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Ten and a half years seroepidemiology of *Mycoplasma pneumoniae* infection in Denmark

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SUMMARY

The study was based on a computerized card index of 9161 patients who had at least one positive blood specimen in the *Mycoplasma pneumoniae* complement fixation test. A total of 12562 specimens from these patients had been sent to Statens Seruminstitut from hospitals and general practitioners during a 10·5-year period. The period encompassed a previously described endemic period in a 30-year study of the epidemiological pattern of *M. pneumoniae* infection in Denmark [1]. The serological data presented support the hypothesis advanced here that a more than sixfold increase of children in day care might have contributed to a change in the epidemiological pattern. The correlation between age and level of specific antibodies, as well as persistence of seropositivity, were also investigated.

INTRODUCTION

Mycoplasma pneumoniae is a common cause of lower respiratory tract disease, especially tracheobronchitis and pneumonia, in the 5- to 40-year age group. In most countries it is prevalent all year round with a relatively higher incidence during winter. In Denmark the disease occurred in a regular pattern of epidemics every 4·5 years during the period 1958–74. Then followed two 'premature' epidemics in 1975 and 1977/8, followed by a 9-year hyper-endemic period of decreasing incidence which ended in the winter 1987/8 when the biggest epidemic ever was recorded (Fig. 1) [1].

One of the aims of this survey was to investigate possible changes in incidence by correlating the serological responses with age groups at various periods of time, and with specimens from hospitals and general practitioners. The other aim was to examine the correlation between age and titre level and the duration of seropositivity.

MATERIAL AND METHODS

Material

The basis of this study was a card index of 9161 patients who had at least one positive blood specimen in the *M. pneumoniae* complement fixation (MPCF) test. A total of 12562 specimens from these patients had been sent to the Mycoplasma Laboratory, Statens Seruminstitut, from hospitals and general practitioners all

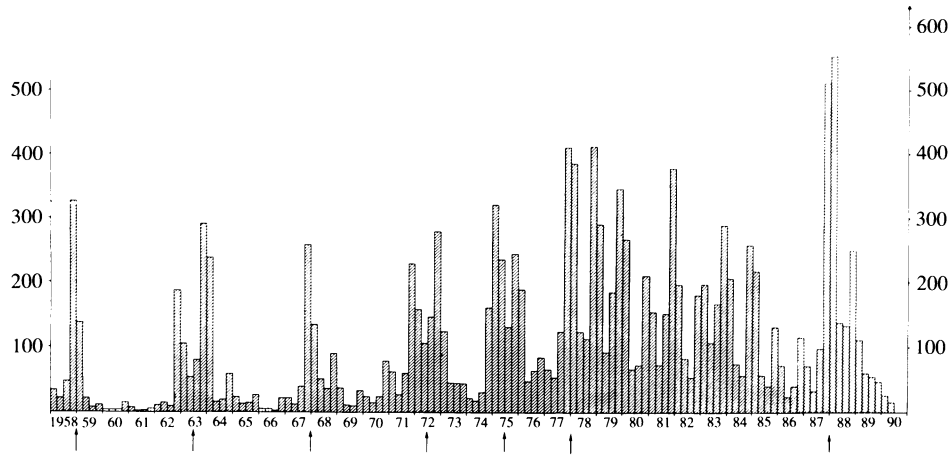

Fig. 1. Quarterly number of anti-*M. pneumoniae* positive and cold agglutinin positive sera. Arrows indicate epidemics. The endemic period is 1978–87.

over Denmark during a 10·5-year period (1 July 1976–31 December 1986). The Mycoplasma Laboratory receives from the whole country all specimens for which the MPCF test has been requested for the confirmation or otherwise of the clinical diagnosis of *M. pneumoniae* infection. No MPCF tests are made in hospital laboratories. Clinical data was not generally available.

Some of the analyses were based on 12-month periods from 1st July one year to 30th June the next year, because of the usual high incidence in the winter. The patients were grouped according to age: 0–4; 5–19; 20–39; and $\geqslant 40$ years of age. A patient was referred to as a hospitalized case if any of the specimens were received from a hospital to which the patient had been admitted, or where the patient was seen for follow up after discharge. Hospital laboratories do not serve general practitioners, who most often refer patients to medical laboratories associated with their own organization, and from where blood specimens are then sent to the Mycoplasma Laboratory.

Serological methods

The MPCF test and the cold agglutinin (CA) test were performed as described previously. In the MPCF test a chloroform-methanol extract of *M. pneumoniae* was used as antigen [1, 2]. Both tests were carried out on all blood specimens. For the MPCF test titres were recorded as the reciprocal of twofold dilutions from $\leqslant 16$ to $\geqslant 4096$, and for cold agglutinins (CA) from $\leqslant 16$ to $\geqslant 1024$. In both tests a titre of $\geqslant 64$ was considered positive [1]. In one period consecutive serum samples from 1874 patients were screened for CA at a dilution of 1:64, and results recorded as negative (< 64) or positive ($\geqslant 64$).

Registration

The major proportion of the patients were identified by their Central Person Register (CPR) number. This number gives the day, month and year of birth, together with a four-cipher number which uniquely identifies a person. This was needed to link samples taken at long intervals. In the remaining patients, sex, date

of birth and initials of name were used. When necessary, the hospital's case sheet was checked. For each sample, information about hospital or practitioner and municipality was registered together with sampling date, sample number and test results.

RESULTS

Table 1 shows the total number of specimens received for *M. pneumoniae* serodiagnosis in each of the 12 month periods over 10·5 years, the number of specimens both from seronegative and seropositive cases, and the number of seropositive cases. We have no information on the number of cases with seronegative specimens only as they were neither in the index nor registered elsewhere. The whole study comprised 4673 women with 6438 specimens (average: 1·38 per case) and 4470 men with 6103 specimens (average: 1·37 per case). There was no information as to the sex in 18 persons with 21 specimens (Table 2).

Fig 2 shows the age and sex distribution of 9143 (4673+4470) patients, each 3-year age group column representing the percentage of the total for each sex. The top of the columns forms a curve with two major peaks, one of the 3–14 year age group, the other, somewhat smaller, of the 27–38 year age group. In the first peak boys predominate by 5·3% ($P < 0·0001$) while in the second peak there were 9·2% more women than men ($P < 0·0001$).

Only one blood specimen was received from 73·8% of the patients. From 19·7% we received 2 specimens, and from 600 or 6·5% of the patients 3 or more specimens were taken (Table 2). Two or more specimens from a patient were most often sent from hospitals. The Table also shows that 57% had been hospitalized, and 52% of these were men, while out of the 43% non-hospitalized patients only 45% were men. In 23 of a total of 9161 patients we had no information as to who had requested the test.

In order to investigate whether a possible change in herd immunity had taken place during the 10·5 years, the data was examined for three periods stratified by age group as shown in Table 3. The total number of seropositive cases decreased by 30·6% from 3810 in the first 4-year period to 2643 in the last 3·5-year period. Patients aged 0–19 years represented 49·3% of the total in the first period, but only 38·5% in the last period, a 10·8% decrease. The Table shows that the relative decrease was most pronounced in children aged 0–4 years, (from 9·1% to 5·2%), but they comprised only 7·3% of all patients, while the 5–19 year age group, representing 37·4% of all patients, decreased from 40·2% to 33·3%.

Table 3 also demonstrates that the ratio between hospitals and general practitioners of the number of patients they had investigated had changed over the same three periods. While the total number seen by general practitioners was unchanged from the first to the last period, the number of patients admitted to hospital had dropped by 47% from 2456 to 1299 (Table 3). The male/female ratio changed only little with time. The proportion of female patients, both of total and of hospitalized cases, rose by about 4% from the first to the last period (data not shown).

When the incidence recorded according to municipality in various parts of the country was followed over the years, minor temporary variations were seen, but there was no trend indicating routes of spread of the disease.

Table 1. *Number of specimens and seropositive cases in the 12 month periods over 10·5 years*

Period	Total number of specimens received for MPCF test*	Number of specimens from seropositive† cases	Per cent specimens from seropositive cases out of total	Number of seropositive† cases	Average number of specimens per seropositive case
1 July 1976–30 June 1977	11506	649	6·0	322	2·02
1 July 1977–30 June 1978	16666	1714	11·5	1170	1·46
1 July 1978–30 June 1979	15906	1527	10·6	1139	1·34
1 July 1979–30 June 1980	17760	1554	9·6	1182	1·31
1 July 1980–30 June 1981	15309	959	6·6	697	1·38
1 July 1981–30 June 1982	17190	1614	10·1	1201	1·34
1 July 1982–30 June 1983	18586	1059	6·0	790	1·34
1 July 1983–30 June 1984	17449	1318	8·0	982	1·34
1 July 1984–30 June 1985	18020	1175	6·7	875	1·34
1 July 1985–30 June 1986	19165	724	3·9	567	1·27
1 July 1986–31 Dec 1986	8488	269	3·3	226	1·19
1 July 1976–31 Dec 1986	176045	12562	7·6	9161	1·37

* MPCF test, *Mycoplasma pneumoniae* complement fixation test.
† Seropositive = a titre of $\geqslant 64$.

Table 2. *Distribution of hospitalized and non-hospitalized cases with regard to number of specimens and sex*

Number of specimens per case	Number of cases with specimens: From hospitals	From practitioners	Total	Per cent of total
1	3614	3126	6740	73·8
2	1144	654	1798	19·7
3	302	94	396	
4	93	22	115	
5	28	7	35	6·5
6	28	1	29	
> 7	24	1	25	
Total	5233 (57%)	3905 (43%)	9138*	100
Male cases	2714 (52%)	1742 (45%)	4456	49
Female cases	2510 (48%)	2154 (55%)	4664	51
Sex unknown (cases)	9	9	18	

* In 23 (14 males and 9 females) of a total of 9161 patients there was no information of who requested the test.

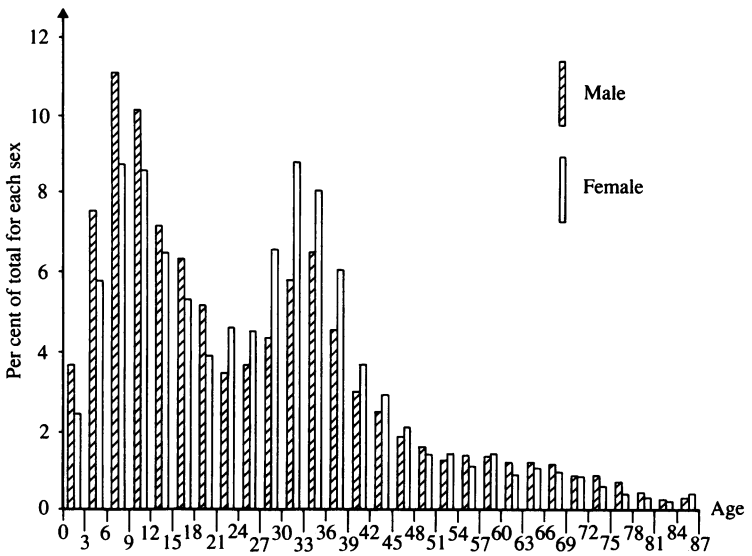

Fig. 2. Age distribution of 4470 male and 4673 female patients with a positive *M. pneumoniae* CF test. The sex was unknown in 18 cases, which are not included. In each 3-year age group the columns indicate the percentages of the total for each sex.

To investigate how long a positive MPCF titre could persist, all patients whose first titre was at least 128 had further antibody titres recorded. Table 4 shows that 48 (96%) out of 50 patients still had positive titres 100 and 199 days after the first sample was drawn, and 75 (87%) out of 86 were still seropositive after day 200. There were 181 patients who had at least three specimens tested over a period of 60 days or more and who showed at least a fourfold change in titre. Among these,

Table 3. *Number of seropositive patients in three periods of the 10·5 years, recorded by age group and by hospital (Hosp) or general practitioner (GP) requesting the* Mycoplasma pneumoniae *CF-test*

	Age groups (years)									
	0–4		5–19		20–39		⩾ 40		Total	
Periods of study	Hosp	GP	Hosp	GP	Hosp	GP	Hosp	GP	Hosp	GP
July 1976–June 80 (4 years)	317	30	967	564	672	564	500	196	2456	1354
Total	347		1531		1236		696		3810	
(per cent)	(9·1)		(40·2)		(32·4)		(18·3)		(100)	
July 1980–June 83 (3 years)	142	40	572	431	472	536	292	200	1478	1207
Total	182		1003		1008		492		2685	
(per cent)	(6·8)		(37·4)		(37·5)		(18·3)		(100)	
July 1983–Dec 86 (3·5 years)	107	30	458	422	427	637	307	255	1299	1344
Total	137		880		1064		562		2643	
(per cent)	(5·2)		(33·3)		(40·3)		(21·3)		(100)	
July 1976–Dec 86 (10·5 years)	566	100	1997	1417	1571	1737	1099	651	5233	3905
Total	666		3414		3308		1750		9138*	
(per cent)	(7·3)		(37·4)		(36·2)		(19·2)		(100)	

* In 23 of the total of 9161 patients there was no information as to who requested the test.

Table 4. *Persistence of a positive MPCF test (titre ⩾ 64) after the first sample with maximal positive titre as indicated*

First maximal titre...	128–256		512–4096		128–4096 Total number of patients	
	Number of patients		Number of patients			
Last blood specimen drawn on day	Tested	Positive (%)	Tested	Positive (%)	Tested	Positive (%)
0–99	94	81 (86)	158	155 (98)	252	236 (94)
100–199	16	15 (94)	34	33 (97)	50	48 (96)
> 200	30	28 (93)	56	47 (84)	86	75 (87)
Total	140	124 (89)	248	235 (95)	388	359 (93)

Table 5. *Distribution of low and high positive MPCF titre levels among four age groups*

Titre range	Age groups 0–4	5–19	20–39	⩾ 40	Total
64–256	432	1706	1743	1214	5095
512–⩾ 4096	234 (35·1 %)*	1717 (50·2 %)	1571 (47·4 %)	544 (30·9 %)	4066 (44·4 %)
64–⩾ 4096	666	3423	3314	1758	9161

* Per cent within the age group.

88 were selected whose first maximal titre was ⩾ 1024. Of these, 24 (28 %) had decreased but still remained positive (range 256–64) after 2 years. Seven patients were still positive after 3 years, one after 4, and five after 5 years (data not shown).

Examination of titre levels by age group (Table 5) shows that MPCF titres between 512 and ⩾ 4096 were found in about half of the 5–39 year age group, but only in about one third of both the 0–4 and the ⩾ 40-year age groups. In the four age groups there was an even distribution between titre levels within the two titre ranges except for the age group ⩾ 40, where titres of 64 were most common (not shown).

Correspondingly, a positive CA test (titre ⩾ 64) was recorded in about two thirds of the patients aged 5–39 years. Half of the 0–4 year-old group had a positive CA test, while this was the case in only one third of those above 39 years of age. Among the 7287 patients who had their sera titrated for CA, high titres (⩾ 512) were also most common in the 5–39 age group, representing 75 % of those with a positive CA test (data not included).

DISCUSSION

The MPCF test has been the most commonly used diagnostic test for antibodies to *M. pneumoniae* since it was described by Kenny & Grayston in 1965 [3]. The CA test for the diagnosis of primary atypical pneumonia was introduced in 1943 before *M. pneumoniae* was known as an aetiological agent [4]. Though non-specific, this test has been retained as a supplement to the MPCF test, because CA develop

in more than half the patients with *M. pneumoniae* infection, and they are usually the first antibodies detected and also the first to disappear [5].

We have not investigated the possibility that a proportion of the patients might have been infected with *M. genitalium* alone or in association with *M. pneumoniae* [6]. The tests which we have applied do not discriminate between antibodies to the two serologically cross-reacting species [7].

An estimation of the diagnostic value of the two tests has not been possible in this study because sufficient clinical information was only available for a few of the patients, and culture for *M. pneumoniae* was not performed. However, this serological survey must be viewed against the background that the specimens were from patients in whom the physician suspected a current *M. pneumoniae* illness, and 59% of these MPCF-positive patients also had a positive CA test supporting the diagnosis [1, 5]. Furthermore, 4066 or 44% of the patients had MPCF titres of $\geqslant 512$, and 10% of the total had significant changes in titre. The sensitivity and specificity of the MPCF test in the diagnosis of *M. pneumoniae* pneumonia was recently evaluated by Kenny and coworkers on the basis of isolations of the organism. Fourfold antibody increases combined with a titre of $\geqslant 32$ showed a sensitivity of 90% in culture – positive persons, while the specificity was 94% for fourfold antibody increases found in 6% of culture-negative persons [8]. We have not compared the sensitivity and specificity of our and Kenny's MPCF tests, which are comparable in principle using the same preparation of antigen. With the awareness of minor inter-laboratory technical differences we assume that our cut-off titre of $\geqslant 64$ for positivity is reasonably comparable to Kenny's $\geqslant 32$.

While the average ratio between female and male patients was close to 50%, there were significantly more boys than girls in this seropositive population, and women predominated in the age group 27–38 years (Fig. 2). It is possible that the lower rates of infection in young girls might provide a greater pool of susceptible young women. It may also be suggested that mothers are more exposed to transmission of the disease due to a closer contact with their sick children than fathers. We have no explanation for the finding that boys were apparently more susceptible than girls, which is similar to the trend shown by Foy and colleagues [9]. Grayston and colleagues [10] isolated *M. pneumoniae* and found seropositivity about 50% more frequently in males than in females. In our study 51·9% of hospitalized patients were males, and they constituted 56% of the 0–19-year old hospitalized patients (data not shown).

An interesting shift was observed in the course of this 10·5 years study, namely a significant decrease in the number of seropositive patients, suggesting an overall decreasing *M. pneumoniae* morbidity (Table 3). This decrease was mainly in those who had been hospitalized in contrast to the constant number seen by general practitioners, which presumably reflects an increasing mildness of *M. pneumoniae* illness during the years of the study. In the same period a striking shift was observed in age composition of these seropositive patients. The number in the 0–19 years age group decreased from 1878 to 1017, i.e. by 10·8% of the total from the first 4-year period to the last 3·5-year period. Between the same two periods the number of patients over 19 years of age decreased only by 306 with a corresponding 10·8% increase of the total. This shift from younger to older

Table 6. *Number of children registered in day-care institutions and day care in private homes in Denmark 1961–86*

1961	42816
1965	48769
1970	85381
1975	162647
1980	245196
1986	266434

Population 0–14 years old: ca. 1000000.

patients, coincident with a decreasing number of hospitalized cases, occurred during the 9 years which we described previously as a period of hyper-endemic *M. pneumoniae* infections in Denmark [1]. In that study we put forward the suggestion that a more than sixfold increase of children in day care from 1961–86 might have contributed to the change in the epidemiological pattern. The increase was most steep in the years of change from an epidemic to a hyper-endemic situation (Table 6, Fig. 1). This well-known risk factor for transmission of the disease among children may have resulted in an increasing protective immunity in a significant proportion of the population. This indication of a shift in herd immunity between age groups is in agreement with our hypothesis if we accept that protective immunity may last for only a few years with a range of 1–10 years [5, 11, 12, unpublished observations]. Hence the balance between protected and susceptible individuals may have tipped towards the end of the endemic period, so that the infection could spread sufficiently rapidly to develop into the following large epidemic which occurred in the winter 1987/88 [1].

Pönkä and Ukkonen [13], who correlated the prevalence of CF antibodies against *M. pneumoniae* with age, found that titres of $\geqslant 32$ were rather frequent in the age group 4–7 months to 3 years. This is in accordance with findings of Fernald and colleagues [11] and Brunner and colleagues [14], who reported that the infection is often asymptomatic in infants and young children. In their review, Denny and colleagues [5] state that *M. pneumoniae* disease is recognized infrequently in patients younger than 5 years of age.

In the present study 666 (7·3%) of the MPCF-positive patients were below the age of 5 years and 566 or 85% of them had been admitted to hospital. Although we have no clinical data, this observation, together with the serological results, indicates that most of them may have had symptoms due to *M. pneumoniae*. However, a minor proportion could still have had a diagnostic titre of mycoplasma antibodies when subsequently tested for another respiratory infection. This proportion is unknown but hardly likely to be significant. Since the studies cited above are from before 1972, we cannot exclude the possibility that the clinical manifestations of the disease may have changed in the course of time, perhaps concurrent with the increased number of children in day care [1].

McCormick and co-workers [15] presented evidence that high levels of CF antibodies to *M. pneumoniae* were associated with resistance to *M. pneumoniae* infection and severe disease, while lower levels protected against mild disease only. On the other hand, Brunner and colleagues [14] who measured the antibodies by more sensitive techniques found indications that moderately high levels of serum

antibody were not directly linked to host resistance. These different correlations probably depend on the nature and concentration of the specific antibodies measured.

The finding that the highest titre levels of specific MPCF antibodies and of CA were present in the age group 5–39 years is in agreement with data published by Grayston and co-workers [10], by Pönkä and Ukkonen [13], and by Biberfeld [16]. However, our data are dependent to an unknown degree on the physicians' motivation for requesting an MPCF test.

Previous long-term investigations have indicated that antibodies to *M. pneumoniae* may persist for 2–4 years [16, 17]. This was confirmed in our study. Patients were recorded whose titres decreased but remained positive in the range 256–64 after 2 years, in a few patients even after 3–5 years. Unfortunately, we had no information on why the patients were followed for that long period of time. A few cases had changes in antibody titres compatible with reinfection with *M. pneumoniae* after 1–6 years but without supporting clinical data. In the majority of long-term cases studied too few consecutive blood specimens had been taken to reveal reinfection.

Because there is long persistence of antibodies following an *M. pneumoniae* infection, the diagnosis should only be considered 'confirmed' on the basis of a significant rise in titre of specific antibodies. For the same reason a presumptive diagnosis of *M. pneumoniae* infection should be made only in the presence of compatible symptoms plus a high titre as defined by the laboratory performing the MPCF test. A positive CA titre may also persist long after convalescence, making a rising titre of greatest value in diagnosis.

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