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Original Article

Secretory IgA as a diagnostic tool for *Pseudomonas aeruginosa* respiratory colonization

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Abstract

Background: Pseudomonas aeruginosa sinusitis may be the focus for intermittent lung colonization in patients with cystic fibrosis (CF). The sinusitis may induce elevated IgA levels in nasal secretion and saliva against *P. aeruginosa*.
Methods: 120 CF patients chronically infected, intermittently colonized or without *P. aeruginosa* in the lungs participated in this cross-sectional study. IgA and IgG against *P. aeruginosa* sonicate and alginate were measured in nasal secretions, saliva, and in serum by ELISA.
Results: The intermittently colonized patients had significantly higher IgA levels in nasal secretions and saliva than those without *P. aeruginosa* in the lungs, indicating that *P. aeruginosa* sinusitis may precede intermittent colonization and chronic infection of the lungs.
Conclusions: Specific IgA against *P. aeruginosa* in nasal secretions and saliva can contribute to differentiation between patients chronically infected, intermittently colonized, and without *P. aeruginosa* in the lungs. The diagnostic value of the IgA ELISA awaits a prospective study.

Keywords: Pseudomonas aeruginosa; Cystic fibrosis; Colonization; IgA antibodies; Diagnosis; Sinusitis

1. Introduction

Patients with Cystic Fibrosis (CF) have increased susceptibility to infections, and *Pseudomonas aeruginosa* is the cause of most of the morbidity and mortality in these patients [1]. Lung infections with *P. aeruginosa* cause inflammation resulting in gradually declining lung function and a systemic increase of antibodies against a polyvalent *P. aeruginosa* antigen (Standard Antigen (St-Ag)) [2] and the mucoid extracellular polysaccharide alginate [3,4]. In addition to serum, specific antibodies are present in tears and upper airways such as saliva and sputum; secretory immunoglobulin A being the dominant antibody on mucosal surfaces [4,5].

The immune system of the upper airways differs from that of the lower airways, by generating an early local response with a high plasma cell production of IgA when infected with *P. aeruginosa* [6,7]. The non-phlogistic IgA, binds *P. aeruginosa* on the mucosal surface, reducing the inflammatory response, inhibiting complement activation and the recruitment of polymorphonuclear leucocytes (PMN), and diminishing their oxidative burst by preventing fagocytosis [6,8]. *P.aeruginosa*

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probably colonizes and adapts to the paranasal sinuses acting as a reservoir, even before intermittently colonizing and chronically infecting the lungs [9]. The reduced inflammation contributes to this rather 'silent' adaptation, allowing *P. aeruginosa* to evolve to the chronic biofilm phenotype in the paranasal sinuses [9,10].

When CF patients become intermittently colonized in the lungs with *P. aeruginosa*, elevated levels of specific IgG against *P. aeruginosa*, measured by ELISA, can be detected 1–3 years before onset of the chronic lung infection, maybe reflecting a 'hidden' focus for subsequent intermittent lung colonization and chronic infection [8]. The 'hidden focus' may be the paranasal sinuses, which in CF patients frequently contain *P. aeruginosa* and are filled with mucus and pus [6,10]. In fact, re-infection of the lungs of transplanted CF patients often originates from the paranasal sinuses [11–14]. Chronic lung infection can be postponed by early antibiotic treatment of intermittent colonization [15]. Serological assays (IgG) have high sensitivity and specificity in distinguishing between CF patients chronically and not chronically *P. aeruginosa* infected in the lungs [16,17]. However, these methods have not been useful to distinguish between CF patients with intermittent colonization and patients without *P. aeruginosa* in the lungs [16–19], but the possible presence of *P. aeruginosa* sinusitis as a focus for subsequent lung colonization and chronic infection was not considered at the time of these publications. A diagnostic antibody assay for early detection of *P. aeruginosa* infection in the paranasal sinuses would therefore be useful [20]. As secretory IgA antibodies are detectable in CF patients with chronic *P. aeruginosa* lung infection [4], we hypothesized that *P. aeruginosa* sinusitis may cause a local rise in the specific IgA in nasal secretions and saliva, which could be used to diagnose *P.aeruginosa* sinusitis in addition to detection of *P. aeruginosa* by cultures from the sinuses. The clinical consequence could, hopefully, be successful attempts to eradicate *P. aeruginosa* from the paranasal sinuses and thereby prevent subsequent intermittent colonization and chronic infection of the lungs.

2. Material and methods

2.1. Patients

All CF patients above the age of seven, who were treated at the CF centre Copenhagen, were eligible for this cross-sectional study. The CF-diagnoses were based on characteristic clinical features, abnormal sweat electrolytes and genotypes. The CF patients are followed in the out-patient clinic every month, and the examinations are composed of sputum samples taken for microbiological examinations and regularly blood samples are analyzed for anti-bacterial antibodies [8]. In total 120 CF patients (60/60 male/female, mean age 22 years) participated in the study; saliva and nasal secretions were obtained from 73 patients, 24 patients had only saliva samples obtained and 23 patients had only nasal secretions obtained. Nasal secretions and new blood samples were obtained approximately six months later than the saliva samples. All patients had blood samples analyzed. Patients who both had sputum and nasal secretions obtained had two separate blood samples analyzed.

Twelve healthy employees from the Department of Clinical Microbiology Rigshospitalet participated as healthy controls.

2.2. Infection status

As reported previously, we defined chronic *P. aeruginosa* lung infection (CF+P (c)) as growth of this bacteria in six consecutive monthly samples taken from lower respiratory tract secretions, or a shorter period if there were two or more precipitating antibodies against *P. aeruginosa* [16,19]. Lung transplanted patients were categorized according to their infection status before transplantation.

Intermittent lung colonization was defined as growth of *P. aeruginosa* for less than 6 months, but normal levels of precipitating antibodies (0–1) against *P. aeruginosa*. If the monthly samples had never contained *P. aeruginosa*, patients were classified as without *P. aeruginosa*.

Based on these criteria the patients were divided into four groups: 1: CF patients without *P. aeruginosa* in the lungs (CF-P) 2: intermittently colonized with *P. aeruginosa* (CF+P(i)) in the lungs, 3: chronically infected in the lungs with *P. aeruginosa* (CF+P(c)) or 4: colonized/infected in the lungs with other CF pathogenic Gram-negative bacteria (*Stenotrophomonas maltho-philia*, *Achromobacter xylosoxidans* or *Burkholderia cepacia complex*)(CF+GNB).

2.3. Collection of serum, saliva, and nasal secretions

The blood samples and secretions were obtained simultaneously. Mixed saliva was collected by using four sterile 6 mm diameter paper discs (Antibiotica Testblættchen, Struers, Copenhagen, Denmark) which were placed on the oral mucosa for 30 s as reported previously [5].

Nasal secretions were obtained by using four sterile 6 mm paper discs as mentioned above (Fig. 1B). With forceps, the discs where gently stroked against the mucosa for five seconds, each absorbing nasal secretions. Depending on the patients' anatomy and co-operation the anterior part of the medial meatus could be reached, being the anatomical target for the discs (Fig. 1B). No decongestion was used. The patients decided if the samples were taken from the left, right or both sides. The saliva/nasal secretion-containing paper discs were stored at room temperature until analyzed.

2.4. IgA and IgG against P. aeruginosa

Serum and eluates of saliva and nasal secretions from the paper discs were examined for IgA and IgG antibodies against *P. aeruginosa* alginate and *P. aeruginosa* sonicate ((St-Ag) (serogroups 1–17)) using enzyme-linked immunosorbent assays (ELISA) as reported previously [4,5]. Saliva- and nasal secretion impregnated paper-discs (mean: 25 μl/disc as reported previously [5]) were incubated on a shaker for one hour at 35 °C in 175 μl dilution buffer to elute IgG and IgA antibodies (1:8 dilution). Serum was diluted 1:100 for the St-Ag ELISAs and 1:4000 for the alginate ELISAs. The volume of diluted serum samples for the ELISA was 100 μl, and 50 μl for the saliva and nasal

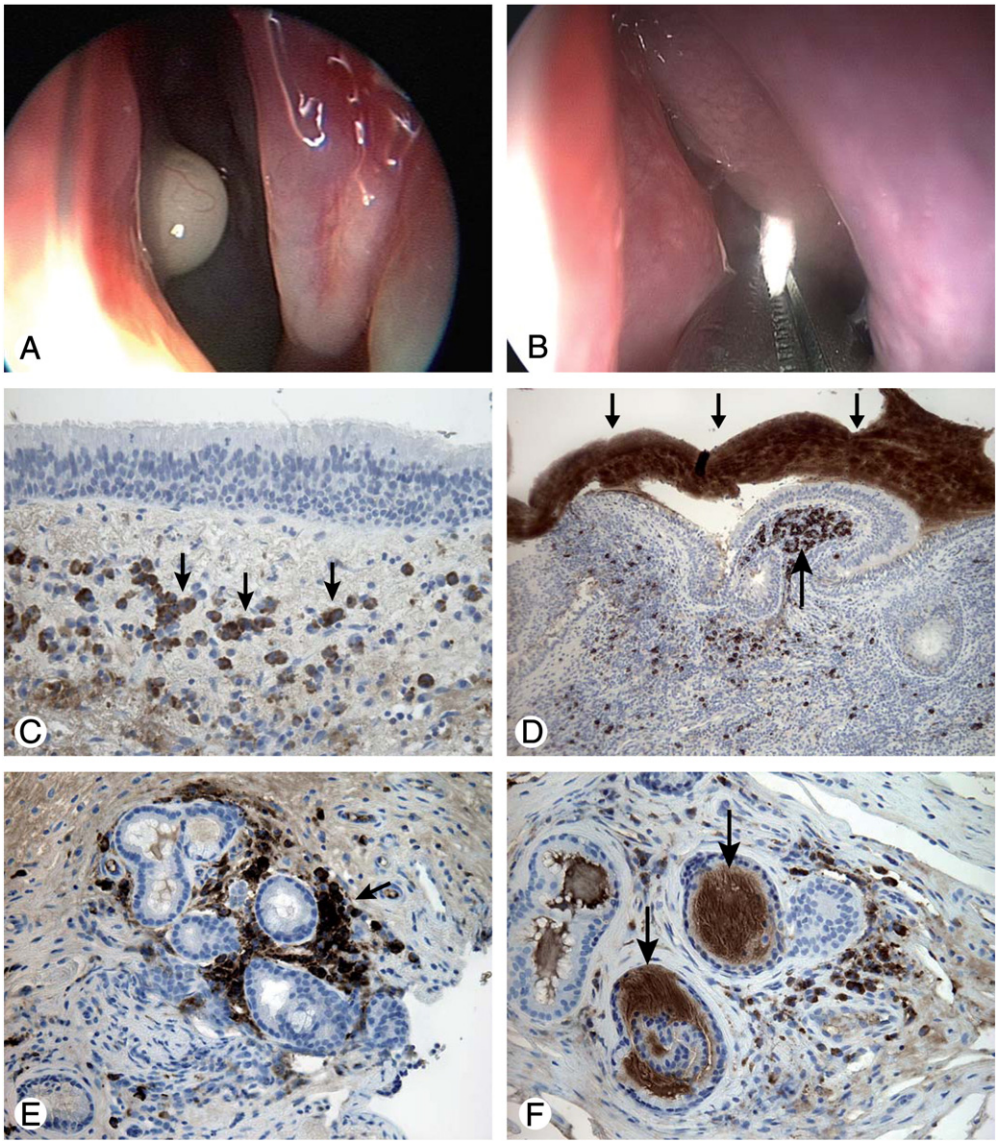

Fig. 1. A–F. A: A structure we thought was an abscess, but it turned out to be a sterile sinus-associated-lymphoid-tissue with plasma cells and IgA. B: Blotting paper within the right middle meatus absorbing nasal secretions. C: 09-33224 IgA x 40. Immunoreaction visualizing IgA immunoreactive (brown) plasma cells (arrows) in the lamina propria close to the pseudostratified respiratory surface epithelium. D: 09-32541 IgA x 10. An accumulation of IgA containing plasma cells (brown, large arrow) in the lamina propria close to the mucosal surface and also a strong IgA immunoreaction in the luminal mucosal layer (brown, smaller arrows). E and F: 01-586 IgA x 25 IgA immunoreactive plasma cells (brown) concentrated around secretory acini of the glands of the sinus tissue and also positive immunoreaction in secretions in the excretory ducts (arrows).

secretion ELISAs. Phosphate-buffered saline (PBS pH 7.2)+ 0.1% Tween-20 (Sigma) (washing buffer)+NaCl 15 g/l (= dilution buffer) was used and the plates were washed three times.

2.4.1. Antibodies against alginate (IgA-alginate, IgG-alginate)

Ninety-six-well microtiter plates (Mikrowell, BiotechLine A/S, Denmark) were coated with alginate (10 μg/ml) purified from a mucoid CF *P. aeruginosa* strain (6680NH) as previously reported [7]. The plates were coated overnight at 35 °C and blocked for one hour at 35 °C in dilution buffer. Diluted serum, saliva and nasal secretions (see above) were added and allowed to react for one hour at 35 °C. After washing, horseradish peroxidase (HRP)-conjugated rabbit anti-human IgA (P0216) and anti-human IgG (P0214) (Dako A/S, Glostrup, Denmark) both diluted 1:10,000 were added and left to react for one hour at 35 °C.

2.4.2. Antibodies against P. aeruginosa standard antigen (IgA-St-Ag, IgG-St-Ag)

A sonicated cell extract of *P. aeruginosa* serogroups 1–17 was used as standard antigen (St-Ag, protein concentration 16 mg/ml) [4,5] and coated onto irrigated 96-well polystyrene plates (Maxisorb, BiotechLine A/S, Denmark) at a dilution of 1:2000. The plates were incubated for one hour at room temperature and blocked overnight with dilution buffer at 4 °C. Serum was diluted 1:100, saliva and nasal secretion 1:8 and allowed to react for one hour at room temperature. After washing, horseradish peroxidase (HRP)-conjugated rabbit anti-human IgA (P0216) and anti-human

IgG (P0214) (Dako A/S, Glostrup, Denmark)) diluted 1:20,000 were added and left to react for one hour at room temperature.

For all ELISAs TMB Plus was added (KemEnTec Diagnostics). The reactions were stopped after one hour at room temperature by adding 1 M H_2SO_4. The absorbance was measured at 450 nm on a plate reader (Multiscan EX, Bie & Berntsen, Denmark). The results were expressed as optical density values (OD).

2.5. Immunohistochemistry

The presence and localization of IgA in mucus and plasma cells in the mucosal tissue were visualized by means of immunohistochemistry. Resected nasal polyps and mucosa from the maxillary sinus from two CF+P(c) and two CF+P(i) (Fig. 1A) were fixed in 4% buffered paraformaldehyde and embedded in paraffin and 4 μm sections were cut on a microtome. The sections were incubated for 10 min in 2% bovine serum albumine followed by 18 h at 4 °C after addition of a polyclonal rabbit anti-human IgA (IMGENEX, IMG-80368) diluted 1:100. For visualization of the immunoreaction the sections were incubated for 1 h with biotinylated goat anti-rabbit immunoglobulin (BA-1000, Vector) diluted 1:200, followed by StreptABComplex/horseradish peroxidase (Vectastain, PK-4000), and finally visualized by means of 3,3-diaminobenzidine for 15 min. The sections were counterstained with hematoxylin (Fig. 1C–F).

2.6. Sinus and nasal bacterial colonization/infection

We randomly chose 20 of our (CF-P) patients (13/7 male/female, mean age 16 years) and cultured their nasal cavity to determine if they were also free from *P. aeruginosa* (and other CF pathogens) in the nose and sinuses. Wearing a headlight and using a nasal speculum, the nasal cavities were examined by one ENT surgeon (one of the authors, K.A.). In each side of the nose a thin cotton swab was wiped within the middle meatus. Furthermore, a nasal irrigation was performed using a plastic Neti Pot (Yogaprosess A/S) containing 100 ml of sterile saline. The saline was poured through one nostril, passed through the nasal cavity and out of the other nostril where 10 ml were collected. Finally, a sputum sample or an endolaryngeal suction was taken [21]. This provided four different samples which were cultured the same day.

2.7. Ethics

The study was approved by the local ethics committee (H-A-2008-141). All patients gave informed consent. In patients <18 years a consent was also obtained from their parents. Obtaining the saliva samples did not involve any discomfort for the patients. Obtaining nasal secretions could result in a little discomfort; therefore in a couple of cases fewer samples were taken. The serum and saliva samples were part of the routine, thus no extra blood samples were taken. The antibody results did not result in any change of the treatment modality for the involved patients.

2.8. Statistics

SAS 9.1.3 was used for analyzing data, making receiver operating characteristic (ROC) curves and Spearman rank coefficient test. The St-Ag and alginate data were unpaired, continuous and positively skewed distributed why Log_{10} transformations were made. The transformed data had an approximately normal distribution justifying an unpaired two-sample *t*-test for the means and a one way analysis of variance. The level of significance was set to ≤0.05 (two-tailed).

3. Results

The results of the antibody measurements are shown in Table 1A and B.

CF+P(c) had the highest IgA and IgG antibody levels against St-Ag and alginate in serum, nasal secretions (Table 1) and saliva (Table 2) compared with CF+P(i) and with CF-P (all p-values<0.0001 except IgA against St-Ag which was not significantly different between CF+P(c) and CF+P(i)).

Generally, the nasal/serum (Table 1) and the saliva/serum (Table 2) IgA ratios were high in CF+P(c) in accordance with a local mucosal production of IgA, in contrast to the low IgG ratios being in accordance with a high systemic production and subsequent transudation of IgG to the mucosa secretions.

CF+P(i) had higher IgA and IgG antibody levels against St-Ag and alginate than CF-P (all p values<0.0001) (Tables 1 & 2). Nasal IgA against alginate was also higher in CF+P(i) compared to CF-P (Fig. 2G).

Table 1
Mean nasal and serum antibodies against *P. aeruginosa* (PA).

	Nasal IgA	Serum IgA	Nasal IgG	Serum IgG	Nasal IgA / Serum IgA	Nasal IgG / Serum IgG
Non-CF control group, n=9	0.17	0.05	0.09	0.12	3.29	0.92
CF-P, n=32	0.63	0.51	0.28	0.96	1.74	0.24
	0.28‡	0.13	0.09‡	0.12 *	2.57‡	0.84 *
	(0.20)	(0.08)	(0.05)	(0.09)	(1.83)	(0.40)
	0.77‡	0.73 *	0.19‡	1.01‡	1.99	0.19¤
	(0.28)	(0.57)	(0.13)	(0.45)	(2.28)	(0.13)
CF+P(i), n=24	1.03	0.14	0.50	0.30	27.92	2.80
	(0.68)	(0.14)	(0.50)	(0.26)	(83.42)	(3.64)
	1.33	1.21	0.64	1.68	1.99	0.38
	(0.35)	(0.87)	(0.59)	(0.64)	(1.87)	(0.27)
CF+P(c), n=25	1.70¤	0.45‡	0.63 *	0.85‡	4.84 *	1.16
	(0.60)	(0.31)	(0.50)	(0.71)	(2.79)	(1.41)
	1.46	1.65 *	0.96¤	2.52‡	1.06 *	0.38
	(0.47)	(0.68)	(0.41)	(0.77)	(0.58)	(0.16)

The standard deviations are shown in brackets. The ratios were first calculated for each individual and following the mean values were calculated. The white marked numbers refer to alginate; the grey marked numbers refer to standard antigen.

* :P<0.05, ¤:P<0.01 and ‡:P<0.0001 and when the CF+P(i) are compared with CF-P or when the CF+P(i) are compared with CF+P(c). None of the values form the control group differed significantly from the CF-P group.

Table 2
Mean sputum and serum antibodies against *P. aeruginosa*.

	Saliva IgA	Serum IgA	Saliva IgG	Serum IgG	Saliva IgA / Serum IgA	Saliva IgG / Serum IgG
Non-CF control group,	0.19	0.05	0.10	0.12	4.00	1.07
n=12	0.48	0.52	0.07	0.98	1.21	0.12
CF-P, n=19	0.23	0.09	0.07	0.17	2.88 *	0.58
	(0.22)	(0.06)	(0.03)	(0.17)	(2.49)	(0.29)
	0.26 *	0.54 *	0.07	1.51	0.80	0.07
	(0.21)	(0.49)	(0.03)	(0.86)	(0.75)	(0.06)
CF+P(i), n=47	0.38	0.08	0.08	0.26	5.62	0.51
	(0.39)	(0.04)	(0.06)	(0.34)	(5.87)	(0.27)
	0.45	0.92	0.07	1.68	0.77	0.05
	(0.33)	(0.73)	(0.02)	(0.72)	(0.72)	(0.02)
CF+P(c), n=23	0.82 ‡	0.20 ‡	0.21 ‡	0.74 ‡	5,06	0.35 *
	(0.48)	(0.14)	(0.22)	(0.66)	(3.99)	(0.18)
	0.71 ‡	1.23 ‡	0.25 ‡	2.59 *	0.67	0.09 ¤
	(0.33)	(0.56)	(0.21)	(0.37)	(0.44)	(0.07)

The standard deviations are shown in brackets. The ratios were first calculated for each individual and following the mean values were calculated. The white marked numbers refer to alginate; the grey marked numbers refer to standard antigen.

* :P<0.05, ¤:P<0.01 and ‡:P<0.0001 and when the CF+P(i) are compared with CF-P or when the CF+P(i) are compared with CF+P(c). None of the values form the control group differed significantly from the CF-P group.

CF+P(i) also had significantly higher ratios between alginate IgA in nasal secretion/serum (Table 1) and in saliva/serum (Table 2) compared with CF-P.

ROC-curves were used for finding the best cut-off values between CF+P(i) and CF-P:

Nasal IgA: A cut-off value of 1.09 for nasal IgA-St-Ag gave 76% sensitivity and 87% specificity. A cut-off value of 0.54 for nasal IgA-alginate gave 75% sensitivity and 94% specificity. When these cut-off values of the two nasal IgA tests (IgA-alginate and IgA-St-Ag) were combined, CF+P(i) could be discriminated from CF-P with 96%, sensitivity, 81% specificity, 79% predictive value of a positive test (pvpos), and 96% predictive value of a negative test (pvneg) .

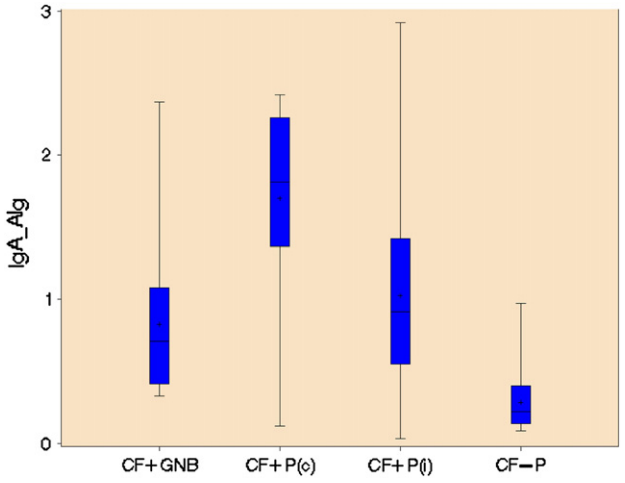

Fig. 2. A Box-and-whisker plot of the IgA values (OD) against alginate in nasal secretions distributed on four infection categories, showing the 2½, 25, 50, 75 and 97½% percentiles. CF+P(c), CF+P(i) and CF-P all differed significantly (P<0.0001) from each other.

Saliva IgA: A cut-off value of 0.27 for saliva IgA-St-Ag gave 64% sensitivity of and 74% specificity. A cut-off value of 0.24 for saliva IgA-alginate gave 55% sensitivity and 74% specificity. When the cut-off values of the two saliva IgA tests were combined, CF+P(i) could be discriminated from CF-P with 72%, sensitivity, 74%, specificity, 87% pvpos, and 52% pvneg .

Nine sputum samples and 17 nasal secretions were obtained from CF-P. In general, the antibody levels were higher in CF-P than in CF-GNB but lower than in CF+P(c).

There was a significant correlation between IgA-alginate in nasal secretions and saliva (rho=0.60) and between IgA-St-Ag in nasal secretions and saliva (rho=0.51) from the seventy-five CF patients where these values were available (P-values<0.0001).

In eighteen of 20 CF-P patients (90%), sputum samples with *S. aureus*, *H. influenzae*, *S. pneumoniae* or Aspergillus spp. were cultured. There was agreement in 12/20 (60%) of the nasal irrigation cultures and in 9/20 (45%) of the nasal swabs from the middle meatus cultures when compared with the sputum bacteriology. In one CF-P patient, *S. malthophilia* were found in sputum and nasal irrigation but these bacteria were not found by culture from the middle meatus swabs.

By immunohistochemistry examinations we found excessive amounts of IgA producing plasma cells in the sino-nasal tissue. They were mostly found in the connective tissue of the lamina propria (Fig. 1C), with higher concentration in focal areas close to the mucosal surface (Fig. 1D). Accumulations of the IgA producing cells were also seen around the secretory acini (Fig. 1E). IgA was also detected in the layer of mucus on the luminal mucosal surface (Fig. 1D) and in the secretions in the excretory ducts from the submucosal glands (Fig. 1F).

4. Discussion

We have previously reported that CF+P(c), in contrast to healthy persons, had high levels of IgA against *P. aeruginosa* alginate and St-Ag in sputum, saliva and tears and that the IgA contained a secretory component (sIgA) [4]. The high ratio of IgA in saliva, tears, and sputum versus serum also showed a local IgA production in contrast to the low ratio of IgG in the secretions versus serum in accordance with the systemic production of this immunoglobulin class [4]. The specific IgA response to *P. aeruginosa* in these secretions reflects the common mucosal immune response to offending microbes. Since our previous report (4;), *P. aeruginosa* sinusitis has attracted increasing attention as a focus for subsequent lung colonization and chronic infection [6,9,11–14,20,22]. Thus, we have expanded the study [4] in order to evaluate whether sIgA against *P. aeruginosa* can contribute to the diagnosis of *P. aeruginosa* sinusitis before onset of the chronic lung infection. In another article, more details are given about the bacteriological and clinical results from functional endoscopic sinus surgery on a large number of CF patients with sinusitis [6].

In the present study we also investigate IgA against *P. aeruginosa* alginate and St-Ag in saliva and serum, but in contrast to our earlier study [4] we now include nasal secretions and a greater number of CF patients, now subdivided according

to the lung infection status. Significant differences of IgA levels to the *P. aeruginosa* antigens were seen between both CF+P(c), CF+P(i) and CF-P. Accordingly, we also found an abundance of local IgA producing plasma cells in the nasal mucosa of CF+P(c) CF+P(i). Many plasma cells have previously been found in sinonasal tissue from CF patients [23]. Importantly, our results allow a differentiation between CF+P(i) and CF-P. Nevertheless, the test is limited by the possibility of cross-reaction with other pathogenic Gram-negative bacteria (e.g. *S. malthophilia*, *A. xylosoxidans* or *B. cepacia complex*) [15]. We conclude that the sensitivity, specificity, pvpos, and pvneg of each of the results of the IgA ELISAs or combined ELISA results are high enough to be evaluated as a diagnostic tool for *P. aeruginosa* sinusitis in a prospective study in our CF centre.

IgA concentration is in general elevated as a result of the local mucosal antibody response, whereas IgG is elevated as a consequence of the systemic inflammatory response [4,7]. Our results indicate an early, local production of IgA in the sinuses when these are infected with *P. aeruginosa*. This assumption is fortified, as we did not find *P.aeruginosa* in the nasal irrigation or middle meatus cultures from CF-P, which indicates that the sinuses are also free from *P.aeruginosa* [22,24]. This result can be compared with our previous findings of pathogenic bacteria in the majority of the sinuses in CF+P(i) and CF+P(c) [6].

Our results are therefore in accordance with our previous findings of high IgA levels in saliva, tears and sputum [4] and additionally show that nasal secretions contain even higher levels. This is in agreement with the increasing evidence of *P. aeruginosa* sinusitis being a focus for subsequently intermittent lung colonization and infection [6,9,11–14,22]. When *P. aeruginosa* settles in the paranasal sinuses, a local increase of mucosal IgA response is initiated. However, the inflammatory response is reduced compared with that of chronic lung infection since the secretory IgA is non-phlogistic in contrast to IgG which forms immune complexes with *P. aeruginosa* antigens and activate complement and attract PMNs in the respiratory zone of the lungs [1,6,10]. The result may be that IgA keeps *P. aeruginosa* at a distance from the cells of mucosal membranes [25] and thereby diminishing the clinical symptoms of sinusitis [10]. The same mechanism may be working in the conductive airways of CF+P(c), where most *P. aeruginosa* are located inside sputum together with the highly active PMNs [1,26,27], probably also due to IgA in mucosa and mucus in the conductive airways [4]. Keeping the bacteria at a distance from the mucosal membrane cells may be a common strategy in the respiratory and intestinal tracts [25] by means of the mucus layer and the anti-bacterial molecules such as IgA which prevent inflammation. *P. aeruginosa* sinusitis in CF may therefore resemble secretory otitis media with effusion where bacterial biofilms and IgA have also been found [28–30].

There are no standardized guidelines for detection of upper airway *P. aeruginosa* colonization or how or when to eradicate CF-pathogenic bacteria from the CF sinuses by sinus surgery and/or with antibiotic treatment [24,31]. The potential of surgical and/or conservative antibiotic treatment for eradication of *P.aeruginosa* sinusitis requires further prospective evaluation; for now an early stage attempt of eradicating *P. aeruginosa* may be considered when detected in the sinus [6,9,11,12,22,32].

A prospective study is planned to examine if the IgA response in nasal secretion or saliva is helpful for early diagnosis of *P.aeruginosa* sinusitis. It would also be relevant to investigate whether IgA in nasal secretions, collected through a gentle nasal lavage, is an easier way to collect IgA or if the method is complicated by dilution problems. Nasal lavage is already used as a supplementary diagnostic tool for CF infections [22], and as a research tool for rhinitis [33].

5. Conclusions

Both CF+P(c) and CF+P(i) have significantly higher IgA antibody levels in nasal secretions and saliva against *P. aeruginosa* alginate and St-Ag compared to CF-P. In CF+P(i) the elevated IgA probably reflect *P. aeruginosa* sinusitis where the bacteria adapt to the environment of CF airways CF patients; sinusitis may then be the focus for subsequent intermittent *P. aeruginosa* colonization and chronic infection of the lungs. The diagnostic value of IgA in saliva and nasal secretions for sinusitis will be studied prospectively.

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