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Is the Presence of *Chlamydophila pneumoniae*-Specific Antibodies and Human IL-10 Linked with Obstructive Pulmonary Disease?

Czy istnieje związek między obecnością swoistych przeciwciał anty-*Chlamydophila pneumoniae* i ludzkiej interleukiny 10 a obturacyjnymi chorobami płuc?

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Abstract

Background. *Chlamydophila pneumoniae* infections seem to be associated with many non-infectious diseases.
Objectives. To determine whether *Chlamydophila pneumoniae* antibodies are connected with chronic airway infections and obstructive pulmonary disease, and to establish whether there is any correlation between the presence of IL-10 and bronchial asthma in patients infected with *Chlamydophila pneumoniae*.
Material and Methods. The study involved two groups of subjects. The first consisted of 97 adults: 46 with bronchial asthma and bronchial asthma exacerbation, 19 with chronic obstructive pulmonary disease (COPD) and a control group of 32 adults without any signs of respiratory problems. The second group consisted of 92 children: 15 with bronchial asthma, 26 with pneumonia, 17 with recurrent respiratory tract infections and a control group of 34 children without any respiratory symptoms. The materials for the study were blood samples to evaluate the concentrations of specific IgM, IgG and IgA antibodies and IL-10. Serological studies were performed using the enzyme linked immunosorbent assay (ELISA).
Results. In adults with bronchial asthma, *C. pneumoniae*-specific IgM antibodies occurred more frequently than in patients with COPD. In patients with bronchial asthma, IL-10 was detected in serum in 44.3%. IL-10 was not detected in either the adult or the juvenile control group.
Conclusions. A high percentage of positive results for specific antibodies may suggest that *Chlamydophila pneumoniae* plays a role in obstructive pulmonary disease exacerbations (**Adv Clin Exp Med 2010, 19, 5, 579–584**).

Key words: interleukin 10, *C. pneumoniae* antibodies, bronchial asthma, COPD.

Streszczenie

Wprowadzenie. Obecnie zakażenia wywołane przez *C. pneumoniae* wiążą się coraz częściej z wieloma chorobami o etiologii niezakaźnej.
Cel pracy. Ustalenie związku między obecnością przeciwciał anty-*C. pneumoniae* a przewlekłymi zakażeniami dróg oddechowych i obturacyjnymi chorobami płuc oraz ustalenie, czy istnieje jakakolwiek zależność między obecnością IL-10 a astmą oskrzelową u pacjentów zakażonych chlamydiami.
Materiały i metody. Grupę badaną stanowiło 97 osób dorosłych, w tym 46 pacjentów z rozpoznaniem astmy oskrzelowej lub jej zaostrzeniami, 19 pacjentów z przewlekłą obturacyjną chorobą płuc (p.o.ch.p.) oraz 32 osoby zdrowe stanowiące grupę kontrolną. Grupa II obejmowała 92 dzieci, w tym 15 dzieci z astmą oskrzelową, 26 z zapaleniem płuc i 17 z nawracającymi zakażeniami dróg oddechowych oraz 34 dzieci stanowiących grupę kontrolną. Materiałem do badań były próbki krwi, które pobierano w celu oznaczenia stężenia swoistych przeciwciał klasy IgG, IgM, IgA oraz stężenia IL-10. Badania serologiczne w kierunku *Chlamydophila pneumoniae* przeprowadzono metodą immunoenzymatyczną ELISA.
Wyniki. U dorosłych chorych na astmę oskrzelową stwierdzono znacznie częstsze występowanie przeciwciał klasy IgM anty-*C. pneumoniae* w porównaniu z chorymi na p.o.ch.p. W grupie pacjentów chorych na astmę oskrzelową obecność IL-10 w surowicy stwierdzono u 44,3% badanych. W grupie kontrolnej zarówno u dorosłych, jak i dzieci IL-10 nie stwierdzono.
Wnioski. Duży odsetek wyników dodatnich w kierunku swoistych przeciwciał anty-*C. pneumoniae* może wskazywać na udział tego drobnoustroju w zaostrzaniu objawów u chorych z obturacyjnymi chorobami płuc (**Adv Clin Exp Med 2010, 19, 5, 579–584**).

Słowa kluczowe: interleukina 10, przeciwciała anty-*C. pneumoniae*, astma oskrzelowa, p.o.ch.p.

Chlamydiae are microorganisms that have features of both bacteria and viruses. *Chlamydophila pneumoniae* causes infections only in humans; animal reservoirs of this microorganism have not been found. Among humans, it is transmitted through the air. *Chlamydophila pneumoniae* is a cause of various clinical forms of infections, including asymptomatic infections; various mild upper respiratory tract infections such as pharyngitis, laryngitis and sinusitis; and lower respiratory tract inflammations such as bronchitis and pneumonia [1–3]. Infections develop most frequently in children from 5 to 14 years of age. It has been estimated that about 10% of *Chlamydophila pneumoniae* infections develop into pneumonia. The average incubation period is about one to two weeks. Usually general symptoms and upper respiratory tract symptoms appear first: fatigue, fever, muscle pain and sore throat. Pharyngitis may be diagnosed in an objective examination. In most cases, no other symptoms develop, but in some cases the infection proceeds in two phases: It starts with upper respiratory tract infections such as pharyngitis or laryngitis, and then pneumonia symptoms develop, with a dry cough and occasionally dyspnoea. Pneumonia can also develop without the initial upper respiratory tract symptoms. In chlamydial pneumonia, a radiological examination reveals the presence of irregular or segmentary maculate inflammatory infiltration or interstitial density. These microorganisms are located mainly in the middle and inferior lobes of the lungs. Severe cases of pneumonia with respiratory failure have been observed [1, 4].

C. pneumoniae infections also seem to be linked with various non-infectious diseases. Many studies indicate that this microorganism may play a role in asthma, chronic obstructive pulmonary disease (COPD), lung cancer, coronary heart disease, atheromatosis, arthritis, Alzheimer's disease, multiple sclerosis, sarcoidosis and erythema nodosum however in any of these diseases the role of these microorganisms has not been proven [5–7].

In adults, *C. pneumoniae* antibodies are detected about five to ten times more frequently than *C. trachomatis* antibodies. *C. pneumoniae* IgM antibodies appear about three weeks after the appearance of clinical symptoms, and are present in the infected organism from two to three months. *C. pneumoniae* IgG antibodies appear six to eight weeks after the initial infection and persist for many years. In cases of reinfection, an increase in the concentration of IgG antibodies is observed in the second week of disease.

Serological methods have limited application in diagnosing acute chlamydial infections. Antibodies remain present in the organism for a few years, and their presence does not indicate an active infection. The results of a single blood sample taken in the acute phase of the disease do not suffice to confirm or exclude acute infection. In some tests false positive results are caused by the presence of other microorganisms' antigens in the research material. Nonspecific reactions with *Staphylococcus aureus, Streptococcus* groups A, C and G, *Acinetobacter, Enterobacter cloaceae* and *Escherichia coli* have been observed. It has been found that rheumatoid factor circulating in the blood can influence the results of IgM determination using microimmunofluorescence methods [8, 9].

The aim of this study was to determine whether *Chlamydophila pneumoniae* antibodies are connected with chronic airway infections and obstructive pulmonary disease, and to establish whether there is any correlation between the presence of IL-10 and bronchial asthma in patients infected with *Chlamydophila pneumoniae*.

Material and Methods

The study involved two groups of subjects, both made up of patients hospitalized in three of Wroclaw Medical University's research hospitals. The first group consisted of 97 adults aged from 18 to 88: 46 patients with bronchial asthma and asthma exacerbation, 19 patients with COPD and a control group of 32 adults with no respiratory problems. The second group consisted of 92 children aged from 2 to 18: 15 with bronchial asthma, 26 with pneumonia, 17 with recurrent respiratory tract infections and a control group of 34 children with no respiratory signs. The materials for the study were blood samples, which were taken to evaluate the concentration of *C. pneumoniae*-specific IgG, IgM and IgA antibodies and human IL-10. The 5-ml blood samples were centrifuged and the serum was stored at 4°C until the determination was performed. Serological testing for *C. pneumoniae* IgG, IgM and IgA was performed using ELISA kits (Vircell S.L., Spain) [10]. To determine the concentration of human IL-10, Hu IL-10 kits were used (Biosource Europe S.A., Belgium) [11].

The concentration of antibodies was calculated in comparison with the absorbance value of samples with the absorbance value cut off:

$$\text{Antibody index} = (\text{sample O.D.}/\text{cut off serum mean O.D.}) \times 10$$

Samples with indexes below 9 were regarded as lacking *C. pneumoniae*-specific antibodies, while samples with indexes above 11 were considered

to have specific antibodies against *C. pneumoniae*. Samples with equivocal results were retested and/ /or a new sample was obtained for confirmation.

The results of the tests for human IL-10 were read with a spectrophotometer. On the basis of absorbance values of samples and absorbance values of each standard dilution the standard curve was drawn and concentrations of all samples were read.

Results

The results of the tests for *C. pneumoniae*-specific antibodies and human IL-10 are presented in Tables 1 and 2. IgM antibodies occurred more frequently in adults with bronchial asthma than in patients with COPD. A high percentage of IgG positive results (62.5%) in the adult control group may suggest previous contact with the *C. pneumoniae* antigen – a past infection and/or the very common carrier state. IL-10 was found in 44.3% of the patients with bronchial asthma; the concentration of this cytokine was, however, very low. Stabilization of usefulness of serum IL-10 concentration determination in patients with bronchial asthma infected with *C. pneumoniae* requires further investigation. IL-10 was not found in either the adult or the juvenile control group.

Discussion

Infections caused by *Chlamydophila pneumoniae* are very common around the world: About 50% of the population has antibodies against *C. pneumoniae,* from an early age. The only source of infection is another human [12]. *C. pneumoniae* often causes asymptomatic infections with no characteristic clinical picture, and in general they are mild and self-limiting. About 70–90% of *C. pneumoniae* infections are subclinical [13]. *C. pneumoniae* is connected with both upper and lower respiratory tract symptoms. It is often a cause of asthma and COPD exacerbations, and may cause 6–9% of post-hospital pneumonia. It seems that one person in 1000 suffers from chlamydial pneumonia [13]. In children, the most common lower airway infection is bronchitis, and in adults – pneumonia. It is estimated that about 10% of pneumonia and 5% of bronchitis in outpatients or hospital patients are caused by *C. pneumoniae*, which makes it the leading microorganism causing lower airway infections (ahead of *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Mycoplasma pneumoniae).*

Many authors examining infections caused by *C. pneumoniae* have noted the role of this microorganism in the pathogenesis of asthma and asthma and COPD exacerbations. Many efforts have been

Table 1. The frequency of *Chlamydophila pneumoniae*-specific antibodies and IL-10 occurence in adults with obstructive pulmonary disease

Tabela 1. Częstość występowania swoistych przeciwciał anty-*Chlamydophila pneumoniae* oraz IL-10 u dorosłych z obturacyjnymi chorobami płuc

Diagnosis (Rozpoznanie)	IgM N (%)	IgG N (%)	IgA N (%)	IL-10 N (%)
Bronchial asthma (n = 46)	11 (23.9)	26 (56.5)	20 (43.5)	24 (52.2)
COPD (n = 19)	1 (5.3)	16 (84.2)	16 (84.2)	2 (10.5)
Control group (n = 34)	2 (6.2)	20 (62.5)	12 (37.5)	0

COPD – chronic obstructive pulmonary disease.

Table 2. The frequency of *Chlamydophila pneumoniae*-specific antibodies and IL-10 occurence in children with bronchial asthma, pneumonia and recurrent respiratory tract infections

Tabela 2. Częstość występowania swoistych przeciwciał anty-*Chlamydophila pneumoniae* oraz IL-10 u dzieci z astmą oskrzelową, zapaleniem płuc i nawracającymi zakażeniami dróg oddechowych

Diagnosis (Rozpoznanie)	IgM N (%)	IgG N (%)	IgA N (%)	IL-10 N (%)
Bronchial asthma (n = 15)	0	7 (46.7)	1 (6.7)	3 (20.0)
Pneumonia (n = 26)	0	12 (46.2)	1 (3.8)	6 (23.1)
RRTI (n = 17)	1 (5.9)	4 (23.5)	0	8 (47.1)
Control group (n = 32)	0	7 (20.6)	2 (5.9)	0

RRTI – recurrent respiratory tract infection.

made to identify the best diagnostic methods for *C. pneumoniae*. Serological methods based on the detection of specific antibodies have been found useful. Agarwall et al. examined the connection between *Chlamydophila pneumoniae* and bronchial asthma in adults. They used ELISA to investigate the presence of *C. pneumoniae* IgG, IgA and IgM antibodies in serum samples from 60 adults with bronchial asthma and from a control group of 100 healthy people. The frequency of *C. pneumoniae*-specific IgG antibody was found to be significantly higher in the bronchial asthma patients than in the control group – 61.6% and 38.0% respectively – whereas no significant difference was observed for IgA and IgM chlamydial antibodies. The authors suggested that the more frequent occurrence of IgG antibodies in patients with bronchial asthma indicated chronic infections and a connection between bronchial asthma and chronic chlamydial infections [14].

Niedźwiadek et al. carried out studies on 41 patients with bronchial asthma, aged 23 to 67. The control group consisted of 35 patients with no respiratory symptoms. An indirect microimmunofluorescence test was used to index specific antibodies in sera. Serological evidence of chronic *C. pneumoniae* infections was found in 56.1% of the patients with bronchial asthma and in 11.4% of the control group, suggesting that *C. pneumoniae* may play a role in asthma pathogenesis [15].

In our own studies, *C. pneumonia*-specific IgG antibodies were found in 56.5% of the 46 adults with bronchial asthma, and IgA antibodies in 43.5%. In the control group, specific IgG antibodies were found in 62.5% and IgA antibodies in 37.5%. Specific IgM antibodies were found in 23.9% of the patients with bronchial asthma, which is a low rate in comparison with other classes of *C. pneumoniae* antibodies.

A link between chronic *C. pneumoniae* infection and asthma exacerbation in children was found by Cunningham et al., who investigated 108 children (aged 9 to 11) with asthma symptoms. Observations were carried out for 13 months. The ELISA method was used to determine the level of *C. pneumoniae*-specific IgA in sera; it was times higher in patients who reported four or more exacerbations than in those who reported just one. The authors suggested that „the immune response to chronic *C. pneumoniae* infection may interact with allergic inflammation to increase asthma symptoms" [16].

Jiang et al. studied 120 asthmatic children and 82 healthy ones. Serum levels of *C. pneumoniae*-specific IgM and IgG antibodies were detected by ELISA. *C. pneumoniae*-specific IgM was demonstrated in 18.3% of the asthmatic patients and *C. pneumoniae* IgG in 26.7%. The incidence of *C. pneumoniae* infection in asthmatic children was significantly higher than in the healthy control group (3.9%) [17].

In our own study, *C. pneumoniae*-specific IgM antibodies were not found in asthmatic children, and there were significantly higher percentages of IgG-positive results both in asthmatic children (46.7%) and in the control group (20.6%).

Wlazlowski et al. evaluated the frequency of *C. pneumoniae* occurrence in children with bronchial asthma exacerbations. The study group consisted of 29 children (13 girls and 16 boys) aged from nine months to 16 years old. Serological studies were performed by the ELISA method. IgG antibodies were found in 10.3% of the children, and IgM in 17.2%. The authors observed that infections caused by atypical bacteria such as *C. pneumoniae* often accompany bronchial asthma exacerbations, and should be considered when treating children with this condition [18].

Communications from congresses and our own observations suggest that *C. pneumoniae* infections were chronic or recurrent.

Wazir et al. carried out studies using ELISA on 54 children with asthma, 10 with asthma exacerbations and a control group of 34 healthy children. IgG antibodies were found in 40.6% of the patients with asthma and in 11.4% of the control group. IgM antibodies were evaluated only in patients with asthma exacerbations in which positive results weren't detected. The research indicated more frequent occurrences of chronic *C. pneumoniae* infections in patients with asthma than in the control group [19].

In our own study, the serum of 15 children with bronchial asthma aged from two to 18 was examined. IgG antibodies were detected in 46.7% of the subjects and IgA antibodies in 6.7%; antibodies of the IgM class were not found in children with bronchial asthma. These findings demonstrate the occurrence of the chronic *C. pneumoniae* infection in this group of children. In the control group of children with no respiratory ailments, antibodies of the IgG isotype were detected at a much lower frequency (20.6%) than in the subjects with bronchial asthma; IgA antibodies were found in 5.9% of the subjects. An analysis of our own findings confirms the previous observations of other authors concerning a correlation between an elevated concentration of the antibodies of the IgA isotype and the development of bronchial asthma [17, 19, 20].

The vast majority of researchers have used the immunoenzymatic ELISA method to determine the concentration of the specific antibodies in serum; only a few have used the microimmunofluorescence test (MIF) [21, 22].

Using the MIF method, Szczepanik et al. studied the occurrence of antibodies of the IgG and IgA isotypes in a group of 30 children with bronchial asthma (aged three to 14) and in a group of 10 children with pneumonia (aged five to 11). Antibodies of the IgG isotype specific for *C. pneumoniae* were detected in 10% of the children with asthma and 10% of the children with pneumonia. Antibodies of the IgA isotype were detected in 3.3% of the children with asthma; in the children with pneumonia antibodies of this isotype were not found at all [22].

In our own research, using the ELISA method, 46.2% of the specific IgG antibodies and 3.8% of IgA antibodies were found in children with pneumonia; antibodies of the IgM isotype were not detected in this group of subjects. These are significantly higher percentages of IgG antibodies than Szczepanik et al. encountered in children with pneumonia [22].

Podsiadły et al. used the ELISA method to determine the levels of *C. pneumoniae*-specific IgM, IgG and IgA antibodies of in a group of 123 children with the infection of the respiratory system aged from one week to three years. In children over 12 months old, IgM antibodies were found in 13% of the subjects and IgG antibodies in 8.9%; IgA antibodies were not found. Moreover, IgG antibodies were not encountered in subjects in whom IgM antibodies were found. In the control group of healthy children, antibodies of the IgM isotypes were detected in 7.4% of the children, and neither IgA nor IgG anitbodies were found. *C. pneumoniae*-specific antibodies were detected more frequently in children from two to three years old (46.7%) [23].

In our own study, in a group of children with recurrent infections of the respiratory system, IgM antibodies were found in one child and IgG antibodies in four; IgA antibodies were not encountered. In terms of age, the one subject in whom IgM antibodies were detected was from the group aged from two to five; IgG antibodies were found in two subjects from the group aged from two to five, in one from the 10–13 age group and in one from the 14–18 age group. Antibodies of IgG isotype were not found in children aged from six to nine.

Machura et al. examined a group of 30 children with recurrent obstructive bronchitis, aged from seven to 36 months (10 girls, 20 boys). The control group was comprised of healthy children aged from five to 36 months. IL-10 concentration in the serum was determined by the ELISA method. IL-10 concentration was significantly higher in children with recurrent obstructive bronchitis (average concentration: 55.87 pg/ml) than in healthy children (average concentration: 4.05 pg/ml); moreover, it correlated with the number of recurrent obstructive bronchitis episodes suffered beforehand [24].

In our own study, the serum of 58 children was examined using the ELISA method, and IL-10 was discovered in 29.3% (average concentration: 0.75 pg/ml). In the control group of healthy children, IL-10 was not detected.

The data found in the literature point to a relationship between *C. pneumoniae* infection and chronic obstructive pulmonary disease (COPD). A factor encouraging frequent chlamydial infections in patients with COPD is tobacco smoke, which facilitates the microbes' penetration of the pulmonary tissue.

Seoung et al. researched the role of *C. pneumoniae* in 36 patients with asthma and 59 with COPD, and in a control group of 45 healthy subjects. Levels of IgG and IgM antibodies were determined by the MIF method. The number of patients with bronchial asthma that tested positive for these antibodies was higher (8.3%) than in the control group (2.2%). Among the patients with COPD, the percentage testing positive for *C. pneumoniae*-specific antibodies (3.4%) was slightly higher than in the control group (2.2%) [21].

Branden et al. carried out research in 199 patients with COPD. The MIF method was used to detect IgG and IgA antibodies in blood serum. Chronic *C. pneumoniae* infection – i.e., infection in which the level of IgA antibodies is ≥ 1/64 – was detected in 85 subjects (42.7%). Chronic *C. pneumoniae* infection was connected with smoking cigarettes and age, but there was no relation with the sex of the subjects. These results indicate that chronic *C. pneumoniae* infection may be an independent factor of the development of COPD [25].

Siritantikorn et al. examined 127 elderly patients with COPD and 131 subjects in a control group. Using the immunoenzymatic (EIA) method, antibodies of the IgG isotype were found in 85.8% of the patients with COPD and in 66.4% of the patients from the control group; antibodies of the IgA isotype were detected in 85.0% and 51.1% of the two groups respectively; antibodies of IgM isotype were found in 3.9% of the subjects with COPD, but were not detected in the control group. This research indicates that both acute and chronic infections of *C. pneumoniae* play an important role in COPD. Due to the high percentage of the positive results, the authors suggested that antibiotic therapy should be considered when treating patients with COPD [26].

In our own study with COPD patients, *C. pneumoniae*-specific antibodies were detected using the ELISA method. IgM antibodies were detected in 5.3% of the subjects with COPD and in 6.2% of the individuals in the control group; IgG and IgA an-

tibodies were found with equal frequency in both groups: 16 persons, or 84.2%. When treating COPD exacerbations we recommend using (among other therapies) macrolides, which are not only effective in treating *C. pneumoniae* infections, but also have unique anti-inflammatory properties.

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