

## The meningococcal antibody test: how useful in the diagnosis of meningococcal disease?

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### SUMMARY

Based on 92537 blood samples received from 7365 patients with a request for a meningococcal antibody test (MAT) during a 10-year period (1986–1995), the usefulness of the test in the diagnosis of meningococcal disease was assessed. Of 635 patients with culture-confirmed meningococcal disease, 88% were seronegative on admittance to hospital and 90% were seropositive 10–15 days after onset of disease. The humoral immune response in children <2 years of age was weaker than in older children and adults. Among 893 MAT-positive patients without culture-confirmed meningococcal disease, 261 (29%) had been notified as cases of meningococcal disease. Among 228 patients notified as serologically confirmed the MAT results were consistent with the clinical diagnosis in 86%. MAT is a reliable tool for establishing a diagnosis in patients with suspected meningococcal disease. Key factors facilitating appropriate interpretation of negative as well as positive test results were: time(s) of sampling(s) after onset of disease, age of the patient and clinical features.

### INTRODUCTION

Meningococcal disease is a serious but uncommon event for which immediate diagnosis by culture of *Neisseria meningitidis* from normally sterile sites is still the gold standard. Outside periods or settings (e.g. military camps) with epidemics, prospective studies of the immune response in patients with meningococcal disease have proved to be difficult. We analysed results generated in the routine serology laboratory at the Neisseria Unit, Statens Serum Institut over a 10-year period from 1986 to 1995, in conjunction with information available through the National Laboratory Surveillance System for meningococcal disease (Neisseria Unit, Statens Serum Institut), as well as the Clinical Notification System

for Communicable Diseases (Department of Epidemiology, Statens Serum Institut). Within the study period the incidence of meningococcal disease in Denmark varied between 4.5 and 5.6/100 000 population per year corresponding to between 216 and 297 notified cases per year. On average, 80% of notified cases had been confirmed by culture of *N. meningitidis* from cerebrospinal fluid (CSF) and/or blood. Within the study period serogroup B accounted for 68–82%, serogroup C for 16–31% and other serogroups for 1–4% of the annual number of culture-confirmed cases of meningococcal disease.

Commencement of antibiotic therapy before hospitalization increases the proportion of clinically diagnosed meningococcal disease cases. The potential of nucleic acid amplification techniques for improved detection of *N. meningitidis* was recently reviewed by Fox [1]. The use of serological markers of infection adds another dimension to the surveillance of meningococcal disease. Furthermore, during vaccine

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field trials, the ascertainment of suspected cases is often difficult due to lack of confirmatory laboratory tests, thus hampering the interpretation of vaccine efficacy. Since the complement fixation test does not detect antibodies induced by vaccination with the meningococcal A and C polysaccharides [2, 3] its use for case ascertainment in vaccine field trials will remove one source of bias. It has been suggested that the complement fixation test may be a useful tool for estimating the efficacy of the serogroup C vaccine in reducing the acquisition rate of serogroup C strains [3]. A meningococcal antibody test (MAT) designed for diagnosis of disease may, therefore, still have a role to play [4].

In Denmark, the MAT became routinely available at the Statens Serum Institut in 1941 prompted by the first signs of the epidemic occurring during World War II [5]. The aim of the present study was to assess the diagnostic performance of the MAT when available as a routine test during a period of hyperendemic meningococcal disease dominated by sporadic cases of serogroup B infection.

## METHODS

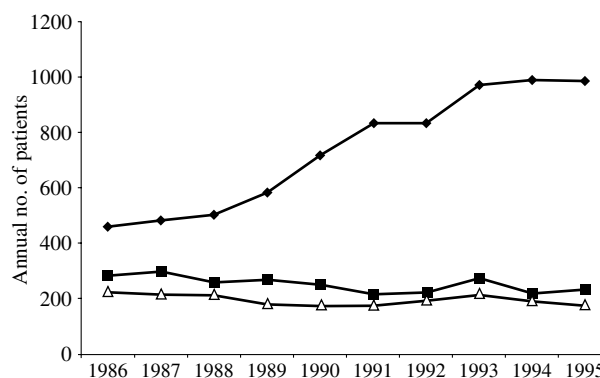
### Serum samples

The material comprised 9257 blood samples received during a 10-year period (1986–1995) with a request for examination with the MAT at the Neisseria Unit, Statens Serum Institut, which serves all hospitals and practitioners in Denmark. The sera originated from 7365 patients. From the written request form the following information was available: age and gender, name or civil code, date of sampling and the health service involved. The annual number of patients tested by MAT increased significantly during the study period (from 459 to 986) even though the annual numbers of notified and bacteriologically verified cases of meningococcal disease decreased from 282 to 235, and 225 to 178 respectively (Fig. 1).

### Assessment of the study population

Two sources of information were available:

- (1) All *N. meningitidis* isolates from patients hospitalized with invasive meningococcal disease are referred to the Neisseria Unit for determination of epidemiological markers (serogroup, serotype, serosubtype and antimicrobial susceptibility pattern). For each individual patient information



**Fig. 1.** Annual numbers of patients from whom one or more serum samples were tested for meningococcal antibodies (—◆—), numbers of notified cases of meningococcal disease (—■—), and numbers of culture-confirmed cases of meningococcal meningitis and/or septicemia (—△—).

is given about date(s) of sampling(s) and type of material (CSF, blood, etc.) from which *N. meningitidis* has been cultured. Isolates from 95–98% of all cases notified as culture confirmed are referred to the Unit.

- (2) The Clinical Notification System for Communicable Diseases. All cases of clinical or suspected meningococcal disease are notifiable on an individual basis. The cases are coded according to their clinical manifestations as meningococcal meningitis, meningococcal septicemia or meningococcal meningitis plus septicemia. Each of these groups is subdivided according to the evidence on which the aetiological diagnosis is based: microscopy, antigen detection, culture, serology, etc. For each notified case of meningococcal disease, the Department of Epidemiology routinely makes a request for a copy of the hospital's discharge letter to the general practitioner. Cases of other forms of purulent meningitis are grouped according to aetiology, e.g. *Streptococcus pneumoniae* or *Haemophilus influenzae* type b, or as purulent meningitis of unknown aetiology.

### MAT

The preparation of antigen and the complement fixation test employed was analogous to that described for the detection of gonococcal antibodies [6], except that phosphate-buffered saline was replaced by veronal-buffered saline (pH 7.2) with optimal concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . To avoid false-positive results caused by human rheumatoid factor reacting with determinants assessable on complexed rabbit

IgG, the amboceptor used was rabbit IgM antibody to sheep red cell ghosts. Until 1992 a pool of heat-treated (56 °C for 20 min) whole cells from meningococcal strains of serogroups A, B and C and from 1993 a pool of serogroup B and serogroup C strains, including current outbreak strains was used as antigen. Guinea-pig serum was used as complement source. Fresh guinea-pig serum was kept as small aliquots in liquid nitrogen and once thawed, used immediately. The MAT titres are expressed as degrees of potency (DP) calculated as described by Kristensen [7], i.e. the DP being defined as that value of  $n$ , which when substituted in the formula  $a = 0.025 \times 3^{-n/3}$  gives a value exactly equal to the volume of serum containing enough antibody (one unit) to give a minimum reaction, i.e. 60% inhibition of haemolysis. Before testing all sera were heat-inactivated (56 °C for 20 min). The initial serum dilution was 1:12. Seroconversion was defined as a change from negative to positive at a level  $\geq 3$  DP. A difference of  $\geq 3$  DP, which corresponds to  $\geq$  three-fold change in antibody titre was the criterion for serological response. If two or more tubes of the three-fold dilution row of a serum showed partial haemolysis the result was designated an atypical positive test result.

The intra- and inter-assay variations of the performance of MAT were determined by triplicate testing on three different days of one negative (just below cut-off), one moderately positive and one strongly positive serum sample. Using the same sera, these experiments were performed independently by two technicians. One of the technicians performed a second experiment using a further set of four sera (three positive and one with an atypical positive test result) and another source of sheep red cells.

### Statistics

To determine the intra- and inter-assay variation of MAT titres an analysis of variance was performed. Wilcoxon's test was used for comparison of distribution of patients with serogroup B and serogroup C meningococcal disease according to age. The distribution of MAT titres according to days of sampling for patients  $\leq 2$  years of age and those  $\geq 3$  years of age was assessed by the LMS method [8]. The effect of gender, serogroup and age on the level of MAT titres was estimated by a linear model of log (MAT) using these factors and 'day of sampling' as qualitative explanatory variables. Estimates of negative fraction of MAT titres according to days after onset of disease

and of differences in immune response (MAT titres) were done by logistic regression analysis. The software package SAS, version 8.2 (SAS Institute Inc., Cary, NC, USA) was used for analysis of the data. A  $P$  value of  $< 0.05$  was considered to imply statistical significance.

## RESULTS

### MAT

Based on limited data the repeatability of the test results, including those of sera with a negative and an atypical positive reaction, was 100%. Without any observed variation in test results for the individual sera the coefficient of variation was zero. The standard deviation of the repeatability was estimated to be 0.49 DP. From a practical point of view this means that the variation of a MAT result is a maximum  $\pm 1$  DP and that a change in titre of  $\geq 3$  DP is statistically significant.

### Laboratory results

A total of 9257 samples from 7365 patients were tested by MAT; from 5755 patients only one, from 1399 patients two, and from 211 patients three or more samples were available. Of the 9257 sera 1496 (16%) were MAT positive. In addition, 80 sera (0.86%) exhibited an atypical positive reaction. These latter sera were excluded from the analysis unless mentioned specifically. Of the 1496 MAT-positive sera, 340 (23%) were from patients with culture-confirmed meningococcal disease (Table 1) and 1156 (77%) from patients without culture-confirmed meningococcal disease (Table 2).

### Patients with culture-confirmed meningococcal disease

By comparison with the files of the National Laboratory Surveillance System it was found that 858 serum samples (9.3%) originated from 635 patients with culture-confirmed meningococcal disease.

For culture-confirmed cases onset of disease (day 0) was defined as the day on which the first sample of CSF and/or blood with positive culture for *N. meningitidis* was taken.

A single serum sample only was available from 450 patients (Table 1); of these, 131 (29%) were MAT positive. Six sera (1%) showed atypical positive test results. Of 260 samples drawn within 48 h after admittance to hospital 228 (88%) were MAT negative,

Table 1. MAT results of 858 serum samples from 635 patients with culture-confirmed meningococcal disease 1986–1995

No. of samples per patient	Total number		MAT test results		
	Patients	Samples	Negative	Positive	Atypical
1	450	450	313	131	6
2	155	310	146	152	12
≥3	30	98	41	57	0
Total	635	858	500	340	18

MAT, Meningococcal antibody test.

Table 2. MAT results of 8399 serum samples from 6730 patients without culture-confirmed meningococcal disease 1986–1995

No. of samples per patient	Total number		Test results in MAT		
	Patients	Samples	Negative	Positive	Atypical
1	5305	5305	4825	451	29
2	1244	2488	1965	499	24
≥3	181	606	391	206	9
Total	6730	8399	7181	1156	62

MAT, Meningococcal antibody test.

16 (6%) weakly positive and 16 (6%) moderately or strongly positive. Of 29 samples drawn during the second week after onset of disease, two (7%) were MAT negative, three (10%) weakly positive, 22 (76%) moderately or strongly positive and two (7%) showed an atypical positive test result.

In total, 408 sera were available from 185 patients in whom two or more samples had been drawn (Table 1). Of 118 patients from whom samples were drawn within 48 h after admittance to hospital, 103 (87%) were MAT negative at that point. Seroconversion was demonstrated in 84 (45%), a significant change in titre in 11 (6%), static titres in 40 (22%) and other reaction patterns in the remaining 12 seropositive patients. Of 32 seronegative patients, 13 had been tested during the second or third week after onset of disease. Sera from six patients showed atypical positive MAT results in both samples.

In 629 patients (99%) information about age, sampling dates, test results and serogroup determination of the invasive meningococcal strains were available. Excluding the 18 sera with an atypical reaction

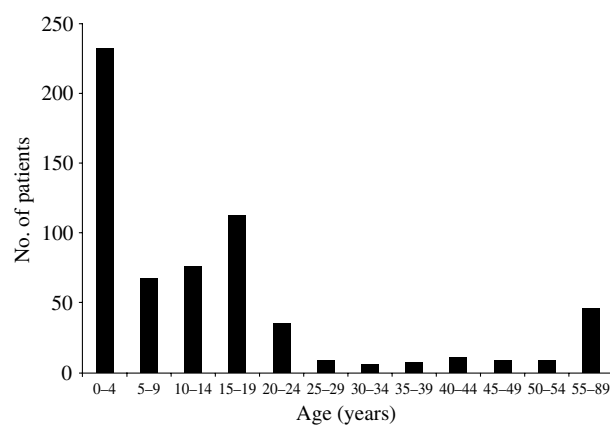


Fig. 2. Age distribution of 629 patients with culture-confirmed meningococcal disease from whom serum samples were tested by MAT.

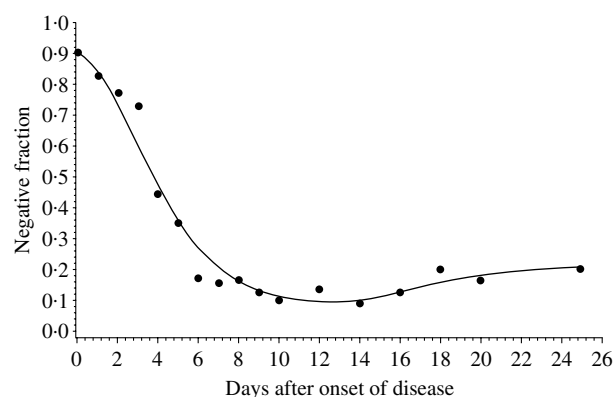
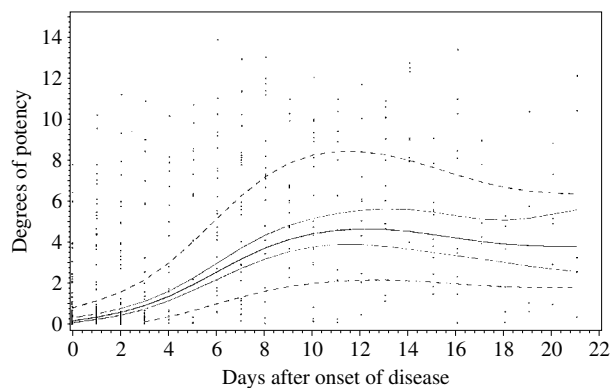


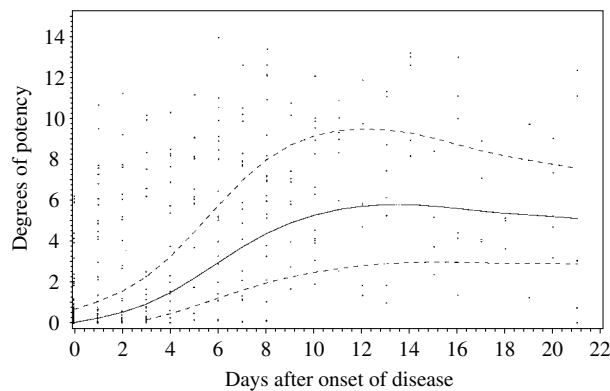
Fig. 3. Observed (●) and estimated (—) negative fraction of MAT results according to day after onset of disease: analysis by logistic regression of 836 serum samples from 629 patients with culture-confirmed meningococcal disease.

(Table 1), 836 sera from these 629 patients were available for the assessment of the humoral immune response as reflected in the MAT results. The age distribution is shown in Figure 2. The median age was 11 years. For patients with serogroup B infection ( $n=472$ ) the median age was 9 years (range 0–88 years) and for those with non-serogroup B infections ( $n=157$ ) the median age was 14 years (range 0–77 years). This difference is significant ( $P=0.03$ ). Serogroup C accounted for 90% (142/157) of non-serogroup B infections.

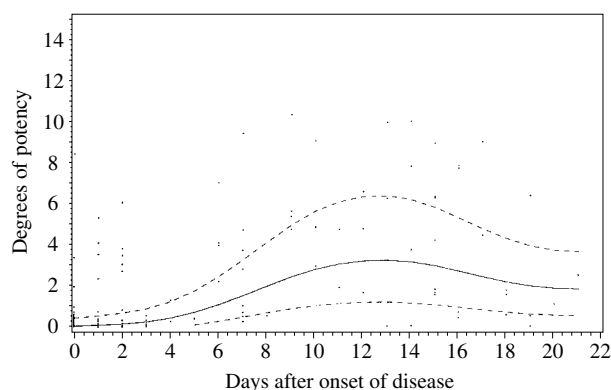
The majority of negative MAT results occurred in samples drawn shortly after onset of disease (Fig. 3). Between days 10 and 15, 90% of patients had become seropositive. Thus, the reason why a high proportion of sera from patients with meningococcal disease was MAT negative was that 75% of all sera were samples collected early after onset of disease. No difference in



**Fig. 4.** Smoothed 25th, 50th (median) and 75th percentile curves of MAT titres (degrees of potency, DP) found from 0 to 21 days after onset of disease; 95% point-wise confidence limits for the median. Analysis of 836 serum samples from 629 patients with culture-confirmed meningococcal disease.



**Fig. 6.** Smoothed 25th, 50th (median) and 75th percentile curves of MAT titres (degrees of potency, DP) found from 0 to 21 days after onset of disease in 452 patients  $\geq 3$  years of age (605 serum samples).



**Fig. 5.** Smoothed 25th, 50th (median) and 75th percentile curves of MAT titres (degrees of potency, DP) found from 0 to 21 days after onset of disease in 177 patients  $\leq 2$  years of age (231 serum samples).

distribution according to day of sampling for patients aged  $\leq 2$  years and those  $\geq 3$  years could be demonstrated.

Figure 4 shows 25th, 50th (median) and 75th percentile curves of estimated MAT titres according to number of days after onset of disease. The immune response in children  $\leq 2$  years of age (Fig. 5) resulted in lower MAT titres than those found in older children and adults (Fig. 6). The overall relative level of antibodies (DP) was estimated as 1.31 (95% CI 1.18–1.44,  $P < 0.0001$ ). No difference in the humoral immune response according to gender ( $P = 0.42$ ) or serogroup B or C disease ( $P = 0.49$ ) could be demonstrated.

In aggregate, MAT results obtained for 858 sera from 635 patients with culture-confirmed meningococcal disease showed that  $\sim 88\%$  of the patients were seronegative on admittance to hospital and that  $\sim 90\%$  were seropositive 10–15 days after onset of

disease. Within the first week of illness weakly positive or atypical positive test results occurred regularly.

#### Patients without culture-confirmed meningococcal disease

A positive test result was found in 1156 (14%) of 8399 serum samples from 6730 patients without culture-confirmed meningococcal disease; 62 results were designated atypical. The majority 5305 (79%), was only examined by a single serum sample of which 451 (9%) were MAT positive. Among 3094 samples from 1425 patients tested at least twice, 705 (23%) samples from 501 patients were MAT positive (Table 2).

Supplementary information about the clinical diagnosis was available for those patients who had been notified as cases of meningococcal disease or as purulent meningitis of other or unknown aetiology. On 1 January 1987 the codes used for classification of identified cases of bacterial meningitis were changed. For this reason patients registered in 1986 have been omitted from the assessment based on data obtained from the Clinical Notification System for Communicable Diseases. During the period 1987–1995, 893 seropositive patients without culture-confirmed meningococcal disease were recorded. Information about signs and symptoms of disease useful for the assessment of the positive test result was available in 261 cases notified as meningococcal disease (29%). A total of 288 (87%) had been recorded as serologically confirmed, whereas 13 (5%) were categorized as positive by direct microscopy and 20 (8%) as clinical meningococcal disease (Table 3); 12 MAT-positive patients had been notified as cases of purulent meningitis of unknown aetiology.

Table 3. Numbers of notified cases of meningococcal disease among 893 MAT-positive patients without culture-confirmed meningococcal disease 1987–1995

No. of samples per patient	Culture-negative seropositive patients			No. of patients notified under code		
	Total number	Number	(%)	Positive MAT	Positive microscopy	Clinical diagnosis
1	410	68	(17)	54	6	8
2	341	158	(46)	139	7	12
≥3	142	35	(25)	35	0	0
Total	893	261	(29)	228	13	20

MAT, Meningococcal antibody test.

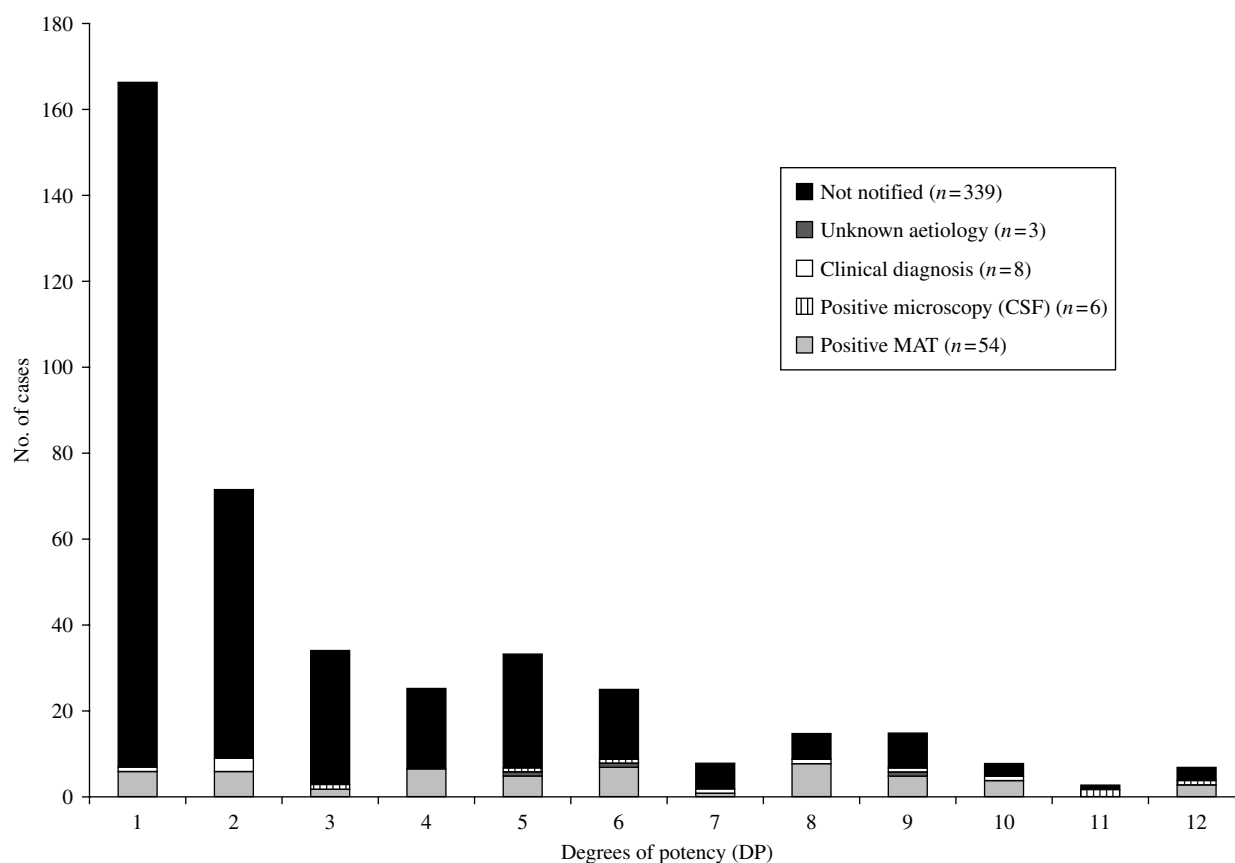


Fig. 7. Assessment of positive MAT results in single serum samples from 410 patients without culture-confirmed diagnosis of meningococcal disease.

The 893 MAT-positive patients were divided into three groups according to the individual number of sera tested: (a) one sample ( $n=410$ ), (b) two samples ( $n=341$ ) and (c) three or more samples ( $n=142$ ). For patients from whom two or more serum samples were available, the day on which the first blood sample was drawn was registered as 'day 0'.

*Patients with a single sample positive in MAT ( $n=410$ )*

Weakly positive test results ( $\leq 3$  DP) were found in 66% (271/410) of cases. The notified cases made up 7% (19/271) of those with titres  $\leq 3$  DP, 28% (23/83) of those with titres of 4–6 DP and 52% (29/56) of the strongly seropositive cases ( $\geq 6$  DP) (Fig. 7). In total,

71 (17%) had been notified, namely 68 as meningococcal disease and three as meningitis of unknown aetiology. The 68 notified cases of meningococcal disease were coded as serologically confirmed ( $n=54$ ), positive by microscopy ( $n=6$ ) or as clinical cases ( $n=8$ ).

In the three patients notified as meningitis cases of unknown aetiology, the MAT results (5, 6, 8 DP) indicated that *N. meningitidis* was probably the cause of disease.

*Seropositive patients from whom two samples were tested (n=341)*

Within this group 158 patients had been notified as cases of meningococcal disease: 139 as serologically confirmed, seven as confirmed by positive microscopy and 12 as clinical cases (Table 3).

A change from MAT negative to  $\geq 4$  DP or a significant change in titre was demonstrated in 83 out of the 139 patients notified as serologically confirmed cases of meningococcal disease; 20 patients showed static titres  $\geq 4$  DP and 36 patients were weakly seropositive ( $\leq 3$  DP). Among the 19 patients notified under the code positive microscopy and/or clinical diagnosis eight patients showed seroconversion, two showed static titres  $\geq 4$  DP and nine were weakly seropositive.

Since 1992 registration of seroconversion or significant change in MAT titres in patients without culture-confirmed diagnosis has been part of the surveillance system, i.e. information about the serological evidence of meningococcal disease is given to the Department of Epidemiology and to the local medical officer of health; in total 50 out of 63 patients (in this category) had been or were subsequently notified. Of the remaining 13 patients seven had substantial serological evidence of meningococcal disease and four did not fulfil the serological criteria set up (misinformation).

Among seven MAT-positive patients notified as purulent meningitis of unknown aetiology; five were weakly seropositive and two had serologically evidence of meningococcal disease (both showed seroconversion to MAT titre 11 DP; one had been treated with antibiotic before sampling for culture of *N. meningitidis*).

*Seropositive patients from whom three or more samples were tested (n=142)*

This group comprised 142 patients of whom 35 were notified as serologically confirmed (Table 3);

Table 4. Proportions of notified cases that were culture-negative and coded as serologically confirmed 1987–1995

Year	No. of notified cases	No. of culture-negative cases		
		Total	No.	(%)
1987	297	78	22	(28)
1988	258	40	15	(38)
1989	268	70	32	(46)
1990	251	78	29	(37)
1991	216	54	21	(39)
1992	224	31	19	(61)
1993	275	55	32	(58)
1994	220	30	18	(60)
1995	234	56	38	(68)

For 1986 these data are not available (see text).

seroconversion or significant change in titres were demonstrated in 20 patients, high static titres in one and low-level antibody response in 10.

Two patients had been notified as purulent meningitis of unknown aetiology; both were weakly seropositive.

**Patients notified as serologically confirmed cases of meningococcal disease (1987–1995) (n=228)**

In 1987 the proportion of culture-negative cases of meningococcal disease coded as serologically confirmed was 28% (Table 4). In 1995 the percentage had increased to 68% and since then it has been around 80%. In 106 patients with seroconversion or significant changes in MAT titres to or from an antibody level  $\geq 4$  DP the MAT results were considered to support the diagnosis of meningococcal disease strongly. An attempt to assess the diagnostic significance of MAT by means of the hospital records was, therefore, only made for patients whose sera showed MAT titres  $\leq 3$  DP or a single or static MAT titre of  $\geq 4$  DP.

*Weekly seropositive patients (MAT  $\leq 3$  DP) (n=59)*

The case histories of 37 patients (63%) were compatible with a clinical diagnosis of meningococcal disease (fever, neck stiffness and/or petechiae); nine of these patients were  $\leq 2$  years of age. The clinical manifestations in the remaining 22 patients (37%) among whom three were  $\leq 2$  years of age included diarrhoea, dermatitis atopica, peritonsillar abscess and other

conditions, not supporting a diagnosis of meningococcal disease.

*Seropositive patients (MAT  $\geq$  4 DP) without seroconversion or significant changes in titres (n=63)*

In 54 (86%) of the 63 patients with a single or static MAT titre of  $\geq$ 4 DP the clinical manifestations strongly supported the serological evidence of the presence of meningococcal disease. In the remaining nine patients the notification form did not contain any or insufficient information to support or exclude a clinical diagnosis. Five patients had been treated with antibiotics before sampling.

In summary, among seropositive patients without culture-confirmed meningococcal disease but notified as cases of meningococcal disease the serological response confirmed or supported the clinical diagnosis in 86% (197/228) of cases.

**Patients notified as cases of purulent meningitis of unknown aetiology**

From 1987 to 1995 there were 344 patients notified as cases of purulent meningitis of unknown aetiology; of these, 179 (52%) had one or more serum samples tested by MAT. Among 92 patients from whom a single sample had been tested only three (3%) were seropositive, all with MAT titres indicative of meningococcal disease. Among 87 patients from whom two or more sera had been tested, two patients (mentioned above) showed seroconversion and seven were very weakly and intermittently seropositive (1 DP). In 85 patients (98%) at least one sample had been taken during the second or third week after onset of illness; the hospital records mention clinical meningitis in 68 patients and the simultaneous presence of petechiae in eight patients (all seronegative); antibiotic therapy had been given prior to sampling in 22 patients. Thus, only two patients had serological evidence of meningococcal disease.

**Patients notified as cases of purulent meningitis of other known aetiology**

Over a 4-year period sera from patients with other types of purulent meningitis were investigated. These included cases of *H. influenzae* type b meningitis (n=129), *S. pneumoniae* meningitis (n=212) or infection with beta-haemolytic streptococci (n=22). A single serum sample had been received from 38 patients of whom four with pneumococcal infection

and one with streptococcal infection were weakly MAT positive.

**Atypical positive reactions in MAT**

Overall, atypical positive reactions were rare. Among 635 sera from patients with culture-confirmed meningococcal disease 18 (2.8%) and among 8399 sera from patients without culture-confirmed disease 62 (0.7%) showed atypical positive reactions in MAT (Tables 1 and 2). Thus, nearly a quarter (22.5%, 18/80) of these reactions were found in sera from patients with proved meningococcal infection. Six of the remaining patients were notified as cases of meningococcal disease. Atypical reactions occurred primarily early or late in the immune response, i.e. in sera obtained before and after those with typical positive reactions. In some cases the atypical reaction was associated with the presence of heterophilic antibodies to sheep erythrocytes (Lind, I., unpublished results).

**DISCUSSION**

The crucial role of humoral antibodies in protective immunity to, and natural restoration to health from meningococcal disease was recognized shortly after the first isolation of the causative agent, *Neisseria meningitidis*, in 1887 [9]. Serum therapy had already been developed before World War I and was the only treatment available until the introduction of sulphonamides in 1937. Several serological methods for diagnosis as well as for the assessment of natural immunity were developed [2, 5, 10–13] and since the unique work of Goldschneider and colleagues [14, 15] the complement-dependent bactericidal activity of serum has been considered the primary correlate of protective immunity. At that time the occurrence of meningococcal disease was related more to the unique susceptibility of the individual host than to the innate virulence of the infecting organism [14]. Later development of sophisticated serological and molecular methods for determining the relationships between meningococcal isolates substantiated the view that meningococcal populations are antigenically and genetically diverse and that only a few ‘hypervirulent’ strains cause most cases of invasive disease [4].

The potential of a meningococcal complement fixation test as a diagnostic tool was pointed out in the 1940s [5, 10, 12] but it has not been widely used. The genus specific test described by Edwards and Devine



in 1968 [3] has been used in studies of the immune response in vaccinees and carriers [2, 3] and in a prospective study carried throughout 1970–1971 among 87 recruits with serogroup C meningococcal disease [16]. The latter study showed that the induction phase of the immune response was shorter in persons without detectable antigen in their serum than in those with antigen present in their sera (median, 24 vs. 60 h). The clearance of antigen from serum appeared to relate to the formation of immune complexes. Antibody activity may also be absent from serum due to blocking by IgA [17]. These findings offer explanations for the observed variability in the length of the period before antibodies are detectable.

Another factor influencing the capacity of the MAT to detect a significant level of meningococcal antibodies is the composition of the antigen. In the individual patient the most reliable measure of the induction of complement-fixing antibodies has been obtained using the homologous strain as test antigen [18]. However, we have shown that the use of an antigen prepared from a pool of epidemiologically relevant strains allows the detection of an antibody response in approximately 90% of patients with culture-confirmed meningococcal disease, from whom samples were drawn during the second week after onset of disease. This finding is in agreement with studies on the kinetics of the immune response in individual patients followed by consecutive sampling during the cause of illness. The maximum response in adult patients was recorded between days 7–15 and then, in general, falling to below cut-off within 3 months [18, 19]. For samples taken at this point a recent study [19] has shown that single-point IgM enzyme immunoassay offers a tool for the laboratory confirmation of meningococcal disease. Such an assay might be supplemented with assays determining IgM antibodies against capsular polysaccharides B and C [20], thus enhancing the value of seroepidemiological surveillance. The immune response to capsular polysaccharides cannot be assessed by complement fixation tests [2, 3]. In vaccinees seroconversion or a significant increase in antibody titres measured by a complement fixation test is thought to result from acquisition of a carrier strain [2].

In the present study the duration of the immune response and the mean levels of meningococcal antibodies detectable by the complement fixation test were dependent on the age of the patient, but unrelated to gender and to serogroup of the invasive strain. In children <2 years of age the MAT response

was weak and transitory when compared to that in older children and adults. Physiological hypogammaglobulinaemia has been demonstrated in infants from 2 to 4 months up to 2 years of age [21]. This pronounced temporary drop correlates with a high incidence of invasive disease caused by encapsulated bacteria. Further, the complement system, which is a major effector mechanism for the expression of antibody-dependent activity is still immature at that age [22]. Densen hypothesized that the absence of specific antibody to initiate classical pathway activation, coupled with capsular sialic acid-mediated inhibition of alternative pathway activity, might contribute to the high prevalence of group B disease in young children [22].

Studies on the immune response in patients with meningococcal disease using others methods and performed during epidemics or vaccine trials [2, 5, 10, 12, 16, 18, 23–25] have shown a pattern similar to that found in the present study – seroconversion in 80–90% of the patients within the second week after onset of disease and a weaker and more transient response in infants than that seen in older children and adults.

Natural immunity to meningococcal disease appears to reflect the development of antibody to sub-capsular, cross-reactive antigens following acquisition of *N. meningitidis* carrier strains or *N. lactamica* [15, 26]. The induction of these antibodies does not confer immunity in complement-deficient individuals [27]. In infants pharyngeal colonization with *N. lactamica* precedes that by *N. meningitidis* by several years [15, 28, 29]. Olsen et al. [29] studied pharyngeal carriage of *N. meningitidis* and *N. lactamica* in households with infants within areas with high and low incidences of meningococcal disease. They demonstrated a negative association between *N. lactamica* carriage and incidence of meningococcal disease and a negative association between *N. lactamica* carriage and *N. meningitidis* carriage in the high incidence areas.

These findings support the view that *N. lactamica* protects against meningococcal disease and pharyngeal colonization with *N. meningitidis*. Gold et al. [28] studied asymptomatic carriage of *N. meningitidis* and *N. lactamica* and showed that 66% of children who acquired *N. lactamica* developed four-fold or greater rises in titre of IgG antibodies to group A, B and/or C meningococci compared to only 5% of control children. Older children and adults in whom carriage of *N. lactamica* is rare, develop antibodies by pharyngeal acquisition of non-pathogenic meningococci.

A Danish study of the immune response following acquisition of *N. meningitidis* among military recruits demonstrated seroconversion to, or increase in MAT titre at a low to moderate level, in 86% of cases [30]. Among Danish teenagers and young adults meningococcal carriage rates between 30% [31] and 40% [30] and MAT seropositivity rate, between 11% [31] and 22% [30] have been demonstrated. Among 225 infants with haemorrhagic rash and fever, but without confirmed or probable meningococcal disease [32], only nine (4%) were weakly seropositive (Nielsen, H.E. et al., unpublished observations). Current or previous gonococcal infection [5, 10, 24] may induce antibodies detected by MAT due to cross-reactive antigenic determinants common to *N. gonorrhoeae* and *N. meningitidis*.

In this study the gold standard for the diagnosis of meningococcal disease was culture of *N. meningitidis* from CSF and/or blood (or other sterile body fluid). The Danish laboratory surveillance system was established in 1974 and has documented that the proportion of notified cases being culture confirmed has remained constant at around 80%. An increasing proportion of the remaining cases are now confirmed serologically. In countries such as the United Kingdom in which the practice of early antibiotic treatment is growing, the discrepancy between numbers of notified and culture-confirmed cases may increase. This discrepancy could be further accentuated by a growing reluctance to perform lumbar punctures on suspected cases [1]. Under these circumstances the impact of the introduction of nucleic acid amplification techniques (e.g. PCR) for the diagnosis of meningococcal disease is considerable.

Two recent studies include PCR as well as serology among non-culture methods for a confirmatory diagnosis [33, 34]. Analysis of data on the laboratory confirmation of 1749 notified cases of meningococcal disease in Scotland during 1993–1999 showed that after the introduction of these methods the proportion of notified cases confirmed by culture fell from 58% to 38% and that those confirmed by non-culture methods increased from 1% to 35%. In 1999 16.5% of all confirmed cases and 34% of cases confirmed by non-culture methods had been verified only by serology. In addition, most PCR-positive cases were confirmed by antibody testing [33]. The other study [34] assessed 79 culture-negative cases classified by physicians as suspicious for meningococcal disease during a meningococcal vaccine field trial. The combined use of PCR and antibody testing reduced the number of

suspicious cases from 79 to six, thereby improving the potential of assessment of vaccine efficacy.

In conclusion, moderately to strongly positive MAT results confirmed or supported the diagnosis of meningococcal disease in patients with symptoms compatible with meningococcal disease from whom blood samples were drawn during the second or third week after onset of disease. However, weakly positive MAT results, especially in adult patients and especially if only a single test result was available could not be interpreted reliably. The antibody response in infants was weaker and more transient than in older children and adults. Key factors enabling appropriate interpretation of negative as well as positive test results were time(s) of sampling(s) after onset of disease, age of the patient and clinical features.

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