Bacterial biofilms in chronic rhinosinusitis; distribution and prevalence

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Introduction

Chronic rhinosinusitis (CRS) is today understood as a multifaceted group of diseases. The most established differentiation is between CRS with nasal polyps (CRSwNP) and without nasal polyps (CRSsNP).

Patients with CRSwNP have the worst quality-of-life scores, and they have frequent recurrences of their symptoms after surgery. The pathophysiology of nasal polyps is poorly understood [1,2].

Bacterial infection, in the form of biofilms, is proposed as a major drive behind the inflammation in CRS. Bacterial biofilms are identified as the agent behind an ever increasing number of chronic infectious diseases, ranging from endocarditis to dental caries. Bacterial biofilms are communities of bacteria in their sessile form, and can be extremely difficult to eradicate with conventional antibiotic therapy [3–7].

There are relatively few articles published concerning CRSwNP and bacterial biofilms. One major drawback is the lack of a common methodology to detect biofilms. Methods ranging from light microscopy to staining with crystal violet to nasal cytology are utilized.

Ha et al. [8] concluded that, in the setting of CRS, confocal laser scanning microscopy is the best suited modality for imaging of biofilm formations.

Using a protocol with confocal laser scanning microscopy detailed in a prior article [9], we have investigated the point prevalence of biofilms in patients with CRSwNP vs CRSsNP and controls. We have also investigated the occurrence of biofilms in different locations within the nasal cavity.

Materials and methods

The methodological details have been thoroughly described previously, initially by the Wormald group and also by this group [9–11].

Eighty-six consecutive patients undergoing primary endonasal surgery were included. Sixty-one patients with CRS undergoing functional endoscopic sinus surgery (FESS) were included in the experimental group, and 25 patients undergoing septoplasty without CRS were included as controls.

Inclusion and exclusion criteria are detailed in Table I. Patients were diagnosed with CRS as defined by the EPOS group in 2007. Medical and surgical history, including the use of nasal and systemic corticosteroids, smoking, asthma, and allergy were obtained.

In the CRS group, tissue samples were harvested from the anterior part of the middle turbinate, the uncinate process, and the ethmoid bulla during primary sinus surgery. Mucosal

Conclusion: Biofilms were more prevalent in patients with CRSwNP compared to both CRSsNP and controls, and also on the ethmoid bulla compared to the middle turbinate, supporting a biofilm-related pathogenesis of CRSwNP.

Objective: To investigate the prevalence of biofilms in patients with chronic rhinosinusitis with nasal polyps (CRSwNP) compared to patients with chronic rhinosinusitis without nasal polyps (CRSsNP) and controls. To examine the prevalence of biofilms in different anatomical localizations.

Study design: Cross-sectional.

Methods: This study comprised 27 patients with CRSsNP, 34 patients with CRSwNP, and 25 controls. Biopsies from the middle turbinate, the uncinate process, and the ethmoid bulla were harvested pre-operatively, snap frozen in isopentane, cooled, and stored at −80°C. Prepared with Invitrogens’ Baclight LiveDead kit and investigated with confocal scanning laser microscopy.

Results: Biofilms were studied in 33/34 (97%) CRSwNP, 22/27 (82%) CRSsNP, and 14/25 (56%) controls. The difference in point prevalence between patients with CRSwNP vs CRSsNP (p = 0.042, χ² = 4.12), CRSwNP vs Controls (p < 0.001, χ² = 15.0), and CRSsNP vs controls (p = 0.047, χ² = 3.96) were all significant. Biofilms were found in 43/54 (80%) ethmoid bulla, 39/55 (71%) uncinate process, and 31/50 (62%) middle turbinate. The difference between the ethmoid bulla and the middle turbinate locations (p = 0.047, χ² = 3.93) was significant.

ABSTRACT

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samples in the control group were obtained from the anterior part of the middle turbinate.

The samples were prepared for confocal scanning laser microscopy by fluorescence incubation with Invitrogen’s LIVE/DEAD BacLight Bacterial Viability Kit (Invitrogen, Burlington, Canada).

In this study we included the same patients as in our previous article on CRS and biofilm [9], but performed microscopy on ~ 100 additional tissue samples to stratify for localization and the presence or absence of nasal polyps.

**Statistical analyses**

The data obtained in this study were analysed with SPSS 19 (IBM Corp. SPSS, Armonk, NY). Chi-square with continuity correction and odds ratio, when comparing dichotomous data and student t-test for continuous data, were used. For all results a two-sided significance level of 5% and 95% confidence interval were used.

**Results**

The total number of patients in the CRS group was 61, 23 females and 38 males, and median age was 40 years. Twenty-seven of the patients in the experimental group had CRSsNP, and the rest, 34, had CRSwNP. There were 25 individuals in the control group, eight females and 17 males, with a median age of 40 years.

Bacterial biofilms (Figure 1) were detected in 97.1% of patients with CRSwNP, 81.5% of patients with CRSsNP, and 56% of controls. Patients with CRSwNP had highly significantly increased prevalence of biofilms compared to controls ($p < 0.001$, $\chi^2 = 15.0$). The increased prevalence of biofilms in patients with CRSsNP compared to controls was also significant ($p = 0.047$, $\chi^2 = 3.96$). Patients with CRSwNP also had significantly higher prevalence of biofilms compared to CRSsNP ($p = 0.042$, $\chi^2 = 4.12$).

The prevalence of biofilms in different anatomical locations within the nasal cavity differed. In total, 159 samples from patients with CRSsNP and CRSwNP were collected from the middle turbinate, uncinated process, and ethmoid bulla. Biofilms were detected in 79.6% of the samples from the ethmoid bulla, 70.9% of the samples from the uncinated process, and 62.0% of the samples from the middle turbinate. Biofilms were significantly more prevalent on the ethmoid bulla compared to the middle turbinate ($p = 0.047$, $\chi^2 = 3.93$). The difference in biofilm prevalence between the other locations was non-significant.

**Discussion**

In this study a significantly increased prevalence of biofilms were found in patients with CRSwNP compared to controls, but also compared to CRSsNP (Figure 2). Indeed only one of the patients with CRSwNP was biofilm negative. This indicates a role for biofilms in the pathogenesis of CRS, but specifically in CRSwNP.

The pathophysiological mechanisms underlying nasal polyps are still poorly understood. Biofilms are shown to be heterogeneous and can be composed of both bacteria and fungi [12]. *Staphylococcus Aureus* feature prominently in most biofilms found in the sinonasal cavity, being isolated in ~50% of the samples, and can possibly facilitate co-colonization with fungi. This pathway is also shown, in *in-vitro* studies, to exist intracellularly in mucosal cells, possibly acting

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**Table I. Inclusion and exclusion criteria.**

| CRS group included | CRS excluded | Controls included | Controls excluded |
|--------------------|--------------|------------------|-------------------|
| Primary FESS       | Pregnant     | Septal deviation and/or concha media bullosa requiring primary surgery | Same as CRS group plus septal perforation |
| Bilateral disease  | Immunodeficiency | Reduced mucociliary clearance (e.g. Kartagener) | Above 18 years old |
| Above 18 years old | Antibiotics within 2 weeks of operation | Non-invasive fungal balls or invasive fungal disease | Lund-Mackay CT score of zero |
|                    | Non-invasive fungal balls or invasive fungal disease | Systemic vasculitis | |
|                    | Granulomatous disease | Cocaine abuse | |
|                    | Neoplasm | Aspirin exacerbated respiratory disease | |
as a reservoir. Bacteria in a biofilm are shown to have up to a 1000-fold increased resistance to antibiotics compared to planktonic bacteria [13]. These features of biofilms make them notoriously hard to eradicate, e.g. bacterial endocarditis with the need for prolonged intravenous antibiotic treatment. In the setting of CRS we have the opportunity of direct local treatment which gives us a greater range of potential treatment options. To date many different modalities have been tested, from Manuka honey to ultrasound and surfactant, but none have been shown to be very efficient. Even surgery is debated in the setting of biofilm [14]. In regards to nasal polyps, further studies are needed to investigate why some patients with biofilms develop nasal polyps while others do not.

Other studies investigating CRSwNP and biofilms showed mixed results. Sun et al. [15], Toth et al. [16], and Karosi et al. [17] all reported significant associations, while Bezerra et al. [18], Mladina et al. [19], and Mladina and Skitarelic [20] found no association between biofilms and nasal polyps. This can, at least partially, be explained by different methodologies. The modalities employed ranged from scanning electron microscopy to ordinary light microscopy with gram staining. The advantages of the method in this study, first described by Psaltis et al. [11], later improved by Foreman et al. [10], and described in detail in an earlier article by this group [9], is that the BacLight kit distinguishes between cells with uncompromised and compromised cell membranes. In addition, the confocal laser microscopy allows the examiner to scan the suspected biofilms in three dimensions. Both confocal and electron microscopy have higher resolution than light microscopy. Indeed, Ha et al. [8] concluded that confocal microscopy is the best suited modality to examine biofilms in the sinonasal mucosa.

In this study the controls were patients undergoing septoplasty. Only patients with a Lund-Mackay CT score of zero and no signs of CRS on nasal endoscopy were included as controls. There is a higher prevalence of biofilms in our control group compared to studies with patients without rhinologic pathology undergoing skull base surgery as controls [10–12]. Bezerra et al. [18], who also used septoplasty patients as controls, reported comparable prevalence of biofilms to this study.

The other major finding in this study was the trend for increased prevalence of biofilms deeper into the sinonasal cavity (Figure 3). There was a significantly higher prevalence between the ethmoid bulla compared to the front of the middle turbinate.

To our knowledge the prevalence of biofilms at different anatomical locations within the nasal cavity has not been investigated before. Biofilms thrive in moist areas without too much turbulence [13], conditions found deep in the middle meatus. This may also explain why there were a higher number of biofilm positive CRSwNP patients, as regular nasal polyps originate in the ethmoid.

In the opinion of the authors the findings in this article suggest a role for biofilms in CRSwNP. Further studies are needed to investigate the inflammatory response to biofilms in CRS and also possibly to the location of biofilms within the nasal cavity. We are also conducting a follow-up study to determine the treatment results of our CRS patients with biofilms compared to the CRS patients without biofilms.
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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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