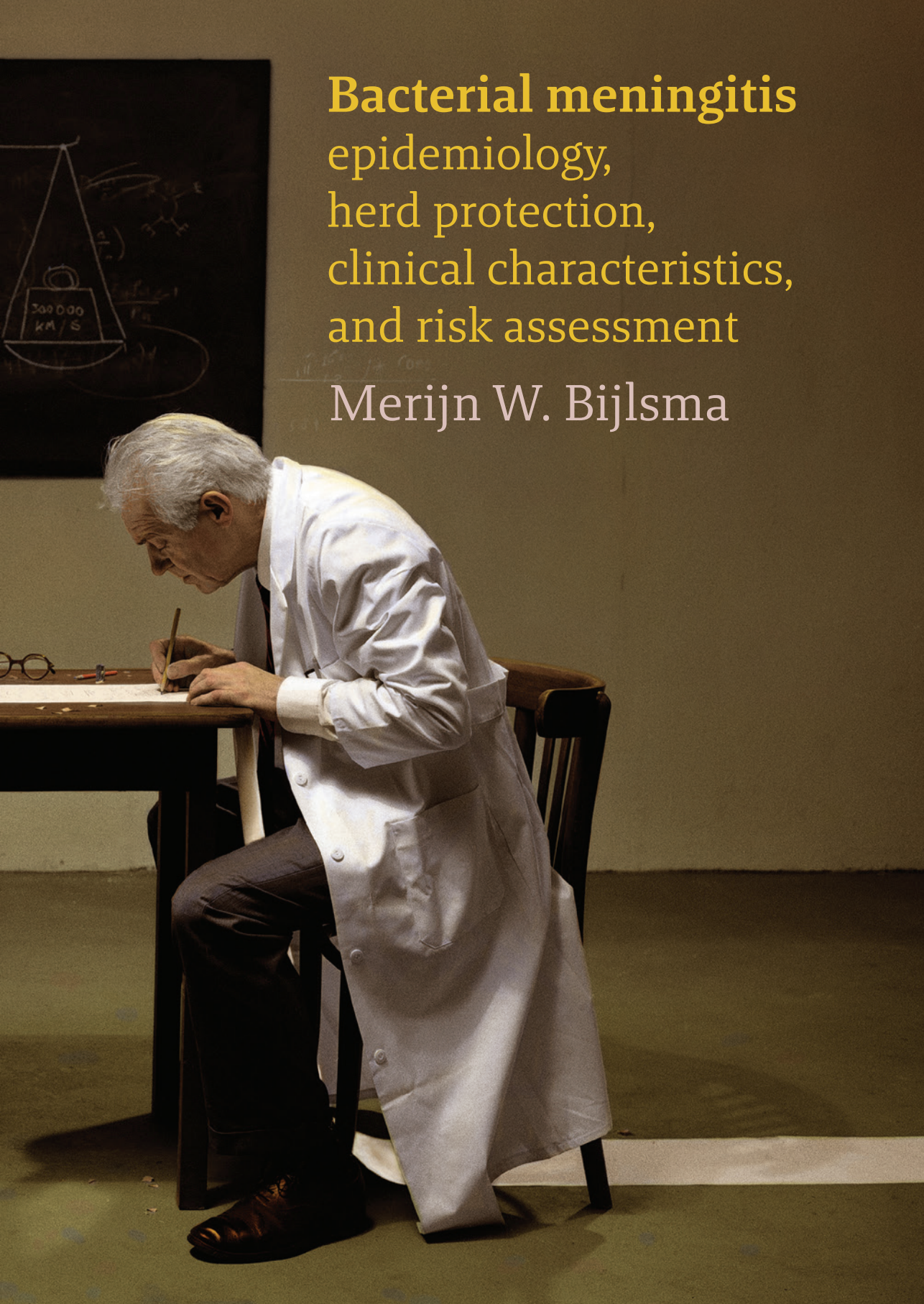
Bacterial meningitis
epidemiology,
herd protection,
clinical characteristics,
and risk assessment
Merijn W. Bijlsma

Bacterial meningitis: epidemiology, herd protection, clinical characteristics, and risk assessment

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Bacterial meningitis: epidemiology, herd protection, clinical characteristics, and risk assessment

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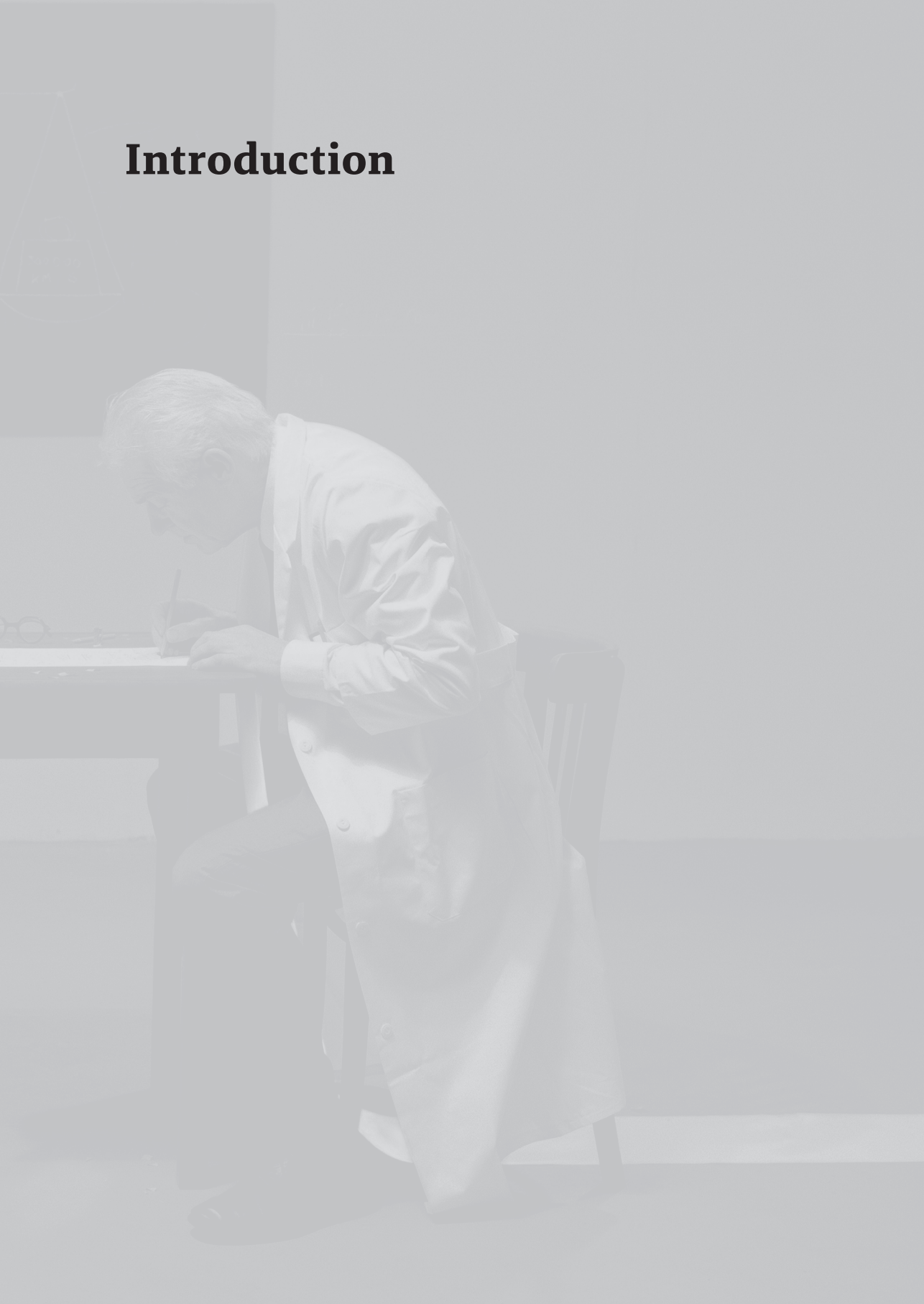
CHAPTER 1

Introduction



Meningitis is an infection of the meninges and the subarachnoid space that can also involve the brain parenchyma (meningo-encephalitis).[1] Bacterial meningitis is a devastating disease that is associated with substantial mortality and morbidity. An estimated 303,500 deaths, and 21 million disability adjusted life years were attributed to bacterial meningitis in 2013 worldwide.[2,3]

The clinical characteristics of bacterial meningitis have been described by Hippocrates in the 5th century B.C. Gaspard Vieusseux was the first to give a detailed description of the clinical syndrome during an epidemic of meningococcal meningitis in Geneva in 1805. Adult patients with community-acquired bacterial meningitis usually complain of headache, nausea, vomiting and photophobia. Almost all patients will present with at least two of the signs and symptoms headache, fever, neck stiffness, and altered mental status.[4]

The prognosis of meningitis before the twentieth century was dismal; more than three quarters of patients with meningococcal meningitis and nearly all with pneumococcal or *Haemophilus influenzae* meningitis died.[5] The introduction of intravenous and intrathecal administration of specific antisera in the 1920s greatly improved outcome. After the introduction of sulphonamides in the 1930s, over three quarters of patients survived *H. influenzae* and meningococcal meningitis. The use of penicillin therapy for pneumococcal meningitis began in the mid-1940s, reducing the case fatality rate from nearly 100% to less than 50%. At the turn of the century the case fatality rate in the Netherlands had declined to 7% for meningococcal meningitis and 30% for pneumococcal meningitis patients.[4]

The most common causative pathogens of bacterial meningitis worldwide are *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *H. influenzae* type b, respectively causing 26%, 22% and 21% of global cases in 2013.[3] These bacteria are transmitted from person to person through droplets of respiratory or throat secretions.[6] Humans are the main reservoir for pneumococci, and *N. meningitidis*, and *H. influenzae* are exclusively human pathogens.[6,7] Transmission usually results in a period of asymptomatic colonization of the nasopharynx, before they are cleared by the host, or supplanted by other pathogens.[6] Asymptomatic carriage is much more common than invasive disease. The mechanism whereby colonization in the host progresses to disease is not fully understood, but is thought to depend on the interaction of environmental, host susceptibility and bacterial virulence factors.

The main virulence factor of the most common causative bacteria of meningitis is the polysaccharide capsule. Whereas many carried isolates are unencapsulated, isolates that cause disease almost invariably express the polysaccharide capsule.[6,7] Differences between capsules are used to clasiffy meningococci into 13 serogroups, *H. influenzae* into six serotypes and pneumococci into more than ninety serotypes. Most meningococcal disease is causes by six serogroups (A, B, C, W, X, Y), and serotype b causes the majority of *H. influenzae* disease.[6,7] Meningococci are further classified based on serological and genetic differences of outer membrane proteins (e.g. porA, porB).

Another typing scheme, Multi Locus Sequence Typing (MLST), identifies differences in seven genes required for the maintenance of basic cellular function.[6,8] Based on allelic variants at these seven loci, isolates are classified into sequence types that can subsequently be grouped into clonal complexes. Isolates in these clonal complexes can share important clinical characteristics, such as the propensity to cause invasive disease.[6]

Large scale immunization programs against *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b are among the most effective public health interventions of the last 50 years.[6] The observation that anti-capsular bactericidal antibodies protect against disease led to the use of purified capsular polysaccharides in vaccine formulations. Because polysaccharides are T-cell-independent antigens that cannot be presented to T cells in conjunction with MHC class II molecules, plain polysaccharide vaccines do not stimulate the development of memory B cells. Consequently, the vaccine works poorly or not at all in young children, and no memory response is generated in adults. Conjugation of the bacterial polysaccharide to a carrier protein induces a T-cell-dependent immune response. Conjugate vaccines are, in general, immunogenic from early infancy and induce a longer lasting immune response.

Conjugate vaccines against *H. influenzae* type b were introduced in the late 1980s.[9] A conjugate vaccine against serogroup C meningococci was introduced in 1999,[10] followed by vaccines against serogroups A, W and Y.[11] Because the serogroup B polysaccharide is identical to a polysialic acid of human glycoproteins and poorly immunogenic, there is no vaccine against the serogroup B polysaccharide capsule.[11] Pneumococcal conjugate vaccines against a limited number of the most common serotypes became available in the year 2000.[12]

Although initially developed for individual protection against disease, conjugate vaccines proved effective against nasopharyngeal carriage as well.[6,11] Reduced carriage leads to reduced transmission, thereby protecting the unvaccinated population. The impact of herd protection elicited by conjugate vaccines was largely unexpected. Randomized controlled trials were too small to elicit herd protection, or had not been performed at all.[7,13]

The long-term effectiveness of conjugate vaccines is uncertain. Because vaccines are available against a limited subset of meningococcal serogroups and pneumococcal serotypes only, non -vaccine types could replace vaccine types.[14] Long-term surveillance data offers invaluable information for the post licensure evaluation of vaccine impact, and the planning of future vaccine development and implementation. It can be used to evaluate herd protection and serogroup or serotype replacement.

In the Netherlands, Charlotte Ruys, professor of Bacteriology, Epidemiology and Immunity at the Laboratory of Hygiene of the University of Amsterdam, started to systematically collect *N. meningitis* isolates from patients with meningitis in 1959. This was the basis for the establishment of the Netherlands Reference Laboratory for Bacterial Meningitis in 1975 by the Department of Medical Microbiology of the University of Amsterdam and the National Institute for Public Health

and the Environment (RIVM). Nationwide, an estimated 85% of isolates cultured from blood or cerebrospinal fluid from patients with (suspected) meningitis are sent to this reference laboratory.[15,16] The Netherlands Reference Laboratory has one of the largest and oldest collections of meningococcal, pneumococcal, and Haemophilus isolates from patients with (suspected) meningitis in the world.

In **chapter two** we describe the epidemiology, clinical characteristics and outcome of adult community-acquired bacterial meningitis after the introduction of adjunctive dexamethasone therapy and nationwide implementation of paediatric conjugate vaccines. 1,412 episodes of community-acquired bacterial meningitis identified between January 2006 and July 2013 through the National Reference Laboratory for Bacterial Meningitis or individual physicians, were prospectively evaluated.

In **chapter three** we present national surveillance data from the Netherlands Reference Laboratory of Bacterial Meningitis (NRLBM) for invasive meningococcal disease from Jan 1, 1960, to Jan 1, 2013. The variability and distribution of serogroups, serosubtypes and *porA* sequencing data over time are described. This is valuable information for future vaccine implementation strategies and serogroup B vaccine development and post licensure evaluation of vaccine impact. There was a 99% decline in serogroup C meningococcal disease after the introduction of serogroup C conjugate vaccine. In **chapter four** we show how 36% of the reduction in serogroup C cases occurred in unvaccinated age groups, and was most profound for meningococcal sequence types that have a high propensity to express the serogroup C polysaccharide capsule during colonization. This illustrates the importance of herd protection for serogroup C conjugate vaccine efficacy.

In 2013 the European Centre for Disease Prevention and Control called for enhanced surveillance and retrospective investigation of serogroup C cases in young men, in response to the clusters of invasive meningococcal disease among men who have sex with men (MSM) that were reported in several cities in North America and Europe. In **chapter five** we show that there was no evidence of serogroup C clusters in young men in the Netherlands. The National Institute of Public Health and the Environment (RIVM) convened a meeting to discuss the reported clusters of IMD among MSM. Because no case of serogroup C IMD had been reported in the Dutch MSM community and because of the high vaccine coverage of young males in the Netherlands, the RIVM made the recommendation to the Ministry of Health, Welfare and Sport (VWS) to take no specific action at this time.

Group B streptococcus is the most common cause of neonatal infections. In **chapter six** we studied the clinical and molecular epidemiology of invasive group B streptococcus infection in children younger than 3 months in the Netherlands over 25 years. We found that the introduction of prevention guidelines for invasive group B streptococcus disease in 1999, consisting of intravenous antibiotic prophylaxis during labour in case of premature labour, prolonged rupture

of membranes, or fever during delivery, did not reduce the incidence of disease in neonates. These guidelines should be reassessed and alternative approaches to prevent infant invasive group B streptococcus disease should be sought.

Clinical deterioration can occur rapidly in bacterial meningitis and is often difficult to predict. Identifying patients at high risk of an adverse clinical outcome is important for counselling patients and their families, as well as deciding upon optimal patient management. We developed a risk score in **chapter seven** that identifies adults with cerebrospinal fluid (CSF) pleocytosis and a negative CSF Gram stain at low risk of an urgent treatable cause. In **chapter eight** we performed an external validation study of risk scores that predict adverse clinical outcome in bacterial meningitis. Risk scores were identified through a systematic review of the literature.

This thesis concludes with a general discussion (**Chapter 9**) in which the implications of the presented studies are discussed and suggestions for future research are proposed.

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CHAPTER 2

Community-acquired bacterial meningitis in adults in the Netherlands, 2006-14: a prospective cohort study

Merijn W Bijlsma, Matthijs C Brouwer[1*], E Soemirien Kasanmoentalib[*], Anne T Kloek[*], Marjolein J Lucas[*], Michael W Tanck[*], Arie van der Ende[*], Diederik van de Beek

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1 [*] in alphabetical order

Abstract

We studied causative pathogens, clinical characteristics and outcome of adult community-acquired bacterial meningitis after the introduction of adjunctive dexamethasone treatment and nationwide implementation of paediatric conjugate vaccines. In this cohort study, we prospectively assessed adults (age >16 years) with community-acquired bacterial meningitis in the Netherlands, identified through the National Reference Laboratory for Bacterial Meningitis or individual physicians between Jan 1, 2006, and July 1, 2014. We identified independent predictors of an unfavourable outcome (Glasgow Outcome Scale score 1–4) by logistic-regression. We assessed 1412 episodes of community-acquired bacterial meningitis. Incidence declined from 1.72 cases per 100,000 adults per year in 2007–08 to 0.94 per 100,000 per year in 2013–14. *Streptococcus pneumoniae* caused 1017 (72%) of 1412 episodes. Rates of adult bacterial meningitis decreased most sharply among pneumococcal serotypes included in paediatric conjugate vaccines, and in meningococcal meningitis. We found no evidence of serotype or serogroup replacement. The overall case fatality rate was 244 (17%) of 1412 episodes and unfavourable outcome occurred in 531 (38%) of 1412 episodes. Predictors of unfavourable outcome were advanced age, absence of otitis or sinusitis, alcoholism, tachycardia, lower score on the Glasgow Coma Scale, cranial nerve palsy, a cerebrospinal fluid white-cell count lower than 1000 cells per µL, a positive blood culture, and a high serum C-reactive protein concentration. Adjunctive dexamethasone was administered in 1234 (89%) of 1384 episodes. The multivariable adjusted odds ratio of dexamethasone treatment for unfavourable outcome was 0.54 (95% CI, 0.39–0.73). The incidence of adult bacterial meningitis has decreased substantially which is partly explained by herd protection by paediatric conjugate vaccines. Adjunctive dexamethasone treatment was associated with substantially improved outcome.

Introduction

Bacterial meningitis is associated with substantial mortality and morbidity.[1] The epidemiology and treatment of bacterial meningitis has changed over the last 15 years.[2,3] The routine use of protein-polysaccharide conjugate vaccines in childhood against common causative pathogens of bacterial meningitis has reduced the overall incidence, affecting the distributions of causative pathogens and the age groups most often affected.[2,4] The introduction of new treatments, such as dexamethasone as adjunctive treatment, might have affected national outcomes of bacterial meningitis.[5,6] In 2006, we started a prospective cohort study to identify and characterise host genetic traits and bacterial virulence factors controlling occurrence and outcome of bacterial meningitis (MeninGene).[7-19] Here, we report data from this study, including the incidence, causative pathogens, clinical features, and prognostic factors in adults with community-acquired bacterial meningitis in the Netherlands from 2006 to 2014.

Methods

Study population

We identified adults (patients older than age 16 years) who had bacterial meningitis in the Netherlands between Jan 1, 2006, and July 1, 2014, and who were listed in the database of the Netherlands Reference Laboratory for Bacterial Meningitis. This laboratory receives bacterial isolates, cultured from cerebrospinal fluid or blood, from roughly 85% of all patients with bacterial meningitis in the Netherlands (population, 16.9 million).[20,21] The laboratory provided daily updates of the names of hospitals where patients with bacterial meningitis had been admitted in the preceding 2–6 days, and the names of the physicians, usually neurologists. Physicians were informed about the study by telephone. Physicians could also contact investigators at any time to include patients, without preceding report by the reference laboratory. Subsequently, patients or their legal representatives received written information concerning the study and were asked to give written informed consent for participation. Online case-record forms were used to collect data on patients' history, symptoms and signs on admission, laboratory findings at admission, clinical course, outcome and neurologic findings at discharge, and treatment. Before the study, all Dutch neurologists received information about the study, which was followed by periodic reminders.

We defined bacterial meningitis as a bacterial pathogen cultured in cerebrospinal fluid, or the combination of a positive PCR or antigen test in cerebrospinal fluid for *Streptococcus pneumoniae* or *Neisseria meningitidis* with at least one specific cerebrospinal fluid finding predictive of bacterial meningitis (according to the criteria of Spanos and colleagues:[22] glucose concentration <340 mg/L [1.9 mmol/L], cerebrospinal fluid glucose:blood glucose ratio <0.23, protein concentration >2200 mg/L, white-cell count >2000 cells per µL, or >1180 polymorphonuclear leucocytes per

µL). We excluded episodes of hospital acquired meningitis, defined as bacterial meningitis that occurred while in hospital or within one week after discharge.[23] We also excluded. patients with head trauma or neurosurgery in the previous month, or those with a neurosurgical device or missing outcome.

Procedures

Neurological examination were done at admission and at discharge. Outcome was scored by the Glasgow Outcome Scale score.[24] 1=death, 2=vegetative state (unable to interact with the environment), 3=severe disability (unable to live independently but can follow commands), 4=moderate disability (capable of living independently but unable to return to work or school), and 5=mild or no disability (able to return to work or school). A favourable outcome was defined as a score of 5, and an unfavourable outcome as a score of 1–4.

Statistical analysis

We calculated the incidence of community acquired meningitis as the number of new episodes per epidemiological year (July 1– June 30) and per 100,000 adult patients (>16 years old on Jan 1). We tested bacterial susceptibility as previously described.[25] *N. meningitidis* was considered susceptible to penicillin if the minimum inhibitory concentration (MIC) was 0.06 µg/ml or less. Reduced susceptibility was defined as a MIC of 0.06–1.0 µg/ml before 2010, and 0.06–0.25 µg/ml after 2010. Penicillin resistance was defined as a MIC of more than 1.0 µg/ml before 2010, and more than 0.25 µg/ml after 2010. *S. pneumoniae* was considered to be sensitive to penicillin if the MIC 0.06 µg/ml or less and resistant if the MIC was more than 0.06 µg/ml. We did meningococcal serogrouping and multilocus sequence typing, and pneumococcal serotyping, as previously described.[21,26,27]

Categorical variables are expressed as counts (percentage) and we compared frequency distributions with the Fisher exact test. Continuous variables are expressed as median (IQR). We tested differences with the independent *t* test for normally distributed variables or the Mann-Whitney U test otherwise. We tested trends in the incidence of pneumococcal serotypes with linear regression using incidence per 100,000 adults as the dependent and epidemiological year as the independent variable. We chose possible predictors for an unfavourable outcome on the basis of previous research, pathophysiologic interest, and availability early in the course of the illness. We investigated the association between these predictors and outcome with logistic regression, providing odds ratios (ORs) and 95% CIs. We assessed the linearity of the association between continuous predictors and outcome with the Hosmer-Lemeshow goodness of fit test and by visual inspection. If there was no linear relationship, the continuous predictor was categorized for further analyses. We estimated both univariable crude ORs and multivariable ORs corrected for all other variables in the model. We used multiple imputation for missing data in the multivar-

iable analysis. We used all predictors together to impute missing values using the mice package (version 2.22). We combined the coefficients of 60 rounds of imputation to obtain the final estimates for the multivariable model. The statistical tests were two-tailed, and we deemed p values of less than 0.05 as statistically significant. We did the imputation and all statistical analyses in R (version 3.0.1).

Results

1887 episodes of bacterial meningitis were identified, 1736 (92%) by the reference laboratory and 151 (8%) by physicians (figure 1). 240 (13%) of 1887 episodes were excluded from the cohort, and 235 (12%) met exclusion criteria, resulting in 1412 (75%) episodes in 1391 patients (figure 1).

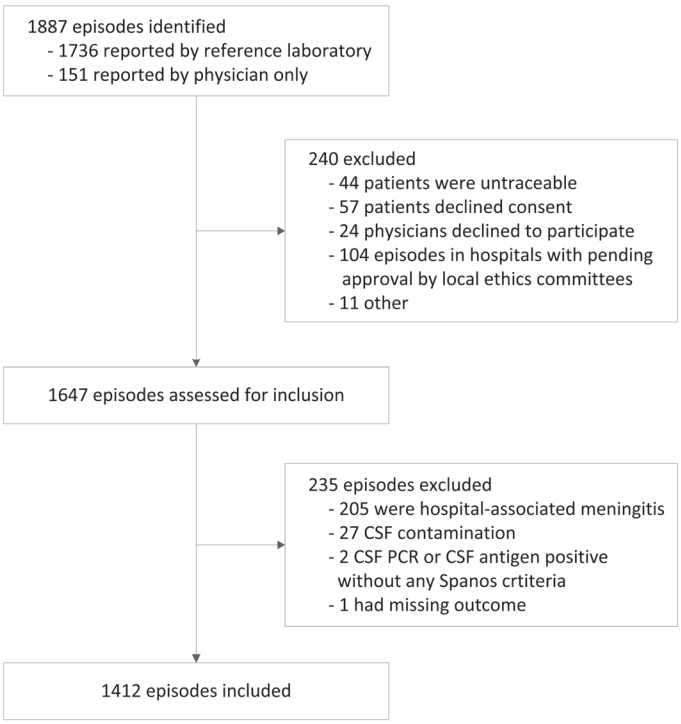

Figure 1. Selection of patients

Median age was 61 years (table 1). A history of splenectomy or cerebrospinal fluid leak was present in 71 (5%) of 1374 episodes. For 457 (33%) of 1380 episodes the patient had used immunosuppressive drugs or had a history of cancer, diabetes, HIV or alcoholism. Extra-meningeal foci of infection (otitis, sinusitis, pneumonia or endocarditis) were present in 598 (42%) of 1412 episodes, and were more likely to occur in pneumococcal meningitis (518 [51%] of 1008), than in meningococcal meningitis (nine [6%] of 150) or listeria meningitis (eight [11%] of 74). Headache, neck stiffness, fever, and a change in mental status were common symptoms at presentation (table 1). The classic triad of fever, altered mental status and neck stiffness was present in 563 (41%) of 1389 episodes. Rash was noted in 116 (8%) of 1412 episodes, of which 70 (60%) episodes were meningococcal meningitis and 34 (29%) were pneumococcal meningitis. The rash was petechial or consisted of purpura or ecchymosis in 69 (99%) of the 70 meningococcal cases and in 25 (74%) of 34 pneumococcal cases with a rash.

At least one specific cerebrospinal fluid finding predictive of bacterial meningitis was present in 1229 (96%) of 1277 episodes.[22] Cranial imaging on admission was performed in 1206 (86%) of 1402 episodes and abnormalities were recorded in 561 (47%) of 1206 episodes, most commonly sinusitis or mastoiditis (386 [34%] of 1125 episodes in which otitis or sinusitis was evaluated), brain oedema (118 [10%] of 1165 episodes), and hydrocephalus (58 [5%] of 1177 episodes). Cranial imaging preceded lumbar puncture in 936 (86%) of 1092 episodes with neuroimaging. Treatment was started before imaging in 304 (36%) of 855 episodes for which we had data.

The most common pathogen was *S. pneumoniae,* accounting for 1017 (72%) of 1412 isolates identified (figure 2, table 2). Pneumococcal serotype was available for 930 (91%) of 1017 episodes. Serotypes included those covered by the the seven-valent, ten-valent, and 13-valent conjugate vaccines (figure 2, table 2). 763 (82%) of 930 episodes were due to a pneumococcal serotype included in the 23-valent polysaccharide vaccine. In patients aged older than 65 years, 79 (22%) of 359 episodes were of serotypes covered by the seven-valent, 129 (36%) were of serotypes covered by the ten-valent vaccine, 181 (50%) were of serotypes covered by the 13-valent vaccine, and 283 (79%) were of serotypes covered by the 23-valent vaccine.

The incidence of community-acquired bacterial meningitis was highest in 2007–08, with 1.72 cases per 100,000 adults per year, and subsequently declined to 0.94 per 100,000 adults per year in 2013–14 (figure 2). There was a reduction in both the absolute number of pneumococcal cases per year and the proportion of cases caused by the seven-valent vaccine serotypes (figure 2). The incidence of pneumococcal serotypes included in the seven-valent vaccine decreased from 0.42 per 100 000 adults per year in 2006, to 0.02 in 2013. There was no evidence of serotype replacement. The mean incidence of pneumococcal serotypes not included in the seven-valent vaccine was 0.68 per 100,000 adults per year excluding missing serotypes, and 0.76 per 100,000 adults per year including missing serotypes. The mean incidence of pneumococcal serotypes not included in the ten-valent vaccine was 0.55 per 100,000 adults per year excluding missing

serotypes, and 0.63 including missing serotypes. There was no increasing trend in the incidence of non-seven-valent serotypes (β –0.01, p=0.45) or non-ten-valent serotypes (β 0.004, p=0.69) during the observation period.

Table 1. Characteristics of the study population

Characteristic – no. /no.evaluated (%)	Episodes of Meningitis (N=1412)	Characteristic	Episodes of Meningitis (N=1412)
Age (years)	61 (47–69)	Triad fever, neck stiffness, altered mental status	563 /1389 (41%)
Men	707/1412 (50%)	Cranial nerve palsy	109/ 1245 (9%)
History of meningitis	93/1396 (7%)	Aphasia, hemiparesis, or monoparesis	268 /1221 (22%)
Symptoms <24 h	636/1353 (47%)	*Indices of CSF inflammation*	
Seizures	98 /1353 (7%)	Opening pressure > 400 mm water	253/480 (53%)
Pretreatment with antibiotics	152 /1377 (11%)	White cell count (cells per µL)	2310 (547–6840)
Otitis or sinusitis	480 /1404 (34%)	<100	149 /1352 (11%)
Pneumonia	122 /1347 (9%)	100–999	316 /1352 (23%)
Endocarditis	17 /1346 (1%)	>999	887 /1352 (66%)
Cerebrospinal fluid leak	39 /1374 (3%)	Protein (g/L)‡	3.9 (2.3–6.0)
Immunosuppressive drugs	107/1391 (8%)	CSF: blood glucose ratio§	0.04 (0.0–0.3)
History of splenectomy	32 (2%)	Positive Gram stain	1057 /1245 (85%)
History of cancer	173/1407 (12%)	Positive blood culture	927/1243 (75%)
Diabetes	171/1394 (12%)	*Blood chemical tests*	
HIV positive	12 (1%)	C-reactive protein (mg/L)	194 (87–311)
Alcoholism	82 (6%)	< 40	162 /1353 (12%)
Symptoms and signs on presentation		> 80	1040 /1353 (77%)
Headache	1015/1223 (83%)	Thrombocyte count (per µL)	199 (151–253)
Nausea	713/1159 (62%)	< 150	322 /1345 (24%)
Neck stiffness	977 /1322 (74%)	*Clinical course*	
Rash	116 (8%)	Seizures	185 /1353 (14%)
Heart rate (beats per min)*	100 (84–112)	Pneumonia	221/1311 (17%)
Systolic blood pressure (mmHg)†	142 (125–163)	Cardiorespiratory failure	530 (38%)
Diastolic blood pressure(mmHg)†	80 (69–90)		
Body temperature (°C)	38.9 (37.9–39.6)	*Score on Glasgow Outcome Scale*	
≥38°C	1033/1391 (74%)	1 (death)	244 /1412 (17%)
Score on Glasgow Coma Scale	11 (9–14)	2 (vegetative state)	2 /1412 (<1%)
< 14 (altered mental status)	996 /1403 (71%)	3 (severe disability)	64 /1412 (5%)
< 8 (coma)	185 /1403 (13%)	4 (moderate disability	221 /1412 (16%)
		5 (mild or no disability)	881 /1412 (62%)

Data are median (IQR) or n/N (%). *Evaluated in 1363 episodes. †Evaluated in 1381 episodes. ‡Evaluated in 1344 episodes. §Evaluated in 1309 episodes. CSF=cerebrospinal fluid.

N. meningitidis was responsible for 150 (11%) of 1412 episodes (table 2). Meningococcal clonal complex by multilocus sequence typing was available for 119 (79%) of 150 episodes and the most common clonal complexes were ST-41/44 (42 [35%] of 119 episodes), ST-32 (28 [24%] of 119 episodes), and ST-269 (12 [10%] of 119 episodes). Patients with meningococcal meningitis were generally younger (median age 32 years, IQR 19–54) than were patients with other causative bacteria (median 61 years, IQR 46–71; p<0.0001).

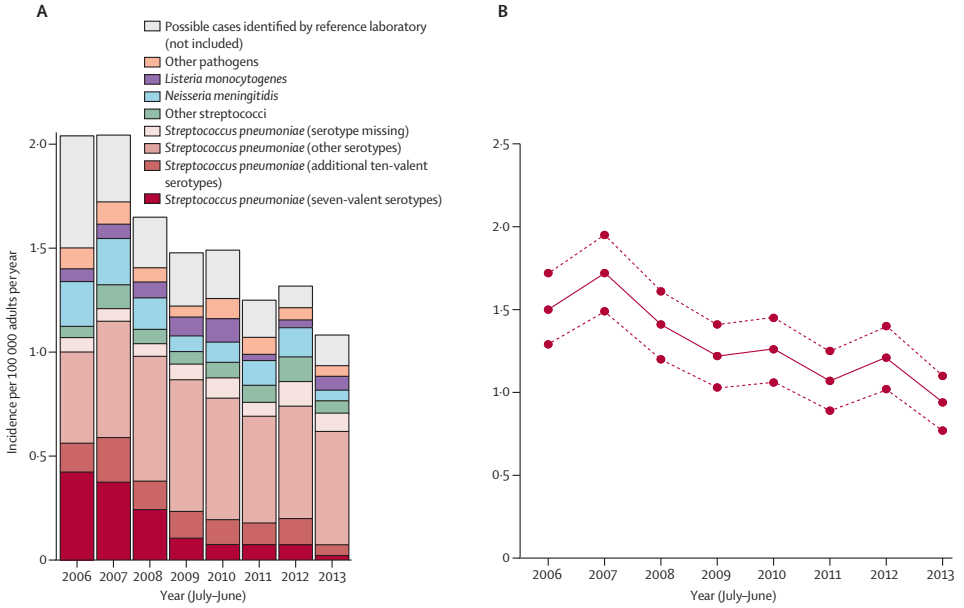

Figure 2: Incidence of community-acquired bacterial meningitis in the Netherlands for 2006–14

(A) Incidence rate per 100,000 adults per year of all episodes reported to the Netherlands Reference Laboratory for Bacterial Meningitis. (B) Incidence rate with 95% CIs of all included episodes of community-acquired meningitis per 100 000 adults per year. Not all patients could be included in the first months of the study because of pending ethical approval in several hospitals. Routine vaccination against *S. pneumoniae* at 2 months, 3 months, 4 months, and 11 months of age with the seven-valent conjugate vaccine was started in 2006, and replaced by a ten-valent conjugate vaccine in 2011. Children aged 1–19 years were offered a single meningococcal serogroup C vaccination in 2002, and routine vaccination at 14 months was subsequently introduced.

Gram staining of cerebrospinal fluid yielded a positive result in 848 (92%) of 919 episodes of pneumococcal meningitis, 109 (83%) of 132 episodes of meningococcal meningitis and in 24 (41%) of 59 episodes of listeria meningitis in which Gram staining was performed. Gram stain results led to a change in antimicrobial treatment for 560 (43%) of 1316 episodes. Blood cultures were positive for 927 (75%) of 1243 episodes in which cultures were done. Penicillin susceptibility was tested in 928 (91%) of 1017 episodes of pneumococcal meningitis; 15 (2%) of 928 pneumococcal isolates showed penicillin-resistance, and three (20%) of these 15 isolates showed reduced

susceptibility to ceftriaxone. Antibiotic susceptibility was tested in 134 (89%) of 150 episodes of meningococcal meningitis; 16 (12%) isolates showed intermediate penicillin resistance, all were susceptible to ceftriaxone and rifampicin.

Table 2. Typing and subtyping of causative pathogens.

Microorganism	**n/N (%)**
*Streptococcus pneumoniae**	1017 (72)
7F	110/930 (12)
3	106/930 (11)
8	78/930 (8)
22F	68/930 (7)
23F	44/930 (5)
19A	40/930 (4)
19F	34/930 (4)
39 other serotypes †	450/930 (48)
Neisseria meningitidis	150 (11)
Serogroup B	113/137 (83)
Serogroup Y	10/137 (7)
Serogroup C	10/137 (7)
Other serogroup	4/137 (3)
Listeria monocytogenes	74 (5)
Haemophilus influenzae	47 (3)
Streptococcus pyogenes	24 (2)
Streptococcus agalactiae	21 (2)
other streptococcal species ‡	35 (2)
Staphylococcus aureus	21 (1)
other ¶	23 (2)

*Of the 930 episodes with an identified pneumococcal serotype, 193 (21%) episodes were due to serotypes included in the seven-valent pneumococcal conjugate vaccine and 329 (35%) episodes were due to episodes included in the ten-valent vaccine. †serotype (number): 10A(30), 23B(30), 4(28), 12F(27), 6B(25), 14(23), 18C(23), 1(23), 9N(21), 11A(20), 23A(20), 6A(18), 24F(17), 33F(17), 9V(16), 15B(15), 6C (12), 35F(10), 16F(10), 15A(8), 17F(8), 15C(6), 31(6), 38(6), 18B(4), 20(4), 37(4), 5(3), 34(3), 35B(3), 22A(2), 7A(1), 7B(1), 10B(1), 13(1), 24B(1), 25A(1), 27(1), 28F(1).
‡*Streptococcus suis* (n=7), *Streptococcus salivarius* (n=5), *Streptococcus mitis* (n=4), *Streptococcus anginosus* (n=3), *Streptococcus dysgalactiae ssp equisimilis* (n=3), *Streptococcus intermedius* (n=3), *Streptococcus equi ssp zooepidemicus* (n=2), *Streptococcus parasanguinis* (n=2), *Streptococcus constellatus ssp constellatus* (n=1), *Streptococcus gallolyticus ssp gallolyticus* (n=1), *Streptococcus gallolyticus ssp pasteurianus* (n=1), *Streptococcus gordonii* (n=1), *Streptococcus oralis* (n=1), *Streptococcus sanguinis* (n=1), *Escherichia coli* (n=10), *Capnocytophaga canimorsus* (n=3), *Klebsiella pneumoniae* (n=3), *Haemophilus parainfluenzae* (n=2), *Aggregatibacter aphrophilus* (n=1), *Campylobacter fetus* (n=1), *Nocardia farcinica* (n=1), *Pseudomonas aeruginosa* (n=1), *Salmonella enterica* (n=1).

Initial antibiotic treatment included a combination of amoxicillin with a third-generation cephalosporin in 459 (36%) of 1273 episodes. Monotherapy was started with a third-generation cephalosporin in 365 (29%), and with either penicillin or amoxicillin in 259 (20%) of 1273 episodes. Other

regimens were used in 190 (15%) of 1273 episodes. Initial antimicrobial treatment was appropriate for the pathogen cultured in 1247 (98%) of 1273 episodes; eight (11%) of 74 patients with listeria meningitis were initially treated with cephalosporins alone. Adjunctive dexamethasone was administered in 1234 (89%) of 1384 assessed episodes. Dexamethasone, 10 mg intravenously, every 6 h for 4 days, was started before or with the first dose of parenteral antibiotics in 1075 (78%) of 1384 episodes.[5,28]

The overall case fatality rate was 244 (17%) of 1412 episodes and varied with the causative organism: 179 (18%) in 1017 episodes of pneumococcal meningitis, five (3%) in 150 episodes of meningococcal meningitis, and 26 (35%) in 74 episodes of listeria meningitis. An unfavourable outcome occurred in 531 (38%) of 1412 episodes: 413 (41%) of 1017 episodes of pneumococcal meningitis, 19 (13%) of 150 episodes of meningococcal meningitis, and 40 (54%) of 74 episodes of listeria meningitis. Characteristics of listeria meningitis episodes are shown in supplementary table 1. In a multivariable analysis, several characteristics were associated with an unfavourable outcome in bacterial meningitis due to any pathogen: older age, absence of otitis or sinusitis, alcoholism, tachycardia, lower score on the Glasgow Coma Scale, cranial nerve palsy, a cerebrospinal fluid white-cell count of fewer than 1000 cells per µL, a positive blood culture, and a high serum C-reactive protein concentration (table 3).

Pneumococcal serotype was not associated with outcome after correcting for multiple testing, neither with use of serotype 7F as a reference category, nor in dichotomized analyses between high-virulence and low virulence serotypes or between serotypes included in conjugate vaccines versus those not included (data not shown).[29,30]

The proportion of patients with unfavourable outcome was lower in those treated with adjunctive dexamethasone according to guideline recommendations (10mg QID four days) than those who did not receive dexamethasone treatment according to guideline recommendations (360 of 1075 [34%] vs. 157 of 309 [51%]; P<0.0001). In a multivariable analysis including all baseline variables, the adjusted OR of dexamethasone treatment for unfavourable outcome was 0.54 (95% CI, 0.39–0.73) and the adjusted OR for death was 0.46 (95% CI, 0.32–0.66). The adjusted OR for the association between dexamethasone treatment and unfavourable outcome was 0.55 (95%CI, 0.38–0.80) in pneumococcal meningitis and 0.44 (95% CI, 0.23–0.85) in episodes due to other pathogens. Hearing loss at discharge was present in 144 (16%) of 902 surviving patients and was not significantly affected by dexamethasone use (OR 1.32, 95% CI 0.80–2.23; P=0.30).

Table 3. Multivariable Analysis of Factors Associated with an Unfavourable Outcome

Characteristic no. /no.evaluated (%)	Favourable Outcome (n=881)	Unfavourable Outcome (n=531)	Univariable OR for Unfavourable Outcome (95% CI)	Multivariable OR for Unfavourable Outcome (95% CI)	P Value of Multivariable analysis
Age (years)	58 (42–67)	64 (55–75)			
16– 39 years	194/881 (22)	43/513 (8)	Ref	Ref	
40 – 70 years	542/881 (62)	297/513 (56)	2.47 (1.73–3.54)	1.55 (1.02–2.35)	0.041
> 70 years	145/881 (17)	191/513 (36)	5.94 (4.01–8.81)	3.04 (1.89–4.89)	<0.0001
Symptoms <24 h	428/855 (50)	208/498 (42)	0.72 (0.57–0.90)	0.84 (0.65–1.10)	0.22
Seizures	48/857 (6)	50/496 (10)	1.89 (1.22–2.92)	1.50 (0.85–2.63)	0.16
Pre-treated antibiotics	97/858 (11)	55/519 (11)	0.93 (0.64–1.34)	1.10 (0.72–1.68)	0.65
Otitis or sinusitis	338/876 (39)	142/528 (27)	0.59 (0.46–0.75)	0.74 (0.55–0.99)	0.041
Pneumonia	54/860 (6)	68/487 (14)	2.42 (1.63–3.60)	1.36 (0.81–2.27)	0.23
Immunosuppressive drugs	57/870 (7)	50/521 (10)	1.51 (1.00–2.29)	1.02 (0.62–1.67)	0.94
History of splenectomy	15/881 (2)	17/531 (3)	1.91 (0.89–4.14)	1.08 (0.47–2.48)	0.85
History of cancer	83/881 (9)	90/526 (17)	1.98 (1.42–2.77)	1.17 (0.80–1.72)	0.42
Diabetes mellitus	94/874 (11)	77/520 (15)	1.44 (1.03–2.02)	1.38 (0.93–2.04)	0.11
HIV	9/881 (1)	3/531 (1)	0.55 (0.10–2.22)	0.62 (0.14–2.63)	0.51
Alcoholism	34/881 (4)	48/531 (9)	2.47 (1.54–4.02)	1.98 (1.15–3.41)	0.013
Headache	716/819 (87)	299/404 (74)	0.41 (0.30–0.56)	0.71 (0.49–1.01)	0.058
Nausea	495/762 (65)	218/397 (55)	0.66 (0.51–0.85)	0.86 (0.63–1.16)	0.32
Neck stiffness	636/837 (76)	341/485 (70)	0.75 (0.58–0.97)	0.82 (0.54–1.25)	0.36
Rash	85/881 (10)	31/531 (6)	0.58 (0.37–0.90)	0.88 (0.53–1.46)	0.63
Heart rate (beats per min)*	98 (82–110)	102 (89–120)	1.18 (1.12–1.24)	1.10 (1.03–1.17)	0.0030
Diastolic blood pressure (mmHg)†	79 (68–88)	80 (70–92)	1.10 (1.03–1.17)	1.03 (0.95–1.11)	0.51
Temperature (°C)‡	39.0 (38.0–39.7)	38.8 (37.6–39.5)	0.87 (0.81–0.95)	0.89 (0.79–1.01)	0.070

Table 3. Multivariable Analysis of Factors Associated with an Unfavourable Outcome (Continued)

Characteristic no. /no.evaluated (%)	Favourable Outcome (n=881)	Unfavourable Outcome (n=531)	Univariable OR for Unfavourable Outcome (95% CI)	Multivariable OR for Unfavourable Outcome (95% CI)	P Value of Multivariable analysis
Score on Glasgow Coma Scale§	12 (9–14)	10 (8–13)	0.85 (0.82–0.88)	0.91 (0.87–0.96)	0.00085
Triad of fever, neck stiffness and GCS <14	354/851 (42)	209/496 (42)	1.02 (0.81–1.29)	1.03 (0.68–1.56)	0.88
Aphasia, hemiparesis or monoparesis	148/547 (27)	120/257 (47)	2.36 (1.71–3.25)	1.15 (0.83–1.58)	0.40
Cranial nerve palsy	43/747 (5)	60/449 (13)	2.68 (1.74–4.14)	2.55 (1.59–4.09)	0.00013
Cerebrospinal fluid White cell count (cells per µL)	3183 (950–7983)	1186 (249–5010)			
<100	60/846 (7)	89/506 (18)	3.61 (2.50–5.22)	2.10 (1.34–3.29)	0.0012
100 – 999	157/846 (19)	159/506 (31)	2.47 (1.87–3.25)	2.04 (1.46–2.84)	<0.0001
1000–10,000	470/846 (56)	193/506 (38)	Ref	Ref	
>10,000	159/846 (19)	65/506 (13)	1.00 (0.71–1.39)	0.81 (0.56–1.19)	0.29
CSF Protein (g/L)¶	3.5 (2.1–5.7)	4.5 (2.7–6.5)	1.08 (1.04–1.11)	1.03 (0.99–1.07)	0.13
CSF:blood glucose ratio‖	0.1 (0–0.3)	0.01 (0–0.1)			
< 0.25	560/825 (68)	407/484 (84)	1.53 (0.88–2.64)	1.28 (0.63–2.58)	0.49
0.25–0.5	223/825(27)	57/484 (12)	0.54 (0.29–0.98)	0.71 (0.34–1.50)	0.36
> 0.5	42/825 (5)	20/484 (4)	Ref	Ref	
Positive blood culture	550/777 (71)	377/466 (81)	1.75 (1.31–2.34)	1.44 (1.02–2.05)	0.040
Thrombocyte count	207 (160–257)	185 (135–237)			
< 150	163/845 (19)	159/500 (32)	1.94 (1.50–2.50)	1.32 (0.96–1.81)	0.088
150–450	664/845 (79)	334/500 (67)	Ref	Ref	
>450	18/845 (2)	7/500 (1)	0.77 (0.32–1.87)	0.46 (0.17–1.26)	0.13
C-reactive protein (mg/L)**	162 (72–272)	249 (134–370)	1.04 (1.03–1.05)	1.02 (1.01–1.03)	<0.0001

The study included 1412 episodes of community-acquired meningitis in 1391 patients; data are median (IQR) or n/N (%), unless stated otherwise. The multivariable analysis used an imputed dataset with sixty imputation rounds, all variables in the table were entered in the multivariable logistic regression model simultaneously. *Evaluated in 1363 episodes; odds ratio is for an increase of 10 beats per min. †Evaluated in 1381 episodes; odds ratio is for a 10 mm Hg increase. ‡Evaluated in 1391 episodes. §Evaluated in 1403 episodes; odds ratio is for a one point increase. ¶Evaluated in 1344 episodes. ‖Evaluated in 1309 episodes. **Evaluated in 1353 episodes; odds ratio is for a 10 mg/L increase. CSF=cerebrospinal fluid.

Discussion

Our findings show that the incidence of adult bacterial meningitis has decreased since the introduction of conjugate vaccines, primarily because of falls in pneumococcal and meningococcal meningitis. Incidence decreased most sharply among pneumococcal serotypes included in the seven-valent and ten-valent conjugate vaccines; these vaccines were introduced in the Netherlands in 2006 and 2011. A surveillance study[4] of 17.4 million people in the USA during 1998–2007, showed a similar effect of herd protection in adults. Our study included 16.8 million people, and by contrast with the US study, we observed no serotype replacement of non-vaccine serotypes. This difference between studies might only partly be explained by age differences. The US study showed that serotype replacement was age dependent, with an increase of 90% in children aged younger than five years, 61% at any age, and 18% in patients older than 65 years.[4] Dutch surveillance data has shown evidence of serotype replacement,[31,32] but only for all invasive pneumococcal disease combined, and not in the subgroup of patients with meningitis. Pneumococcal meningitis due to non-vaccine types did not increase during the observation period in adults or children aged 0–16 years (data not shown). The incidence of adult meningitis due to non-seven-valent or non-ten-valent serotypes in 2006–14 was not higher than in 1998–2002.[1]

Serogroup C meningococcal meningitis virtually disappeared after routine vaccination against this bacterium in 2002.[33] Herd protection was responsible for more than 36% of the effect of meningococcal conjugate serogroup C vaccine and lasted for more than 10 years.[34] Serogroup B meningococcal meningitis has probably decreased because of a natural fluctuation in incidence.[21]

The relative contribution of pneumococcal meningitis to adult bacterial meningitis has increased and has led to a change in population characteristics of those with bacterial meningitis. Patient with bacterial meningitis are older and more likely to have risk factors for pneumococcal meningitis, such as otitis, sinusitis, and an immunocompromised state, than were patients in 1998–2002.[1] For the subgroup of pneumococcal meningitis, the proportions of patients with an unfavourable outcome or death have decreased substantially over this period (unfavourable outcome from 50% to 41%, absolute risk reduction, -9%, 95% CI -7 to -11; death from 30% to 18%, absolute risk reduction -12%, 95% CI -10 to-14).[1] The strongest risk factors for an unfavourable outcome were those that suggested systemic compromise, a low level of consciousness, and infection with *S. pneumoniae*.

Dexamethasone therapy has been used widely as adjunctive treatment for adults with bacterial meningitis. In our study, it was administered to about 90% of patients, irrespective of the causative pathogen. Dexamethasone treatment was independently associated with favourable outcome and increased survival in patients with both pneumococcal and non-pneumococcal meningitis. These findings are consistent with the results of a Cochrane review.[35] We previously

wrote that it seems unlikely that a study could have enough power to prove or disprove an effect of adjunctive dexamethasone treatment on meningococcal meningitis.[18] The use of observational data precludes making strong conclusions about treatment effects, but the present findings in combination with a randomised study in the same population,[5] and meta-analysis of randomized clinical trials[35,36] showing a similar effect, suggest that implementation of dexamethasone treatment has improved the prognosis of bacterial meningitis, both pneumococcal and non-pneumococcal.

A history of meningitis was reported for seven percent of episodes. This proportion is similar to previous studies.[37,38] Causes of recurrent meningitis are cerebrospinal fluid leakage and immunodeficiency, which should carefully be assessed in patients with recurrent meningitis.[37–39] Because of the large number of patients with a history of meningitis in our cohort, physicians could consider vaccinating against the most common causes of bacterial meningitis in their region in any patient with bacterial meningitis.

Our study has several limitations. Patients who underwent lumbar puncture and who had a positive cerebrospinal fluid culture were over-represented. 11–22% of patients with bacterial meningitis have negative cerebrospinal fluid cultures.[40] Lumbar puncture might be postponed, in patients with coagulation disorders or severe septic shock, which can result in negative cerebrospinal fluid cultures. Additionally, patients with bacterial meningitis who have space-occupying lesions on CT might not undergo lumbar puncture. Another limitation of our study was that we had little information about comorbidity, and the timing of systemic and neurological complications. The vaccination status of patients was also not available. Theoretically, decreased incidence rates among serotypes included in the seven-valent vaccine could be, at least partly, due to vaccination of Dutch adults. However, in the Netherlands, routine pneumococcal vaccination for adults is not advised by the Health Council of the Netherlands, with the exception of high-risk groups (eg, those with hyposplenia or asplenia, sickle cell disease, and cerebrospinal fluid leakage). Based on sales records from Dutch pharmacies pneumococcal vaccine coverage among adults over 65 years old in the Netherlands is low.[32] Finally, antibiotic resistance among pneumococcal isolates was rare. Adding dexamethasone has the potential to reduce CSF penetration of vancomycin, a drug that has become the standard empirical antimicrobial therapy for pneumococcal strains that, on the basis of local epidemiology, are likely to be highly resistant to penicillin or cephalosporin.[41] Although a prospective multicentre observational study[42] showed that appropriate concentrations of vancomycin in CSF may be obtained even when concomitant steroids are used, some experts have advised addition of rifampicin to vancomycin and ceftriaxone or cefotaxime regimens in areas with high rates of pneumococcal drug resistance.[3,41]

Our findings show the substantial improvement in the prognosis of pneumococcal meningitis over the past two decades and the effect of paediatric conjugate vaccines on adult bacterial meningitis. Herd protection is a major part of the effectiveness of conjugate vaccines and can protect those with poor immunological response to vaccination- eg, infants and elderly people. The development of vaccines covering more pneumococcal serotypes, new potent anti-inflammatory treatments,[14] starting treatment immediately after blood cultures are obtained, and aggressive supportive care might further improve prognosis of patients with bacterial meningitis.

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Supplementary material

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Supplementary table 1. Clinical characteristics of patients with ***L. monocytogenes*** meningitis compared to meningitis due to other pathogens.

Characteristic	***L. monocytogenes*** meningitis (n=74)	Meningitis due to other pathogens (n=1338)	P -value
Age – yr	69 (62-76)	60 (46-69)	< 0.0001
Male sex	49/74 (66)	658/1338 (49)	0.006
Otitis or sinusitis	2/74 (3)	478/1330 (36)	< 0.0001
Immunosuppressive drugs	34/74 (46)	73/1317 (6)	< 0.0001
History of cancer	21/74 (28)	152/1333 (11)	0.0001
Diabetes mellitus	11/74 (15)	160/1320 (12)	0.47
Alcoholism	7/74 (10)	75/1338 (6)	0.19
Heart rate – beats /min ‡	96 (86-110)	100 (84-112)	0.70
Score on Glasgow Coma Scale	12 (10-14)	11 (9-14)	0.03
Cranial nerve palsy	6/67 (9)	97/1172 (8)	0.82
C-reactive protein	116 (55-184)	201 (91-317)	< 0.0001
CSF white cell count - cells/mm3	720 (356-1499)	2560 (581-7307)	< 0.0001
CSF Protein – g/litre ‡	2.6 (1.8-3.7)	4.0 (2.3-6.1)	< 0.0001
Positive blood culture	44/68 (65)	883/1175 (75)	0.06
Any adjunctive dexamethasone	50/73 (69)	1184/1311 (90)	< 0.0001
Adjunctive dexamethasone according to guideline recommendation	37/73 (51)	1038/1311 (79)	< 0.0001
Unfavourable outcome	40/74 (54)	491/1338 (37)	0.004
Death	26/74 (35)	218/1338 (16)	0.0002

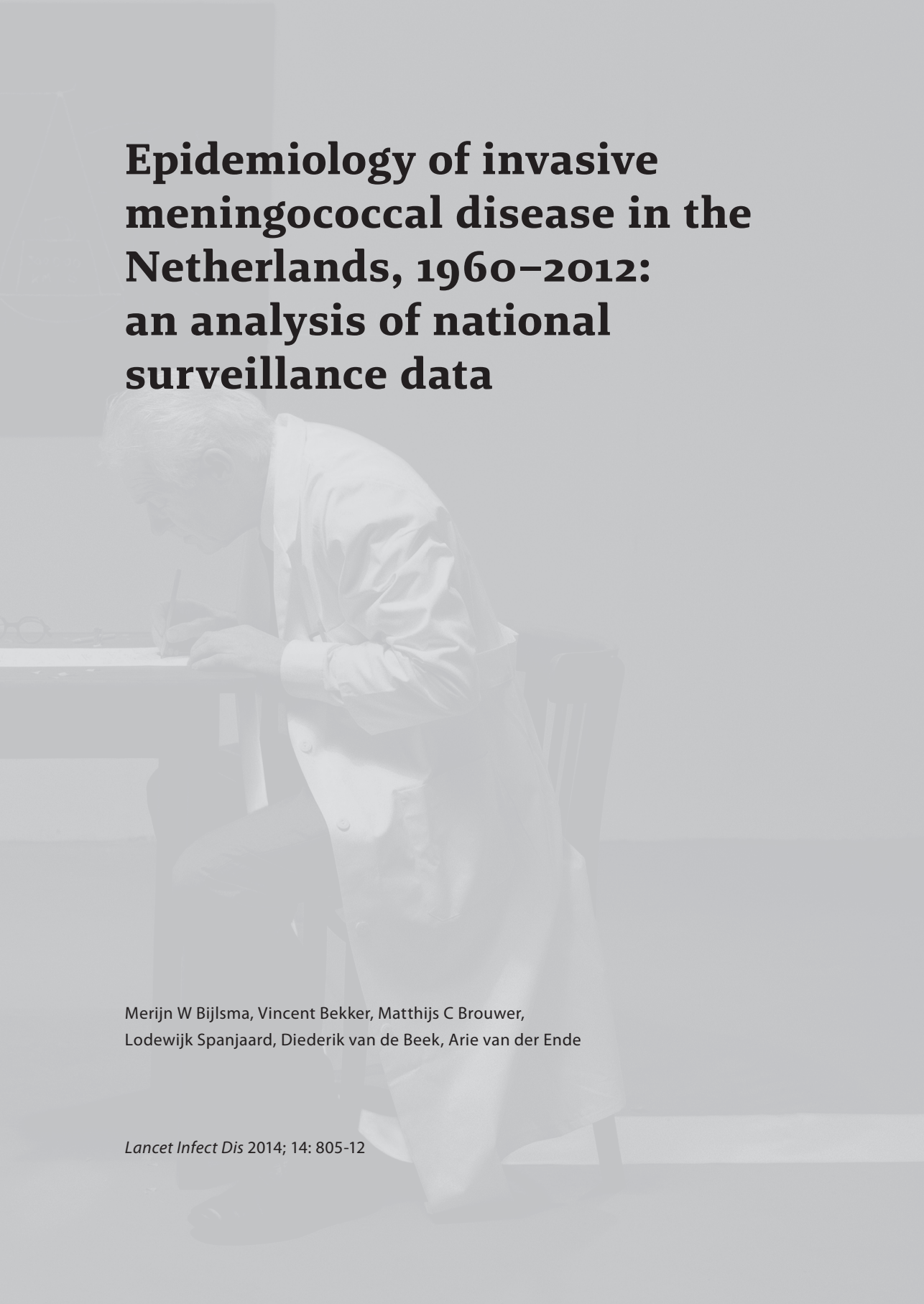
Median (interquartile range), statistical tests: Fisher exact for categorical and Mann-Whitney U Test for continuous data.

Appendix 1. Local investigators (participating hospitals)

P. Admiraal (Gemini Ziekenhuis), J.C. Baart (Ziekenhuisgroep twente), R.J. Beukers (Medisch Centrum Alkmaar), H.P. Bienfait (Gelre Ziekenhuizen), H.J. Bökkerink (Zkh Nij Smellinghe), A.E. Bollen (Wilhelmina Ziekenhuis Assen), H.M. Bos (St. Anna Ziekenhuis), D. Broere (Westfries Gasthuis), M.H. Christiaans (Diakonessenhuis Utrecht), S.F.T.M. de Bruijn (Haga Ziekenhuis), K. de Gans (Groene Hart Ziekenhuis), R.J. de Graaf (Amphia Ziekenhuis), L. de Lau (Slotervaart Ziekenhuis), M.C. de Rijk (Catharina Ziekenhuis), P. de Roos (Ziekenhuis Rivierenland), J.P. de Ruiter (Streekziekenhuis Koningin Beatrix), R.F. Duyff (Zkh de Tjongerschans), J.L.A. Eekhof (Diaconessenhuis Leiden), J. Engelsman (Spaarne Ziekenhuis), R.H. Enting (UMC Groningen), B. Feenstra (Zkh. Lievensberg), E. Geiger (Beatrix ziekenhuis Gorinchem), P. Groot (St. Jansdal Ziekenhuis Harderwijk), W.J.H.M. Grosveld (Reinier de Graaf Gasthuis), G. Hageman (Medisch Spectrum Twente), S.G.B. Heckenberg (Kennemer Gasthuis), D. Herderscheê (Tergooi ziekenhuizen), W. Hoefnagels (Zorgsaam Ziekenhuis), R.S. Holscher (Antonius Ziekenhuis Sneek), E.M. Hoogerwaard (Rijnstate Ziekenhuis), U.W. Huisman (Van Wheel Bethesda), B.C. Jacobs (Erasmus Medisch Centrum), C. Jansen (Gelderse Vallei Ziekenhuis), K Jellema (Medisch centrum Haaglanden), H. Kerkhoff (Albert Schweizer Zkh), E.J.W. Keuter (Diaconessenhuis Meppel), J.G.M. Knibbeler (Ropke-Zweers ziekenhuis), A.J.M. Kok (Elkerliek Ziekenhuis), N.D. Kruyt (LUMC), M.J.H. Langedijk (Refaja ziekenhuis), M. Liedorp (Havenziekenhuis), H.J.M.M. Lohmann (Deventer ziekenhuis), H. Lövenich (St Jans Gasthuis Weert), N.K. Maliepaard (Waterland Ziekenhuis), D.S.M. Molenaar (Ziekenhuis Amstelland), W.G.H. Oerlemans (Meander Medisch Centrum), E.W. Peters (Admiraal de Ruyter ziekenhuis), P.H.M. Pop (Viecurie Ziekenhuis), P. Portegies (OLVG), B. Post (UMC St Radboud), F.M. Reesink (Ommelander Ziekenhuis Groep), J.C. Reijneveld (Vumc), A.M.G. Sas (Vlietland ziekenhuis), R. Saxena (Maasstad Ziekenhuis), P.R. Schiphof (Ziekenhuis Bernhoven), J.P. Schipper (Bethesda Ziekenhuis), A. Schreuder (Atrium Medisch Centrum), A. Schuitemaker (Hofpoort ziekenhuis), E.S. Schut (Martini Ziekenhuis), T.H. Sie (Rode Kruis Ziekenhuis), A.L. Strikwerda (t lange land ziekenhuis), G.A. Sulter (Ziekenhuis de Sionsberg), R.J.J. Tans (MC groep), M. Te Linteloo (Franciscus Ziekenhuis), L.L. Teunissen (Sint Antonius Ziekenhuis), M.T. Tonk (Ziekenhuis Bronovo), J.T.H. van Asseldonk (Elisabeth-TweeSteden Ziekenhuis), C.J.W. van de Vlasakker (Slingeland ziekenhuis), J. van de Vlekkert (Flevoziekenhuis), J.S.P. van den Berg (Isala Klinieken), M.M. van der Graaff (Boven-IJ Ziekenhuis), G.W. van Dijk (Canisius-Wilhelmina Ziekenhuis), M.P.J. van Goor (Laurentius Ziekenhuis), B. van Harten (Medisch Centrum Leeuwarden), R.J. van Oostenbrugge (Academisch ziekenhuis Maastricht), N.P. van Orshoven (Orbis Medisch Centrum), L. van Winsen (Maasziekenhuis), M.D.I. Vergouwen (UMC Utrecht), F.H. Vermeij (Sint Franciscus Gasthuis), H.F. Visee (Jeroen Bosch Ziekenhuis), L.J.J.C. Wagener-Schimmel (Maxima Medisch Centrum), A.D. Wijnhoud (IJsselland Ziekenhuis), R.J.W. Witteveen (Rijnland Ziekenhuis), E.J. Wouda (St. Lucas Andreas Ziekenhuis), E.V. Zuilen (Scheper ziekenhuis).

CHAPTER 3

Epidemiology of invasive meningococcal disease in the Netherlands, 1960–2012: an analysis of national surveillance data



Merijn W Bijlsma, Vincent Bekker, Matthijs C Brouwer,
Lodewijk Spanjaard, Diederik van de Beek, Arie van der Ende

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Abstract

Epidemiological data for invasive meningococcal disease is essential for public health policy and vaccine development. We analysed national surveillance data from the Netherlands for PorA coverage of two PorA-based meningococcal serogroup B vaccines to describe the epidemiology of invasive meningococcal disease. We examined national surveillance data from the Netherlands Reference Laboratory of Bacterial Meningitis (NRLBM) for cases of culture-positive invasive meningococcal disease from Jan 1, 1960, to Jan 1, 2013. We included cases with a meningococcal isolate cultured from cerebrospinal fluid, blood, skin biopsy, or all, and cases with a positive cerebrospinal fluid latex agglutination test, counter current electrophoresis test, or positive cerebrospinal fluid PCR for *Neisseria meningitidis*. To test for completeness of case ascertainment, we compared data of the NRLBM with those of the Dutch National Institute for Public Health and the Environment (RIVM) to which notification was compulsory. We did serogrouping by ouchterlony gel diffusion. We tested susceptibility of meningococcal strains by agar dilution and E test. We tested differences between proportions with the Pearson χ^2 test or Fisher's exact test, and differences in frequencies between time periods with the Mann-Whitney U test. We defined penicillin resistance as MIC of 1.0 µg/mL or higher. Annual incidence rates of invasive meningococcal disease per 100,000 population increased from 0.5 in 1960, to 4.5 in 2001, and subsequently decreased to 0.6 in 2012. Median age increased from 1.8 years in 1960, to 6.1 years in 2012 for all serogroups. The proportion of blood culture positive cases increased from 4% in 1960, to 60% in 2012 ($p<0.0001$). Serogroup B was the most common serogroup over time, 64% of isolates were from ST-41/44 complex. We established PorA finetype of 4133 isolates, 19 of 252 variable regions covered 99% of sequenced serogroup B cases. Coverage of the 4CMenB PorA component was 4% in 1960-65, and 36% in 2006-12. In response to a serogroup C epidemic (1999–2001), serogroup C conjugate vaccine was introduced, which reduced serogroup C disease by 95%. Since 2003, serogroup Y disease emerged and serogroup A disease disappeared. We identified evidence of capsular switching, but not of serogroup replacement. The rate of reduced penicillin susceptibility increased to 37% from 1993 to 2012, but penicillin resistance was not recorded. Incidence of invasive meningococcal disease has decreased, but decreasing rates of penicillin susceptibility and the possible resurgence of this devastating disease remain a threat to public health. Our long-term serosubtyping and porA sequencing data is valuable for the assessment of vaccine coverage and future serogroup B vaccine development.

Introduction

Neisseria meningitidis is a major cause of bacterial meningitis, severe sepsis, and septic shock.[1] The case fatality rate of invasive meningococcal disease is roughly 7%.[2] The main determinant of virulence is the meningococcal polysaccharide capsule, which is used to classify this species into 12 serogroups.[1,3] Meningococci are further classified on the basis of serological differences in outer membrane proteins-eg, PorB (serotype) and PorA (serosubtype).[1,2,4] Molecular finetyping is based on differences in variable regions of the *porA* and *fetA* gene.[5] Multilocus sequence typing identifies genetic subgroups in sequence types and clonal complexes on the basis of variation in seven housekeeping genes.[5,6]

Meningococcal vaccines are based on capsular polysaccharides of serogroup A, C, W, and Y meningococci.[7] Polysaccharide vaccines are not effective against serogroup B disease, which is prevalent in Europe, America, and Australia.[7] The group B polysaccharide is poorly immunogenic because it is identical to polysialic acid of human glycoproteins. Serosubtype specific outer-membrane vesicle vaccines have been used to control serogroup B epidemic outbreaks in Brazil, Chile, Cuba, Norway, and New Zealand.[8] The immune dominant antigen of these vaccines is the outer-membrane protein PorA. Multivalent PorA outer-membrane vesicle vaccines have been developed, such as the nonavalent vaccine NonaMen.[9] Another outer-membrane vesicle vaccine (MenBvac) decreased carriage in Normandy, France.[10] In 2013, the 4CMenB serogroup B vaccine, which contains three recombinant proteins and one PorA protein was licensed in Europe and Australia.[11,12] In March 2014, the Joint Committee on Vaccination and Immunisation from the UK Department of Health recommended the 4CMenB vaccine for children at ages 2, 4, and 12 months if made available at an affordable price.[13]

Assessment of vaccine effect is complicated by variability in meningococcal disease incidence even without vaccination. Knowledge of the prevalence and variability of antigen distribution in invasive meningococci is essential for the design of outer-membrane vesicle vaccines.[2,14] We aimed to describe the epidemiology of invasive meningococcal disease in a western European country with national surveillance data from 1960 to 2012, from the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) and to assess potential coverage of PorA-based meningococcal serogroup B vaccines during this time.

Methods

Data sources

Clinical microbiology laboratories throughout the Netherlands send meningococcal isolates collected from cerebrospinal fluid, blood, or skin biopsy to the NRLBM. Some patient characteristics and information about the source of the isolate are collected. We included cases that arose

between Jan 1, 1960, and Jan 1, 2013, with a meningococcal isolate cultured from cerebrospinal fluid, blood, skin biopsy, or all, and cases with a positive cerebrospinal fluid latex agglutination test, counter current electrophoresis test, or positive cerebrospinal fluid PCR for *N. meningitidis*. The source of isolation was judged as blood if the blood culture or skin biopsy grew *N. meningitidis*. Data for positive PCR in serum or skin biopsies were not available. The source of isolation was cerebrospinal fluid if *N. meningitidis* was detected by culture, PCR, latex agglutination, or counter current electrophoresis in cerebrospinal fluid. To test for completeness of case ascertainment, we compared data of the NRLBM with those of the Dutch National Institute for Public Health and the Environment (RIVM) to which notification was compulsory.

Procedures

We did serogrouping by ouchterlony gel diffusion.[15] Isolates were routinely serosubtyped between 1985 and 2005 as previously described.[4,16] Samples from isolates received in 1965, 1970, 1975, and 1980 were serotyped and subtyped for a previous study.[17] From 2000 onwards, we established subtype by DNA sequencing of the *porA* variable regions, according to established subtyping schemes.[5] We compared putative amino acid sequences with those in the database. PorA finetype was retrospectively sequenced in random samples from 1960–65, 1970, 1980, 1985, and 1990.[18] We calculated coverage of the serogroup B outer-membrane vesicle vaccine NonaMen, containing nine different PorA variants with 18 different variable region 1 and variable region 2 regions (ie, P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13; P1.7-2,4; P1.22,14; P1.7-1,1; and P1.18-1,3,6), and the Bexsero PorA component P1.7-2,4, by dividing the number of finetyped isolates that expressed one or more of these variable regions by all finetyped isolates per period.[9,11] Multilocus sequence typing was routinely done from 2000 to 2010 as described by Martin Maiden and colleagues.[6] Multilocus sequence typing was retrospectively established in random samples. We included years with more than 15% of isolates retrospectively sequenced (1961–64, 1970, 1980, and 1985). We tested susceptibility of meningococcal strains for ceftriaxone resistance by agar dilution and E test. We judged strains as susceptible to penicillin if the minimum inhibitory concentration (MIC) was 0.1 µg/mL or less before 2001, and 0.06 µg/mL or less after 2001. Reduced susceptibility was defined as an MIC between 0.2 µg/mL and 1.0 µg/mL before 2001, and a MIC between 0.12 µg/mL and 1.0 µg/mL after 2001. We defined penicillin resistance as MIC of 1.0 µg/mL or higher.

Statistical analysis

We obtained demographic data on the age and sex distribution of the Dutch population during the observation period from the Central Bureau of Statistics.[19] Data are presented as median with IQR unless otherwise stated. We tested differences between proportions with the Pearson χ^2 test or Fisher's exact test. We tested differences in frequencies between time periods with the

Mann-Whitney U test. Statistical tests were two-sided and a p value of 0.05 or less was deemed significant.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 16,366 entries with *N. meningitidis*, 329 were excluded as double entries and 625 were excluded because the source of isolation was not cerebrospinal fluid, blood, or skin biopsy (15,412 cases [94%] were included; appendix). We identified recurrent meningococcal disease in 30 (0.2%) of 15,412 episodes. *N. meningitidis* was detected in cerebrospinal fluid only in 8,629 (56%) of 15,412 episodes, blood only in 3,861 (25%), and in cerebrospinal fluid and blood in 2,922 (19%). The proportion of cases identified in cerebrospinal fluid only decreased from 96% in 1960 to 40% in 2012 ($p<0.0001$; figure 1). Comparison of our data with the mandatory notification data of the RIVM showed under-reporting of collaborative laboratories between 1960 and 1972, after which the reporting of the NRLBM was similar (figure 1).[20]

The annual incidence rate increased from 0.5 per 100,000 population per year in 1960, to 4.5 in 2001, and subsequently decreased to 0.6 in 2012 (figure 1). Patient age was available for 15,042 (98%) of 15,412 cases (figure 2). Most cases (7,815 [52%] of 15,042) arose in the age group 0-5 years. In 2,408 (16%) of 15,042 cases the patient was younger than 1 year, with a peak incidence at 6 months. Median age increased from 1.8 years in 1960 (IQR 1.1-3.7), to 6.1 years (IQR 1.4-29.9) in 2012. We noted a slight male predominance (7647 [53%] of 14,410 participants were male; $p<0.0001$). Serogroup data was available for 15 313 cases (99%). Serogroup B caused most cases during the observation period (11,523 [75%] of 15,313 cases) followed by serogroup C (2,610 [17%]); serogroup A (656 [4%]); serogroup W (244 [2%]); serogroup Y (191 [1%]); and serogroup X, Z, E, or non-groupable (89 [1%]). We identified a seasonal pattern with peaks of serogroup B and C incidence between January and March, and for serogroup A between March and April (figure 2). PorA serosubtype was available in 9,655 cases (99%) that arose between1983 and 2003. Serosubtype was retrospectively determined in 377 cases (61%) occurring in 1965, 1970, 1975, and 1980. PorA finetype was determined in 3,566 cases (97%) from 2000 to 2012. PorA finetype was retrospectively sequenced in 567 cases (36%) from 1960-64, 1970, 1980, 1985, and 1990.

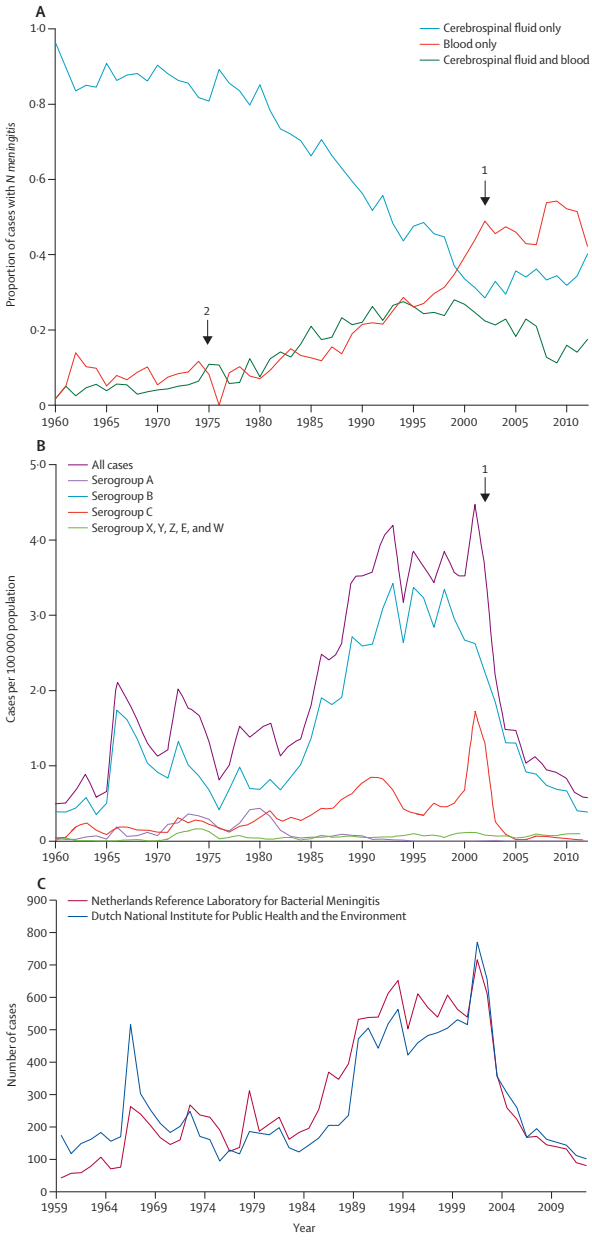

Figure 1. Annual incidence and source of isolation of *Neisseria meningitidis* invasive disease

(A) Proportion of isolates per year from cerebrospinal fluid, blood, or cerebrospinal fluid and blood. (B) Annual incidence per 100 000 population per year. (C) Comparison of data from the Netherlands Reference Laboratory for Bacterial Meningitis and compulsory notification to the registration of the Dutch National Institute for Public Health and the Environment. Arrow 1 shows the start of serogroup C vaccination. From arrow 2 onwards blood isolates were specifically requested.

A hyperendemic period of serogroup B disease started in 1982, reached a peak yearly incidence rate of 3.43 per 100,000 population in 1993, and subsequently decreased to 0.39 per 100,000 per year in 2012 (figure 2). Median patient age of serogroup B cases was 4.5 years (IQR 1.5-15.8; figure 2). Most cases arose in children younger than 5 years, with a second peak at roughly age 16 years. Between the start of the hyperendemic period and peak incidence, the incidence rate increased 4.5 times in children aged 0-9 years, 10.3 times in children aged 10-19 years, and 6.7 times in patients older than 19 years.

The most common PorA serosubtype during the first decade of the hyperendemic period (1982-91) was P1.4 (n=748, 30%), followed by P1.16 (n=216, 9%), and P1.2 (n=192, 8%). During the second decade (1992-2001), most common serosubtypes were P1.4 (n=1999, 43%), P1.10 (n=319, 7%), and P1.16 (n=275, 6%; appendix). PorA finetype was available for the last decade of the epidemic (2002–12; appendix); most common finetypes were P1.7-2,4 (n=517, 28%), P1.22,14 (n=175, 10%), and P1.5-2,10 (n=127, 7%).

We identified 252 different PorA variable region 1 and variable region 2 combinations, but a few variable region types were expressed by most serogroup B isolates. At least one of 18 variable region types included in the NonaMen vaccine was expressed by 2,926 (95%) of 3,087 finetyped serogroup B isolates; 71% in 1960-65, 100% in 1970, 92% in 1980, 94% in 1985, 90% in 1990, 96% in 2000-05, and 96% in 2006-12. If we added variable region 2 type 2 to the NonaMen vaccine, PorA coverage would be higher than 99% in all periods. The percentage of sequenced strains per time period that expressed the 4CMenB PorA component (variable region 7-2 or variable region 4) was 4% in 1960-65, 4% in 1970, 13% in 1980, 38% in 1985, 58% in 1990, 52% in 2000-05, and 36% in 2006-12.

The second most common meningococcal serogroup was C. In 1999, a serogroup C epidemic started reaching an incidence rate of 1.73 per 100,000 population in 2001. During the 1999-2001 epidemic, the incidence rate per 100,000 of same age increased 3.0 times in children aged 0-9 years, 4.9 times in children aged 10-19 years, and 2.7 times in children older than 19 years (figure 2). In response, children aged 1-19 years were offered one meningococcal C conjugate vaccination in 2002, and routine vaccination at age 14 months was subsequently introduced.[21] Serogroup C invasive disease subsequently decreased 95% to 0.09 per 100,000 population in 2012. Median age of serogroup C invasive disease cases increased from 10.8 years (IQR 2.9-19.7) before meningococcal C conjugate vaccination (1960-2001), to 24.3 (IQR 5.9-49.0) in 2002-12. Most common serogroup C serosubtypes were P1.2,5 (23%), P1.5 (20%), and P1.2 (15%; appendix). Most common PorA types were P1.5,2 (40%), P1.5-1,10-8 (30%), and P1.5-1,10-4 (3%; appendix).

For other meningococcal serogroups, the peak incidence of serogroup A disease was 0.44 per 100,000 population in 1980. Only 163 cases have been reported since 1980, and no serogroup A cases have been reported after 2003. We noted 244 (1.6%) of 15,313 episodes due to serogroup W

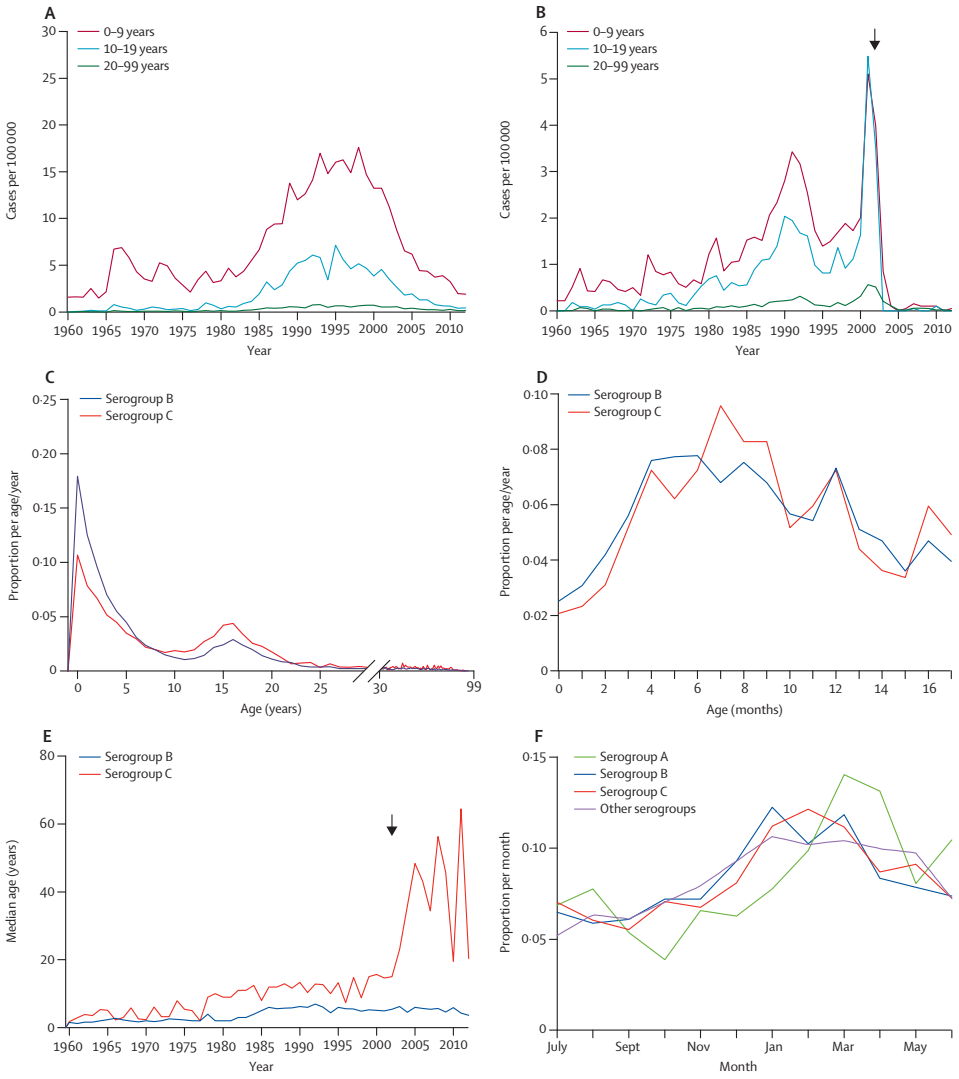

Figure 2. Age distribution of serogroup B and serogroup C, and seasonal distribution for all cases.

(A) Serogroup B age-specific incidence rate per 100 000 of age group per year. (B) Serogroup C age-specific incidence rate per 100 000 of age group per year. (C) Proportion of cases per year of age. (D) Proportion of patients aged 0–17 months per month of age. (E) Median age per calendar year. (F) Proportion of cases per month of infection. Arrow shows the start of serogroup C vaccination.

and 191 (1.2%) of 15,313 episodes due to serogroup Y between 1960 and 2012. The median number of serogroup W was 4 (IQR 3-6), there were 11 serogroup W cases in 1999 and 14 serogroup W cases per year in both 2000 and 2001. The median number of serogroup W cases from 2002 to 2012 returned to 4 (IQR 3-6). Sequencing data was available for 10 of the 28 serogroup W cases that occurred in 2000 and 2001. Seven were ST-11 complex with PorA type 5,2 and serosubtype P1.2,5. Serosubtyping, but no sequencing data was available for 1999. One of the 11 cases that occurred in 1999, compared to 10 of the 14 cases in 2000 were of serosubtype P1.2,5 (p=0.004).

We did multilocus sequence typing in 2,238 (64%) of 3,489 cases reported between 2000 and 2010. We established typing retrospectively in 542 isolates (51%) from 1960-64, 1970, 1980, and 1985. In these 19 years, we identified 700 different sequence types. The median number of years that a particular sequence type was identified was 2 (IQR 1-5) and the median number of different sequence types identified per year was 86 (IQR 72-99). The median number of cases per sequence type was one (IQR 1-2).

We could assign clonal complexes in 2,651 (95%) of 2,780 sequenced cases. Most common clonal complexes were ST-41/44 complex (1,355 [51%]), ST-32 complex (368 [14%]), and ST-11 complex (317 [12%]). Incidence rates of each of these clonal complexes was higher in 2000-02 than before the start of the serogroup B hyperendemic period. Between 2002 and 2012, these clonal complexes decreased simultaneously (figure 3). ST-32 complex was first identified in the Netherlands in 1980, and ST-41/44 and ST-11 complex were present in the Dutch population in 1960.

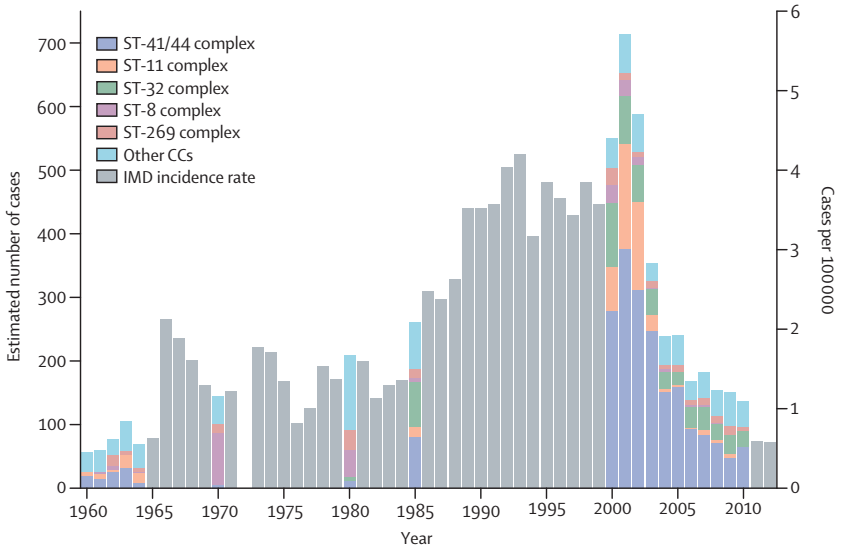

Figure 3. Estimated number of cases of invasive meningococcal disease due to common clonal complexes

We calculated the estimated number of cases by dividing the number of cases due to a particular clonal complex by the proportion of cases sequenced.

The most common clonal complexes in serogroup B were ST-41/44 complex (1,335 of 2,102 [64%]), ST-32 complex (364 [17%]), and ST-269 complex (101 [5%]). Most common clonal complexes in serogroup C were ST-11 complex (283 [66%] of 427), ST-8 complex (49 [12%]) and ST-41/44 complex (19 [4%]).

Most cases of serogroup Y in 1960–64, 1980, and 1985 were caused by the ST-167 complex; seven (78%) of nine sequenced isolates. Between 2000 and 2010 the proportion of serogroup Y cases due to ST-167 had decreased with seven (21%) of 34 cases (p=0.003). ST-23 and ST-174 complex were the most common serogroup Y clonal complexes since 2000, each comprising ten (29%) of 34 isolates.

Different isolates from a particular clonal complex can express different polysaccharide capsules (serogroup). Capsule switching, defined as relative change in the proportion of a clonal complex that expressed a particular serogroup polysaccharide capsule, happened in several clonal complexes (table 1). For example, all isolates from ST-8 complex in 1960–64 expressed the serogroup B capsule polysaccharide, whereas all isolates from ST-8 complex from 2005 onwards were serogroup C. We noted no evidence of capsular switching in clonal complexes that caused serogroup Y disease (appendix).

Data for antibiotic resistance were available for 7628 meningococcal isolates (99%) received from 1993 to 2012. The number of isolates with reduced penicillin susceptibility gradually increased from six (0.9%) of 636 in 1993, to 30 (37%) of 81 in 2012 (p<0.0001). The proportion of isolates from blood only with reduced susceptibility to penicillin was higher than the proportion of isolates from cerebrospinal fluid only and cerebrospinal fluid and blood (107/2889 [4%] *vs* 107/4739 [2%]; p=0.0002). No isolates were resistant to penicillin. Three of 7,628 isolates were resistant to rifampicin and two of these were susceptible to penicillin.

Table 1. Polysaccharide capsule expression of common clonal complexes in different time periods.

Clonal Complex	Serogroup % (N)	Period		
		1960-64	1980 & 1985	2005-10
ST-8 Complex	B	100 (5)	100 (16)	-
	C	-	-	100 (8)
ST-11 Complex	B	44 (15)	20 (3)	5 (1)
	C	56 (19)	67 (10)	79 (15)
	W-135	-	13 (2)	16 (3)
ST-32 Complex	B	-	99 (70)	100 (118)
	C	-	1 (1)	-
ST-41/44 Complex	A	2 (1)	-	-
	B	96 (51)	83 (66)	100 (374)
	C	2 (2)	17 (13)	0 (1)
ST-269 Complex	A	-	4 (1)	-
	B	43 (6)	73 (16)	95 (41)
	C	57 (8)	23 (5)	5 (2)

Discussion

Large-scale vaccination with a meningococcal C conjugate vaccine has resulted in the near disappearance of serogroup C invasive meningococcal disease in the Netherlands. A similar reduction in serogroup C cases was noted after the vaccine was introduced in the UK (1999), Spain (2000), Ireland (2000), Belgium (2002), and Iceland (2002).[22] We identified evidence of meningococcal capsular switching, but not of serogroup replacement. Incidence rates of all serogroups except for serogroup Y decreased after meningococcal C conjugate vaccine introduction, and clonal complexes responsible for the increase in serogroup Y did not previously express the serogroup C capsule.

Serogroup B was the most common cause of meningococcal invasive disease in the Netherlands. This finding accords with other studies in Europe, Australia, and the Americas.[2,23] Although no vaccine against serogroup B with broad coverage and established efficacy on disease incidence exists, the multicomponent meningococcal B protein vaccine candidate (4CMenB) is immunogenic in infants (given at 2, 4, and 6 or 2, 3, and 4 months of age), adolescents (aged 11-17 years), and adults (aged 18–50 years).[11,24,25] Our findings show that at least one of the variable regions of the 4CMenB PorA component was expressed by 4-51% of sequenced isolates in different periods between 1960 and 2012. Persistence of other components of the 4CMenB vaccine in the Dutch population has also been reported; isolates expressing fHbp-1.1 and NadA-3.8 circulated for 30 years and NHBA-2 for 50 years.[18] A previous study[12] estimated that 20% of serogroup B isolates submitted to reference laboratories in England and Wales, France, Germany, Norway, and

Italy in 2007, expressed the 4CMenB PorA type. On the basis of the prevalence of the 4CMenB PorA component or reactivity against the other 4CMenB components in a meningococcal antigen typing system, 78% coverage was predicted in these countries in 2007. Outer-membrane vesicle vaccines against a particular serogroup B clone have been used to control serogroup B epidemics, but do not offer broad coverage. Our data suggest the possibility of a serogroup B outer-membrane vesicle vaccine with broad coverage;[19] PorA variable region types would have covered more than 99% of all serogroup B strains in the study period. The longitudinal information about serogroup B PorA subtype distribution is especially valuable in relation to the interest in serogroup B vaccines.

The emergence of serogroup Y and disappearance of serogroup A disease also accords with previous studies.[2,24] During the second half of the 20th century, serogroup A incidence decreased in western Europe, the Americas, and Australia. Although rare in Europe before 2000, serogroup Y caused more than 5% of invasive disease in 2010.[26]

An outbreak of W135 meningococcal disease happened in the spring of 2000 in pilgrims returning from Saudi Arabia and their contacts.[27] Hajj-associated cases were caused by meningococcal strains of serogroup W, ST-11 complex, PorA type 5,2 and serosubtype P1.2,5.27,28 Our finding that most serogroup W cases in 2000 and 2001 were caused by ST-11 complex with PorA type 5,2 and that serogroup W cases with serosubtype P1.2,5 increased from 1999 to 2000, are in line with these previous findings.

We noted a strong increase in the proportion of cases with *N. meningitidis* identified in blood from 4% in 1960, to 60% in 2012. This finding can partly be explained by a difference in NRLBM policy; from 1960 to 1975, laboratories were requested to submit isolates only from patients with meningitis, whereas blood isolates were specifically requested from 1975 onwards. Furthermore, clinicians nowadays take blood cultures more readily than in the 1960s, and the blood culture media and techniques have been improved resulting in an increased yield of fastidious microorganisms such as the meningococcus.[29]

We recorded an increase in median age of patients with invasive meningococcal diseases over time. An increase in median age during an epidemic has been previously described in serogroup A disease.[30] Consistent with this report, we recorded a disproportionate increase of invasive meningococcal disease during the hyperendemic period in adolescents. The disproportionte increase can be explained by age-dependent nasopharyngeal carriage rates, which have been attributed to changing contact patterns and social behaviour.[31] The continuing increase in median age after the hyperendemic years might be explained by a combination of large-scale meningococcal C conjugate vaccination and ageing of the population.

The longevity and nationwide coverage of our study with serotyping and sequencing data make this, to our knowledge, the most comprehensive report of meningococcal epidemiology in a western European country. One study[32] from the Czech Republic (1970-2004) presented serological and sequencing data for 1320 invasive meningococcal isolates. Nationwide surveillance studies from Denmark (1974-99) and the UK (1993-2003) presented serological data.[33,34] The findings of our study show the highly variable epidemiology of invasive meningococcal disease. The introduction of conjugate vaccines have decreased the rate of invasive meningococcal disease but decreasing susceptibility to penicillin, capsular switching, and the possible resurgence of serogroup B disease remain a threat to public health.

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Supplementary material

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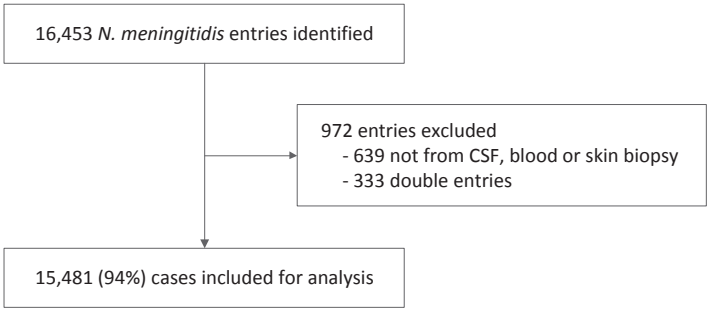


Supplementary figure 1. Flowchart of case inclusion.

Supplementary table 1A. Common[1] serogroup B serosubtypes in different time periods[2].

Serosubtype (% (N))[3]	Period						
	1965	1970	1975	1980	1983-1987	1988-2003	All years
% (N) subtyped[4]	70 (43)	56 (47)	36 (33)	95 (92)	98 (993)	90 (7181)	89 (8398)
P1.4	2 (1)	-	-	3 (3)	19 (189)	41 (2906)	37 (3099)
P1.16	5 (2)	-	3 (1)	1 (1)	8 (83)	7 (467)	7 (554)
P1.10	1(2)	2 (1)	-	9 (8)	3 (26)	6 (450)	6 (486)
P1.15	9 (4)	2 (1)	6 (2)	12 (11)	11 (108)	5 (356)	6 (482)
P1.7,16	2 (1)	-	-	1 (1)	11 (107)	4 (264)	4 (373)
P1.2	42 (18)	71 (40)	33 (11)	22 (20)	9 (85)	2 (138)	4 (312)
P1.14	-	2 (1)	-	1 (1)	2 (19)	3 (247)	3 (268)
P1.7	2 (1)	2 (1)	3(1)	1(1)	3 (27)	3 (233)	3 (264)
P1.9	-	2 (1)	-	2 (2)	2 (17)	3 (178)	2 (198)
P1.6	16 (7)	5 (3)	9 (3)	8 (7)	3 (30)	2 (139)	2 (189)
P1.2,5	-	-	-	1 (1)	-	2 (146)	2 (147)
P1.12	5 (2)	2 (1)	9 (3)	8 (7)	3 (27)	1 (82)	2 (122)
P1.1,7	14 (6)	5 (3)	6 (2)	3 (3)	2 (24)	1 (66)	1 (104)
Not typeable	-	-	24 (8)	26 (24)	24 (240)	18 (1293)	19 (1565)

[1] Serosubtypes found 100 times or more from 1960-2012, most common in all years (top) to least common (bottom).
[2] From 1983-2003 serosubtype was prospectively determined, isolates from 1965, 1970, 1975 and 1980 were retrospectively serosubtyped.
[3] The percentage and number of serosubtype per subtyped cases per time period.
[4] Percentage of cases serosubtyped per time period

Supplementary table 1B. Common[1] serogroup B PorA sequence variants in different time periods[2].

PorA (% (N))[3] VR1, VR2	Period							
	1960-65	**1970**	**1980**	**1985**	**1990**	**2000-05**	**2006-12**	**All years**
% (N) sequenced[4]	44 (139)	20 (24)	25 (24)	98 (194)	5(19)	99 (1914)	100 (773)	81 (3087)
7-2, 4	-	-	-	21 (40)	42 (8)	33 (639)	24 (182)	28 (869)
7-2, 13-2	1 (1)	-	-	1 (2)	5 (1)	9 (179)	4 (28)	7 (211)
22, 14	-	-	-	-	-	6 (107)	12 (95)	7 (202)
5-2, 10	-	4 (1)	-	2 (3)	5 (1)	7 (128)	8 (65)	6 (198)
19, 15	3 (4)	-	4 (1)	11 (3)	-	2 (36)	3 (24)	3 (87)
21, 16	4 (6)	-	-	2 (4)	-	3 (50)	3 (26)	3 (86)
7-1, 1	21 (29)	13 (3)	-	3 (5)	-	1 (26)	2 (14)	3 (77)
7-2, 16	-	-	-	10 (19)	11 (2)	2 (45)	1 (11)	3 (77)
5, 2	12 (16)	-	-	2 (4)	11 (2)	2 (40)	2 (14)	3 (76)
7, 16	-	-	4 (1)	10 (20)	11 (2)	2 (41)	1 (10)	2 (74)
5-1, 2-2	12 (16)	63 (15)	33 (8)	3 (6)	5 (1)	1 (9)	1 (4)	2 (59)
18, 25	-	-	-	-	-	1 (25)	1 (11)	2 (55)
7, 16-32	-	-	-	-	-	2 (39)	2 (13)	2 (52)
22, 9	-	-	4 (1)	1 (2)	-	1 (25)	3 (23)	2 (51)
18-7, 9	3 (2)	4 (1)	4 (1)	-	-	2 (34)	1 (8)	2 (47)
7-2, 13-1	1 (1)	-	13 (3)	1 (1)	-	2 (28)	1 (11)	1 (44)
22-1, 14	-	-	-	1 (2)	5 (1)	2 (29)	1 (7)	1 (39)
22, 14-6	-	4 (1)	-	-	-	1 (24)	2 (13)	1 (38)
18-1, 3	-	-	-	1 (2)	-	1 (17)	2 (15)	1 (34)
19-1, 15-11	-	-	-	-	-	1 (20)	2 (12)	1 (32)

[1] PorA variants found 25 times or more from 1969-2012, most common in all years (top) to least common (bottom).
[2] From 2000-2012 PorA finetype was prospectively determined, isolates from 1960-65, 1970, 1980 and 1990 were retrospectively sequenced.
[3] The percentage and number of sequence type per sequenced cases per time period.
[4] Percentage and number of cases sequenced per time period

Supplementary table 2a. Common[1] serogroup C serosubtypes in different time periods[2].

Serosubtype (% (N))[3]	Period						
	1965	1970	1975	1980	1983-1987	1988-2003	All years
% (N) subtyped[4]	64 (7)	50 (8)	19 (6)	87 (39)	82 (214)	97 (1706)	93 (1980)
P1.2,5	-	-	-	-	-	27 (454)	23 (454)
P1.5	-	-	-	-	-	23 (393)	20 (393)
P1.2	43 (3)	50 (4)	-	10 (4)	15 (32)	15 (261)	15 (304)
P1.1	-	-	-	3 (1)	4 (9)	3 (46)	3 (56)
P1.10	-	-	17 (1)	-	1 (3)	3 (51)	3 (55)
P1.1,7	-	13 (1)	17 (1)	5 (2)	9 (20)	2 (28)	3 (52)
P1.6	-	-	-	15 (6)	8 (18)	2 (26)	3 (50)
P1.16	-	13 (1)	-	-	4 (9)	2 (32)	2 (42)
P1.14	29 (2)	-	-	21 (8)	8 (17)	1 (10)	2 (37)
P1.15	-	(0) 0	17 (1)	3 (1)	6 (12)	1 (20)	2 (34)
P1.7	-	-	-	-	1 (2)	2 (25)	1 (27)
Not typable	-	-	33 (2)	31 (12)	33 (71)	17 (291)	19 (376)

[1] Serosubtypes found 25 times or more from 1960-2012, most common in all years (top) to least common (bottom).
[2] From 1983-2003 serosubtype was prospectively determined, isolates from 1965, 1970, 1975 and 1980 were retrospectively serosubtyped.
[3] The percentage and number of serosubtype per subtyped cases per time period.
[4] Percentage of cases serosubtyped per time period

Supplementary table 2b. Common[1] serogroup C porA sequence variants in different time periods[2].

PorA (% (N))[3] VR1, VR2	Period							
	1960-65	1970	1980	1985	1990	2000-05	2006-12	All years
% (N) sequenced[4]	48 (44)	6 (1)	27 (12)	100 (50)	5 (6)	99 (650)	98 (44)	79 (807)
5,2	39 (17)	-	8 (1)	8 (4)	17 (1)	44 (283)	36 (16)	40 (322)
5-1, 10-8	-	-	-	-	17 (1)	34 (223)	34 (15)	30 (239)
5-1, 10-4	7 (3)	-	-	-	-	3 (19)	2 (1)	3 (23)
5-1, 10-1	-	-	-	2 (1)	-	3 (17)	2 (1)	2 (19)
5, 2-1	-	-	-	12 (6)	33 (2)	2 (11)	-	2 (19)
21, 16	7 (3)	-	-	4 (2)	17 (1)	2 (13)	-	2 (19)
7-1, 1	5 (2)	-	17 (2)	8 (4)	-	1 (7)	2 (1)	2 (16)
5, 2-3	-	-	-	-	-	2 (11)	()	1 (11)

[1] PorA variants found 10 times or more from 1969-2012, most common in all years (top) to least common (bottom).
[2] From 2000-2012 PorA finetype was prospectively determined, isolates from 1960-65, 1970, 1980 and 1990 were retrospectively sequenced.
[3] The percentage and number of sequence type per sequenced cases per time period.
[4] Percentage and number of cases sequenced per time period

Supplementary table 3. Serogroup per time period in common serogroup B clonal complex and clonal complexes associated [1] with the serogroup C or serogroup Y polysaccharide capsule.

Clonal Complex	Serogroup % (N)	Period			
		1960-64	1980 & 1985	2000-02	2003-10
Clonal complexes associated with serogroup B					
ST-32 Complex	B	-	99 (70)	98 (129)	100 (165)
	C	-	1 (1)	2 (3)	-
ST-41/44 Complex	A	2 (1)	-	-	-
	B	96 (51)	83 (66)	100 (571)	100 (646)
	C	2 (1)	17 (13)	0 (2)	0 (3)
Clonal complexes associated with serogroup C					
ST-8 Complex	C			97 (38)	92 (11)
	B	100 (5)	100 (16)	3 (1)	8 (1)
ST-11 Complex	C	56 (19)	67 (10)	96 (219)	88 (35)
	B	44 (15)	20 (3)	1 (2)	5 (2)
	W-135	-	13 (2)	3 (7)	7 (3)
ST-22 Complex	C	-	57 (4)	-	6 (1)
	B	-		43 (3)	13 (2)
	W-135	-	43 (3)	57 (4)	69 (11)
	Y	-	-	-	6 (1)
	Non-groupable	-	-	-	6 (1)
ST-103 Complex	C	-	-	100 (1)	-
	B	-	-	-	43 (3)
	Y	-	-	-	57 (4)
ST-254 Complex	C	67 (6)	71 (5)	-	50 (1)
	B	33 (3)	29 (2)	100 (4)	50 (1)
ST-269 Complex	C	57 (8)	23 (5)	8 (2)	4 (2)
	A	-	5 (1)	-	-
	B	43 (6)	73 (16)	92 (22)	96 (54)
ST-334 Complex	C	29 (2)	100 (7)	100 (1)	50 (1)
	B	71 (5)	-	-	50 (1)
ST-364 Complex	C	100 (3)	38 (3)	-	-
	B	-	62 (5)	100 (1)	100 (1)
ST-376 Complex	C	-	100 (2)	-	-
	B	71 (5)	-	100 (1)	-
ST-461 Complex	C	67 (2)	38 (3)	43 (3)	-
	B	33 (1)	62 (5)	57 (4)	90 (9)
	Non-groupable	-	-	-	10 (1)
ST-1157 Complex	C	-	-	100 (1)	-
	B	-	-	-	67 (2)
	X	-	-	-	33 (1)

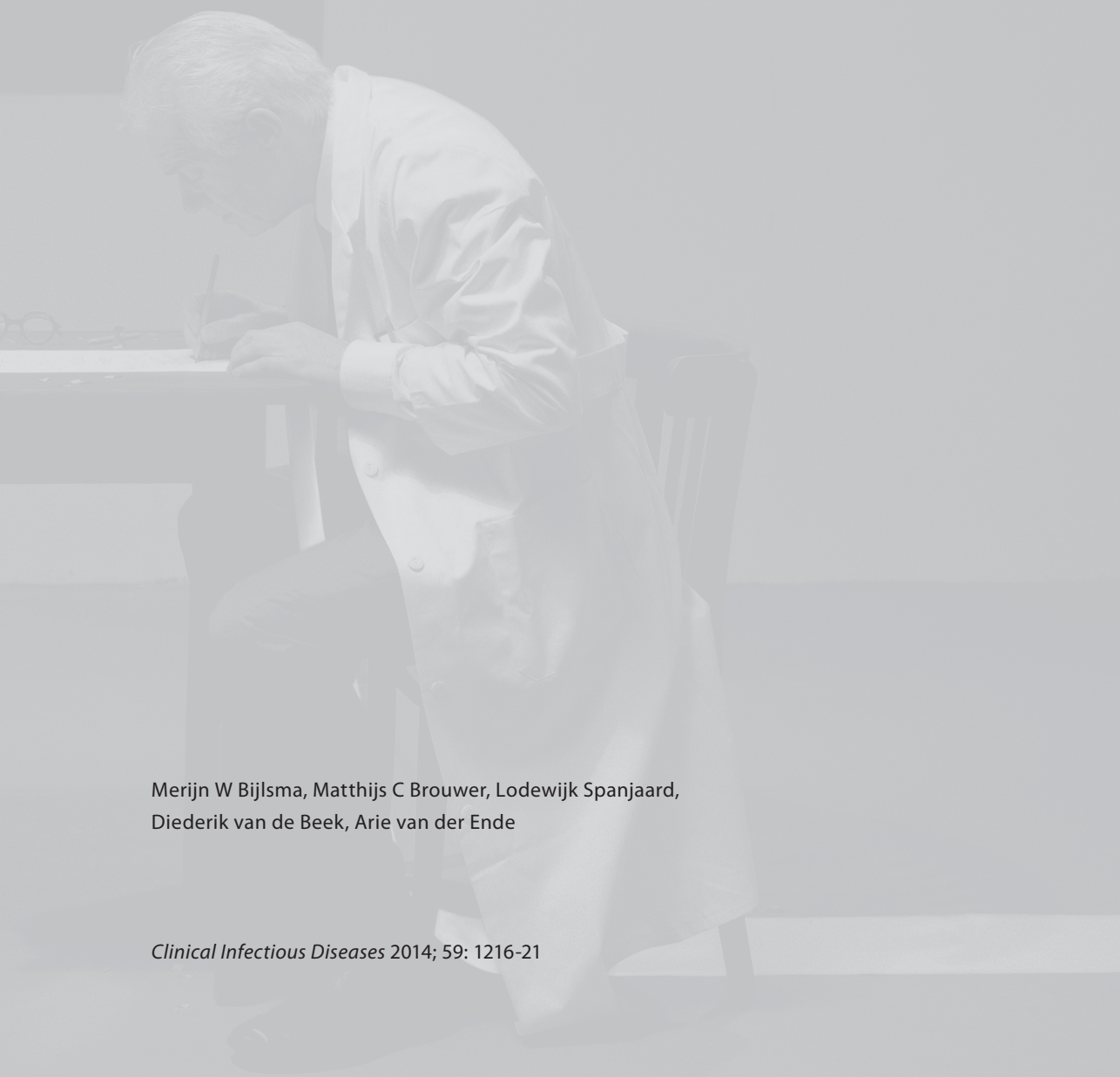
Supplementary table 3. Serogroup per time period in common serogroup B clonal complex and clonal complexes associated [1] with the serogroup C or serogroup Y polysaccharide capsule. (Contiuned)

Clonal Complex	Serogroup % (N)	Period			
		1960-64	1980 & 1985	2000-02	2003-10
Clonal complexes associated with serogroup Y					
ST-23 Complex	Y	100 (1)	-	-	
ST-92 Complex	Y	-	-	-	100 (2)
ST-167 Complex	Y	100 (5)	100 (2)	67 (2)	100 (5)
	W-135	-	-	33 (1)	-
ST-174 Complex	Y	-	-	25 (1)	64 (9)
	B	-	-	25 (1)	-
	W-135	-	-	50 (2)	36 (5)
ST-175 Complex	Y	100 (1)	-	-	-

[1] Association defined as a clonal complex with more than 50% of isolates belonging to serogroup C or serogroup Y in one of the time periods.
Serogroup C vaccination was introduced in the summer of 2002. The statistical significance of changes in serogroup proportions between 2000-02 and 2003-10 in serogroup C associated clonal complexes was tested with the Fisher Exact test. Only the change in the proportion of serogroup B or W-135 combined in ST-11 complex showed a P value below 0.05. No significant changes were found after correcting for multiple testing.
Clonal complexes associated with serogroup Y did not express other polysaccharide capsules before the year 2000.

CHAPTER 4

A decade of herd protection after introduction of meningococcal serogroup c conjugate vaccination



Merijn W Bijlsma, Matthijs C Brouwer, Lodewijk Spanjaard,
Diederik van de Beek, Arie van der Ende

Clinical Infectious Diseases 2014; 59: 1216-21

Abstract

Vaccination with meningococcal serogroup C (MenC) conjugate (MCC) polysaccharide vaccines led to a substantial decline in MenC disease in the vaccinated and the unvaccinated population. The decline in the unvaccinated population can be explained by herd protection by reduced colonization of meningococci expressing the MenC capsule. The duration of such herd protection is unknown. In a nationwide study from the Netherlands, we compared MenC invasive disease between 1998 and the introduction of MCC vaccination (2002) with that from 2002 to 2012, in age groups eligible and not eligible for vaccination. The proportion of isolates from clonal complexes with high serogroup C capsule expression rates during carriage (sequence type [ST] 11 and ST-8 complex) was compared between the pre- and post-vaccination periods. A total of 814 patients with invasive MenC disease were included for analysis. There was a 99% decline in MenC disease in patients eligible for vaccination and a 93% decline in those not eligible. Thirty-six percent of the overall MenC reduction between the first and last 4 years of the observation period occurred in the unvaccinated population. Clonal complex was determined in 350 (43%) isolates. The proportion of cases caused by clonal complex ST-11 and ST-8 serogroup C meningococci decreased from 251 of 268 (94%) before, to 46 of 57 (81%) after MCC vaccine introduction (P = .004). Our findings provide further evidence that herd protection results from reduced carriage of virulent meningococci. Herd protection was responsible for >36% of MCC vaccine impact and lasted for ≥10 years.

Introduction

The introduction of meningococcal serogroup C (MenC) conjugate (MCC) polysaccharide vaccines has greatly contributed to invasive meningococcal disease control.[1] In the Netherlands, in response to a sharp increase in MenC cases that started in the fall of 1998, children aged 1–18 years were offered a single MCC vaccination in 2002.[2] Routine vaccination at 14 months was subsequently introduced.[3] Vaccine coverage of the target population has been estimated at 94%.[4] After MCC vaccine introduction there was a large decline in the MenC incidence rate in the unvaccinated population.[3-5] Indirect protection is thought to be the result of herd protection, whereby MCC vaccination protects the unvaccinated population by reducing carriage and transmission of virulent meningococci in the vaccinated population.[6,7] Reduced carriage in the vaccinated population results in reduced transmission to the unvaccinated population. A computer simulation study in the United Kingdom has suggested that protection against carriage might last 3–10 years.[8] It is unknown how long herd protection after MCC vaccination will last. Herd protection is especially relevant for countries that opted for routine MCC vaccination of infants only, because it has become clear that protective antibody titres do not last for more than a few years after infant immunization.[9,10]

The majority of meningococcal invasive cases are caused by a limited number of hyperinvasive bacterial lineages.[11] Multilocus sequence typing can identify genetically related meningococcal isolates, based on similarities in 7 conserved housekeeping genes. A sequence type (ST) is defined as a combination of unique sequences of the 7 loci. Genetically similar isolates can be grouped into clonal complexes.[12] During nasopharyngeal carriage, meningococci of different clonal complexes vary in their propensity to express their polysaccharide capsule, which is the main virulence factor of the meningococcus. Carriage studies in the United Kingdom and Germany have shown that that between 78% and 88% of the clonal complexes ST-11 and ST-8 serogroup C meningococci expressed their polysaccharide capsule compared with <28% of serogroup C meningococci of other clonal complexes combined.[5,13]

In a nationwide surveillance study, we determined the long-term effect of MCC vaccination on the incidence rate of MenC disease in both the vaccinated and unvaccinated population. To evaluate the proposed mechanism of herd protection, we studied the impact of MCC vaccination on the occurrence of invasive MenC disease due to isolates from clonal complexes that are known to have a high expression rate of their polysaccharide capsule during nasopharyngeal carriage.

Methods

The Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) started a nationwide surveillance on meningococcal disease in the Netherlands in 1958. Clinical microbiology laboratories throughout the country send patient characteristics and meningococcal isolates to the NRLBM. Studies have estimated that isolates and clinical information on >85% of patients with meningococcal disease in the Netherlands is reported to the NRLBM.[14,15] For this study, patients were included if the NRLBM received a MenC isolate cultured from blood, skin biopsy, and/or cerebrospinal fluid (CSF), or if polymerase chain reaction findings were positive for MenC in CSF from 1 June 1998 to 1 June 2012. Serogrouping was performed by Ouchterlony gel diffusion.[16] Multilocus sequence typing was performed as described by Maiden et al.[17] Statistics about the Dutch population were obtained from the Dutch Central Bureau of Statistics (available at: http://www.cbs.nl).

Patients were categorized as eligible or ineligible for MCC vaccination based on the earliest recorded date of the illness and the patient's age at that time. Incidence rates were calculated per 100 000 inhabitants of the same age group, both per 3 months and per 12 months, starting 1 June 1998. Based on previous carriage studies, MenC isolates from ST-11 and ST-8 complexes were considered to express their polysaccharide capsule frequently during nasopharyngeal carriage, and MenC isolates from other clonal complexes were considered to express their capsule infrequently.[5,13] The proportion of sequence typed isolates from ST-11 and ST-8 complexes before the start of MCC vaccination (1 June 1998 to 1 June 2002) was compared with that from ST-11 and

ST-8 after completion of MCC catch-up campaign (1 December 2002 to 1 June 2012). Differences between proportions were tested with the Fisher exact test. Differences were considered statistically significant at $P < .05$, and statistical tests were 2 tailed.

Results

In total 900 MenC episodes were identified; 86 episodes (10%) were excluded (52 were identified as double entries, and 34 were excluded because the source of isolation was not blood, skin biopsy, or CSF or was not reported; figure 1), leaving 814 patients. Age, date of illness, and date of birth were available for all 814 included cases; sex was available for 788 of 814 patients (97%). *Neisseria meningitidis* was detected in both CSF and blood in 218 patients (27%), in CSF only in 212 (26%), and in blood only in 384 (47%).

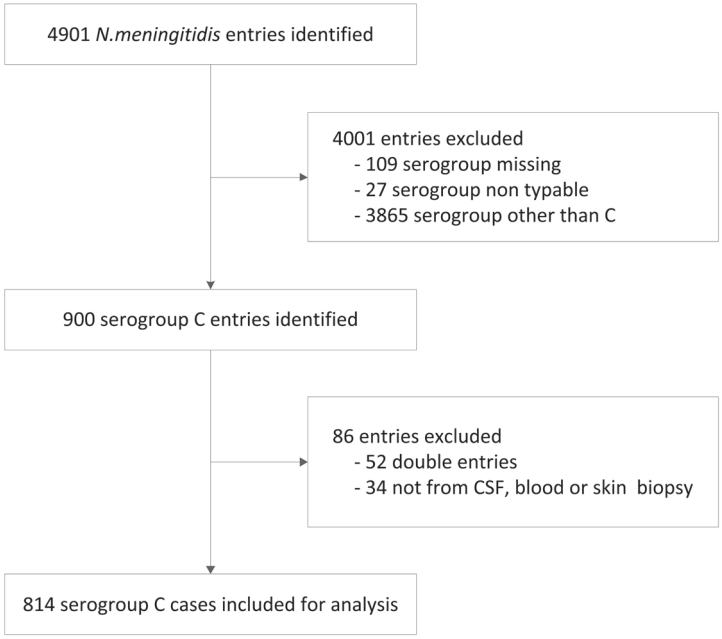


Figure 1. Flowchart of case inclusion.

Abbreviations:
CSF, cerebrospinal fluid;
N. meningitidis, *Neisseria meningitidis*.

The median age was 16 years (interquartile range, 5–29 years; figure 2) and 51% of patients were male. The seasonal distribution showed that the most cases occurred in January (12%) and the fewest in September (5%). The incidence rate per 100 000 persons per 12 months increased from

0.48 in 1998 to 1.99 in 2001 and declined after the introduction of the MCC vaccine to 0.006 in 2011. The epidemic did not affect age groups equally. Peak incidence in 2001 was 7.4 per 100 000 infants and children aged 0–5 years, 5.3 per 100 000 children and adolescents aged 6–18 years, and 1.8 per 100 000 young adults aged 19–28 years. The relative increase of the incidence rate was highest for young adults. Compared with the start of the epidemic (1998), the incidence rates increased 10-, 5-, and 3-fold, respectively, for those aged 19–28, 6–18, or 0–5 years. The median age increased, from 15 years before the introduction of MCC vaccination, to 35 years after the catch-up campaign.

In June and July 2002 children aged 1–5 years and 15–18 years received the MCC vaccination.[3,18] Children aged 6–14 years were vaccinated in September, October and November 2002.[18] During the first 3 months of the vaccination campaign, the incidence rates per 3 months for all age groups decreased (table 1). Compared with the same period in the previous year, the incidence rate decreased 65% in patients aged 15–18 years and 23% in those aged 1–5 years (table 2). Interestingly, in the same period the incidence rate for age groups that were not (yet) eligible for vaccination also decreased; this decrease was 49% in infants <1 year old, 41% in 6–14-year-olds, 24% in 19–28-year-olds, and 50% in 29–99-year-olds. The decrease tended to occur later in infants <1 year old, but numbers were small.

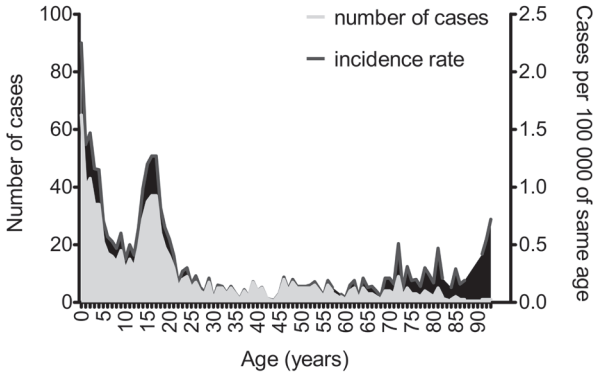


Figure 2. Age distribution.

Light grey: number of cases per year of age.
Dark grey: incidence rate per 100,000 population of same year of age.

Herd Protection

The reduction in MenC cases was most pronounced in patients eligible for MCC vaccination, but the incidence declined in all age groups (figure 3). During the 48 months before the introduction of MCC vaccination (June 1 1998 to June 1 2002) there were 413 cases in patients eligible for vaccination and 249 cases in patients not eligible for vaccination. During the last 48 months of

the observation period (June 1 2008 to June 1 2012) only four cases occurred in vaccinated age groups and 18 cases in the unvaccinated age groups; a reduction of respectively 99% and 93%. Thirty-six percent of the reduction of cases between these 2 periods occurred in the unvaccinated age groups.

Multilocus Sequence Typing

Multilocus sequence typing was performed in 350 of 814 isolates (43%). Fifty-three STs were identified; the median number of cases per ST was 1, and the maximum was 234. Six STs had not yet been assigned to a clonal complex at the time of writing. The most common clonal complex was ST-11 complex with 264 of 350 isolates (77%), followed by ST-8 complex with 51 of 350 (15%). Although the MenC incidence rate declined in all clonal complexes after the introduction of MCC vaccination, the decline was most pronounced among ST-11 and ST-8 complex serogroup C meningococci, which frequently express their capsule during nasopharyngeal carriage. The proportion of ST-8 and ST-11 isolates decreased from 251 of 268 isolates (94%) before the start of MCC vaccination to 46 of 57 (81%) after completion of the MCC catch-up campaign ($P = .004$; table 3). A similar pattern was observed in unvaccinated patients: the proportion of ST-11 and ST-8 complex isolates decreased from 103 of 111 cases (93%) before to 42 of 52 (81%) after completion of the MCC catch-up campaign ($P = .03$; table 3).

Table 1. Serogroup C meningococcal disease incidence rates in different age groups, before and after the introduction of MCC vaccination.[a]

	Incidence rate per 3 months per 100,000 of the same age						
	Age group (years)	**<1**	**1-5**	**6-14**	**15-18**	**19-28**	**29-99**
Quarter	Jun - Aug 01	1.95	.90	.95	1.81	.40	.12
	Sep - Nov 01	1.95	1.20	.67	2.19	.35	.11
	Dec - Feb 02	3.43	2.49	1.12	2.95	.60	.23
	Mar - May 02	2.46	2.28	1.06	1.79	.45	.13
	Jun - Aug 02	.98	**.69**	.56	**.64**	.30	.06
	Sep - Nov 02	.49	**.10**	**.06**	**.13**	.10	.03
	Dec - Feb 03	1.00	**.20**	**0**	**.13**	.20	.05
	Mar - May 03	2.48	**.29**	**0**	**0**	.05	.07
	Jun - Aug 03	.50	**0**	**0**	**0**	.05	.04
	Sep - Nov 03	0	**.10**	**0**	**0**	.05	0
	Dec - Feb 04	1.00	**.10**	**0**	**0**	.10	.09
	Mar - May 04	0	**0**	**0**	**0**	0	.01

[a] Bold-faced incidence rates indicate vaccinated groups. Abbreviation: MCC, meningococcal serogroup C conjugate.

Table 2. Serogroup C meningococcal disease incidence rate per age group compared to the same period in the peak year 2001-2002.[a]

	Percentage of incidence rate in the same quarter in 2001-2002						
	Age group (years)	**<1**	**1-5**	**6-14**	**15-18**	**19-28**	**29-99**
Quarter	Jun - Aug 02	51	**77**	59	**35**	76	50
	Sep - Nov 02	25	**8**	**8**	**6**	29	27
	Dec - Feb 03	29	**8**	**0**	**4**	34	21
	Mar - May 03	101	**13**	**0**	**0**	11	53
	Jun - Aug 03	26	**0**	**0**	**0**	13	33
	Sep - Nov 03	0	**8**	**0**	**0**	14	0
	Dec - Feb 04	29	**4**	**0**	**0**	16	39
	Mar - May 04	0	**0**	**0**	**0**	0	8

[a] Bold-faced incidence rates indicate vaccinated groups.

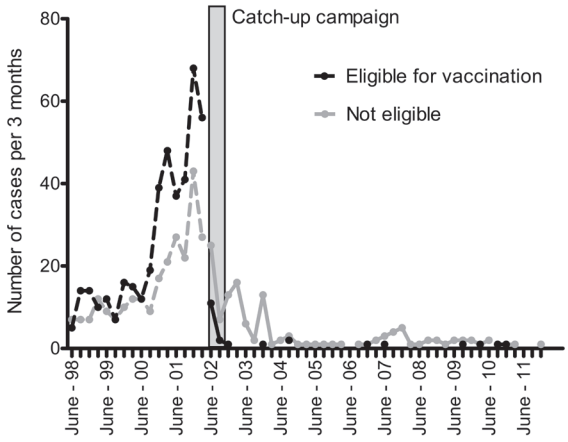


Figure 3. Number of serogroup C cases per 3 months in patients eligible and not eligible for meningococcal serogroup C conjugate vaccination.

Table 3. Proportion of isolates from clonal complexes with frequent capsule expression during nasopharyngeal carriage (ST-11 and ST-8 complex) before and after the introduction of MCC vaccination.

	ST-11 and ST-8 Isolates/All Isolates, No. (%)		**P Value[a]**
	Before	**After**	
All age groups	251/268 (94%)	46/57 (81%)	P=0.004
Unvaccinated	103/111 (93%)	42/52 (81%)	P=0.03

Abbreviations:
MCC, meningococcal serogroup C conjugate;
ST, sequence type.
[a] Fisher exact test.

Discussion

Our data show that a decade after MCC vaccine introduction, MenC disease has been reduced by >93%. These results are in accordance with previous findings.[1,6] The United Kingdom, Spain, Ireland, Iceland, and Belgium experienced a substantial decline in MenC disease after the introduction of routine MCC vaccination between 1999 and 2002.[1]

Our findings provide further evidence for the conclusion that herd protection is an important part of MCC vaccine effectiveness. We found that at least a third of the reduction of cases occurred in unvaccinated age groups. Herd protection was probably responsible for more than the 36% decrease in serogroup C cases because reduced transmission will also have prevented serogroup C cases in the vaccinated age groups. Previous work has demonstrated that MCC vaccination has a disproportionate impact on the carriage of serogroup C meningococci belonging to the ST-11 complex, which showed a high frequency of capsule expression during nasopharyngeal colonization.[5] We now show that MCC mass vaccination also has the highest impact on reducing disease caused by clonal complexes with a high capsule expression rate, both in all patients and in the subgroup of unvaccinated patients. This finding supports the proposed mechanism of herd protection in meningococcal disease whereby reduced nasopharyngeal carriage in immunized individuals leads to less disease transmission to the unvaccinated population .[19,20] The magnitude of the herd effects after MCC vaccination was largely unanticipated.[6] Reliable estimates of the impact and duration of herd protection are important for policy deliberations about vaccine cost-effectiveness and the design of future immunization strategies.[6]

It is unknown how long this herd protection after MCC vaccination will last. Computer models that were developed based on data from the United Kingdom have predicted stabilization of low levels of MenC disease for >15 years after mass vaccination.[8] Our national surveillance data together with postlicensure surveillance studies from the United Kingdom have now confirmed that herd protection has persisted for at least a decade.[8] National surveillance studies are crucial to estimate MCC vaccine effectiveness and herd protection.[21]

The MCC vaccines became available at a time of increased MenC incidence in Europe. Because large randomized controlled trials were considered to be unattainable, MCC vaccines were licensed on the basis of safety and immunogenicity studies only.[21] Even if randomized controlled trials had been performed, they would have been of limited use to investigate herd protection.[20] The relatively small numbers of study participants in randomized controlled trials are unlikely to confer herd protection. Even if herd protection is elicited, the traditional calculation of vaccine efficacy is insufficient.[22] Vaccine efficacy is calculated as $[(Iu - Iv)/Iu] \times 100\%$, where Iu and Iv represent for disease incidence in the unvaccinated and vaccinated study arms, respectively. Herd protection can affect both Iu and Iv. If disease frequency is equally reduced in both groups, the herd protection effect is cancelled out. Bias is introduced if the protective effect is unevenly distributed between the vaccinated and unvaccinated study arm. Methodological advances in the

design and analysis of cluster randomized trials have been proposed that would make it possible to assess herd protection before introducing a vaccine into public health programs.[23]

Lasting herd protection is especially relevant, because it has become clear in recent years that children who are vaccinated before the age of 5 years lose their serological protective antibody levels within a few years.[9] However, most countries that have implemented MCC vaccination opted for a vaccination schedules with either 2 doses in the first year of life and a booster in the second year or a single dose in the second year of life.[4] As the catch-up cohort ages, protection against carriage and disease in the adolescent population will diminish in the coming years. Meningococcal carriage is age dependent, with the highest prevalence in teenagers and young adults.[24] Furthermore, natural immunity in the population has declined because MCC vaccination has reduced MenC circulation.[25] Dutch children <14 months of age and nonimmunized adults have lower serogroup C specific immunoglobulin G levels at present compared with the prevaccination era.[9] This might actually put these individuals at high risk for invasive meningococcal disease if MenC circulation were to increase again.

Our data suggest a delay of herd protection in infants <1 year of age but offer no clear explanation for this finding. Seasonal variation may be more important in this age group, perhaps owing to a higher incidence of respiratory infections.[26]

In case of re-emergence of MenC, additional vaccination of children between age 5 years (adequate immunological response) and adolescence (high carriage rate) should be considered. Because of age-dependent carriage, an additional booster vaccination in teenagers and young adults would probably improve herd protection.

The capability of meningococci to switch to a different polysaccharide capsule poses another potentially limiting factor for long-term effectiveness of MCC mass vaccination.[27] Capsular switching under the selective pressure of MCC vaccination could in theory explain part of the reduction of serogroup C disease in our study. However, in that case one would expect a concurrent increase in the occurrence of other serogroup disease. Since the introduction of MCC mass vaccination, the incidence rates of all serogroups except serogroup Y have declined in the Netherlands. Clonal complexes responsible for the rise in serogroup Y disease did not previously express the serogroup C capsule.[28]

A limitation of our study is its observational design. The observed decrease in MenC may have been caused by other factors than MCC vaccination. However, the consistent high coverage of the reference laboratory, the temporal relationship between the mass vaccination campaign, and the decline in the MenC incidence rate, as well as the consistency with the experience in other countries, make a causal effect likely.[1]

Our findings provide further evidence that herd protection results mostly from reduced carriage of meningococci with a high capsule expression rate and that it is responsible for >36% of MCC vaccine impact. The observed herd protection lasted for ≥10 years.

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Supplementary material

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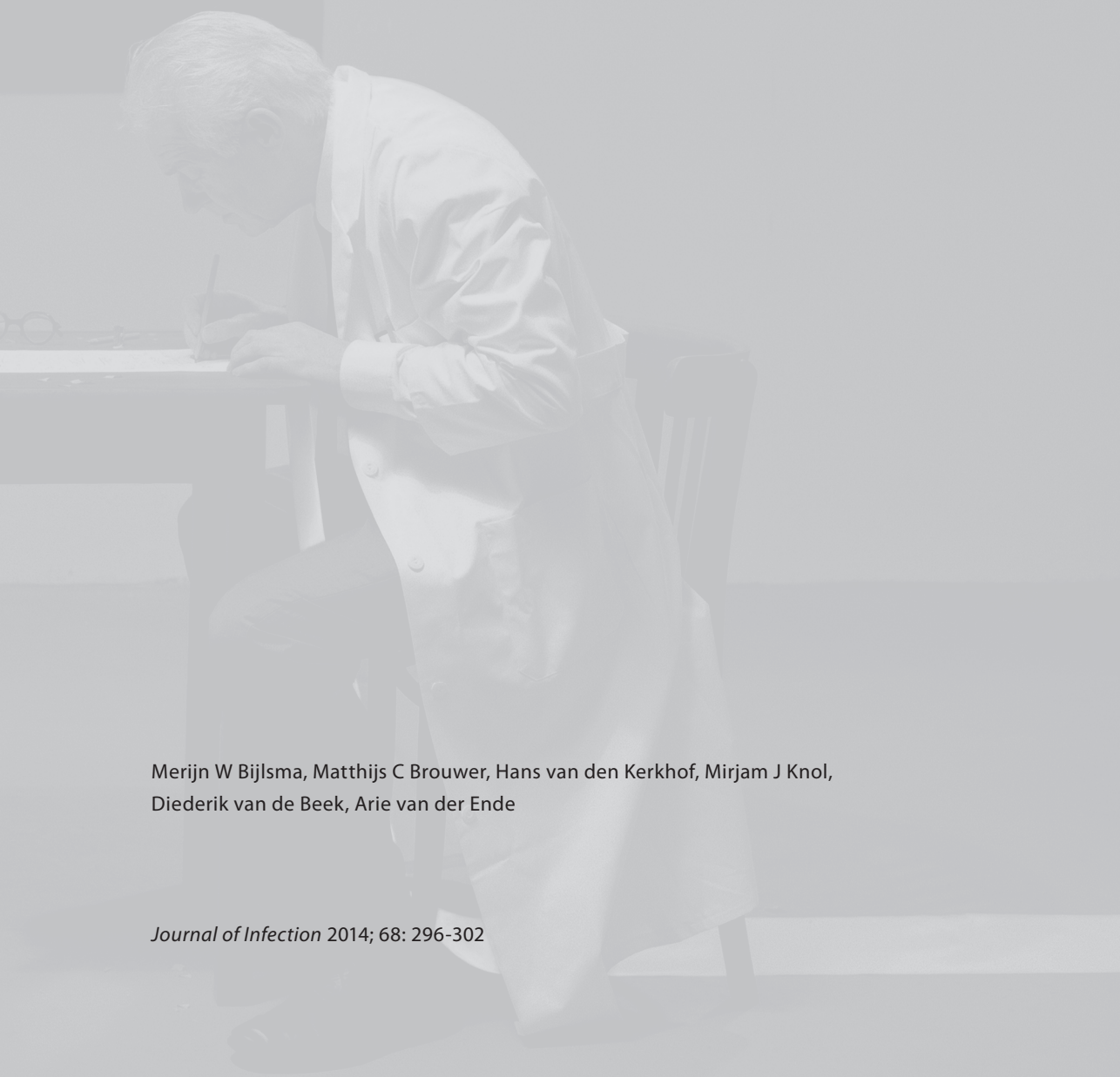
CHAPTER 1

No evidence of clusters of serogroup C meningococcal disease in the Dutch MSM community



Merijn W Bijlsma, Matthijs C Brouwer, Hans van den Kerkhof, Mirjam J Knol, Diederik van de Beek, Arie van der Ende

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To the Editor

Clusters of invasive meningococcal disease (IMD) among men who have sex with men (MSM) have been reported in Toronto (2001), Chicago (2003), New York (2010-13), Paris (2013), Belgium (2013) and Berlin (2013).[1-6] All cases occurred in men aged 20-45 years and were caused by *Neisseria meningitidis* serogroup C, sequence type 11 (ST-11). Fine typing of both PorA and FetA was available for the meningococcal isolates in Berlin and Paris and were of PorA-VR1 5-1, PorA-VR2 10-8: FetA F3-6 type. In response, the European centre for Disease Prevention and Control has called for enhanced surveillance and retrospective investigation of serogroup C cases in young men, and to consider targeted vaccination campaigns as a means of outbreak control.[7]

Amsterdam is a popular destination for MSM, hosting the only waterborne gay-pride parade in the world, held on the canals on the first Saturday in August. We hypothesized that similar outbreaks of serogroup C IMD in MSM may have occurred in the Netherlands. Therefore, we retrospectively evaluated the occurrence of serogroup C IMD cases in particular Nm:C:P1.5-1,10-8:F3-6 in the Netherlands in the database of the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM), with special attention to cases occurring in men aged 20-45. The NRLBM receives meningococcal isolates of more than 90% of IMD patients nationwide.[8]

Between 1960 and 2013, 2610 cases of serogroup C IMD were reported to the NRLBM. Patient age and sex were available for 2430 (93%). The incidence rate increased from 0.05 in 1960 to 1.73 per 100,000 inhabitants in 2001. In response, routine immunization with a serogroup C polysaccharide conjugate vaccine at 14 months and a catch up campaign for children aged 1e19 was initiated in 2002. PorA and FetA fine typing data were available for 69 (91%) of serogroup C cases that occurred from September 2003 to August 2013.

Throughout the observation period of the NRLBM (1960-2013), the proportion of male patients aged 20-45 in all serogroup C cases fluctuated between 0% and 50% per year, with a mean of 9% and a standard deviation (SD) of 7%. The mean proportion per year of woman aged 20-45 was 8% (SD 6%), ranging from 0 to 25%. There were seven years during the observation period in which the proportion of male patients aged 20-45 in all serogroup C cases was more than two standard deviations above the mean yearly proportion. In these seven years no more than four cases per year occurred in men aged 20-45 and there was a minimum of 5 weeks between cases. The Surveillance data of serogroup C cases offer no evidence to suggest that outbreaks of serogroup C disease have previously occurred in Dutch males aged of 20-45.

We subsequently evaluated the occurrence of serogroup C:P1.5-1,10-8:F3-6. In the last decade, 19 (28%) of fully fine typed serogroup C isolates were of P1.5-1,10-8:F3-6 type. Overall, 6 of 19 (32%) cases were male. However, five of the seven (71%) patients in the subgroup of 20e45 year olds were male. These 5 patients lived in different cities and the time interval between cases ranged from 6 weeks to 3 years. All five cases occurred before 2010. From 2010 to 2013, only one patient

with Nm:C:P1.5-1,10-8:F3-6 IMD was identified: a 1-year-old girl. Because of the geographical distribution and time interval between cases, we conclude that there is no evidence that a serogroup C:P1.5-1,10-8:F3-6 IMD epidemic among young males has occurred in the Netherlands.

The Dutch MSM population may be protected from serogroup C meningococcal infections because of the introduction of serogroup C conjugate vaccination in 2002, after which the number of reported cases of serogroup C has declined to around to around 4-11 cases per year.[9] Because of the concurrent catch-up campaign an estimated 95% of Dutch men up to the age of 30 have presently been vaccinated.

In July 2013 the National Institute of Public Health and the Environment (RIVM) convened a meeting to discuss the reported clusters of IMD among MSM. Because no case of serogroup C IMD had been reported in the Dutch MSM community and because of the high vaccine coverage of young males in the Netherlands, The RIVM made the recommendation to the Ministry of Health, Welfare and Sport (VWS) to take no specific action at this time. MSM over 30 years old who intend to visit a city where outbreaks have been reported with the intention to participate actively in the MSM community are advised to consider vaccination before departure.[10]

Our data show that serogroup C:P1.5-1,10-8:F3-6 has so far spared the MSM community in the Netherlands.

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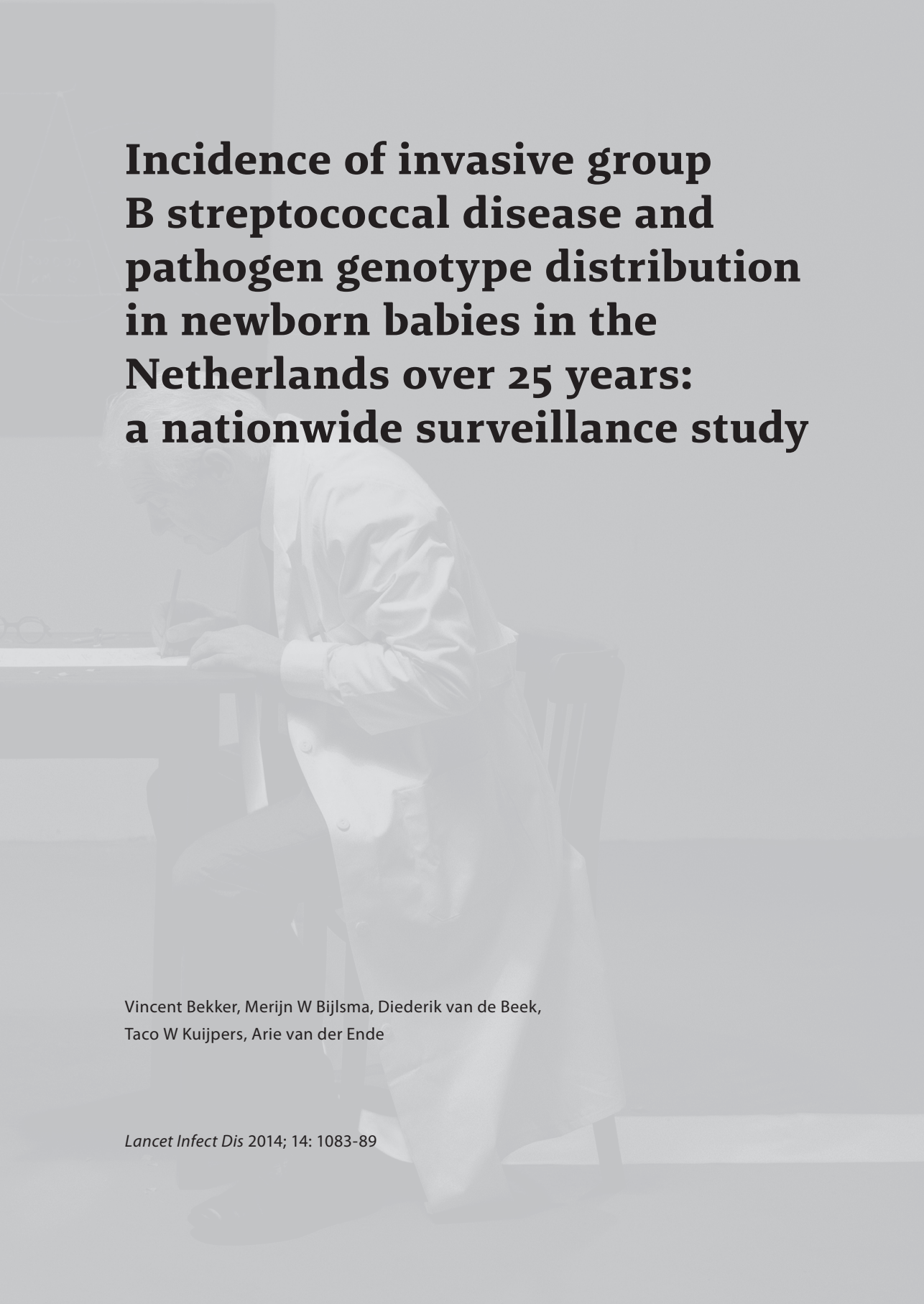
CHAPTER 6

Incidence of invasive group B streptococcal disease and pathogen genotype distribution in newborn babies in the Netherlands over 25 years: a nationwide surveillance study



Vincent Bekker, Merijn W Bijlsma, Diederik van de Beek,
Taco W Kuijpers, Arie van der Ende

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Abstract

Group B streptococcus is the most common cause of neonatal infections. We studied the clinical and molecular epidemiology of invasive group B streptococcus infection in children younger than 3 months in the Netherlands over 25 years. We assessed the effect of the Dutch guidelines, introduced in 1999, for prevention of group B streptococcus, consisting of intravenous antibiotic prophylaxis during labour in cases of premature labour, prolonged rupture of membranes, or fever during delivery. We did this nationwide surveillance study with data from 1987 to 2011, from the Netherlands Reference Laboratory for Bacterial Meningitis. We included data for patients aged 3 months or younger with positive blood culture or cerebrospinal fluid culture for group B streptococcus and Escherichia coli infection. Early onset was defined as less than 7 days after birth and late onset was defined as 7 or more days after birth. We did multilocus sequence typing of a random subset of group B streptococcus samples to assess changes in sequence type (Mann-Kendall trend test) and the distribution of clonal complexes ($\chi 2$ and Fisher exact test) before the introduction of prevention guidelines (1987-99) and afterwards (2000-11). We compared incidences and the distribution of clonal complexes before and after the introduction of guidelines. Most cases of group B streptococcus had early onset (696/1075; 65%). The incidence of invasive group B streptococcus infection increased from 0.20 per 1000 livebirths in 1987, to 0.32 per 1000 livebirths in 2011 ($p<0.0001$). The incidence of early-onset disease increased from 0.11 per 1000 livebirths to 0.19 per 1000 livebirths ($p<0.0001$). The incidence of invasive Escherichia coli infection was 0.05 in 1987, and 0.16 in 2011 ($p=0.17$). Early-onset group B streptococcus infection caused by isolates belonging to clonal complex 17 was more common in the post-implementation period than in the pre-implementation period ($p=0.002$). The introduction of prevention guidelines for invasive group B streptococcus disease in 1999 did not reduce the incidence of disease in neonates. The guidelines should be reassessed and alternative approaches to prevent infant invasive group B streptococcus disease should be sought.

Introduction

Group B streptococcus (*Streptococcus agalactiae*) is the most common cause of neonatal infections.[1] Perinatal group B streptococcus infections are usually classified as early-onset disease, occurring in the first week of life, or late-onset disease, occurring between 1 week and 3 months of age.[2] Early-onset disease is thought to develop in the foetus after aspiration of amniotic fluid infected with bacteria that have ascended from the colonised genital tract of the mother.[3] The pathogenesis of late-onset infections is less well understood.[3] Pathogens can be acquired during passage through the birth canal, but nosocomial and community sources are also probably involved.[3]

The incidence of group B streptococcus in high-income countries varies geographically and over time.[2] Karen Edmond and colleagues[4] did a systematic review to define the present worldwide incidence of group B streptococcus disease in infants younger than 3 months. Limited to data for 2000-11, the estimated overall incidence was 0.53 per 1000 livebirths (95% CI 0.44-0.62) in Europe, 0.67 per 1000 livebirths (0.54-0.80) in North America, and 0.00 per 1000 livebirths (0.00-0.44) in Australia. The incidence in low-income countries, as systematically reviewed by Alemnew Dagnew and coworkers,[5] ranged from 0.00 to 3.06 per 1000 livebirths with variation within and between geographic regions. Risk factors associated with neonatal group B streptococcus disease include maternal colonisation, male sex, black ethnic origin, low concentrations of maternal antibodies against group B streptococcus, prematurity, prolonged rupture of membranes, and intrapartum fever.[2]

In 1986, a randomised controlled trial[6] involving 160 women with group B streptococcus colonisation and various perinatal risk factors (premature labour, prolonged rupture of membranes, or intrapartum fever) showed that intravenous ampicillin prophylaxis during labour significantly reduced neonatal group B streptococcus disease. On the basis of these results, various prevention programmes have been initiated. Two major strategies have been adopted in high-income countries. The first approach is based on risk stratification of women at the time of delivery. Prophylaxis with antibiotics is recommended for women in labour with clinical risk factors for disease transmission, such as intrapartum fever, heavy colonisation with group B streptococcus (ie, bacteriuria), having previously had a child with group B streptococcus disease, preterm delivery, or an interval between rupture of membranes and time of delivery of 18 h or more. The second approach is based on universal screening of pregnant women by vaginal and rectal swabs for group B streptococcus. Intrapartum antibiotic prophylaxis is offered to carriers. In those who were not tested before 35 weeks of gestation, antibiotic prophylaxis is offered to women with any of the clinical risk factors. In the Netherlands, narrow-spectrum penicillin or amoxicillin are preferentially used (intravenously, starting 4 h before delivery if possible). In case of penicillin allergy, macrolides or clindamycin are recommended. Investigators cautioned that, although intrapartum antibiotics are effective for prevention of group B streptococcus disease, they might

select for other virulent and more drug-resistant pathogens, such as *Escherichia coli*.[7] In 1999, the National Society of Obstetrics and Gynaecology and the National Society of Paediatrics in the Netherlands introduced an approach based on risk factors to reduce the occurrence of invasive group B streptococcus disease.[8]

Multilocus sequence typing enables the identification of genetically related group B streptococcus isolates on the basis of similarities in seven conserved housekeeping genes. A combination of unique sequences of the seven loci defines a sequence type. Genetically similar isolates can be grouped into clonal complexes.[9] Five major clonal complexes have been identified in neonates and adults: cc1, cc12, cc19, cc17, and cc23.[9,10,11] Sequence types 17 and 19 cause more neonatal group B streptococcus disease than would be expected on the basis of the proportion of pregnant women with asymptomatic carriage.[10] However, changes in the incidence of different genotypes over more than 10 years have not been studied. We assessed the clinical and molecular epidemiology of invasive group B streptococcus infection over 25 years and assess the effect of the introduction of the prevention programme in Netherlands on the incidence of neonatal group B streptococcus disease.

Methods

Study design and patients

We used data from a nationwide surveillance study of bacterial meningitis and infant bacteraemia that is done by the Netherlands Reference Laboratory for Bacterial Meningitis. From 1975 onwards, medical microbiology laboratories throughout the country have submitted to the laboratory samples of cerebrospinal fluid and blood from all children with invasive group B streptococcus or *E coli* infection. Information about the probable underlying disease and the immunological status of patients is not included. The study covers 84% of patients with meningitis in the Netherlands.[12-14] We included all patients aged 3 months or younger for whom an isolate was received between Jan 1, 1987, and Dec 31, 2011. Patient age was calculated as the number of days between the date of birth and earliest known date of the illness, which mostly was the date of culture from the first positive sample culture. If the culture date was missing, we used the date that the material was sent to or received by the laboratory. If no date of birth or early date of illness or age was recorded, the patient was excluded.

We did this study with anonymous patient data and in accordance with Dutch privacy legislation. Additional institutional review board approval is not required for the assessment of anonymised laboratory surveillance data.

Procedures

We did serogrouping and serotyping as previously described,[15] using monospecific antiserum samples against group B streptococcus serotypes IA, IB, II, III, IV, V, VI, VII, and VIII.

Invasive infection was defined as a positive culture from cerebrospinal fluid or blood. Early-onset disease was defined as invasive infection within 7 days after birth. Late-onset disease was defined as invasive infection between 7 days and 3 months after birth. On the basis of implementation of the guidelines for the prevention of neonatal group B streptococcus infections in the Netherlands, the study period was divided into a pre-implementation period (1987-99) and post-implementation period (2000-11).

For multilocus sequence typing, we selected a random sample of all available strains, stratified by calendar year, with the function "sample" from the R-base package. We did multilocus sequence typing as previously described.[9] Briefly, we used PCR to amplify fragments from seven housekeeping genes (*adhP*, *pheS*, *atr*, *glnA*, *sdhA*, *glcK*, and *tkt*). The seven PCR products were purified and sequenced. We assigned an allele number to each fragment on the basis of its sequence. We then assigned each isolate a sequence type on the basis of the allelic profile of the seven amplicons. We assigned allele and sequence types with the group B streptococcus multilocus sequence typing database. We combined sequence profiles of the Dutch isolates with those of all group B streptococcus isolates in the database with the compare option in eBURST (version 3.0). We assigned isolates to the same group when they shared identical alleles at six of the seven loci with at least one other member of the group. We assigned isolates to a clonal complexes as previously described.[10,16]

Statistical analysis

We calculated incidences with population data obtained from Statistics Netherlands with the use of StatLine. We used R (version 2.15.0) for the statistical analyses. We used Pearson's χ^2 test to assess goodness of fit. We estimated the significance of trends with the Mann-Kendall trend test. For this test, neither normal distribution nor linearity of the trend are needed. This test assesses the increase or decrease of the data elements in the time series. To compare the change in incidence before and after the introduction of prevention guidelines, we calculated the slope of the incidence-time regression line with least-square mean linear regression. We compared the slopes with Student's *t*-test. For the difference in distribution of clonal complexes before and after the introduction of prevention guidelines, we dichotomised values for clonal complexes and analysed them by Pearson's or Fisher exact χ^2 test, as appropriate. We considered a p value of less than 0.05 as statistically significant.

Results

From 1987 to 2011, 1075 cases of invasive group B streptococcus infection (median age 3 days, IQR 1-14) and 474 cases of invasive *E coli* infection (median age 11 days, IQR 6-22) were identified in children aged 3 months or younger. Infection was more common in boys than in girls for both group B streptococcus (p=0.004) and *E. coli* infection (p<0.0001, table 1). The incidence of invasive group B streptococcus infection increased from 0.20 per 1000 livebirths in 1987, to 0.32 per 1000 livebirths in 2011 (p<0.0001), while that of invasive *E. coli* infection was 0.05 cases per 1000 livebirths in 1987 and 0.16 cases per 1000 livebirths in 2011 (p=0.17, figure 1).

Table 1. Patient characteristics.

	Group B streptococcus (n=1075)	***Escherichia coli* (n=474)**
Sex		
Boys	546 (51%)	267 (56%)
Girls	456 (42%)	170 (36%)
Unknown	73 (7%)	37 (8%)
Median age	3 days (1-14)	11 days (6-22)

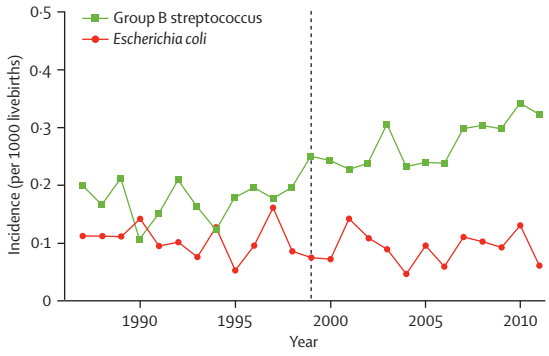

Figure 1. Incidence of group B streptococcus and Escherichia coli invasive disease in the Netherlands among patients aged 3 months or younger.

Vertical dashed line represents the introduction of prevention guidelines in Netherlands in 1999.

Early-onset invasive group B streptococcus infection occurred in 696 neonates (65%) and late-onset invasive group B streptococcus infection occurred in 379 (35%, figure 2). The incidence of early-onset group B streptococcus disease increased from 0.11 to 0.19 per 1000 livebirths (p<0.0001, figure 2). The incidence of late-onset disease increased from 0.03 to 0.13 per 1000 livebirths (p=0.004). The increase in infection in the pre-implementation and post-implementation period were not significantly different for early-onset disease (0.00087 vs 0.0057, p=0.20) or late-onset

disease (0.0002 vs 0.0031, p=0.25, figure 2). Early-onset invasive E. coli occurred in 163 neonates (34%) and late-onset disease in 311 (66%, figure 2).

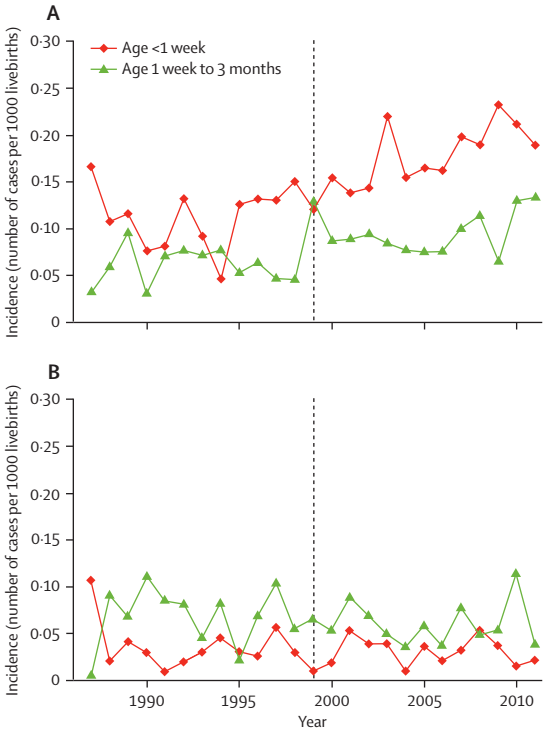

Figure 2. Incidence of group B streptococcus (A) and Escherichia coli (B) by age.
Vertical dashed line represents the introduction of prevention guidelines in Netherlands in 1999.

We did a subgroup analysis of group B streptococcus confirmed by cerebrospinal fluid samples compared with blood culture. Of 1075 confirmed group B streptococcus infections before the age of 3 months, 424 (39%) were cerebrospinal fluid culture positive and 644 (61%) were solely blood culture positive. During the study period, the incidence of blood culture only group B streptococcus increased significantly for both early-onset disease (p<0.0001) and late-onset disease (p<0.0001), but the incidence of cases confirmed from cerebrospinal fluid did not change significantly (appendix).

Of 1075 isolates, 645 (60%) were of serotype III, and 171 (16%) were of serotype IA. The remaining isolates were distributed over eight other serotypes or could not be typed. The distribution of serotypes was not different in the post-implementation period compared with in the pre-implementation period (appendix). Of 167 isolates, we identified 27 different sequence types. Three

sequence types accounted for 66% of the isolates: 44 (26%) isolates were ST17, 41 (25%) were ST19, and 25 (15%) were ST23. The Dutch isolates were mainly distributed in two groups. One group with ST17 as the founder contained 132 (79%) of the Dutch isolates and a second group with ST23 as the founder contained 35 (21%) of the Dutch isolates (figure 3). 51 isolates (31%) of the first group were assigned to clonal complex 19, 49 (29%) to clonal complex 17, 20 (12%) to clonal complex 12, nine (5%) to clonal complex 1, one (1%) to clonal complex 7, and two (1%) to clonal complex 4; the 35 (21%) of the second group to clonal complex 23. Most of the isolates of clonal complex 17 and clonal complex 19 were serotype III (appendix).

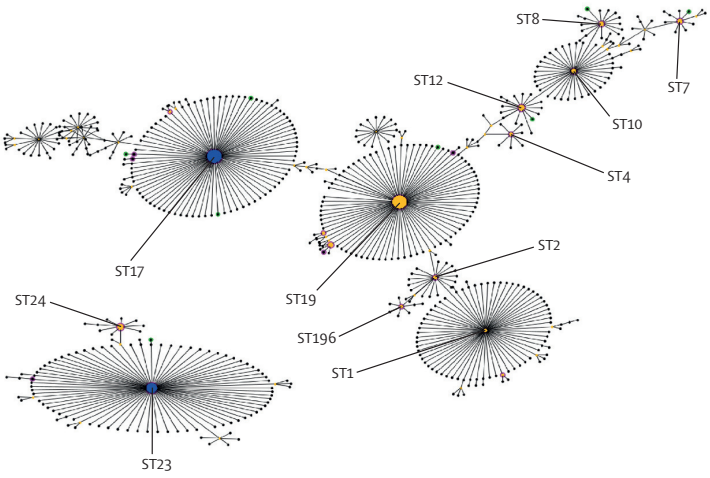

Figure 3. Lineage of group B streptococcus isolates.

Green isolates are unique to the Dutch population. Magenta isolates were found in our Dutch sample and in the database. The size of the dot is proportional to the number of isolates. Sequence types connected by black line are single locus variants of each other. The labelled sequence types were common, central to clonal complexes, or numerous single locus variants. ST17 and ST23, the founders of group 1 and 2, respectively. Yellow dots are secondary founders.

The proportion of isolates belonging to clonal complex 17 was higher in the post-implementation period than in the pre-implementation period (37/99 [37%] vs 12/68 [18%], p=0.006), whereas the isolates belonging to clonal complex 19 were less common in the post-implementation period (21/99 [21%] vs 30/68 [44%], p=0.002, figure 4). The proportion of clonal complex 17 increased among cases of early-onset group B streptococcus disease after implementation (21/63 [33%] post-implementation vs 2/34 [6%] pre-implementation; p=0.002), whereas the proportion of clonal complex 19 decreased among cases of late-onset disease (12/63 [19%] vs 12/34 [35%], p=0.02; figure 4).

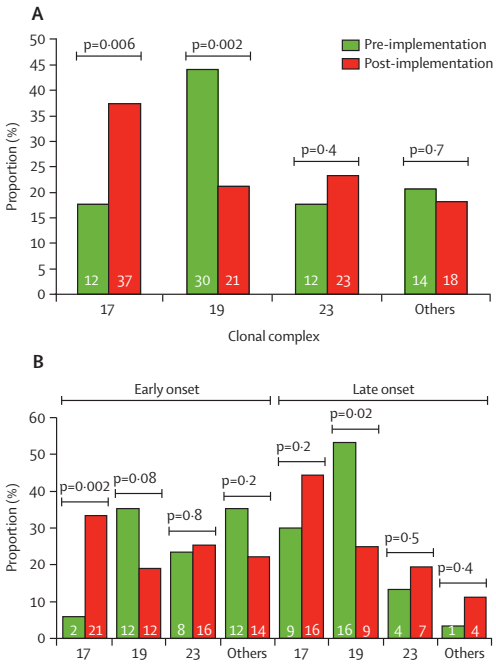

Figure 4. Distribution of clonal complexes.

Overall (A), and stratified by early and late onset (B).

Discussion

We showed a 60% increase in the incidence of infant invasive group B streptococcus infection in the Netherlands over the past 25 years despite the introduction in 1999 of guidelines for prevention of neonatal group B streptococcus disease. Results of a previous study of a shorter period suggested that the introduction of the guidelines stabilised the incidence of neonatal group B streptococcal disease.[17,18] However, we present evidence that, by contrast with what was anticipated, the incidence of invasive group B streptococcus infection has further increased with the same trend as before the introduction of the guidelines. This increase was mainly a result of a rise in the number of cases caused by group B streptococcus belonging to clonal complex 17.

After the guidelines for the use of intrapartum antibiotic prophylaxis were issued in the USA, a large decrease of both group B streptococcal sepsis and meningitis before the age of 7 days occurred, from 1.7 per 1000 livebirths in 1990, to 0.6 per 1000 livebirths in 1998.[19] After the introduction of universal screening in 2002 in the USA, the incidence of early-onset disease fell further, from 0.47 per 1000 livebirth in 1999-2000, to 0.34 per 1000 livebirths in 2003-05, which is still higher than the incidence of 0.2 per 1000 livebirths in Netherlands.[20] Contrary to the USA and most European countries, the UK and Netherlands still use a risk factor-based approach for intrapartum use of antibiotics.[8,21,22]

The implementation of universal screening has led to a substantial increase in the use of antibiotics during labour. Between 1998 and 2002, 35% of mothers who delivered term infants in Utah, USA, received intrapartum antibiotics.[23] A surveillance study[24] in ten US states showed that the proportion of mothers receiving intrapartum antibiotics rose from 27% to 32% from 1998 to 2004. However, preventive intrapartum antibiotics are only effective against early-onset disease.[19,25]

Our findings offer no explanation for the increase in incidence over the past 25 years in Netherlands. Possible explanations include changes in the host, medical practice, increased submission of isolates to the National Laboratory, or the pathogen itself.

Population structure probably plays a part in differences in incidences between regions and countries. A meta-analysis showed large regional differences in the incidence of group B streptococcus disease, ranging from 0.15 cases per 1000 livebirths in the western Pacific, to 1.21 cases per 1000 livebirths in Africa.[4] In the USA, the incidence of group B streptococcus disease is higher in African-American than in European-American neonates.[4,19] This disparity could explain the differences in incidence between these reports and our study.

Additionally, increased recognition of symptoms and a lower threshold for diagnostic tests might have increased registration and so resulted in higher incidence, but this possibility cannot account for the stable incidence of *E. coli* infection. Another potential explanation is the change

in survival over time of pre-term and immature neonates, who are more vulnerable to group B streptococcus infection. However, prematurely born neonates more often have *E. coli* infection than group B streptococcus infection.[1]

Our study is based on historical data. We cannot rule out a reduction in under-reporting by improved submission of isolates to the national laboratory, resulting in an apparent increase in the incidence of group B streptococcus. The incidence of both early-onset and late-onset cerebrospinal fluid-based culture-confirmed infections did not change during the study period (39% of all cases). However, changes in diagnostic practice—eg, blood cultures are increasingly common when sepsis in neonates is suspected—might have increased the number of submitted isolates from blood. Additionally, the incidence of *E. coli* disease among children younger than 3 months did not change during our observation period. Furthermore, our findings are consistent with observations from elsewhere. In the UK, the incidence of disease in children up to 90 days of age also increased, from 0.3 cases per 1000 livebirths in 1977-78, to 0.72 cases per 1000 livebirths in 2000-01.[28] Results of an epidemiological study [27] of England and Wales showed a steady increase for both early-onset and late-onset group B streptococcus infection of up to 5% per year during a 20-year surveillance period. In the USA, the incidence of early-onset of group B streptococcus disease decreased between 2000 and 2003, but increased significantly from 2003 to 2006.[29] By contrast, in Denmark, the incidence of early-onset disease among neonates decreased during 1992-2001.[30] In Denmark, a modified regimen of the risk-based approach for prevention of early-onset group B streptococcus disease was recommended in 1999-2000 and fully implemented during 1999-2001.

Finally, the emergence of new and more virulent group B streptococcus types could be another explanation.[31] The increase of group B streptococcus was associated with a concomitant change in the distribution of clonal complexes. In the post-implementation period more cases were caused by clonal complex 17 and fewer by clonal complex 19, compared with the pre-implementation period. Isolates of clonal complex 17 have been associated with invasive disease in neonates.[9,11,32] Isolates of clonal complex 17 express a unique serine-rich repeat protein (Ssr-2) and, in a mouse model of neonatal sepsis,[33] have a significantly lower lethal dose for 90% of mice than do group B streptococcus isolates that do not express Ssr-2.

Most strains in clonal complexes 1, 17, 19, and 23, cluster into a dominant capsular serotype (cps): cpsV for clonal complex 1, cpsIII for clonal complex 17, cpsIII for clonal complex 19, and cpsIA for clonal complex 23.[34] In our study, almost all isolates of clonal complex 17 and clonal complex 19 were of serotype III. Serotype III is the main cause of group B streptococcus disease in Netherlands, which is relevant for the development of vaccines based on capsular polysaccharides. Comparison of the serotype distribution before and after the introduction of the prevention guidelines showed a similar proportion of serotype III group B streptococcus in both periods, caused by an increase in clonal complex 17 and a decrease in clonal complex 19.

A limitation of our study is that patient age was measured as the difference between the date of birth and the earliest known date of the illness, mostly the date of cerebrospinal fluid or blood culture. This approach might have resulted in an underestimation of cases. Because incidence of group B streptococcus decreases with age, underestimation would be more likely for the group with early-onset disease. However, we calculated age in the same way for patients with *E. coli* and those with group B streptococcus. Moreover, the incidence of both early-onset and late-onset group B streptococcus disease increased. Therefore, this possible underestimation of the true incidence cannot explain the difference in trends between both pathogens.

Furthermore, our incidence estimates might be underestimated because we included only culture-confirmed cases. Also, although we describe the most extensive series published thus far, this study was limited by the fact that the surveillance system does not collect data for underlying diseases or for outcome of the disease.

This study, based on surveillance data, cannot establish the effectiveness of neonatal group B streptococcus prevention based on risk stratification of women at the time of delivery. The incidence of group B streptococcus would have probably been higher in the absence of prevention. However, even if current practice is (partly) effective, and rising incidence is (partly) caused by changes in diagnostic or reporting practices, the number of neonates with positive group B streptococcus cultures is not decreasing. We believe that our study should lead to a reassessment of current practices. Whether the Netherlands should move to a culture-based screening programme is beyond the scope of this study. Results from a cost-effectiveness study[35] in 2006 in the Netherlands showed a much higher cost-effectiveness ratio per quality-adjusted life-year gained for the screening-based strategy compared with the risk-based strategy. The effect of an increasing incidence of neonatal group B streptococcus disease on the cost-effectiveness of both strategies remains to be assessed. Vaccination against group B streptococcus is a promising alternative to be seriously considered for prevention of neonatal invasive group B streptococcus infection, although vaccines are still at an early stage of development.

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Supplementary material

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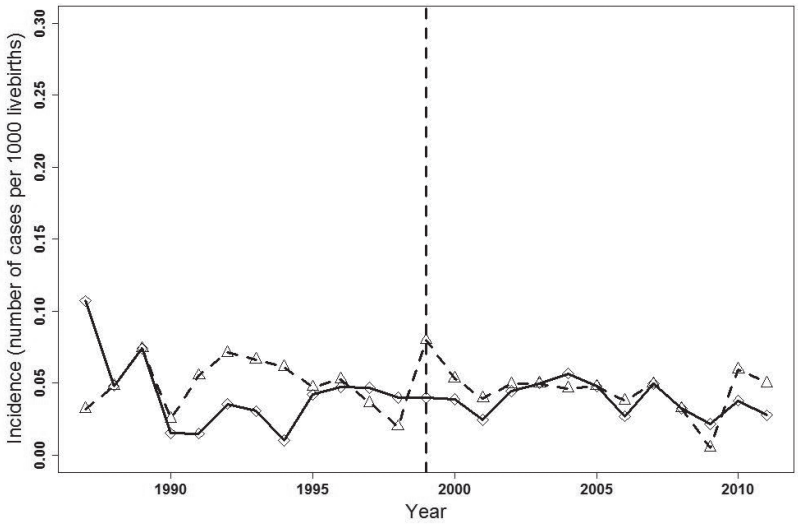
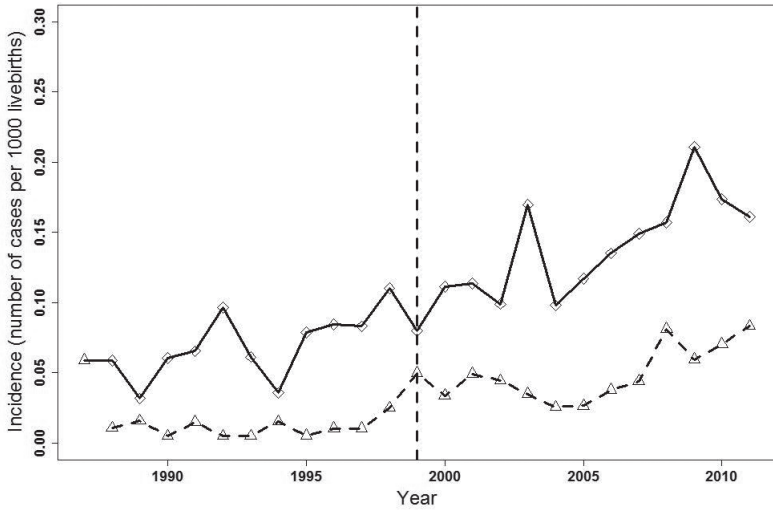


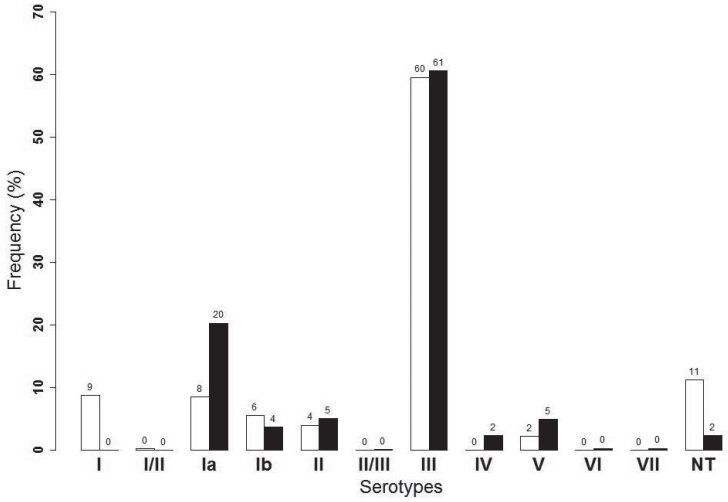
Supplementary figure 1. Incidence rates of GBS invasive disease in the Netherlands among patients aged 3 months or less. (A) CSF culture confirmed; (B) Blood culture confirmed.

Supplementary figure 1A.
Age less than 1 week (early-onset disease), diamonds with solid line and age between 1 week and 3 months (late-onset disease), dashed line.



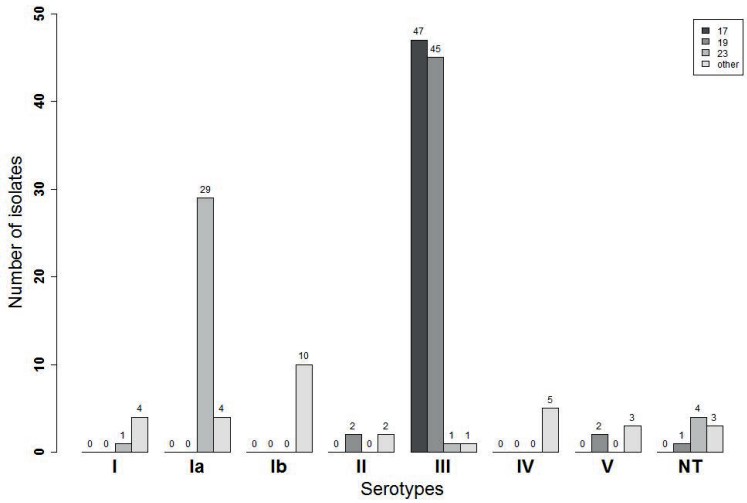
Supplementary figure 1B.

Age less than 1 week (early-onset disease), diamonds with solid line and age between 1 week and 3 months (late-onset disease), dashed line.



Supplementary figure 2. GBS serotypes distribution. Serotypes of all G BS isolates stratified by pre- and post-implementation period.

White bars: guidelines' pre-implementation period; black bars: guidelines' post-implementation period. Numbers indicate absolute numbers of isolates.



Supplementary figure 3. Association between GBS serotypes and clonal complex. Serotype distribution among the 167 randomly selected samples for clonal complex analysis.

CHAPTER 7

Risk score for identifying adults with CSF pleocytosis and negative CSF Gram stain at low risk for an urgent treatable cause

Rodrigo Hasbun, Merijn Bijlsma, Matthijs C Brouwer, Nabil Khoury, Christiane M Hadi, Arie van der Ende, Susan H Wootton, Lucrecia Salazar, Md Monir Hossain, Mark Beilke, Diederik van de Beek

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Abstract

We aimed to derive and validate a risk score that identifies adults with cerebrospinal fluid (CSF) pleocytosis and a negative CSF Gram stain at low risk for an urgent treatable cause. Patients with CSF pleocytosis and a negative CSF Gram stain were stratified into a prospective derivation (n = 193) and a retrospective validation (n = 567) cohort. Clinically related baseline characteristics were grouped into three composite variables, each independently associated with a set of predefined urgent treatable causes. We subsequently derived a risk score classifying patients into low (0 composite variables present) or high (≥1 composite variables present) risk for an urgent treatable cause. The sensitivity of the risk score was determined in the validation cohort and in a prospective case series of 214 adults with CSF culture proven bacterial meningitis, CSF pleocytosis and a negative Gram stain. A total of 41 of 193 patients (21%) in the derivation cohort and 71 of 567 (13%) in the validation cohort had an urgent treatable cause. Sensitivity of the dichotomized risk score to detect an urgent treatable cause was 100.0% (95% CI 93.9-100.0%) in the validation cohort and 100.0% (95% CI 97.8-100.0%) in bacterial meningitis patients. The risk score can be used to identify adults with CSF pleocytosis and a negative CSF Gram stain at low risk for an urgent treatable cause.

Introduction

Inflammation of the central nervous system is a medical emergency that is associated with high mortality and morbidity. It is generally confirmed if the cerebrospinal fluid (CSF) shows an elevated white blood cell count (pleocytosis). The epidemiology of infectious meningitis and/or encephalitis has changed in the last decades due to routine vaccination, an increase in conditions causing an impaired immune response and the emergence of "new" pathogens, such as the West Nile virus.[1-3,5] Furthermore, advances in diagnostic microbiology, virology, and neuroradiology have expanded the etiological differential diagnosis of inflammation of the central nervous system.[2]

The primary concern of physicians treating patients with CSF pleocytosis is to distinguish between an urgent and treatable cause, *e.g.*, bacterial meningitis, and conditions of less concerning aetiologies (*e.g.*, viral meningitis). An important diagnostic test is CSF Gram staining, which is rapid, inexpensive and well validated for detecting bacteria. Although CSF Gram stain has good specificity, its sensitivity is highly variable depending on the patient population, pathogen and previous treatment with antibiotics.[5-7] Consequently, a negative CSF Gram stain makes viral meningitis likely, but does not rule out bacterial meningitis or another urgent treatable cause. Most patients with CSF pleocytosis and negative CSF Gram stain are therefore admitted and treated empirically with intravenous antibiotic therapy and anti-viral therapy until CSF and blood cultures are negative and the clinical condition improves, even though only a small minority will turn out to have an urgent treatable cause.[1] This uncertainty about the probability of an urgent treatable cause in an individual patient thus leads to costly diagnostic testing, admission and treatment.

The purpose of this observational study was to identify predictors of an urgent treatable cause, and subsequently derive and validate a risk score that can identify patients at very low risk of an urgent treatable cause in adults with CSF pleocytosis and negative CSF Gram stain.

Methods

Case definition and data collection

Cases were defined as adult patients (age >16 yrs) presenting to an emergency department with community-acquired symptoms of meningitis, CSF white cell count >5 cells/mm^3, and negative CSF Gram stain. Patients with neurosurgical procedures, previous treatment with antibiotics for >48 h and unknown aetiology, and recurrent meningitis were excluded from the analysis.

The derivation cohort was assembled prospectively at Tulane University Hospital and Clinic and the Medical Center of Louisiana in New Orleans between 1999 and 2008 – interrupted between 2005 and 2006 due to Hurricane Katrina. The study was approved by the Institutional

Review Board and written informed consent was obtained. The validation cohort was assembled retrospectively at eight Memorial Hermann hospitals (Texas Medical Center, Katy, Woodlands, Memorial City, Sugarland, Northwest, Southwest, Southeast) in the Greater Houston area and surroundings between 2005 and 2010. This validation study was approved by the University of Texas Health in Houston Committee for the Protection of Human Subjects and by the Memorial Hermann Hospital Research Review Committee. Baseline characteristics were identified when the patient was in the emergency department. Sociodemographic data, comorbid conditions (Charlson comorbidity scale), immunocompetence, exposures, clinical features and Glasgow coma scale, laboratory results and management decisions were recorded.[8,9]

To further evaluate the risk of missing bacterial meningitis cases, a second validation study was performed in two nationwide prospective cohort studies on patients with bacterial meningitis in the Netherlands. Patients were prospectively included between 1998–2002 and 2006–2012 if they were over 16 years old and had CSF culture proven community-acquired bacterial meningitis. In- and exclusion criteria have previously been published.[7,10] Patients were considered immunocompromised if they used immunosuppressive medication, or had HIV/AIDS. The Charlson comorbidity scale and data on IV drug use were not routinely recorded. For those variables necessary for risk categorization, missing data were assumed to be normal; this will lead to an underestimation of the sensitivity of the risk score. All local ethics committees of participating hospitals approved the studies. Written informed consent was obtained from all participating patients or their legally authorized representatives.

Laboratory testing and diagnostic criteria

CSF from all patients was tested for glucose, protein, cell count, and was sent for bacterial culture. Further diagnostic testing for causative microorganisms by additional cultures (fungal, acid fast bacilli), polymerase chain reaction (PCR) and serologic tests were performed at the discretion of the treating physician. For the diagnosis of bacterial or fungal meningitis a positive CSF culture or antigen detection was required. The diagnosis of *Enterovirus*, HSV, and CMV meningitis required either positive CSF culture or PCR. Diagnosis of Varicella Zoster Virus (VZV) required either a positive culture or PCR in the CSF or isolation of VZV (viral culture or a positive VZV Direct fluorescent antibody) from a coexisting vesicular skin lesion. Diagnosis of rickettsial disease required evidence of specific serum antibody or demonstration of *Rickettsia* from a skin biopsy. Bacteraemia required isolation of the pathogen from at least two blood culture samples. Diagnosis of meningeal carcinomatosis required positive CSF cytology. Parameningeal and intracranial mass lesions and intracranial haemorrhages required documentation by either cranial or spinal imaging.

Classification of aetiology and outcome

Cases were divided into four etiological categories: (1) unknown cause; (2) untreatable cause; (3) treatable but not urgent cause; (4) urgent treatable cause. Urgent treatable cause was defined as bacterial, fungal, or tuberculous meningitis; Herpes simplex virus (HSV), VZV, or Cytomegalovirus (CMV) meningoencephalitis, rickettsial meningoencephalitis; bacteraemia; central nervous system vasculitis; intracranial haemorrhages; or parameningeal or intracranial abscess.[2] HSV aseptic meningitis was considered a treatable but not urgent cause as there is no data to suggest that early acyclovir therapy in this syndrome improves clinical outcomes. The primary study endpoint was the presence of an urgent treatable cause. In the derivation, validation and bacterial meningitis cohorts, outcome was graded according to the Glasgow Outcome Scale.[11]

Statistical analysis

A sample size of at least 175 patients in the derivation sample was needed to achieve 80% power to detect significant differences in the proportions of clinically plausible variables using a two-tailed test with a *p*-value of <0.05. Baseline characteristics having a clinically plausible association

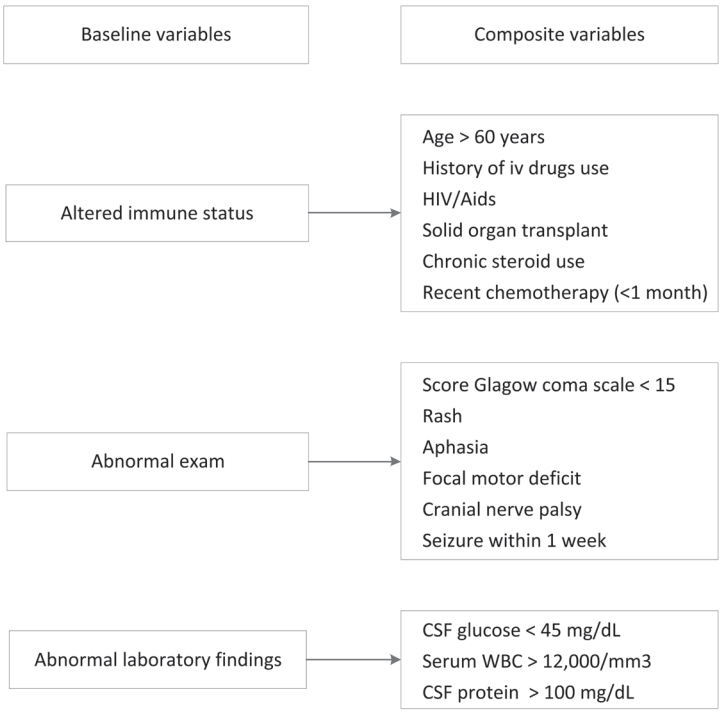

Figure 1. Grouping of baseline variables into composite variables.

with an urgent treatable cause were examined in bivariate analysis. To avoid overfitting in the regression modelling, no more than one variable was entered per 10 outcome events.[12,13] As a variable reduction strategy, clinically related baseline variables showing a bivariate association were grouped into three composite variables: altered immune status, abnormal exam, and abnormal laboratory findings (figure 1). The composite variables were entered into a logistic regression model to verify independent associations with an urgent treatable cause.

Using these composite variables, we subsequently developed a dichotomized risk score to classify patients into low (0 composite variables present) or high (≥1 composite variables present) risk for an urgent treatable cause.

Sensitivity of the risk score was defined as the proportion of patients with an elevated risk score out of all patients with a UTC. Specificity of the risk score was defined as the proportion of low risk patients out of all patients without a UTC. We performed a second external validation study in the Dutch bacterial meningitis cohort. Finally we compared our risk score to a previously published prediction model, developed to predict an urgent treatable cause (bacterial meningitis) in adult patients with acute meningitis and a negative CSF Gram stain.[14]

Results

Derivation and validation cohorts

Two hundred and forty-two patients were screened for inclusion in the derivation study, 49 (20%) were excluded, leaving 193 patients to be enrolled in the derivation study (figure 2A). For the validation study, 747 patients with meningitis were screened, of which 180 patients (24%) were excluded, leaving 567 patients for the clinical prediction score (figure 2B). The derivation and validation cohorts were similar regarding age, gender, duration of illness, physical and neurological examination, and mortality rate. Ethnicity, medical history, and cranial imaging results were different between cohorts. In the validation cohort a higher rate of patients were admitted, treated with empiric acyclovir, and received cranial imaging (table 1).

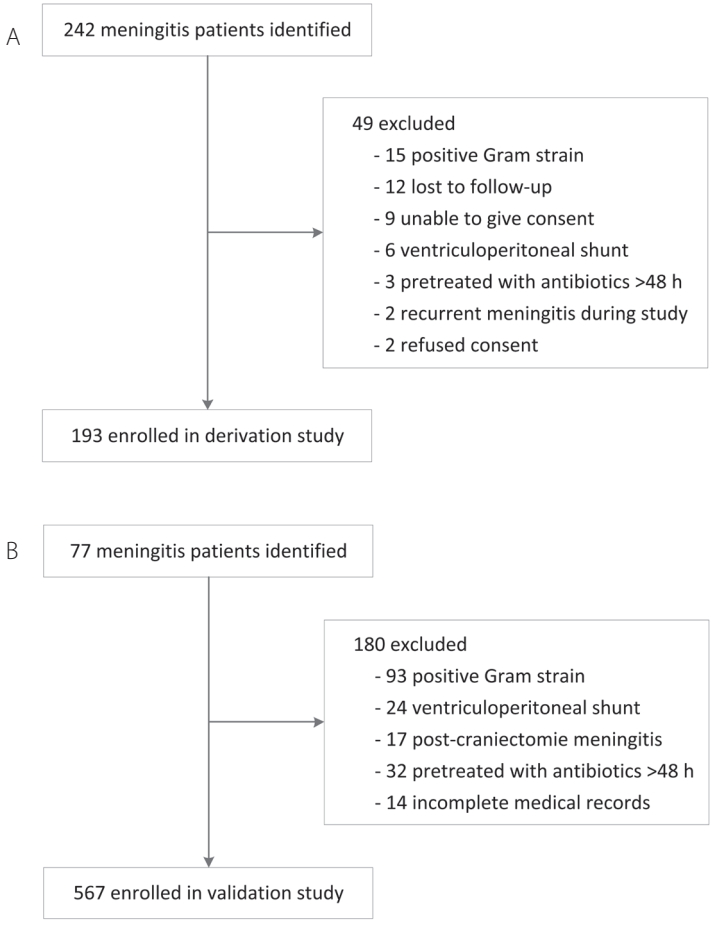

Figure 2. Flowcharts of patient inclusion in derivation cohort (a) and validation cohort (b).

Table 1. Clinical and laboratory characteristics of adults with CSF pleocytosis and a negative Gram stain.[a]

Variable	Derivation cohort (*n* = 193)	Validation cohort (*n* = 567)	Variable	Derivation cohort (*n* = 193)	Validation cohort (*n* = 567)
Age, years	38 (18–96)	36 (18–92)	Neck stiffness	52 (27%)	160 (18%)
Female	102 (53%)	312 (55%)	Altered mental status[b]	38 (21%)	106 (19%)
Ethnicity			Focal neurologic deficits[c]	20 (11%)	57 (10%)
African American*	142 (74%)	143 (26%)	Vesicular or petechial rash*	10 (6%)	10 (2%)
White*	43 (22%)	265 (47%)	*Laboratory results*		
Hispanic*	7 (4%)	138 (24%)	Serum leukocyte count >12,000/mm^3	52 (27%)	133 (23%)
Asian*	0 (0%)	17 (3%)	CSF leukocyte count >1000/mm^3	23 (12%)	43 (8%)
			CSF protein >100 mg/dL	57 (30%)	204 (36%)
Coexisting medical condition			CSF glucose <45 mg/dL*	44 (23%)	80 (14%)
Charlson comorbidity index >1*	63 (33%)	70 (12%)	*Management decisions*		
I mmunodeficiency*	63 (33%)	62 (11%)	Admission to hospital*	157 (88%)	546 (97%)
HIV/AIDS*	56 (29%)	44 (8%)	Empiric antibiotic therapy*	153 (86%)	415 (73%)
Intravenous drug use*	26 (13%)	13 (2%)	Median duration of antibiotics (days)	3 (0–44)	2 (0–45)
Presenting history			Empiric acyclovir therapy*	34 (19%)	155 (27%)
Headache	163 (84%)	510 (93%)	Cranial CT*	166 (86%)	512 (90%)
Fever*	142 (74%)	363 (64%)	Abnormal[d]	20 (10%)	36 (7%)
Photophobia	95 (49%)	280 (57%)	Cranial MRI*	39 (20%)	228 (40%)
Respiratory symptoms*	51 (26%)	63 (11%)	Abnormal[e]	24 (62%)	77 (34%)
Seizure <1 week of presentation	14 (7%)	25 (4%)	*Clinical status at discharge*		
Duration of illness, days (range)	3 (0–30)	3 (0–30)	Normal	168 (87%)	503 (89%)
Presenting signs, n (%)			Morbidity[f]	16 (9%)	56 (10%)
Temperature >100.4° F*	94 (49%)	170 (30%)	Death	5 (3%)	6 (1%)
			Unknown	4 (2%)	1 (0%)

*Significant difference between derivation and validation cohort $p < 0.05$.
[a] Data are n/N (%), or median (range).
[b] Disorientation or Glasgow coma scale <15.
[c] Focal motor deficit, cranial nerve abnormality or aphasia.
[d] Focal (i.e., mass lesions, strokes, or bleeds) or nonfocal (i.e., hydrocephalus, white matter changes) intracranial abnormalities.
[e] Mass lesions, strokes, hypoattenuations, meningeal enhancement, bleeds, white matter abnormalities.
[f] Glasgow Outcome Scale from 2 to 4.

Characteristics of patients with CSF pleocytosis and negative Gram stain

Clinical and laboratory characteristics of adults with CSF pleocytosis and negative Gram stain are summarized in table 1. The total cohort (n = 760) consisted primarily of young adults (median age 36 years) with 54% of the patients being female and 59% belonging to a minority (Hispanic, African-American, or Asian). Co-morbidity was present in 133 patients (18%) and 100 patients (13%) were HIV positive; 39 (5%) had a history of intravenous drug use. Presenting symptoms included headache (89%), fever (66%), photophobia (49%), and stiff neck (47%). Thirty-nine patients (5%) had a seizure within 1 week prior to arrival to the emergency department. On examination 77 patients (10%) had focal neurologic deficits, 144 individuals (19%) had a reduced level of consciousness and 20 (3%) had a vesicular or petechial rash. A cranial CT scan was done in 678 (89%) patients as part of their initial evaluation in the emergency department, being abnormal in 56 (7%). Additionally, 263 (35%) also underwent a cranial MRI during admission, with 95 (35%) of them showing mass lesions, strokes, hypo-attenuations, meningeal enhancement, bleeds, or white matter abnormalities (table 1).

Diagnostic causes (table 2) for the episode of meningitis were identified in 215 patients (28%) of the total cohort, the most common diagnoses were: HSV type 2 (5%), West Nile virus (4%), cryptococcal (3%), bacterial (3%) and enterovirus meningitis (3%). No cause was identified in 545 patients (72%). In the subgroup of 108 patients (14%) with urgent treatable causes the most common diagnoses were bacterial meningitis (32%), and cryptococcal meningitis (20%). Untreatable and non-urgent treatable conditions were both identified in 7% each.

Seven hundred and three patients (93%) were admitted to the hospital and 568 patients (75%) received empirical antibiotic therapy, while waiting for culture results. The median duration of antibiotic therapy was 2 days. Empiric intravenous antiviral therapy with acyclovir was administered in 189 patients (25%). Follow up was available for 755 (99%) of the patients. The majority of patients (671; 89%) had no residual neurological morbidity; 72 patients (9%) had persistent neurological deficits or seizures and required transfer to a rehabilitation facility. Additional one-month follow up was available in the derivation cohort only, 143 out of 167 (87%) had no residual morbidity or deficits.

The overall mortality rate was 1.4% (11 of 760 patients). Death was caused by encephalitis of unknown aetiology in two patients, and by methicillin-sensitive Staphylococcus aureus (MSSA) bacteraemia, MSSA endocarditis, HSV encephalitis, gliomatosis cerebri, AIDS-dementia-complex, end-stage AIDS, CMV encephalitis, cerebral toxoplasmosis, and Mycobacterium tuberculosis meningitis in one patient each.

Table 2. Etiologies of meningitis and negative Gram stain n (%).

Aetiology	Total cohort (*n* = 760)	Derivation cohort (*n* = 193)	Validation cohort (*n* = 567)
Urgent treatable cause	108 (14.2%)	41 (21.2%)	71 (12.5%)
Bacterial meningitis[a]	31 (4.1%)	15 (7.7%)	16 (2.8%)
Cryptococcus neoformans	22 (2.9%)	8 (4.1%)	14 (2.5%)
Other serious bacterial infections[b]	18 (2.4%)	7 (3.6%)	11 (1.9%)
Herpes simplex encephalitis	15 (2.0%)	2 (1.0%)	13 (2.3%)
Varicella zoster virus	8 (1.1%)	3 (1.6%)	5 (0.9%)
Mycobacterium tuberculosis	7 (0.9%)	2 (1.0%)	5 (0.9%)
Toxoplasma gondii	3 (0.4%)	2 (1.0%)	1 (0.2%)
CNS vasculitis	3 (0.4%)	0 (0.0%)	3 (0.5%)
Other[c]	7 (0.9%)	3 (1.5%)	4 (0.7%)
Untreatable causes	54 (7.1%)	10 (5.2%)	44 (7.8%)
West Nile Virus	30 (3.9%)	1 (0.5%)	29 (5.1%)
Enterovirus	19 (2.5%)	8 (4.1%)	11 (1.9%)
St. Louis Encephalitis	3 (0.4%)	0 (0.0%)	3 (0.5%)
Epstein Barr virus	2 (0.3%)	1 (0.5%)	1 (0.2%)
Non-urgent treatable causes	53 (7.0%)	9 (4.6%)	44 (7.7%)
HSV type 2 [d]	37 (4.9%)	2 (1.0%)	34 (6.0%)
CNS lymphoma/carcinomatosis	5 (0.7%)	3 (1.0%)	3 (0.5%)
Neurosyphilis	2 (0.3%)	1 (0.5%)	1 (0.2%)
Multiple sclerosis	2 (0.3%)	1 (0.5%)	1 (0.2%)
Other[e]	4 (0.5%)	2 (1.0%)	2 (0.4%)
Unknown aetiology[f]	545 (71.7%)	134 (69.4%)	407 (71.8%)

Bold values represent that cases were divided into four etiological categories: 1) unknown cause; 2) untreatable cause; 3) treatable but not urgent cause; 4) urgent treatable cause. Urgent treatable cause was defined as bacterial, fungal, or tuberculous meningitis; Herpes simplex virus (HSV), VZV, or Cytomegalovirus (CMV) meningoencephalitis, rickettsial meningoencephalitis; bacteraemia; meningeal carcinomatosis; central nervous system vasculitis; intracranial haemorrhages; or parameningeal or intracranial mass lesions (e.g., tumor, abscess).

[a] Cerebrospinal fluid culture positive (20): *Streptococcus pneumoniae* (8), *Haemophilus influenzae* (3), *Listeria monocytogenes* (2), Methicillin-resistant *Staphylococcus aureus* (2), *Neisseria meningitidis* (1), *Streptococcus agalactiae* (2) and *Enterococcus* (2). Blood culture positive and cerebrospinal fluid culture negative (11): Methicillin-susceptible *Staphylococcus aureus* (2), Methicillin-resistant *Staphylococcus aureus* (2), *Enterococcus faecalis* (1), Vancomycin-resistant *Enterococcus* (1), *Streptococcus agalactiae* (2), Alpha haemolytic *Streptococcus* (1), *Streptococcus pyogenes* (1), *Listeria monocytogenes* (1).

[b] *taphylococcus aureus* infective endocarditis (3), *Escherichia coli* urosepsis (3), Brain abscess (2), Lemierre's syndrome with *Fusobacterium necrophorum* (1).

[c] *Cytomegalovirus* encephalitis (1), *Taenia solium* (1), *Histoplasma capsulatum* (1), subarachnoid haemorrhage (1), transverse myelitis (1), epidural abscess (1), gliomatosis cerebri (1).

[d] Herpes simplex virus aseptic meningitis was considered a treatable but not urgent cause.

[e] Neurosarcoidosis, Lyme disease, *Influenza A*, *Cytomegalovirus* was diagnosed in one patient each.

[f] Patients with unknown causes had less cerebrospinal fluid polymerase chain reaction for enterovirus and herpes viruses performed than those with known causes ($p < 0.05$).

Development and validation of a risk score for an urgent treatable cause

Bivariate analyses relating clinically cogent variables to an urgent treatable cause in the derivation cohort are shown in table 3. As a variable reduction strategy and to avoid overfitting, baseline variables showing a bivariate association ($p < 0.05$) were grouped into three composite variables: altered immune status, abnormal exam, and abnormal laboratory exam (figure 1). A composite value was present if one or more of the baseline variables applied to the patient. The composite variables were all independently associated with an urgent treatable cause (adjusted odds ratios (OR): altered immune status 2.86, 95% confidence interval (CI) 1.28–6.38; abnormal examination 2.63, 95% CI 1.20–5.77; abnormal laboratory exam 10.22, 95% CI 3.94–26.50). We subsequently derived a risk score classifying patients into low (0 composite variables present) or high (≥1 composite variables present) risk for an urgent treatable cause. In the New Orleans derivation cohort, none of the 55 patients in the low risk group had an UTC, while 41 out of 138 (30%) in the high risk had an UTC (sensitivity 100% (95% CI 0.89–1.0), specificity 36% (95% CI, 0.29–0.44), negative predictive value 100% (95% CI, 0.92–1.0), positive predictive value 29.7% (95% CI, 0.22–0.38)).

Table 3. Baseline variables associated with an urgent treatable cause in 193 adults with CSF pleocytosis and a negative CSF Gram stain.

Baseline characteristics	Patients with UTC *n/N* (%)	Odds ratio (95% CI)	*p*-Value
Host variables			
Age >60 years	7/14 (50%)	3.59 (1.19–10.85)	0.02
Intravenous drug use	13/26 (50%)	4.44 (1.86–10.60)	0.0004
Immunosuppression[a]	21/63 (33%)	2.10 (1.06–4.16)	0.03
Comorbidity[b]	19/61 (31%)	1.76 (0.88–3.49)	0.11
Physical examination			
Fever[c]	19/89 (21%)	0.89 (0.44–1.78)	0.74
Altered mental status[d]	19/41 (46%)	3.99 (1.91–8.39)	0.0001
Abnormal neurologic exam[d]	16/43 (37%)	2.37 (1.13–4.95)	0.02
Rash	6/12 (50%)	3.52 (1.08–11.53)	0.03
Laboratory variables			
CSF protein >100 mg/dL	24/53 (45%)	4.44 (2.19–9.00)	<0.0001
CSF glucose <45 mg/dL	26/47 (55%)	7.80 (3.71–16.41)	<0.0001
CSF WBC >1000/mm^3	26/87 (30%)	1.83 (0.94–3.58)	0.07
CSF ANC[e] >500/mm^3	9/22 (41%)	2.34 (0.93–5.89)	0.06
Serum WBC >12,000/μL	22/52 (44%)	3.58 (1.77–7.23)	0.0003

[a] HIV/AIDS, solid organ transplant, recent chemotherapy, or chronic use of steroids.
[b] Charlson's comorbidity score >1.
[c] Temperature >38.4 °C or <35.0 °C.
[d] See figure. 1.
[e] Absolute neutrophilic count (Cerebrospinal fluid cell count × neutrophilic percent).

Validation of the risk score to identify patients at low risk for an urgent treatable cause

A false negative test result was defined as a patient with an urgent treatable cause, classified as low risk, by the risk score. In the Houston validation cohort, none of the 222 patients in the low risk group had an UTC, while 71 out of 345 (20.6%) in the high risk had an UTC (sensitivity 100% (95% CI 0.94–1.0), specificity 45% (95% CI, 0.41–0.50), negative predictive value 100% (95% CI, 0.98–1.0), positive predictive value 21.8% (95% CI, 0.18–0.26)) (table 4).

Table 4. Risk score for an urgent treatable cause of CSF pleocytosis and a negative Gram stain.

Risk group	Composite variablesa	Patients with an UTC (n/N, %)
Derivation sample (n = 193)[b]		
Low	0	0/55 (0%)
High	≥1	41/138 (30%)
Validation sample (n = 567)[c]		
Low	0	0/222 (0%)
High	≥1	71/345 (20.6%)

[a] See figure. 1.
[b] Sensitivity 100% (95% CI 0.89–1.0), specificity 36% (95% CI, 0.29–0.44), negative predictive value 100% (95% CI, 0.92–1.0), positive predictive value 29.7% (95% CI, 0.22–0.38).
[c] Sensitivity 100% (95% CI 0.94–1.0), specificity 45% (95% CI, 0.40–0.49), negative predictive value 100% (95% CI, 0.98–1.0), positive predictive value 20.6% (95% CI, 0.16–0.25).

Although bacterial meningitis was the second most common urgent treatable cause in the validation cohort, the absolute number of cases was low. To further evaluate performance of the risk score in this clinically import subgroup, we subsequently assessed sensitivity of the risk score in a cohort of adults with bacterial meningitis. Out of a total of 1728 episodes of culture proven and community acquired bacterial meningitis, we identified 214 (12%) patients with a CSF white cell count >5 cells/mm3 and a negative CSF Gram stain (supplemental fig. 1). Of these 214 patients, 66 (31%) had pneumococcal meningitis and 44 (18%) meningococcal meningitis. Variables included in the risk score were present in the majority of patients (table 5). Sensitivity of the risk score to detect bacterial meningitis in patients with a CSF pleocytosis and a negative Gram stain was 100.0% (95% CI 97.8–100.0%).

Table 5. Baseline characteristics, composite variables, and risk scores in patients with bacterial meningitis.

Baseline variables	Bacterial meningitis with pleocytosis and a negative CSF Gram stain (*n* = 214)
Host variables[a]	
Age >60 years	95/214 (44%)
Immunosuppression[b]	18/133 (14%)
Exam variables	
Fever[c]	134/209 (64%)
Altered mental status[d]	147/214 (69%)
Abnormal neurological exam[e]	55/214 (26%)
Vesicular or petechial rash	26/213 (12%)
Laboratory variables	
CSF protein >100 mg/dL	172/203 (85%)
CSF glucose <45 mg/dL	123/206 (60%)
CSF WBC >1000/mm^3	138/214 (65%)
Serum WBC >12,000/μL	145/213 (68%)
Composite variables[f]	
Altered immune status	100/214 (47%)
Abnormal examination	169/214 (79%)
Abnormal laboratory findings	192/214 (90%)
Risk score[g]	
Low (0)	0/214 (0%)
High (≥1)	214/214 (100%)

[a] History of intravenous drug use and Charlson's comorbidity index not assessed.
[b] HIV infection cohort 1998. Immunosuppressive drugs or HIV infection in cohort 2006.
[c] T > 38.4 C or <35.0 C.
[d] Glasgow coma scale <15.
[e] Aphasia or cranial nerve palsy or paresis of one or more arms or legs or (recent) seizure. Missing data are assumed to be normal.
[f] See figure. 1. Missing data are assumed to be normal.
[g] Sensitivity 100% (95% CI 0.98–1.0), positive predictive value 100% (95% CI, 0.98–1.0).

We know of one previously published risk score that was developed to predict bacterial meningitis in adult patients with acute meningitis and a negative CSF Gram stain.[14] We compared this previously published risk score to our risk score in the subgroup of 214 bacterial meningitis patients with community acquired bacterial meningitis, a CSF pleocytosis and a negative CSF gram. After exclusion of 96 of the 214 (49%) cases because of missing data for 1 or more variables, we found a sensitivity of the previously published risk score of 92.2% (95% CI 84.7–96.0). Ten (7.8%) bacterial meningitis cases were incorrectly classified as viral meningitis. None of these cases were classified as low risk by our risk score. In an analysis with missing data assumed to be abnormal, the sensitivity was 95.3% (95% CI 91.3–97.6). To our knowledge there are no other clinical prediction models except in children.[15,16]

Discussion

We have derived and validated a risk score that helps physicians to reliably stratify patients with CSF pleocytosis and negative CSF Gram stain with respect to the risk for an urgent treatable cause. Risk assessment helps physicians take decisions about the level of care, plan initial therapy, and to inform the patient and his or her relatives about prognosis. About one-third of patients was categorized as of low risk for an urgent treatable cause and could potentially be considered for outpatient management and follow-up. Patients with an elevated risk score should be hospitalized, with further diagnostic testing and therapy depending on individual characteristics. Although our model shows good external validity, it should be regarded only as a piece of diagnostic assessment among others and should not replace a careful diagnostic evaluation on an individual level for all cases. Our risk score had an excellent sensitivity for detecting bacterial meningitis, but for other urgent treatable causes this is less certain. Therefore, future studies should validate our model in other geographical areas, and evaluate the impact of our model on the management of this common clinical dilemma.

A previous model validated in adults (and children) predicted the probability of bacterial meningitis as opposed to viral meningitis.[19,20] A slightly altered version was evaluated in 500 consecutive cases with paediatric and adult community–acquired meningitis, with c-statistics, and negative and positive predictive values of 0.99, 0.99 and 0.85 respectively.[21] In the initial study, cases with a negative CSF Gram stain had been excluded from the derivation and validation cohorts. However, performance of the model was subsequently evaluated in 109 consecutive patients, both children and adults (mean age 30 years, range 1–85), with acute meningitis and a negative CSF Gram stain.[14] Negative and positive predictive values and accuracy of this model to predict bacterial versus viral meningitis were 98.7%, 66.7%, and 96.5%, respectively. The model missed one patient with bacterial meningitis due to Leptospira spp. resulting in a sensitivity of 80%. Comparing both prediction models in the Dutch cohort of adult patients with bacterial meningitis, CSF pleocytosis and a negative Gram stain, our risk score had better sensitivity 100.0% (95% CI 97.8–100.0%) than a previously published prediction model 92.2% (95% CI 84.7–96.0).

The "Bacterial Meningitis Score" was developed to differentiate bacterial from "aseptic" meningitis in children.[17] It showed good external validity; classification of children as of low risk had a negative predictive value of 99.9% (95% CI 99.4–100.0%) for bacterial meningitis.[18] However, the Bacterial Meningitis Score has not been used in adults with meningitis. Interestingly, one of the five components of the Bacterial Meningitis Score is a positive Gram stain.

Bacterial meningitis constituted 32% of all urgent treatable causes in our cohorts. Our score did identify all patients with CSF pleocytosis and community-acquired bacterial meningitis in the largest prospective meningitis cohort to date. A major advantage of our model over previously

published prediction models is that it does not solely focus on bacterial meningitis but on all urgent and treatable causes.

Many features of our cohort of meningitis cases with a negative Gram stain were similar to previous studies, however, there were several noteworthy observations. First, the differential diagnosis of this syndrome is very broad and includes a wide range of infectious and non-infectious causes, some of which require urgent treatment. Second, the majority of patients were hospitalized (93%), underwent cranial imaging (89%), and received empiric intravenous antibiotic therapy (75%), even though the minority of patients (15%) had an urgent treatable cause. Third, despite extensive diagnostic testing with current techniques and similar to other studies, the majority of the patients (72%) had an unknown aetiology.[1,2,4] Lastly, even though the overall mortality of this syndrome is low (1.4%); 72 (9.6%) of surviving patients had residual functional impairment at discharge.

There are several limitations to our study. First, although the majority of patients in the derivation and validation cohort had a CSF bacterial culture performed (96%), only 338 patients (44%) had polymerase chain reaction tests done in it. By using PCR, the causative microorganism can be identified in a substantial proportion of culture negative cases.[5] Furthermore, PCR for enterovirus and for herpes virus was done less frequently in the unknown category ($p < 0.05$). Therefore, part of the CSF culture negative cases, now classified as "unknown cause" may have had positive viral CSF PCR. As this study was observational and resembles clinical practice, use of PCR was at the discretion of the treating physician. Second, we excluded a total of 35 patients that received oral antibiotics before lumbar puncture or were treated with intravenous antibiotics for more than 48 h and had no identifiable aetiology. This was done to avoid misclassification bias, as these patients could have had bacterial meningitis. Third, the majority of patients (72%) had meningitis of unknown cause. This is the frustrating reality of this syndrome and we hope that novel molecular tools and standardized diagnostic algorithms will be able to improve our current understanding of patients with CSF pleocytosis and negative CSF Gram stain. A further limitation was that data on IV drug use was not available in the bacterial meningitis cohorts and information on the use of immunosuppressive medication was only routinely available for the 2006 bacterial meningitis cohort. We do not think, however, that this weakens our conclusion that the risk model has a high sensitivity to detect bacterial meningitis, as using fewer variables in the risk model can only lead to an underestimation of sensitivity. Even if we assumed all patients in the bacterial meningitis cohort to be immunocompetent, no additional bacterial meningitis patients were classified as low risk.

In conclusion, we developed and validated a risk score that can be used to identify adults with CSF pleocytosis and a negative CSF Gram stain at low risk for an urgent treatable cause. Using this model up to one-third of patients with this common clinical dilemma can be considered for outpatient management.

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Supplementary material

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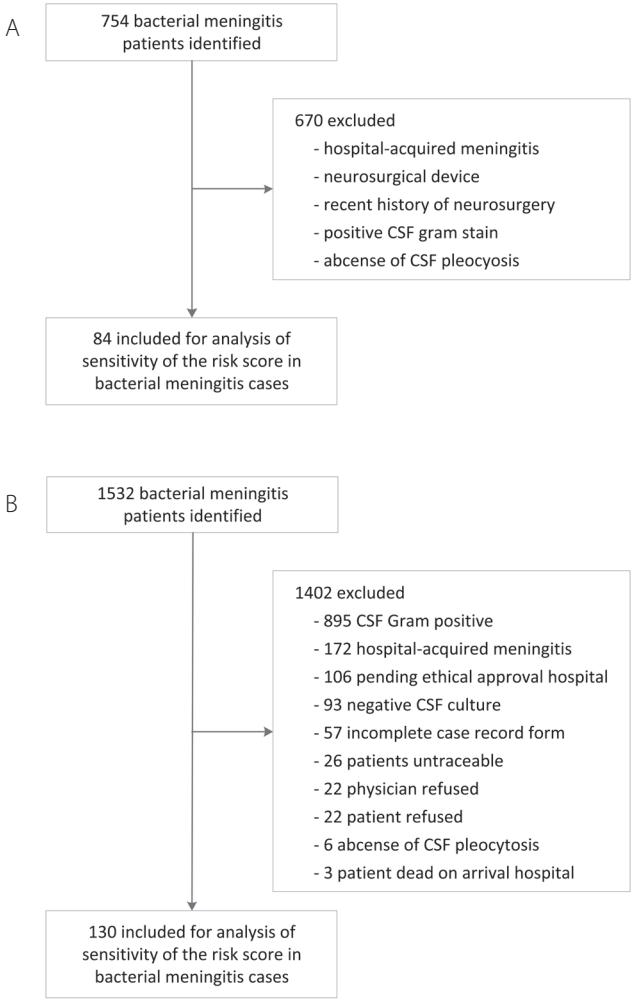

Supplemental figure 1. Flowcharts of patient inclusion in bacterial meningitis cohort I (A) and bacterial meningitis cohort II (B).

CHAPTER 8

Risk Scores for Outcome in Bacterial Meningitis: Systematic Review and External Validation Study

Merijn W Bijlsma, Matthijs C Brouwer, Patrick M Bossuyt, Martijn W Heymans, Arie van der Ende, Michael W T Tanck, Diederik van de Beek

Submitted

Abstract

In bacterial meningitis, many risk scores have been developed, but few have been externally validated. We compared the performance of nine risk scores, identified through a systematic review predicting outcome in community-acquired bacterial meningitis. MEDLINE and EMBASE were searched for articles published between January 1960 and August 2014. Performance was evaluated in 2,108 episodes of adult community-acquired bacterial meningitis from two nationwide prospective cohort studies. Performance was estimated by the area under the receiver operating characteristic curve (AUC), the calibration curve, calibration slope or Hosmer-Lemeshow test, and the distribution of calculated risks. Nine risk scores were identified predicting death, neurological deficit at discharge or unfavourable outcome (Glasgow Outcome Scale 1-4) in bacterial meningitis, pneumococcal meningitis and invasive meningococcal diseases. Most studies had shortcomings in design, analyses, and reporting. The evaluation showed AUCs of 0.59 (0.57-0.61) and 0.74 (0.71-0.76) in bacterial meningitis, 0.67 (0.64-0.70) in pneumococcal meningitis, and 0.81 (0.73-0.90), 0.82 (0.74-0.91), 0.84 (0.75-0.93), 0.84 (0.76-0.93), 0.85 (0.75-0.95), and 0.90 (0.83-0.98) in meningococcal meningitis. Calibration curves showed adequate agreement between predicted and observed outcomes for four scores, but statistical tests indicated poor calibration of all risk scores. The usefulness for individual patient management was probably limited, for all scores, due to low proportions of predictions high or low enough to justify specific counseling or treatment options. None of the existing scores performed well enough to recommend use in individual patient management. One score could be of value in future trials.

Introduction

Bacterial meningitis kills about a fifth of people with the disease and up to half of the survivors suffer debilitating sequelae.[1,2] In bacterial meningitis, clinical deterioration can occur rapidly and is often difficult to predict.[3] Identifying patients at high risk of an unfavourable outcome may be important for counselling patients and their families, as well as deciding upon optimal patient management such as level of care. Accurate prognostic stratification can also be a valuable tool in evaluating and correcting for case mix in clinical research and for targeting intervention strategies.[4,5]

Risk scores can help physicians estimate the likelihood of a particular outcome, by combining multiple predictors from a patient's history, physical examination, or laboratory tests.[4,5] To be useful in clinical practice, the calculated risk has to match observed risk in patients under investigation. To justify treatment or counselling options that differ from standard practice, the calculated risk should differ substantially from baseline risk. A very high (or low) calculated risk should occur often enough to recommend calculation of the score in all patients.

In bacterial meningitis, many risk scores have been developed but few have been externally validated in separate datasets, and their applicability to new patients is not guaranteed.[4-6] After a systematic review of the literature to identify risk scores in community-acquired bacterial meningitis we performed an external evaluation of the performance of existing risk scores, using data collected in a prospective cohort of 2,108 Dutch adult patients with community-acquired bacterial meningitis.

Methods

Systematic review

We performed a systematic search in MEDLINE and EMBASE to identify scores to predict outcome in adults with community acquired bacterial meningitis (supplemental appendix 1). The search strategy included both MeSH terms and search terms in titles and abstracts. Terms for meningitis and common pathogens of community acquired bacterial meningitis were combined with a previously validated filter for risk scores.[7] We searched for studies report in full in scientific peer-reviewed journals between January first 1960 and August first 2014, without language restrictions.

A risk score was defined as a decision-making tool that provides probabilities, or risk categories, for particular patient outcomes, based on three or more variables obtained from history, physical examination, or simple diagnostic tests.[8] Derivation or validation studies predicting mortality or neurologic deficit in adult patients (defined as 16 years of age or older), or in patients without age restrictions, with community-acquired bacterial meningitis were eligible. We included studies

based on cohorts with various pathogens, as well as those on specific pathogens. For invasive meningococcal disease, studies were selected if at least a third of patients in the cohort were reported to have meningitis. Studies focusing on tuberculous meningitis were excluded. If several risk scores had been developed in a single dataset, we only extracted the score with the highest sensitivity and specificity reported in the original publication.

Two reviewers (MWB and MCB) independently screened abstracts. Papers potentially eligible for inclusion bases on the title and abstract were read in full. The risk of bias of included studies was assessed with a list of criteria, based on a number of quality systems for prognostic studies (supplemental table 1).[9] Disagreement between reviewers (MWB, MCB) was resolved by inviting a third reviewer (DvdB).

Evaluation of performance

The performance of identified risk scores was evaluated using data from 2,108 episodes of community-acquired bacterial meningitis collected in two nationwide prospective cohort studies on community-acquired bacterial meningitis. The Dutch Meningitis Study was performed between 1998 and 2002. It included 696 episodes of community-acquired bacterial meningitis.[1] The MeninGene study is still ongoing.[10-13] We used 1,412 episodes included from 2006 to 2014.[14]

Both studies have a similar design and methods have been described in detail elsewhere.[1,14] Included patients were older than 16 years, had bacterial meningitis confirmed by cerebrospinal fluid (CSF) culture, or the combination of a positive polymerase chain reaction or antigen test in cerebrospinal fluid for *Streptococcus pneumoniae* or *Neisseria meningitidis* with at least one specific cerebrospinal fluid finding predictive of bacterial meningitis.[15] Patients were prospectively identified through continuous surveillance of the Netherlands Reference Laboratory for Bacterial Meningitis.

We evaluated risk scores developed for a specific bacterial pathogen and outcome in the subgroup of patients with that particular pathogen and outcome. Whenever specific predictors or outcome data were not available, proxy variables were used. If a reasonable proxy could not be identified, the risk score was validated without that particular variable and the suggested cut-off for defining high risk was adjusted accordingly. One risk score had been developed in the Dutch Meningitis Study cohort; this score was evaluated using data from the MeninGene cohort only.[23]

Statistical analysis

The performance of the identified risk scores was evaluated by evaluating discrimination and calibration.[4,16] Discrimination was assessed by building receiver operating characteristic (ROC) curves and estimating the area under these ROC-curves (AUC) with 95% confidence intervals. Higher AUC values were considered to indicate to better discriminatory ability, as follows: "excellent discrimination" with an AUC of ≥ 0.90; "good discrimination" for $0.80 \leq \text{AUC} < 0.90$; "fair

discrimination" with 0.70 ≤ AUC < 0.80; and "poor discrimination" whenever AUC < 0.70.[17]

Calibration was evaluated by constructing a calibration curve, estimation of the calibration slope, and through calculating the difference between the mean observed proportion and mean predicted proportion of patients with poor outcome (calibration-in-the-large).

Some risk scores were not based on a multivariable logistic regression model; in other cases not all beta-coefficients of the model had been reported in the original publication. For these risk scores we used the observed proportion in the respective risk categories, as reported in the original publication, as the expected proportion for that risk category in the evaluation data. The calibration slope could not be estimated for these risk scores. Instead, we calculated the Hosmer-Lemeshow (HL)-test with the ordinal risk categories for which a predicted probability was available as groups, and with the number of risk categories minus one degrees of freedom.[18]

Whenever cut-off values for defining high risk had been provided in the original study report we calculated estimates of sensitivity, specificity, and predictive values for the high-risk categories. Spread and variability of the calculated risks were also evaluated.

The median number of missing values per imputed variable was 1% (interquartile range 0% to 5%). Missing values were handled using multiple imputation based on the MICE algorithm.[19] We used 27 variables from history, physical examination, laboratory and microbiological testing as predictors in the R package MICE . We used R packages pROC and predictABEL for evaluating discrimination and calibration.[19-21] Rubin's rule was used to estimate proportions and c-statistics based on the five imputation sets. All statistical tests were two-tailed, and P values of less than 0.05 were considered to indicate statistical significance.

Results

Systematic review

Our literature search retrieved 3,468 potentially eligible publications (supplemental figure 1); 3,265 had to be excluded based on the title or abstract, 203 articles were read in full. One additional relevant study was found through reference checking. We identified 11 reports on outcome prediction in adult bacterial meningitis. Eight reports described the development of a novel risk score,[22-29] two studies had evaluated a previously developed risk scores in an external dataset,[30,31] and one study did both.[32]

The evaluation of the risk of bias and completeness of reporting in the included studies is shown in supplemental table 1. Common limitations were the use of a historical cohorts, little information on inclusion and exclusion criteria, dichotomization of continuous predictors, and insufficient numbers of subjects per predictor variable. Multivariable models of prognostic factors were reported in five studies, and only three reported all regression coefficients of the multivariate regression model. Internal techniques for validation were used in one study.

Table 1. Description of the derivation cohort and previously reported performance measures of identified risk scores.

Name	Description of derivation cohort	Previously reported performance measures
Bacterial meningitis (all pathogens)		*Death or neurologic deficit at discharge*
Aronin (1998)	*S. pneumoniae* 47%, *N. meningitidis* 12% USA, 1970-95. N =176 Age: median 56, range 17-91 years CSF culture positive 81% Fatality rate 28%	AUC = 0.73 (0.65-0.81), HL-test: p = 0.08 Sens = 0.43, spec = 0.83 External validation (Aronin 1998): AUC = 0.81 (0.71-0.92) Sens = 0.33, spec = 0.81 HL-test: p = 0.2
Bacterial meningitis (all pathogens)		*Unfavourable outcome*
Weisfelt (2008)	*S. pneumoniae* 51 %, *N. meningitidis* 37% Netherlands, 1998-02, N =696 Age: mean 50, standard deviation 20 years CSF culture positive 100% Fatality rate 21%	AUC = 0.84 (0.80-0.87), HL-test: p = 0.70 External validation: *The Netherlands* (Weisfelt 2008): AUC =0.81 (0.74-0.87), HL-test: p = 0.89 *Vietnam* (Schut 2012): AUC = 0.70 (0.65-0.75), HL-test: p = 0.005 *Malawi* (Schut 2012): AUC = 0.68 (0.63-0.73), HL-test: p < 0.0001
Pneumococcal meningitis		*Death*
Hoen (1993)	France, 1975-90, N = 105 Age: mean 43, range 1-87 years CSF culture or antigen positive CSF Gram stain 73% Fatality rate 27%	Sens = 0.39, spec = 0.97
Invasive meningococcal disease		*Death*
Ajayi-Obe (1998)	Nigeria, 1996, N = 132 Age: mean 9, range 0-60 years Fever and nuchal rigidity 85% Mortality 11%	Sens = 0.39, spec = 0.97
Barquet (1997)	Spain, 1987-90, N =651 Age: median 5, range 0-89 years Meningeal signs 47% Fatality rate 5%	AUC = 0.89 (0.83-0.95), HL-test: p=0.73 Sens = 0.47, spec = 0.99 External validation (Barquet 1997) AUC = 0.91 (0.83-0.99), Sens = 0.47, spec = 0.99
Gardlund (1986)	Denmark, 1971-83, N = 115 Age: mean 19, range 0-85 years CSF culture positive 70% Fatality rate 10%	Sens = 0.67, spec = 0.97
Ged-de-Dahl (1990)	Norway, 1981-1982 N = 113, Age: range 0-76 years Meningitis 62% Fatality rate 10%	Sens = 1.00, spec = 0.95
Niklasson (1971)	Norway, 1959-68, N = 80 Age: mean 22, range 1-67 years CSF culture positive 88% Fatality rate 11%	Sens = 1.00, spec = 0.82 External validation (Andersen 1978) Sens = 0.73, spec = 0.93
Turini (1979)	Brazil, 1972-76, N = 254 Age: range 0- over 40, 28% over 15 years Meningitis 100% Fatality rate 10%	Sens = 0.85, spec = 0.82

Abbreviations: AUC = area under the receiver operating curve, HL-test = Hosmer-Lemeshow test, CSF = cerebrospinal fluid, N = number, sens = sensitivity, spec = specificity.

We selected nine risk scores for the current evaluation (supplemental table 2).[22-29,32] Two risk scores were developed for bacterial meningitis due to any pathogen (table 1).[22,23] Predicted outcomes for these two scores were the combination of death or neurological deficit at discharge (Aronin),[22] and unfavourable outcome, defined as a score of 1 to 4 on the Glasgow Outcome Scale (Weisfelt).[23]

The derivation cohort of the Aronin score was assembled between 1970 and 1995. The proportion of pneumococcal meningitis was 47% and the case fatality rate was 28%. Three independent predictors of outcome were identified in a logistic regression analysis. A risk score was subsequently derived based on the number of risk factors present at presentation. The derivation cohort of the Weisfelt rule (1998-2002) had a case fatality rate of 21%, and 51% of episodes were pneumococcal meningitis. The risk score consists of a logistic regression model of six predictor variables that are available within one hour after presentation. External validation was reported in the original publications, showing adequate calibration and an AUC of 0.81 for both risk scores (table 1). A subsequent external evaluation of the Weisfelt score in two developing countries showed limited generalizability.[31]

One risk score had been developed for pneumococcal meningitis (Hoen) with death as predicted outcome.[24] The authors had developed a logistic regression model with four baseline variables. The AUC and HL-test statistic were not provided in the original publication. Sensitivity of the risk score, at a 0.7 cut-off for defining high risk, was 0.39 at a specificity of 0.97 (table 1). The rule has not previously been evaluated in an external dataset.

The other six risk scores predict death in invasive meningococcal disease.[25-29,32] All were developed in cohorts of both pediatric and adult patients with invasive meningococcal disease (table 1). Mortality rates ranged from 5% to 11%. Scores are calculated by looking at the presence or absence of between three to seven variables. Only one of these risk scores was based on logistic regression modelling, the intercept of the model was not reported.[26] That model showed good to excellent discrimination in the derivation and validation set (table 1). All risk scores provided high-risk categories. Reported sensitivities ranged from 33% to 100%, and specificities varied between 81% and 97% (table 1). One of these risk scores [28] has previously been evaluated in two unrelated patient cohorts.[30,32]

External validation

Baseline and outcome characteristics of the 2,108 patients in our evaluation dataset are shown in supplemental table 3. A predictor identical to the one used in development, or a proxy, was available for all variables of the risk scores for meningitis due to any pathogen or due to pneumococcal meningitis (supplemental table 4). Predictors for poor clinical outcome common in all risk scores were altered mental status, decreased systolic blood pressure, other signs of circulatory

compromise, signs of disseminated intravascular coagulation, and age. Analysis in our dataset showed that each one of these variables was significantly associated with poor outcome even when adjusted for the other variables (supplemental table 4).

The discriminative ability of Aronin's risk score was poor (AUC 0.59; 95% CI: 0.57 to 0.61; Figure 1a). Seventy-five percent of episodes fell into the risk category of a 33% chance of death or neurologic deficit at discharge. The observed proportion with that outcome ranged from 21% to 55% in the risk categories. The calibration curve showed reasonable agreement between predicted and observed proportions of death across risk groups (figure 1b). Calibration-in-the-large showed an overall underestimation of outcome of 12%, and the HL-test indicated poor fit (table 2).

The Weisfelt score, developed for all pathogens, had fair discriminative ability (AUC 0.74; 95% CI: 0.71 to 0.76). Median calculated risk of an unfavourable outcome was 39%, with an interquartile range of 22% to 63%. A calculated risk of less than 5%, or more than 95% was seen in 5% of episodes. The calibration curve showed good calibration (figure 1b), but the calibration slope was significantly lower than one (0.73; 95% CI: 0.63 to 0.84; $p<0.001$). The AUC of the Weisfelt score for predicting death in the subgroup of pneumococcal episodes from the MeninGene cohort was 0.73 (95% CI: 0.69 to 0.77). The Weisfelt score showed good to excellent discrimination in the subgroup of meningococcal episodes, but overestimated the mean risk of death in meningococcal episodes from the MeninGene cohort by 26% (95% CI: 18% to 33%; supplemental table 5).

The discriminative ability of the risk score for pneumococcal meningitis proposed by Hoen was poor (AUC 0.67; 95% CI: 0.64 to 0.70). Median risk of death was 22% (interquartile range 7% to 33%). In 10 % of episodes the calculated risk was less than 5%; in only 1% of episodes the calculated risk exceeded 90%. The calibration curve showed overestimation of the probability of death (figure 1b); the calibration slope was significantly lower than one (0.41; 95% CI: 0.32-0.50; $p<0.001$).

Discrimination of the six risk scores predicting death in meningococcal meningitis was good to excellent; AUC's ranged from 0.81 (Ajayi-Obe) to 0.90 (Niklasson). Inspection of the calibration curves (figure 1b) and the probability of death in each risk score category (supplemental figure 2) showed good agreement of the predicted and observed proportion of deaths for the scores of Gardlund and Gedde-Dahl, and overestimation by the scores of Barquet, Niklasson and Turini. The HL-test showed poor calibration of all models (table 2). Specificity of the high-risk categories was greater or equal to 0.9 for five scores, but sensitivity was below 0.9 for all, and below 0.6 for four scores (table 3). The risk score of Niklasson had the highest combination of both sensitivity and specificity.

The overall case fatality rate of meningococcal meningitis was 6%. For three scores (Ajayi-Obe, Gardlund, Gedde-Dahl) episodes classified as high risk had a mortality rate above 50%, and up to 90% for one risk score (Ajayi-Obe). However, the number of episodes categorized as high risk de-

creased for higher calculated risks of death. The scores of Ajayi-Obe, Gardlund and Gedde-Dahl classified 0.3%, 2%, and 6% of episodes as high risk. Episodes not classified as high risk had a case-fatality rate between 1% and 6%.

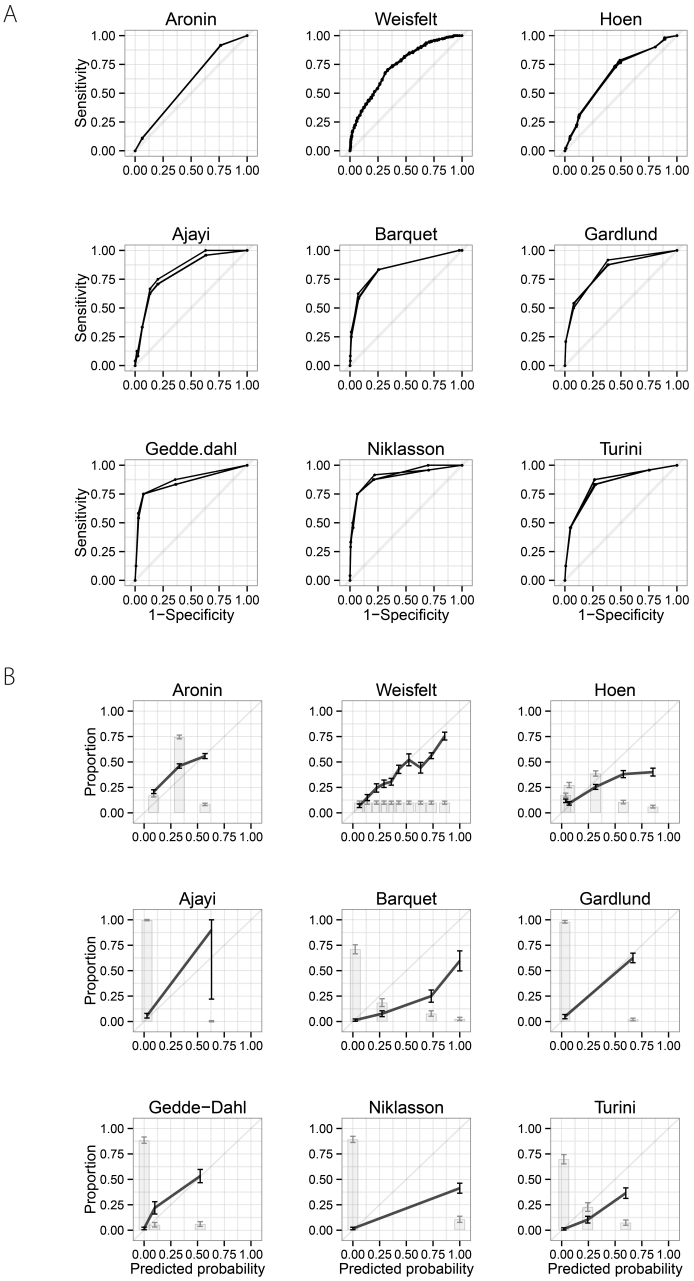

Figure 1. ROC curves (a) and calibration curves (b) of identified risk scores in the Dutch Bacterial Meningitis Cohorts.

Footnote figure 1: Aronin: Dutch Meningitis Study and MeninGene cohorts for outcome death or neurologic deficit, Weisfelt: MeninGene cohort for unfavourable outcome (GOS 1-4), Hoen: pneumococcal meningitis episodes from Dutch Meningitis Study and MeninGene cohorts for outcome death. All others: meningococcal meningitis episodes from Dutch Meningitis Study and MeninGene cohorts for outcome death. Different lines represent ROC curves of different imputation sets. Histograms depict the distribution of patients over the risk categories.

Table 2. External validation of Risk Scores*

Name	AUC (95%-CI)	Calibration in the large ¥ (95%-CI)	Calibration slope (95%-CI) or HL-test
Bacterial meningitis		*Death or neurologic deficit at discharge*	
Aronin	0.59 (0.57-0.61)	12% (10 to 15%)	HL-test: p<0.001
Bacterial meningitis		*Unfavourable outcome*	
Weisfelt	0.74 (0.71-0.76)	-5% (-8% to -1%)	Slope: 0.73 (0.63-0.84), p<0.001
Pneumococcal meningitis		*Death*	
Hoen	0.67 (0.64-0.70)	-5% (-8% to -2%)	Slope: 0.41 (0.32-0.50), p<0.001
Meningococcal meningitis		*Death*	
Ajayi-Obe	0.81 (0.73-0.90)	3% (0% to 6%)	HL-test: p<0.001
Barquet	0.84 (0.76-0.93)	-9% (-13% to -5%)	HL-test: p<0.001
Gardlund	0.82 (0.74-0.91)	2% (-1% to 5%)	HL-test: p=0.03
Gedde-Dahl	0.85 (0.75-0.95)	2% (0% to 5%)	HL-test: p<0.001
Niklasson	0.90 (0.83-0.98)	-5% (-9% to -1%)	HL-test: p<0.001
Turini	0.84 (0.75-0.93)	-6% (-9% to 2%)	HL-test: p<0.001

*****Aronin: Dutch Meningitis Study and MeninGene cohorts, Weisfelt: MeninGene cohort, Hoen: pneumococcal meningitis episodes from the Dutch Meningitis Study and MeninGene cohorts, All others: meningococcal meningitis episodes from the Dutch Meningitis Study and MeninGene cohorts. ¥ Calibration in the large defined as mean observed minus mean predicted outcome

Table 3. Test characteristics of high risk category for outcome*

Name	Sensitivity (95%-CI)	Specificity (95%-CI)	Overall proportion with outcome	Probability high risk (PPV, 95%-CI)	Proportion of episodes in high risk category (95%-CI)	Probability not high risk (1-PPV, 95%-CI)
Bacterial meningitis (all pathogens)			*Death or neurologic deficit at discharge*			
Aronin	0.11 (0.10-0.12)	0.94 (0.93-0.95)	0.43	0.56 (0.54-0.58)	0.08 (0.07-0.09)	0.42 (0.39-0.43)
Pneumococcal meningitis			*Death*			
Hoen	0.12 (0.10-0.13)	0.95 (0.94-0.96)	0.21	0.40 (0.37-0.43)	0.06 (0.05-0.07)	0.20 (0.18-0.22)
Invasive meningococcal disease			*Death*			
Ajayi-Obe	0.04 (0.02-0.06)	1.00 (1.00-1.00)	0.06	0.90 (0.73-1.00)	0.00 (0.00-0.01)	0.06 (0.03-0.08)
Barquet	0.59 (0.54-0.64)	0.93 (0.90-0.95)	0.06	0.34 (0.29-0.38)	0.10 (0.07-0.13)	0.03 (0.01-0.04)
Gardlund	0.21 (0.17-0.25)	0.99 (0.98-1.00)	0.06	0.63 (0.58-0.67)	0.02 (0.01-0.03)	0.05 (0.03-0.07)
Gedde-Dahl	0.55 (0.50-0.60)	0.97 (0.95-0.99)	0.06	0.53 (0.48-0.58)	0.06 (0.04-0.08)	0.03 (0.01-0.04)
Niklasson	0.75 (0.71-0.79)	0.93 (0.91-0.96)	0.06	0.41 (0.37-0.46)	0.11 (0.08-0.14)	0.02 (0.00-0.03)
Turini	0.86 (0.82-0.89)	0.73 (0.69-0.78)	0.06	0.17 (0.13-0.20)	0.30 (0.26-0.35)	0.01 (0.00-0.02)

* The score of Weisfelt did not define cut-off values for high risk. PPV=Positive Predictive Value.

Discussion

In this external evaluation we compared the performance of nine risk scores predicting outcome in community-acquired bacterial meningitis in a variety of settings and populations. The risk scores were identified through a systematic review. Deficiencies in the design, analyses and reporting of studies on the development of risk scores were common. Our analysis in more than 2,000 patients with bacterial meningitis showed fair discrimination for one risk score for community acquired bacterial meningitis (Weisfelt) and good discrimination for all six risk scores in meningococcal meningitis. Inspection of the calibration curves showed adequate agreement between predicted and observed proportions with poor outcome for the risk scores of Aronin, Weisfelt, Gardlund and Gedde-Dahl. However, statistical tests indicted poor calibration of all risk scores. An important limitation of all identified risk scores was the low proportion of cases that were assigned a risk high or low enough to justify specific counselling or treatment options that differ from standard treatment.

Our analysis has a number of potential limitations. First, and most importantly, the quality of reporting of the identified studies did not conform to current standards. Most identified studies, gave little information on calibration of the model and multivariable regression analyses were either not performed, or regression coefficients were not fully reported. The absence of these data limited our evaluation of calibration. Guidelines to improve the quality of reporting of prediction model studies have recently been published.[33] Adherence to these guidelines would not only improve interpretability of study results, but would also facilitate subsequent validation studies.

The Weisfelt risk score was developed in the Dutch Meningitis Study cohort and validated in the MeninGene cohort. Causative pathogens, treatment and outcome differed considerably between these two cohorts. However, the similar research design may have provided an advantage for an evaluation of the Weisfelt score compared to other risk scores.

Because of the different outcomes predicted by the identified risk scores, the variable predictors included in the scores and the heterogeneity of patients, pooling of risk scores or individual predictors was not performed. Furthermore a few original predictor variables were not available in our validation set. Although a reasonable proxy was available for most of these, two risk scores could not be validated with all original variables.

There are many potential pitfalls in the development of prognostic models. Poor generalizability of identified risk scores can be explained in part by overfitting, and differences in characteristics between the target population and the development cohort.[4] Overfitting occurs when risk scores are not only based on associations between predictors and outcome in the population, but also on idiosyncrasies and random variations in the development sample.[4,34] Such overfitting leads to accurate predictions in the development set, but poor performance in new patients. The risk of overfitting may be reduced to some extent, for instance by internal validation tech-

niques and limiting the number of potential predictors in relation to the number of patients. However, only one identified risk score was derived from a cohort in which there were more than 10 cases per predictor variable and for which performance was evaluated in the development set. Another strategy to reduce overfitting is to select potential predictors on the basis of previous literature instead of analysis of the development cohort.[4] As such, our findings offer an overview of possible predictor variables to be used in future risk score development or updating.

Differences between the target population and the development cohort may also lead to poor performance of identified risk scores.[4] The relatively low mortality rate in pneumococcal meningitis episodes from the MeninGene cohort, for example, compared to the derivation cohort of Hoen's score for pneumococcal meningitis (18% versus 27%) might explain the overestimation of death by Hoen's score. Improved prognosis for patients with pneumococcal meningitis may be explained by the introduction of adjunctive dexamethasone therapy.[14,35,36] The prognosis of meningococcal meningitis has not become much better over the past fifty years.[37] Because the epidemiology of bacterial meningitis is changing continuously, a framework for continuous improvement and updating of prediction models is required.[34]

The performance of the Weisfelt score, developed for all pathogens, has now been evaluated in four external cohorts. It showed fair to good discrimination and calibration in the present study as well as in a previous Dutch cohort.[23,31] Discrimination of the Weisfelt score in the subgroups of either meningococcal or pneumococcal meningitis episodes was similar to that of risk scores developed specifically for these pathogens. However, calibration of the Weisfelt score in meningococcal meningitis was poor, and it performed less well in two developing countries.[31] The risk score of Niklasson, developed for invasive meningococcal disease, has now been evaluated in three external datasets. It showed excellent discrimination in our study, and its high-risk category had the highest combination of sensitivity and specificity of all risk scores for meningococcal meningitis.

None of the scores performed well enough to recommend routine use in individual patient management. Either predicted outcomes differed too much from observed outcomes, or calculated risks were too similar to baseline risk, with extreme risk prediction occurring too infrequently to justify calculation of the risk score for all patients. In our evaluation the risk scores for meningococcal meningitis assigned a risk of death that was close to the overall mortality rate in most episodes. Although the few patients classified as high risk had substantially higher mortality rates, most of the patients who died were not categorized as high risk. Extreme risk of an adverse clinical outcome were infrequently calculated by the risk scores for pneumococcal meningitis or meningitis due to all pathogen.

The risk scores that we identified could be of value in the design and interpretation of future clinical trials. The necessary sample size of a trial could be reduced; for example, by only including patients with an intermediate risk of adverse outcome. In other cases, a limited number of

patients with a very poor prognosis could be selected for a costly or invasive intervention. Differences in severity of illness between study populations could be evaluated by comparing the mean calculated risk of adverse outcome in the study groups sampled from these populations. We would recommend the Weisfelt score for risk stratification in scientific research on community acquired bacterial meningitis in developed countries. It has shown adequate performance in two evaluations in external cohorts in the Netherlands, and has an even distribution of patients across the whole range of predicted likelihoods.

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Supplementary material

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Supplemental table 1. Quality* of Identified Studies

	Ajayi--Obe	Andersen	Aronin	Barquet	Garlund	Gedde-Dahl	Hoen	Niklasson	Schut	Turini	Weisfelt
Study design											
Inception cohort	1	1	1	1	1	1	1	1	1	1	1
Source population	0	0	1	0	0	1	0	0	1	0	1
Inclusion and exclusion criteria	0	0	1	0	0	0	0	0	1	0	1
Prospective design	0	0	0	1	0	1	0	0	1	0	1
Study attrition											
Number of drop-outs	?	?	?	1	?	?	?	?	1	?	1
Information given on method how they deal with missing data	0	0	0	0	0	0	0	0	0	0	1
Prognostic factors											
All prognostic factors described used to develop the model	1	1	1	1	1	1	1	1	1	1	1
Standardized or valid measurements	1	1	1	1	1	1	1	1	1	1	1
Linearity assumption studied	0	0	0	0	0	0	0	0	0	0	0
No dichotomization of prognostic variables	0	0	0	0	0	0	0	0	0	0	0
Data presentation all prognostic factors	1	1	1	1	1	0	1	1	1	1	1
Outcome measures											
Description of outcome measures	1	1	1	1	1	1	1	1	1	1	1
Standardized or valid measurements	1	1	0	1	1	1	1	1	1	1	1

Supplemental table 1. Quality* of Identified Studies (continued)

	Ajayi--Obe	Andersen	Aronin	Barquet	Garlund	Gedde-Dahl	Hoen	Niklasson	Schut	Turini	Weisfelt
Data presentation of most important outcome measures	1	1	1	1	1	1	1	1	1	1	1
Analysis											
Presentation of univariate crude estimates	1	0	1	1	0	0	1	0	1	1	1
Sufficient numbers of subjects per variable	0	0	0	0	0	0	0	0	1	0	1
Selection method of variables explained	0	1	0	1	0	0	0	0	1	1	1
Presentation of multivariate estimates	0	0	1	1	0	0	1	0	1	0	1
Clinical performance / validity											
Clinical performance	0	1	1	1	0	1	1	0	1	1	1
Internal validation	0	0	0	0	0	0	0	0	0	0	1
External validation	0	1	1	1	0	1	0	0	1	0	1

The number 1 indicates high, and the number 0 low quality. A question mark signifies that no relevant information was reported. Quality was independently scored by MB and MWB, Cohens kappa was 0.80, DvdB scored items without consensus between MB and MWB

***Operationalization of items.**

a. Inception cohort: positive when patients were identified at an early uniform point (inception cohort) in the course of their complaints (e.g. first point at which symptoms were first noticed or first consultation at physiotherapy practice). Also positive in case of a heterogeneous population (survival cohort) for which subgroups of patients were identified and analysed (first episode of complaints or first consultation at physiotherapy practice). Negative when no inception cohort was used.

b. Source population: positive when population was described in terms of sampling frame (primary care, general population, physiotherapy practice) and recruitment procedure (place and time-period of recruitment and type of methods used to identify the sample). Negative when not both of these features are given. Also negative when it is likely that the recruitment procedure led to selection of participants that are systematically different from eligible non-participants.

c. Inclusion and exclusion criteria: positive when criteria were formulated for at least 4 out of 5 of the (for the study) most relevant characteristics, mostly:

1. Age
2. Sex
3. Relevant co-morbidity
4. Duration of complaints
5, Type of complaints

Negative when ≤3 criteria were formulated. Also negative when it is likely that the criteria used for inclusion/exclusion led to selection of participants that are systematically different from eligible non-participants.

a. Prospective design: positive when a prospective design was used. Also positive in case of a historical cohort of which the predictor variables (prognostic factors) are measured before the outcome was determined. Negative if a historical cohort is used, considering prognostic factors at time zero which are not related to the primary research question for which the cohort is created or in case of an ambispective design.

b. Drop-outs: positive when total number of drop-outs (loss to follow-up) was ≤20% at 12 months. Also positive when appropriate procedures were used to deal with missing values (e.g. use of multiple imputation). Negative when the total number of drop-outs exceeds the 20% cut-off point and no appropriate procedures were used to deal with missing values.

c. Positive if method is described. Negative if not.

d. Clinical relevant potential prognostic factors: positive when the article describes at least one of the following factors at baseline:

    1. Physical/disease factors (e.g. severity of pain, range of motion, duration of complaints, localization of complaints)
    2. Psychosocial factors (e.g. live events, anxiety, depression)
    3. Sociodemographic factors, other than gender and age (e.g. employment status, occupation, co-morbidity)

Negative when the article does not describe at least one of the factors mentioned above at baseline.

a. Standardized or valid measurements: positive if at least one of the factors of g), excluding age and gender, are measured in a standardized, valid and reliable way.

b. Positive if studied (and accounted for if necessary) or not relevant (in case of no continuous predictors used), negative if not.

c. Positive if prognostic variable isn't dichotomized (in the univariate or multivariate analysis) or dichotomization is sensible to do. Negative if prognostic variable is dichotomized (in the univariate or multivariate analysis).

d. Data presentation of most important prognostic factors: positive when frequencies, percentages or mean (and standard deviation or CI), or median (and Inter Quartile Range) are reported for all prognostic factors in the final model. In all other cases: negative.

e. Clinical relevant outcome measure(s): positive if at least one clinical relevant outcome criteria for recovery is reported. In all other cases: negative.

f. Standardized or valid measurements: positive if one or more of the main outcome measures are measured in a standardized, valid and reliable way. In all other cases: negative.

g. Data presentation of most important outcome measures: positive if frequencies, percentages or mean (and standard deviation/CI), or median (and Inter Quartile Range) are reported for one or more of the main outcome measures for the most important follow-up measurements. In all other cases: negative.

h. Univariate crude estimates presented: positive if univariate crude estimates (RR, OR, HRR) between prognostic factors separately and outcome are provided. Negative if only p-values or wrong association values (Spearman, Pearson, sensitivity) are given, or if no tests are performed at all.

i. Sufficient numbers of subjects per variable: positive if it is mentioned (or easy derivable) that the number of cases (and non-cases) in the study was at least 10 times the number of candidate variables. In all other cases: negative.

j. Positive if references are used to explain the selection method of variables. Also positive if an appropriate rationale is given. Negative if not.

k. Multivariate estimates presented: positive if multivariate estimates (with CI or p-values) are presented of all prognostic factors that are part of the final risk score. Negative if not.

l. Performance measurement: positive if the study provides information about performance measurement (e.g. discrimination, calibration, explained variance). In all other cases: negative.

m. Internal validation: positive if appropriate techniques are used to assess internal validity of the prognostic model (e.g. cross-validation or bootstrapping). In all other cases: negative.

n. External validation: positive if the prognostic model is tested in a different population. Negative if not.

Supplemental table 2. Calculation of identified risk scores and suggested cut-off values for high risk

Name	Score calculation*	Cut-off for high risk
Bacterial meningitis (all pathogens)		*Death or neurologic deficit at discharge*
Aronin	(systolic BP ≤ 90 mmHg or a > 40 mm Hg decrease) + (altered mental status) + (baseline seizures)	high risk ≥ 2
Bacterial meningitis (all pathogens)		*Unfavourable outcome*
Weisfelt	1/[1 + exp(-lp)], where lp = -1.83 + 0.02*(age in years) + 1.09*(pulse >120/min) -0.13*(GCS) + 0.91*(cranial nerve palsy) + 1.37*(CSF leucocytes < 1,000/ul) + 0*(gram-negative cocci)+1.29*(gram- positive cocci) + +0.19*(no bacteria) + 0.24*(other gram result)	Not provided
Pneumococcal meningitis		*Death*
Hoen	1/[1 + exp(-lp)], where lp = -2.36*(GCS score ≥ 7) + 1.90*(age > 45 years) -1.89*(CSF glucose ≥ 0.6 mmol/l) + 1.30*(concomitant pneumonia) -0.24	high risk > 0.7
Invasive meningococcal disease		*Death*
Ajayi-Obe	3*(systolic BP ≤ 85 mmHg) + (heart rate ≥ 140/min) + (respiratory rate ≥ 50/min) + (capillary refill time > 3 sec) + 3*(GCS score < 8) + 2*(no neck stiffness) + (petechiae or purpura)	high risk ≥ 9
Barquet	-1*(preadmission antibiotics) + (age ≥ 60 years) + (focal neurologic signs) + 2*(haemorrhagic diathesis)	high risk ≥ 2
Gardlund	(systolic BP < 100 mmHg) + (thrombocytes < 125 x109/l) + (rectal temperature > 39*c)	high risk >2
Gedde-Dahl	100*[(thrombocytes < 100 x 10^9/l) + (systolic BP < 100 mmHg) + (ecchymosis) + (blood leucocytes < 10 x 10^9/l) + (pCO2< 3.7 kPA) + (no nuchal rigidity)] / number of available predictors	high risk > 45%
Niklasson	(CSF leucocytes < 100/ ul) + (systolic BP ≤ 100 mmHg) + (petechiae less than 12 hours) + (rectal temperature ≥ 40*c) + (blood leucocytes < 15 x 10^9/l) + (thrombocytes < 100 x 10^9/l)	high risk ≥ 3
Turini	(age > 40 years) + (duration of symptoms < 48 hours) + (coma) + (shock on admission) + (blood leucocytes ≤ 10.000/mm3)	high risk ≥ 2

* the presence of a dichotomous variable is scored as 1, absence as 0. Abbreviations used BP = blood pressure, GCS = Glasgow Coma Score, CSF = cerebrospinal fluid, lp = linear predictor.

Supplemental table 3. Baseline characteristics of validation cohort

Characteristic – no. /no.evaluated (%)	All pathogens (N=2108)	Pneumococcal meningitis (N=1369)	Meningococcal meningitis (n=407)
Age – yr	59 (42-69)	62 (50-70)	30 (19-52)
Male sex	1052/2108 (50)	700/1369 (51)	205/407 (50)
Symptoms <24 hr	953/2014 (47)	645/1301 (50)	200/393 (51)
Seizures	130/2019 (6)	107/1297 (8)	8/402 (2)
Predisposing condition *	1126/2108 (58)	954/1369 (70)	64/407 (16)
Heart rate – beats /min	100 (84-112)	100 (87-115)	92 (80-106)
Systolic blood pressure – mmHg	140 (122-160)	147 (130-169)	125 (110-140)
Diastolic blood pressure – mmHg	80 (69-90)	80 (70-90)	70 (60-81)
Body temperature – °C	38.9 (38.0-39.6)	39.0 (38.1-39.7)	38.4 (37.3-39.1)
Rash	292/2108 (14)	42/1369 (3)	175/407 (43)
Score on Glasgow Coma Scale	11 (9-14)	10 (8-13)	14 (10-15)
Neck stiffness	1546/2007 (77)	987/1290 (77)	62/400 (16)
Cranial nerve palsy	192/1807 (11)	139/1144 (12)	24/374 (6)
Aphasia, hemiparesis, or monoparesis	425/1774 (24)	309/1108 (28)	48/366 (13)
White cell count - cells/mm3	2500 (582-7600)	2389 (500-6784)	5478 (1653-12430)
Protein – g/liter	4.0 (2.3-6.2)	4.3 (2.5-6.4)	4.3 (2.2-6.7)
CSF: blood glucose ratio‡	0.06 (0-0.26)	0.03 (0-0.2)	0.09 (0-0.3)
Positive blood culture	1331/1854 (72%)	951/1198 (79)	193/358 (54)
C-reactive protein - mg/l	200 (94-311)	205 (97-323)	228 (153-308)
Thrombocyte count - /mm3	194 (147-250)	200 (154-255)	175 (137-226)
Any adjunctive dexamethasone	1352/2080 (65)	972/1348 (72)	178 (44)
Unfavourable outcome (GOS<5)	768/2108 (36)	590/1369 (43)	49/407 (12)
Death	387/2108 (18)	286/1369 (21)	24/407 (6)

Data are number/number assessed (%) or median (25th–75th percentile). * defined as otitis or sinusitis, pneumonia, endocarditis, CSF leak, use of immunosuppressive medication, history of splenectomy or cancer, HIV, diabetes mellitus, alcoholism noted in case record form.

Supplemental table 4. Outcome predictors in bacterial meningitis

Risk Score	Predictors and outcome	Proxy	no./no. evaluated (%)*	Multivariable odds ratio (95% CI)†	P-value
All pathogens			*Death or neurologic deficit at discharge*		
Aronin	Systolic BP ≤ 90 mmHg or a > 40 mm Hg decrease	Systolic BP ≤ 90 mmHg	62/2051 (3)	2.83 (1.62-4.94)	<0.001
	Altered mental status	GCS ≤ 14	1709/2099 (81)	3.17 (2.43-4.13)	<0.001
	Seizures at presentation		130/2019 (6)	1.17 (0.82-1.68)	0.39
Bacterial meningitis			*Unfavourable outcome*		
Weisfelt	Age, years		61 (47-69) ¥	1.03 (1.02-1.04)	<0.001
	Heart rate >120/minute		198/1363 (15)	1.90 (1.33-2.72)	<0.001
	Glasgow Coma Scale score, per point increase		11 (9-14) ¥	0.89 (0.86-0.93)	<0.001
	Cranial nerve palsy		103/1239 (8)	2.62 (1.68-4.09)	<0.001
	CSF white-cell count < 1,000/ul		465 /1352 (34)	2.55 (1.95-3.33)	<0.001
	Gram stain				
	Gram positive		887/1245 (71)	2.18 (1.16-4.26)	0.02
	Gram negative		112/1245 (9)	Ref	
	other		188/1245 (15)	1.76 (0.76-4.20)	0.18
	no bacteria		58/1245 (5)	1.35 (0.66-2.85)	0.41
Pneumococcal meningitis			*Death*		
Hoen	GCS < 7		140/1363 (10)	3.15 (2.12-4.68)	<0.001
	Age > 45 years		1102/1363 (81)	1.58 (1.06-2.36)	0.02
	CSF glucose < 0.6 mmol/l		426/967 (44)	2.39 (1.75- 3.27)	<0.001
	Concomitant pneumonia		166/1315 (13)	1.61 (1.10-2.36)	0.01
Meningococcal meningitis			*Death*		
Ajayi-Obe	Systolic BP ≤ 85 mmHg		10/392 (3)	8.47 (2.01-35.79)	<0.001
	Heart rate ≥ 140/minute		9/384 (2)	17.70 (3.14-99.79)	0.001
	Respiratory rate ≥ 50/minute	NA			
	Cap. refill time > 3 seconds	NA			
	GCS score < 8		28/404 (7)	2.47 (0.54-11.33)	0.24
	No nuchal rigidity		62/400 (16)	3.64 (1.33-9.96)	0.01
	Petechiae or purpura		227/407 (56)	7.37 (1.62-33.49)	0.01
Barquet	Preadmission antibiotics		12/402 (3)	0.00 (0-inf)	0.99
	Age	Age ≥ 60 years	61/407 (15)	7.23 (2.28-22.98)	<0.001
	0-14 years				
	15-59 years				
	≥ 60 years				
	Focal neurologic signs		34/366 (9)	1.06 (0.21-5.27)	0.95

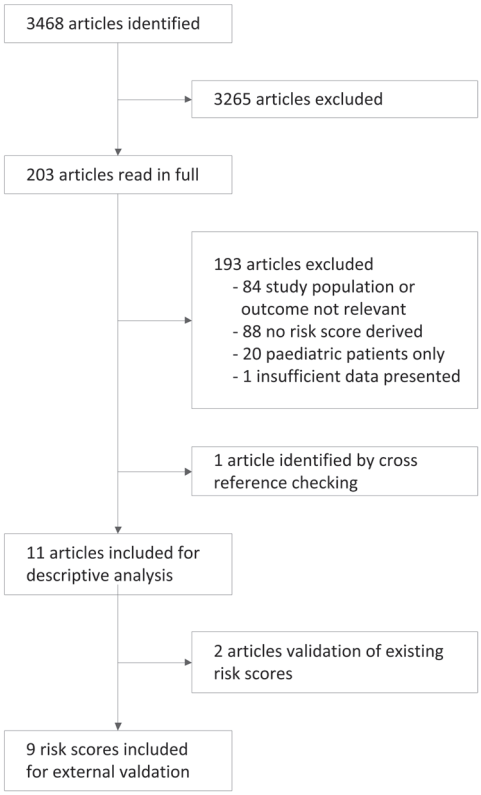
Supplemental table 4. Outcome predictors in bacterial meningitis (continued)

Risk Score	Predictors and outcome	Proxy	no./no. evaluated (%)*	Multivariable odds ratio (95% CI)†	P-value
	Haemorrhagic diathesis	Thrombo-cytes < 100*10e9/l	36/388 (9)	19.57 (6.42-59.67)	<0.001
Gardlund	Systolic BP < 100 mmHg		31/392 (8)	5.74 (2.06-15.98)	<0.001
	Thrombocytes < 125 x10^9/l		81/388 (21)	7.79 (2.93-20.68)	<0.001
	Rectal temperature > 39*c		104/399 (26)	2.33 (0.92-5.92)	0.07
Gedde-Dahl	Thrombocytes < 100 x10^9/l		36/388 (9)	10.76 (3.31-34.94)	<0.001
	Systolic BP < 100 mmHg		31/392 (8)	2.55 (0.75-8.72)	0.13
	Ecchymosis	Purpura	60/407 (15)	0.79 (0.22-2.82)	0.72
	Blood leucocytes < 10 x10^9/l		44/404 (11)	10.95 (3.50-34.27)	<0.001
	pCO2 < 3.7 kPa	NA			
	No nuchal rigidity		62/400 (16)	4.21 (1.32-13.42)	0.02
Niklasson	CSF leucocytes < 100/ ul		30/377 (8)	4.02 (1.16-13.88)	0.03
	Systolic BP ≤ 100 mmHg		41/382 (11)	3.29 (1.06-10.25)	0.04
	Petechiae less than 12 hours	Petechiae at presentation	230/399 (58)	3.77 (0.77-18.44)	0.10
	Temperature ≥ 40*c		25/390 (6)	3.91 (0.93-16.51)	0.06
	Blood leucocytes < 15 x10^9/l		98/396 (25)	3.17 (0.96-10.48)	0.06
	Thrombocytes < 100 x10^9/l		37/380 (10)	4.63 (1.44-14.85)	0.01
Turini	Age > 40 years		163/407 (40)	3.07 (1.05-8.95)	0.04
	Duration of symptoms < 48 hours	Duration of symptoms < 24 hours	193/393 (49)	1.85 (0.71-4.87)	0.21
	Coma	GCS score ≤ 8	49/404 (12)	3.21 (0.97-10.60)	0.06
	Shock on admission	Systolic BP < 90 mmHg	12/392 (3)	4.79 (1.01-22.71)	0.05
	Blood leucocytes ≤ 10 x10^9/l		44/404 (11)	10.69 (3.72-30.74)	<0.001

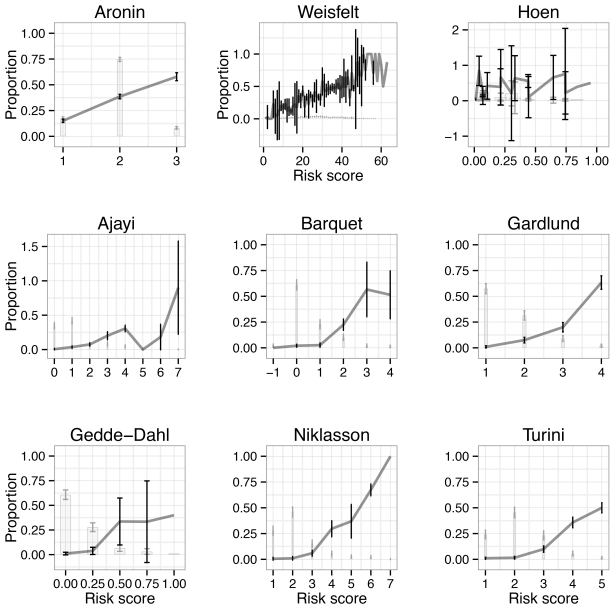
Aronin: Dutch Meningitis Study and MeninGene cohorts, Weisfelt: MeninGene cohort, Hoen: pneumococcal meningitis episodes from the Dutch Meningitis Study and MeninGene cohorts, All others: meningococcal meningitis episodes from the Dutch Meningitis Study and MeninGene cohorts. Abbreviations used BP = blood pressure, GCS = Glasgow Coma Score, CSF = cerebrospinal fluid, NA=not available. *non imputed data. †imputed data, ORs were derived in a multivariate analysis with the other variables in the risk score. ¥median (interquartile range)

Supplemental table 5. Performance of the Weisfelt score in pneumococcal and meningococcal meningitis episodes from the Dutch Meningitis Study and MeninGene cohorts.

Cohort	AUC (95%-CI)	Calibration in the large (95%-CI)
Unfavourable outcome		
Pneumococcal meningitis, both cohorts	0.73 (0.70-0.76)	2% (-1% to 6%)
Pneumococcal meningitis, MeninGene only	0.72 (0.69-0.75)	-1% (-5% to 4%)
Meningococcal meningitis, both cohorts	0.80 (0.73-0.87)	-16% (-22% to -11%)
Meningococcal meningitis, MeninGene only	0.78 (0.67-0.89)	-16% (-25% to -7%)
Death		
Pneumococcal meningitis, both cohorts	0.73 (0.70-0.76)	-20% (-23% to -17%)
Pneumococcal meningitis, MeninGene only	0.73 (0.69-0.77)	-24% (-27% to -20%)
Meningococcal meningitis, both cohorts	0.89 (0.83-0.95)	-22% (-27% to -18%)
Meningococcal meningitis, MeninGene only	0.95 (0.88-1)	-26% (-33% to -18%)



Supplemental figure 1. Inclusion process.


Aronin
Weisfelt
Hoen
Ajayi
Barquet
Gardlund
Gedde−Dahl
Niklasson
Turini
Proportion
Risk score

Supplemental figure 2. Patient outcome per risk category in the in Dutch Bacterial Meningitis Cohorts.

Aronin: Dutch Meningitis Study and MeninGene cohorts for outcome death or neurologic deficit, Weisfelt: MeninGene cohort for unfavourable outcome (GOS 1-4), Hoen: pneumococcal meningitis episodes from Dutch Meningitis Study and MeninGene cohorts for outcome death. All others: meningococcal meningitis episodes from Dutch Meningitis Study and MeninGene cohorts for outcome death. Histograms depict the distribution of patients over the risk categories.

Supplemental appendix 1. Search strategy in Medline (OVID 2)

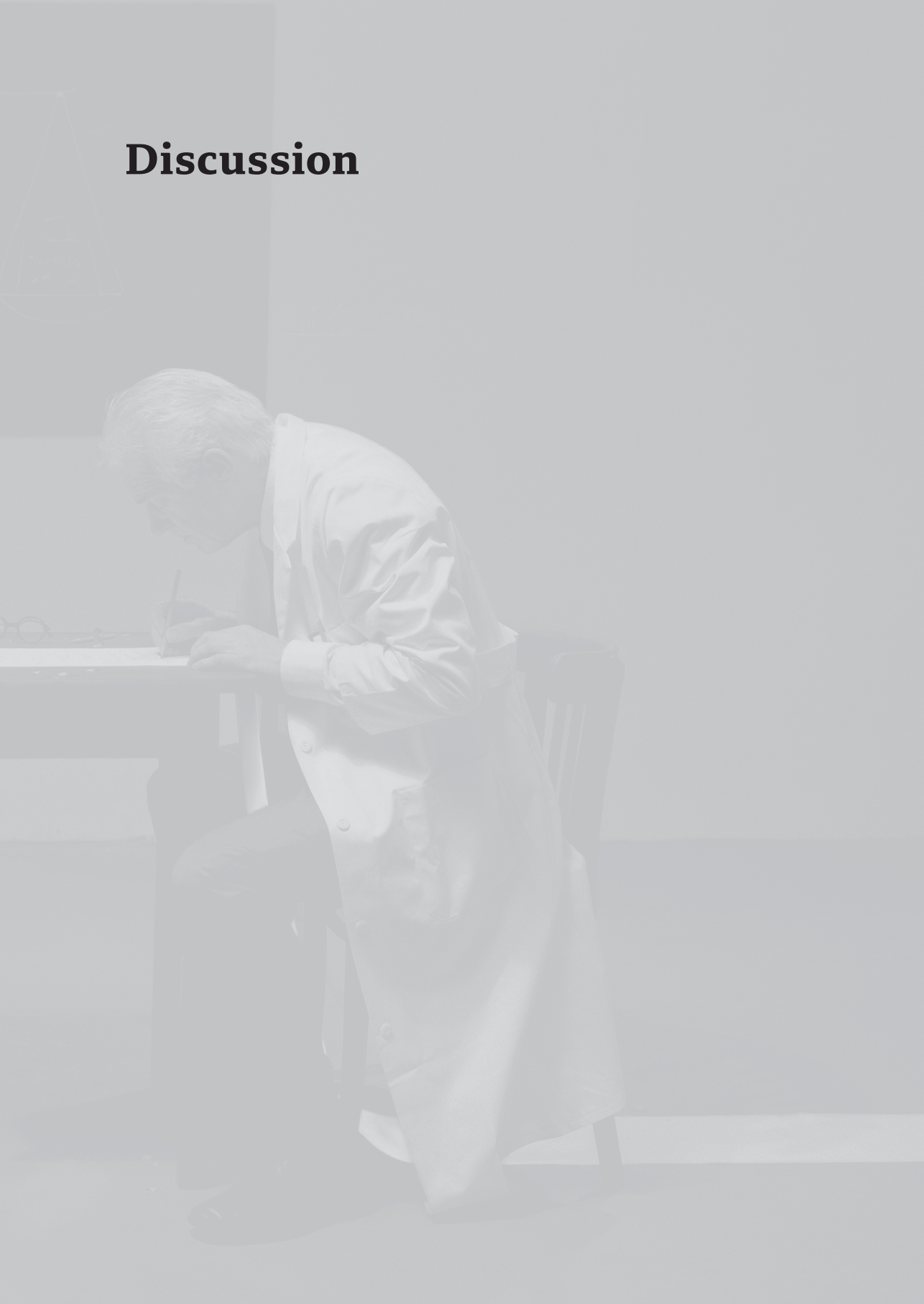
(exp Meningitis, Bacterial/
OR
Bacterial Meningiti*.ti,ab.
OR
((bacterial or meningococcal or pneumococcal or Neisseria or meningitidis or Streptococcus or pneumoniae or Haemophilus or Hib or influenzae or Listeria or monocytogenes or Escherichia or coli or agalactiae or pyogenes or Staphylococcus or aureus or Cryptococcus or neoformans) adj5 meningiti*).ti,ab.)
AND
(exp models, statistical/
OR
(((History or Variable* or Criteria or Scor* or Characteristic* or Finding* or Factor*) and (Predict* or Model* or Decision* or Identif* or Prognos*)) or (Decision* and (Model* or Clinical* or Logistic Model*)) or (Prognostic and (History or Variable* or Criteria or Scor* or Characteristic* or Finding* or Factor* or Model*))).ti,ab.)

Supplemental appendix 2. Local investigators (participating hospitals)

P. Admiraal (Gemini Ziekenhuis), J.C. Baart (Ziekenhuisgroep twente), R.J. Beukers (Medisch Centrum Alkmaar), H.P. Bienfait (Gelre Ziekenhuizen), H.J. Bökkerink (Zkh Nij Smellinghe), A.E. Bollen (Wilhelmina Ziekenhuis Assen), H.M. Bos (St. Anna Ziekenhuis), D. Broere (Westfries Gasthuis), M.H. Christiaans (Diakonessenhuis Utrecht), S.F.T.M. de Bruijn (Haga Ziekenhuis), K. de Gans (Groene Hart Ziekenhuis), R.J. de Graaf (Amphia Ziekenhuis), L. de Lau (Slotervaart Ziekenhuis), M.C. de Rijk (Catharina Ziekenhuis), P. de Roos (Ziekenhuis Rivierenland), J.P. de Ruiter (Streekziekenhuis Koningin Beatrix), R.F. Duyff (Zkh de Tjongerschans), J.L.A. Eekhof (Diaconessenhuis Leiden), J. Engelsman (Spaarne Ziekenhuis), R.H. Enting (UMC Groningen), B. Feenstra (Zkh. Lievensberg), E. Geiger (Beatrix ziekenhuis Gorinchem), P. Groot (St. Jansdal Ziekenhuis Harderwijk), W.J.H.M. Grosveld (Reinier de Graaf Gasthuis), G. Hageman (Medisch Spectrum Twente), S.G.B. Heckenberg (Kennemer Gasthuis), D. Herderscheê (Tergooi ziekenhuizen), W. Hoefnagels (Zorgsaam Ziekenhuis), R.S. Holscher (Antonius Ziekenhuis Sneek), E.M. Hoogerwaard (Rijnstate Ziekenhuis), U.W. Huisman (Van Wheel Bethesda), B.C. Jacobs (Erasmus Medisch Centrum), C. Jansen (Gelderse Vallei Ziekenhuis), K Jellema (Medisch centrum Haaglanden), H. Kerkhoff (Albert Schweizer Zkh), E.J.W. Keuter (Diaconessenhuis Meppel), J.G.M. Knibbeler (Ropke-Zweers ziekenhuis), A.J.M. Kok (Elkerliek Ziekenhuis), N.D. Kruyt (LUMC), M.J.H. Langedijk (Refaja ziekenhuis), M. Liedorp (Havenziekenhuis), H.J.M.M. Lohmann (Deventer ziekenhuis), H. Lövenich (St Jans Gasthuis Weert), N.K. Maliepaard (Waterland Ziekenhuis), D.S.M. Molenaar (Ziekenhuis Amstelland), W.G.H. Oerlemans (Meander Medisch Centrum), E.W. Peters (Admiraal de Ruyter ziekenhuis), P.H.M. Pop (Viecurie Ziekenhuis), P. Portegies (OLVG), B. Post (UMC St Radboud), F.M. Reesink (Ommelander Ziekenhuis Groep), J.C. Reijneveld (Vumc), A.M.G. Sas (Vlietland ziekenhuis), R. Saxena (Maasstad Ziekenhuis),

CHAPTER 9

Discussion



Introduction

The first objective of this thesis was to study the epidemiology of community-acquired bacterial meningitis, invasive meningococcal disease and neonatal group B streptococcal disease in the Netherlands. In **chapter two** we described 1412 episodes of community-acquired meningitis included in the nationwide prospective MeninGene cohort from 2006 to 2014. In **chapter three** laboratory surveillance data on invasive meningococcal disease from 1960 to 2012 is presented. In **chapter four** post-licensure evidence is shown of the impact of the nationwide vaccination campaign against serogroup C meningococcal disease. Serogroup C has largely disappeared after vaccine introduction, and at least a third of the effectiveness was due to herd protection. In **chapter five** we evaluated if there was evidence of increased serogroup C disease in men who have sex with men, after several outbreaks in the gay community had been described in Germany, Belgium, France, and North America. We found no evidence of clusters of serogroup C disease in Men who have sex with men in the Netherlands. In **chapter six** we described the incidence rates and genetic epidemiology of invasive disease due to *Streptococcus agalactiae* (group B streptococcus, GBS) in newborns from 1987 to 2011. We found that the introduction of guidelines for the prevention of perinatal group B streptococcal disease did not reduce neonatal meningitis or sepsis due to GBS.

The second objective was to describe the clinical features of adult community-acquired bacterial meningitis after the introduction of adjunctive dexamethasone therapy and routine paediatric conjugate vaccines against *H. influenzae* type b, *S. pneumoniae* and *N. meningitidis* serogroup C. This was done by an analysis of clinical features from 1412 episodes from the MeninGene cohort (**chapter two**). We found that outcome improved substantially after the widespread introduction of adjunctive dexamethasone treatment, both in pneumococcal and non-pneumococcal meningitis.

The third objective of this thesis was to identify predictors of severe illness, both in adults with bacterial meningitis as well as in patients presenting with cerebrospinal pleocytosis and a negative cerebrospinal fluid gram stain. We developed a risk score in **chapter seven** that identifies adults with cerebrospinal fluid (CSF) pleocytosis and a negative CSF Gram stain at low risk of an urgent treatable cause. In **chapter eight** we performed an external validation study of risk scores that predict adverse clinical outcome in bacterial meningitis. Risk scores were identified through a systematic review of the literature.

In this chapter we will discuss our main findings in a more general perspective, discuss methodological strengths and limitations and provide recommendations for future research.

Epidemiology

The Dutch Meningitis Study cohort included patients from 1998 to 2002,[1] and had a similar research design to the MeninGene cohort described in **chapter two**. The mean incidence rate of community acquired bacterial meningitis in this previous study was 2.6 per 100,000 adults per year. We found a lower incidence rate of 1.72 cases per 100,000 adults in 2007-08, that further declined to 0.94 per 100,000 adults in 2013-14 (figure 1). Rates of adult bacterial meningitis decreased most sharply among pneumococcal serotypes included in paediatric conjugate vaccine, and in meningococcal meningitis.

Cases in both cohorts were identified through the Netherlands Reference Laboratory for Bacterial Meningitis and by individual physicians. With any surveillance system, the possibility for bias exists if case reporting changes over time. However, comparison of our data to the mandatory notification data from National Institute for Public Health and the Environment (RIVM) was similar over time, as was the proportion of additional patients reported by treating physicians between the two cohorts.

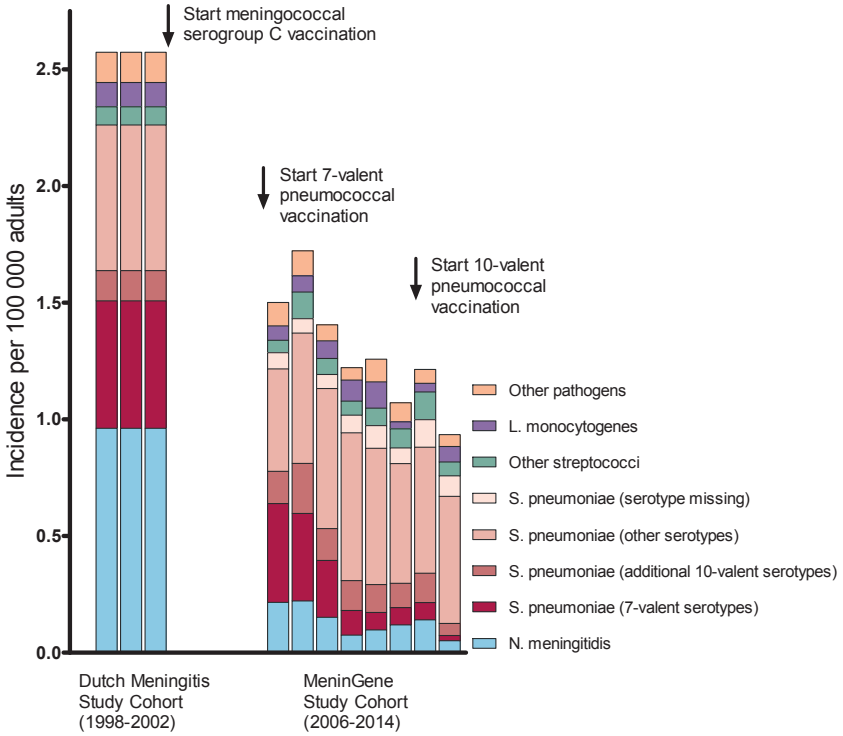


Figure 1. Incidence rates of bacterial meningitis in the Netherlands

The first bars represent mean incidence during the Dutch Meningitis Study cohort.

The incidence rate of meningococcal meningitis was 95% lower in 2013-14 than during the Dutch Meningitis Study cohort of 1998-2002. The introduction of vaccination against *N. meningitidis* serogroup C in 2002 can only party explain this reduction, because most disease was caused by serogroup B. After 15 years of hyper endemic incidence rates in the Netherlands, serogroup B incidence has been steadily declining since 1993 (**chapter three**). A systematic review of global serogroup B incidence rates found that most countries had low incidence rates during the last 15 years, and that serogroup B incidence tended to decrease in countries that collected data consistently.[2] The reasons for this decline in serogroup B disease are unknown, but could include population immunity to strains currently circulating, and changes in the prevalence of behavioural risk factors.[2] Our finding that several genetic meningococcal types increased and decreased simultaneously during the hyperendemic period suggests that changes in the risk factors for meningococcal carriage and diseases play an important role.

Meningococcal incidence rates and serogroup distribution are unpredictable and vary over time and by geographic region.[3] Endemic meningococcal disease at an annual attack rates of around 1 to 3 per 100,000 of the population are found in many countries around the word. Endemic disease can be interrupted by hyperendemic periods, local outbreaks and large epidemics. In hyper-endemic periods the incidence rate increases simultaneously in the whole population for several years. In high-income countries, localised outbreaks occur in small communities like colleges or nursing homes, but the majority of cases are sporadic. The largest epidemics occur in the "meningitis belt", an area extending from Senegal in the west to Ethiopia in the east, and can exceed 1000 cases per 100,000 population.[3] The mechanisms underlying these epidemic patterns are poorly understood. A recent systematic review of incidence and carriage data from the meningitis belt found that carriage prevalence did not substantially increase between endemic and hyperendemic periods.[4] This suggests that either hosts become more susceptible to invasion, or meningococci express more virulence factors during carriage. In contrast, the occurrence of epidemics was associated with a substantial increase in meningococcal transmission and colonisation, and to a lesser extent with increased risk of meningitis given carriage.[4]

Vaccination and herd protection

The implementation of conjugate polysaccharide vaccines against *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b has been one of the most effective public health innovations of the last decades.[5] Although these three bacteria still cause most cases of bacterial meningitis worldwide, incidence and case-fatality rates have decreased substantially.[6] Worldwide, deaths due to pneumococcal, meningococcal and *H. influenzae* type b meningitis, decreased respectively by 29%, 25% and 45% between 1990 and 2013.[7]

A conjugate vaccine against *H. influenzae* type b was introduced into the Dutch National Immunization Program in 1993, and a seven-valent pneumococcal conjugate vaccine has been offered

to children since 2006.[8,9] In response to a sharp increase in serogroup C meningococcal disease in 1998, children aged 1-18 years were offered a single meningococcal serogroup C polysaccharide conjugate vaccination in 2002.[10] Vaccine coverage during the mass vaccination campaign against serogroup C was 89% to 94% depending on age.[11] Routine vaccination at 14 months was subsequently introduced.

In the Netherlands *H. influenzae* type b meningitis decreased from over 200 episodes per year before vaccine introduction to less than 20 episodes per year in recent years.[12] We found a strong reduction in adult pneumococcal meningitis due to serotypes included in the seven-valent conjugate vaccine between 2006 and 2014 (figure 1), and only 10 episodes of serogroup C meningitis occurred in the MeninGene cohort.

The temporal relationship between the introduction of these paediatric conjugate vaccines and the strong decline in adult cases due to bacteria covered by these vaccines is suggestive of a causal relationship. However, the majority of adults are not directly protected by these vaccines. The first children vaccinated against *H. influenzae* type b were 22 years old at the end of the observation period of the current cohort, and only adults below the age of 30 years in 2014 had been eligible for routine vaccination against serogroup C. Routine pneumococcal vaccination for adults is not advised by the Health Council of the Netherlands, with the exception of high-risk groups (e.g., those with hyposplenia or asplenia, sickle cell disease, and cerebrospinal fluid leakage). Based on sales records from Dutch pharmacies pneumococcal vaccine coverage among adults over 65 years old in the Netherlands is low.[13]

The impact of conjugate vaccines on adult bacterial meningitis is therefore likely due to herd protection, whereby reduced nasopharyngeal carriage and transmission in the vaccinated part of the population protects the unvaccinated, by limiting their exposure to the bacteria covered by the vaccine.[14] This is illustrated by our finding in **chapter four.** We found that genetic meningococcal types that are known to frequently express a capsule during nasopharyngeal carriage were more affected by the introduction of the serogroup C conjugate vaccine, than sequence types that express the capsule infrequently. This finding provides further evidence that reduced carriage is an important factor of vaccine impact. Thirty-six percent of the overall reduction in serogroup C cases occurred in the unvaccinated population. Because many cases will have been prevented by reduced carriage and transmission in the vaccinated population as well, the true impact of herd protection will have been even higher.

The long-term effectiveness of conjugate polysaccharide vaccines against *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b is by no means guaranteed. Currently available vaccines do not include all capsule types and these bacteria are capable of capsular switching. Capsular switching occurs through horizontal gene transfer and makes it possible for a genetic lineage to change its capsular phenotype.[15] The selective pressure that is introduced by mass vaccination, using vaccines that do not offer protection against all capsule types, could lead to an increase in

strains not covered by the vaccines, replacing the strains that were covered by the vaccines. The possibility of capsular switching has been clearly established.[5,15,16] The evidence of serotype or serogroup replacement after mass immunization is less clear.[16-18]

A temporary increase in *H. influenzae* meningitis due to serotype a has been reported in Brazil,[19,20] and Portugal experienced a transitory increase in unencapsulated disease.[21] Canadian surveillance data on *H. influenzae* disease from 1989 and 2007 identified some replacement by serotype f and non-typeable strains in children under 5 years old.[22] However, an analysis of three separate surveillance systems in the United States between 1987 and 1995 showed no increase in the incidence of non-type-b disease following vaccine introduction.[23] The European Union Invasive Bacterial Infections Surveillance Network collects surveillance data from 29 European countries and did not find evidence of serotype replacement between 1999 and 2004.[23] In the Netherlands, there was a transitory rise in *H. influenzae* type b disease between 1999 and 2004.[8] The number of non-typeable isolates cultured from blood has increased over the last twenty years, and serotype f seems to be on the rise as well.[12] The incidence rate of *H. influenzae* type b meningitis has remained low after vaccine introduction in 1993, causing 3% of episodes in both the Dutch Meningitis Study and MeninGene cohorts.[1]

Conjugate vaccines against serogroup C meningococcal disease were introduced in the United Kingdom in 1999. No evidence of serogroup replacement has been reported.[17] An affordable conjugate vaccine against serogroup A has been developed to combat meningococcal disease in the meningitis belt. It has recently been introduced in several sub-Saharan countries, and has proven highly effective against serogroup A carriage and disease.[24] A major serogroup C outbreak in Nigeria, the first in the African meningitis belt since 1979, started in 2013 and was still showing rapid expansion at the time of writing.[25]

There is strong evidence in pneumococcal nasopharyngeal carriage that non-vaccine types have increased among carriers in vaccinated populations.[18] Studies on serotype replacement in invasive pneumococcal disease are more heterogeneous, with some studies reporting complete replacement resulting in no net indirect benefit, whereas others report relatively little replacement.[18] The large magnitude of serotype replacement in carriage compared to disease might indicate lower invasiveness of non-vaccine serotypes.[18]

In pneumococcal meningitis, a surveillance study in the United States covering approximately 17.4 million persons during 1998-2007, found evidence of serotype replacement. The increase of non-vaccine types was age dependent, increasing 90% in children under five, 61% at any age, and 18% in patients over 65 years old.[26] We found no evidence of serotype replacement in adult pneumococcal meningitis in the Netherlands between 2006 and 2014. Dutch surveillance data did show serotype replacement in invasive pneumococcal disease, but not in meningitis.[9,13]

Nationwide post-licensure observational studies are suitable to identify the population-wide effect of mass vaccination.[18] However, these observational studies are more prone to biases in-

troduced by changes in clinical practice and case ascertainment. These biases tend to be less for a well-defined disease like meningitis, compared to other pneumococcal disease.[18] This might partly explain the larger estimates of serotype replacement in all invasive pneumococcal disease compared to meningitis only.

Future directions

The large impact of conjugate vaccines on carriage and the strong reduction of cases in the unvaccinated population were largely unexpected, and mostly identified by post-licensure surveillance studies. Randomized controlled trials had not been performed, or the number of study participants was unlikely to confer herd protection. Continuous, long-term, and high quality surveillance studies will be essential for the evaluation and planning of future public health interventions.

Capsular polysaccharide vaccines against serogroup B are not available. However, two serogroup B vaccines (4CMenB, Trumenba), have recently been licenced.[27] Both use outer-membrane proteins as their antigens. Licensure was based on safety and immunogenicity. Efficacy data are not available because of the difficulty in conducting a clinical trial for a low incidence infection. The 4CMenB vaccine has been used in several outbreaks in Canada and the United States, and The Joint Committee on Vaccination and Immunization, which advises U.K. health departments on immunization issues, has recommended an infant 4CMenB schedule, with doses to be given at 2, 4, and 12 months of age. Because of the historically low incidence rate of serogroup B disease in the Netherlands, vaccine introduction at this time does not seem likely. Due to the unpredictable nature of meningococcal epidemiology, this could change in the near future. Post-licensure evaluation studies in the North America and the UK will first have to establish the impact of these vaccines on carriage and disease. The results of these studies are eagerly awaited.

Surveillance data are essential for the early detection of the re-emergence of vaccine type strains, or the replacement by non-vaccine type strains. Age groups with the highest burden of disease are usually targeted for vaccination. However, these groups also tend to have the poorest vaccination response. It has for instance become clear in recent years that antibody levels against serogroup C decline rapidly in the majority of children vaccinated before the age of five years.[28] The immune response of children who were over five years old during the catch-up campaign in 2002 persist much longer.[28] However, as these children become adults, protection against carriage and disease in the adolescent population will diminish. Because meningococcal carriage has the highest prevalence in teenagers and young adults,[29] lasting herd protection is unlikely. The current single serogroup C vaccine at 14 months of age in insufficient, and a booster vaccination at the age of 12-15 years has recently been suggested.[30]

Although speculative, the magnitude of herd protection elicited by conjugate vaccines could possibly open the door to protection of infants, the elderly, and the immunocompromised by only vaccinating the part of the population with both high carriage rates and a good vaccination response.

For instance, maternal vaccination against GBS has been suggested as the most promising strategy to reduce perinatal GBS disease.[31,32] GBS is the leading cause of neonatal sepsis and meningitis in high-income countries.[33] GBS frequently colonizes the human genital and gastrointestinal tracts, and usually results in asymptomatic carriage. In resource-rich countries 20–30% of pregnant women are colonized with GBS, approximately 50% of their babies become colonized and 1% will develop invasive disease.[33,34] Early onset disease, occurring in the first week of life, occurs after aspiration of amniotic fluid infected with bacteria that have ascended from the colonised genital tract of the mother.[33,34] Late-onset disease (day 8 to 90 after birth) can be acquired from the mother or from environmental sources. Currently, many high-income countries have implemented prevention strategies for perinatal GBS disease that include intrapartum antibiotic prophylaxis for all pregnant woman identified to be colonized with GBS or intrapartum antibiotic prophylaxis for woman with specific risk factors.[35] Although these guidelines were based on studies of poor methodological quality, surveillance studies from several countries found reduced incidence of early onset disease after guideline implementation.[31,36] Intrapartum antibiotic prophylaxis has no impact on late onset GBS infection.[33] Despite the introduction of perinatal GBS prevention guidelines in the Netherlands in 1999, the incidence of invasive group B streptococcus infection has steadily increased (**chapter six**). Maternal vaccination could result in direct protection of the newborn by the placental transfer of GBS antibodies, or could provide protection against maternal GBS colonization and reduce transmission. Phase I and phase II clinical trials of maternal GBS vaccines have shown promising results.[37] GBS vaccines are expected to become available in coming years. To evaluate the possible benefit of maternal GBS vaccination in the Netherlands, a prospective cohort study should be set up to determine the current burden of GBS disease, and a nationwide cross-sectional carriage study of GBS isolates from pregnant woman could be used to evaluate possible coverage of GBS vaccines.

Clinical features

Although the epidemiology of community bacterial meningitis has changed after the introduction of paediatric conjugate vaccines, over 95% of patients still present with at least two of the four symptoms of headache, fever, neck stiffness, and altered mental status. Median age in adult bacterial meningitis increased from 52 years in the Dutch Meningitis Study cohort (1998-2002),[1] to 61 years in the present cohort. As patients became older, the proportion of patients with comorbidity increased.

Cranial imaging on admission was performed in 86% of episodes in the MeninGene cohort, compared to 71% in the 1998-2002 cohort. Abnormalities were recorded in 47% of episodes from the current cohort, and 34% in the previous cohort. In adults with suspected meningitis, clinical features have been shown to identify those who are unlikely to have abnormal findings on cranial CT.[38] Clinical features at base line that were associated with an abnormal finding on CT of the

head were an age of at least 60 years, immunocompromised state, a history of central nervous system disease, and a history of seizure within one week before presentation, as well as the following neurologic abnormalities: an abnormal level of consciousness, an inability to answer two consecutive questions correctly or to follow two consecutive commands, gaze palsy, abnormal visual fields, facial palsy, arm drift, leg drift, and abnormal language (e.g., aphasia).[38] Absence of all of these factors occurred in 41% of patients who underwent cranial imaging and had a negative predictive value of 97%.[38]

An important reason to perform imaging before lumbar puncture is to identify patients with brain shift, who are at increased risk of acute herniation after lumbar puncture. Dutch guidelines recommend cranial imaging before lumbar puncture in all patients suspected of bacterial meningitis with aphasia, hemiparesis, monoparesis, seizures, papilledema, score on the Glasgow Coma Scale below 10, active cancer or HIV at admission.[39] We found that imaging before lumbar puncture was performed in 88% of episodes with a guideline indication, as well as 83% of episodes without a documented guideline indication. Overall, imaging before lumbar puncture increased from 67% in 1998-2002 to 86% in the present cohort.

Dutch guidelines recommend empiric treatment with a combination of amoxicillin and a third generation cephalosporin in adults with (suspected) community acquired bacterial meningitis.[39] Adjunctive dexamethasone therapy should be added before or together with antibiotic treatment. If lumbar puncture is postponed because of cranial imaging, treatment should be initiated before the patient is sent to radiology, but after blood cultures are drawn. We found that a combination of amoxicillin with a third generation cephalosporin was started in only a third of episodes. In episodes where cranial imaging preceded lumbar puncture, antibiotics therapy was delayed in almost two thirds of patients. The use of adjunctive dexamethasone, increased from 17% in the previous cohort to 89% of episodes in current study.

Overall outcome did not improve between the two cohorts, but this is due to the relative increase of the more severe pneumococcal compared to meningococcal meningitis. For the subgroup of pneumococcal meningitis, the proportions of patients with an unfavourable outcome or death have decreased substantially; unfavourable outcome from 50% to 41% and death from 30% to 18%. In meningococcal meningitis, the case fatality rate decreased from 7% to 3%.

Adjunctive dexamethasone treatment was independently associated with a favourable outcome and increased survival. The use of observational data precludes strong conclusions in the evaluation of treatment effects, but our current finding in combination with a randomized study in the same population,[40] suggests that implementation of dexamethasone therapy has improved the prognosis of bacterial meningitis in the Netherlands. A recent Cochrane review found that adjunctive corticosteroid use in bacterial meningitis was associated with a non-significant decrease in mortality (RR 0.90, 95% CI 0.80 to 1.01).[41] Use of adjunctive corticosteroids was

not associated with a decrease in long-term neurological sequelae (RR 0.90, 95% CI 0.74 to 1.10). Subgroup analysis on *S. pneumoniae* showed a favourable effect of corticosteroids on mortality (RR 0.84, 95% CI 0.72 to 0.98). A non-significant reduction in mortality was found in *N. meningitidis* (RR 0.71, 95% CI 0.35 to 1.46).

Future directions

Efforts should be made to improve guideline adherence. Both the impact of these interventions on patient management, as well as the possible effect on patient outcome should be studied. Previously identified characteristics of patients who are unlikely to have abnormal findings on cranial CT should be validated in external cohorts. Our study found a previous history of meningitis in 7% of episodes. Causes of recurrent meningitis are cerebrospinal fluid leakage and immunodeficiency, which should carefully be evaluated in patients with recurrent meningitis. Given the high rate of recurrence, vaccination against common causative pathogens of bacterial meningitis could be considered in patient with a first episode of bacterial meningitis.

Risk stratification

When a patients presents with symptoms and signs that are suggestive of meningitis, the differential diagnosis is broad and includes both life threatening and self-limiting diseases. Being able to quickly and accurately identify patients with a self-limiting illness could reduce the number of admissions, diagnostic tests and empiric antibiotic treatment. In **chapter seven** we have developed a risk score that could possibly help physicians to identify adults with CSF pleocytosis and a negative CSF Gram stain at low risk of an urgent treatable cause. The negative predictive value of the low risk category was excellent in the derivation and validation cohorts.

Clinical deterioration can occur rapidly in bacterial meningitis, and poor outcome is common. An accurate prognosis, early on in the course of the disease, could help physicians to better counsel patients and their families, as well as decide upon optimal patient management. Accurate prognostic stratification can also be a valuable tool in evaluating and correcting for case mix in clinical research and for targeting intervention strategies.

Several risk scores have previously been developed that predict outcome in bacterial meningitis. Adequate performance of these scores in new patients is not guaranteed. Poor generalizability of risk scores is common, and can be partly explained by overfitting, and differences in characteristics between the target population and the development cohort. Overfitting can occur when risk scores are not only based on associations between predictors and outcome in the population, but also on idiosyncrasies and random variations in the development sample.[42]

In **chapter eight** we evaluated the performance of nine risk scores that predict outcome in bacterial meningitis in 2108 episodes of bacterial meningitis from the Netherlands. Risk scores

were identified by a systematic review of the literature. Seven risk scores had not been previously evaluated in a new study. We found that these risk scores were able to identify some patients at high risk of an adverse outcome, although many patients with a poor prognosis were missed. Inspection of the calibration curves showed adequate agreement between predicted and observed outcomes for four scores.

To be useful in clinical practice the calculated risks should be accurate, but also differ enough from baseline risk to justify specific treatment or counselling options. For instance, the baseline risk of an unfavourable outcome in bacterial meningitis is 38%, a prediction of 25% might be accurate, but would not be helpful for the treating physician. The baseline case fatality rate in meningococcal meningitis is 6%. Not being classified as high-risk reduced the probability of death to between one and five percent. A high-risk classification was rare, and almost always corresponded to an observed probability of death around 25 to 50%.

Compared to the original study, performance of all scores was reduced in our validation cohort. There were clear differences between the development cohorts of the risk scores and our validation cohort. Six risk scores were developed for invasive meningococcal disease, both meningitis, sepsis and meningitis or sepsis only. Meningococcal sepsis has a worse overall outcome than meningococcal meningitis, which is likely reflected in the higher cases fatality rates in five of the six developmental cohorts compared to our validation cohort. The high rate of adjunctive dexamethasone treatment in our validation might explain the overestimation of the risk score for death in pneumococcal meningitis.

None of the risk scores could be recommended for routine use at this time. However, these scores could be of use in the design and interpretation of scientific studies. For instance, a trial that wanted to evaluate the effect on outcome of an experimental treatment that is expensive or burdensome, could reduce the number of patients that would have to undergo the novel treatment by excluding patients with a very high or very low risk of a clinical adverse outcome. Also, these scores could be used as a measure of severity of illness and be used to correct for case mix between studies or study sites.

Future directions

The risk score that predicts an urgent treatable cause in patients with cerebrospinal fluid pleocytosis and a negative Gram stain could possibly be useful in clinical practice. Over a third of patients in the American validation cohort were categorized as low risk of any urgent treatable cause, and could be considered for outpatient management. However, future studies should first establish if these results are generalizable to new patient populations, whether physicians using the score are better at recognizing patients at low risk of an urgent treatable cause than physicians that do not use the score, and if implementation of the score leads to a safe reduction of healthcare expenditure or improved patient outcome.

An important limitation of our external validation study on outcome in bacterial meningitis was that the quality of reporting of the studies on prediction of outcome in bacterial meningitis did not conform to current standards. Most identified studies, gave little information on calibration of the model and multivariable regression analyses were either not performed, or regression coefficients were not fully reported. In 2015 The Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) Initiative published a set of recommendations for the reporting of studies developing, validating, or updating of a prediction model, whether for diagnostic or prognostic purposes.[43] Adherence to this guideline would aid the interpretation and subsequent validation of future risk scores.

One strategy to reduce overfitting of prediction models, and thereby possibly improve the generalizability of the model, is to select potential predictors on the basis of previous literature instead of analysis of the development cohort.[42] We are currently updating a previously reported risk score for outcome in the cohort described in chapter two, using our systematic review as an overview of possible additional predictor variables.

To facilitate the use of risk sores in clinical practice, the number of predictors is generally kept to a minimum. Generally, only predictors with a strong and independent association with outcome in the derivation cohort are included in the score. However, a strong association identified in a single dataset tends to be weaker, or not even replicable in another dataset.[44] Furthermore, reproducible associations may change over time as clinical practice changes. The widespread introduction of electronic medical records could pave the way for risk scores that include a multitude of predictors with small individual associations, which are automatically updated to changes in disease epidemiology.

In conclusion, although bacterial meningitis is still a formidable disease with high mortality and morbidity, progress has been made. The improvement in outcome that is associated with adjunctive dexamethasone treatment is the largest step forward in decades. Conjugate paediatric vaccines have greatly reduced the incidence of meningococcal, pneumococcal and *H. influenzae* type b meningitis worldwide. These vaccines have been unexpectedly effective against colonization, thereby making it possible to protect infants, immunocompromised patients, and the elderly by vaccinating healthy carriers. High quality and long-term surveillance studies have been essential for vaccine development, implementation and evaluation.

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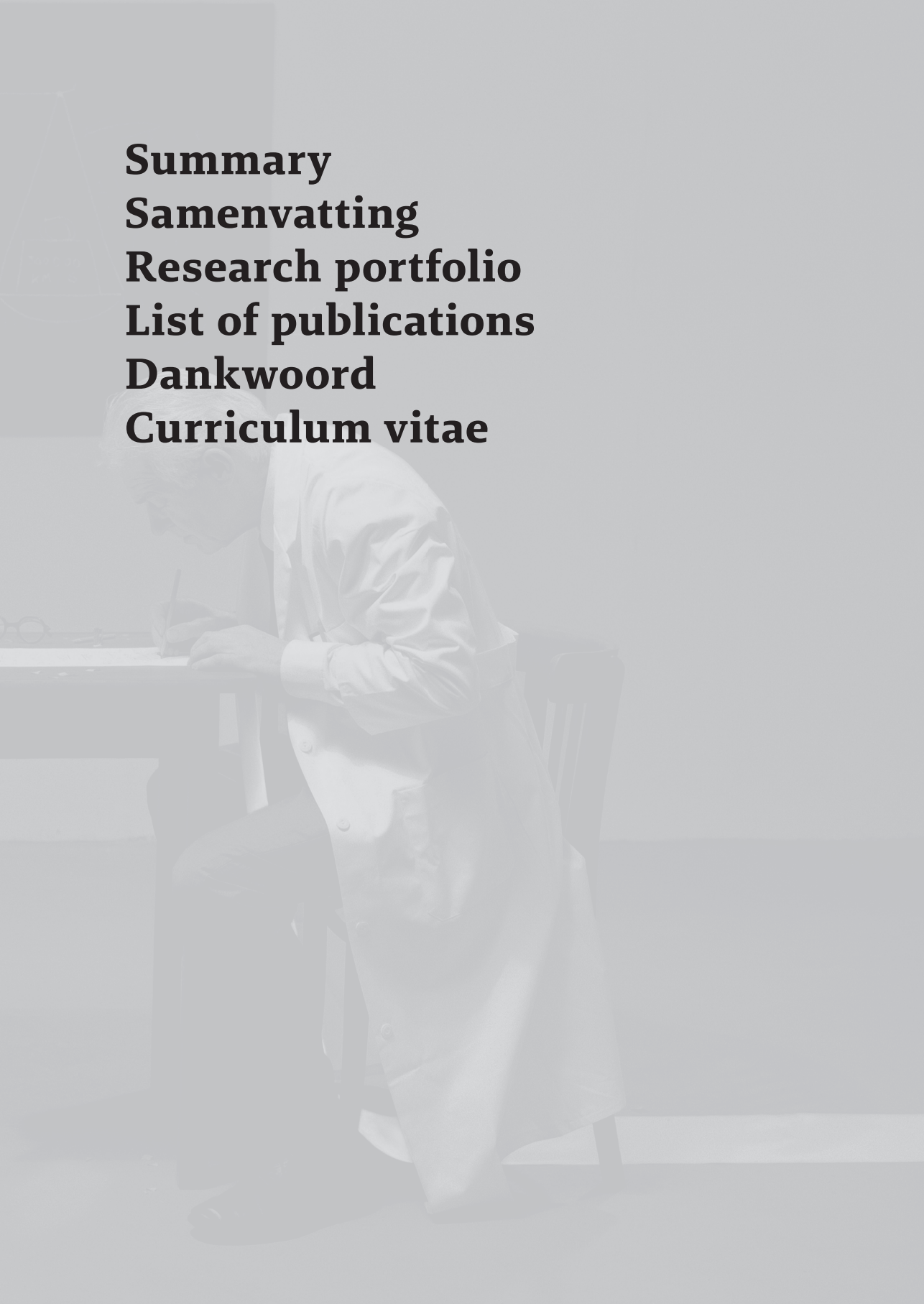
ADDENDUM



Summary

Bacterial meningitis is a devastating disease that is associated with substantial mortality and morbidity. An estimated 303,500 deaths and 21 million disability adjusted life years were attributed to bacterial meningitis in 2013 worldwide. The most common causative pathogens of bacterial meningitis worldwide are *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b, causing 26%, 22% and 21% of global cases in 2013. Large-scale paediatric vaccination programs against these bacteria have been implemented by many countries.

The first objective of this thesis was to study the epidemiology of community-acquired bacterial meningitis after the nationwide implementation of paediatric conjugate vaccines, as well as the long-term epidemiology of invasive meningococcal disease and neonatal group B streptococcal disease in the Netherlands. In a nationwide prospective study on adult bacterial meningitis, we found that the incidence rate declined from 1.72 cases per 100,000 adults per year in 2007–08 to 0.94 per 100,000 per year in 2013–14. Rates of adult bacterial meningitis decreased most in pneumococcal and meningococcal meningitis. Although adults were not eligible for pneumococcal vaccination, pneumococcal incidence rates decreased mostly among pneumococcal serotypes included in paediatric conjugate vaccines. We found no evidence of pneumococcal serotype replacement.

National surveillance data from the Netherlands Reference Laboratory of Bacterial Meningitis from 1960 to 2013, showed that the annual incidence rates of invasive meningococcal disease per 100,000 population increased from 0.5 in 1960, to 4.5 in 2001, and subsequently decreased to 0.6 in 2012. Serogroup B was the most common serogroup over time, causing a hyperendemic period that started in 1982, reached a peak yearly incidence rate of 3.43 per 100,000 population in 1993, and subsequently decreased to 0.39 in 2012. The mechanisms underlying this spontaneous fluctuation of serogroup B disease are poorly understood. A polysaccharide vaccine against serogroup B is not available, but outer-membrane protein vaccines (4CMenB, Trumenba®) have recently been licensed. We established that 19 of 252 PorA variable regions covered 99% of sequenced serogroup B cases over time, and that coverage of the 4CMenB PorA component was 4% in 1960-65, and 36% in 2006-12.

In response to a sharp increase in serogroup C disease, which started in the fall of 1998, children aged 1–18 years were offered a single MCC vaccination in 2002. Routine vaccination at 14 months was subsequently introduced. Vaccine coverage of the target population has been estimated at 94%. Serogroup C has largely disappeared after vaccine introduction. We found that 36% of the overall reduction in serogroup C cases occurred in the unvaccinated population. Meningococci belonging to sequence types that are known to frequently express a capsule during nasopharyngeal carriage were more affected by the introduction of the serogroup C conjugate

vaccine than sequence types that express the capsule infrequently. This finding provides further evidence that reduced carriage is an important factor of vaccine impact. Because many cases will have been prevented by reduced carriage and transmission in the vaccinated population as well, the true impact of herd protection is probably even higher. We found evidence of meningococcal capsular switching, but not of serogroup replacement.

Group B streptococcus is the most common cause of neonatal infections. We studied the clinical and molecular epidemiology of invasive group B streptococcus infection in children younger than 3 months in the Netherlands over 25 years. We found that the introduction of guidelines for the prevention of perinatal group B streptococcal disease did not reduce neonatal meningitis or sepsis due to GBS. Early-onset group B streptococcus infection caused by isolates belonging to clonal complex 17 was more common in the post-implementation period than in the pre-implementation period.

The second objective was to describe the clinical features of adult community acquired bacterial meningitis after the introduction of adjunctive dexamethasone therapy and routine paediatric conjugate vaccines against *H. influenzae* type b, *S. pneumoniae* and *N. meningitidis* serogroup C. This was done by an analysis of clinical features from 1412 episodes from the nationwide prospective MeninGene cohort included from 2006 to 2014. Median age was 61 years. Ninety-five percent of patients presented with at least two of four symptoms consisting of headache, fever, neck stiffness, and altered mental status.

Dutch guidelines recommend empiric treatment with a combination of amoxicillin and a third generation cephalosporin in adults with (suspected) community acquired bacterial meningitis.[39] Adjunctive dexamethasone therapy should be administered together with antibiotic treatment. Cranial imaging before lumbar puncture is recommended for all patients with aphasia, hemiparesis, monoparesis, seizures, papilledema, score on the Glasgow Coma Scale below 10, active cancer or HIV at admission. If lumbar puncture is postponed because of cranial imaging, treatment should be initiated before the patient is sent to radiology, but after blood cultures are drawn. We found that the combination of amoxicillin with a third generation cephalosporin was started in only a third of episodes. In episodes where cranial imaging preceded lumbar puncture, antibiotic therapy was delayed in almost two thirds of patients. Adjunctive dexamethasone treatment was started in 89% of episodes.

Compared to a previous Dutch cohort from 1998 to 2002, the outcome of pneumococcal meningitis improved substantially. The proportions of patients with an unfavourable outcome decreased from 50% to 41%, and the case fatality rate declined from 30% to 18%. In meningococcal meningitis, the case fatality rate decreased from 7% to 3%. Adjunctive dexamethasone treatment was independently associated with a favourable outcome and increased survival. The adjusted odds ratio for the association between dexamethasone treatment and unfavourable

outcome was 0.55 (95%CI, 0.38–0.80) in pneumococcal meningitis and 0.44 (95% CI, 0.23–0.85) in episodes due to other pathogens.

The third objective of this thesis was to identify predictors of severe illness, both in adults with bacterial meningitis as well as in patients presenting with cerebrospinal pleocytosis and a negative cerebrospinal fluid gram stain. We developed a risk score that identifies adults with cerebrospinal fluid pleocytosis and a negative cerebrospinal fluid Gram stain at low risk of an urgent treatable cause. The negative predictive value of the low risk category was excellent in the derivation and validation cohorts. The score could possibly be useful in clinical practice. Over a third of patients in the American validation cohort were categorized as low risk of any urgent treatable cause, and could be considered for outpatient management. However, future studies should first establish if these results are generalizable to new patients, and if implementation of the score leads to a safe reduction of healthcare expenditure or improved patient outcome.

Clinical deterioration can occur rapidly in bacterial meningitis, and poor outcome is common. An accurate prognosis, early on in the course of the disease, could help physicians to better counsel patients and their families, as well as decide upon optimal patient management. Accurate prognostic stratification can also be a valuable tool in evaluating and correcting for case mix in clinical research and for targeting intervention strategies. We performed an external validation study of nine risk scores that predict adverse clinical outcome in bacterial meningitis. Risk scores were identified through a systematic review of the literature and evaluated in 2108 episodes of bacterial meningitis from the Netherlands. Seven risk scores had not been previously evaluated in a new study.

We found that these risk scores were able to identify some patients at high risk of an adverse outcome, although many patients with a poor prognosis were missed. Inspection of the calibration curves showed adequate agreement between predicted and observed outcomes for four scores, but predicted probabilities that differ enough from baseline risk to justify specific treatment or counselling options, were uncommon. We therefore concluded that none of the risk scores could be recommended for routine use in patient care at this time. One risk score showed fair to good discrimination and calibration in two external cohorts in the Netherlands, and could be of value for the design or interpretation of scientific studies on bacterial meningitis in high-income countries.

Bacterial meningitis is a formidable disease with high mortality and morbidity, but progress has been made over the last decades. Outcome has been improved substantially by the introduction of adjunctive dexamethasone. Conjugate paediatric vaccines have greatly reduced the incidence of meningococcal, pneumococcal and *H. influenzae* type b meningitis worldwide. These vaccines have been unexpectedly effective against colonization, thereby making it possible to protect infants, immunocompromised patients, and the elderly by vaccinating healthy carriers.

High quality and long-term surveillance studies will remain essential for vaccine development, implementation and evaluation.

Samenvatting

Bacteriële meningitis (hersenvliesontsteking) is een ernstige infectieziekte met een hoge morbiditeit en mortaliteit. Wereldwijd stierven in 2013 meer dan 300,000 mensen aan de gevolgen van bacteriële meningitis en was het berekende verlies aan gezonde levensjaren naar schatting 21 miljoen. De meest voorkomende verwekkers van bacteriële meningitis zijn *Streptococcus pneumoniae*, *Neisseria meningitidis*, en *Haemophilus influenzae* type b, die werden geïdentificeerd bij 26%, 22% en 21% van alle ziektegevallen in 2013. In Nederlands zijn vaccins tegen deze meest voorkomende bacteriën opgenomen in het Rijksvaccinatieprogramma.

Het eerste doel van dit proefschrift was het beschrijven van de epidemiologie van bacteriële meningitis bij volwassenen na de invoering van deze vaccins bij kinderen, van meningitis of sepsis ten gevolge van *N. meningitidis* in de gehele Nederlandse bevolking tussen 1960 en 2012 en het vóórkomen van meningitis of sepsis veroorzaakt door groep B streptokokken (GBS) bij pasgeborenen van 1987 tot 2012. In een landelijk prospectief cohort vonden wij dat de incidentie van bacteriële meningitis afnam van 1.72 gevallen per 100,000 volwassenen per jaar in 2007-08 tot 0,94 per 100,000 in 2013-14. Deze afname kwam met name door een daling in het aantal gevallen van meningokokken- en pneumokokkenmeningitis. Alhoewel volwassenen in Nederland niet gevaccineerd worden tegen pneumokokken, werd de afname van pneumokokkenmeningitis voornamelijk gezien in serotypen waartegen kinderen worden gevaccineerd. We vonden geen aanwijzingen voor een gelijktijdige toename van serotypen waartegen niet wordt gevaccineerd.

Data van het Nederlands Referentie Laboratorium voor Bacteriële Meningitis van 1960 tot 2013 toonde dat de jaarlijkse incidentie van meningitis of sepsis ten gevolge van *N. meningitidis* opliep van 0.5 per 100,000 inwoners in 1960, tot 4.5 per 100,000 in 2001. Sindsdien is de incidentie weer afgenomen tot 0.6 per 100,000 in 2012. De meeste gevallen werden veroorzaakt door serogroup B tijdens een hyperendemische periode 1982 tot 2012 met een maximale incidentie van 3.43 per 100,000 in 1993. De oorzaken voor deze spontane fluctuatie in serogroup B zieke zijn grotendeels onbekend. Er bestaan geen vaccins tegen het polysaccharide kapsel van serogroep B. Recentelijk zijn er wel twee vaccins geregistreerd tegen membraan eiwitten van serogroep B (4CMenB, Trumenba®). Wij vonden 252 verschillende variabele regio's van het PorA membraan eiwit. Een vaccin tegen 21 van 252 regio's zou 99% van alle getypeerde meningokokken isolaten kunnen dekken. Vier procent van de isolaten uit 1960-65 en 36% van de isolaten uit 2006-2012 hadden het PorA type dat is opgenomen in het 4CMenB vaccin.

In de herfst van 1998 begon het aantal gevallen van serogroup C meningokokken ziekte in Nederland sterk toe te nemen. In 2002 werden alle kinderen van 1 tot 19 jaar opgeroepen voor een eenmalige vaccinatie met het polysaccharide conjugaat vaccin tegen serogroup C. Kinderen worden sindsdien op de leeftijd van 14 maanden tegen serogroep C gevaccineerd binnen

het Rijksvaccinatie Programma. De vaccinatiegraad wordt geschat op 94% van de doelgroep. Serogroep C is sindsdien grotendeels uit Nederland verdwenen. Wij vonden dat 36% van de afname zich voordeed in het deel van de bevolking dat niet voor vaccinatie in aanmerking kwam. Ook zagen wij dat MLST typen die het serogroep C kapsel frequent tot expressie brengen tijdens dragerschap sterker afnamen dan MLST typen die dit minder vaak doen. Deze bevindingen illustreren hoe een groot deel van de effectiviteit van dit vaccin wordt veroorzaakt door verminderde kolonisatie en transmissie. Omdat deze zogenaamde 'herd protection' ook voor een afname zal hebben gezorgd in het gevaccineerde deel van de bevolking is dit aandeel waarschijnlijk nog hoger.

GBS is de meest voorkomende oorzaak van sepsis en meningitis bij pasgeborenen. In Nederland werden in 1999 richtlijnen ingevoerd om perinatale GBS ziekte te voorkomen. Wij vonden dat de incidentie van GBS ziekte bij kinderen onder de 3 maanden tussen 1987 en 2012 echter niet was afgenomen. Sinds het invoeren van de richtlijn kwamen isolaten behorende tot clonal complex ST-17 meer voor.

Het tweede doel van dit proefschrift was het beschrijven van de kliniek van bacteriële meningitis sinds het landelijk invoeren van adjuvante therapie met dexamethason en de vaccinatie van kinderen tegen *S. pneumoniae*, *N. meningitidis*, en *H. influenzae* type b. Hiertoe werden klinische gegeven geanalyseerd van 1412 gevallen van bacteriële meningitis die van 2006 tot 2014 werden geïncludeerd in het landelijke prospectieve MeninGene cohort. De gemiddelde leeftijd in het cohort was 61 jaar. Vijfennegentig procent van de patiënten presenteerde zich met minimaal twee van de symptomen koorts, hoofdpijn, nekstijfheid of een gedaald bewustzijn.

De landelijke richtlijn Bacteriële Meningitis adviseert empirische behandeling met een combinatie van amoxicilline en een 3e generatie cefalosporine. Tevens dient dexamethason gestart te worden, bij voorkeur met de eerste gift antibiotica. Een CT-scan van de hersenen is geïndiceerd vóór het verrichten van een lumbaalpunctie bij (de verdenking op) bacteriële meningitis en focale neurologische uitval (exclusief hersenzenuwuitval), insulten, papiloedeem, gedaald bewustzijn (Glasgow Coma Scale score <10) of een ernstige immunodeficiëntie. Behandeling met antibiotica en dexamethason dient dan direct gestart te worden. Wij vonden dat slechts een derde van de patiënten tijdens de observatie periode werd behandeld met zowel amoxicilline als een 3e generatie cefalosporine. Wanneer beeldvorming van de hersenen werd verricht vóór de lumbaalpunctie leidde dit in bijna twee derde van de gevallen tot het uitstellen van adequate behandeling. Dexamethason werd in 89% van de gevallen gestart.

De uitkomst van bacteriële meningitis is verbeterd in vergelijking met een Nederlands cohort uit 1998-2002. Het aantal patiënten met pneumokokkenmeningitis en onvolledig herstel (Glasgow Outcome Scale score 1-4) nam af van 50% tot 41%, en het overlevingspercentage steeg van 70% naar 82%. In meningokokkenmeningitis nam de kans op overlijden af van 7% naar 3%. Behande-

ling met dexamethason bleek een onafhankelijk voorspeller voor een goede uitkomst, met een odds ratio van 0.55 (95% BI, 0.38–0.80) in pneumokokkenmeningitis en 0.44 (95% BI, 0.23–0.85) in meningitis veroorzaakt door andere bacteriën.

Het derde doel van dit proefschrift was het vinden van voorspellers van ernst van ziekte, zowel voor patiënten met bacteriële meningitis als voor patiënten die zich presenteren met een cel verhoging in de liquor bij wie geen bacteriën worden gezien in het Gram preparaat. Voor deze laatste groep maakten wij een risicoscore waarmee patiënten kunnen worden geïdentificeerd met een zeer lage kans op een ernstige of behandelbare aandoening. De score zou mogelijk van waarde kunnen zijn in de patiëntenzorg. Meer dan een derde van de patiënten die zich op een Amerikaanse spoedeisende hulp presenteerden met een pleiocytose en negatief Gram preparaat werd door de score geclassificeerd als laag risico en zouden mogelijk poliklinisch vervolgd kunnen worden. Andere studies dienen deze bevinding echter eerst te bevestigen in andere patiëntengroepen en vervolgens moet worden aangetoond dat het gebruik ook daadwerkelijk leidt tot een veilige en kosteneffectieve afname of verbetering van patiëntenzorg.

Patiënten met bacteriële meningitis kunnen snel verslechteren en een slechte uitkomst komt vaak voor. Een betrouwbare prognose in het begin van de ziekte zou kunnen helpen bij het begeleiden van patiënten en hun familie en het aanpassen van de behandeling aan de ernst van ziekte. Het kunnen voorspellen van de kans op een slechte uitkomst op basis van meerdere patiënten-karakteristieken kan ook waardevol zijn bij het opzetten, vergelijken en interpreteren van wetenschappelijk onderzoek. Wij verrichtten een externe validatie studie van negen bestaande risico scores in 2108 patiënten met bacteriële meningitis. Wij vonden dat meerdere scores goed in staat waren om patiënten met een hoger of lager risico op een slechte uitkomst van elkaar te onderscheiden, en vier scores vertoonden een redelijke overeenkomst tussen de voorspelde kans op een slechte uitkomst en de geobserveerde uitkomst. Veel patiënten met een slechte uitkomst werden echter door de scores gemist, en een voorspelde kans die genoeg afweek van het a priori risico om een beleidswijziging te rechtvaardigen kwam niet vaak voor. Wij verwachten niet dat het standaard gebruik van een van deze scores de patiëntenzorg zal verbeteren. Een score had een redelijke tot goede kalibratie en discriminatie in twee Nederlandse validatie cohorten, en zou mogelijk gebruikt kunnen worden in wetenschappelijk onderzoek naar bacteriële meningitis in ontwikkelde landen.

Dit promotieonderzoek illustreert de vooruitgang die er de laatste decennia is geboekt in de preventie en behandeling van bacteriële meningitis. Het gebruik van dexamethason is sterk geassocieerd met een beter herstel en een betere overleving. Vaccinatie heeft de incidentie van meningitis door *S. pneumoniae*, *N. meningitidis*, en *H. influenzae* type b doen afnemen. Het induceren van 'herd protection' (kudde bescherming) heeft het mogelijk gemaakt om mensen met een slechte immuunrespons, zoals pasgeboren, ouderen en patiënten met een immuunstoornis, te beschermen door het vaccineren van genoeg gezonde dragers.

Research portfolio

Name PhD student:	M. W. Bijlsma
PhD period:	September 2012 - January 2016
Name PhD supervisor:	Prof. dr. D. van de Beek

1. PhD training	Year	Workload (ECTS)
General courses		
Master's Programme in Epidemiology (EpidM)	2012 - 2015	56
BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2013	0,9
Specific courses		
Clinical Prediction Models (EpidM)	2013	2
Systematic Reviews and meta-analysis (EpidM)	2013	2
Seminars, workshops and master classes		
Weekly Department Seminars	2012 - 2016	8
Presentations		
"*Aanhoudende groepsimmuniteit 10 jaar na introductie van menin-gokokken-C-vaccinatie in Nederland*", oral presentation at Wetenschappelijke Vergadering NVN	2013	0,5
"*Herd Immunity following Meningococcal Vaccine*", oral presentation at 53rd ICAAC conference	2013	0,5
(Inter)national conferences		
53rd ICAAC conference in Denver, Colorado	2013	1
Wetenschappelijke Vergadering, Nederlandse Vereniging voor Neurologie	2013	0,3
Other		
Expert meeting National Institute of Public Health and the Environment (RIVM) on invasive meningococcal disease among men who have sex with men	2013	0,5

2. Teaching	Year	Workload (ECTS)
Lecturing		
"*Epidemiologie invasieve meningokokken ziekte in Nederland 1960-2012*", Regionale Academische Avond Kindergeneeskunde	2014	0,5
"*Pleiocytose met een negatief Gram preparaat, is een onschuldige aandoening te voorspellen?*", Klinische Avond Amsterdamsche Neurologen Vereeniging	2012	0,5
"*Bacterial meningitis, differential diagnosis and treatment*", paediatric specialty training program	2015	0,5
Tutoring, Mentoring		
Residents in paediatric specialty training	2012 - 2015	12
Supervising		
PhD student M.M. Koopmans, project "Listeria meningitis and genetics"	2015	1

3. Parameters of Esteem	Year
Grants	
ICAAC Infectious Diseases Fellows Grant Program	2013
ASM Infectious Disease (ID) Fellow Travel Grant	2013

List of publications

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Bijlsma MW. Korte geschiedenis van de jeugd-ggz. In: Leerboek Psychiatrie Kinderen en Adolescenten. Utrecht: De Tijdstroom Uitgever

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Curriculum vitae

Merijn Bijlsma was born on October 2, 1978 in Amsterdam. He finished secondary school in 1997 at the Fons Vitae Lyceum in Amsterdam. After studying physics for a year at the University of Amsterdam, he decided to switch to Medicine at the same university and obtained his medical degree in 2006. During his studies he was a member of the study program committee, and he was involved in promoting global health education through the International Federation of Medical Students' Associations. He did a clinical internship at Shriners Hospital for Children in Boston and a research internship in Nepal, studying referral patterns in tuberculosis treatment.

After graduation he worked in the Sint Lucas Andreas Ziekenhuis in Amsterdam at the department of Paediatrics. He completed the Paediatrics residency training programme of the VU University Medical Center in 2012, also having worked at Onze Lieve Vrouwe Gasthuis in Amsterdam. He joined the Neuroinfections Amsterdam research group in 2012, and worked on this thesis under supervision of Professor Diederik van de Beek. He is a member of the Guidelines Committee of Dutch Society for Paediatrics (NVK) and completed a Master's programme in epidemiology in 2015. He is currently working as a general paediatrician at the VU University Medical Center.

Merijn is married to Maartje Kester and they have a one-year old son Midas.


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