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Distribution of antibodies to selected antigens of *Pseudomonas aeruginosa* in children and young adults with cystic fibrosis

Występowanie przeciwciał dla wybranych antygenów *Pseudomonas aeruginosa* u dzieci i młodych dorosłych chorych na mukowiscydozę

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Abstract

Introduction: Eradication of *Pseudomonas aeruginosa* (*P.a.*) in patients with cystic fibrosis (CF) is possible if it is initiated in the early course of infection. Therefore, the detection of *P.a.* as early as possible is an important goal of care. Regular determination of antibodies to *P.a.* antigens in serum may be useful in patients who have not yet been infected or were infected intermittently. The aim of the present study was to assess the concentrations of antibodies to selected antigens of *P. aeruginosa* in the serum of children with CF and with known status of *P.a.* infection.

Material and methods: The study was performed in 111 CF patients (27 not infected with *P. aeruginosa*, 29 with intermittent infection and 55 with chronic infection). The concentrations of IgG antibodies to the alkaline protease (AP), elastase (ELA) and exotoxin A (Exo-A) were measured. The increased concentration of antibodies was defined as exceeding 500 units (according to the manufacturer). The results of antibodies assessment were analysed according to previous infection status and the results of present culture.

Results: At the time of the study, *P.a.* was cultured from sputum of 57 patients: 9 out of 29 (31%) with intermittent infection, and 48 out of 55 (87%) with chronic infection. Increased concentrations of antibodies to one or more *P.a.* antigens were found in 60 patients, and to all three types of antigens in 30 patients. Increased serum antibody concentration was found significantly more often in the patients with chronic *P.a.* infection compared to those with intermittent infection (82% vs. 35%, $p = 0.0001$). In the patients with chronic *P.a.* infection (especially with mucoid type), serum antibody concentrations were significantly higher than in other patients. Higher concentrations of antibodies were also found in the patients with positive result of *P.a.* culture at the time of the study, compared to those with negative culture. In 19% of patients not infected with *P.a.*, increased serum antibodies to at least one *P.a.* antigen were found. The clinical significance of such findings is unclear and needs further investigation.

Conclusions: In the present study, the increased serum concentrations of IgG antibodies to *P. aeruginosa* antigens (AP, ELA and Exo-A) were found most often in the patients with chronic *P.a.* infection and in those in whom *P.a.* (especially mucoid type) was cultured at the time of the study. The clinical significance of the elevated antipseudomonal antibodies level in 19% of the patients never infected with *P.a.* is unclear and needs further investigation.

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Key words: antibodies IgG, *Pseudomonas aeruginosa*, alkaline protease, elastase, exotoxin A, cystic fibrosis, ELISA assay

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Streszczenie

Wstęp: Eradykacja *Pseudomonas aeruginosa (P.a.)* u pacjentów z mukowiscydozą jest możliwa, jeśli zostanie rozpoczęta na wczesnym etapie zakażenia. Z tego powodu wykrycie *P.a.* stanowi ważny element opieki nad chorymi. Oznaczanie przeciwciał dla antygenów *P.a.* w surowicy może być użyteczne u pacjentów, którzy dotychczas nie byli zakażeni *Pseudomonas aeruginosa* lub byli zakażeni w sposób przerywany.

Celem badania była ocena stężenia przeciwciał dla wybranych antygenów *Pseudomonas aeruginosa* w surowicy chorych na mukowiscydozę (CF, *cystic fibrosis*), ze znaną historią zakażenia.

Materiał i metody: Badania wykonano u 111 pacjentów (27 dotychczas nie zakażonych, 29 z okresowym zakażeniem i 55 przewlekle zakażonych), u których oznaczano stężenia przeciwciał IgG dla alkalicznej proteazy (AP), elastazy (ELA) i egzotoksyny A (Exo-A) metodą immunoenzymatyczną. Za podwyższone stężenie przeciwciał uznawano wartość powyżej 500 jednostek producenta.

Wyniki zestawiono z aktualnym badaniem mikrobiologicznym plwociny oraz z wywiadem dotyczącym zakażenia *P.a.*

Wyniki: Aktualny wzrost *P.a.* z plwociny stwierdzono u 57 chorych: 9 spośród 29 (31%) z okresowym zakażeniem i 48 spośród 55 (87%) z zakażeniem przewlekłym. U 60 chorych stwierdzono podwyższone stężenie przeciwciał dla co najmniej jednego antygenu *P.a*., u 30 – dla wszystkich badanych antygenów.

Odsetek chorych z podwyższonym surowiczym stężeniem przeciwciał był istotnie wyższy wśród chorych przewlekle zakażonych w porównaniu z zakażonymi okresowo (82% *v.* 35%, p = 0,0001). U chorych przewlekle zakażonych *P.a.* (zwłaszcza szczepem śluzowym) stężenia przeciwciał były wyższe niż w pozostałych grupach.

U chorych aktualnie zakażonych *P.a.* stwierdzono istotnie wyższe stężenie przeciwciał antypseudomonalnych w porównaniu z chorymi, u których aktualnie nie stwierdzano wzrostu *P.a.*

U 19% badanych z dotychczas i aktualnie ujemnymi wynikami mikrobiologicznego badania w kierunku obecności *P.a* uzyskano dodatnie wyniki oznaczenia przeciwciał dla co najmniej 1 antygenu tej bakterii. Zjawisko to ma niejasne znaczenie kliniczne i wymaga dalszej obserwacji.

Wnioski: W badanej grupie chorych na mukowiscydozę odpowiedź immunologiczna na zakażenie *P.a.* zależała w większości przypadków od czasu i intensywności zakażenia. Stężenie przeciwciał było również związane z aktualną obecnością szczepu *P.a.* w hodowli – najwyższe stężenie przeciwciał obserwowano wśród aktualnie zakażonych typem śluzowym *P.a.*. Znaczenie kliniczne przeciwciał przeciwko *P.a.* u 19% chorych dotychczas nie zakażonych tym patogenem jest niejasne i wymaga dalszych badań.

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Słowa kluczowe: przeciwciała IgG, *Pseudomonas aeruginosa,* proteaza alkaliczna, elastaza, egzotoksyna A, mukowiscydoza, ELISA

Introduction

Cystic fibrosis (CF) is a genetically determined, multiorgan, autosomal recessive disease. It cannot be cured (similarly as other genetic diseases), but knowledge about its pathogenesis, the course of the disease, early detection of complications and the improvement of treatment methods have significantly extended survival time and ameliorated its quality.

Cystic fibrosis develops due to mutation of the gene encoding the CFTR protein (cystic fibrosis transmembrane conductance regulator), which is found in the apical membrane of the surface of epithelial cells. The effect of inappropriate functioning of CFTR is dehydration and the condensation of secretion in excretory ducts of the exocrine glands. Excessive density of secretion causes disturbances in the transport through the excretory ducts, with its progressive damage. The epithelium of the airways is particularly exposed to the development of bacterial infections, whereas the impairment of ciliary clearance hinders the elimination of pathogens. At the initial stage of the disease, the airways are the most frequently infected with *Haemophilus influenzae* and *Staphylococcus aureus*. As the disease progresses, *Pseudomonas aeruginosa* infections develop. Over time, *P.a.* is the dominating pathogen with a negative influence on the respiratory system. At the beginning, non-mucoid, *in vitro* susceptible to antibiotics *P. aeruginosa* strains are usually cultured [1, 2]. Together with the progression of the disease, the frequency of isolation of the mucoid form of *P. aeruginosa* increases.

Eradication of *P.a.* in CF patients is possible if it is initiated early in the course of infection [2, 3]. Therefore, early detection of *P.a.* is an important target in monitoring CF patients. The application of serological methods may be useful in early detection of infection [4, 5].

The objective of the study was to evaluate the concentration of antibodies to selected antigens of *P.a.* (alkaline protease, elastase and exotoxin A) in serum of cystic fibrosis patients with known history of infection, and to compare the obtained results with microbiological tests.

Material and methods

The analysis included 111 CF patients diagnosed and treated at the Institute of Tuberculosis and Lung Diseases in Rabka Zdrój. Depending on the results of the previous sputum or swab cultures for *P. aeruginosa* taken at least every 3 months during the previous 12 months, patients were divided into three groups, according to the definition by Lee et al. [6]:

1. Group 1. Free of infection: *P a.* never cultured from sputum or swab.
2. Group 2. Intermittent infection: the growth of *P.a.* in less than 50% of samples.
3. Group 3. Chronic infection: the growth of *P.a.* in more than 50% of samples.

The concentrations of IgG antibodies to selected *P.a.* antigens [alkaline protease (AP), elastase (ELA), exotoxin A (Exo-A)] were examined using enzyme-linked immuno ELISA assay, based on sets from the Mediagnost GmbH, Germany. Values exceeding 500 arbitrary units were assumed as a positive concentration (according to the manufacturer).

Material from the airways (sputum collected from older children and adults, or swabs from the throat of younger children) were analysed according to the manufacturer's guidelines. In patients with exacerbations, material was taken prior to the start of antibiotic therapy. In patients treated with inhaled antibiotics, material was taken at least 12 hours after the last dose. Media produced by the company Graso were used. The cultured *P. aeruginosa* strains were identified with the help of ID 32 GN strips, BioMerieux Polska, using miniAPI apparatus.

Results

The mean age of patients with CF was 11.2 ± 6.0 years, range from 3 months to 28.1 years. The mean age in the study groups was as follows:

1. Group 1 (27 patients) — 7.9 ± 4.8 years; range: 0.3–18.1 years.
2. Group 2 (29 patients) — 10.4 ± 4.0 years; range: 0.9–18.4 years.
3. Group 3 (55 patients) — 13.1 ± 4.3 years; range: 0.3–20.0 years.

Table 1 shows the relationship between the current results of *P. a.* culture and the data concerning the type of infection. The frequency of currently positive cultures of *P. a.* and its mucoid morphotype increased together with the infection chronicity. Among patients with intermittent infection (Group 2), the growth of *P. a.* was found in 9/29 patients (31%), including the growth of mucoid strain in 2 patients (7%), non-mucoid in 6 patients (21%) and both types in 1 patient (3%). Among patients with chronic infection (Group 3), the growth of *P.a.* was found in 48/55 patients (87%), including the growth of mucoid strain in 15 patients (27%), non-mucoid in 13 patients (24%) and both types in 20 patients (36%).

In 60 patients, elevated concentration of IgG antibodies was found (> 500 U) for at least one *P. aeruginosa* antigen, and in 30 patients — for all examined antigens. Positive results were obtained in 40 patients (36%) for alkaline protease (AP), in 46 patients (41%) for elastase (ELA), and in 47 patients (42%) for exotoxin A (Exo-A). For AP antigen, the frequency of elevated antibody concentration was 14% among intermittently infected patients (Group 2) and 62% among chronically infected patients (Group 3). Similar dependencies were found for the remaining antigens: for ELA — 17% and 71% in Groups 2 and 3, respectively, and for Exo-A — 24% and 65%, respectively (Table 2).

Elevated antipseudomonal antibody concentrations were also found in serum from the patients free of *P.a.* infection — for AP and ELA

Table 1. Actual results of culture according to chronicity of *Pseudomonas aeruginosa* infection in 111 children with cystic fibrosis

Type of infection	Number of patients	*P.a.* negative culture No (%)	*P.a.* positive culture No (%)	Nonmucoid *P.a.* No	Mucoid *P.a.* No	Both types of *P.a.* No
No infection	27	27 (100)	0 (0)	0	0	0
Periodic infection	29	20 (69)	9 (31)	6	2	1
Chronic infection	55	7 (13)	48 (87)	13	15	20
Total	111	54 (49)	57 (51)	19	17	21

Table 2. The frequency of detection of antibodies against *Pseudomonas aeruginosa* according to chronicity of infection. Antibodies to the alkaline protease (AP), elastase (ELA) and exotoxin A (Exo-A)

Type of infection	Number of patients	Increased concentration of antibodies					
		No (%)	≥ 1 No (%)	AP (+) No (%)	ELA (+) No (%)	Exo-A (+) No (%)	All types No (%)
No infection	27	22 (81)	5 (19)	2 (7)	2 (7)	4 (15)	1 (4)
Periodic infection	29	19 (65)	10 (35)	4 (14)	5 (17)	7 (24)	3 (10)
Chronic infection	55	10 (18)	45 (82)	34 (62)	39 (71)	36 (65)	26 (47)
Total	111	51 (46)	60 (54)	40 (36)	46 (41)	47 (42)	30 (27)

(+) — positive result

Table 3. The concentration of antibodies against *Pseudomonas aeruginosa* according to chronicity of infection. Antibodies to the alkaline protease (AP), elastase (ELA) and exotoxin A (Exo-A)

Type of infection	Number of patients	AP x ± SD	ELA x ± SD	Exo-A x ± SD
No infection	27	70 ± 221	136 ± 288	305 ± 902
Periodic infection	29	280 ± 807	527 ± 1446	519 ± 910
Chronic infection	55	1359 ± 1283*	2483 ± 2494*	1622 ± 1559*

*$p < 0.001$ compared to groups with periodic infection and without infection

Table 4. Concentration of antibodies against *Pseudomonas aeruginosa* according to actual culture results. Antibodies to the alkaline protease (AP), elastase (ELA) and exotoxin A (Exo-A)

Current culture result	Number of patients	Antibodies concentration		
		AP	ELA	Exo-A
		$\bar{\chi}$ ± SD	$\bar{\chi}$ ± SD	$\bar{\chi}$ ± SD
Negative	54	92 ± 240	216 ± 401	276 ± 670
Only non-mucoid *P.a.*	19	807 ± 1134$^{\#}$	1316 ± 1998$^{\#}$	774 ± 1001*
Only mucoid *P.a*	17	1684 ± 1397$^{\#\wedge}$	2959 ± 2824$^{\#\wedge}$	2364 ± 1738$^{\#\wedge}$
Wzrost *P.a*. nieśluzowy + śluzowy *Both types of P.a.*	21	1731 ± 1273$^{\#\wedge}$	3320 ± 2521$^{\#\wedge}$	2071 ± 1468$^{\#\wedge}$

$^{\#}p < 0.001$ significant difference comparing to the group with negative culture
$^{*}p < 0.05$ significant difference comparing to the group with negative culture
$^{\wedge}p < 0.05$ significant difference comparing to the group with non-mucoid type of *P.a.*

antigens — in 7% of patients for each antigen, for Exo-A — in 15% of patients, and for at least one antigen — in 5 patients (19%) (Table 2).

Normal antibodies concentration (< 500 U) was found in 50 patients: including 81% of patients free of infection to date, in 65% of patients with intermittent infection, and in 18% of chronically infected patients.

Table 3 presents the absolute values of antipseudomonal antibodies depending on infection chronicity. Significantly higher concentrations of the examined antibodies were observed in chronically infected patients in comparison to other groups ($p < 0.001$).

In 57 patients with currently *P.a.*-positive culture, elevated levels of antibodies to AP, ELA and Exo-A antigens were found in 91%, 87% and 85% of patients, respectively.

Table 4 shows antipseudomonal antibody concentrations depending on the current growth of *P.a.* from sputum. Significantly higher antibodies concentrations were found in the case of the current growth of mucoid type of *P. aeruginosa*, compared to the non-mucoid type of *P.a.*

Discussion

In the presented group of children and young adults with cystic fibrosis, it was shown that the frequency of isolation of *P. aeruginosa* increases together with age, in accordance with data published by other authors [4].

Negative results of culture in 13% of chronically infected patients with *P.a.*, and in 69% of intermittently infected patients, illustrate the difficulties concerning culturing methods in the examined population. Potential reasons include, among others, the problems with obtaining material from the lower airways, particularly in younger patients (in some of them a pharyngeal swab was collected). The other possible cause of negative culture in chronically infected patients is the growth of *P. aeruginosa* in the airways in the form of biofilm. In such cases the secretion includes only single colonies of *P.a.* that leave biofilm [5]. In the present study some patients received the therapy with an inhaled antibiotic. In such circumstances the material was taken at least 12 hours after the last dose, so it was unlikely that it influenced the obtained results, as the concentration of inhaled antibiotic in the secretion decreases rapidly below MIC [7].

The main exoproducts of *P. aeruginosa* are: alginate capsule (which acts as an adhesive and antiphagocytic factor), pilin (important during initial colonisation of the epithelium), adhesins, toxins [among others lipopolysaccharide, enterotoxin, exotoxin S (exoenzyme S), exotoxin A — the main toxin of *P. aeruginosa*], hemolysins (which degrade surfactant) and proteases (e.g. elastase, alkaline protease). The production of proteases that decompose fibronectin and expose receptors plays a crucial role in the invasion and favours the spread of infection. Moreover, elastase decomposes collagen, IgG, IgA and the components of the complement, whereas alkaline protease degrades fibrin. They both participate in inactivation of interferon and TNF [8].

Immunologic response to *P.a.* infection results in the production of antibodies directed against antigens and toxins of the bacteria [4, 6].

In the study groups, the increase in the prevalence and concentration of IgG antibodies to the three antigens (AP, ELA and Exo-A), with increasing chronicity of infection by *P. aeruginosa*, was found. The obtained results confirm the possibility of monitoring the response of the immune system to *P.a.* antigens by measuring the concentration of antibodies to antigens of the bacteria. The highest antibodies concentrations were found in patients infected with mucoid strain of *P. aeruginosa*. As mucoid strains appear mostly in patients with chronic infection, it suggests the role of time (and possibly the intensity) of stimulation with *P.a.* antigens in provoking immune response. Other researchers also showed that in CF patients, the response of the immune system is greater in the case of infection with mucoid phenotype of *P.a.* These antibodies are not able to inhibit bacteria growth, but they participate in the development of immune complexes that activate specific and non-specific inflammatory defence mechanisms of the lungs. Chronic inflammatory process is the main cause of destruction, worsening of lung function and developing progressive respiratory insufficiency [7, 9]. Therefore, it is possible that detection of specific antipseudomonal antibodies in CF may help in the identification of patients with aggressive forms of chronic infection [10].

In the study group, antipseudomonal antibodies concentration was highest in chronically infected patients. However, in 18% of chronically infected patients, elevated antibody concentration was not found. The clinical meaning of this finding was unclear, but the possible reasons could be corticotherapy in some patients, low sensitivity of serological test or the lack of current *P. aeruginosa* growth from sputum.

In the present study, in 19% of patients, increased concentration of antibodies for at least one *P. aeruginosa* antigen was found — in spite of permanently negative results of cultures. This finding might be caused by latent or intermittent *P.a.* infection and requires further clinical observation, in particular, repeated cultures of material from the airways.

It should be emphasised that current indications for *P. aeruginosa* eradication depend on microbiological confirmation of the presence of bacteria in the airways. According to current knowledge, treatment based only on antibody concentration cannot be recommended, but it may be an indication for more intensive microbiological testing (e.g. verification through culturing the material obtained from bronchoscopy) [10–12].

West et al. found that in CF patients diagnosed through screening tests of newborn infants, *P. aeruginosa* infection of the lungs had occurred between the 6^{th} and 12^{th} months [13]. Monitoring the titre of anti *P.a.* antibodies could perhaps facilitate recognition and treatment of *P.a.* infections in such population.

Serological diagnosis of *P.a.* infection in CF has been applied in clinical practice in Europe for

many years. In the USA and Canada it is used for scientific purposes only [14]. Lowering of antibody titre in response to treatment with tobramycin inhalations in patients with early *P.a.* infection has been shown, but in patients with chronic *P.a.* infection the level of antibodies rarely decreased in response to antibacterial treatment.

The results of the present study are in accordance with data of Kappler et al. [1], who used the same commercialised sets to determine the three serum IgG antibodies against *P. aeruginosa* (AP, ELA and Exo-A). Positive antibody titre was indicative for the presence of *P. aeruginosa* in the airways with a sensitivity of 86% and specificity of 90%, whereas a negative result indicated lack of *P. aeruginosa* infection with a positive predictive value of 97%, (especially in younger children). From a clinical perspective, regular determination of the serum level of antibodies to *P.a.* may be useful in patients with negative microbiological *P. aeruginosa* status. If the antibody titre rises, *P. aeruginosa* infection is suspected, which should lead to intensive microbiological testing and a possible eradication treatment.

The obtained results imply that periodic serum antibody determination may be useful in CF patients who were to date free of *P. aeruginosa* infection, or who were infected intermittently.

Conclusions

In the study group of patients with cystic fibrosis, immune response to infection with *P. aeruginosa* in the majority of cases depended on the time and intensity of infection. In the patients with chronic *P.a.* infection, higher antibody concentration was found, in comparison with intermittently infected patients.

Anti *P.a.* antibody concentration was also dependent of the current presence of *P. aeruginosa* strain in the culture and its type — the highest values were observed in patients currently infected with mucoid type of *P.a.*

Elevated antibody concentration for at least one out of three *P. aeruginosa* antigens was also found in 19% of patients free of the pathogen. It may imply latent *P.a.* infection in this group of patients and needs further investigation and correlation with clinical data.

Low concentrations of antibodies against *P. aeruginosa* that were found in 18% of patients chronically infected with *P.a.* is an interesting finding, the interpretation of which is possible only in the context of the clinical course of the disease.

Conflict of interest

The authors declare no conflict of interest.

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