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DIAGNOSTIC AND PROGNOSTIC VALUE OF SERUM ANTIBODIES AGAINST PSEUDOMONAS AERUGINOSA IN CYSTIC FIBROSIS

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ABSTRACT

Background: Eradication of *Pseudomonas aeruginosa* in CF patients is possible if initiated early in the course of colonization. To detect *Pseudomonas aeruginosa* as early as possible thus is a major goal. Aim of our study was to validate a commercialised test for the detection of serum *Pseudomonas* antibodies in CF patients.

Methods: Representative cross sectional analysis of serum antibodies against three *Pseudomonas* antigens (i.e. alkaline protease, elastase, and exotoxin A) in 183 CF patients (mean age 16.7, FEV-1 predicted 85.9%) and correlation to microbiological results of the previous two years to calculate sensitivity, specificity, positive and negative predictive values. The following two years were assessed to determine prognostic predictive values.

Results: A combination of all three tested antibodies yielded the best results with a sensitivity of 86%, a specificity of 96%, and a positive predictive value of 97%. These values were higher if only patients were considered in whom sputum cultures were available (n=76, sensitivity: 95%, specificity: 100%, positive predictive value: 100%). The prognostic positive predictive value was high in intermittently infected patients (83%) but low in patients free of infection (33%), whereas the prognostic negative predictive value was high in patients free of infection (78%) and low in intermittently infected patients (58%).

Conclusions: Regular determination of serum antibodies is reasonable in CF patients with negative or intermittent but not with positive *Pseudomonas aeruginosa* status. Rise of antibody titers indicates probable infection and eradication therapy may be initiated even in the absence of microbiological detection of *Pseudomonas aeruginosa*.

List of abbreviations

CF	Cystic fibrosis
ELISA	Enzyme linked immunoassay
P. aeruginosa	*Pseudomonas aeruginosa*
FEV-1	Forced expiratory volume
PPV	Positive predictive value
NPV	Negative predictive value

INTRODUCTION

Cystic fibrosis (CF) is the most frequent life-threatening autosomal recessive disorder in Caucasians, caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, leading to disturbed ion transport across epithelial cells. The clinical consequences of this basic defect become manifest in exocrine glands, comprising pancreatic insufficiency and progressive pulmonary insufficiency which is the major cause of morbidity and mortality in CF patients. The airway epithelium is susceptible to lower respiratory tract colonization involving infection and inflammation even in patients with clinically mild lung disease.[1] The most frequently reported respiratory pathogen is *Pseudomonas aeruginosa* (*P. aeruginosa*) [2], cultured in specimens from as much as 21% of CF patients less than one year of age and in the absence of a policy of early eradication treatment increasing to >80% at 26 years or older. [2] [3] Identification of mucoid forms of *P. aeruginosa* thereby is an unfavourable prognostic factor in regard to survival. [4]

Since eradication of this organism is usually not possible in cases of chronic colonization and infection, but evidence is found that early antibiotic treatment reduces the rate of positive cultures in CF patients with newly isolated *P. aeruginosa*, [5] a major goal is to detect *P. aeruginosa* as early as possible, to use the window of opportunity for possible eradication. [6] From this point of view broncho alveolar lavage or spontaneous or induced sputum cultures obtained at regular intervals would be the desirable "gold-standard" methods for the microbiological detection of *P. aeruginosa*. [7] However, in clinical practice we often have to rely on the results from oropharyngeal swab cultures, because the patients are too young or too healthy to expectorate, and this method is the only direct and readily non-invasive available technique to obtain respiratory secretions for culture.

An alternative approach is to track *P. aeruginosa* colonization by testing for serum antibodies against this organism, especially as they may be detected clearly before the organism is isolated from respiratory samples. [2] [8] Since serum precipitins and antibodies against *P. aeruginosa* have been described [2] [9] [10] [11] and the first ELISA against *Pseudomonas* antigens was developed, [12] attempts have been made to correlate the acquisition of *P. aeruginosa* with the production of an antibody response against this organism. [13] [14] [15] [16] [17] [18] [19] Reviewing the published data, a correlation between microbiological findings, clinical state and results of antibody determination is definite for cohorts of CF patients, whereas the impact of an individual result of serum antibodies against *P. aeruginosa* remains difficult to interpret.

Therefore the aim of our study was to assess the diagnostic accuracy of a commercial antibody ELISA test in relation to the microbiological findings from respiratory secretions and to estimate the prognostic value of these antibody test results to anticipate the future trend of microbiological results. The particular test is a further development of a radioimmunoassay developed by Döring and Hoiby [18], which permits the determination of three major extracellular proteins of *P. aeruginosa*, namely alkaline protease (AP), elastase (E), and exotoxin A (EA). These proteins show high-grade immunogenicity and are expressed by nearly all strains of *P. aeruginosa*.

MATERIAL AND METHODS

Subjects and study design:

In 2000 routine regular determination of serum antibodies against *P. aeruginosa* was introduced in our CF centre. To conduct a representative cross sectional analysis of *P. aeruginosa* antibody titers in CF patients, we extracted antibody test results, initially obtained between 2000 and 2002. We correlated the antibody test results, firstly, to known microbiological data from the previous two years and, secondly, to microbiological results from the following two years.

Of 421 CF patients attending our centre for Cystic Fibrosis at the Children's University Hospital in Munich between 2000 and 2002 (mean age 16.3 years, median 15.4, range 0.4-41), 212 patients continuously were seen at three monthly intervals by one physician whereof 187 patients agreed to participate in this study. Three patients were excluded from the study because, after vaccination against *P. aeruginosa*, they had known serum antibodies against exotoxin A. One patient was excluded because, after 14 years of chronic *P. aeruginosa* infection, he underwent lung transplantation 3 years before the study was started and consequently changed microbiologically to not infected with this organism. Mean age of the remaining 183 patients was 16.7 years (15.7, 2-41), mean FEV-1 was 85.9% predicted (89; 20-147), mean weight for height 105% (104; 62-158). 181 patients (99%) were on continuous (>300 days/year) oral antibiotic therapy against *Staphylococcus aureus*. Weight and height were measured at the beginning of the study and the weight for height predicted was calculated using height percentile standards given by Prader et al.. [20]

According to the microbiological results of the previous two years, the patients were divided into three groups considering their *P. aeruginosa* status which was defined solely by the number of positive microbiological cultures of *P. aeruginosa*: *Free* of *P. aeruginosa* (0 positive of 8 cultures), *intermittently* colonized (1 to 6 positive of 8 cultures) and *chronically* colonized (≥7 positive of 8 cultures). The clinical details of these subjects are shown in table 1, their age distribution and *P. aeruginosa* status are given in figure 1. Complete microbiological data over the following two years with at least 7 of 8 possible microbiological samples was available in 162/183 patients (overall rate: 88.5%, 66/68 patients in the *P. aeruginosa* free group, 24/27 patients in the intermittently infected group, and 68/88 patients in the chronically infected group).

Table 1: Clinical data of 183 CF patients according to their *P. aeruginosa* status

	Free		**Intermittent**		**Chronic ****	
Number of subjects (n)	68		27		88	
Classified according to sputum (n)	11		5		60	
Classified according to swab (n)	57		22		28	
Age (years)*	11.4	(2-29)	14.5	(2-41)	21.5	(3-38)
Weight for height (%)*	103	(62-130)	107	(88-148)	105	(78-158)
FEV-1 predicted (%)*	97	(62-147)	94	(48-143)	75	(21-128)
IgG (mg/dl)*	842	(294-1962)	966	(296-2440)	1293	(654-2575)
C-reactive protein (mg/dl)*	0.18	(0.00-1.36)	0.14	(0.01-0.58)	0.64	(0.00-3.90)
Detection of mucoid strains (n/N)	0/68		15/27		84/87	

** Data are expressed as means with range*

*** Duration of infection for a medium of 11.5 years (range 1.5-26 years)*

Statistics: Two way ANOVA explains the differences between the groups considering FEV-1 by influence of age (p=0.025) but not group membership (p=0.35)

Microbiology and serology

Sputum samples or deep oropharyngeal swabs were routinely obtained every three months and, after homogenization of sputa, were cultured on blood-agar plates and McConkey-agar-plates for at least 72 hours aerobically at 37°C without the use of additional selective media for *P. aeruginosa*.

Antibody titers against the three purified *P. aeruginosa* antigens, alkaline protease (AP), elastase (E), and exotoxin A (EA) were determined, using a commercially available ELISA test system (Mediagnost, Germany) according to the manufacturers instructions. Two replicates were used for each sample. The antibody titers were expressed in arbitrary units and were categorized as 0 (titer negative <1:500), and 1 (titer positive > 1:500). The calibration curves of the test system are linear between optical density (OD) values between 0.1 and 1.7, corresponding to titers of 1:10 to 1:2000. Negative and positive controls were provided by the manufacturer and consisted of pooled sera from *P. aeruginosa* positive CF patients and from normal children, respectively. All measurements were conducted in duplicate for each ELISA panel. For determination of high titers (>2000), dilutions of 1:10 to 1:100 were used. The cut off, depending on the slope of the calibration curve, was reached at a mean OD of 0.35 ± 0.046 standard deviation (SD) for all three antibodies.

Intra- and inter-assay-variability of serological assays

The intra-assay-variability (correlation between the results of duplicate measurements) was excellent for the three different antibodies with a coefficient of correlation between 0.988 and 0.995 (Spearman, 100% correlation is 1.000) and a coefficient of repeatability between 0.091 and 0.096 (Bland and Altmann, 100% correlation is 0.000).

The inter-assay-variability (correlation between the results obtained to generate the ELISA calibration curves) was satisfying with a relative coefficient of variation between 7.3 and 20.3, whereas a coefficient of 0 would characterise complete concordance and 100 would describe no agreement.

Statistical analysis

Sensitivity, specificity, 95% confidence intervals (95% CI), positive predictive value (PPV) and negative predictive value (NPV) were calculated from contingence tables, with confidence intervals calculated using the binominal exact method.

To briefly review the concept of diagnostic accuracy with respect to serum antibodies against *P. aeruginosa*, *sensitivity* is the probability that a patient who is colonized with *P. aeruginosa* shows detectable antibodies against this organism. *Specificity* is the probability that a patient who is not colonized with *P. aeruginosa* shows no detectable antibodies against this organism. The sensitivity and specificity are properties of the test and are not determined by the prevalence of *P. aeruginosa* colonization in the population studied. Therefore neither the sensitivity nor the specificity answer the most important questions: If the antibody result is positive, what is the chance that the patient is really colonized with *P. aeruginosa*, and if the antibody result is negative, what is the chance that the patient is not colonized with this organism? The answers to these questions are given by the predictive values, which take the prevalence into account: The *positive predictive value* is the probability that a subject with positive antibodies against *P. aeruginosa* is colonized with that organism. The *negative predictive value* is the probability that a subject with negative antibody result is not colonized with *P. aeruginosa*.

In this study, we calculated the predictive values accounting for the *P. aeruginosa* prevalence retrospectively determined in our study population according to microbiological results. Furthermore, we calculated the prospective predictive values, using the antibody test results and the microbiological results of the following two years. The later values state the

probability for a single CF patient to shift his or her *P. aeruginosa* status dependent on his or her initial antibody titer.

For comparison of paired data the coefficient of correlation according to Spearman and the relative coefficient of variation were calculated and the coefficient of repeatability was calculated according to the method proposed by Bland and Altmann. [21] For comparison of unrelated data the Mann-Whitney Test was used, for categorical variables the Fishers exact test was applied, corrected for multiple comparisons by Bonferroni method. Analyses were performed using SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL). Data are given as mean (median, range).

Informed consent was obtained from all subjects. The study has been approved by the local institutional review board for human studies of the university children´s hospital, Munich.

RESULTS

Serum antibodies against *P. aeruginosa* in correlation to *P. aeruginosa* status as retrospectively defined

The levels and positivity of the three antibodies determined, i.e. alkaline protease (AP), elastase (E), and exotoxin A (EA), differed substantially among the three defined groups of CF patients (table 1). A negative single test was scored as 0 and a positive test as 1, thus giving a cumulative score (AP or E or EA) from 0 to maximum of 3. The patients free of infection, as determined by culture results from the previous two years, had positive antibody results only in a very small fraction (3/68 patients) and the mean cumulative score was low (0.06). The patients with chronic infection had a very high rate of positive antibody results (86/88 patients) and the mean cumulative score was high (2.22). The patients with intermittent infection were in between (13/27 patients positive) with a medium mean cumulative score of 0.81 (table 2).

Table 2: Anti-*P. aeruginosa* antibodies in cystic fibrosis patients correlated to *P. aeruginosa* status as defined retrospectively.

	P. aeruginosa status according to culture results					
	Free (N=68)	**Intermittent** (N=27)	**Chronic** (N=88)	**Comparison* (p-values)**		
Antibodies against	**F**	**I**	**C**	**F** vs **I**	**I** vs **C**	**C** vs **F**
Alkaline Protease						
Frequency of antibody positive (n/N)	1/68	7/27	66/88	<0.001	<0.001	<0.001
Elastase						
Frequency of antibody positive (n/N)	0/68	4/27	57/88	<0.001	<0.001	<0.001
Exotoxin A						
Frequency of antibody positive (n/N)	3/68	11/27	73/88	<0.001	<0.001	<0.001
Cumulative-Score (AP/E/EA)						
Frequency of antibody score ≥1 (n/N)	3/68	13/27	86/88	<0.001	<0.001	<0.001

Antibody titers were scored as 0 (titer negative <1:500), and 1 (titer positive > 1:500)
**Mann-Whitney Test for comparison of antibody levels and Fishers exact test for comparison of frequency of patients with antibody score ≥1, corrected for multiple comparisons by Bonferroni method.*

Sensitivity and specificity of serum antibodies against *P. aeruginosa* compared to *P. aeruginosa* status as retrospectively defined

To calculate sensitivity and specificity from the above data, it was necessary to categorize intermittently infected patients as either negative or as positive. Since *P. aeruginosa* had been detected at least once, they were all defined as positive. Thus, two groups of patients were defined: 68 patients free of *P. aeruginosa* and 115 patients with *P. aeruginosa* detection within the previous two years.

Overall, specificity was satisfying (AP: 98.5, E: 100, EA: 95.6) but sensitivity partially was very low (AP: 63.5, E: 53.0, EA: 73.0). Results for sensitivity were better if only patients with sputum cultures were considered for calculation (AP: 76.9, E: 67.7, EA: 78.5), and were worse, if only patients with swabs were considered (AP: 48.0, E: 34.0, EA: 66.0). Results for specificity were excellent if only patients with sputum cultures were considered for calculation (AP: 100, E: 100, EA: 100), and were only very little worse, if only patients with swabs were considered (AP: 98.2, E: 100, EA: 94.7).

Combination of antibody results by counting exclusively multiple positive results as positive (AP+E, AP+EA, E+EA, AP+E+EA) generated unsatisfactory results for sensitivity (41.7% to 52.2%) with good results for specificity (98.6 to 100%). Combination of antibody results by assessing a patient as positive with one positive of two results (AP/E, AP/EA, E/EA) generated somewhat better results for sensitivity (70.4 to 84.3%) but worse results for specificity (90.3 to 97.2%).

A combination of all three antibodies (AP/E/EA, cumulative antibody score) yielded the best results. Patients were counted as positive if any of the three antibodies was positive and negative if no positive antibody result was obtained (table 3).

Table 3: Sensitivity and specificity of cumulative anti-*P. aeruginosa* antibody scores differ in regard to the method by which material was obtained for microbiological culture.

	Sputum only	**All (sputum and swab)**	**Swab only**
Number of subjects (n)	76	183	107
Sensitivity % (95% CI)	**95.4** [87.1-99.0]	**86.1** [78.4-91.8]	**74.0** [59.7-85.4]
(n/N)	62/65	99/115	37/50
Specificity % (95% CI)	**100** [71.5-100]	**95.6** [87.6-99.1]	**94.7** [85.4-98.9]
(n/N)	11/11	65/68	54/57
Positive predictive value % (95% CI)	**100** [94.2-100]	**97.1** [91.6-99.4]	**92.5** [79.6-98.4]
Negative predictive value % (95% CI)	**78.6** [49.2-95.3]	**80.2** [69.9-88.3]	**80.6** [69.1-89.2]

95% CI: 95% confidence interval

Predictive values were calculated for the given prevalence, i.e. 61.5% for all patients (115/183), 85.5% for patients with sputum cultures (65/76), and 45.0% for patients with swabs (50/107).

Prognostic value of antibody results

Of 183 patients a cohort of 162 patients was studied at three monthly intervals for microbiological status over a period of the two years, following first antibody determination. This allowed to calculate the prognostic values of the antibody determinations separately for patients free of *P. aeruginosa* and for those with intermittent *P. aeruginosa* colonization. Of 63 patients free of *P. aeruginosa* and with *negative* antibody results, 14 patients turned intermittently infected within the following two years and 49 patients remained free of *P. aeruginosa*. Of three patients free of *P. aeruginosa* but with *positive* antibody results, one patient changed to intermittent infection and two patients remained free of *P. aeruginosa*. In the intermittently infected group of 24 patients, 12 patients had no antibodies. In nine of them microbiological follow up was negative and in three of them positive. Of the other 12 patients with positive serum antibodies two became free of *P. aeruginosa* within the following two years and ten stayed intermittently infected.

Consequently the prognostic positive predictive value was low in patients free of *P. aeruginosa* (33.3%) and high in intermittently infected patients (83%). The prognostic negative predictive value was high in patients free of *P. aeruginosa* (77.8%) and 58% in patients with intermittent infection (table 4).

Table 4: Prognostic values of serum antibody determination. Results from the evaluation over the two years following the initial determination of antibody titers.

	***P. aeruginosa* status according to culture results**	
	Free (N=66)	**Intermittent** (N=24)
Positive predictive value % (95% CI)	**33.3** [0.8-90.6]	**83.0** [51.6-97.9]
(n/N)	1/3	10/12
Negative predictive value % (95% CI)	**77.8** [76.5-94.4]	**58.0** [27.7-84.8]
(n/N)	49/63	7/12

95% CI: 95% confidence interval

Predictive values were calculated on the basis of the *P. aeruginosa* prevalence after two years (15/66 in the *P. aeruginosa* free group =22.7% and 13/24 in the intermittent infected group =54.2%) based on the serum antibody results, to state the probability for a CF patient to shift his or her *P. aeruginosa* status depending on the antibody titer.

DISCUSSION

The *first* part of our study demonstrated a close correlation between microbiologically defined status of *P. aeruginosa* and serum antibodies against this organism in CF patients, as high values for sensitivity and specificity of serum antibodies were obtained. This confirms similar observations described by others. [13] [14] [15] [16] [17] [18] [19] However, the clinical relevance of a test can best be derived from the positive and negative predictive values which, in our case, showed that a positive antibody result indicated the presence of *P. aeruginosa* in the airways of a CF patient with >93% probability. For CF patients, chronically infected with *P. aeruginosa* and positive antibody test results, routine follow up antibody determinations thus are of no use. On the other hand, a negative antibody result made the presence of *P. aeruginosa* unlikely (80%).

In the *second part* we tested the prognostic predictive values of antibody determinations, assessing the time period two years after initial antibody determination. Unexpectedly, the *prognostic positive predictive value* was low for patients who were not infected with *P. aeruginosa* (table 4). Although the number of patients in this case is too small to yield any significance of this value, we tried to explain this surprising finding: Of 3 patients with positive antibody results despite negative retrospective microbiology, only one patient became microbiologically positive within the following two years, but the other two remained *P. aeruginosa* free. This finding was not consistent with the very high positive predictive value calculated from the first observation period. Detailed analysis of the two patients with the unexpected positive antibody titers provided possible clues: one patient had been colonized with *P. aeruginosa* five years before our study for 18 months and the bacteria were successfully eradicated. The other patient was put on inhaled anti-pseudomonal therapy using tobramycin for four years before our study because of initial clinical instability without any microbiologically detected *P. aeruginosa*. His subsequent clinical improvement might reflect successful eradication of *P. aeruginosa*, which had been present in the lungs and had escaped microbiological detection.

The *prognostic negative predictive value* in the group free of *P. aeruginosa* was 78%, suggesting that in patients with negative antibody results, *P. aeruginosa* could be detected within the next two years with a probability of around 20%, i.e. 10% per year. This value was in the order of the annual rate of new infections of about 10% per year. [22] Thus the prognostic negative predictive value of the test was excellent.

The *prognostic predictive values* calculated for the group of intermittently infected patients can be applied to patients, in whom *P. aeruginosa* is detected for the first time in swab or sputum culture. If the serum antibodies were positive, it was very likely (83%) that the patient maintained the *P. aeruginosa* infection, if the serum antibodies were negative, the chance of elimination of *P. aeruginosa* was 58% (table 4).

The microbiological status of a patient in our study was retrospectively defined by sputum samples or oropharyngeal swabs taken regularly every three months. According to this, we can not differentiate between colonization and infection, since we believe these two conditions to be a continuum in CF patients. Although this widespread regimen is feasible in everyday patient care and approximates the recommendations for surveillance of CF-patients, [23] the results of swab analyses may be erroneous [24] and the impact of swab results in previous studies lead to controversial conclusions, yielding either a high positive predictive value, [25] or a high negative predictive value. [26] [27] In part relying on swab culture results may thus introduce some inaccuracy into the interpretation of our serological data. Therefore we separately considered sputum or swab culture results. For patients in whom *sputum cultures* were available, the positive predictive value of the cumulative antibody test results was 100%, meaning that a CF patient with positive results in any antibody determination actually has been colonized with *P. aeruginosa* within the previous two years.

As sputum is considered the reference method, the same conclusion must be drawn for patients in whom only oropharyngeal swabs were available. This means that independently of the method by which material for microbiological testing was obtained, a positive cumulative serum antibody result indicates *P. aeruginosa* colonization within the previous two years.

Interestingly, the negative predictive value of the cumulative antibody results of around 80% did not change if sputum or swab culture results were calculated independently, demonstrating that negative results of antibody determination did not rule out future colonization with *P. aeruginosa*.

Our calculations of positive and negative predictive values depend on the prevalence of *P. aeruginosa* and thereby on successful early therapeutic intervention strategies and consequently on the age distribution of our cohort so that our conclusions may not be applicable to other populations of CF patients. Overall, the prevalence of *P. aeruginosa* in our patients (61.5%) was high and comparable to published data. [28] Among the adult patients, the rate of chronic *P. aeruginosa* colonization was 67% and thus is comparable with data from adult CF centres, showing a chronic *P. aeruginosa* infection rate of about 70% of patients. [22] The rate of *P. aeruginosa* detection among children under the age of six years was 18% in our group and thus lower than the 30% reported elsewhere. [29]

As the predictive values depend on the prevalence of *P. aeruginosa*, approximate adjustments regarding a single patient may be made: The younger the patients are, the lower is the prevalence of *P. aeruginosa*, leading to a worsening of positive predictive values but to an improvement of negative predictive values. Using our results for the sensitivity (86%) and the specificity (90%) of the test, the negative predictive value is only 80% for the *P. aeruginosa* prevalence of 61% in our collective, whereas it is 96% at a prevalence of 18%, as we found in children younger than six years of age. This means that negative serum antibodies against *P. aeruginosa* make an infection with this organism the less likely, the younger the child is.

The determination of a collection of three serum antibodies against *P. aeruginosa*, i.e. antibodies against alkaline protease, elastase, and exotoxin A in CF patients is reliable if cumulative antibody scores are utilized. Positive antibody results almost prove colonization with *P. aeruginosa*, negative test results indicate the absence of *P. aeruginosa* with increasing probability, the younger the patient is. We conclude from our results that from a clinical perspective a regular determination of serum antibodies against *P. aeruginosa* may make sense in patients with negative microbiological *P. aeruginosa* status. If under these conditions antibody titers rise, we have a high suspicion for possible infection with *P. aeruginosa* and we suggest eradication therapy even in the absence of microbiological detection of *P. aeruginosa*. Presently, our regime for eradication of *P. aeruginosa* relies solely on the microbiological detection of the organism. In the future, early initiation and possibly the intensity and duration of anti-pseudomonal treatment may also be determined by antibody test results, however more longitudinal data will be necessary to achieve this goal.

Competing interest: None. The corresponding author has checked with all authors that they have read the competing interest. All authors declare that the answer to the questions on your competing interest declaration form are all No and therefore have nothing to declare.

These data are part of the medical thesis of Angelika Kraxner. Parts of the study have been presented in abstract form during the annual North American Cystic Fibrosis Conference, Los Angeles, 2003.

Legends to figures

Figure 1: Age distribution of 183 CF-patients divided into three groups according to their microbiological *P. aeruginosa* status. *Free* of *P. aeruginosa* (0 positive of 8 cultures): white columns, *intermittently* colonized (1 to 6 positive of 8 cultures): dark columns, and *chronically* colonized (≥7 positive of 8 cultures): grey columns.

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Appendix

Cumulative serum antibodies against *P. aeruginosa* compared to microbiological results as „gold-standard“ FLOW DIAGRAM