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Original Article

Decreased mucosal oxygen tension in the maxillary sinuses in patients with cystic fibrosis

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Abstract

Background: Pseudomonas aeruginosa in the sinuses plays a role in the lungs in cystic fibrosis (CF) patients, but little is known about the sinus environment where the bacteria adapt. Anoxic areas are found in the lower respiratory airways but it is unknown if the same conditions exist in the sinuses.
Methods: The oxygen tension (pO_2) was measured, using a novel *in vivo* method, in the maxillary sinus in a group of 20 CF patients.
Results: The CF patients had a significant lower pO_2 on the mucosa but not in the sinus lumen as compared with a control group of non-CF patients. Anoxic conditions were found in 7/39 (18%) of the sinuses from where we cultured *P. aeruginosa, Stenotrophomonas maltophilia* and/or coagulase negative staphylococci.
Conclusion: These findings support our hypothesis that *P. aeruginosa* can adapt or acclimate to the environment in the lungs, during growth in anoxic parts of the paranasal sinuses.

Keywords: Pseudomonas aeruginosa; Cystic fibrosis; Maxillary sinuses; Oxygen tension; Sinus surgery; Catheter optode

1. Background

Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene for the CF transmembrane conductance regulator (CFTR) protein, resulting in altered chloride transport, in multiple organs, which comprises the mucociliary function and renders the mucus viscosity and the mucosa more susceptible to infections [1]. Nasal and sinus inflammation is a frequent condition in patients with CF, commonly leading to findings as congestion, mucopurulent material in the nose cavity, polyposis, abnormalities of the lateral nasal wall, mucoceles, and hypoplasia of the paranasal sinuses [2]. Such conditions might affect the O_2 exchange and the O_2 content in the sinuses and thus the microenvironment of its bacterial community.

The gas exchange in the maxillary sinus takes place via the ostium and the mucosa that absorbs and consumes O_2. The diffusion through the ostium obeys simple physical laws and depends on the patency of the ostium, the volume of the sinus and the respiratory work in the nose.

The absorption of O_2 from the sinuses as well as the change in the O_2 content in the paranasal sinuses after obstruction of the ostium, are thought to be important factors in the pathogenesis of sinusitis. There is a significant decrease in the luminal O_2 content in persons with acute sinusitis or allergic rhinitis compared with patients without symptoms [3,4]. Aust and Drettner [4] also found decreased O_2 tension (pO_2) in a group of patients

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with recurrent sinusitis and in a group of patients with obstructed ostia (all non-CF). They did not find pO_2 to be related to the presence or absence of antral pus or mucus. Carenfelt and Lundberg [5] reported a pO_2 close to zero in some purulent sinus secretions with *Streptococcus pneumoniae* or *Haemophilus influenza* compared with 96 mmHg in non-purulent secretions. They conclude that the gas composition in the sinuses influences the bacterial growth, as well as the bactericidal function of the granulocytes, and that the O_2 levels also might be of importance to the mucociliary activity. Purulent sinusitis should thus be treated by drainage of the sinus cavity, not only to reduce the debris, but also to improve the condition for the local host defense mechanisms of the sinus.

The limited research that has been published regarding pO_2 in the sinus deals with non-CF patients. CF patients have a different inflammatory response in their sinus mucosa as compared with non-CF related rhinosinusitis [6,7], and the O_2 conditions and their importance for rhinosinusitis in these patients are unknown. However, it has been shown that hypoxia contributes to a reduction of cell surface CFTR [8], which might have an additional negative impact in the sinuses of CF patients.

Chronic *Pseudomonas aeruginosa* lung infection develops in most patients with CF. Once the bacteria have established a chronic infection in the lungs, they cannot be eradicated. *P. aeruginosa* is a facultative anaerobe, which can proliferate and adapt to anaerobic environments, when thick layers of mucoid exopolysaccharide surround the bacteria (biofilm). Such biofilms are known to exist in the sinuses [6] and in the lungs, and the presence of such biofilms limit the diffusive supply of O_2 which then can lead to total O_2 depletion in the sputum [9]. In addition, numerous of polymorphonuclear leucocytes (PMNs) in the infected bronchi exhibit strong consumption of O_2 for production of reactive oxygen species (ROS) [9–13].

Under such anoxic conditions *P. aeruginosa* can achieve anaerobic growth either based on denitrification using nitrate as a terminal electron acceptor or by fermentation of arginine [9–12,14].

It has been indicated that *P. aeruginosa* responds to hypoxic mucus with an upregulation of alginate production, which may decrease the susceptibility to some antibiotics. Also, novel therapies for CF include removal of hypoxic mucus plaques and the use of antibiotics effective against *P. aeruginosa* adapted to anaerobic environments [10].

In the mucus with low pO_2, *P. aeruginosa* can make alterations due to mutations caused by the ROS or conversion in phenotype, e.g. becoming mucoid and developing antibiotic resistance, in order to adapt to different focal niches [15,16]. Studies of *P. aeruginosa* suggest a correlation between nutrient limitation, growth rates and conversion to mucoidy [17], and we speculate that the same applies for anoxic conditions.

The upper airways are shown to be a gateway for acquisition of opportunistic bacteria like *P. aeruginosa*, where the paranasal sinuses can act as a reservoir. Concordant genotypes have been found in the sinuses and in the lungs [18]. Our hypothesis is that *P. aeruginosa* adapts to the environment in the paranasal sinuses where some bacteria mutate or converse their phenotype [19]. This results in bacterial strains that are fit for spreading to the lungs, where they can maintain an ongoing deleterious infection.

Our present knowledge about *P. aeruginosa* is primarily related to the lungs. The pattern of inflammation differs in the sinus from the findings in the lower airway specimens of chronically infected patients with CF [20]. There is a Th2 dominated response in the lungs, while there is a significantly reduced PMN response in the sinuses, probably due to the higher concentration of IgA in the sinuses then in the lungs [21]. The latter statement combined with the fact that antibiotics more difficultly penetrates and achieves therapeutic levels in the sinus cavity than in the lungs, are some of the reasons why the immune response in the sinuses is less challenging than in the lungs. Based on the above mentioned knowledge, it is important to determine whether *P. aeruginosa* can adapt to anaerobic environments in the sinuses. In this study we determined the pO_2 in the maxillary sinuses in CF patients never infected, intermittently infected and chronically infected with *P. aeruginosa* in their lungs as a first step towards determining under which conditions *P.aeruginosa* adapts in the sinuses.

2. Materials and methods

The patients were recruited at the CF Centre in Copenhagen. The CF-diagnosis was based on characteristic clinical features, abnormal sweat electrolytes and the genotype. CF patients planned for sinus surgery were invited to participate in our study. As a control group, we asked non-CF patients who underwent surgery under general anesthesia because their nasal septum needed correction. Patients suffering of acute or chronic rhinosinusitis were excluded from the control group. All invited CF-patients accepted to be included in the study, while two patients who were invited to join the control group denied.

The CF-patients follows a routine with monthly medical examinations including lung function tests and cultures taken from the lower airways. At least every third month blood samples are taken for measurements including antibodies against *P. aeruginosa* (precipitating antibodies).

No standardized guidelines comprising criteria and motivations for sinus surgery in CF patients exist [22]. At our institution we select patients based on the following criteria in descending order:

1. Patients with declining lung function despite intensive antibiotic-chemotherapy and/or increasing antibodies against Gram-negative bacteria despite negative bacteriology in their sputum samples. Especially patients with unknown focus and increasing antibodies against *P. aeruginosa, Achromobacter xylosoxidans* or *Burkholderia multivorans* are given priority. The majority of patients has been operated due to criteria 1, but may also fulfill criteria 2 or 3 as well.
2. Patients who have undergone lung transplantation within the last year.
3. Patients with severe symptoms of rhinosinusitis according to EPOS guidelines [22].

2.1. Ethics

All the measurements were done during anesthesia, which the patients underwent for other reasons. The measurements were done through the natural ostia to the maxillary sinus, so no permanent damage was done to the control group, and the CF patients all had their sinuses opened during surgery afterwards. The study was approved by the local ethics committee (H-A-2008-141), and all patients gave informed consent. In patients <18 years of age, consent was also obtained from their parents.

2.2. Preparation of optodes

The pO_2 in the sinus was measured with a new type of catheter O_2 optode. See [23] for a recent review of fiber-optic O_2 sensor technology. The optode was manufactured from an Endonasal Suction Tip (EST)(Medioplast, Malmö, Sweden, 3.0×150 mm 11 G) and a 3 m length of a plastic PMMA optical fiber (POF, step index, 2 mm diameter with a polymethyl methacrylate core and a fluorinated polymer cladding; Laser Components GmbH, Olching, Germany). The end of the POF was glued inside the EST with two components Super Epoxy (Plastic Padding, Henkel Technologies) with ~1 mm protruding from the tip of the EST. The other part was covered with 2.4 mm shrink tubing (Low Shrink Temperature (LSTT) polyolefin tubing; RS Components, Denmark) and a SMA-connector (Laser Components GmbH, Germany, SMA-B2100) was glued at the end. Both ends were subsequently polished down to the metal. At the EST end, a disc (2 mm diameter) of a 0.125 mm thick transparent Mylar®-foil (Goodfellow, UK) was glued on the fiber tip with 1:1 diluted contact glue (Bostik Kontaktlim A3). Subsequently, the foil was covered with a thin layer of a luminescent O_2 indicator layer composed of a solution of 1 g polystyrene (Goodfellow), 500 mg TiO_2, and 25 mg Pt(II) meso Tetra(pentafluorophenyl)porphine (PtTFPP) (Frontier Scientific, USA), in 29 g $CHCl_3$.

3. Calibration and measurements

Prior to use, the catheter O_2 optodes were sterilized in a plasma oven (Sterrad 100 S), which enables sterilization at low temperatures using hydrogen peroxide. Calibration and measurements were done with the catheter O_2 optodes connected to a fiber-optic O_2 meter (Fibox 3, Minisensor Oxygen Meter, Presens Precision Sensing GmbH, Regensburg, Germany)[24]. The optode meters used in this study measures the luminescence lifetime with a phase-modulation technique [22], where the O_2 dpendent luminescence lifetime of the PtTFPP indicator, τ, can be calculated from the measured phase angle shift, Φ, between the sinusoidal intensity modulated (at frequency, fmod) excitation and emission signals:$\tan(\Phi)=2\pi\cdot f_{\mathrm{mod}}\cdot\tau$

The sensor response can be described by a modified Stern-Volmer equation (x):

$$\tan(\Phi)=\tan(\Phi_0)\left(\frac{1-\alpha}{1+K_{SV}[O_2]}+\alpha\right) \quad (1)$$

where, Φ_0 is the phase angle of the indicator in the absence of O_2, Φ is the phase angle of the indicator at a given O_2 concentration, K_{SV} is a characteristic temperature dependent quenching coefficient of the immobilized indicator, and α is the non-quenchable fraction of the indicator ($\alpha=0.11$ in this study) in the carrier foil. For a given mixture of indicator and matrix material, α is usually constant over the dynamic range (x). The Stern-Volmer constant (K_{sv}) was calculated from calibration values measured in anoxic and atmospheric air. The Φ_0 was measured in an anoxic bench and the Φ_{Sat} was measured in a thermostated climate room with atmospheric air temperature of 37 °C. The values were not corrected for air pressure:

$$K_{SV}=\left(\frac{1-\alpha}{\frac{\tan(\Phi_{Sat})}{\tan(\Phi_0)}-\alpha}-1\right)\frac{1}{[O_2]_{Sat}} \quad (2)$$

The pO_2 for a given experimental phase angle measurement was calculated according to:

$$[O_2]=\left(\frac{1-\alpha}{\frac{\tan(\Phi)}{\tan(\Phi_0)}-\alpha}-1\right)\frac{1}{K_{SV}} \quad (3)$$

3.1. Measurement

The patients (CF and non-CF) were anaesthetized using an oral tracheal tube and intravenous solutions. The patients were pre-oxygenated with 100% O_2 before intubation. We waited at least 15 min before onset of the O_2-measurements. During anesthesia, all patients were given ~40% O_2 of supplementary O_2. At the beginning of the general anesthesia but prior to surgery the catheter was introduced, through the nasal cavity, into the maxillary sinus through the natural maxillary ostium (Figs. 1 and 2). The pO_2 in the maxillary sinuses was continuously recorded, first in the lumen of the sinus, and then with the catheter touching the mucosa in the bottom of the sinus. It was noted if the patient had an occluded or a patent maxillary ostium. Temperature, pH and the volume of the sinuses were not recorded. After the pO_2 was measured, a regularly sinus surgery was performed, where each maxillary sinus was cultured separately.

All CF patients were CT scanned within 6 weeks prior to surgery and staged according to the Lund McKay staging system, where every sinus scores from 0 (no opacification) to 2 (total opacified).

3.2. Statistical methods and definitions

Statistical significance was evaluated by unpaired *t*-test for observations with parametric data. Catagorical data were analysed by Fisher's exact test. A p-value <0.05 was considered statistically significant. The tests were performed with Prism 4.0c (GraphPad Software, La Jolla, California, USA).

We define anoxia as pO_2 values <0.8 mmHg.

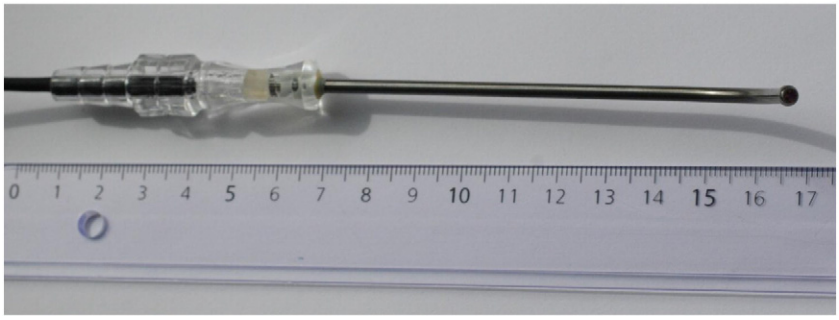

Fig. 1. The catheter with the incorporated optode.

4. Results

4.1. Patients

The pO_2 was measured in 20 CF patients, representing 39 maxillary sinuses (one measurement failed). The mean age of the CF patients was 18 years (range 6–39 years.), 12 of the patients were under 18 years of age. One patient had previously undergone sinus surgery but his ostia to the maxillary sinuses were totally obstructed.

Sixteen of the 20 CF patients were categorized as intermittently infected [25], two were chronically infected and two were lung transplanted (former chronically *P.aeruginosa* infected).

Four out of 40 CF-sinuses had a Lund McKay score [26] of 0 (no opacification) seen on the CT scan. The average score of all sinuses was 1.2. The control group contained 11 patients representing 22 sinuses. The mean age was 31 years (range 22–66 years.)

4.2. Microbiology

In nine CF-patients representing 13 sinuses, mucoid and/or non-mucoid *P. aeruginosa* were found. In only one maxillary sinus no bacteria were detected. Besides *P. aeruginosa*, the results from the cultivation were as follows: *3* sinuses contained *Stenotrophomonas maltophilia, 3 A. xylosoxidans, 23* coagulase negative staphylococci (CNS)*, 3 Staphylococcus aureus, 3 Haemophilus influenzae, 1 Enterobacter cloacae and 3* sinus contained *Candida albicans.* (Several sinuses had growth of more than one microorganism).

4.3. pO_2 measurements

A significantly ($p<0.03$) lower pO_2 on the maxillary mucosa was found in CF patients, as compared with the control group (Fig. 3). There was no difference between the patients above and under 18 years of age within the CF group.

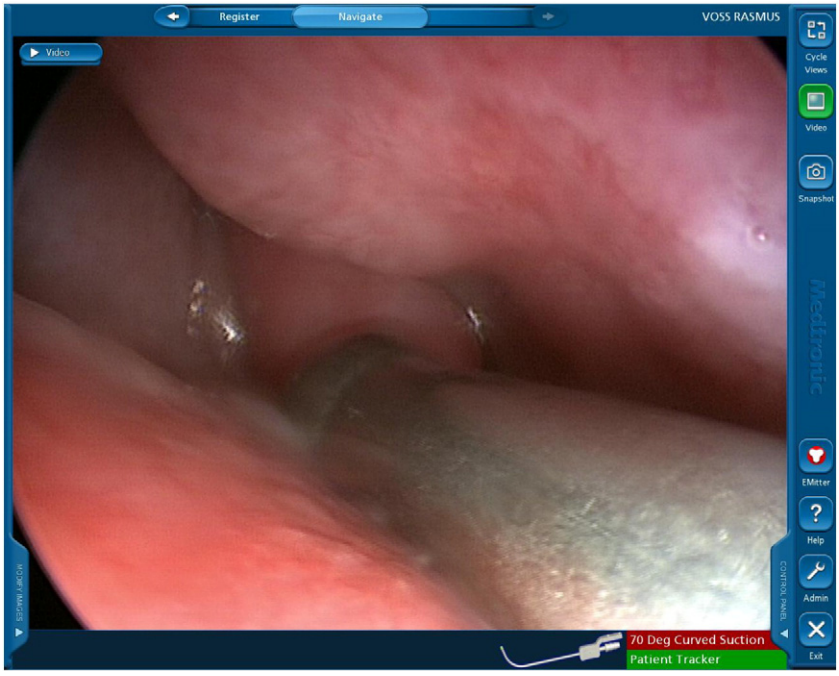

Fig. 2. The catheter on its way to the right maxillary sinus.

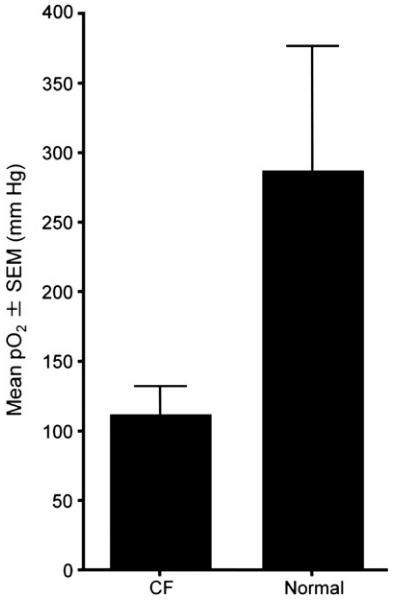

Fig. 3. PO_2 on the mucosa of all maxillary sinuses (*t*-test) $P<0.0263$.

In contrary to what we expected, there were only two measurements in the lumen of the sinuses below 20 mmHg O_2 (10.0 and 13.8 mmHg) and none with an anoxic lumen. There was no correlation between the types of bacteria cultured from the sinuses, the presence of pus or visible enlarged ostia, or the pO_2 content in the maxillary lumen. The pO_2 was not significantly lower in the CF patients' lumen compared to the controls either.

We found anoxic conditions on the mucosa in five CF-patients, comprising three patients with unilateral anoxia and two patients with bilateral anoxia. Thus, anoxia was found at a higher frequency in the CF patients than in non-CF patients ($p<0.02$). Four of the sinuses harbored *P. aeruginosa* and/or *S. maltophilia* but we also diagnosed total anoxia in three sinuses that only harbored CNS.

In eight sinuses in five patients no macroscopically pus was found. These sinuses were not anoxic. However, we also found high pO_2 in sinuses with pus and *P. aeruginosa*.

In three sinuses from three patients a large visible natural ostium to the maxillary sinus existed in accordance with high pO_2 values in the lumen (average of 128.3 mmHg). Unexpectedly, anoxia on the mucosa was demonstrated in one of these patients. No significant difference was found between the luminal pO_2.

5. Discussion

Little is known about how O_2 influences the immune system and the bacterial community in the sinuses, and to our knowledge no research has previously been done in relation to either the pO_2 lumen or the pO_2 mucosa in the sinuses of CF patients. We found a significantly lower pO_2 on the mucosa in CF patients than in the healthy control group, and also found some CF patients with anoxic conditions. However, we suspect that our study has a tendency to overestimate the pO_2 values. The reasons are: 1: If the sensitive optode comes in contact with a lot of blood it will measure the pO_2 in the blood; 2: The fact that all patients were given supplementary O_2 which could increase the pO_2 in the sinuses; 3: We only measured a little area in the bottom of the sinus mucosa, from which we cannot exclude the possibility that other areas of the mucosa had lower or higher pO_2. Our measurements were done in the maxillary sinuses, but we expect our findings to be representative for the other paranasal sinuses as well.

No follow up study was made, but additional measurements in patients with anoxic conditions could have determined whether the pO_2 increased after surgery. In principle, it is possible to re-measure some patients only using local anesthetics, as long as the sinus ostium exceeds 4 mm and the patient is capable of lying still for about two minutes while the optode is in contact with the mucosa. Due to unfortunate circumstances as severe nasal septum deviation, young age, and two patients dropping out of our follow up study, we have not, so far, had patients for re-measuring their pO_2.

The CF-sinus-anatomy varies, probably due to the associated chronic rhinosinusitis when the sinus development takes place. Consequently the natural maxillary ostium is often obstructed. However, among the minority a very enlarged ostium is observed. Opacified (blurred) sinuses and viscous mucus complicates the O_2 diffusion in the sinuses (Fig. 4). CF patients often have opacified sinuses diagnosed by a CT-scan. In physical examination or during sinus surgery, the nose and sinuses often present clinical signs of inflammation and infection, but this is less commonly related to rhinosinusitis symptoms than in non-CF patients. We have shown some maxillary sinuses with

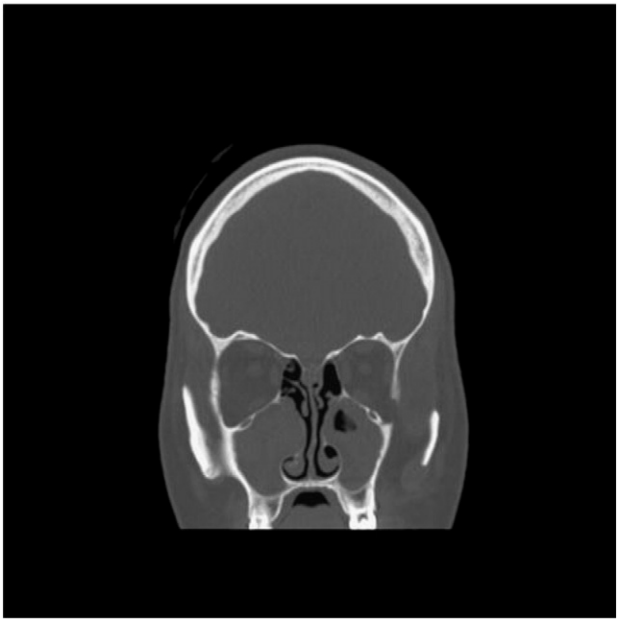

Fig. 4. A typical coronal CT scan of a CF patient. A right total opacified maxillary sinus is seen. The left maxillary sinus shows a little air (black) medially in the top between the natural ostium and the middle turbinate.

aerobic areas and some with anaerobic areas. We hypothesize that some sinuses may contain aerobic as well as anaerobic areas, probably hosting two different niches of *P.aeruginosa*, as seen in CF-sputum [9], which could enter the lower airways. In the anoxic areas, we suspect a lower growth rate and an adaption to the environment as seen in the endobronchial mucus [9]. Our bacterial findings and the observed O_2 depletion confirms that *P. aeruginosa*, as well as other bacteria, can adapt to and live under anaerobic conditions in the maxillary sinuses. We suspect that the sinus epithelium, the bacteria and the PMNs along with other inflammatory mechanisms competes to use the limited amount of O_2. These results support the hypothesis that *P. aeruginosa* can adapt to and change phenotype in the sinuses where there is O_2 depletion, a lover antibiotic concentration and a less active immune system/PMN response than in the lungs.

No significant difference was found in the lumen pO_2, which we suspect is due to O_2 consumption primarily taking place in the mucus/mucosa, and due to the fact that O_2 in the lumen represent a large O_2 reservoir compared with the mucus, wherein O_2 is less soluble as in air. Full depletion of lumen O_2 is thus unlikely.

No correlation between the pO_2 values on the left and right sinuses was shown. This compares with the fact that we often found different microbiological flora in the two maxillary sinuses, and that the anatomic findings in the two sides often differ. This supports the theory that bacteria, the size of the sinus, and the size of the ostium influence the pO_2.

Since the function of the CFTR gene is reduced by hypoxia [8] and because it has been suggested that the immune system is less functional in O_2 depleted areas [5], we speculate whether anoxia itself should be an indication for sinus surgery, and if harmless bacteria, such as CNS, can induce unfavorable conditions with anoxic pus. We have not been able to show a significant difference between the pO_2 in the maxillary lumen, but we hypothesize that surgery gives the possibility for sinus irrigations with saline and antibiotics and hence a better pO_2 besides the removal of pus and bacteria.

6. Conclusion

We present a new method for determining the pO_2in the maxillary sinuses. In this first study, it was only used during sinus surgery, but the new method can, in some cases, be used after surgery without anesthesia. We show that patients with CF have a lower pO_2 on the mucosa as compared to a control group. Some CF patients have anoxic conditions, probably due to the mucosa's and the bacteria's O_2 consumption. In contrast, there was no anoxia in the sinus lumen. Both *P. aeruginosa* and CNS can thrive under anoxic conditions in the sinuses. These findings support our hypothesis that *P. aeruginosa* can adapt to the environment, mutate or converse phenotype in the paranasal sinuses. In addition, hypoxia influences the regulation of the CFTR protein, may facilitate biofilm formation and may influence the immune system. It is not known, whether surgery alone improves the anoxia in the mucosa, but surgery is often necessary if one should attempt to remove the anoxic secretions buy suction and nasal irrigations.

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