Chlamydophila pneumoniae serological epidemiology

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Key words: *C. pneumoniae,* MIF, Antibodies

SUMMARY

Chlamydophila pneumoniae is an obligate intracellular pathogens, that causes multiple types of respiratory tract infections. The exposure to this pathogen is quite common. *C. pneumoniae* may cause a persistent infection in multiple organs and may be associated with several chronic inflammatory conditions. The aim of this study is the evaluation of the serological epidemiology of *C. pneumoniae* infection in four heterogeneus groups of patients. The detection of antibodies anti-*C. pneumoniae* was conducted using an, in-house, microimmunofluorescence (MIF) test, as indirect diagnostic technique.

INTRODUCTION

C. pneumoniae, strict obbligate intracellular bacterium, was first isolated in 1965 from the conjunctiva of a Taiwanese child, participating in a *Trachoma* vaccine trial (8). Since then, numerous scientific studies have been conducted against this bacterium, which still fascinates many researchers for possible clinical implications in various pathologies (2, 5, 9, 14). The *C. pneumoniae* infection is ubiquitous, causing acute respiratory diseases, including atypical pneumonia (10% of all cases of atypical community-acquired pneumonia), bronchitis, sinusitis, and pharyngitis (6). The infection by this obligatory intracellular pathogen may remain chronic in some individuals, and may be associated with several serious disorders such as exacerbations of chronic bronchitis and asthma (14). Moreover recent studies, have reported that *C. pneumoniae* is a possible cause of reactive arthritis (1), atherosclerosis (4), primary biliary cirrhosis (PBC) (10) and central nervous system disorders (Alzheimer, multiple sclerosis) (2, 5, 13). The persistence of this infection is caused by the fact that *C. pneumoniae* has developed mechanisms to survive into macrophagic cells (7). The infection by this pathogen is common: by age of 20 yr, 50% of the population has evidence of past infection (7). The objective of this study is to measure the serum levels (titre) of IgM and IgG anti-*C. pneumoniae*, to evaluate in different patient groups the serological epidemiology of *C. pneumoniae*.

MATERIALS AND METHODS

In 2008, 366 patients were investigated for detection antibodies anti-*C. pneumoniae*. The patients were divided in four groups: 100 patients with STDs (*C. trachomatis*, *T. pallidum* and *HIV*), 100 patients with suspicion of atypical acute respiratory infections (AARI), 66 patients with liver diseases (LDs) (criptic liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune liver disease) and 100 blood donors (Bds). The patients were 183 men and 183 women, with age between 20-65. The four groups under study were similar for age and sex. The patients with clinical history of chronic respiratory disease (asthma and COPD) were excluded from this study, because *C. pneumoniae* might be implicated in these pathologies (12, 14). Serological diagnosis of a *C. pneumoniae* infection was based on the following criteria: presence of *C. pneumoniae*-specific IgM (titer ≥1:16) in sera or 4-fold increase in IgG. Positive IgG, associated to IgM <1:16 are presumed past infection (12).

The detection of antibodies anti-*C. pneumoniae* was conducted using the in-house microimmunofluorescence (MIF) technique (6), based on an *C. pneumoniae* indirect detection (IgG and IgM) (16).

SELECTION OF STUDY GROUP

The patients were selected according to the following criteria:

BDs group: only patients with negative serology for HIV (immunoassay, bioMerieux, Boxtel and Immunoblot, The Netherlands and Chiron RIBA, HIV-1/HIV-2, Chiron Co., Emerville, CA, USA), HCV/HBV (Chemiluminescent assay, Abbott Diagnostic, Wiesbaden, Germany and Molecular biology assay, Cobas Amplicor, Roche Diagnostics, Mannheim, Germany) and Syphilis (Chemiluminescent assay, Abbott Diagnostic, Wiesbaden, Germany and in-house whole-cell lysate antigen-based immunoblotting as confirmatory test) .

LDS group: only patients with clinical (Child-Pugh stage) and laboratory evidence for liver disease.

STDs group: only patients with positive serology for HIV (Immunoassay, bioMerieux, Boxtel and immunoblot, The Netherlands and Chiron RIBA, HIV-1/HIV-2, Chiron Co., Emerville, CA, USA), Syphilis (Chemiluminescent immunoassay, Abbott Diagnostic, Wiesbaden, Germany and in-house whole-cell lysate antigen-based immunoblotting as confirmatory tests) and patients infected with *C. trachomatis* infection, detected by isolation in cell cultures (LLCMK2, Rhesus monkey kidney cells).

AARI group: only patients with acute respiratory symptoms (nonproductive cough and low-grade fever).

The study group showed the following risk factors for acute atypical respiratory infection: 54.6% (200/366) smoke, 2.4% (9/366) immunodepression, 87.4% (320/366) person-to-person contact work and 9.3% (34/366) viral respiratory infection. Furthermore, the group of patients, during this study, showed the following suggestive symptoms of acute respiratory infection: 28.1% (103/366) low-grade fever, 26.2% cough (96/366), 20.5% (75/366) vague feeling of sore throat, 20.2% (74/366) headache, 9.0% (33/366) ear pain. Patients with asthma were excluded from this study.

ANTIGEN PREPARATION

Elementary bodies (EBs) of *C. pneumoniae* were prepared and purified as described in other studies (15), with some modifications. Briefly, a culture fluid was centrifuged at 3000 rpm for 10 minutes at 4°C. The supernatant was saved and sonicated for a few seconds, and centrifuged at 18000 rpm for 35 minutes. Precipitated crude EBs were suspended in a small volume of 0.01 M Tris hydrochloride, pH 7.4. The suspension was sonicated for a few seconds and centrifuged through 30% sucrose-0.01 M Tris hydrochloride, pH 7.4 at 18000 rpm for 40 minutes at 4°C. The precipitate was resuspended in Tris hydrochloride pH 7.4 and loaded on 30 to 60% sucrose linear density gradients. After ultracentrifugation at 18000 rpm for 50 minutes at 4°C, a white band located at the

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middle of the gradient was collected with a Pasteur pipette. The fraction was diluted with 0.01 M Tris hydrochloride pH 7.4, and centrifuged at 18.000 rpm for 35 minutes at 4°C. The precipitate of purified EBs was finally suspended in a small volume of in 0.25 M sucrose-10 mM potassium phosphate-5 mM glutamic acid (SPG) pH 7.4 and stored at -80°C until use. The MIF assay, used in this study, was home made with crude lysate of the EBs purified. Likewise, the EBs of *C. trachomatis* (D serovar) and *C. psittaci* (6BC strain) were purified to prepared the home made MIF slide.
The quality control of the MIF slides was performed using a monoclonal antibody anti-chamydial LPS with Evans blue (Bioscence Meridian Inc., Cincinnati, USA) and a monoclonal antibody specific for *C. pneumoniae* without Evans blu (Argene SA, Varilhes, France).

SERUM PREPARATION
The venous blood was centrifuged at 1.000 rpm for 10 minutes and the serum was collected and stored at -20°C until serologic examination for antibodies against *C. pneumoniae*. All Sera were collected during the four seasons of the year to assess any increase of spread season-dependent. The sera were diluted in PBS pH 7.2 additioned of 2% Serum Bovine Fetal inactivated (SBF) at 56 °C for 45 minutes. The dilution were: 1:16, 1:32, 1:64, for quantitative detection of IgM and 1:16, 1:32, 1:64, 1:128, 1:256, 1:512 for quantitative detection of IgG. MIF slide wells dotted with inactivated *C. pneumoniae*, *C. psittaci*, *C. trachomatis* EBs. The IgG slide were incubated for 45 minutes and the IgM slides for 2 hours at 37°C in a moist chamber. The slides were washed with PBS at pH 7.2 for 10 minutes for three times, rinsed in distilled water and dried properly. Specific chlamydial antibodies were detected by using fluorescein isothiocyanate-conjugated anti-human IgM (dilution 1:30 in PBS pH 7.2) and IgG (dilution 1:40 in PBS pH 7.2) (Dako A/S, Glostrup, Denmark), were added to the wells of corresponding slides. The slides were placed in a moist chamber and incubated at 37°C for 30 minutes. The slides were washed and dried as after incubation. The slides were examined under fluorescent microscope Zeiss.

RESULTS
The four patient groups object of this study have presented similar distribution of the infection from *C. pneumoniae*, except the groups with acute infection in AARI and BDs (Tabella 1). In the LDs group the IgM prevalence was 15.1% (10/66) and the IgG prevalence was 56.0% (37/66); in the STDs group the IgM prevalence was 11.0% (11/100) and the IgG prevalence was 50% (50/100); in the AARI group the IgM prevalence was 19.0% (19/100) and the IgG prevalence was 59.0% (59/100); in BDs group the IgM prevalence was 4.0% (4/100) and the IgG prevalence was 51.0% (51/100). The global prevalence in all patient groups of the antibodies anti-*C. pneumoniae* was: IgM 12.0% (44/366) and IgG 53.8% (197/366). It is interesting to note that the prevalence of antibodies anti-*C. pneumoniae*, specially the IgG, grows in direct proportion with age (Figura 1) and that *C. pneumoniae* infection often occurred in winter and in the transition period between one season and the other (specially in winter-spring) (Figura 2). Furthermore specific antibodies anti-C. trachomatis were detected only in population with sexually transmitted infection by *C. trachomatis* and were not detectable in other groups of patients. Specific antibodies anti-*C. psittaci* were not detected.

DISCUSSION
This study confirms the high prevalence of infection by *C. pneumoniae* in all patient groups. The four groups of patients did not differ in the distribution of IgM and IgG anti-*C. pneumoniae*. The high diffusion of antibodies against *C. pneumoniae* are a limit for the serological studies that assess the correlation between this infection and some diseases (atherosclerosis, CBP, Alzheimer, etc). The high spread of this infection involves a considerable difficulty in solving the rebus of a chlamydial possible clinical implication in some clinical pathologies (3, 13). It seems clear in this study that the distribution of the antibodies among the group with liver disease and the other groups under study was similar. Therefore to evaluate a possible correlation (direct or indirect) between *C. pneumoniae* infection and liver disease is necessary to make a multiparametric laboratory analysis through biochemical markers (e. g. AST, ALT, AP, ?GT, total and fractional bilirubin), serological markers (autoantibodies), clinical evidence (Child-Pugh stages). Also these data must be related at the direct research (polymerase chain reaction) of the bacterium, at the genetic predisposition (e. g. HLA-B27) and finally at

Tabella 1. *Distribution of antibodies IgM and IgG anti-*C. pneumoniae

Population	Modal titre of IgG	Positive IgM (%)	Modal titre of IgG	Positive IgG (%)	Acute infection (%)	Past infection (%)
LDs	1:32	15.1	1:64	56.0	15.1	53.0
STDs	1:32	11.0	1:32	50.0	11.0	49.0
AARI	1:64	19.0	1:32	59.0	19.0	57.0
BDs	1:32	4.0	1:64	51.0	4.0	48.0

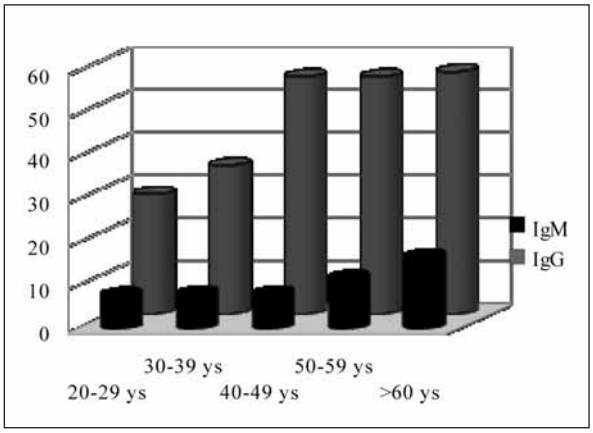

Figura 1. *Distribution antibodies anti-*C pneumoniae *for age.*

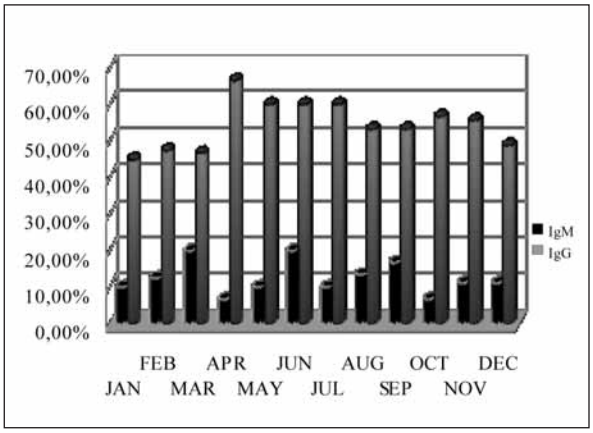

Figura 2. *Incidence of antibodies anti-*C. pneumoniae *in 2008.*

the titre of anti-*C. pneumoniae* (9, 15, 5, 16). In conclusion, the groups of patients without LDs were free of liver disease at this time, although they showed a high incidence of IgG anti-*C. pneumoniae*.

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