Clinical and laboratory correlations in patients with cystic fibrosis and *Pseudomonas aeruginosa* infection: the experience of a regional centre

Corelații clinico-paraclinice la pacienții cu fibroză chistică și infecție cu Pseudomonas aeruginosa*: experiența unui Centru Regional*

Roxana Smădeanu[1,2], Simona Moșescu[1], Carmen Zăpucioiu[1,2], Dumitru Orășeanu[1,2]

1. "Grigore Alexandrescu" Emergency Clinical Hospital for Children, Bucharest

2. "Carol Davila" University of Medicine and Pharmacy, Bucharest

Corresponding author:
Smădeanu Elena Roxana, MD, pediatric specialist, PhD student and university assistant at "Carol Davila" MPhU Bucharest
Adresss: "Grigore Alexandrescu" Emergency Clinical Hospital for Children, Bucharest, No. 30-32 Iancu de Hunedoara Boulevard
E-mail: rsmadeanu@yahoo.com

Abstract

Pseudomonas aeruginosa (Pa) is the most important pathogen affecting the prognosis of patients with cystic fibrosis (CF). Distinguishing the correlations associated with Pa infection may change the way in which investigation and therapeutic plans are approached. This study aims to evaluate the correlations between various factors such as age at diagnosis, nutritional status, homozygous status of delF508 mutation, the number of exacerbations per year, imagistic modifications, the presence of chronic infection with Staphylococcus aureus, serum levels of total immunoglobulins G (IgG) and gammaglobulins and ventilation parameters - forced vital capacity (FVC) and one-second forced expired volume (FEV1) and their association with Pa infection. Methods. The associations between the analyzed parameters have been made by logistic regression; the comparisons between means of continuous variables were performed using Student t-test; the variables distribution was tested using chi-square test, and correlations between variables were assessed using Pearson coefficient. Results. The mean age at the acquisition of Pa was 5.26 (±5.3 years). Of the 50 patients included in the study, 21 acquired the Pa infection having the median age of primo-infection of 3 years. The study identified a greater number of exacerbations per year in those infected with Pa (2.71 compared to 1.38 exacerbations per year; p=0.002); the increase of radiological score with 1 unit associated with an increased risk of infection with Pa (OR 1.31; 95% CI 1.10-1.56; p = 0.002), the mean Brasfield radiologic score is lower for uninfected children (6.9 vs. 11 points, p=0.001); increasing the degree of bronchiectasis associated with Pa infection (OR 3.75; CI 1.77-7.96; p=0.001); gammaglobulin (15.91 vs. 19.42%; p=0.01) or immunoglobulin G values (IgG) (9.8 vs. 11.9 g/L; p=0.05) were higher for those infected. Conclusions. The increased number of exacerbations per year, the severity of imagistic modifications, and IgG and gammaglobulins values are associated with Pa infection. Tracking these parameters guides the therapeutic conduct.

Keywords: ***Pseudomonas aeruginosa, risk factors, cystic fibrosis***

Rezumat

Pseudomonas aeruginosa (Pa) este cel mai important patogen care influențează prognosticul pacienților cu fibroză chistică (FC). Decelarea corelațiilor asociate infecției cu Pa poate schimba modul în care se abordează planul de investigații și cel terapeutic. Acest studiu își propune să evalueze corelațiile dintre diverși factori precum vârsta la diagnostic, starea de nutriție, starea de homozigot al mutației delF508, numărul de exacerbări pe an, modificările imagistice, prezența infecției cronice cu Stafilococcus aureus, nivelul seric al imunoglobulinelor G totale (IgG) și al gamaglobulinelor și parametrii ventilatori - capacitatea vitală forțată (CVF) și volumul expirator maxim în prima secundă (VEMS1) și asocierea infecției cu Pa. Metode. Asocierile dintre parametrii analizați s-au realizat prin regresie logistică; comparațiile dintre mediile variabilelor continue s-au efectuat cu testul t-student; distribuția variabilelor s-a testat cu ajutorul testului Chi-pătrat, iar corelațiile dintre variabile s-au apreciat cu ajutorul coeficientului Pearson. Rezultate. Vârsta medie la achiziția Pa a fost de 5,26 ani (±5,3 ani). Din cei 50 de pacienți incluși în studiu, 21 au contractat infecția cu Pa la o mediană a vârstei la primoinfecție de 3 ani. Am identificat un număr mai mare de exacerbări pe an la cei cu infecție cu Pa (2,71 față de 1,38 exacerbări pe an, p=0,002); creșterea scorului radiologic cu o unitate se asociază cu creșterea riscului de infecție cu Pa (OR 1,31; 95% CI 1,10-1,56; p=0,002), media scorului radiologic Brasfield fiind mai mică pentru cei neinfectați (6,9 vs. 11 puncte, p=0,001); creșterea gradului bronșiectaziilor se asociază infecției cu Pa (OR 3,75; CI 1,77-7,96; p=0,001); valorile gamaglobulinelor (15,91 vs. 19,42%, p=0,01), respectiv cele ale imunoglobulinelor G (IgG) (9,8 vs. 11,9 g/l; p=0,05) fiind mai mari în cazul celor infectați. Concluzii. Numărul crescut de exacerbări pe an, severitatea modificărilor imagistice, nivelul IgG și cel al gamaglobulinelor se asociază infecției cu Pa. Urmărirea acestor parametri ghidează conduita terapeutică.

Cuvinte-cheie: ***Pseudomonas aeruginosa, factori de risc, fibroză chistică***

Introduction

There is a characteristic group of pathogens in cystic fibrosis (CF) which are acquired in an age-dependent sequence. Among the microorganisms that may cause infections in CF there are: *Staphylococcus aureus,* a pathogen for immunocompetent individuals, and *Pseudomonas aeruginosa (Pa), Burkholderia cepacia, nontypable Haemophilius influenzae, Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans*, which are considered opportunistic agents. Of these, although Pa is not the first pathogen to colonize respiratory tract of patients with CF[1], it is by far the most important pathogen in CF[2], Pa respiratory infection being the main cause of mortality and morbidity[3]. This may occur early in the disease, and the prevalence increases with age, reaching 80% in adults. Primo-infection with Pa is characterized by isolation of non-mucoid colonies with high sensitivity to antibiotic, which provides a "window of opportunity" for prompt intervention in order to delay or to prevent the progression to chronic infection[4]. Between the two situations, there is an intermittent isolation of the infection with Pa. The most important feature of Pa is its ability to persist in the lung as a result of its extensive genetic palette of gaining antibiotic resistance. The development of biofilm characterizes the development of chronic infection. Chronic infection with Pa is associated with poor outcome in terms of life expectancy for those infected, the risk of death increases 2.6 times[5], pulmonary function, radiographic scores, pulmonary exacerbations and nutritional status[6,7]. The aim of this paper is to evaluate the factors associated with Pa infection and clinical and laboratory evolution after Pa respiratory infection. The detection of such factors provides additional data in order to select groups of patients at risk for poor outcome.

Material and method

The present study is an observational cross-sectional study. It was conducted on hospitalized patients in the Pulmonary Clinic from "Grigore Alexandrescu" Emergency Clinical Hospital for Children Bucharest, between 2000 and 2016. The study was approved by the Ethics Committee of the same institution. CF was diagnosed based on typical clinical manifestations and using Wescor sweat conductivity test method and genetic testing. Pa infection was established based on cultures of tracheobronchial aspirate. For each patient, clinical and demographic data were processed: gender, area of origin, age at diagnosis of CF, age of primo-infection with Pa, the presence of chronic infection with PA[8], the association of chronic respiratory infection with *S. aureus*, nutritional status (agreed by common consensus of nutritional status assessment[9]), *delF508* genotype; lung function was assessed using ventilation parameters - forced vital capacity (FVC) and one-second forced expired volume (FEV1) expressed as a percentage of the expected values in healthy population (according to the "Knudson" equation - device Jaeger Flow Screen 2005) to those over 6 years of age who could cooperate to perform spirometry. Radiographic modifications were quantified using Brasfield[10] score, and the degree of bronchiectasis was established using Bhalla[11] score. Exacerbations were defined by increases in respiratory symptoms: cough, sputum, fever, associated with weight loss and decrease of spirometric values, independent events if they were separated by at least 21 days, events which required hospitalization and intravenous antibiotics. Laboratory data, which included serum levels of immunoglobulin G and the gammaglobulin (GG) depending on age and sex, were collected from the medical database of "Grigore Alexandrescu" Emergency Clinical Hospital (Hippocrates interface).

Statistical processing

Baseline measurements of investigated parameters for each patient were recorded at the time of enrollment (March 2016). Data were summarized using descriptive statistics. Continuous variables are expressed as means ± standard deviation (SD). To compare the means of continuous variables, T-student test was used, and to analyze the distribution of non-parametric variables we used the Chi-square test. The Receiver Operating Curve (ROC) was used to test the accuracy of Brasfield score in determining Pa infection. The independent associations of tested variables and the occurrence of Pa primo-infection were assessed by logistic regression; Odds Ratio was calculated with 95% confidence interval (95% CI). Correlations between continuous variables were assessed using Pearson coefficient (r^2). The statistical significance threshold (p) was set at ≤0.05. Statistical processing was performed using the program SPSS Statistics version 20.

Results

Seventy-six patients were identified with CF. Of these, 26 were excluded from the study because they did not have enough data to be statistically processed (they were hospitalized in other clinics or they didn't come for subsequent follow-ups). The remaining 50 patients were subjects to statistical processing; they come for regular checkups in the clinic and they receive a special treatment. The average age at CF diagnosis was 1.4 (± 3.1) years with a median of 0.4 years. Of these, 21 (42%) patients acquired the infection with Pa at some point, the median age at primo-infection being of three years, and 29 (58%) remained uninfected by the time of enrollment. Of all patients, the infection became chronic in six (12%) of them, and in four patients (8%) was isolated mucoid Pa. The small number of patients in the group of those with chronic infection, with mucoid strains of Pa, does not allow a proper statistical analysis, so that the possible associations remain the subject for a further study. The results reveal a low rate of chronicity of Pa infection, showing that aggressive treatment of primo-infection decreases the rate of occurrence of chronic infection.

Table 1 General characteristics of patients (N=50)

Infection with Pa (+/-)	21/29 (42/58%)
Mean age (years) at baseline	8±5.2
Mean age (years) at diagnosis	1.4± 3.1
Mean age (years) at primo-infection	5.26± 5.3
Area of origin (U/R)	19/31(38/62%)
Gender (F/B)	27/23 (54/46%)
Genotype At least one delF508 mutation delF508 homozygous	 37 (74%) 23 (46%)
Nutritional deficiency (with/without)	28/22 (56/44%)
The mean Brasfield radiologic score	8.38 ±4
GG mean values (%)	17.1± 4.6
Ig G mean values (g/L)	10.5 ± 3.4

The mean age at initial acquisition of Pa was 5.26 (± 5.3 years) with a mean of 6.03 ± 5.16 years for girls and 3.9 ± 5.8 years for boys, p=0.428.

After analyzing the association of each potential risk factor and the presence of infection with Pa, several correlations were identified. A higher number of exacerbations per year is significantly associated more frequent with Pa infection, 2.71 versus 1.38 exacerbations per year (p=0.002) for each increase of one unit/year, the risk of acquiring the infection being 2.95 times higher (95% CI; 1.44-6.06; p=0.003). Increasing radiological score by 1 is associated with increasing risk of acquiring the infection (OR 1.31; 95% CI 1.10-1.56; p=0.002). The mean Brasfield radiologic score was 6 ± 2.38 points for uninfected ones, respectively 9.8 ± 3.9 points for those infected with Pa (p=0.000). The accuracy with which Brasfield radiological score may establish the presence of Pa infection in the studied population was estimated to be good, the area under the ROC curve was 0.813 (p=0.000). In this regard, the best threshold value able to predict Pa infection was of 6.5 points, with a sensitivity of 95% and a specificity of 65% (Figure 1).

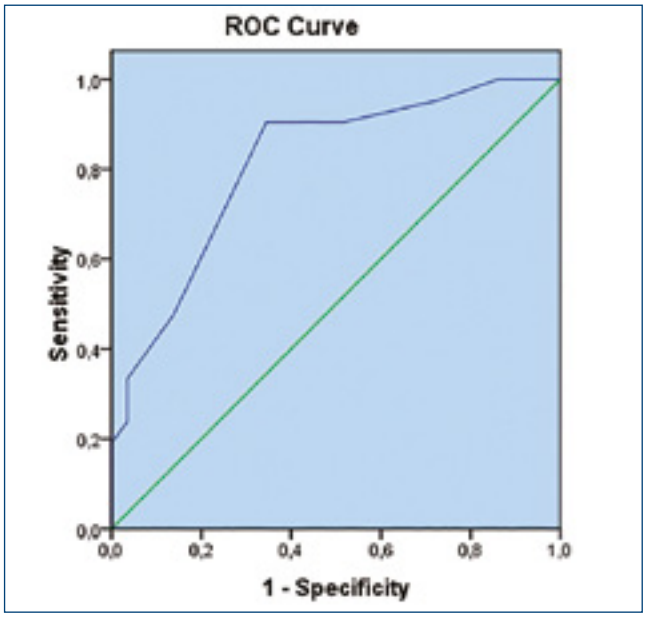

Figure 1. *Characteristics of ROC curve obtained for all 50 enrolled patients, which estimates the ability of Brasfield score to predict Pa infection*

Table 2 Characteristics of patients infected with Pa (N=21)

Parameter		P value
Gender (F/B)	13/8	0.340
Nutritional deficiency (with/without)	12/9	0.890
Concomitant chronic infection with *S. aureus*	11/10	0.827
DelF508 homozygous (+/-)	8/13	0.340
Area of origin (U/R)	6/15	0.242

Table 3 Correlations between ventilation and biological parameters

Correlation	Pearson coefficient r_2	P value
IgG/CVF	-0.362	0.045
Serum Gammaglobulins /FVC	-0.358	0.048
IgG/FEV1	-0.515	0.003
Gammaglobulins/FEV1	-0.539	0.002

In the same way, increasing the degree of bronchiectasis is associated with Pa infection (OR 3.75; CI 1.77-7.96; p=0.001). The presence of Pa is associated with elevated values of GG in serum, the mean value of GG in serum for uninfected patients is lower than for the infected ones (15.91±3.96 vs. 19.18±4.23%; p=0.01), their growth by one unit increases the risk of association of Pa infection (OR 1.18; 95% CI 1.03-1.35; p=0.01).

A significant difference was also found in the case of IgG levels, the mean value of IgG in the non Pa group being of 9.84±3 mg/dl, while in patients positive for Pa it was 11.9±4.23 mg/dl (p=0.05) for each increase of one unit, increasing the risk of an association with Pa infection (OR 1.17; 95% CI 1-1.39; p=0.05). The presence of at least one *delF508* mutation is more commonly associated with Pa infection – 16 patients (76.19%) vs. 5 patients (23.8%), p=0.016. There has been also identified a positive correlation between the value of radiologic score and IgG levels (r^2=0.531, p=0.000) and between radiologic score and GG (r^2=0.552, p=0.000). Factors such as the area of origin, gender, nutritional status, homozygous status of *delF508* mutation, concomitant chronic infection with *S. aureus* have not been identified as factors that are more frequently associated with Pa infection (Table 2).

Pulmonary function tests were performed on 31 patients (62%). Ventilation parameters (FVC, FEV1) were not worse in patients infected with PA compared to uninfected ones; mean values of FEV1 and those of FVC were similar between uninfected and infected patients (96.18±17.32 vs. 94.33±16.9%; p=0.765, and 97.43±16.1 vs. 95.73±15.4%, p=0.766, respectively).

However, for chronically infected patients, both FVC and FEV1 values were lower than in patients who succeeded the eradication (permanent or intermittent) of Pa infection (84.4±15.6 vs. 101.4±12.39%, p=0.003, and 17.64±78.6 vs. 102.2±12.39%, p=0.005, respectively). The study also determined the inverse correlations regarding ventilation parameters and serum levels of IgG and GG (Table 3).

Discussions

The study analyzed all demographic, clinical and laboratory data associated with Pa infection in patients

with CF. Twenty-one (42%) of the children included in the study had acquired the infection with Pa, the values being similar to the international ones. Data from national registers in France reported a prevalence of Pa infection of 39.7%[12]; England indicated a cumulative incidence of Pa infection (chronic or intermittent) of 49%[13]; in Australia, Pa infection prevalence was 53.3%[14]. Although females were incriminated to be associated more often with Pa infection[15], in the current study this association was not evident.

The age at wich patients were infected with Pa was influenced by a complex relationship between factors related to the host and environmental factors. In a study by Collaco *et al*., it is shown the influence of temperature on Pa infection, the average age of acquisition ranging between 9 and 15 months, earlier for those in hot areas, compared to cold areas. Rosenfeld *et al*.[16] established a relationship between the severity class of CFTR mutation and the Pa primo-infection age, these varying between 2.9 years for those with minimal CFTR function and 10.3 years for those with residual CFTR function and a median age at diagnosis of 3.25 years, this being the classic example of how the host-dependent factors influence the moment when Pa infection is acquired. This study indicates a mean age of 5.26 years for Pa primo-infection.

Early recognition and vigorous management of pulmonary exacerbations in CF are crucial in maintaining lung function, quality of life and survival. Most respiratory exacerbations are caused by infections in the lower respiratory tract with Pa[17]; this association is also found in the population studied, but there are also described other additional factors which act as triggers of exacerbations, such as the lack of adherence to treatment, respiratory viruses (respiratory syncytial virus, influenza), fungal infections / allergic bronchopulmonary aspergillosis, atypical mycobacteria etc. [18]. Retrospective studies have investigated the associations between pulmonary exacerbations and factors such as age, gender, treatment, socioeconomic status[19], but the present study does not aim to analyze these issues.

Chest radiography is the most used tool for assessing lung damage, together with microbiological monitoring. Pedrosa *et al*. report a significant deterioration of Brasfield score after Pa infection[20], this result being similar to that in our study. Moreover, Kosorok *et al*. indicate that radiological changes are more sensitive than the ratio FEV1/FVC in the detection of early changes in the lung disease associated with CF[21]. Patients with CF have a wide range of lung abnormalities that can be visualized on computed tomography scans (CT), of which the most common (76%) is bronchiectasis, which correlates with pathophysiological factors such as inflammation and infection[22]. As reported by Robinson *et al*., the degree of bronchiectasis extension is strongly correlated with Pa infection, this having a score of 9.6 for uninfected patients and of 29.9 for those with Pa[23]. In the current study, assessing changes on lung CT scan was limited to the degree and extension of bronchiectasis (according to Bhalla score), the results obtained indicating an increased severity of bronchiectasis in patients infected with Pa.

As in the case of specialty literature data[24], the findings show higher values of both serum IgG and GG in the group of infected children, reflecting the inflammatory and immunological response to Pa infection.

The study of Maselli et *al*., conducted on 180 children with CF, has identified a more frequent association of Pa infection in homozygous patients with *delF508* mutation[25]. The current research has not found such an association, but has identified a higher rate of Pa infection in patients having at least one *delF508* allele. Moreover, Levy *et al*.[1] indicate an increased association with the transition to mucoid Pa in the presence of at least one *delF508* mutation, contrary to Martha *et al*.[26] who indicates a decrease in the risk of transition to mucoid Pa for those who have at least one *delF508* allele. Also, chronic infection with *S.aureus* was not a risk factor associated with Pa infection, although previous studies show contradictory results. More authors[25,27] reported that both chronic infection with *S.aureus* and a recent history of infection are associated with a lower risk of acquiring Pa infection, while Rosenfeld *et al*.[15] reported younger ages for Pa infection in those patients in whom *S. aureus* was isolated. Thus, the role of *S. aureus* remains unclear. It is possible that the high incidence of *S. aureus* infection at an early age may create this protection against Pa infection, however, this is a short-term protection and the transition from being infected with *S. aureus* to uninfected status may create a "window of opportunity" for Pa colonization, *S. aureus* pulmonary infection being synonymous to a less advanced lung injury, Pa being absent or "non-mucoid". To support even more this hypothesis, Ratjen *et al*. and Stutman *et al*. found that patients receiving continuous anti-staphylococcal therapy had higher Pa infection rates than those receiving intermittent or no antibiotics[28,29].

The study failed to establish a relationship between being infected with Pa and poor nutritional status, although several studies report the opposite[15,30]. Similar results were reported by Ranganathan *et al*.[31]. Instead, they identified an association of early infection with *S. aureus* in those with lower values for BMI Z-score.

The study of Amin *et al*.[32], who followed 116 children with CF for five years, has identified an association between infection with Pa and a more severe impairment of lung function. FVC and FEV1 decline was not different in patients who have never been infected with Pa compared to those intermittently infected or those chronically infected. Similarly, Zemanick *et al*.[16] reported the same associations. Contrary to these studies, Kozlowski *et al*.[33] argue that those who were infected at some point with Pa, even if the eradication was successful, have a higher deterioration of lung function compared to those at whom Pa was never isolated.

In the presented study, lung function assessed by the same parameters (FVC, FEV1) did not significantly differ between uninfected and infected patients, but these parameters were significantly altered in patients whose

infection was chronic. These findings could be explained by the fact that the inflammatory response of the host acts as a "double edged sword": in the first step, mediators of inflammation are produced in a physiological manner in response to infection in CF: viscous mucus, altered mucociliary clearance, increased adhesion for Pa; failure of these mechanisms lead to chronic infection and decline in the lung function. Going forward, Sanders *et al*. report only mucoid Pa infection as a prognostic factor for the impairment of the lung function[34].

Conclusion

In conclusion, the study identified clinical and laboratory correlations with Pa infection. The increased number of exacerbations per year, the deterioration of radiological scores (X-ray and CT), the elevated values of serum GG and IgG represent the main parameters correlated with Pa infection. In the studied group of children, nutritional deficiencies, homozygous *delF508* status or gender were not associated more frequently with Pa infection. Also, only chronic infection with Pa was associated with significantly lower values of spirometry parameters. Although observational, these results provide support for determining a profile of patients with CF from the perspective of the most important moment of the natural course of this disease, namely the infection with Pa.

The rigorous follow-up of patients with CF through these parameters, along with efforts to isolate Pa in respiratory secretions (sometimes difficult to perform in case of children), constitutes a guide in determining therapeutic conduct. ■

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