ANNALES
UNIVERSITATIS MARIE CURIE-SKŁODOWSKA
LUBLIN-POLONIA
VOL. LVIII, N 1, 82 SECTIO D 2003

Pulmonary Department, Medical University of Lublin
Department of Clinical Microbiology, Medical University of Lublin

BEATA GRYGLICKA, IRENA WĘGRZYN-SZKUTNIK,
MAREK MICHNAR, ELŻBIETA MAZUR, JUSTYNA NIEDŹWIADEK,
JANUSZ MILANOWSKI

Evaluation of an anti-chlamydial antibiotic therapy influence on asthma patients

Asthma is a chronic lung disease characterized by airway obstruction, inflammation and bronchial hyperresponsiveness to a variety of stimuli, including infections. Virus infections have been identified in 10 to 90% of children and adults affected by acute exacerbation of asthma (1, 17, 19). Other atypical pathogens, such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, have also been associated with acute asthma exacerbation (7, 17).

C. pneumoniae is one of the most common human pathogens. *Chlamydiaceae* family includes obligate intracellular pathogens that cause both acute and chronic infections in human target tissues. Several studies have estimated the population prevalence of antibodies against *C. pneumoniae* to be between 40 and 60%. Up to 90% of infected subjects have only a few or no symptoms at all (1, 2) and an infection shows a tendency to persist. Both persistent and repeated infections may cause intense inflammatory reactions. Chronic chlamydial infections have been associated with asthma (4, 8, 15, 20), Chronic Obstructive Pulmonary Disease (COPD) (11, 12, 15, 16), arteriosclerosis, coronary heart disease (2, 4, 13, 14, 20), abdominal aortic aneurysm (13), sarcoidosis, as well as with reactive arthritis and lung cancer (12).

Hähn et al. first reported an association between antibodies against *C. pneumoniae* and asthma in 1991. Afterwards, a link between *C. pneumoniae*, asthma and COPD was described owing to seroepidemic and experimental research conducted by laboratories in several countries. These studies suggested a supporting role of *C. pneumoniae* infection in acute wheezing as well as in exacerbation, initiation and promotion of asthma in both children and adults (1, 3, 6, 7, 11, 12, 15, 16, 18-20).

Numerous studies have reported an improvement in the course of asthma after antibiotic theraphy of culture-positive asthma patients (6, 10, 18). Contrary to acute *C. pneumoniae* infection treatment, in case of chronic infection longer courses of antibiotic therapy have been recommended (5, 6, 9, 10). Persisting clinical asthma improvement has been observed in culture–positive male patients after administration of a prolonged course of clarythromycin (500 mg, twice a day), azithromycin (1000 mg per week, orally) or doxycyclini (100 mg, twice a day) given for four to six weeks (5, 9, 10). The therapy disadvantage is that the duration of extracellular continued existence of the elementary bodies (EBs) remains unknown (9). If EBs survival time was longer than the treatment period, *C. pneumoniae* would not be eradicated.

The aim of the study was to examine whether appropriate treatment of *C.* pneumoniae infection in a group of patients with a prolonged course of severe

atopic asthma would give an improvement of symptoms and pulmonary functions.

MATERIAL AND METHODS

The examined group of patients consisted of 13 subjects suffering from bronchial asthma, who were given medical treatment in the Ambulatory Chest Clinic of the Pulmonary Department of the University Medical School in Lublin. The patients were diagnosed to have moderate to severe stable stage of chronic atopic asthma, without its exacerbation during the last three months. The illness duration was more than 20 years. The subjects were examined in order to find serological markers of chlamydial chronic infections. The mean patient's age was 48.5 (ranging from 28 to 65 year-old subjects). The group consisted of nine female and four male patients. The initial mean percentage of the predicted forced expiratory volume in one second (FEV_1) was 54.85% of predicted normal values (PV) (ranging from 34 to 96% of PV). The patients were regularly treated with bronchodilators and inhaled steroids. The mean duration of a follow-up was 6 weeks.

Serologic studies. Microimmunofluorescence (MIF) method was applied. *Chlamydia pneumoniae* Micro IF test (Labsystem, Finland) was used in accordance with the manufacturer's instructions. Patients' sera analyzed for the presence of IgA and IgM against *C. pneumoniae* were diluted in IgG blocker (Labsystems, Finland) to remove possible interference with IgG. In agreement with the reference data, the following criteria were adopted: 1. Chronic infection: IgG ≥ 1:128, IgA ≥ 1:16, IgM = 0. 2. Acute infection: IgG≥ 1:512, IgM ≥ 1:8, IgA = 0.

Spirometry testing. Spirometry testing was performed using LUNG TEST 1000. The values of the forced vital capacity (FVC) and FEV_1 were analyzed, before and after treatment.

Patient general improvement score. The disease symptoms were examined before and after treatment using the following criteria: 1. The dyspnoea intensification was evaluated basing on the ten-point visual Borg's scale at the beginning of each visit in the clinic. 2. The level of physical efficiency was estimated basing on a one-to-five scale with the following meaning of each number: a) entirely limited; b)considerably limited; c)limited on an average; d)slightly limited; e)unlimited. 3. The medication dose administered on patient's demand.4. The evaluation of life quality using the standard SF-36 and Saint George Hospital questionnaires.

Patient treatment. Patients were treated with azithromycin in a dose of 1000 mg administered once a week for six weeks. They were randomized into two groups, one group was receiving placebo and the other one – azithromycin. The research was conducted using the double blind trial method. The patients were acquainted with the examination protocol giving their written consent.

Statistical methods. Continuous variables with normal distribution (FEV_1 and FVC) were analyzed using the t-test for dependent and independent samples. Variables distributed differently were examined using the Wilcoxon matched pair test. To analyze the nominal variables the Kruskal-Wallis ANOVA by ranks test, the Median test and the sign test were used.

RESULTS

The group of patients receiving placebo ('the placebo group') consisted of six subjects, while the group treated with azithromycin ('the treated group') – of seven. The mean age in the placebo group was 45.3 (ranging from 28 to 54 year-old patients), whereas in the treated group – 50.2 (ranging from 38 to 65). In the first group there were four female and two male patients and in the other one – five female and two male subjects. All of them were non-smokers in the course of examination. When initiating the research, there were no differences regarding the evaluated spirometry parameters (FEV_1 and FVC), disease duration and the degree of disease progression between those two groups.

PULMONARY FUNCTION RESULTS

Table 1. Spirometry parameters

	Placebo group		Treated group	
	before treatment	after treatment	before treatment	after treatment
FEV_1 (ml)	1533 (950-2677)	1803 (970-3430)	1653 (890-3200)	1820 (930-3480)
FEV_1 (% of PV)	52.7 % SD ±23.9	59.8% SD ±22.5	56.7% SD± 16.8	61.1% SD± 14.5
FVC (% of PV)	67.7% SD± 24.1	71.8% SD± 26.1	70.4 % SD ±12.5	71.4% SD ±11.8

Despite the observed improvement of FEV_1 value of 4.4% in the treated group and of 7.1% in the placebo group, the results were not significantly different from the initial values. Spirometry parameters of both groups, before and after the treatment, are presented in Table 1. Figures 1 and 2 show graphic comparison of the mean FEV_1 values and of standard deviation in those two groups.

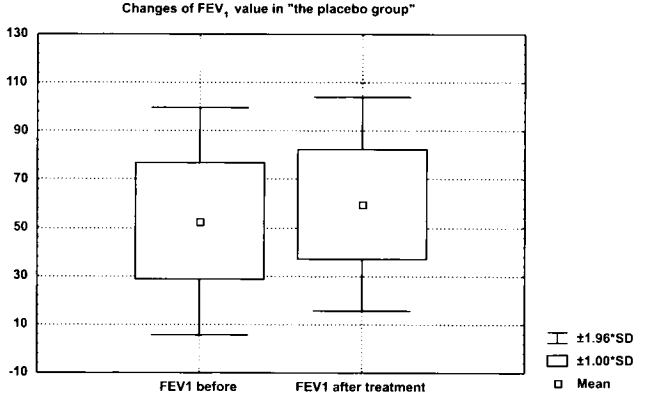

Fig. 1

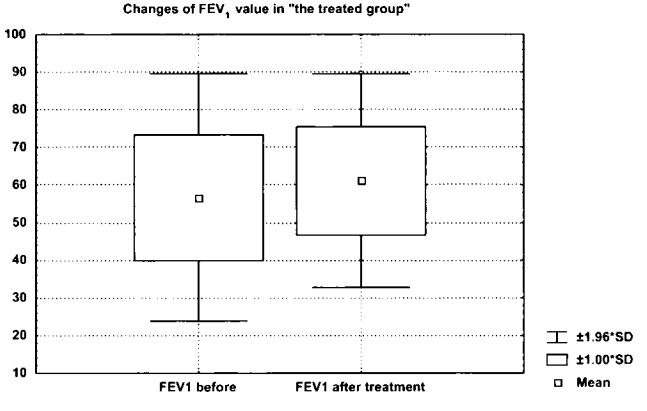

Fig. 2

EVALUATION OF SEROLOGIC RESULTS

Reduction of IgG level was observed in both groups. Table 2 presents the median values obtained for the examined subjects. This diminution was more statistically significant in the treated group (Fig. 3). The IgA level did not change in the placebo group, whereas in the treated group no statistical significance was observed in the reduction of the value.

Table 2. IgG and IgA levels

	Placebo group		Treated group	
	before reatment	after treatment	before treatment	after treatment
IgA the median value and its range	32 (16-64)	64 (0-64)	16 (16-32)	16 (0-32)
IgA the mean value ± SD	37.3 ± 21.9	37.3 ± 31.5	20.6 ± 7.8	13.7 ± 14.4
IgG the median value and its range	128 (128-512)	128 (0-512)	512 (128-512)	128 (0-512)
IgG the mean value ± SD	256.0 ± 198.3	149.3 ± 188.4	347.4 ± 205.3	182.9± 232.0

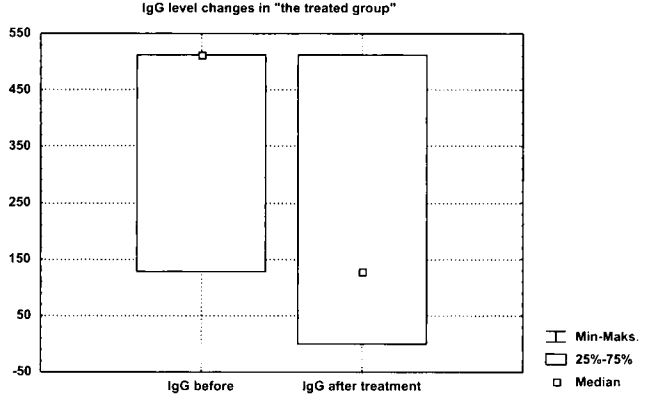

Fig. 3

POINT EVALUATION RESULTS

Dyspnoea intensity, evaluated after treatment basing on the ten-point visual Borg's scale, did not alter significantly in any group, although patients belonging to the treated group reported the reduction of dyspnoea intensification (i.e. the median value decreased from seven to five). In the placebo group the median value increase from 3.5 to 4.5 was noticed (Tab. 3). The treatment also had no effect on physical efficiency of patients from both groups evaluated on a scale from one to five. The amount of medications administrated on demand decreased in those groups, although insignificantly (Tab. 4). The quality of life was estimated basing on SF-

36 and Saint George Hospital standard questionnaires given the patients to complete at the beginning of the treatment. In the treated group, patients considered themselves to have poorer chances of recovery ($p<0.05$). As far as other questionnaire statements are considered, there were no significant differences between the groups.

Table 3. Borg's dyspnoea scale (median value)

Placebo group		Treated group	
before treatment	after treatment	before treatment	after treatment
3.	4.5	7	5

Table 4. Mean medication usage administrated on demand (median value of dose number)

	Placebo group	Treated group
before treatment	2	4
after treatment	1	3

DISCUSSION

Bacteria belonging to *Chlamydiales* sp. are often responsible for chronic infections characterized by progressive formation of excessive fibrous tissue and its cicatrization (e.g. trachoma, pelvic inflammatory disease or venereal lymphogranuloma caused by *C. trachomatis*). It is suggested that an analogous mechanism may be associated with chronic *C. pneumoniae* infection (2,12,16). Although clear evidence has not yet been found, *C. pneumoniae* infection and asthma coexistence is thought to bring about remodelling of the airways (9).

Owing to our co-operation with the Department of Clinical Microbiology, we obtained the results that indicate the coexistence of *C. pneumoniae* infection with asthma and COPD (15,16,19). As serologic examination revealed, chronic infection caused by this pathogen was significantly more frequently diagnosed in asthma and COPD patients in comparison with healthy subjects. The presence of antibodies against *C. pneumoniae* was more often detected in patients with a severe course of illness and in the elderly, which is consistent with other reports (9,18).

In 1999, Hahn analyzed the Medline accessible papers, which referred to 'obstructive lung disease' and '*Chlamydia pneumoniae*' (9). From 18 controlled epidemic studies (over 4 000 cases/controls), 15 found significant associations between *C. pneumoniae* infection and asthma. Eight case reports and 13 case series of *C. pneumoniae* infections in asthma (over 1 000 patients) also included descriptions of improvement or even complete disappearance of asthma symptoms after a prolonged antibiotic therapy directed against this pathogen. These findings confirm the necessity of prolonged antibiotic therapy when dealing with chronic infection. As *C. pneumoniae* infection usually has an asymptomatic or oligosymptomatic course, it was determined (7,18) that the presence of anti-chlamydial IgA in serum should be considered as a chronic *C. pneumoniae* infection indicator. The half-life of serum IgA is less than one week. Its continuous presence may therefore indicate persistent antigenic stimulation to the immune system.

Other methods, like the polymerase chain reaction (PCR), IgE against chlamydia level testing (7) and immunohistochemical assay are more expensive and technically demanding. Our previous studies, conducted in the Department of Clinical Microbiology, showed the positive

PCR results for nosopharyngeal swab specimens in 6 of 23 patients (26%) with increased anti-chlamydial IgA level (15). Miyashita et al. obtained the positive PCR results for 9% of the examined asthma patients in relation to 47.6% of positive serologic results indicating chronic infection (18). Bacterial culture in a cellular medium (Hep-2, HL) is challenging and less sensitive than PCR assay (18). Immunohistochemical (ICC) staining of fresh and/or formalin fixed paraffin-embedded tissues, using either genus-specific or species-specific monoclonal antibodies, has been used to detect chronic *C. pneumoniae* tissue infection in upper respiratory and cardiovascular diseases (4,9,13). MIF test has become the serological "gold standard" for diagnosis of *C. pneumoniae* infections, being highly specific and sensitive when compared with culture (7,18).

Twenty-three patients suffering from asthma, treated in the Ambulatory Chest Clinic, were invited to take part in the research after having been diagnosed as having serologic indicators of chronic *C. pneumoniae* infection. The examined group consisted of 13 subjects, who gave their consent to be included in the studies. They were divided into two groups with the double blind trial usage. There were no age differences, function test results, disease duration and its progression between the groups. Both azithromycin (250 mg, capsules) and placebo were kindly provided by PLIVA. The reason for choosing azithromycin was its relatively low cost and convenient way of administering (suggested dose of 1000 mg, once a week).

Anti-chlamydial antibiotic therapy duration may differ. The cycle lasting for three to six weeks is mainly recommended, but it has been prolonged in some cases (6,10). Our patient underwent a six-week antibiotic theraphy. Numerous studies consider the necessity of anti-chlamydial antibiotic therapy in non-atopic asthma patients with chronic *C. pneumoniae* infection and short disease duration (10,16,19). The results indicated a marked improvement in asthma progression. The aim of our research was to evaluate whether analogous positive effects could be observed for patients with severe, long-lasting atopic asthma.

Although in the examined patient groups spirometry result improvement was observed, it was not of statistical significance. Analogously, expected significant improvement of asthma symptoms and life quality was not noticed. Some patients, however, reported decrease on dyspnoea intensity, still of no statistical significance. The observed lack of noticeable improvement may result from the patient selection. Hahn examined 46 subjects with bronchial asthma and chronic *C. pneumoniae* infection describing positive treatment results in 25 patients (10). His research indicated that patients suffering from more severe asthma of longer illness duration and of more intensified functional disturbances had not responded to the therapy. Though chronic *C. pneumoniae* infection appears more often in the elderly (16,18) and in patients with moderate or severe asthma (9,15,16), anti-chlamydial antibiotic therapy is strongly recommended to subjects with minor and non-atopic asthma as well as when treating an early stage of the disease (5,10,16,19).

Our research included patients with severe atopic asthma with illness duration of more than 20 years. Airway remodelling that has already occurred may be the reason for the lack of responsiveness to treatment. Moreover, therapy duration should be settled for each patient separately. A longer antibiotic therapy might probably have resulted in obtaining better clinical results. Compared with the placebo group, where there was no change in IgA level, in the treated group diminution of serologic markers of infection, that is considerable reduction of IgG level and insignificant decrease in IgA concentration, was observed.

CONCLUSIONS

In the examined patients no expected improvement in the course of asthma, neither in evaluated spirometry parameters nor in illness symptoms and the quality of life, was observed. Progressive reduction of serologic

indicators of infection was reported in the treated group (i.e. in three patients IgA level reached the zero mark). It was not, however, associated with spirometry parameter improvement. A low number of the examined subjects, resulting from the selection criteria, was a decidedly limiting factor. Continuation of research is highly recommended.

REFERENCES

1. Björnsson E. et al.: Serology of *Chlamydia* in relation of asthma and bronchial hyperresponsiveness. Scan. J. Infect. Dis., 28, 63, 1996.
2. Cook P. J. et al.: Clinical aspects of *Chlamydia pneumoniae* infection. Press Med., 24, 278, 1995.
3. Daian C. M. et al.: The role of atypical organisms in asthma. Allergy Asthma Proc., 21, 102, 2000.
4. Grayston T. et al.: *Chlamydia pneumoniae* (TWAR) in atherosclerosis of the carotid artery. Circulation, 92, 3397, 1995.
5. Hahn D. et al.: Can acute *Chlamydia pneumoniae* respiratory tract infection initiate chronic asthma? Ann. Allergy Asthma Immunol., 81, 339, 1998.
6. Hahn D. et al.: Evidence for *Chlamydia pneumoniae* infection in steroid- dependent asthma. Ann. Allergy Immunol., 80, 45, 1998.
7. Hahn D. et al.: Serologic markers for *Chlamydia pneumoniae* in asthma. Ann. Allergy Immunol., 84, 227, 2000.
8. Hahn D. L. et al.: Asthma and chlamydial infections: a case series. J. Farm. Pract., 38, 589, 1994.
9. Hahn D. L.: *Chlamydia pneumoniae*, asthma, and COPD: what is the evidence? Ann. Allergy Asthma Immunol., 83, 271, 1999.
10. Hahn D. L.: Treatment of *Chlamydia pneumoniae* infection in adult asthma: a before – after trial. J. Farm. Pract., 41, 345, 1995.
11. Hertzen L. et al.: *Chlamydia pneumoniae* antibodies in chronic obstructive pulmonary disease. Int. J. Epidemiol., 25, 658, 1996.
12. Hertzen L.: *Chlamydia pneumoniae* and its role in chronic obstructive pulmonary disease. Ann. Med., 30, 27, 1998.
13. Karlsson L. et al.: Detection of viable *Chlamydia pneumoniae* in abdominal aortic aneurysms. Eur. J. Vasc. Endovasc. Surg., 19, 630, 2000.
14. Mayr M. I. et al.: Infections, Immunity and Atherosclerosis . Associations of Antibodies to *Chlamydia pneumoniae*, *Helicobacter pylori* and Cytomegalovirus with immune reactions to heat-schock protein 60 and carotid of femoral atherosclerosis. Circulation, 102, 833, 2000.
15. Mazur E. et al.: Chronic *Chlamydia pneumoniae* in patients with asthma and COPD (Chronic Obstructive Pulmonary Disease). Annales UMCS, sectio D,vol.55, 47, 2000.
16. Mazur E. et al.: Przewlekła infekcja *Chlamydia pneumoniae* u chorych na przewlekłą obturacyjną chorobę płuc. Pneumonol. Alergol. Pol., 5, 261, 2000.
17. Micilli E. et al.: Respiratory infections and asthma. Allergy, 55, 42, 2000.
18. Miyashita N. et al.: *Chlamydia pneumoniae* end exacerbations of asthma in adults. Ann. Allergy Asthma Immunol., 80, 405, 1998
19. Niedźwiadek J. et al.: Przewlekłe zakażenie *Chlamydia pneumoniae* u chorych na astmę oskrzelową. Pneumonol. Alergol. Pol., 5, 255, 2000.
20. Saikku P. et al.: Atypical respiratory pathogens. Clin. Microbiol. Infect., 3, 599, 1997.

SUMMARY

Chlamydia pneumoniae is one of the most frequent pathogens causing airways infections. Contribution of chronic chlamydial infection to the following diseases: asthma, POChP, coronary heart disease, abdominal aortic aneurysm, is particularly interesting. The connection between such infection and bronchial asthma was described in the literature in 1991. *C. pneumoniae* often causes asthma exacerbation; it is suggested that it also may be an etiologic factor of the disease. In a group of 55 subjects with chronic, stable bronchial asthma treated in the Pulmonary Department, serologic characteristic of *C. pneumoniae* infection was found in 34 patients (61,8%). Thirteen of these subjects agreed to participate in the study. They were divided into two groups; placebo was administered to the first one and azitromycin in a dose of 1000 mg once a week – to the other one. The research was conducted using the double blind trial method. Anti-chlamydial antibody level was evaluated before and after treatment. Spirometry tests as well as subjective estimation of physical fitness and dyspnoea degree were also determined. In comparison with 'the placebo group', statistically significant improvement in respiratory parameters 'in the treated group' was not ascertained.

Ocena wpływu celowanej antybiotykoterapii przeciwchlamydiowej na przebieg choroby u chorych na astmę oskrzelową

Chlamydia pneumoniae jest jednym z najczęstszych patogenów, będących przyczyną infekcji dróg oddechowych. Szczególnie interesujący jest udział przewlekłego zakażenia tym patogenem w takich chorobach, jak astma, POChP, miażdżyca, choroba wieńcowa, tętniak aorty brzusznej. Związek zakażenia z astmą oskrzelową opisywany jest w literaturze od roku 1991. *C. pneumoniae* jest częstym czynnikiem wywołującym zaostrzenia astmy, istnieją sugestie, że może być również czynnikiem etiologicznym tej choroby. W grupie 55 chorych z przewlekłą stabilną astmą oskrzelową, leczonych pod kontrolą Poradni Przyklinicznej, u 34 (61,8%) stwierdzono serologiczne cechy przewlekłego zakażenia *C. pneumoniae*. Na udział w badaniu zgodziło się 13 osób z tej grupy. Chorych podzielono na dwie grupy – przyjmujących placebo i otrzymujących Azitromycynę 1000 mg 1x w tygodniu. Badanie przeprowadzono metodą podwójnie ślepej próby. Badano poziom przeciwciał przeciwchlamydiowych przed i po zakończeniu leczenia, ponadto przeprowadzono badania czynnościowe dróg oddechowych oraz ocenę subiektywną stopnia wydolności fizycznej i stopnia duszności. Nie stwierdzono statystycznie istotnej poprawy wydolności oddechowej u chorych leczonych w porównaniu z grupą placebo.