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Review

Serum antibodies to *Pseudomonas aeruginosa* in cystic fibrosis as a diagnostic tool: A systematic review

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Abstract

Background: A systematic literature review of the last 40 years on the research of serum antibodies to *Pseudomonas aeruginosa* in cystic fibrosis and its utility as a diagnostic tool.
Methods: Research papers in English, Portuguese, and Spanish were accessed through electronic databases (PubMed, Medline, LILACS, and SciELO).
Results: 26 studies were assessed. ELISA technique was the most commonly used technique to detect serum *P. aeruginosa* antibodies. The most consistent results were those in which the response against the antigen St-Ag:1–17 was evaluated. The accuracy levels of the ELISA technique remain controversial, but most studies showed a good correlation between antibody titers and microbiological culture.
Conclusions: The detection of serum antibodies to *P. aeruginosa* shows capacity for early detection of this pathogen and potential utility and viability of incorporation in the diagnostic routine of patients with cystic fibrosis.

Keywords: *Pseudomonas aeruginosa*; Cystic fibrosis; Serum antibodies; Review

Contents

Abbreviations: CF, Cystic fibrosis; BAL, Bronchoalveolar lavage; ELISA, Enzyme-linked immunosorbent assay; CIE, Crossed immune electrophoresis; RIA, Radioimmunoassay; ExoA, Exotoxin A; ELA, Elastase; AP, Alkaline protease; TTSS, Type three secretion system; PCR, Polymerase chain reaction; PPV, Positive predictive value; NPV, Negative predictive value

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1. Introduction

Pseudomonas aeruginosa pulmonary infection is responsible for elevated morbidity and mortality among cystic fibrosis (CF) patients [1,2]. In childhood, 10 to 30% of these patients are colonized and in adulthood, 80 to 90% are infected with this bacterium. Patients infected with *P. aeruginosa* generally present a reduction of about 10 years in life expectancy when compared with the non-infected ones [3].

When chronic infection [4] is established, *P. aeruginosa* is practically impossible to be eradicated [1,2], although, its clearance from the respiratory tract is possible through early intervention with antibiotic therapy, as soon as the pathogen settles in the organism. Thus, early aggressive treatment is recommended, being possible to delay the chronic colonization and progression of the pulmonary disease [5].

Detection of *P. aeruginosa* in the diagnostic routine is made mostly through sputum culture — spontaneously expectorated or induced by inhalation of hypertonic saline (3–7%); however, many patients – especially children under 7 years – are incapable of producing an expectorated sputum specimen. BAL, the gold standard, is an option, but it is invasive and usually employed only when there is a compelling reason to obtain a respiratory sample and other approaches have failed. For this reason, oropharyngeal (OP) swabs are used as a surrogate for sputum or BAL fluid specimens in these individuals, sampling the microbial flora of the upper respiratory tract, which is assumed to reflect that of the lower respiratory tract [6]. Despite the good specificity reported for this method, in comparison to BAL culture, it is known that a negative culture from the upper airways does not exclude the presence of *P. aeruginosa* in the lower airways [7,8]. Sampling errors during the BAL and the frequent insufficient sample obtained through oropharyngeal swab can lead to false negative results [7]. The difficulty in obtaining representative respiratory specimens from the airways of infants and children indicates the need for the use of methods that can complement or be an alternative to microbiological culture [9].

The detection of serum antibodies against *P. aeruginosa* has emerged as a possible auxiliary method to assess the early eradication therapy [10,11]. Highly sensitive methods for detection of antibodies against several *P. aeruginosa* antigens may complement the monitoring methods currently used [11]. Positive antibody titer results and culture-negative samples of respiratory secretion should alert health professionals to perform a more thorough search for a probable infection, by repeating the test or by using more sensitive and specific methods. In contrast, increasing antibody levels are associated with a greater likelihood of persistent *P. aeruginosa* chronic infection [9]. Elevated antibody titers at the time of initial OP culture may be a useful tool for CF clinicians and researchers monitoring patients at risk for subsequent infection. However, their use in routine practice remains controversial [9].

Thus, the aim of this systematic review was to collect studies published in the last 40 years addressing the detection of serum antibodies to *P. aeruginosa* and to evaluate its utility for early detection of this bacterium and the diagnostic and prognostic value in CF patients.

2. Methods

Research papers in English, Portuguese, and Spanish in the period from 1973 to 2013 were reviewed. The search for references was made through electronic database exploration (PubMed, Medline, LILACS and SciELO), using the keywords "Cystic Fibrosis", "*Pseudomonas aeruginosa*", "serology", and "serum antibodies", and their correspondent translations in varied combinations, from November to August of 2013. In addition, the references of all papers were consulted in the search of new papers to include.

Then, we began the paper selection process, by reviewing titles and abstracts. The first inclusion criterion was the identification of potentially relevant papers, considering those in which the study assessed the utility of serum antibody detection in the diagnostic routine of pulmonary disease. The recuperation criteria for complete papers were the following types of study: cohort, longitudinal, case–control, descriptive, experimental and cross-sectional, whose results addressed such subject. The selection was based on the limits of the compliance of issues in relation to objectives, excluding those in which, despite appearing in the search results, did not address the issue from the diagnostic point of view of serum antibody detection. We also excluded review articles, studies addressing the immune response to *P. aeruginosa* but not related to CF, or related to CF but without clinical application or applied to the diagnosis and studies not related to the CF pulmonary disease.

3. Data synthesis

3.1. Studies addressed, casuistries and methods used

In the first search, a total of 29 research papers were found, being submitted to a filter process, from which only the papers that could be fully accessed were selected. The review was finished by reading the complete papers and, in the final text, 25 papers were included. The studies were mostly longitudinal [13,14,19,21,28,30,35,37] and experimental [16,18,23,26,32–34], and there were also prospective [12,15,16], case–control [27], cohort [20,23,29] and cross-sectional studies [21,30,36]; all of them were in English (Table 1).

In the last 40 years, a total of 3148 patients were evaluated, aged from 0 to 65 years of age, and seven of the studies did not specify their age range (Table 2). With regard to the diagnostic method (Table 3), the ELISA technique was the most commonly used method (22 studies), followed by CIE (5 studies), Western blot (5 studies), and RIA (one study). Five studies used more than one method. Different antigens were used, being Exotoxin A (ExoA) the most used (13 studies), followed by Elastase (ELA) (10 studies), St-Ag:1–17 (Statens Serum Institute®) (8 studies), Alkaline protease (AP) (8 studies), Lipopolysaccharides (LPS) (3 studies), and other antigens in 8 studies (Table 2). Six studies used the M-15 kit (Mediagnost®) — ExoA, ELA, and AP.

3.2. Diagnostic and prognostic value

The pioneer studies on serological diagnosis of *P. aeruginosa* infection in CF patients date from the 70s by Høiby et al. [12],

based on the antibody detection by precipitation tests (precipitins) of CIE. In a 5-year follow-up of 133 patients, high precipitin titers and rapid increase of these were associated with the worst prognostic, which was corroborated by Döring et al. [13], who found a higher precipitin production when mucoid *P. aeruginosa* persisted in the lungs, leading to a higher formation of immunocomplexes.

In the 80s, the ELISA technique was included using great antigen diversity. Overall, there was a significant correlation between ELISA and CIE results [13,16,18].

Brett et al. [14] observed higher antibody titers in chronically infected than in intermittent patients, with an overlap between the two groups. However, higher titers against ExoA, ELA, and AP (relative to phospholipase C) were observed among patients colonized/infected with mucoid *P. aeruginosa* than among patients who only presented non-mucoid *P. aeruginosa*. Burns et al. [20] searched for IgG antibodies against *P. aeruginosa* through ExoA ELISA and Western blot for the whole cell antigens. By sputum culture, only 45% of patients would be considered to be infected, while antibody detection elevated this percentage to 97.5%. Da Silva Filho et al. [30] analyzed the serology utility (E-15 kit — Mediagnost®) for 87 patients, comparing with PCR and culture, including 16 patients with negative culture, 20 intermittently colonized and 51 chronically infected patients. They found that the association of the three methods provided the highest positivity percentage (PCR with the higher one), but had no statistical significance. Weisner et al. [28] studied the utility of ELISA for *P. aeruginosa* anti-LPS band A antibodies in oral fluids compared with serum samples in patients with positive culture for *P. aeruginosa* and healthy volunteers. They found significant titers in the oral fluids in 15 of the 17 CF patients and in all serum samples, but in none of the volunteers. Doğru et al. [37] showed significant higher antibody titers against ExoA, ELA and AP in patients colonized with mucoid *P. aeruginosa*. The antibody presence showed varied values, with ELA antibodies presenting the higher titers.

3.3. Early detection of the P. aeruginosa infection

Brett et al. [17], in another ELISA study (for antibodies against cell wall antigens), observed an enhancement in IgG levels until 24.5 months before the first bacterial isolation in culture. Kronborg et al. [19] showed an increase in the serum IgG, IgM and IgA responses to the lipid A, R-LPS, and S-LPS antigens during the chronic infection course, mainly IgG and IgA. Moss et al. [21] found the presence of antibodies against the TTSS proteins in adult CF patients through Western blot. About 70% patients had anti-PopD IgG, 90% had anti-PopB IgG, and 67% had anti-PcrV IgG. The humoral immune response also showed anti-ExoS IgG in about 33% of the cases, and anti-ExoA IgG in 73% of patients. There were evidences that humoral immune response was early for the TTSS components, during the initial stage of infection, as shown by other two studies [24,28,36], being the response to TTSS antigens earlier than to ExoA and PAO1 cell lysate, and the pathogen being found in a mean time of 21 months before the first isolation in the microbiological culture.

West et al. [22] reported a 15-year longitudinal monitoring of antibody response (IgG, IgM e IgA) against several *P. aeruginosa* antigens in sera of 68 patients, detecting the bacterium up to 12 months before the first isolation. Significant titers of anti-cell lysate and anti-ExoA antibodies were detected before or simultaneous to the first *P. aeruginosa* isolation in about 60% of the patients, and antibody titers against ELA were detected until about 40 months after the first isolation.

Pressler et al. [27] followed 89 patients free of infection in a period of 10 years and found 28 patients that acquired chronic infection despite of early antibiotic treatment. This group was compared with 28 patients who did not acquire chronic infection and it was observed that specific *P. aeruginosa* IgG antibodies were significantly higher in the cases than in the control group until three years before the acquisition of chronic infection, but remained at the initial levels in the control group. Later, Pressler et al. [32], following 791 patients, found that, out of 381 patients cultured negative for *P. aeruginosa*, 11 changed status to chronically infected and 24 out of 129 intermittently colonized became chronically infected. The antibody titers in this latter group were significantly higher at the study start and increased significantly during the study period. Elevated levels of specific anti-pseudomonal antibodies showed to be the risk factor for developing chronic infection.

Tramper-Standers et al. [26], in a longitudinal study, using microbiological culture as reference, monitored the IgG response to ExoA, ELA and AP of 220 patients during three consecutive years, performing serological tests annually. All the three individual serological tests discriminated well between the absence and presence of chronic *P. aeruginosa* colonization.

In 2010, Hayes et al. [33] published a longitudinal 6-year study that followed 69 children from the CF diagnosis by neonatal screening. They used serology for anti-cell lysate, ExoA and ELA antibodies. By establishing cut-offs according to the age of the patients, they could early identify *P. aeruginosa* until 2 years before the detection in microbiologic culture.

Anstead et al. [35], using five antigens and two different ELISA tests, found, in recently *P. aeruginosa* colonized children, that baseline positive serology was not significantly associated with failure in bacterial eradication with previous treatment for 10 weeks, but seropositivity to AP and ExoA was significantly associated with increased risk of recurrent isolation 60 weeks after eradication. There was no association between baseline seropositivity and time to pulmonary exacerbation.

3.4. Antibody titers and clinical status

Correlation between antibody titers and clinical status was showed by Høiby et al. [12], which found lower ventilatory function, severe radiographic alterations and worst prognosis associated with high *P. aeruginosa* precipitin levels. Brett et al. [17] observed a correlation between worst Shwachman–Kulczyky and Chrispin–Norman scores and enhancement of IgG antibodies against *P. aeruginosa* in intermittent and chronic patients. West et al. [22] performed chest radiographies at each six months for children under 3 years old and annually

Table 1
Study index, according to author, design, local, objective and results obtained.

Author	Study design	Local	Objective	Main results
Høiby et al. [12]	Prospective	Copenhagen, Denmark	Detection of anti-pseudomonal antibodies by CIE.	Poor prognosis in cystic fibrosis was associated with high numbers of precipitins that were rapidly increasing.
Döring et al. [13]	Longitudinal	Copenhagen, Denmark	To measure humoral antibody titers to AP and ELA and to determine the number of different precipitins to the St–Ag:1–17	After onset of chronic *P. aeruginosa* lung infection, the clinical state of most patients revealed declining and specific antibody production increase in all patients.
Brett et al. [14]	Longitudinal	Leeds, UK	To measure immunological changes associated with colonization and infection of the respiractory tract by *P. aeruginosa* and to assess the value of these measurements in monitoring the progress of infection	High antibody titers associated with worst clinical status and low titers associated with better clinical status both in chronic infected and intermittent patients
Hollsing et al. [15]	Prospective	Danderyd, Sweden	To elucidate the clinical value of the determination of antibodies against various antigens in relation to signs of infection and antimicrobial therapy	Serum antibodies against phospholipase C seem to be a valid indicator of chronic *P. aeruginosa* colonization.
Pedersen et al. [16]	Experimental	Copenhagen, Denmark	Development of an ELISA test to detect anti-pseudomonal antibodies, comparing with CIE	Sensitivity and specificity to detection of infection similar to CIE, but simpler
Brett et al. [17]	Prospective	Leeds, UK	To use an ELISA test for monitoring antibody titers in CF patients from whom *P. aeruginosa* was isolated for the first time during a study period of 3 years	Antibody titers altered even 24 months before the first isolation in culture. Levels decreased after antibiotic therapy
Formsgaard et al. [18]	Experimental	Copenhagen, Denmark	Development of an ELISA test to detect anti-LPS IgG and IgM *P. aeruginosa* antibodies	Enhancement in IgG anti-LPS *P. aeruginosa* antibodies in the onset and course of chronic infection, with marked affinity and specificity
Kronborg et al. [19]	Longitudinal	Copenhagen, Denmark	To investigate the appearance of specific antibodies to endotoxic lipid A as well as to the core and O saccharides of *P. aeruginosa* in serum and sputum samples during lung infection	Enhanced antibody response to all LPS antigens studied during the course of chronic infection
Burns et al. [20]	Cohort	Multicenter (USA)	To examine the agreement among *P. aeruginosa* isolates from upper and low airways; to characterize indirect infection markers	Greater infection evidence when culture and serology were combined than when only culture were performed; 97.5% of the children until 3 years old analyzed were infected.
Moss et al. [21]	Crossectional	Milwaukee, USA	Investigation of TTSS proteins through the measure of immune response against TTSS components	Evidences that antibody response is early to the TTSS components. Type III cytotoxins contribute to the pathogenicity of *P. aeruginosa* in acute lung infections.
West et al. [22]	Longitudinal	Multicenter (USA)	To evaluate the relationship between the production of antibody response against *P. aeruginosa* and clinical factors associated with pulmonary infections in patients with CF diagnosed in early life	Enhanced antibody titers before the first isolation in culture (ExoA until 6 months, cell lysate until 12 months) in patients recently diagnosed with CF
Johansen et al. [23]	Cohort	Copenhagen, Denmark	To investigate the effects of increasingly intensive treatment regimens on the anti-pseudomonas antibody response and survival of patients after acquisition of *P. aeruginosa* chronic lung infection	Lower antibody response and longer survival after acquisition of chronic lung infection in patients who were treated intensively
Corech et al. [24]	Experimental	Milwaukee, USA	To test if identification of the response to TTSS proteins can lead to early detection of *P. aeruginosa* infection than has been measured for the analysis of other pseudomonal antigens	Response to TTSS components earlier than to other antigens in the initial stages of infection, with reduced prevalence during chronic infection.
Kappler et al. [25]	Experimental	Munich, Germany	Validation of an ELISA commercialized test to detect anti-pseudomonas antibodies and *P. aeruginosa* infection	Higher sensitivity, specificity and PPV when the 3 serological assays were combined. Higher PPVs for intermittent patients, but low VPP for patients free of infection.
Tramper-Standers et al. [26]	Experimental	Utrecht, Netherlands	Evaluation of a serological commercialized test to early *P. aeruginosa* detection and chronic colonization	All three individual serological tests discriminated well between the absence and presence of chronic *P. aeruginosa* colonization
Pressler et al. [27]	Case control	Copenhagen, Denmark	To analyze risk factors for the development of chronic *P. aeruginosa* and infection	Specific anti-pseudomonal IgG serum antibodies were significantly higher in the study group than in controls) already 3 years prior to onset of chronic infection
Weisner et al. [28]	Longitudinal	London, UK	To investigate the viability of the use of oral fluid samples to detect anti-LPS *P. aeruginosa* antibodies; to compare these results with serum antibodies	Immunoblotting and ELISA are sensitive procedures for the detection of antibodies to A-band LPS of *P. aeruginosa* in oral fluid and serum from patients with CF.

for children above 4 years old. Evidences of irreversible lung injuries occurred until 5.8 months before the first positive culture, period that was similar to the appearance of anti-ExoA antibodies.

3.5. Antibody response against the infection treatment

Brett et al. [14] observed a considerable variation in IgG titers among CF patients, which decreased after *i.v.* antibiotic

Table 1 (*continued*)

Author	Study design	Local	Objective	Main results
Ratjen et al. [29]	Cohort	Multicenter (USA)	To investigate the antibody response to *P. aeruginosa* antigens in a cohort of patients	Antibody testing against AP, ELA, and ExoA offers high sensitivity and specificity for the presence of *P. aeruginosa*in respiratory cultures and may be useful to monitor response to therapy.
Da Silva Filho et al. [30]	Crossectional	São Paulo, Brazil	To compare the diagnostic value of PCR with that of anti-pseudomonal antibodies and conventional culture techniques for *P. aeruginosa* detection in CF.	The combination of PCR and serology was significantly superior to single methods, and PCR may be the main additive method for *P. aeruginosa* identification.
Milagres et al. [31]	Longitudinal	Rio de Janeiro, Brazil	To assess the value of the measurement of antibodies to *P. aeruginosa* in diagnosing lung infection using cell lysate antigens, recombinant PcrV and a Type III Secretion System protein	When serum reactivity to rPcrV and cell lysate were combined, 94% of CF patients not chronically infected had the first serology positive for *P. aeruginosa* over a mean time of 20 months before the first isolation of *P. aeruginosa*.
Pressler et al. [32]	Experimental	Multicenter (Denmark, Sweden and Norway)	To evaluate three serological methods for their ability to identify CF patients in different infection statuses specially those at risk of developing chronic P. *aeruginosa* infection.	Elevated levels of specific anti-Pseudomonas antibodies showed to be the risk factor for developing chronic Pa infection. Good accuracy of the three methods; antibodies against ExoA, ELA and AP produced several months after antibodies against St-Ag:1-17.
Hayes et al. [33]	Experimental	Multicenter (USA)	To evaluate if serological cutoffs based on age are more useful in assessing *P. aeruginosa* respiratory infections in young children with CF for diagnostic and prognostic purposes.	All the age-specific cutoffs were better than fixed cutoffs previously used, to predict and detect *P. aeruginosa* respiratory infections with a higher sensitivity and specificity.
Douglas et al. [34]	Experimental	Multicenter (Australia)	To investigate the accuracy of serum antibodies against specific and multiple *P. aeruginosa* antigens at predicting lower airway infection in young CF children	Low PPV and elevated NPV of serology, using BAL culture as gold standard
Anstead et al. [35]	Longitudinal	Multicenter (USA)	To assess whether positive serology in CF patients would predict treatment failure, time to pulmonary exacerbation and risk for recurrent *P. aeruginosa* isolation post eradication.	Baseline positive *P. aeruginosa* serology not significantly associated with failure of initial *P. aeruginosa* treatment, but seropositivity AP and ExoA were significantly associated with increased risk for recurrent isolation. No association between baseline seropositivity and time to pulmonary exacerbation.
Cruz et al. [36]	Cross sectional	Rio de Janeiro, Brazil	To investigate whether or not TTSS proteins would be recognized by CF sera.	All patients not chronically infected by *P. aeruginosa* had their first serum positive for TTSS proteins, with weak reactions observed for patients negative to *P. aeruginosa*.
Doğru et al. [37]	Longitudinal	Ankara, Turkey	To evaluate *P. aeruginosa* antibodies, compare them with microbiological culture and determine their role in early diagnosis and follow-up.	The presence of at least one antibody had the highest sensitivity to discriminated chronic colonized patients. The presence of antibodies was much higher than positive *P. aeruginosa* cultures in patients younger than five years of age.

therapy. In another study [17], there was a significant decrease in patients in which antibiotic therapy was well succeeded. Of 15 patients who received *i.v.* antimicrobial therapy, in 5 bacterial eradication and return of the antibody titers to the normal values were observed, while in 10, continuous isolations occurred, besides partial decrease of the titers. Johansen et al. [23] found a decrease in the precipitin levels during the period of most intensive *i.v.* antibiotic therapy regimens, and considerably enhanced survival (after the acquisition of chronic infection). Ratjen et al. [29], with a longitudinal evaluation of antibody titers, before and after inhalatory antibiotic therapy in patients with the first *P. aeruginosa* isolate, showed a significant reduction in the titers of patients clearing *P. aeruginosa* infection, while there was increased titers in patients in which eradication therapy failed. Just one study [15] did not observe significant decrease in antibody titers after antibiotic therapy against the evaluated antigens.

3.6. Accuracy

Eight studies evaluated the accuracy of serology (ELISA) for different antigens in regard to different patterns used (Table 4). Pedersen et al. [16], evaluating 243 patients, found a sensitivity of 93% and specificity of 92% for ELISA when compared to CIE, obtaining a correlation among results of both methods. In advanced stages of infection, the sensitivity was 90% and the specificity was 100%. Kappler et al. [25], in a prospective study with 183 negative cultured, intermittent and chronic patients, reported that the antibody detection against three antigens (ExoA, ELA, AP) resulted in a sensitivity of 86%, specificity of 96% and PPV of 97%, in regard to microbiologic culture. Tramper-Stranders et al. [26] studied 67 patients chronically infected, 60 intermittently colonized and 93 non-colonized with *P. aeruginosa*. The ELISA tests (ExoA, ELA, and AP) using advised cut-off values had a sensitivity of 79% and a specificity of 89% for chronic colonization. Applying new ROC curves to optimize the cut-off values, they obtained a sensitivity of 96% and a specificity of 79% (when all three antigens were evaluated together).

Ratjen et al. [29], studying the response to ExoA, ELA, and AP in 375 patients (and a cohort of 56 patients under antibiotic therapy), observed individual variability in the antibody titers against ExoA, ELA and AP, with good sensitivity and specificity for all the three antigens.

Table 2
Studies listed by number of subjects, age range, methods and antigens used.

Study	n	Age range (years)	Method	Antigen
Høiby et al. [12]	133	NE [1]	CIE	St-Ag:1 –17[3]
Doring et al. [13]	10	8,0 – 29,0	[a]CIE and [b]RIA	[a]St-Ag:1 –17[2], [b]AP and ELA
Brett et al. [14]	75	1,0 – 25,0	ELISA	Cell wall antigens
Hollsing et al. [15]	62	0,32 – 32,0	ELISA	ExoA, AP, ELA and phospholipase C
Pedersen et al. [16]	243	NE [1]	ELISA and CIE	St-Ag:1 –17[2]
Brett et al. [17]	33	0,8 – 27,0	ELISA	Cell wall antigens
Formsgaard et al. [18]	10	NE[1]	[a]ELISA, [b]CIE and [c]Western blot	[a]LPS, [b]St-Ag:1 –17[2] and [c]LPS
Kronborg et al. [19]	12	1,0 – 15,0	ELISA	Different LPS types
Burns et al. [20]	42	2,5 – 15,5	ELISA	ExoA *in house*
Moss et al. [21]	33	NE [1]	Western blot	TTSS proteins
West et al. [22]	68	NE [1]	ELISA	Cell lysate, ELA and ExoA
Johansen et al. [23]	157	NE[1]	CIE	St-Ag:1 -17[2]
Corech et al. [24]	48	NE[1]	ELISA and Western blot	ExoA, cell lysate and TTSS proteins
Kappler et al. [25]	183	2,0 – 38,0	ELISA	ExoA, ELA and AP[3]
Tramper-Standers et al. [26]	220	0,0 – 65,0	ELISA	ExoA, ELA and AP[3]
Pressler et al. [27]	89	NE[1]	ELISA	St-Ag:1 –17
Weisner et al. [28]	17	NE[1]	ELISA and Western blot	LPS
Ratjen et al. [29]	375	1,0 – 52,0	ELISA	ExoA, ELA and AP[3]
Da Silva Filho et al. [30]	87	0,24 – 19,0	ELISA	ExoA, ELA and AP[3]
Milagres et al. [31]	51	1,1 – 16,8	ELISA	Cell lysate and recombinant PcrV
Pressler et al. [32]	719	NE[1]	[a]ELISA and [b]CIE	[a]ExoA and [a,b]St-Ag:1 –17[2]
Hayes et al. [33]	69	NE[1]	ELISA	Cell lysate, ExoA and ELA
Douglas et al. [34]	131	0,1 – 7,1	ELISA	St-Ag:1 –17[2] and ExoA *in house*
Anstead et al. [35]	303	1,0 – 12,0	ELISA	ExoA, ELA, AP[3] and MCW antigens[4]
Cruz et al. [36]	27	2,5 -16,8	Western blot	TTSS proteins
Doğru et al. [37]	90	0,64 – 26,3	ELISA	ExoA, ELA and AP[3]

[1] Non specified.
[2] Antigen commercialized by Statens Serum Institute® (Copenhagen, Denmark).
[3] ExoA, AP and ELA (M-15 kit) – antigens commercialized by Mediagnost® (Reutlingen, Germany).
[4] Antigens produced by the Medical College of Wisconsin (MCW): ExoS+PopB, Cell lysate PAO1 and ExoA.

Pressler et al. [32], for the first time, evaluated the three serological methods most used in the CF follow-up routine and their usefulness in early chronic *P. aeruginosa* detection. They studied 381 patients free of infection, 129 intermittent colonized and 281 chronically infected. The values encountered showed high sensitivity, specificity, PPV and NPV (about 90%) for all methods.

Douglas et al. [34] compared two serological methods (anti-IgG ELISA of the Copenhagen group and an anti-ExoA *in-house* ELISA) between two CF populations in Australia. They observed high PPVs, but low NPVs in both populations, in regard to BAL culture (used as gold standard in the study) — respectively, 14% and 26% for the St-Ag:1–17 and 11% and 19% for ExoA.

Anstead et al. [35] found a varied accuracy employing five different serological methods for antibody research in a multicenter study, obtaining good results of specificity and NPV, despite of oscillating PPV and sensitivity, different from those of Doğru et al. [37] who found high PPV, NPV and sensitivity of serology to ExoA, ELA and AP, but a high number of patients with negative culture presenting high antibody titers, resulting in low specificity.

4. Discussion

This review gathered studies for a period of almost 40 years and evaluated the utility of the antibody detection against *P. aeruginosa* antigens, a technique that has been studied as an alternative to the current gold standard [35], the culture of respiratory samples, which many times is performed through oropharyngeal swab and has several limitations [19], such as no representative samples and occurrence of false-negative results. This makes it crucial to detect early this bacteria in order to avoid chronic infection and the resulting infection progress [38,39].

Infection is commonly distinguished from colonization based on immune and inflammatory responses, despite of inflammatory response being common in CF. *P. aeruginosa* isolation in respiratory samples does not necessarily indicate infection unless there is a specific antibody response [20].

There is a great diversity of results in the studies analyzed herein due to different methodologies, casuistries, times of follow-up, and antigens used. Several studies highlight the

Table 3
Main methods used for detection of serum antibodies to *P. aeruginosa* in CF patients.

Method	Type of assay	Measures	Examples
Enzyme-linked immunosorbent assay (ELISA)	Immunoenzymatic	Antigens or antibodies linked to a conjugate labeled with an enzyme	Immunoglobulins and exoproteins
Western blot	Protein separation followed by immunoenzymatic assay	Antibodies against whole cell proteins	Immunoglobulins
Radioimmunoassay (RIA)	Immunoradiometric	Antigens or antibodies labeled with radioisotopes	Immunoglobulins and exoproteins
Crossed immune electrophoresis (CIE)	Immunoprecipitation	Precipitates resulting from the antigen–antibody interaction	Precipitins

importance of the potential diagnostic value of antibody measure for early *P. aeruginosa* detection, before microbiological culture, in a time period varying from 5 to 30 months [17,22,27,31]. The emergence of the immune response differs according to each employed antigen, being the response to TTSS proteins apparently earlier during the acute stage of infection, declining as the infection progresses to the chronic stage [21,24]. The response to commercialized antigens (ExoA, ELA and AP — M15 Mediagnost and St-Ag:1–17 — Statens Serum Institute) showed good sensitivity and specificity [16,25,26,29,32]. The response to St-Ag:1–17 occurred several months before the first microbiological isolation, and the response to ExoA, ELA and AP

Table 4
Studies evaluating *P. aeruginosa* ELISA serology accuracy for different antigens.

Study	Antigen	Sensitivity	Specificity	PPV	NPV
Pedersen et al. [16]	St–Ag:1–17	93.0%	92.0%	93.0%	72.0%
Kappler et al. [25]	Combination of ExoA, AP and ELA	86.1%	95.6%	97.1%	80.6%
Tramper–Standers et al. [26]	AP	76.0%	97.0%	NI	NI
	ELA	87.0%	89.0%	NI	NI
	ExoA	79.0%	81.0%	NI	NI
Ratjen et al. [29]	AP	85.4%	97.5%	96.6%	88.8%
	ELA	76.2%	97.5%	96.2%	83.2%
	ExoA	72.0%	97.5%	95.9%	80.8%
Pressler et al. [32]	ExoA	89.0%	93.0%	86.0%	95.0%
	St–Ag:1–17	83.0%	97.0%	80.0%	98.0%
Douglas et al. [34]	St–Ag:1–17	91%[a] and 53%[b]	64%[a] and 82%[b]	14%[a] and 26%[b]	99%[a] and 94%[b]
	ExoA in house	82%[a] and 93%[b]	57%[a] and 52[b]	11%[a] and 19%[b]	98,0%[a,b]
Anstead et al. [35]	AP	45.0%	81.0%	85.0%	74.0%
	ELA	52.0%	78.0%	38.0%	86.0%
	ExoA	67.0%	58.0%	29.0%	87.0%
	PopB + ExoS	48.0%	83.0%	42.0%	86.0%
	PAO1	60.0%	72.0%	35.0%	88.0%
Doğru et al. [37]	AP	84.0%	40.0%	100.0%	77.8%
	ELA	80.0%	44.4%	100.0%	80.0%
	ExoA	80.0%	43.3%	100.0%	78.4%

NI: not informed.
[a] Patients submitted to BAL.
[b] Patients of the Australasian Bronchoalveolar Lavage Trial.

occurred several months after the response to St-Ag:1–17, since they are antigens regulated by quorum-sensing, *i.e.*, need a higher microorganism density to lead to the response [13,32,40]. Antibodies to ELA appeared later (a mean of 41.1 months after the first microbiological isolation), being more frequent in chronic infected patients, and may be useful in the diagnostic of chronic infection [22,27]. The enhancement of antibodies against ExoA and AP was associated with the risk of recurrent *P. aeruginosa* isolation, so patients with new acquisition of the pathogen may benefit from a close monitoring of these antibodies [35]. In some cases, antibody response can be transitory and, especially in children between 4 and 6 years, only one positive result may not represent chronic infection or intermittent colonization [26]. Da Silva Filho et al. [30] tested serology for ExoA, ELA, and AP, and compared it to PCR and microbiological culture, finding that the association of the three methods guided to the higher positivity percentage, being PCR the one with higher positivity, but with no statistical significance. As the study was not longitudinal, they could not take any other conclusions, despite considering that serology should not be useful to detect intermittent colonization cases, since the immune response appears just in persistent or recurrent infection cases. Douglas et al. [34] questioned the use of serology for *P. aeruginosa* detection, but recognized that they used BAL culture as the gold standard, and this method may lead to false positive results due to contamination of upper airways and false negatives, resulting from inappropriate samples [7]. In general, good accuracy measures are shown by most studies, being serology for antibody detection recommended as a diagnostic tool in the follow-up routine. It is recommended, for non-colonized patients (in the microbiological point of view), that the antibody monitoring be performed at least annually for patients with negative culture and more frequently for intermittent patients [25,27].

It is observed that there is a relationship between enhanced antibody titers against *P. aeruginosa* antigens (St-Ag:1–17 [12], cell wall antigens [17] and ExoA [22]) and clinical status, where patients presented lower ventilatory function, worst Shwachman–Kulczyki score and severe radiographic alterations, even in a period of almost 6 months before the first positive *P. aeruginosa* culture [22], which is associated with the worst prognosis and deterioration of pulmonary function [41]. There is a proposal to associate the X-ray score and antibody titers for the early diagnosis of *P. aeruginosa* infection [22]. Pressler et al. [32] compared two ELISA tests to distinguish between intermittent colonization and chronic infection. Patients with intermittent culture and higher antibody titers against the St-Ag:1–17 may be indicative of status change to chronically infected, being this response earlier than to ExoA, ELA, and AP. Culture limitations can make it difficult to identify chronically infected patients, which, without immunological results, can be wrongly classified as intermittent or even free of infection or never colonized [9,30,32].

With regard to the response of antibody titers against infection treatment, there was decreasing IgG titers to cell wall antigens and to St-Ag:1–17 after *i.v.* [14,17,23], and inhalatory [29] antibiotic treatments, with survival of patients who presented lower titers being longer, in chronic infection cases [23], and enhanced titers in patients in which eradication therapy failed [29]. When there is a rise in the antibody titers, early antibiotic treatment must be considered, since in 80% of the cases it is possible to prevent chronic infection [21,29,32,42]. However, there is a greater consensus that serology results must be confirmed by a longitudinal follow-up, employing other auxiliary diagnostic resources, and thus, should not be adopted as the only criterion for diagnosis and treatment [28,30,31,33,43].

5. Conclusions

Although we found a low number of papers addressing the diagnostic utility of detection of serum antibodies to *P. aeruginosa*, we recognized consistent results, especially in the studies using the serology for antibody detection against St-Ag:1–17, since the researches employing this antigen had continuity over the course of 30 years, with significant casuistries and early *P. aeruginosa* detection, with good clinical correlation when compared with longitudinal microbiological culture (prospective microbiological analysis). We also highlight the utility of the ELISA technique for anti-pseudomonal antibodies, which is a simple and commercially available method and does not demand great investments. The accuracy values (sensitivity, specificity, PPV and NPV) of the ELISA technique remain controversial, because of the diversity of antigens, cut-off values and casuistries employed; however, considering that most studies showed a good correlation between anti-pseudomonal antibody titers and clinical status, both for success in eradication and for progress to chronic infection or exacerbations, this resource may be useful in early detection or to alert the clinician to seek for other evidences of *P. aeruginosa* colonization or infection, and also to provide parameters to evaluate the colonization/infection status [44,45]. An important gap is the lack of studies comparing serology, microbiological culture and molecular resources for search of evidences of *P. aeruginosa* presence, like PCR and RT-PCR of respiratory samples, mainly in longitudinal studies with significant casuistries, to better elucidate some apparently inconsistent results of serology. The evidences of potential utility of *P. aeruginosa* serology, technical and financial viability, give support to suggest the incorporation of this diagnostic tool in the follow-up routine of CF patients in the effort to reduce the prevalence of chronic *P. aeruginosa* infection and for best understanding of the colonization/infection process.

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