Original Article

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TANAFFOS

Combination of Serologic Tests and Sputum Culture Results as the Best Diagnostic Method of *Pseudomonas aeruginosa* Infection in Cystic Fibrosis Patients

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Background: One of the most common reasons for mortality in patients with cystic fibrosis (CF) is lung infections, among which *Pseudomonas aeruginosa* (*Pa*) infection has the largest share. Diagnosis of *Pa* can be assessed by various methods such as sputum culture results and IgG antibody level via measuring the specific anti-*Pa* antibodies. This study aimed to select the best predictive technique in the diagnosis of *Pa* in CF patients through spirometry, sputum culture, and serum IgG antibody levels.

Materials and Methods: In this cross-sectional study, blood and sputum or pharyngeal samples were taken from 68 patients with cystic fibrosis. Because spirometry was not possible in all patients, 34 patients could do the spirometry. The samples were studied concerning *Pa* infection. The data including variables such as age, sex, and spirometry results were obtained. Then, in the serologic method, 3 serum-specific antibody levels were determined by enzyme-linked immune sorbent assay (ELISA).

Results: The average age of children was 7.4 ± 5.6 (ranging from 0.5 to 23) years. Generally, the percentage of *Pa* infection increased in CF patients with higher ages. A statistically direct significant relationship was observed between the concentration of serum IgG antibodies in patients with CF and *Pa*-positive sputum culture results ($p<0.05$).

Conclusion: Serum IgG antibodies against specific *Pa* antigens could be a diagnostic method against *Pa* infection, especially in patients who cannot expectorate. However, because of the positive and negative predictive value of both serum IgG antibody levels and the results of the sputum culture, we suggested that utilizing the combination of these methods could be beneficial in earlier diagnosis of *Pa*.

Keywords: *Pseudomonas aeruginosa*; Cystic fibrosis; Immunoglobulin G

INTRODUCTION

The most common cause of death in CF patients is pulmonary infections (1, 2). Among these, *Pseudomonas Aeruginosa* (*Pa*) is the prevailing pathogen seen in samples of CF patients of any age. This infection is usually initiated without any specific clinical symptom (3). The epidemiologic data show that many children with CF experience infections caused by *Pa* in their early lives (2). This eventually results in the loss of lung volume (FEV_1) in the infected patients. It is believed that early therapeutic interventions may delay the progressive damage to the lungs caused by *Pa*, or even prevent the onset of chronic infections (4). Therefore, the early diagnosis of *Pa* in CF patients is essential.

Many investigations mentioned the sputum culture and bronchoalveolar lavage (BAL) as diagnostic tests of *Pa*. Currently, BAL is accepted as the gold standard test in the diagnosis of *Pa*. However, BAL is expensive, invasive, and needs expertise.

Sputum culture is also obtained by expectorated or induced sputum as another diagnostic method (5). Unfortunately, in children younger than six, obtaining the expectorated sputum is difficult, and oropharyngeal cultures may also not be able to show the colonization in lower airways (3). In addition, younger patients cannot perform the spirometry (6). Therefore, each of these tests has some limitations and other complementary methods may help detect the *Pa* infection.

In this regard, the results of some studies suggested the measurement of the serum levels of IgG antibodies for specific *Pa* antigens [elastase (ELA), exotoxin A (ExoA), and alkaline protease (AP)] as another diagnostic test for *Pa* infection in CF patients (7). Although these studies manifested that the level of these antibodies could be useful in the diagnosis of early *Pa* infection before positive sputum culture test, and also in elucidating the disease status such as remission of *Pa* or developing to chronic level, suggesting the *Pa* antibodies test as the best diagnostic factor for *Pa* infection is controversial (7).

Considering the lack of evidence about the correlation between *Pa* diagnostic tests such as sputum culture and specific *Pa* antibodies, utilizing the best method for the diagnosis of *Pa* in CF patients is essential. The aims of the present study are the evaluation of the best diagnostic test for the diagnosis of Pa in CF patients between sputum culture and serum IgG antibodies in response to *Pa* antigens and also the correlation between these two tests based on positive and negative predictive values. Moreover, we evaluate the effects of *Pa* in spirometry test in patients who were eligible for this test in addition to assessing the effect of sex and age on *Pa* infection in CF disease.

MATERIALS AND METHODS

Patients

In this cross-sectional study, the patients were chosen from the age range of 0.5 to 23 years who were admitted to Mofid Children's Teaching Hospital of Tehran from April 2013 to May 2014. The diagnosis of CF was confirmed with two sweat chloride tests (5). Mofid Hospital is a referral center and all patients are known as having CF disease.

Ethical standards

The study protocol is consistent with the ethical guidelines of the 1975 Declaration of Helsinki as reflected in prior approval by the institution's human research committee. After telling the purposes of the project to their parents and getting a written consent from them, the eligible patients were included in the study (Ethic No: IR.SBMU.Rec.1394.71).

Method

Non-random samples of blood (2 mL) and sputum samples were taken from 68 patients with CF continuously after the physiotherapy. In case sputum samples cannot be obtained, patients are given salbutamol (100 µg) inhalation followed by nebulization with hypertonic saline 5% for 5 minutes, and then asked to expectorate. If sputum cannot be produced, expert physiotherapy is performed. Standard throat swab cultures are then taken (5).

For the diagnosis of *Pa,* various media were used including Eosin methylene blue (EMB) (for gram negatives), chocolate agar (for *Haemophilus influenza*), blood agar (for gram positives), and Mannitol salt agar (for *staphylococcus aureus*). For suspicious samples, MacConkey culture was used. *Pa* was grown in specific cultures such as blood agar, chocolate agar, and EMB at optimum 37°C in mucoid-positive oxidative colonies with specific colors (5).

Personal information, paraclinical results, and clinical symptoms of the patients who received treatments including antibiotics in the last 3 months were recorded. The blood samples were obtained from a suitable vein and then centrifuged to separate serum from it, and then the serum samples were kept under -20°C until all the samples were collected (8). Afterward, the prevalence of *Pa* in the

patients was determined based on the results of sputum cultures, and patients were divided into two groups: *Pa*-positive and *Pa*-negative. Both groups were assessed for age and sex. Subsequently, to evaluate the relation between *Pa* infection and age, patients of both groups were divided again into 4 different groups: 2 years old and less, 3 to 7 years, 8 to 13 years, and 14 years and above. As exclusion criteria, we did not include patients who were using systemic corticosteroids.

Anti-*Pa*-specific antibodies

To study the relation between serum IgG antibody levels and the results of sputum cultures for *Pa* in patients with CF, serum levels of three specific antigens including elastase (ELA), exotoxin A (ExoA), and alkaline protease (AP) were measured based on the instructions of Mediagnost kit (made in Germany) using the ELISA method with considering the quality control precautions. The antigens titers were analyzed according to the brochure of the mentioned kit (negative 1:1250). Then, patients who were positive for at least one of the antigens were categorized as IgG-positive, and the rest were put in IgG-negative group (8). Specificity, sensitivity, and positive and negative predictive value of the three antigens were determined according to the results.

Spirometry was not possible amongst the patients especially younger children. In this regard, 34 patients could perform spirometry test. They were categorized into four groups based on FEV_1 and FEV_1/FVC percentages: >80%, 60-80%, 40-60%, and <40%. Obtained data were analyzed to assess the effects of *Pa* on spirometry in eligible patients as our investigation's extra findings.

Statistical analysis

The collected data were encoded, entered into SPSS-18, and got analyzed through this statistical software. To evaluate the relation between the qualitative variables, the Chi-square test (Fisher's exact test) was obtained. Normal distribution of data was assessed with Kolmogorov–Smirnov test and the relation between the results of the serum level of immunoglobulin against *Pa* with the results of sputum culture and antibiotic consumption was checked out using the Chi-square test. If the data distribution was not found normal, the Kruskal–Wallis test was applied. The significance level was set at 0.05.

RESULTS

Overall, a group of 68 patients with CF were studied of which, 26 (38.2%) were female and 42 (61.8%) were male.

Table 1. represents the baseline criteria of CF patients with *Pa*. Sputum culture and serum IgG test were done for all patients. In addition, in this table, we showed the median age (7.4±5.6) and also assessed FEV_1 in and FEV_1/FVC in 34 CF patients with *Pa*.

Table 1. The baseline data of CF patients with *Pa*

Characteristic of patients with CF (N=68)		Number (%)
Sex	Male	42 (61.8%)
	Female	26 (38.2%)
Age		7.4±5.6
Sputum culture positive		35 (51.5%)
IgG positive		34 (50%)
FEV_1	>80%	16 (23.5%)
	60-80%	6 (8.8%)
	40-60%	6 (8.8%)
	<40%	6 (8.8%)
FEV_1/FVC	>80%	16 (23.5%)
	60-80%	8 (11.7%)
	40-60%	7 (10.2%)
	<40%	3 (4.4%)

Both of these sub-groups were categorized according to their sputum culture test results for *Pa*. Based on the results of sputum cultures for *Pa*, 35 were *Pa*-positive and 33 were *Pa*-negative.

We also demonstrated the information about age, sex, and spirometry based on the results of sputum culture in Table 2. With regard to this table, there is a significant correlation between age and *Pa* infection in patients with CF (P=0.002). The findings also point out that there is no meaningful correlation between sex and *Pa* infection in patients with CF (P=0.220).

Table 2. Comparison between the two groups of children and young adults with CF, regarding age, sex, and spirometry (According to their sputum culture results for *Pa*)

		Sputum culture positive	Sputum culture negative	P value
		No. (%)	No. (%)	
Sex	Male	19 (27.9%)	23 (33.8%)	0220
	Female	16 (23.5%)	10 (14.7%)	
Age		9.4 (5.9)*	5.4 (4.4)	0.002
FEV_1	>80%	9 (13.2%)	7 (10.2%)	0.698
	60-80%	4 (5.8%)	2 (2.9%)	
	40-60%	4 (5.8%)	2 (2.9%)	
	<40%	5 (7.3%)	1 (1.4%)	
FEV_1/FVC	>80%	7 (10.2%)	9 (13.2%)	0.055
	60-80%	8 (11.7%)	0 (0%)	
	40-60%	5 (7.3%)	2 (2.9%)	
	<40%	2 (2.9%)	1 (1.4%)	

*: *P<0.05*

In terms of categorization of patients with CF based on their sputum culture results for *Pa*, patients of both groups were placed in 4 age groups to investigate the relationship between *Pa* infection and age (Figure 1). According to the obtained data, the frequency of *Pa* in CF patients was significantly higher in the groups aged between 8 to 13 years ($p<0.05$). Generally, the chance of *Pa* infection had increased in older ages, and *Pa*-negative patients were mostly younger. Table 2 also shows the spirometry results of the studied groups: there was no significant difference between the FEV_1 in patients with CF and *Pa* infection rate (P=0.698), neither between the FEV_1/FVC ratio and *Pa* infection rate (P=0.055).

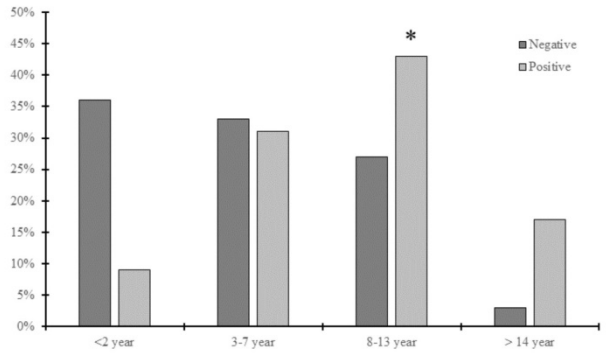

Figure 1. Comparison of frequencies of sputum cultures results for *Pa* in children and older ages with CF, *: *P<0.05*

The results of IgG serum levels in patients with CF are indicated in Table 3. Assessing the sputum cultures concerning *Pa* infection, 33 patients (49%) were *Pa*-negative and 35 (51%) were *Pa*-positive. In terms of serum IgG antibody levels of studied patients, 34 patients (50%) were detected *Pa*-positive, and the other 34 (50%) were detected *Pa*-negative.

Table 3. Comparison of sensitivity, specificity, and positive/negative predictive value in different *Pa*-positive antibodies in serum IgG of children and young adults with CF

IgG serum level	Sputum culture results for PA		Total Number (%)
	Positive number (%)	Negative number (%)	
Positive number (%)	24 (35.3%)	10 (14.7%)	34 (50%)
Negative number (%)	11 (16.2%)	23 (33.8%)	34 (50%)
Total number (%)	35 (51%)	33 (49%)	68 (100%)

Regarding the comparison between sputum culture results and IgG serum levels, 24 people (35.3%) were positive in both tests, 23 (33.8%) were negative in both tests, and the rest had various results for sputum culture test and IgG serum level. There is a direct statistically significant correlation between IgG serum level in patients with CF and sputum culture results for *Pa* (P=0.001). The percentages of sensitivity, specificity, and positive/negative predictive value for different positive anti-*Pa* antibodies of patients are summarized in Table 4. With regards to the data in Table 4, ELA had the highest level of sensitivity, and AP had the highest rate of specificity in the diagnosis of *Pa*. AP had also the highest rate of positive and negative predictive values.

Table 4. Comparison of sensitivity, specificity, and positive/negative predictive value in different *Pa*-positive antibodies in serum IgG of children and young adults with CF

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Exotoxin A	40	79	67	55
Elastase	57	76	71	63
Alkaline protease	54	88	83	64
Presence of at least one anti-body	69	70	71	68

DISCUSSION

One of the most common causes of respiratory infection in CF patients is *Pa* infection which leads to the majority of the related mortality. The present study aims to find the best way for early diagnosis of *Pa* in CF patients. For this aim, we assessed different *Pa* diagnostic methods amongst sputum culture and serum IgG antibody levels via specific antibodies assay. In addition, we investigated the correlation of age and gender with the incidence of *Pa* infection in CF patients. As complementary data, we also showed the effects of *Pa* on spirometry in eligible patients who could do this test.

Spirometry

Several studies have indicated that *Pa* infection raises the hospitalization rate and respiratory insufficiency, as well as increases mortality in CF patients (9). One of the techniques we used in our study was spirometry. Our results demonstrated no meaningful relation between *Pa* infection and FEV_1 and FEV_1/FVC. According to Kosorok et al., one reason for this is the challenge of conducting spirometry tests on children under the age of 7 (6). Furthermore, the study revealed that the initial impact of *Pa* is on the peripheral airways, which does not have an impact on the FEV1 and FEV1/FVC (6). In another study, authors concluded that performing the spirometry is difficult in ages under 5 and also no significant correlation was observed between *Pa* and spirometry. In addition, they suggested that spirometry is more efficient in the diagnosis of early *Pa* in CF patients in combination with serum IgG antibody levels (9). So, based on our results and other investigations, we can deduce that spirometry is not a sufficient method to reveal *Pa* infection in CF patients.

Sputum culture

The most common method to detect *Pa* in CF patients is sputum culture. Our study reveals a significant retaliation between sputum culture and *Pa* infection. But, considering the positive sputum culture as the best method for the early diagnosis of *Pa* is controversial. The study performed by Rosenfeld et al. showed that negative oropharyngeal (OP) culture can predict negative *Pa* infection with 95% accuracy compared with Broncho-alveolar lavage (BAL). However, positive OP cultures do not reliably determine the presence of *Pa* in lower respiratory airways (10). Another study demonstrated that OP culture could successfully predict *Pa* infection in 83% of samples in comparison with BAL but cannot rule out *Pa* infection in negative samples (11). Also, there are some implications in OP culture such as the site of sputum collection and sampling techniques (hypertonic saline, cough induction, etc.). However, compared to BAL which is aggressive, expensive, and needs anesthesia, this method is more common. Hoppe et al, showed that the results of sputum cultures by OP swab are not reliable in children younger than 10 years old (12), and utilizing the sputum induction technique similar to which we used in our study is more accurate to diagnose the infection in CF patients compared to OP swab but needs an expert team and enough facility. Nevertheless, the microbiology of lung infections for this young age group, based on findings in pathology samples taken during intubation, is highly recommended in the case of severe illness along with selective surgery, and bronchoscopy cases (13). As a result, the use of sputum culture is still favorable as an accessible and cost-effective way for the diagnosis of *Pa* in CF patients.

IgG and specific antibodies

In this study, we showed a significant relation between positive serum IgG antibody levels and positive sputum culture in *Pa*-positive patients with CF. The IgG-positives were considered via the presence of at least one of ExoA, ELA, and AP as specific antigens of *Pa* in the serological analysis of the CF patients.

In this regard, a study conducted by Pressler et al. showed that the serum IgG level measured by *Pa*-specific antibodies increased in a group of CF patients who had intermittent infections with *Pa* that were diagnosed by sputum culture. These levels of specific antibodies remained high after these patients acquired *Pa* infection chronically. In this research, it was concluded that specific antibodies could be a good predictive value for the diagnosis of *Pa* infection in CF patients. (14) . The results of

other studies showed that although IgG antibodies against specific *Pa* antigens are favorable markers for diagnosis of *Pa* in every stage of disease (early, intermittent, and chronic), they had some patients with positive antibodies who never acquired *Pa* (8, 15). They concluded that because these antibodies are probably elevated in the serum in response to other antigens, this method has a high rate of false positives (15). They also showed that although the level of these antibodies might be elevated in the chronic phase of the disease, the high level of antibodies did not have a consistent manner and had inconsistency regardless of the results of sputum culture (8).

Tramper-Stranders et al. assessed the diagnostic value of serological tests. They suggested that despite the high sensitivity of serological tests using specific antibodies against *Pa* in CF patients, their unresponsiveness in the early stages of colonization in young patients necessitates the need for microbiological tests and cultures (16). Therefore, Mauch and Levy concluded that the accuracy of *Pa* antibodies is still controversial and attributed this problem to the absence of a study for assessment of the correlation between *Pa* antibodies and the sputum method for diagnosis of *Pa* in CF patients (15).

Based on the studies mentioned above and the result of our study, the serum IgG level is a favorable diagnostic method for the diagnosis of *Pa* in CF patients. In addition, IgG level has a direct significant correlation with the results of positive sputum culture. However, because both of these tests have positive and negative predictive values, it emphasizes that these methods are not diagnostic in all cases of CF. In this regard, we can conclude that utilizing the sputum culture alongside the serum IgG level via specific anti-*Pa* antibodies could be beneficial in the diagnosis of *Pa* in CF patients.

Specific antibody's sensitivity and specificity

In this study, we also investigated the sensitivity and specificity of anti-*Pa* antibodies including ELA, AP, and ExoA. Our results showed that ELA had the highest sensitivity and AP had the highest specificity in the diagnosis of *Pa*. In addition, AP had the highest positive and negative predictive value. Other investigations showed that measuring the magnitude of specific antibodies can be helpful in the diagnosis of *Pa* infection in CF patients (17).

Consistent with our findings, Dogru et al. in 2013, asserted that amongst *Pa*-specific antibodies, AP had the highest specificity rate (84%) and ELA had the highest sensitivity rate (44%) against alginates produced by *Pa*. These findings are in line with the results of our study, but the difference is that our results showed a higher rate of AP in comparison to their study (88% and 57% respectively). They claimed that in children younger than five years old with positive sputum culture results, the antibody concentration was considerably higher than in older ages (8).

Similar to these investigations, our results showed that measuring the amplitude of AP and ELA levels in serologic tests could be used as a favorable diagnostic method, especially in children who are unable to produce the sputum.

Age and gender

In the present study, we also investigated the correlation of age and sex in the development of *Pa* infection in CF patients. According to the results of sputum culture presented in our study, there was a meaningful relationship between the age of patients and *Pa* infection (P=0.002). *Pa* infection significantly increased with age in patients with CF which is compatible with previous studies (18).

Li et al. conducted a study to assess the prevalence of *Pa* in CF patients and its influences on lung function. They found that the prevalence of *Pa* infection was higher in older patients (13). A study by Aebi et al. confirmed the aforementioned study (19). According to the current study, this was valid for ages between 0.5 to 13. However, in the age group above 14, the number of *Pa*-infected CF patients significantly decreased. This finding could be explained by Pittman et al. who conducted a study to evaluate *Pa* infection and its relationship with age in CF patients. It

showed that *Pa* infection has a higher prevalence in pre-school years. This could be connected to changes in lifestyle (20).

Regarding the references, there is some inconsistency that can be explained to some extent. However, more studies are needed because there isn't enough clarity on this issue. The mentioned subject thoroughly approves that the average age of children and adolescents is supposed to be higher in *Pa*-positive group rather than *Pa*-negative infection in CF patients. A study by Olsen et al. in 2010 concluded that the prevalence of *Pa* infection in CF patients has nothing to do with sex which supports the findings of this study (P=0.22) (21). A study by Demko et al., undertaken on 848 patients, also confirms this conclusion and clarifies that women with CF and *Pa* infection survive 3 years less than men (22).

Limitation

It was a cross-sectional study and as the prevalence of *P*a in CF patients is quite low in younger ages, we had some limitations regarding the number of studied cases younger than 2 years old.

CONCLUSION

This study showed that specific antibodies have a significant correlation with positive sputum culture of *Pa* in CF patients. Both of these tests have positive and negative predictive value which emphasized that these methods are not diagnostic in all cases of CF. In this regard, we can conclude that utilizing the sputum culture alongside serum IgG level via specific anti-*Pa* antibodies could be beneficial in the diagnosis of *Pa* in CF patients. In addition, measuring the AP (highest specificity) and ELA (highest sensitivity) amplitude could be used for *Pa* diagnosis in children who are unable to produce sputum.

Acknowledgments

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