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Detection of Antibodies to *Chlamydophila pneumoniae* by Immunoblotting in Patients with Cardiovascular Diseases

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Summary

***Chlamydophila pneumoniae*, one of the most prevalent human pathogens worldwide, is not only a significant cause of pneumonia, but may also be associated with cardiovascular diseases (CVD) as suggested by multiple studies. A total of 228 sera from CVD patients with hypertension, ischemic heart disease or previous reconstructive vascular surgery were screened for the presence of anti-C. pneumoniae IgG and IgA antibodies by ELISA. Out of 150 positive serum samples, 80 with similar IgG and IgA levels were investigated by immunoblot (IB). IgG antibodies were directed predominantly against the 35 kDa and 39 kDa proteins as well as 50-54 and 56-60 kDa proteins of C. pneumoniae. IgA antibodies reacted most frequently with the 50-54 and 56-60 kDa proteins.**

Key words: cardiovascular diseases – Chlamydophila pneumoniae antibodies – immunoblotting.

Summary

Kováčová E., Mongiellová M., Tomka J., Slyško R., Kazár J.: Detection of Antibodies to *Chlamydophila pneumoniae* by Immunoblotting in Patients with Cardiovascular Diseases

***Chlamydophila pneumoniae* patrí k najrozšírenejším ľudským patogénnom na celom svete. Je nielen významnou príčinou pneumónií, ale mnohé štúdie naznačujú možný vzťah medzi infekciou *C. pneumoniae* a kardiovaskulárnymi chorobami (KVCH). Celkove sme vyšetrili 228 pacientov s vybranými KVCH (hypertenzia, ischemická choroba srdca a rekonštrukčný chirurgický zákrok na cievach) na prítomnosť IgG a IgA protilátok proti *C. pneumoniae* metódou ELISA. Zo 150 pozitívne reagujúcich vzoriek sér sme 80, ktoré mali podobné hladiny IgG a IgA protilátok, ďalej vyšetrili metódou imunoblotu. IgG protilátky boli namierené predovšetkým voči 35kDa a 39kDa proteínom, ako aj voči 50-54 a 56-60 kDa proteínom *C. pneumoniae*. Pokiaľ ide o IgA protilátky, tieto reagovali najčastejšie s 50-54 a 56-60 kDa proteínmi.**

Kľúčové slová: Kardiovaskulárne choroby, protilátky proti *Chlamydophila pneumoniae*, metóda imunoblotu.

C. pneumoniae is one of the most frequent pathogens related to respiratory tract infections [8]. It is believed to cause about 10% of the cases of community-acquired pneumonia. It was described also as a risk factor for atherosclerosis based on the antibody detection against this pathogen and the presence of *C. pneumoniae* in atherosclerotic lesions in CVD patients, respectively [3, 15].

Though *C. pneumoniae* infection can be demonstrated by direct detection of the agent or by PCR-based detection of specific nucleotide sequences [19], routine laboratory diagnosis and seroepidemiological studies are performed usually by serological examination [1]. Microimmunofluorescence (MIF) assay considered formerly as a "gold standard" has some disadvantages such as differences in the antigen preparation, experience of the individual investigators, and difficulties in interpretation of

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the test resulting in interlaboratory variations in reporting the IF titers [14]. This evoked the necessity for development of alternative methods, e.g. ELISA and IB, respectively. Of several studies performed to recognize the species-specific antibodies appearing during C. *pneumoniae* infection and thus to improve exact serological diagnosis, IB was the most frequently used [2, 6, 9].

In our previous study we found an association of *Chlamydia pneumoniae* antibodies and markers of inflammation in sera of patients with cardiovascular diseases [11]. The purpose of this study was not only to analyze the anti-*C. pneumoniae* antibodies according to their immunoglobulin (Ig) classes, but also to detect the protein antigens to which the Ig antibody classes were directed.

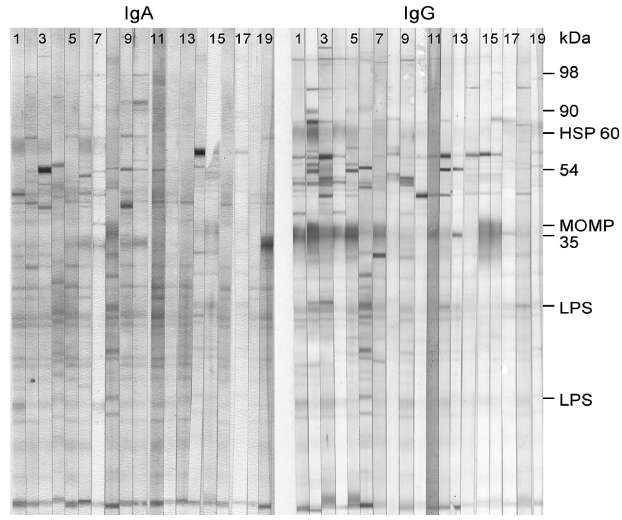

Fig. 1. Typical IB protein profiles with *C. pneumoniae* IgA and IgG, respectively, in sera of CVD patients (lanes No 1-16 correspond to the sera of cardiovascular patients, lanes 17-19 to control sera).

Material and Methods

A total of 228 sera of the patients (62-years-old on the average) with selected CVD, namely coronary heart disease (CHD), hypertension (blood pressure ≥140/90 mm Hg) and those who underwent reconstructive vascular surgery (RVS) – endoarterectomy with removal of intimomedial complex of carotids and excision of the wall of abdominal aorta, respectively, were tested for the presence of anti-*C. pneumoniae* IgG and IgA antibodies by SeroCP-IgG and Sero CP-IgA ELISA kits (Savyon Diagnostics Ltd, Israel) with absorbance (A_{450}) ≥1.1 nm as a cut off value. ELISA was performed and calculated according to the manufacturer instructions. Of 150 positively reacting sera with similar absorbance values, 80 specimens (60 from males and 20 from females) were further tested by .IgG + IgM + IgA IB kit of *C. pneumoniae* (AID GmbH, Germany) to detect Ig classes of antibodies directed to different protein antigens of *C. pneumoniae*. Sera of 15 persons with no evidence of CVD and with the absence or presence of chlamydial antibodies were used as controls.

Results

Analysis of the Ig antibody response to immunodominant protein antigens of *C. pneumoniae* by IB revealed that tested sera reacted with a broad range of antigens (Table 1). As to the IgG, predominant specific antibody activity was directed to the 35 kDa (44 samples), 39 kDa (35 samples), 50-54 kDa proteins (50 samples) and 56-60 kDa (25 samples) proteins, 39 kDa corresponding to the major outer membrane protein (MOMP) and 56-60 kDa to the heat-shock protein (HSP). Of the proteins with high molecular mass, reactivity was observed only in one sample reacting with 90 kDa protein. Most of serum samples bound also in the low molecular range, but the value of this binding, except for lipopolysaccharide (LPS), is not known and clear.

IgA antibodies reacted most frequently with low molecular weight proteins, often found in LPS region and 50-54 kDa proteins (30 samples), followed by 30 kDa (3 samples), 39 kDa (4 samples), and 56-60 kDa proteins (5 samples). Two samples reacted also with 90-92 kDa proteins. Both IgG and IgA reacting protein bands were evenly distributed irrespective of the clinical status of the patients.

IgM antibodies reaction patterns (not included in Table 1) occurred particularly between 54-60 kDa proteins (7 samples), whereas 39 kDa (MOMP band) and high molecular 98 kDa proteins were recognized only in one the same sample.

Of 15 serum samples of healthy persons, in which the presence of IgG and/or IgA antibodies was detected in 4 by previous ELISA testing, we found with IgG discrete bands in region of 50-54 kDa protein and HSP region, respectively, but only in one sample. As to IgA, we found reactivity with 50-54 kDa protein again in one sample.

As follows from Figs. 1 and 2, presenting the IB results with selected sera, IgG and IgA recognition patterns were similar, but the bands were more intensive in case of IgG.

Discussion

Many studies analyzed the proteins and their reactivity in IB but the importance of individual

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proteins is not clear. When testing sera from *C. pneumoniae* ELISA positive patients by IB, significant antigenic differences among the strains of this agent were observed [18]. It is possible that distinct clinical disease syndrome such as respiratory tract infections or atherosclerosis are associated with different *C. pneumoniae* serovars [10].

In *C. pneumoniae*-infected patients, of the immunodominant proteins, a 39-kDa MOMP was genus specific and 53, 46, and 43 kDa proteins exhibited species specificity [9]. In another study, 160, 97 to 99, 60-62, 40, 27, and 15 kDa protein antigens were found by IB in patients with a current *C. pneumoniae* infection [6]. Two proteins with molecular masses of 43 and 53 kDa have been recognized frequently during human *C. pneumoniae* infection and the 53 kDa protein has potential for its serological diagnosis [4].

As to the patients with cardiovascular diseases, IgG serological responses were directed mostly to 40, 54, 60, 75, and 98 kDa proteins, so that cardiovascular risk from chlamydial infection could be linked to certain antigens [13]. On the other hand, the difference in prevalence of antibodies between the CHD patients and the healthy controls was significant when *Chlamydia* lipopolysaccharide antibodies were measured but not when antibodies to MOMP were tested [16]. In another study, as important antigens were found a 60 kDa HSP and outer membrane protein 2 [5].

In our study we found *C. pneumoniae* IgG antibodies directed mostly to 35 kDa (in 55.0%) and 50-54 kDa (in 62.5%) proteins (Table 1), followed by their reactivity with the MOMP (in 43.3%) and HSP (in 31.3%). IgA antibodies reacted the most frequently with 50-54 kDa protein (in 37.5%). From our results follows that apart from proteins of MOMP and HSP regions, the detection of Ig reactivity with 50-54 kDa proteins can be also of some importance in detecting *C. pneumoniae* infection. Whether it can be used for differentiation of possible participation of *C. pneumoniae* in CVD and other clinical manifestations caused by this agent remains to be elucidated by further studies.

Another problem is the significance of detection of anti-*C. pneumoniae* antibodies in CVD patients. In our recent study [12], the presence of anti-*C. pneumoniae* antibodies in 66.2% of 228 CVD patients corresponded with the presence of inflammatory markers, namely C-reactive protein (in 69.7%) and interleukin-6 (81.1%). Correlation of the chlamydial HSP antibodies and CRP with *C. pneumoniae* in atherosclerotic plaques was described also by Fong et al. [7].

Table 1. The reactivities of sera of CVD patients with C. pneumoniae antigens in IB.

Specific bands of proteins	IgG		IgA	
	No of positive	% positivity	No of positive	% positivity
35 kDa	44	55.0	3	3.7
39 kDa	35	43.7	4	5.0
48 kDa	10	12.5	4	5.0
50-54 kDa	50	62.5	30	37.5
56-60 kDa	25	31.3	5	6,2
90-92kDa	1	12	2	2.5

IB certainly contributes to serological diagnosis of chlamydial infections, but detecting antibodies alone without appropriate clinical symptoms is not sufficient for antibiotic treatment of infection with *C. pneumoniae* [17]. Moreover, the spectrum of possible immundominant protein antigens of *C. pneumoniae* is wide and further studies are necessary to determine their importance not only in chlamydial infections in all, but namely in patients with CVD. To determine the role of serological tests, IB including, in the possible relationship between *C. pneumoniae* infection and atherosclerosis, their strict description and evaluation is mandatory [16].

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