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Extensive endoscopic image-guided sinus surgery decreases BPI-ANCA in patients with cystic fibrosis

Running title: Sinus surgery and BPI-ANCA in cystic fibrosis

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ABSTRACT

**Purpose:** Anti-neutrophil cytoplasm autoantibodies (ANCA) directed against bactericidal/permeability-increasing protein (BPI) are common in patients with cystic fibrosis (CF) and serum levels are correlated with lung colonisation by *Pseudomonas aeruginosa* and the severity of lung damage. The production of BPI-ANCA may be due to the co-stimulation of BPI when mounting an immune response against *P. aeruginosa*. The effect of surgery aiming to eradicate bacteria and infected tissue on BPI-ANCA levels is sparsely described.

**Methods:** A cohort of CF patients were included: 53 patients having extensive image guided sinus surgery (EIGSS) with topical postoperative antibiotic treatment, 131 non-operated controls, and 36 who had double lung transplantation (LTX). In all 219 patients, serum samples before and after surgery or at similar intervals were analysed for IgG and IgA BPI-ANCA.

**Results:** The EIGSS group showed a highly significant decrease in both IgA and IgG BPI-ANCA levels compared with their own pre-operative values and control group values (p < 0.001 to 0.02). The LTX patients also showed a highly significant decrease in both IgA and IgG BPI-ANCA levels (p < 0.001).

**Conclusions:** EIGSS and LTX decreases IgA and IgG BPI-ANCA levels in CF patients, indicating that extensive removal of infected tissue influence the pathogenic process of autoantibody production. The results shown herein are in favour of applying EIGSS in selected CF patients, and for using BPI-ANCA as a surrogate marker for guiding further therapeutic interventions.

Keywords: BPI-ANCA, IgG, IgA, sinus surgery, cystic fibrosis, infection, lung transplantation.
INTRODUCTION

The paranasal sinuses in patients with Cystic Fibrosis (CF) are often colonized with CF-lung pathogens, especially *Pseudomonas aeruginosa* [1;2]. Bacteria from the sinuses can be aspirated to the lower airways and thereby initiate or maintain deleterious lung infections [3]. Anti-neutrophil cytoplasm autoantibodies (ANCA) directed against Bactericidal/permeability-increasing protein (BPI) are frequently seen in CF patients [4], especially in those with severe lung damage [5;6]. IgG-BPI-ANCA is common and occur in approximately 70% of CF patients, whereas IgA-BPI-ANCA is found in about 35% [7]. There is a strong association between BPI-ANCA and lung infection by *P. aeruginosa* and BPI-ANCA levels are significantly correlated with the severity of lung damage [5;8]. This correlation may be ascribed to the formation of BPI-ANCA in patients chronically infected with *P. aeruginosa* due to a co-stimulatory mechanism of the dendritic cells involving the complex between BPI and surface antigens from *P. aeruginosa* [8;9]. Apart from a study showing decreased levels of BPI-ANCA in seven CF patients after lung transplantation (LTX) [5], the effect of surgery aiming to eradicate infectious foci and thereby tissue inflammation on levels of BPI-ANCA has not previously been described.

As BPI-ANCA seems to be a biomarker of a detrimental host-pathogen interaction in CF, we chose changes in BPI-ANCA levels as a surrogate marker for the study of potential positive effects of EIGSS. We also compared the effects of EIGSS on BPI-ANCA levels with the effects of LTX as both procedures remove or reduce substantial amounts of *P. aeruginosa* infected and damaged tissue.

MATERIALS AND METHODS
Basic patient population

The CF patients were recruited at the CF Centre in Copenhagen. The diagnosis of CF was based on characteristic clinical features, abnormal sweat electrolytes, and genotype. At least every third month, blood samples are taken for routine measurements.

Serum from a cohort of CF patients (n=237) were examined for the presence of IgA and IgG BPI-ANCA in 2002–2006 [5]. Serum samples from 199 of the 237 previously examined patients were again analysed for BPI-ANCA in February–April 2010. Thirty-eight patients were ineligible for follow-up as they had either died or did not show up for clinical control or blood sampling within the study period (Fig. 1). The patients were divided into three groups: A non-operated control group, a group who had LTX within 2006-2010 and a group who had EIGSS in-between the period where the serum was examined. Our main objective was to compare BPI-ANCA within the EIGSS group pre- and postoperatively. The pre- and postoperative change was also examined in the LTX group and the change over time in the non-operated control group was compared with the EIGSS group.

EIGSS study population

Patients were offered EIGSS based on the following criteria:

1: Patients intermittently lung colonized with increasing frequencies of positive cultures or prolonged declining lung function, despite intensive antibiotic-chemotherapy. Patients with an unknown infectious focus and increasing antibodies against *P. aeruginosa*, *A. xylosoxidans* or *B. cepacia complex* were given highest priority.

2: Patients who had undergone LTX.

3: Patients with severe symptoms of rhinosinusitis according to the European Position Paper guidelines [10].
Of the 199 patients with sera examined before 2006 and again in 2010, 59 underwent EIGSS according to the operative and postoperative procedures described below. Six patients were excluded from the EIGSS group due to having double LTX in between the two blood samples, leaving 53 patients to be evaluated for the isolated effect of EIGSS (Fig. 1). Median time from EIGSS to second blood sample was 301 (IQR: 111–644) days. Thirty-eight patients had surgery due to criterion 1, but 45 patients also had symptoms of chronic rhinosinusitis.

The lung infection status of the 53 EIGSS patients (26 males, 27 females) [11] is shown in Table 1. Thirty-four patients were dF508 homozygous, eighteen were dF508 heterozygous, and one patient had other mutations. The mean age in 2010 was 23 years (8–52 years).

**Non-operated study population**

Of the 131 non-EIGSS CF controls (73 males, 58 females), 77 were chronically lung infected with CF-pathogenic Gram-negative bacteria in 2010. Ninety-nine patients were dF508 homozygous, thirty-one patients were dF508 heterozygous, and one patient had other mutations. The mean age in 2010 was 29 years (8–62 years).

**LTX study population**

The possible effect of LTX on BPI-ANCA levels was examined In addition to the six patients who also underwent EIGSS, a further nine Danish and twenty-one Swedish patients with double LTX had serum samples available for BPI-ANCA testing before and after LTX. Median time from LTX to second blood sample was 275 (IQR: 100–1130). The 36 double LTX CF patients from Denmark and Sweden were essentially diagnosed and treated according to the same criteria [12].

**Surgery and postoperative treatment**
The purpose of surgery was to eradicate sinus bacteria and alleviate symptoms of chronic sinusitis by removing purulent secretions and inflamed tissue, creating ventilation and drainage of the sinuses and to make them accessible for postoperative instrumental cleaning and medical irrigations. Each patient was evaluated for symptoms [10], with a clinical examination including a CT scan of the sinuses. The precise extension of surgery (for instance, exploration of the frontal or sphenoid sinuses) was decided based on these findings. We applied classic EIGSS comprising an uncinectomy, an anterior ethmoidectomy and a medial antrostomy, leaving a significantly enlarged maxillary ostium comprising more than half of the medial maxillary wall. Visible intramucosal abscess looking structures were resected along with other inflamed mucosa when accessible. Following the surgical procedure, the nose was irrigated with saline and Colistimethate sodium in order to irrigate the opened and now accessible sinuses. The majority of patients followed a postoperative regime including 2 weeks of IV antibiotics, 6 months of topical nasal steroids, 6 months of daily nasal irrigations with saline and antibiotics, and five visits to the outpatient clinic where crusts and secretions were endoscopically cleansed.

**Sinus bacteriology**

All EIGSS patients had several sinus samples taken. These were cultured aerobically and anaerobically at 37 °C on standard agar media for 5–7 days [13]. In 52 out of the 53 patients having EIGSS, bacteria were cultured in one or more paranasal sinuses; 45 patients had cultures with CF-pathogenic Gram-negative bacteria, including 37 patients with *P. aeruginosa*, *A. xylosoxidans*, and/or *B. cepacia complex*., representing the bacteria causing most morbidity among CF patients. Of these 37 patients, the 14 latest operated patients had samples cultured six months postoperatively according to a new treatment protocol initiated in June 2009. Using endoscopic guidance, secretions
from the ethmoidal and maxillary sinuses were collected bilaterally using sterile, curved metal suction tubes.

**BPI-ANCA antibodies**

The analyses of both the Danish and the Swedish samples were performed by EuroDiagnostica AB, Sweden, using a direct ELISA as previously described [6]. In short, BPI purified from human granulocytes was coated onto microtiter plates at a concentration of 1 µg/ml in a bicarbonate buffer. Serum samples were diluted and incubated for 1 hour. Bound antibodies were detected using alkaline phosphatase-conjugated goat anti-human IgA and anti-human IgG (EuroDiagnostica, Sweden). BPI-ANCA was quantified from a calibrator curve of serum that was serially diluted. The results were expressed as arbitrary units (U/L). One positive and one negative control were analysed in each ELISA.

According to the manufacturer, BPI-ANCA IgA values below 53 units were regarded as negative and values above 67 units were regarded as positive; BPI-ANCA IgG values below 38 units were regarded as negative and values above 50 units were regarded as positive.

Exact values were used for the data analyses.

In order to show comparability between results from 2002–2006 and 2010, 80 of the 199 blood samples from 2002–2006—all those with high values and random patients with moderate and low values—were re-analysed. We found that the differences between the means of the paired IgA data (267 and 264 (U/L)), were non-significant, and that the differences were normally distributed. The Bland-Altman plot showed no single outlier and systemic errors were therefore not suspected, but the standard deviations were high (424 and 408). The corresponding differences for the paired IgG data (means 235 and 206 U/L) were also non-significant and the means and the plots did not
indicate systemic errors. Based on this, we concluded that the methods were comparable. Re-analysed values were used when available.

**Serum Antibodies**

To assess whether a potential decrease in BPI-ANCA was part of a general decrease in the immune response after EIGSS, the level of precipitating antibodies against the main Gram-negative bacteria (*P. aeruginosa, A. xylosoxidans or B. cepacia complex*) measured by crossed immunoelectrophoresis [14] taken preoperatively closest to FESS was compared with the lowest value found 3–9 months postoperatively. Furthermore, the average level of total anti-Pseudomonas IgG values measured by ELISA twelve months preoperatively were compared with the average level twelve months postoperatively.

**Statistics**

The data were analysed using SAS 9.1.3. The BPI-ANCA data were continuous. As the distribution of data was positively skewed, log10 transformations were performed. Patients with a value of “0” were given a value of 0.1 in order to allow the transformation. The transformed data had an approximately normal distribution justifying two-sample t-tests for the means. The data from the LTX patients and serum antibodies had an approximately normal distribution without transformation.

**Ethics**
The serum samples were obtained as part of the outpatient clinic routine, thus no extra blood samples were taken. The results did not cause any change in the treatment modality for the patients involved.

RESULTS

Sinus surgery and BPI-ANCA levels

The exact BPI-ANCA values in 2010 were compared with the values from 2002–2006 for the EIGSS and the non-operated groups of patients (Table 2): In the EIGSS group, the values before and after EIGSS showed a significant reduction in both BPI-ANCA IgG levels (p < 0.001 (CI: 62%–379%)) and BPI-ANCA IgA levels (p = 0.01 (CI: 15%–202%)). These reductions were due to decreases found in the sub-groups of patients intermittently or chronically colonized in their lungs, as there were no significant differences in the sub-group of non-infected patients (table 3). No significant changes were seen within the non-operated control group (p = 0.55 and p = 0.46).

Thirteen patients had IgA levels above 53 U/L (upper normal limit) before surgery. Eleven patients had IgG levels above 38 U/L (upper normal limit) before surgery. Both groups showed a significant decrease in the values by sub-group analyses (p< 0.05; p < 0.001).

The changes of BPI-ANCA antibodies levels in the EIGSS group were compared with those of the non-operated control group. The EIGSS group showed a significant reduction in both IgG BPI-ANCA (p < 0.001 (CI: 51%–337%)) and IgA BPI-ANCA values (p = 0.02 (CI: 10%–175%)).

Postoperative bacteria burden and BPI-ANCA levels

In the 14 patients who had bilateral sinus samples cultured 6 months postoperatively, 10 patients had negative cultures, 2 showed bilateral growth of *P. aeruginosa*, 1 had bilateral growth of *A.*
*xylosoxidans*, and 1 had unilateral growth of *A. xylosoxidans*. Altogether, the 14 patients showed an average decrease in BPI-ANCA IgG of 51 U/L (range from -11 to +311) and an average decrease in BPI-ANCA IgA of 70 U/L (range from -30 to +680); one chronically lung infected patient had a small increase in BPI-ANCA IgG and one intermittently colonized patient had a small increase in BPI-ANCA IgG and IgA.

**Lung transplantation and BPI-ANCA levels**

The levels of BPI-ANCA IgA were measured pre- and postoperatively in all 35 LTX CF patients; 6 patients had negative IgA values pre and postoperatively, 4 patients had increased postoperative values (mean increase: 89 U/L) and 25 patients showed decreased postoperative values (mean decrease: 620 U/L). Using a two-sample paired t-test for all 35 patients, the total decrease was found to be highly statistically significant (p < 0.001).

The levels of BPI-ANCA IgG were only available pre- and postoperatively in 26 LTX CF patients. Ten patients had negative IgG levels pre- and postoperatively (below 50 U/L), three patients had increased postoperative values (mean: 225 U/L) whereas 13 patients had decreased postoperative values (mean: 713 U/L). Using a two-sample paired t-test for all 26 patients, the total decrease was also found to be statistically significant (p = 0.02).

**Serum Antibodies**

Out of the 53 EIGSS patients, precipitating antibodies were available in 47 patients and total anti-*Pseudomonas* IgG were available in 40 patients. Neither by looking at the whole group or by sub-analyses within the lung infection groups, significantly changes were seen.

**DISCUSSION**
In this case control study, we present novel data from a large group of CF patients with bacterial sinus colonisations treated with EIGSS combined with an intensive peri- and postoperative treatment regimen intending to eradicate the bacteria and prevent re-colonization. We found significantly lower levels of IgA and IgG BPI-ANCA after surgery both compared with the individual values before surgery and compared with CF patients without EIGSS and LTX. We also confirmed the previous finding [5] of decreased IgA and IgG BPI-ANCA levels following double LTX. The decrease in the level of BPI-ANCA following LTX was more pronounced than after EIGSS. This could be ascribed to the immunosuppressive treatment given to the LTX patients as well as the lungs being larger organs with more infected tissue than the sinuses.

Our results strongly suggest that the surgical procedure of EIGSS and LTX with removal of the chronically infected tissue results in decreased BPI-ANCA levels. Our findings of unchanged antibody levels in the EIGSS group indicate that the BPI-ANCA decrease is not caused by a general decrease in immune response. As the CF treatment protocol basically has been unchanged throughout the period of observation, the pre- and post-operative treatment is not expected to influence the results [15]. However, the intensive postoperative local antibiotic treatment regimen in the EIGSS group is presumed to play a role in preventing re-colonization.

There is limited knowledge regarding the mechanisms that determine the levels of BPI-ANCA in CF patients. As BPI-ANCA is strongly correlated with colonisation by *P. aeruginosa* and lung damage in CF patients [5;8], and as BPI-ANCA may be produced due to co-stimulation of the immune system with a complex of BPI and *P. aeruginosa* surface antigens, this could explain our findings and supports the theory that BPI-ANCA may be a useful surrogate marker of the Gram-negative bacterial load in CF patients. Our findings in the 14 patients cultured from the sinuses
during and after EIGSS, showing that the sinus bacterial load in the majority of cases was eradicated or reduced postoperatively, further support this theory.

Apart from reducing/eliminating the bacterial load in the nose and sinuses, it is also possible that our observation, that EIGSS can reduce the frequencies of not only upper but also lower airway cultures positive for Gram-negative bacteria in intermittently colonized patients [16], will contribute to decreasing BPI-ANCA due to the reduction of the bacterial load in the lungs, since intermittent colonization also stimulates an inflammatory response in CF patients [17;18]. The decrease of BPI-ANCA IgA and (not significant) BPI-ANCA IgG in chronically lung infected patients having EIGSS could be due to prevention of further spread of the infection by aspiration from the sinuses to new areas of the lungs after removal of the sinus focus, since it is known that the lung damage in CF patients with chronic infections characteristically is focal [19] leading to a focal loss of alveoles and an annual decline of lung function of about 1-2% [20].

Although the standard deviations were high in all groups, our results are statistically significant in all relevant comparisons: when comparing changes in BPI-ANCA values in patients with or without EIGSS, patients with or without LTX, and BPI-ANCA values before and after EIGSS and LTX. The non-operated group included more chronically infected patients than the EIGSS group and consequently this group had higher BPI-ANCA levels, but those were unchanged over time. Based on experience from patients with Granulomatosis with polyangiitis (Wegener’s) (GPA), where IgG ANCA is also associated with disease activity [21], it must be expected that the levels of BPI-ANCA may depend on the assay methodology [22]. At present, the assays available for the detection of BPI-ANCA have not been standardised. As CF patients have more positive IgA- than
IgG-BPI-ANCA, it may be necessary to further investigate whether or not this difference is real and also whether or not different assays might be more sensitive.

ANCA is a family of autoantibodies directed at different components in the granules of the cytoplasm of human neutrophils. It is of interest that the presumed mechanism for BPI-ANCA production is a co-stimulation of dendritic cells with BPI complexed to *P. aeruginosa* surface antigens [9] and other Gram-negative bacteria, whereas the presumed mechanisms for production of PR3-ANCA include molecular mimicry [23] a disrupted balance between the naturally occurring PR3-ANCA and its anti-idiotypic antibody [24], and epigenetic modifications leading to inappropriate expression of PR3 [25].

Another aspect is the possible pathogenic role of ANCA. In microscopic polyangiitis (MPA), ANCA is mainly directed against myeloperoxidase (MPO). MPO-ANCA and to a lesser extent Pr3-ANCA has been shown to activate TNF-primed neutrophils [26]. Later it has been possible to mimic MPA manifestations in experimental animals by infusing MPO-ANCA [27], and recently a similar observation has been made by the same author, inducing GPA like manifestations in mice with a humanised immune system using PR3-ANCA [28]. So far, a pathogenic role for BPI-ANCA has not been reported, but BPI-ANCA may also play a pathogenic role by neutralising BPI, which is a potent inhibitor of Gram-negative bacteria [5;8].

No standardized guidelines exist regarding the criteria for sinus surgery in CF patients [10;29]. Our results indicate that EIGSS with the intensive postoperative treatment regimen should be performed in selected CF patients with sinus infection. The possible long-term benefit of EIGSS in CF patients
has to await postoperative follow-up studies on the quality of life, frequencies of lung colonisations and the need for LTX. The importance of BPI-ANCA as a surrogate marker for the bacterial load/chronic infection with Gram-negative bacteria, especially *Pseudomonas aeruginosa*, must be elucidated postoperatively by prospective and consecutive correlations of BPI-ANCA levels with microbial findings in the sinuses.

In conclusion, we present novel data showing that extensive endoscopic image-guided sinus surgery followed by antibiotic irrigation without additional immunosuppressive treatment decreases IgA- and IgG BPI-ANCA levels in CF patients and also confirm the same effect following lung transplantation. We hypothesize that extensive surgery eradicating infectious foci and a reduction in mucosal inflammation can influence the pathogenic process of autoantibody production, for example BPI-ANCA. Our results are in favour of applying EIGSS in CF patients with intermittent or chronic sinus infections and also indicate that measuring BPI-ANCA levels in individual patients may be used as a surrogate marker for guiding further therapeutic interventions.

**ACKNOWLEDGEMENTS**

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**CONFLICT OF INTEREST**

Jørgen Wieslander is employed at EuroDiagnostica AB.
Reference List


Figure 1: Flowchart for the inclusion of 184 non lungtransplanted (LTX) Danish CF patients in the cohort study where BPI-ANCA measurements within 2002–2006 were compared with BPI-ANCA measurements in 2010.

<table>
<thead>
<tr>
<th></th>
<th>No Gram-negative bacteria</th>
<th>Pseudomonas aeruginosa</th>
<th>Achromobacter xylosoxidans</th>
<th>Burkholderia cepacia complex</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Colonized/infected</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
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<tr>
<td>Intermittently colonized</td>
<td>-</td>
<td>22</td>
<td>6</td>
<td>1</td>
<td>33</td>
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<tr>
<td>Chronically infected</td>
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<td>-</td>
<td>2</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>29</td>
<td>15</td>
<td>3</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 1: Lung infection status at the time of EIGSS in 53 CF patients.
Table 2: Serum BPI-ANCA (U/L) from a cohort of CF patients having EIGSS, before surgery (2002-2006) and after surgery (2010), from a cohort of non-operated CF controls, and a cohort of LTX CF patients, given as medians with inter-quartile ranges (IQR). The postsurgical decrease of BPI-ANCA IgG and IgA was significant within the EIGSS group and within the LTX group.

<table>
<thead>
<tr>
<th>Group</th>
<th>IgG-BPI-ANCA Median (IQR)</th>
<th>IgA-BPI-ANCA Median (IQR)</th>
<th>patients (2002/2010)</th>
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</thead>
<tbody>
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<td>EIGSS:</td>
<td>20 (9–36)</td>
<td>7 (2–14)</td>
<td>52/53</td>
</tr>
<tr>
<td></td>
<td>15 (7–33)</td>
<td>9 (3–28)</td>
<td>53/53</td>
</tr>
<tr>
<td>Non-operated:</td>
<td>26 (8–108)</td>
<td>24 (8–94)</td>
<td>128/131</td>
</tr>
<tr>
<td></td>
<td>28 (13–100)</td>
<td>26 (10–84)</td>
<td>129/130</td>
</tr>
<tr>
<td>LTX:</td>
<td>182 (19–463)</td>
<td>20 (15–88)</td>
<td>26/26</td>
</tr>
<tr>
<td></td>
<td>198 (83–794)</td>
<td>42 (26–183)</td>
<td>35/35</td>
</tr>
</tbody>
</table>

Table 3: Serum BPI-ANCA (U/L) from a cohort of CF patients having EIGSS, before surgery (2002-2006) and after surgery (2010). The results are categorised according to the lung infection status.

*: significantly postoperative decrease (p<0.05). **: highly significantly postoperative decrease (p<0.01).