THE ROLE OF ATYPICAL PATHOGENS IN COMMUNITY – ACQUIRED PNEUMONIA

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ABSTRACT
The recent microbiological advances have revealed the importance of atypical pathogens such as Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila as a common cause of community-acquired pneumonia (CAP). These microorganisms in western countries caused a third of CAP and there were many dual infections than expected. In our study 40 inpatients with clinical diagnosis CAP were investigated for detection of IgG and IgM specific antibodies to Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila, by commercial ELISA (EUROIMMUN-GERMANY). The results indicated that 62.5% from older patients (mean 46.5 years old) had IgG antibodies to M. pneumoniae and 37.5% - to C. pneumoniae. We obtained reliable serological data, that there was an etiological relationship between diseases and M. pneumoniae – in 10% of the cases, between diseases and C. pneumoniae – in 12.5% of cases and between diseases and L. pneumophila – in 2.5% of the cases. In one of the cases (2.5%) the data showed mixed infection with M. pneumoniae and C. pneumoniae.

Introduction
Atypical pneumonia is a term loosely applied to lower respiratory tract infections that are not characterized by signs and symptoms of lobar consolidation. This description can apply to disease caused by a variety of bacterial, viral and even protozoan organisms. In reality, differentiation as to etiology of CAP cannot be distinguished on the basis of clinical presentation. The atypical pathogens in CAP traditionally have included Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella species. The incidence of pneumonia caused by these pathogens has increased with the development of specific diagnostic techniques. Unfortunately, because many of these pathogens are intracellular, culture systems are either not available or the techniques employed are costly, time consuming or unsafe. Until molecular techniques are standardized and widely available, diagnosis will depend upon serologic confirmation (4, 7).

The aim of this study was to assess the proportion of cases of CAP caused by atypical pathogens as Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila of patients admitted to University hospital “St. Marina” – Varna, Bulgaria and to determine the reliability of commercial serological tests in the diagnosis.

Materials and Methods
Patients. Acute phase serum samples from 40 inpatients with clinical diagnosis CAP, admitted to University hospital “St. Marina” Varna, Bulgaria from November 2002 to May 2003 were examined. Thirty six adults
(21 to 77 years old, mean 46.5 years) and 4 children (6 to 15 years old, mean 10 years) were included in this study.

**Methods.** ELISA (EUROIMMUN - GERMANY) for detecting IgG and IgM class antibodies against Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila was performed. Patient serum samples were diluted 1:101 before tested.

These ELISA test kits provide a quantitative in vitro assay for human antibodies of IgG class and semiquantitative – of IgM class antibodies against Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila respectively. Extinctions values of serum samples exceeding those of the calibration serum 2 (20 RU/ml IgG antibody concentration) or calibration serum only (IgM antibody) were to be considered as positive, those below - as negative (semiquantitative). The standard curve from which the concentration of specific IgG antibodies in the serum samples can be taken was obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (RU/ml).

The antigens used were a detergent extract of Mycoplasma pneumoniae strain “Mac ATCC 15531”, Hep-2 cells infected with the “CDC/CWL-029” strain of Clamydia pneumoniae and antigen LPS from Legionella pneumophila serotype 1, strain “Philadelphia”.

**Results and Discussion**

Serological results are shown in table 1. The data exceeded 100% because some of the patients were infected (had IgG antibodies) to more than one pathogen. Of 40 patients with clinical diagnosis CAP tested, the overall prevalence of IgG antibodies was 62.5% to M. pneumoniae and 37.5% – to C. pneumoniae (range of IgG-concentration 20 – 40 RU/ml). Twelve of the patients were both positive. Four of the positive patients (10%) had serological data of current acute M. pneumoniae infection (IgM antibody positive and IgG antibody positive) and 5 (12.5%) – of persisting acute C. pneumoniae infection (IgM antibody positive and IgG antibody positive) (3). One child (12 years old) had serological data of dual acute infection. One of the patients (43 years old woman) had serological data of acute L. pneumophila infection.

The incidence of CAP caused by atypical pathogens has increased with the development of specific diagnostic techniques. The clinical, radiological, and laboratory manifestations of the disease are similar to those of CAP, caused by other pathogens, and reliable etiological differentiation cannot be based on these factors alone. The rapid diagnosis of pneumonia is important in carrying out chemotherapy in appropriate manner, to be directed against specific organisms earlier in the course of the disease (6, 11).

In our hospital this investigation was performed for the first time. There is no universal agreement upon gold standard serological assay for detection of antibodies to M. pneumoniae. ELISAs are more sensitive and specific than CFT (Complement-fixing test). It is essential to detect the specific IgM. IgM antibody first appears 7 – 9 days after infection, peaks at 4 to 6 weeks, and does not start to decline until 4 to 6 months later. But the IgM response may be non-specific, or absent, particularly in the elderly with reinfection (10). In the present study 2 of the 4 patients with CAP (34 and 56 years old respectively) had IgG and IgM immune response to M. pneumoniae and only IgG specific immune response to C. pneumoniae (previously infected). The remaining 2 (7 and 15 years old respectively) with IgG and IgM immune response to M. pneumoniae, had no serological data of being infected with other atypical pathogens. These data showed the importance of IgM serology test to diagnose M. pneumoniae at the very early phase of infection,
and the specificity of the performed ELISAs for oldest and youngest patients. Other seropositive patients with CAP did not show any significant rise in concentrations of IgG antibodies in paired sera, obtained by time interval of 1 to 3 weeks to confirm reinfection by M. pneumoniae.

Our data showed that 37.5% of the patients with CAP were IgG positive to C. pneumoniae. Five of them had IgM and IgG positive immune response (21, 39, 42, 52 and 77 years old respectively). All of them were only IgG positive to M. pneumoniae (previously infected). Serological criteria for diagnosis of acute C. pneumoniae infection were established, but they were controversial (3). Microimmunofluorescence (MIF), as gold standard was used. The most reliable diagnostic test was enzyme immunoassay (ELISA), that allowed IgG and IgM titration (9), which was proved in our study.

One of the patients (12 years old child) had serological data of dual active current infection with M. pneumoniae and C. pneumoniae, that had been proved by other authors. In children this seem to play important role in causing respiratory-tract infection, than was previously thought (11).

One of the patients (43 years old woman) had serological data of acute L. pneumophila infection. The incidence of Legionella infection, in spite of its world-wide diffusion, was high variable in different studies, ranging from 1% to 27% of CAP (1, 2, 8). Diagnostic serological response to M. pneumoniae and C. pneumoniae were found in 42% of pneumonia patients in Finland (6) According to our data for 40 inpatients with CAP, admitted to the University hospital “St.Marina”, the proportion of the cases of atypical pathogens was assessed up to 27.5%. The prevalence of atypical pathogens varies greatly from study to study, depending on the population and the diagnostic methods used. Infection rates are difficult to determine because many clinicians and investigators do not routinely test for these agents, but reported rates are in the range of up to 8% (C. pneumoniae) and 1.9% to over 30% (M. pneumoniae) of all cases of pneumonia in USA (5, 12). M. pneumoniae infection (16%) occurred in a younger age group (mean age 31,4 years), Legionella infections was common as judged – 11% and was indistinguishable clinically from other pneumonias, C. pneumoniae was 3% of cases in New Zealand (1). In patients with community-acquired pneumonia in South Africa (8), C. pneumoniae was 20,7% of the cases and L. pneumophila - 8,7% of the patients.

Our experience show that IgG positive results with positive IgM specific ELISA results were indicative for a current acute infection requiring appropriate therapy.

Until molecular techniques are standardized and widely available, diagnosis will depend upon serologic confirmation (4, 7), and serology remains the method of choice for laboratory diagnosis.

The possibility of shortening treatment time, at least in some patients, by antimicrobial therapy with the new macrolides has been added to standard therapeutic regimen with erythromycin, tetracyclines, or quinolones.

**REFERENCES**