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Original Article

Predictive values of antibodies against *Pseudomonas aeruginosa* in patients with cystic fibrosis one year after early eradication treatment

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Abstract

Background: Patient dependent parameters to predict the long-term success of early eradication treatment of *Pseudomonas aeruginosa* have not yet been defined. For this purpose we assessed serum antibodies against *P. aeruginosa* in CF patients after early eradication treatment.
Methods: Retrospective analyses of all consecutive patients with first *P. aeruginosa* detection 2005 to 2008. Absence of *P. aeruginosa* in the third year was defined as successful long-term eradication. Main outcome was to determine the predictive value of *P. aeruginosa* antibody results one year after initiation of early eradication treatment using antibodies against alkaline protease, elastase, and exotoxin A with regard to long-term success of eradication treatment.
Results: Antibodies against *P. aeruginosa* correlated well with success of eradication; positive and negative predictive values after one year were 75% and 82% respectively. The incidence of new detection of *P. aeruginosa* was 8.5%. Long-term eradication was successful in 32 of 53 patients (60%).
Conclusions: Determination of serum antibodies against *P. aeruginosa* one year after first detection of *P. aeruginosa* and early eradication treatment can predict success of long-term eradication.

Keywords: Cystic fibrosis; *Pseudomonas aeruginosa*; Serum antibodies; Prognostic value

1. Introduction

In cystic fibrosis (CF), *Pseudomonas aeruginosa* (*P. aeruginosa*) is the most frequent respiratory pathogen [1–3] which is associated with increased morbidity and mortality, particularly if mucoid forms of *P. aeruginosa* develop [4,5]. Eradication of *P. aeruginosa* usually is not possible in the case of chronic infection [6], but early antibiotic therapy has been shown to successfully eliminate *P. aeruginosa* from the respiratory tract of CF patients [7–13]. Today, early eradication policy is recommended [14,15] and has been implemented in most CF centers.

Despite this general acceptance of the principle of early eradication treatment, it is not clear which therapeutic regimen provides the best effect (e.g. the highest rate of eradication) and which patient-assigned factors might be associated with higher or lower eradication success. Defining such prognostic factors

Abbreviations: CF, cystic fibrosis; ELISA, enzyme linked immuno assay; *P. aeruginosa*, *Pseudomonas aeruginosa*; FEV1, forced expiratory volume in one second.

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however might help to conduct eradication therapy in a more specific and target orientated way.

Over the last years, commercialized ELISA antibodies against *P. aeruginosa* have been evaluated in different CF populations, showing a good correlation of antibody titers to chronic infection, but contradictory findings considering the value of antibody determination for early diagnosis of *P. aeruginosa* infection [16–22]. Positive serology has been used to predict treatment failure and the risk for recurrent *P. aeruginosa* isolation post eradication [23]. Antibody titer results however, up to now, were not assessed as surrogate marker to predict long-term success of early eradication therapy or to define the duration of eradication therapy.

Therefore the aim of our retrospective study was to analyze the course of serum antibodies against *P. aeruginosa* in CF patients after early eradication treatment in correlation to the long-term success of eradication. The main outcome was the predictive value of antibodies against *P. aeruginosa* one year after beginning the early eradication treatment with regard to its long-term success. The hypothesis was that the long-term success of a standardized early eradication treatment would be predictable by using antibody results one year after initiation of early eradication treatment. The one year interval was chosen because an early evaluation of serum antibodies includes the possibility of determining antibodies still circulating but a later evaluation does not have an impact on therapeutic eradication efforts. This would offer the possibility to use the course of antibodies against *P. aeruginosa* as a tool to regulate the intensity and duration of antibiotic therapy and thus improve the efficacy of eradication therapy.

2. Material and methods

2.1. Subjects and study design

From the beginning of 2005, a standardized eradication therapy protocol was applied in all CF patients from the Munich CF center in whom *P. aeruginosa* was detected for the first time in respiratory samples. These were patients classified as "never infected" according to the Leeds criteria [24]. All consecutive patients with first *P. aeruginosa* detection between January 2005 and December 2008 were identified and complete microbiological and serological results of the following three years were analyzed retrospectively. The aim of the study was to determine the predictive value of *P. aeruginosa* antibody results one year after initiation of early eradication treatment with regard to long-term success of eradication therapy as primary outcome. Secondary outcome parameters were success of the standardized eradication protocol and the long-term correlation of microbiological results and serum antibodies after first detection.

2.2. Microbiology and serology

Sputum samples or deep oropharyngeal swabs were routinely obtained every three months and were cultured on blood-agar plates and MacConkey-agar-plates for at least 72 h aerobically at 37 °C. After isolation, susceptibility testing was carried out and confirmation was done using Cetrimide agar and growth at 42 °C. Patients were classified according to the microbiological results obtained in the third year after first detection of *P. aeruginosa* as follows: negative (=4 of 4 respiratory samples negative for *P. aeruginosa*), intermittent (<2 of 4 respiratory samples positive) and positive (≥2 of 4 respiratory samples positive). This classification was used to define the long-term success of eradication therapy.

Serum antibody titers against the three purified *P. aeruginosa* antigens, alkaline protease (AP), elastase (E), and exotoxin A (EA) were determined annually, using a commercially available ELISA test system (Mediagnost, Germany) according to the manufacturer's instructions as described before [18]. The antibody titers were expressed in arbitrary units and were categorized as 0 (titer negative <1:500), and 1 (titer positive ≥1:500). The scores of the three antibodies were pooled as cumulative score (giving values between 0 and 3). The cumulative score was defined negative if none of the antibodies was ≥1:500 and positive if any of the antibodies was ≥1:500. In case of more than one determination of antibodies per year, mean annual scores were calculated. "Annual" in this context (microbiology and serology) is referring to the point in time, when *P. aeruginosa* was firstly detected in a patient, starting the first, second, or third year after first detection. For the calculation of predictive values (primary outcome), the results of a single antibody determination one year after first detection of *P. aeruginosa* were used to classify the antibody status as negative (no titer ≥1:500 in any antibody determination) or positive (any titer ≥1:500).

2.3. Standardized eradication therapy protocol

In case of *P. aeruginosa* detection in respiratory samples the following early eradication treatment was initiated immediately after notification by the microbiological laboratory according to antibiotic susceptibility if appropriate:

Three weeks "double therapy"[1] followed by one year "mono therapy".[2]

In case of a new detection of *P. aeruginosa*:

Three months "double therapy"[1] followed by one year "mono therapy".[2]

In any case of a new detection of *P. aeruginosa*:

Additional three weeks intravenous therapy with two antibiotics active according to resistance.

Used dosages:

Inhalative: Tobramycin 300 mg in 5 ml twice a day, colistin 33 mg in 3 ml twice a day. Inhalation device Pari eflow in patients 10 years or older and Pari SX in patients younger than 10 years using the LC plus nebulizer.

[1] "Double therapy": inhalative (tobramycin or colistin) plus oral (ciprofloxacin).
[2] "Mono therapy": inhalative (tobramycin or colistin) or alternating inhalative/oral (ciprofloxacin).