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Patient and strain characteristics in relation to the outcome of meningococcal disease: a multivariate analysis

R. J. P. M. SCHOLTEN[1,2], H. A. BIJLMER[2]*, H. A. VALKENBURG[1] AND J. DANKERT[2]

[1] *Institute for Research in Extramural Medicine, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT, Amsterdam, The Netherlands*

[2] *Netherlands Reference Laboratory for Bacterial Meningitis, WHO Collaborating Centre, University of Amsterdam/National Institute for Public Health and Environmental Protection, Amsterdam, The Netherlands*

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SUMMARY

To investigate the joint association of patient and strain characteristics with the outcome of meningococcal disease (MD), data were collected on 563 consecutive cases of MD reported between 1989 and 1990 in The Netherlands. The meningococcal isolates were characterized with regard to their surface characteristics. Sequelae occurred in 8·5% of the patients, and were only associated with the presence of bacteraemia. The case-fatality rate was 7·7%. Infants aged $\leqslant 5$ months and patients in the age-groups of 10–19 years and $\geqslant 50$ years had an increased risk for a fatal outcome compared with children from 6 months to 9 years old (Odds Ratios [ORs]: 5·1, 3·4 and 9·8, respectively). The OR for females versus males was 2·3. The ORs for patients with bacteraemia, or a combination of bacteraemia and meningitis, compared with meningitic patients, were 2·3 and 3·1. Meningococcal strain characteristics did not influence the case-fatality rate substantially. In conclusion, host factors were found to be determinants for a fatal outcome of MD in The Netherlands from 1989 to 1990.

INTRODUCTION

Meningococcal disease (MD) is a major health issue in both developing and industrialized countries. The reported case-fatality rates (CFRs) of MD range from 2 to 13%, and in 3–11% of the survivors serious sequelae are encountered [1–9]. Despite the improvement of medical care in the past four decades, little progress has been made in reducing the number of fatal cases of MD [7].

Several surface characteristics of *Neisseria meningitidis*, such as the serogroup and serotype, have been found to be related to an unfavourable outcome of MD [3, 6, 10]. In the past decade an increasing number of monoclonal antibodies (moabs) for serotyping, subtyping and lipooligosaccharide (LOS) immunotyping have been developed, enabling us to characterize meningococci more accurately

* Present address: Hospital Bronovo, The Hague, The Netherlands.

and to analyse in more detail the relationship between strain characteristics and disease outcome [11, 12]. The outcome of MD, however, is also determined by host-related factors, such as the age of the patient and underlying diseases compromising the host defence mechanisms [1–6, 8, 9]. When determining the relation between a single variable and the disease outcome, there is always the possibility that this relation is confounded by other determinants. By using multivariate statistical methods it is possible to adjust for the influence of other covariates and to assess the relation of individual variables with the disease outcome, conditional on the other variables.

In this paper we present the results of a multivariate analysis of the association of strain characteristics of *N. meningitidis* and patient characteristics, on the one hand, with the outcome of disease on the other, during a period of high endemicity in The Netherlands [13].

PATIENTS AND METHODS

Patients

The study included 563 consecutive bacteriologically confirmed cases of systemic MD in The Netherlands, reported between 1 April 1989 and 30 April 1990. A case was defined as bacteraemic if *N. meningitidis* was cultured from the blood alone, and as meningitic if there was a positive culture from the cerobrospinal fluid (CSF) alone. In cases of positive cultures from both sources, a patient was defined as having both meningitis and bacteraemia. Data on disease outcome (survived or deceased), the presence of sequelae, age, gender and predisposing factors were collected by means of a questionnaire, completed by the specialist in attendance. The presence of sequelae was assessed at least 4 weeks after admission to hospital. Sequelae were considered to be the occurrence of seizures, hydrocephalus, cerebral atrophy, mental retardation, hearing loss, paralysis of a cranial nerve, disturbance of vision, hemiparesis, peripheral neuropathy, scars after skin necrosis, amputation, behavioural disturbances and hypopituitarism. The following conditions were considered to be predisposing factors for a fatal disease outcome: severe head injury, a CSF leak, diabetes mellitus, chronic obstructive lung disease, chronic renal failure, liver cirrhosis, malignancy, immunosuppressive therapy and intravenous drug-abuse.

Bacterial isolates

Meningococcal isolates of all patients were submitted to The Netherlands Reference Laboratory for Bacterial Meningitis by regional laboratories. Pairs of isolates, cultured from both the CSF and blood of the same patient, were obtained from 123 cases. In 11 pairs, 1 isolate of each pair did not survive the transport to our laboratory. Both isolates of each of the remaining 112 pairs were identical with regard to the surface characteristics, and only 1 of each pair was included in the analysis.

Serogrouping and typing

Serogrouping was performed by means of Ouchterlony gel diffusion [14]. Serotyping, subtyping and LOS immunotyping were performed by means of a whole-cell ELISA, as previously described, with an extensive set of moabs [11].

The assignment of LOS immunotypes is described elsewhere [12]. It was not possible to assign an immunotype to eight serogroup B isolates, due to a non-interpretable moab reaction pattern.

Statistical methods

The chi-square test was used for the analysis of contingency tables. Fisher's exact test was used when appropriate. The relation of the various variables with the outcome of the disease was analysed by means of a multiple logistic regression analysis. All variables which were associated with the disease outcome were included in the initial model. The model was fitted by backward elimination of individual variables. In the final step, interaction terms were added and tested for further improvement of the model. The assessment of the fit was performed as described by Hosmer and Lemeshow [15].

In the statistical analyses the serogroups X, Y, Z, W-135 and 29E and non-groupable isolates were combined, because of their small numbers. For the same reason the serotypes 1, 14, 16 were combined, as were the subtypes P1.1, P1.6, P1.7, P1.7,1, P1.9, P1.10, P1.12, P1.14. The immunotypes were pooled in four categories: L1/8 (L1, L1,8, L8 and L8,10), L2/4 (L2 and L4), L3/1/8 (L3, L3,1, L3,1,8 and L3,8), and 'other ' (L10, L10,11 and non-typable isolates).

Statistical analyses were carried out on a personal computer using the SPSS/PC® package.

RESULTS

During the study-period, 563 patients (295 males, 268 females) with systemic MD were reported. The outcome of disease was known for 562 patients, of whom 43 died (CFR = 7·7%).

The CFR according to serogroup and clinical presentation (meningitis, bacteraemia or both) is shown in Table 1. The lowest CFR was found among meningitic patients ($\chi^2 = 10{\cdot}1$; 2 degrees of freedom [df]: $P = 0{\cdot}006$). The CFR differed among the various serogroups ($\chi^2 = 11{\cdot}7$; 3 df: $P = 0.009$) and was highest in disease due to the uncommon serogroups (21·1%). These differences are at least partly due to an uneven distribution of the clinical presentation among the various serogroups ($\chi^2 = 22{\cdot}8$; 6 df: $P < 0.001$).

No significant association was found between the CFR and serotype or subtype ($\chi^2 = 3{\cdot}1$; 5 df: $P = 0.680$ and $\chi^2 = 13{\cdot}1$; 8 df: $P = 0.109$, respectively; Table 2). The serotypes 2a and 2b and the subtypes P1.2, P1.5, P1.5,2, P1.15 and P1.16 tended to have somewhat higher CFRs. Lower CFRs were found in patients with disease due to serotype 4 (5·4%) and subtype P1.4 (3·5%), which are at present the most prevalent serotype and subtype in The Netherlands.

The association between the CFR and LOS immunotype for each serogroup is shown in Table 3. Immunotypes L2 and L4 were associated with the highest CFR (11·3%), but this association was not statistically significant ($\chi^2 = 3{\cdot}7$; 3 df: $P = 0.296$). Within serogroup B, the CFR in disease due to L2 and L4 (8·5%) was almost twice as high as that in disease due to the L3 group of immunotypes (4·8%), but among serogroup C isolates these CFRs were almost equal (12·8 and 14·0%, respectively).

Table 1. *Distribution of cases and case-fatality rates (in parentheses) of meningococcal disease in The Netherlands, 1989–90, by serogroup and clinical presentation*

Serogroup	Clinical presentation: Meningitis	Bacteraemia	Meningitis and bacteraemia	Total
A	12 (16·7)	1 (0·0)	0 —	13 (15·4)
B	255 (3·9)	74 (8·1)	96 (8·3)	425 (5·6)
C	50 (4·0)	30 (16·7)	25 (24·0)	105 (12·4)
Other	8 (12·5)	9 (22·2)	2 (50·0)	19 (21·1)
Total	325 (4·6)	114 (11·4)	123 (12·2)	562 (7·7)

Table 2. *Distribution of cases and case-fatality rates (in parentheses) of meningococcal disease in The Netherlands, 1989–90, by serotype and subtype according to serogroup*

Variable	Serogroup: A	B	C	Other	Total
Total no. of patients	13 (15·4)	425 (5·6)	105 (12·4)	19 (21·1)	562 (7·7)
Serotype					
2a	0 —	15 (6·7)	44 (11·4)	3 (0·0)	62 (9·7)
2b	0 —	11 (9·1)	13 (15·4)	1 (0·0)	25 (12·0)
4	5 (0·0)	184 (4·3)	9 (22·2)	6 (16·7)	204 (5·4)
15	1 (100·0)	44 (4·5)	2 (0·0)	0 —	47 (6·4)
Other	0 —	18 (5·6)	2 (50·0)	2 (0·0)	22 (9·1)
Non-typable	7 (14·3)	153 (7·2)	35 (8·6)	7 (42·9)	202 (8·9)
Subtype					
P1.2	0 —	7 (14·3)	3 (0·0)	1 (0·0)	11 (9·1)
P1.4	0 —	168 (3·6)	1 (0·0)	2 (0·0)	171 (3·5)
P1.5	0 —	9 (11·1)	16 (12·5)	4 (75·0)	29 (20·7)
P1.5,2	0 —	20 (5·0)	36 (13·9)	3 (0·0)	59 (10·2)
P1.7,16	0 —	24 (8·3)	0 —	0 —	24 (8·3)
P1.15	0 —	24 (12·5)	2 (0·0)	0 —	26 (11·5)
P1.16	7 (14·3)	43 (9·3)	0 —	1 (0·0)	51 (9·8)
Other	5 (0·0)	60 (3·3)	29 (10·3)	5 (20·0)	99 (6·1)
Non-subtypable	1 (100·0)	70 (5·7)	18 (16·7)	3 (0·0)	92 (8·7)

Table 3. *Distribution of cases and case-fatality rates (in parentheses) of meningococcal disease in The Netherlands, 1989–90, by immunotype and serogroup*

Immunotype category	Serogroup: A	B	C	Other	Total
L1/8	0 —	69 (5·8)	5 (0·0)	1 (0·0)	75 (5·3)
L2/4	0 —	59 (8·5)	47 (12·8)	9 (22·2)	115 (11·3)
L3/1/8	8 (25·0)	289 (4·8)	50 (14·0)	8 (25·0)	355 (7·0)
Other	5 (0·0)	0 —	3 (0·0)	1 (0·0)	9 (0·0)
Total	13 (15·4)	417 (5·5)	105 (12·4)	19 (21·1)	554 (7·6)

Note: In eight cases due to serogroup B no immunotype could be assigned.

Table 4. *Distribution of cases and case-fatality rates (in parentheses) of meningococcal disease in The Netherlands, 1989–90, by age-category and gender*

Age-category	Gender		Total
	Male	Female	
0–5 months	12 (16·7)	12 (16·7)	24 (16·7)
6 months–9 years	166 (2·4)	123 (4·9)	289 (3·5)
10–19 years	73 (4·1)	83 (15·7)	156 (10·3)
20–49 years	30 (3·3)	26 (3·8)	56 (3·6)
⩾ 50 years	14 (21·4)	23 (34·8)	37 (29·7)
Total	295 (4·4)	267 (11·2)	562 (7·7)

Table 4 shows the age-specific CFRs, according to gender. The highest CFR is found among patients over 50 years of age (29·7%), followed by infants aged 0–5 months (16·7%) and teenagers (10·3%; $\chi^2 = 38{\cdot}3$; 4 df: $P < 0{\cdot}001$). The CFR among female patients (11·2%) differed significantly from the CFR among males (4·4%; $\chi^2 = 9{\cdot}3$; 1 df: $P = 0{\cdot}002$). Female patients were older on the average ($\chi^2 = 7{\cdot}9$; 4 df: $P = 0{\cdot}094$). The largest gender-specific CFR differences were found in the age-categories 10–19 and ⩾ 50 years of age, in which females outnumbered males (Table 4). There was no significant association between gender and serogroup ($\chi^2 = 0{\cdot}1$; 3 df: $P = 0{\cdot}996$) or clinical presentation ($\chi^2 = 0.0$; 2 df: $P = 0{\cdot}993$).

There were 26 patients (4·8%) with a predisposing factor. The CFR among those patients (19·2%) was almost three times higher than that of patients without a predisposing factor (6·7%; Fisher's exact test: $P = 0{\cdot}034$). Predisposed patients were found to suffer more often from bacteraemia than other patients ($\chi^2 = 11{\cdot}2$; 2 df: $P = 0{\cdot}004$). The proportion of patients with a predisposing factor was highest among patients over 50 years of age (37·8%; $\chi^2 = 100{\cdot}0$; 4 df: $P < 0{\cdot}001$). Predisposed patients and patients over 50 years of age suffered significantly more often from disease due to the uncommon serogroups ($\chi^2 = 21{\cdot}8$; 3 df: $P < 0{\cdot}001$ and $\chi^2 = 49{\cdot}0$; 12 df: $P < 0{\cdot}001$, respectively).

In the multivariate analysis, only the clinical presentation, age and gender turned out to be significantly associated with a fatal disease outcome, and predisposing factors and strain characteristics did not influence the CFR substantially. No significant interactions between the covariates were found. The estimated adjusted Odds Ratios (ORs) for a fatal outcome for each (category of a) variable with respect to a selected reference category in the final model are shown in Table 5. The striking difference in the risk of fatal disease for female versus male patients still stands after adjustment for the other covariates (OR = 2·3; 95%-confidence interval (95%-CI): 1·2–4·7).

Serious sequelae were reported in 44 of the 515 surviving patients (8·5%). In 4 survivors the presence or absence of sequelae was not specified. Five patients had 2 sequelae and 4 patients more than 2 sequelae. Scars after skin necrosis were reported in 20 patients, and amputation in 4. Hearing loss (16 patients), paralysis of a cranial nerve (5), hydrocephalus (3), cerebral atrophy (2) and seizures (2) were the most frequent neurological sequelae. Other sequelae were mental retardation (3 patients), disturbance of vision, hemiparesis, peripheral neuropathy, hypo-

Table 5. *Adjusted Odds Ratios (OR) for fatal outcome for variable versus the reference category of each variable (OR = 1·0) for 562 patients with systemic meningococcal disease in The Netherlands, 1989–90*

Variable	OR (95%-Confidence Interval)
Age of patient	
0–5 months	5·1 (1·4–18·2)
6 months–9 years	1·0
10–19 years	3·4 (1·5–7·9)
20–49 years	1·1 (0·2–5·1)
⩾ 50 years	9·8 (3·6–26·2)
Gender of patient	
Male	1·0
Female	2·3 (1·2–4·7)
Clinical presentation	
Meningitis	1·0
Bacteraemia	2·3 (1·0–5·3)
Meningitis and bacteraemia	3·1 (1·4–6·8)

Table 6. *Sequelae among 515 patients with systemic meningococcal disease in The Netherlands, 1989–90, according to serogroup (percentage of patients with sequelae in indicated serogroup in parentheses)*

		No. of patients (percentage of sequelae)*				
Serogroup	No. of survivors	Total no. of patients with ⩾ 1 sequela	Hearing loss	Damage of other cranial nerve/mot. disturbance	Other neurological or psychological disturbance	Scars and/or amputation
A	11	2 (18·2)	1 (9·1)	1 (9·1)	0 —	0 —
B	399	34 (8·5)	12 (3·0)	4 (1·0)	6 (1·5)	16 (4·0)
C	90	8 (8·9)	3 (3·3)	1 (1·1)	0 —	4 (4·4)
Other	15	0 —	0 —	0 —	0 —	0 —
Total	515	44 (8·5)	16 (3·1)	6 (1·2)	6 (1·2)	20 (3·9)

* Some patients had more than one sequela.

pituitarism and behavioural disturbance (each in 1 patient). The distribution of sequelae among the various serogroups is shown in Table 6. The absence of patients with sequelae in disease due to the uncommon serogroups is noticeable. There was no association of the occurrence of sequelae (either combined or separated into neurological and haemodynamic sequelae) with serogroup, serotype, subtype or immunotype of the causative agent, or with the gender or age of the patient. Sequelae occurred in 6·5% of the meningitic patients, in 9·0% of the bacteraemic patients, and in 13·9% of patients with both meningitis and bacteraemia ($\chi^2 = 5·6$; 2 df: $P = 0·061$). The sequelae rate among patients with a predisposing factor (19·0%) was higher than among those without (8·3%; Fisher's exact test: $P = 0·100$). In a multiple logistic regression model, only the clinical presentation was significantly associated with the occurrence of sequelae. The OR for the occurrence of sequelae for bacteraemic versus meningitic patients was 1·4 (95%-CI: 0·6–3·2), and for patients with both bacteraemia and meningitis versus meningitic cases 2·3 (95%-CI: 1·1–4·7).

DISCUSSION

The overall CFR during the study-period was 7·7%, which is in agreement with the CFRs reported in the literature [1–9]. Our calculation was based on bacteriologically confirmed cases. Because diagnostic procedures concerning severely ill patients are prone to errors, it is likely that, especially in fatal cases, the diagnosis will not be bacteriologically confirmed, and the observed CFR of 7·7% may be an underestimation of the true CFR of MD in The Netherlands. Indeed, during the study-period another 52 patients were reported, of whom the diagnosis was based on clinical grounds ($n = 37$), or from whom the cultured isolate had not been submitted or did not survive during transport to our laboratory ($n = 15$). The CFR in this group was 18%, indicating an under-reporting bias for fatal cases. The CFR calculated on all known patients during the study-period would be 8·5%.

The CFR of 7·7%, which was determined in our prospective study, is higher than the CFR of 5·1%, which was found in an earlier, retrospective study in The Netherlands from 1959 to 1983 [6]. The calculation of the latter CFR was based on hospital records and may be underestimated, because in a retrospective study records of deceased patients may be missing. This might explain the difference.

In our series, age, gender and the clinical presentation were found to be the most important determinants for a fatal outcome of MD. Infants aged 0–5 months, teenagers from 10 to 19 years and adults over 50 years of age have an increased risk for a fatal disease outcome compared with children aged 6 months–9 years, as has also been found in other studies [3, 5, 6]. The increased risk for infants and for adults over 50 years of age might be due to a failure of the immune system, the former due to immaturity and the latter due to normal decline [16]. The increased risk for teenagers might be related to a change in life-style during puberty, which could lead to a temporary deficit of non-specific immunity [16].

Male patients outnumbered female patients, which is usually found among cases of MD, but female patients were found to have an increased risk for a fatal disease outcome, even after controlling for possible confounding by age and the clinical presentation. We have no explanation for this finding. Of course, this association could be an instance of a false positive result, determined by chance alone. An excess of fatalities among females, however, was also found among the additional 52 cases which were reported during the study-period (CFR for males 14·3% and for females 22·7%) and a similar difference was observed among 327 cases which were reported during the 7 months preceding the study-period, and from whom the disease outcome was assessed retrospectively (unpublished observations; CFR for males 8·4% and for females 10·4%). Therefore, this difference of CFRs between males and females seems to be valid for The Netherlands. However, this finding is not supported in the literature [1, 4]. Only in the earlier-cited study concerning the period 1959–83 in The Netherlands, was the CFR for females slightly higher than for males, but this finding was not statistically significant [6]. In our series, the excess of fatalities among females is mainly determined by an excess number of fatal cases in the age-categories 10–19 and $\geqslant 50$ years, in which females also outnumbered males. Women in these age-categories undergo major biological (hormonal) changes. These changes might compromise the non-specific defence

mechanisms among women which, in turn, could lead to an increased risk of contracting MD and of a fatal outcome [16].

The clinical presentation of MD (meningitis, bacteraemia, or both) turns out to be a third important determinant for a fatal disease outcome. The classification of patients into these three categories, which is generally found in reports concerning MD, is based on the source of isolation of the submitted isolate(s). Considerable bias, however, is to be expected, because in most cases it is not known whether specimens of both blood and CSF were investigated in the first place, and, in the case of positive culture from both sources, whether indeed both isolates have been submitted. Therefore, a misclassification could easily occur. Notwithstanding this potential misclassification, major and consistent differences are found in the literature between the CFRs in these three categories, indicating that this 'surrogate' classification is a relatively good estimate of the true clinical presentation [1–6, 8, 9]. The concept underlying the clinical presentation is difficult to understand. It will be determined by both strain and patient characteristics, the latter seeming to be the most important factors. If an individual is suffering from an underlying disease which compromises his defence mechanisms, the invading pathogen can more easily cause bacteraemia, with or without meningitis. Predisposed patients were, indeed, found to suffer more often from bacteraemia than non-predisposed patients. In the multivariate analysis, however, the presence of a predisposing factor did not influence the risk for a fatal outcome of MD after adjustment for the clinical presentation and age. Other (non-specific) predisposing factors, which we did not or could not measure, could account for the strong association of the clinical presentation and age with the outcome [16, 17].

Of the various strain characteristics of *N. meningitidis*, only the serogroup was found to be related to a fatal outcome of MD in the bivariate analysis, but after adjustment for age, gender and the clinical presentation in the multivariate analysis, the influence of this strain characteristic on the disease outcome disappeared, indicating confounding by the other factors. This may also apply to the associations that were found in other studies [3, 6, 10]. It is not very likely that the meningococcal surface characteristics *per se* are determinants of a fatal disease outcome. However, it cannot be excluded that in some circumstances a meningococcal clone exists with another underlying, but yet unknown factor that is associated with a fatal outcome (e.g. the amount of endotoxin-release) [18, 19]. If such a clone is homogenous with regard to the surface characteristics of *N. meningitidis*, an apparent association of one or more of these characteristics with the disease outcome will be found.

Sequelae were found in 8·5% of the survivors, which is similar to the findings of other reports [3, 6, 7]. In a multivariate analysis, the occurrence of sequelae was only related to the clinical presentation and not to strain characteristics, age, gender or predisposing factors.

In conclusion, host factors were found to be the most important determinants for a fatal outcome of MD in The Netherlands from 1989 to 1990. Apart from general measures, like the prompt initiation of antimicrobial treatment [20, 21], the only way to reduce the number of fatalities due to MD is to prevent the disease, i.e. by vaccination. A capsular polysaccharide vaccine against disease due

to meningococci of the serogroups A and C is already available [22, 23]. However, it needs to be improved in order to protect infants as well [22, 23]. Unfortunately, there is no vaccine currently available against meningococci of serogroup B, which is the most prevalent serogroup in industrialized countries [2]. All efforts should be directed towards the further development of a serogroup B meningococcal vaccine and the improvement of the existing serogroup A/C vaccine. Therefore, careful monitoring of the various epidemiological markers of meningococci must continue, with the aim of eventually achieving the development of a vaccine which will give optimal protection against meningococcal disease.

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