

# Prevalence of *Chlamydia pneumoniae* antibodies in patients with acute respiratory infections in Israel

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## Abstract

**Aims**—To evaluate the prevalence of antibodies to *Chlamydia pneumoniae* (TWAR) in relation to other aetiological agents of acute respiratory infections in Israeli patients.

**Method**—Serum samples from 604 patients (183 children and 421 adults) were collected over three years. Antibodies to *C pneumoniae*, *C trachomatis*, and *Legionella sp* were evaluated using the microimmunofluorescence (MIF) assay. Antibodies to *Mycoplasma pneumoniae* were detected using the Serodia Myco II test.

**Results**—Antibodies to TWAR were detected in 319 (51.3%) sera. Twenty one patients had MIF results indicative of recent infection. TWAR prevalence and antibody titres in children (aged 1–10 years) were low, gradually increased in teenagers (11–18 years), and were highest in adults and elderly patients. In contrast to the consistently noted TWAR antibody prevalence and serological evidence of recent infection during the study period, a significant decrease in those variables was recorded for *C trachomatis*. Six patients had serological evidence of recent infection with both *C pneumoniae* and *C trachomatis*. The presence of antibodies to *Mycoplasma pneumoniae* and *Legionella sp* was tested in 473 of the patients; 29 had antibodies to *M pneumoniae* and 23 to *Legionella sp*. Six patients (including five children) had serological evidence of recent infection with *M pneumoniae* and four with *Legionella sp*. **Conclusion**—*C pneumoniae* should be considered in patients with acute respiratory diseases. MIF is the preferred method for monitoring the presence of antibodies to this organism.

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*Chlamydia pneumoniae* (TWAR), a newly recognised chlamydial organism, is now regarded as a common cause of pneumonia and other respiratory tract infections.<sup>1,2</sup> *Chlamydia*, together with *Mycoplasma pneumoniae*, *Legionellae*, and viruses, are the aetiological agents of atypical pneumonia.

Seroepidemiological studies from different areas of the world indicate that TWAR is distributed worldwide.<sup>2-13</sup> Antibodies to it are rare before the age of 5 years. An increase in

seropositivity is observed with age, and it is reported that by the age of 13–17 a third to three quarters of teenagers have antibodies to TWAR.<sup>2,5,7,9,11,12</sup> Prevalence then further increases to 40–80% and persists into old age.<sup>2,11-13</sup>

This three year study was undertaken to investigate the prevalence of *C pneumoniae* antibodies in Israeli patients, as well as the prevalence of antibodies to other microorganisms associated with lower respiratory tract infections like *M pneumoniae*, *Legionella* and *C trachomatis*.

## Methods

Serum samples taken between June 1989 and May 1992, from 183 children up to the age of 18 years and 421 adults with undefined acute respiratory diseases, mostly atypical pneumonias, were sent to us for chlamydial serodiagnosis. A second serum sample was collected from 18 patients three weeks after the first sample, while only one serum sample was available from 586 patients. The sera were tested immediately and stored at –20°C until further use.

## ANTIBODIES TO CHLAMYDIA

### MIF test

The prevalence of antibodies to *C pneumoniae* was determined using formalin fixed elementary bodies of *C pneumoniae* TWAR (Washington Research Foundation, Seattle, USA) as antigen. The antigen reacted with monoclonal antibody specific for *C pneumoniae* (a gift from Dr Persson, Sweden) but not *C trachomatis*. The sera were diluted twofold starting at 1 in 16. Specific chlamydial antibodies were measured using fluorescein isothiocyanate (FITC) conjugated sheep anti-human immunoglobulin (Institut Pasteur, France) and positive sera of  $\geq 1/16$  were further analysed using FITC conjugated goat antihuman IgG and IgM (Institut Pasteur, France). Antibodies to *C trachomatis* were detected using formalin fixed elementary bodies of *C trachomatis* L2/434 as antigen and FITC conjugated anti-human Ig, IgG, IgA, and IgM.

A titre of  $\geq 1/512$  in the IgG fraction,  $\geq 1/16$  in the IgM fraction, or a four-fold titre change in the IgM or IgG antibodies (seroconversion) was considered indicative of recent infection. *Chlamydia* antibody titres of 1/16 to 1/256 in the IgG fraction were considered indicative of past infection. Sera were tested for the presence of IgM antibodies after

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a strip-treatment with reagents from the IPAzyme chlamydia "true" IgM kit (Savyon Diagnostics, Israel) to avoid false results introduced by the presence of IgG or rheumatoid factor (RF) in the serum.<sup>14</sup>

#### IPAzyme assay

The IPAzyme immunoperoxidase assay was used to detect chlamydial IgG, IgA, and IgM antibodies, according to the manufacturer's instructions. IgG antibodies at a titre of  $\geq 1/128$ , an IgM titre of  $\geq 1/16$ , and an IgA titre of  $\geq 1/16$  were considered indicative of recent infection. A titre of 1/64 in the IgG fraction was considered indicative of past infection.

#### Antibodies to *Legionella* sp

Antibodies to different serogroups of *Legionella* sp were detected using the indirect immunofluorescence test, as described before.<sup>15</sup> Nine groups of formalin fixed *Legionella* sp (pooled or single) served as antigens:

- pool I—*L pneumophila* serogroups 1–4;
- pool II—*L pneumophila* serogroups 5–6;
- pool III—*L pneumophila* serogroups 7–10;
- pool IV—*L longbeachae* serogroups 1–2;
- pool V—*L bozemanii* serogroups 1–2;
- pool VI—*L feeleii*, *L wadsworthi*, *L jordanis*,  
*L oakridgensis*;

*L dumoffii*

*L gormanii*

*L micdadei*

A titre of  $\geq 1/256$  in the IgG fraction or a lower titre, together with IgM antibody of  $\geq 1/64$  was considered indicative of recent infection.<sup>16</sup>

#### Antibodies to *M pneumoniae*

Antibodies to *M pneumoniae* were detected using the Serodia Myco II particle agglutination test kit (Fujirebio Inc, Japan).<sup>17,18</sup> A titre of  $\geq 1/320$  was considered indicative of recent infection.

#### Rheumatoid factor

The presence of RF was detected using the Arthi Slidex technique (BioMérieux, France).

The significance of data was determined using the  $\chi^2$  test. A probability value ( $p$ ) of  $< 0.05$  was considered significant.

## Results

### PREVALENCE OF ANTIBODIES TO *C pneumoniae*

The presence of IgG antibodies to *C pneumoniae* was detected by the MIF test in 319 (51.3%) of the 622 tested sera with titres ranging from 1/16 to  $\geq 1/512$  (table 1). Using the IPAzyme assay, 66 out of 187 (35.3%) sera with anti-TWAR MIF titres of  $\geq 1/64$  and 93 out of 435 (21.4%) sera with anti-TWAR MIF titres of  $< 1/64$  showed a positive reaction. All MIF IgG reactive sera and 175 negative sera were tested for IgM class antibodies in the MIF test. Fifteen patients with an IgG reaction had a positive IgM reaction, 12 patients had an IgM titre of 1/16, and three patients had a titre of 1/32, but no correlation with IgG titres was noticed.

Twenty one adults had MIF results indica-

Table 1 Prevalence of IgG antibodies to *C pneumoniae* in 622 sera of patients with acute respiratory disease

Endpoint titre (IgG)	No (%)
$< 1/16$	303(48.7)
1/16	53(8.5)
1/32	79(12.7)
1/64	91(14.6)
1/128	49(7.8)
1/256	38(6.1)
$\geq 1/512$	9(1.4)

Table 2 Age dependent prevalence of IgG antibodies to *C pneumoniae*

Age (years)	No of patients	No (%) positive	MGT
$< 1$	57	16 (28)	1/52
1–5	39	8 (21)	1/22
6–10	16	4 (25)	1/32
11–18	12	4 (33)	1/32
19–30	25	11 (44)	1/75
31–50	29	16 (55)	1/101
51–70	44	29 (66)	1/91
$\geq 71$	32	22 (69)	1/111

Positive = titre of  $\geq 1/16$ ; MGT = mean geometric titre of positives

tive of recent infection. Of the 586 patients with only one available serum sample, 12 had a positive IgM titre (1/16–1/32), and four had a high IgG titre ( $\geq 1/512$ ), as the indicative criteria for their recent infection with *C pneumoniae*. Of the 18 patients with two available serum samples, two with early negative or low positive sera had seroconverted. For three patients with an early positive serum (IgM titre 1/16–1/32 and/or an IgG titre of 1/512) the diagnosis was confirmed by testing the second serum sample. (A fourfold titre change and/or an IgG titre of  $\geq 1/512$ .)

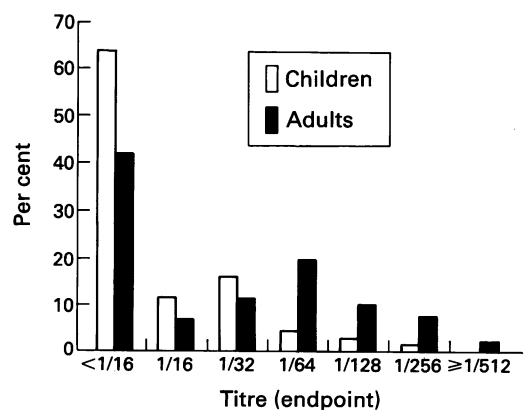
### AGE RELATED PREVALENCE OF IgG CLASS ANTIBODIES TO *C pneumoniae*

The prevalence of antibodies to *C pneumoniae* in the sera of patients whose age was known was evaluated using the MIF test (table 2). There was a significant difference ( $p < 0.05$ ) in prevalence as well as in mean geometric titre (MGT) between children ( $n = 124$ ) and adults ( $n = 130$ ), but differences within the groups were not significant ( $p > 0.05$ ).

An analysis of the titres showed that 66 children out of 183 had moderate MIF titres of 1/16–1/256, while 234 out of 421 adult patients had titres of 1/16–1/256 and seven had IgG titres of  $\geq 1/512$  (figure).

### PREVALENCE OF ANTIBODIES TO *C pneumoniae* AND *C trachomatis* DURING THE STUDY PERIOD

A comparison of the three study years for prevalence of antibodies to *C pneumoniae* and *C trachomatis* showed an even distribution of seropositive results for *C pneumoniae*, but a decline for *C trachomatis* (table 3). This decline was significant ( $p < 0.05$ ). A parallel decline was observed in the prevalence of



TWAR IgG endpoint titres in 183 children and 421 adults with acute respiratory infections measured by the MIF test.

Table 3 Comparison of prevalence of antibodies to *C pneumoniae* and *C trachomatis* during study period

Study period	No	<i>C pneumoniae</i>		<i>C trachomatis</i>	
		Seropositive No (%)	Recent infection No (%)	Seropositive No (%)	Recent infection No (%)
I	76	43 (56.6)	1 (1.3)	44 (58.0)	15 (19.7)
II	164	81 (49.4)	6 (3.7)	38 (23.2)	15 (9.1)
III	364	172 (47.5)	14 (3.9)	52 (14.4)	15 (4.1)

Study period: 12 months (June to May) (I) 1989-90, (II) 1990-91, (III) 1991-92

Seropositive:

*C pneumoniae* titre IgG  $\geq$  1/16

*C trachomatis* titre IgG  $\geq$  1/16

Recent infection (indicative of) fourfold titre change or

*C pneumoniae* titre IgG  $\geq$  1/512, IgM  $\geq$  1/16

*C trachomatis* titre IgG  $\geq$  1/512, IgA  $\geq$  1/16, IgM  $\geq$  1/16

patients with serological evidence of recent infection ( $p < 0.05$ ).

PREVALENCE OF ANTIBODIES TO *C pneumoniae*, *C trachomatis*, *M pneumoniae* AND *Legionella sp*  
A retrospective analysis of our computed laboratory data provided information on 473 (78.6%) of the patients whose sera were also processed for the detection of antibodies to *Legionella sp* and *M pneumoniae* (table 4).

Evidence of recent infection with *C trachomatis* and *M pneumoniae* was detected in four and five children, respectively. Recent infection with *C pneumoniae* or *Legionella sp* was undetectable in children included in our study group.

Sixty three of the 473 patients had antibodies indicative of recent infection with one of the tested groups of antigens. Eight patients had antibodies indicating recent infection with two aetiological agents (six patients with both *C pneumoniae* and *C trachomatis*, one patient with both *C trachomatis* and *M pneumoniae*, and one patient with both *C pneumoniae* and *Legionella sp*).

## Discussion

Prevalence of antibodies to *C pneumoniae* in the Middle East has not yet been thoroughly investigated. It was therefore of interest to explore this aspect of respiratory infections especially as we were able to analyse the same sera for the prevalence of antibodies to other

Table 4 Prevalence of antibodies to *C pneumoniae*, *C trachomatis*, *M pneumoniae* and *Legionella sp* in 473 patients

	Positive results No(%)
<i>C pneumoniae</i>	
Past infection	230(48.6)
Recent infection	21(4.4)
<i>C trachomatis</i>	
Past infection	68(14.4)
Recent infection	32(6.8)
<i>M pneumoniae</i>	
Past infection	23(4.8)
Recent infection	6(1.3)
<i>Legionellae</i>	
Past infection	19(4.0)
Recent infection	4(0.8)

Past infection (indicative of):

*C pneumoniae* titre IgG 1/16-1/256

*C trachomatis* titre IgG 1/16-1/256

*M pneumoniae* titre 1/80-1/160

*Legionellae* titre IgG 1/128

Recent infection (indicative of):

fourfold titre change or

*C pneumoniae* titre IgG  $\geq$  1/512, IgM  $\geq$  1/16

*C trachomatis* titre IgG  $\geq$  1/512, IgM  $\geq$  1/16, IgA  $\geq$  1/16

*M pneumoniae* titre  $\geq$  1/320

*Legionellae* titre IgG  $\geq$  1/256, IgM  $\geq$  1/64

causes of respiratory infections, such as *M pneumoniae*, *Legionella sp*, and *C trachomatis*.

The patients included children and adults with acute respiratory diseases attending the Health Center, the casualty Department, or in paediatric or other departments. The overall antibody prevalence to *C pneumoniae* proved similar to reported values from other parts of the world.<sup>2,4,6-8,11-13</sup> About half the patients had antibodies to *C pneumoniae* in their sera, with IgG titres of 1/16 to  $\geq$  1/512. Only 35.3% of the sera positive to *C pneumoniae* by MIF were positive by the IPAzyme method. IPAzyme is therefore not suitable for the detection of antibodies to *C pneumoniae*.

Only 21 patients showed evidence of recent infection—in the MIF test (high IgG titre  $\geq$  1/512, IgM titre of  $\geq$  1/16, or seroconversion)—but the limited numbers of paired specimens available in this study must be borne in mind. The need for a larger proportion of patients with available paired sera is obvious, although in only two out of the 18 (11%) patients with paired sera could seroconversion from negative or low positive early serum be shown.

In our study infants under 1 year old showed a relative high prevalence and titre (28%, MGT 1/52) of anti-TWAR IgG antibodies. This could probably be attributable to transfer of maternal antibodies, as a 50% prevalence of TWAR antibodies was reported in cord blood.<sup>11</sup> A 3% prevalence was reported for Filipino infants under the age of 1 year with acute respiratory infections.<sup>4</sup>

In our study the prevalence of TWAR seropositive children increased with age, from 21% in the 1-5 year age group to 33% in teenagers, but titres were still low (mean geometric titre 1/22 and 1/32, respectively). The reported prevalence of TWAR antibodies in young children (under 10) was relatively low, ranging from 12% to 25%. The prevalence was much higher in teenagers (30%-76%) (Shemer-Avni Y, Gonen R, Sarov B, *et al.* Abstract presented at the 2nd National Scientific Congress on STD, 1992).<sup>2,8,11,12</sup> In our study prevalence increased with age and reached 70% by old age. Mean titres of 1/100 were measured after the age of 30. Antibody persistence into old age might be a result of infection and reinfection during life.<sup>2,11-13</sup> The incidence and level of antibody titres probably depend on exposure and time elapsed between the last encounter and monitoring for the organism. It is obvious from our results (table 4) that though high antibody prevalence to *C pneumoniae*, indicating exposure to this organism, was detected in 48.6%, only 21 (4.4%) patients had evidence of a recent infection. Although a lower exposure was measured in 14.4% for *C trachomatis*, 32 (6.8%) patients had evidence of recent infection.

The decline in prevalence of antibodies to *C trachomatis* during the study period was significant, and could be attributed, in part, to changes in sexual behaviour, due to AIDS, though our patients were admitted primarily for acute respiratory diseases. A parallel decline in the isolation rate of *C trachomatis* from the genital tract has been noticed in

Israel (Ben-Yaakov *et al*, unpublished data). A similar trend has recently been reported.<sup>19</sup>

When sera react to more than one antigen the question of specificity of each of the detection methods and of cross-reactivity always arises.<sup>20-23</sup> The MIF test depends mainly on type specific epitopes residing on the major outer membrane protein (MOMP) exposed on the surfaces of elementary bodies, but the use of chlamydial inclusions in the IPzyme method is based predominantly on a non-specific genus reaction, presumably involving lipopolysaccharide (LPS) reactivity. As possible cross-reactivity might be caused by the presence of high anti-LPS titres, even the specificity of the MIF assay is debatable.<sup>24 25</sup> The prevalence of chronic TWAR antibodies in contrast to the relatively low occurrence of *C trachomatis* antibodies supports those who claim specificity.<sup>1</sup> Recently serological evidence of infection with *C pneumoniae* and with at least one other chlamydial species was reported,<sup>26</sup> as a result of either heterotypic antibodies or mixed related infections.

In our study serological cross-reactivity could not be excluded as only one serum sample was available for most patients.

Infection with more than one respiratory pathogen has been reported by several groups,<sup>3 10</sup> while others have not detected such mixed infection.<sup>1 5</sup>

We detected antibodies to *M pneumoniae* in 23 (4.8%) patients with only six (1.3%) patients, mostly children, showing evidence of recent infection. Higher prevalences have been reported before.<sup>27 28</sup>

Previous infection with *Legionella sp* was serologically diagnosed in 4% of the patients, with 0.8% showing evidence of recent infection. Cases of legionellosis have been reported in Israel.<sup>29-33</sup>

Our study points to serological evidence of recent infection with *C pneumoniae*, *M pneumoniae*, or *Legionella sp* in 6.5% (31 out of 473) of our patients. However, the contribution of *C trachomatis* (6.8%) to the diagnosis of respiratory diseases remains inconclusive as in our patients genitourinary infection could not be excluded.

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