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# Epidemiology of infections caused by *Chlamydophila pneumoniae* in patients with chronic cough

## Epidemiologia zakażeń wywołanych przez *Chlamydophila pneumoniae* u pacjentów z przewlekłym kaszlem

**Authors' Contribution:**
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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## Summary

**Background:** *Chlamydophila pneumoniae* is an important etiological agent in respiratory system infections. The aim of study was to analyze the rate of *Chlamydophila pneumoniae* infection in adults and children and also to determine a correlation between the presence of this pathogen and symptoms of chronic cough.

**Material/Methods:** The material for the study included swabs from the posterior pharyngeal wall taken on an empty stomach without cleaning the mouth. The diagnostic method was indirect immunofluorescence test (IIFT), which uses two types of antibodies: monoclonal mouse antibodies, which link specifically with the antigen that is present in the tested material and goat anti-mouse antibodies linked to fluorescein isothiocyanate, providing the colour reaction with *C. pneumoniae* antigen.

**Results:** In our research, 593 patients, including 319 women, 175 men, aged from 18 to 87 years and a group of 99 children aged from 2 to 17 years with symptoms of chronic cough n=432 and other respiratory manifestations n=161 were studied. In the group of studied women with cough, 28.2% (64/227) of results were positive. In the group of men with cough, 22.3% (27/121) of results were positive. In the group of children with a cough, 28.6% (24/84) of the results were positive.

**Conclusions:** In the examined group of children and adults with a chronic cough, the *C. pneumoniae* antigen was detected. The frequency of detection of *C. pneumoniae* antigen differed depending on the age group of both children and adults with symptoms of chronic cough.

**Keywords:** **pharyngeal swab • diagnostics • immunofluorescence**

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## INTRODUCTION

*Chlamydophila pneumoniae* is an important etiological agent in respiratory system infections. It is the cause of approx. 10% of pneumonia cases and approx. 5% of bronchitis and sinusitis cases. Infection is transmitted through the droplet route, the course of infection is usually mild; however, it can sometimes have a severe course and lead to respiratory failure. The incubation period of the disease is 3 to 4 weeks. Initially, a sore throat and hoarseness occur, which is rarely observed in respiratory system inflammation of a different etiology. Then, a dry cough occurs which continues for several weeks. The body temperature is normal or slightly increased. More severe course of the infection is observed in infants and children from the younger age groups, which often have dyspnoea and upper airway bronchial tree obstruction. In laboratory studies, the deviations from the normal state are rarely found and the parameters of inflammation are usually normal or slightly increased. The radiographic image, in the course of pneumonia of the *C. pneumoniae* etiology does not show any specific changes; interstitial inflammatory changes are most common [2, 7]. Laboratory diagnostics of *Chlamydophila* infections is based on breeding method, molecular biology techniques, serological surveys and indirect immunofluorescence methods which enable the detection of the bacterial antigen.

The standard of diagnostic procedure for infections caused by *C. pneumoniae* has not been developed so far. This constitutes a major obstacle, especially since the diagnosis must take into account the three possible states of infection: acute, chronic and past acute infection [4]. The lack of diagnostic standard hinders also a comparative assessment of the results of various studies. Materials for the micro-organism identification are throat or larynx swabs, swabs from the epiglottis taken with the use of laryngoscope, bronchial lavage or bronchoalveolar lavage fluid (BALF). When using the serological testing we should be aware of the persistent positive IgG antibody titre in a significant percentage of the healthy population. Therefore, the single test can show a new infection only when the titre is very high (>1:512). The demonstration of a 4-fold increase in antibody titre in two consecutive detections is more important. It is necessary to have knowledge of the course of immunological phenomena in order to interpret the serological test result appropriately. The antibodies are found in many people without prior symptoms of disease (asymptomatic infection). Infection leaves no lasting immunity. Cases of asymptomatic carrying in nasopharynx after the infection have been reported [6]. In the treatment of *Chlamydophila pneumoniae* infections, three groups of antibiotics are used: macrolides, tetracyclines and some fluoroquinolones. The lack of peptidoglycan in the cell wall causes the resistance of *C. pneumoniae* to beta-lactam antibiotics. The second most important factor determining the effectiveness of treatment is the intracellular location of the microorganisms. Macrolides, tetracyclines and fluoroquinolones are characterized by good penetration to the interior of cells. Due to the severe course of some infections and consequences of ineffective eradication of microorganisms as well as the risk of developing a chronic form of infection, it is necessary to treat the *C. pneumoniae* infections effectively. Most of the recommendations indicate the macrolides as the first-line of drugs for the treatment of infections caused by *C. pneumoniae*. Considering the fairly long recommended time of treatment (14-21 days), new generations of macrolides exhibit an advantage over erythromycin, which results from their better tolerance. Clarithromycin seems to be the most active from among the macrolides. A too short period of treatment can lead to the recurrence of symptoms or prolonged symptoms, which sometimes are characterized by a cough lasting several months [11].

## MATERIAL AND METHOD

In 2016 from January to December the studies involved 593 adults including 319 women, 175 men and 99 children with symptoms of chronic cough n=432 and other respiratory manifestations n=161. The subjects consisted of two age groups. The age of women and men examined during the study was 18-87 years and the average age in the group of adults was 49.7 ± 16.6. The age of children was 2-17 years and the average age of this group was 7.4 ± 4.3. In the study group, 146 adults (92 women and 54 men) had cough symptoms. In the group of children 84 had a cough. Other patients without a cough exhibited other symptoms, i.e. sore throat, burning, dry throat and redness, hoarseness and dyspnoea. The material for the study included swabs from the posterior pharyngeal wall taken on an empty stomach without cleaning the mouth. After collecting, the swab was smeared onto a slide, fixed in acetone for 5 minutes and allowed to air-dried. This material can be stored for 48 hours in a refrigerator. The diagnostic method used was indirect immunofluorescence test (IIFT) (Chlamydia Cell PN testing, CellabsPty Ltd., Australia), which uses two types of antibodies: monoclonal mouse antibodies, which link specifically with the antigen that is present in the tested material and goat anti-mouse antibodies linked to fluorescein isothiocyanate, providing the color reaction with *C. pneumoniae* antigen. The specimens were examined with the use of a fluorescent microscope. In the case of 4 or more visible elementary bodies of a similar shape, size and fluorescence in the examined preparation, as in the control specimens, the result was evaluated as positive.

Studies were carried out according to the guiding principles of the Declaration of Helsinki.

The research was carried out from statutory funds of Wroclaw Medical University No. ST-966.

The aim of the study was to analyze the rate of *C. pneumoniae* infection in adults and children and also to determine a correlation between the presence of this pathogen and symptoms of chronic cough.

## RESULTS

In our research, 593 patients, including 319 women, 175 men, aged from 18 to 87 years and a group of 99 children aged from 2 to 17 years were studied.

In the group of studied women with cough, 28.2% (64/227) of the results were positive. Women aged 18-37 constituted the group in which *C.pneumoniae* was found most often, i.e. 32.0%. In the group of men with cough, 22.3% (27/121) of the results were positive. Men aged 38-57 constituted the group in which the infection with this atypical pathogen was found most often, i.e. 29.8 %.

The distribution of the results in different age groups in patients with cough is shown in Table 1.

In the group of women without cough, 23.9% (22/92) of the results were positive. In the group of men without cough, 37.0% (20/54) of the results were positive. The distribution of the results in different age groups in patients without cough is shown in Table 2.

Children aged 2-17 years constituted a separate group of the subjects. In the group of children with cough, 28.6 % (24/84) of the results were positive. In the group of children without cough, 33.3% (5/15) of the results were positive.

The distribution of the results in different age groups and the correlation between the positive results from the throat swab and the occurrence of cough in children is shown in Table 3.

The most frequent symptom in adults was a dry cough, which was found in 70.4% (348/494) of the subjects. Other patients exhibited another symptoms, i.e. sore throat, burning, dry throat and redness, hoarseness and

**Table 1.** The distribution of the results of immunofluorescence method in different age groups in adults with cough IF – immunofluorescence

| Age | Number of women in the age group | Positive result IF (%) | Number of men in the age group | Positive result IF (%) |
|---|---|---|---|---|
| 18-37 | 50 | 16 (32.0) | 30 | 6 (20.0) |
| 38-57 | 69 | 20 (29.0) | 47 | 14 (29.8) |
| 58-77 | 93 | 24 (25.8) | 38 | 6 (15.8) |
| 78-87 | 15 | 4 (26.7) | 6 | 1 (16.7) |
| Total | 227 | 64 (28.2) | 121 | 27 (22.3) |

**Table 2.** The distribution of the results of immunofluorescence method in different age groups in adults with respiratory infections without cough

| Age | Number of women in the age group | Positive result IF (%) | Number of men in the age group | Positive result IF (%) |
|---|---|---|---|---|
| 18-37 | 33 | 7 (21.2) | 24 | 7 (29.2) |
| 38-57 | 27 | 6 (22.2) | 17 | 9 (52.9) |
| 58-77 | 29 | 8 (27.6) | 13 | 4 (30.8) |
| 78-87 | 3 | 1 (26.7) | 0 | 0 |
| Total | 92 | 64 (28.2) | 54 | 27 (22.3) |

**Table 3**. The distribution of the results of immunofluorescence method in different age groups in all children

| Age | Total number of children | Number of children with cough | Positive results IF (%) | | Pearson' correlation coefficient r |
|---|---|---|---|---|---|
| | | | With cough | Without cough | |
| 2-9 | 65 | 56 | 14 (25.0) | 4 (44.4) | 0,9 |
| 10-17 | 34 | 28 | 10 (35.7) | 1 (16.7) | 0,9 |
| Total | 99 | 84 | 24 (28.6) | 5 (33.3) | 0,9 |

**Table 4.** The correlation between the positive results of immunofluorescence method and the occurrence of cough in adults

| Age | Positive results in women | Positive results in women with cough (Pearson's correlation coefficient r) | Positive results in men | Positive results in men with cough (Pearson's correlation coefficient r) |
|---|---|---|---|---|
| 18-37 | 23 | 16 (0.7) | 13 | 6 (0.6) |
| 38-57 | 26 | 20 (0.8) | 23 | 14 (0.7) |
| 58-77 | 32 | 24 (0.8) | 10 | 6 (0,7) |
| 78-87 | 5 | 4 (0.9) | 1 | 1 (1) |
| Total | 86 | 64 (0.8) | 47 | 27 (0.7) |

dyspnoea. The correlation between the positive result from the throat swab and the occurrence of cough in adults is shown in Table 4. The highest Pearson' correlation coefficient occurred in the group of women aged 78-87 years, r = 0.9. Statistica 13.1 program was used for statistical calculations.

## DISCUSSION

The efficient functioning of the immune system of the host has a decisive influence on the image and development of the clinical form of infections caused by *C. pneumoniae* in the respiratory system, starting from inflammation of the throat, larynx and ear, ending with the acute cases of pneumonia. Apart from the diversity of infections, also a wide spectrum of symptoms of these infections is observed, which most frequently includes the chronic cough [9]. A cough is a symptom that occurs in the infection of respiratory system, both in the course of viral and bacterial infections [12]. In Lima (Peru), in the period from May 2009 to September 2010, the screening of the group of 675 children hospitalized due to acute respiratory system infections was carried out. Diagnostics of these infections included detection with the use of PCR method, from the material obtained from patients by means of nasopharynx swabs, of two species of atypical bacteria: *Mycoplasma pneumoniae* and *C. pneumoniae* with the frequency of isolation of, respectively, 25.19% and 10.52%. As the authors point out, a significant limitation of these studies is the fact that at the same time other etiological agents, both bacterial and viral, which are responsible for respiratory system infections, the spectrum of symptoms coinciding with that presented by atypical bacteria, were not detected [4]. The *C. pneumoniae* is transmitted through the air in droplets and aerosols which facilitates the spread of the micro-organism in such gatherings as kindergartens, schools and military units. The latest reports also confirm that *C. pneumoniae* causes incidences of an epidemic nature. In the period from August 2013 to May 2014 in the laboratory of the American Air Force Academy, diagnostic tests were conducted in a group of aviation cadets with symptoms of acute respiratory system infections and 102 cases of pneumonia were found in this group of patients. Nasal swabs were used for the study, which were then analyzed genetically with the use of RT-PCR test for influenza viruses, and negative samples were again tested using the multiplex PCR test in order to identify a wide panel of typical pathogens responsible for respiratory system infections, including *C. pneumoniae*. In a group of cadets with pneumonia of bacterial etiology, *C. pneumoniae* was isolated with the frequency of 73%, and in the group of subjects with symptoms of acute infection of upper respiratory tract, but without pneumonia, the percentage was 36%. It is worth noting that in a group of cadets with the infection of upper respiratory tract, symptoms subsided spontaneously, without antibacterial treatment [5]. This is confirmed by the typical pathomechanism of infections caused by *C. pneumoniae*, in which the acute infection in young people is usually characterized by a mild course of the disease and is not accompanied by any additional conditions. A more acute course is usually found in older people with a chronic illnesses or immune disorders. Respiratory tract infections occur also with a high frequency in children worldwide, which, in the case of younger kids, is usually associated with the physiological dysfunction of the immune system. Epidemiological data shows that infections caused by *C. pneumoniae* affect all age groups, including children under 5 years of age [10]. The description for Sasaoka et al. involves a male newborn born at 37 weeks with normal weight by a healthy mother without episodes of infection during pregnancy, or any health problems in the family, in whom the cough occurred 24 days after the date of birth and after three days the child was hospitalized because of apnea. In connection with the presented case of infection, it is important to note two important issues: for the first time, *C. pneumoniae*, contrary to *C. trachomatis*, was identified as the etiologic agent of apnea in the newborn. The authors of the report suggest that because of this fact, the detection of *C. pneumoniae* in the differential diagnosis of apnea in newborns and infants should be considered. Secondly, the difficulties with the diagnosis and detection of infections caused by *C. pneumoniae* were pointed out. In the case of positive results of the serological tests, however, an infection with *C. pneumoniae* was confirmed both in bacteria culture and by means of PCR method. The difficulty in diagnosing the *C. pneumoniae* infection may lead to the underestimation of the incidence of this infection type for newborns and infants [14]. The analysis of the results of own research in children with symptoms of chronic cough, performed with the use of indirect immunofluorescence technique showed that *C. pneumoniae* was more often detected from the throat swabs in younger children. The studies of Sarowska et al. from 2014 conducted in 694 children

with symptoms of chronic cough, the *C. pneumoniae* antigen was detected in 122/694 subjects (17.6%). Due to the lack of explicit clinical criteria and, above all, standardized reference tests, in the case of the diagnosis of infections caused by *C. pneumoniae* the epidemiological data concerning the detection of etiological agent in different groups of patients, both adults and children are very diverse [13]. Aydie et al. conducted research in a group of 85 adult patients hospitalized due to the suspected atypical pneumonia of the *C. pneumoniae* etiology in Jordan. Moreover, 110 healthy people, who formed the control group, were examined. The research was carried out by means of two methods: micro-immunofluorescence test and PCR method. Using the MIF test, the acute infection is determined at the level of 16.3% in patients with pneumonia, compared to 5.5% in the control group, while the results obtained with the use of PCR method in both groups were less diverse and were, respectively, 8.8% and 8.2% [1]. In the research conducted by Jama-Kmiecik et al. in 2016, on the other hand, using the IFT method, the *C. pneumoniae* antigen was detected in throat swabs in 19.1% of non-hospital patients with the symptoms of upper respiratory tract infections, including in 20.5% of women and in 18.1% of men [8].

In a study conducted by Choroszy-Krol et al. [3] in 2014, a group of 100 adults (26 men and 74 women) aged 20-69 years without any complaints regarding their respiratory system were examined. The presence of C. pneumoniae antigen was detected in 15% of respondents (including 14.9% of women and 15.4% of men). The lowest percentage of positive results (4%) was observed in the group of people aged 20-29. The highest percentage of positive results (27.8%) was found in people in the 40-49 age group.

## CONCLUSIONS

• In the examined group of children and adults with a chronic cough the *C. pneumoniae* antigen was detected.

• The frequency of detection of *C. pneumoniae* antigen was differentiated depending on the age group of both children and adults with symptoms of chronic cough.

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