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Chronic Chlamydia pneumoniae *infection in patients with asthma and COPD (Chronic Obstructive Pulmonary Disease)*

Przewlekłe zakażenie *Chlamydia pneumoniae* u chorych na astmę i przewlekłą obturacyjną chorobę płuc (POChP)

INTRODUCTION

Chlamydia pneumoniae (*C. p.*) was originally discovered as a human pathogen in patients with pneumonia. Later this intracellular bacterium was associated with other acute respiratory diseases, such as pharyngitis, sinusitis and acute bronchitis. However, the major significance of *C. p.* as a human pathogen lies in its propensity to cause chronic infections. Its persistence and the resulting immune response of the host have been recognised as the major factors in the pathogenesis of chlamydial disease. Since the first observation of the association between *C. p.* and heart diseases in 1988, an increasing amount of evidence has indicated that *C. p.* is associated with many more diseases than originally expected. The following diseases have been associated with chronic or persistent *C. p.* infection: coronary heart disease, atherosclerosis, carotid artery disease, abdominal aortic aneurysm, sarcoidosis, reactive arthritis, asthma, COPD, lung cancer [5].

The aim of our study was to evaluate the frequency of persistent *C. p.* infection in asthma and COPD patients by measuring *C. p.*-specific serum IgG, IgM and IgA

levels. We were also trying to detect the presence of *C. p.* in upper respiratory tract of asthma patients using PCR method.

MATERIALS

1. Asthma

The group consisted of 41 patients treated in Ambulatory Chest Clinic by the Department of Pulmonary Medicine. The mean patients' age was 44.4 (range 23–67) years of age. Patients have been divided into 3 groups according to the severity of the disease:

1. mild asthma (11 patients),
2. moderate asthma (14 patients),
3. severe asthma (16 patients).

The control group comprised 35 age-matched volunteers (without cardiac and pulmonary diseases).

2. COPD

The group comprised 40 patients with exacerbated COPD, who have been hospitalised at the Department of Pulmonary Medicine and 24 patients with stable COPD, who have visited an Ambulatory Chest Clinic by the Department of Pulmonary Medicine. According to COPD severity patients have been divided into 3 groups:

1. severe COPD (44 patients),
2. moderate COPD (14 patients),
3. mild COPD (6 patients).

The mean age in patients' group was 62.9 (range 27–79) years of age. 39 healthy controls took part in the research. The control group was age, sex and smoker/non-smoker ratio matched to the patients' group.

METHODS

A. Serologic studies

Microimmunofluorescence (MIF) method was applied. *Chlamydia pneumoniae* Micro-IF test (Labsystems, Finland) was used according to the manufacturer's instructions. Sera analysed for *C. p.* IgA and IgM were diluted in IgG blocker (Labsystems, Finland) to remove possible interference with IgG.

According to the reference data, the following criteria have been adopted [3, 8]:

1. chronic infection: IgG $\geq$ 1:128, IgA $\geq$ 1:32, IgM = 0
2. acute infection: IgG $\geq$ 1:512, IgM $\geq$ 1:8, IgA = 0.

B. PCR

Specimens were taken from the retropharyngeal wall with plastic swabs (Unipath, Great Britain), which were frozen at –70°C until the preparation of DNA has been done. Specimen preparation was done using QIAamp Tissue Kit (Qiagen). The primers used for PCR have been HR-1 and HL-1 according to Campbell et al. [2]. Amplification products in agarose gel with added ethidium bromide were visualised after electrophoresis on an UV transilluminator.

C. Statistical analysis

Statistical analysis was carried out using SPSS for Windows. A p-value of 0.001 was taken as indicative of statistical significance.

RESULTS

1. Asthma

Serologic markers of chronic *C. p.* infection appeared to be present in 23 (56.1%) patients and in 4 (11.4%) healthy controls (Figure 1). The difference was statistically significant ($p < 0.001$). Taking in account asthma severity, chronic *C. p.* infection occurred more frequently in patients with moderate and severe asthma (71.4% and 56.3%, respectively) than in patients with mild asthma (36.4%) (Figure 2).

DNA of *C. p.* was detected by PCR in 7 asthma patients and in 1 healthy control. Among patients positive in PCR, 6 had serologic markers of chronic and 1 of primary acute infection. 1 healthy control positive in PCR had the serologic markers of acute infection.

2. COPD

According to serologic criteria, chronic *C. p.* infection occurred in 41 (64.1%) patients and in 8 (20.5%) healthy controls (Figure 3). The difference was statistically significant ($p < 0.001$). Serologic markers of chronic *C. p.* infection appeared to be more frequent in patients with severe COPD (68.2%) than in those with moderate (57.1%) and mild (50%) disease (Figure 4).

DISCUSSION

In the recent review current evidence linking *C. p.* infection to obstructive lung diseases (asthma and COPD) has been summarised. Of 18 controlled epidemiological studies (over 4000 cases/controls) 15 found significant associations between *C. p.*

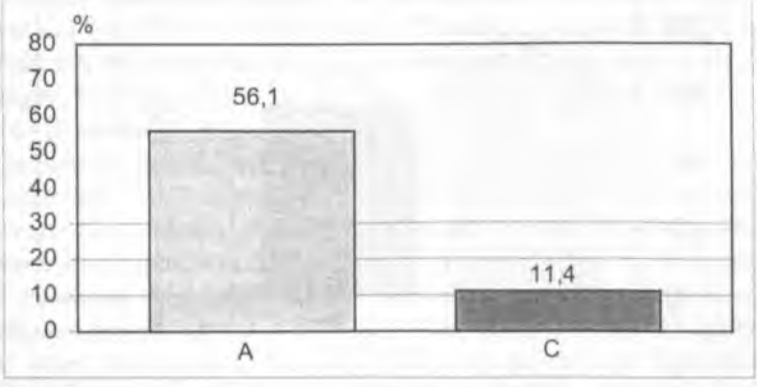

Figure 1. Chronic *C.p.* infection in patients with asthma (A) and healthy controls (C)

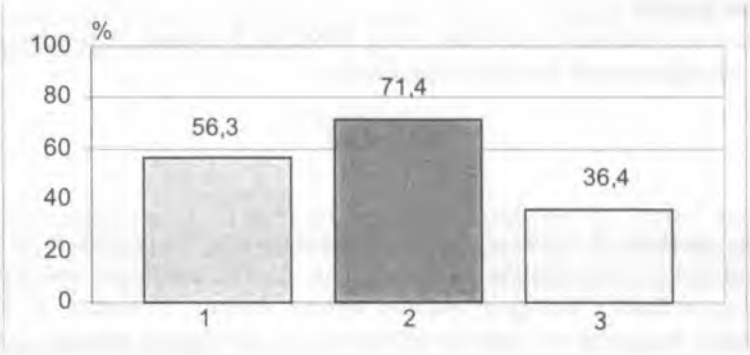

Figure 2. Chronic *C.p.* infection in severe (1), moderate (2), and mild (3) asthma

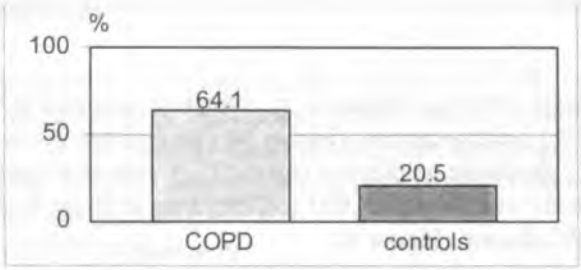

Figure 3. Chronic *C.p.* infection in COPD patients and in healthy controls

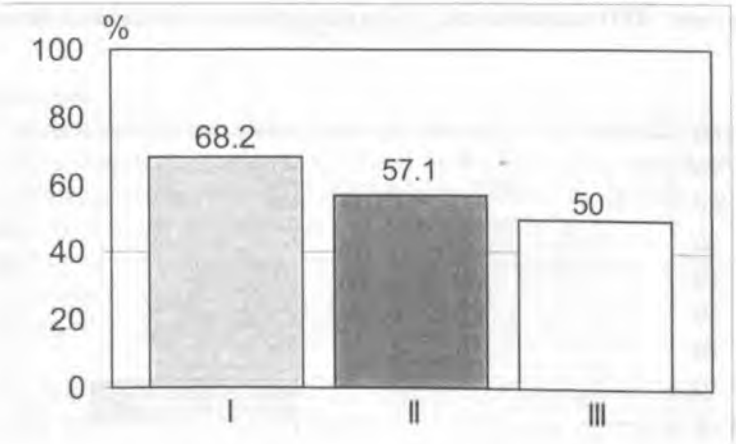

Figure 4. Chronic *C.p.* infection in severe (I), moderate (II), and mild (III) COPD

infection and asthma using organism detection (PCR or fluorescent antigen testing), *C. p.*-specific secretory IgA antibody testing, and/or specific serum IgE, IgA, IgG or other antibody criteria. 8 case reports and 13 case series of *C. p.* infection in asthma (over 100 cases) also include descriptions of improvement or complete disappearance of asthma symptoms after prolonged antibiotic therapy directed against *C. p.* Significant associations with COPD (over 1000 cases/controls) were reported in 5 of 6 studies. Results of treating chronic chlamydial infections in COPD patients have not yet been reported [4].

Our study confirms that chronic *C. p.* infection occurs more frequently in patients with asthma and COPD than in healthy controls. It is also more frequent in patients with severe and moderate asthma and COPD than in ones with mild types of these diseases. Our results concerning COPD patients are in accordance with the ones obtained by L. von Hertzen et al. [7]. In that study the overall prevalence of chronic *C. p.* infection in patients with severe COPD was 71% and in patients with milder disease — 46%. In our work the incidences in severe, moderate and mild COPD, according to serologic criteria, were 68.2%, 57.1% and 50.0%, respectively. To our knowledge we are the first to publish data concerning the frequency of chronic *C. p.* infection in relation to the degree of asthma — the prevalences in severe, moderate and mild asthma were 56.3%, 71.4% and 36.4%, respectively.

It is well known that isolation of chlamydia is difficult in chronic disease [13]. An antigen shedding in the chronic stages may be minimal; the organism can persist quiescently for long periods inside the host cells [14]. Since the cultures in chronic chlamydia infections frequently remain negative (there are suggestions that specific "cryptic", uncultivable form of chlamydia exists) [10], the polymerase chain reaction (PCR) technique might be a useful method for detecting chlamydial nucleic acids directly. However, there is no generally accepted "gold standard" criterion for detecting chronic infections by this method. A recent multicenter study revealed the urgent need of PCR methods standardization [1].

In our work serum MIF was in accordance with PCR results only in 30.4% of asthma patients. Discrepant results in the two methods were also obtained by L. von Hertzen et al. [7] in 37% of COPD patients. The authors suggest that PCR and serum MIF should not be compared because it is probable that they detect infection at different stages; PCR may detect cases at an earlier stage whereas in more advanced cases DNA detection by PCR is probably unsuccessful.

The persistence of elevated antibody titres is generally considered to be a sign of chronic infection [9, 13]. There are no means to distinguish between chronic and recurrent infections reliably, although the constant presence of short-lived IgA antibodies has been proposed to indicate chronic infection, whereas constantly elevated levels of more long-lived IgG antibodies may reflect recurrent infections in the past. IgM antibodies are considered to indicate primary acute infection [6]. Serology by the MIF test, when properly performed, is considered to be the most sensitive diagnostic method for *C. p.* to date, it still remains "the method of choice" [7, 11, 12].

CONCLUSIONS

1. Chronic *C. p.* infection occurs statistically more frequently in patients with asthma and COPD than in healthy controls, so it is possible that it participates in the pathogenesis of both diseases.

2. Chronic *C. p.* infection is more frequent in patients with severe and moderate asthma and COPD than in ones with mild types of these diseases, so it is possible that it amplifies inflammation in patients' respiratory tract.

3. The results suggest a need to diagnose chronic *C. p.* infection in asthma and COPD patients and, if confirmed, to take an attempt of antimicrobial therapy.

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STRESZCZENIE

Celem naszej pracy była ocena częstości występowania przewlekłego zakażenia *Chlamydia pneumoniae* (*C.p.*) u chorych na astmę i POChP poprzez ocenę swoistych przeciwciał klasy IgA, IgG i IgM w surowicy metodą MIF oraz próba detekcji DNA *C.p.* w wymazach z gardła u chorych na astmę metodą PCR. Na podstawie kryteriów serologicznych przewlekłą infekcję *C.p.* stwierdzono u 56,1% chorych na astmę i u 64,1% chorych na POChP. Występowanie przewlekłego zakażenia *C.p.* było statystycznie częstsze u chorych niż w grupie kontrolnej ($p < 0,001$). Przewlekła infekcja *C.p.* obecna była częściej u chorych z ciężką i umiarkowaną postacią astmy i POChP niż u pacjentów z lekkimi postaciami tych chorób. U 7 chorych na astmę i u jednej osoby z grupy kontrolnej wykryto DNA *C.p.* w wymazach z gardła.