

Effects of intranasal steroid treatment on the presence of biofilms in non-allergic patients with chronic rhinosinusitis with nasal polyposis

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Received: 30 March 2013 / Accepted: 16 August 2013 / Published online: 25 August 2013
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Abstract Microbial biofilms have been implicated in the pathogenesis of chronic rhinosinusitis with nasal polyposis (CRSwNP). Intranasal application of corticosteroids (INCS) is a reliable option in the management of CRSwNP. INCS medication has been suspected to influence the presence and thickness of microbial biofilms and inflammatory cell patterns in CRSwNP. Two series of identical nasal polyps obtained from non-allergic patients with CRSwNP ($n = 56$), who underwent endoscopic sinus surgery (ESS), were processed to hematoxylin–eosin (H.E.) and Gram staining, respectively. Patients were recruited into three groups. Group A ($n = 21$) consisted of patients with continuous preoperative INCS treatment. In group B ($n = 17$), patients were never treated by INCS, while in group C ($n = 18$) INCS medication was stopped at least 6 months before ESS. Biofilm positivity varied from 76.4 to 88.8 % in different subject groups. These values and average thickness of biofilms did not reach statistically significant levels (Mann–Whitney’s U probe, $p > 0.05$) in different patient groups. In contrast, microscopic pattern and numbers of predominant inflammatory cell populations displayed obvious differences according to INCS treatment (Mann–Whitney’s U probe, $p < 0.001$). According to these

observations, INCS treatment does not affect the presence and thickness of microbial biofilms in CRSwNP. In contrast, it has significant effects on the pattern of inflammatory cells infiltrating the subepithelial layer, which might result in beneficially altered extracellular matrix production and cytokine release.

Keywords Biofilm · Chronic rhinosinusitis · Inflammatory cells · Intranasal corticosteroid · Nasal polyp

Introduction

Chronic rhinosinusitis (CRS) is a common inflammatory disorder of the respiratory mucosa of nasal cavity and paranasal sinuses, which leads to various symptoms such as nasal obstruction, olfactory dysfunction, nasal discharge, facial tension and pain [1, 2]. According to the high incidence and prevalence rates, CRS is a serious health problem causing significant morbidity and decreased quality of life resulting in considerable financial costs [1, 2]. Clinical diagnosis of CRS can be established, when two or more symptoms persist over 12 weeks [1–3]. CRS with nasal polyposis (CRSwNP) represents a separate diagnostic and therapeutic entity within CRS cases [1–3]. Etiopathogenesis of CRSwNP is poorly understood [1, 2, 4]. CRSwNP is supposed to be an immunologic disease affected by multiple factors, such as immunologic and genetic disorders, environmental factors, allergic rhinitis, bronchial asthma, aspirin intolerance, staphylococcus superantigens, fungal and viral infections and the presence of bacterial biofilms [1–7]. The antigen-mediated immunologic pathways and cell-to-cell interactions may form the basis of nasal polyp formation in CRS [4, 5, 8]. CRSwNP is supposed to be a heterogeneous

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disease with distinct cytokine expression profiles and different subpopulations of immune cells in the subepithelial layer [4, 8–10]. This heterogeneity can also be observed in the clinical practice [3]. Several cases of CRSwNP are characterized by high recurrence rates and therapy resistance, while other cases have a relatively benign clinical behavior [1–3]. It can be explained by biofilm presence or negativity, co-existence or absence of aspirin intolerance, allergic rhinitis, bronchial asthma, atopic character and genetic disorders, respectively [1–5, 7, 11].

The presence of microbial biofilms and consecutive inflammatory reactions has been suspected to play an important role in the pathogenesis of CRSwNP [5, 6]. Bacterial and fungal biofilm forms the natural environment of non-planktonic microbial proliferation that is characterized by a quorum-sensing induced, self-produced three-dimensional extracellular matrix that consists of polysaccharides, proteins, nucleic acids and water [5, 6, 10, 11]. From the clinical point of view, biofilms have evolutionary advantages for the pathogen microorganisms, since they are characterized by extreme high resistance against chemical agents and host immune reactions due to the physical barrier formed by polysaccharide matrix that blocks the diffusion of antibiotics, superoxides, immunoglobulins and complement components [5, 6, 10, 11]. Biofilm-induced chronic inflammatory reactions may play a promoting role in epithelial disintegration and subsequent hyperplasia of the subepithelial layer [5, 10, 12]. Biofilm-positive cases of CRSwNP histologically differ from biofilm-negatives, since they are characterized by a predominant pattern of neutrophil granulocytes, mononuclear and dendritic cells [8, 10, 13]. These features of microbial biofilms and complexity of disease might explain surgical failures and high recurrence rates in CRSwNP [1–3].

At this time, we do not have disease-specific therapeutic agents targeting nasal polyp formation, therefore advanced cases of CRSwNP with serious nasal obliteration fundamentally requires surgical treatment [1–3]. Functional endoscopic sinus surgery (FESS); however, cannot solve olfactory dysfunction and high recurrence rate of nasal polyposis [1–3]. In the clinical practice, INCS medication serves as a potent supportive therapeutic option in the pre- and postoperative treatment of CRSwNP [1–3, 14, 15]. In case of early stages of CRSwNP, INCS treatment is an effective and well-tolerated therapy for uni- or bilateral nasal polyposis in adults or in children that reduces nasal polyp size and symptoms of nasal congestion and obstruction [1–3, 14–16]. It has been reported that INCS treatment prevents regrowth of nasal polyps after FESS in acetylsalicylic acid tolerant patients with CRSwNP [3, 7, 14–16]. It has also been confirmed that INCS treatment does not influence the rate of bacterial culture or antibiotic sensitivity in patients with CRSwNP [17]. However, at this

time, we do not have information about the effects of INCS medication on the presence or thickness of microbial biofilms in CRSwNP [3, 5, 8, 13, 17].

This study investigates the presence of bacterial biofilms and predominant subepithelial inflammatory cell populations in nasal polyps obtained from different patient groups with CRSwNP according to the clinical history of intra-nasal steroid treatment.

Materials and methods

Patients and controls

The group of patients consisted of 27 men and 29 women ($n = 56$, mean age 50.31 years, range 29–69 years) with the diagnosis of CRSwNP that was confirmed by nasal endoscopy and computed tomography (CT) scans of paranasal sinuses. The Lund-Mackay scores of coronal reconstructed CT scans varied from 10 to 24 with an average score of 18.65 indicating serious involvement of paranasal sinuses by CRS. The score was higher than 20 in 73.2 % of patients ($n = 41$). Clinical information on bronchial asthma, allergic rhinitis, aspirin intolerance, previous ESS, topical steroid treatment and systemic antimicrobial therapy was recorded before surgery. Seven patients (12.5 %) had bronchial asthma, 5.3 % ($n = 3$) had the diagnosis of aspirin intolerance; however, no patients had allergic rhinitis. Exclusion diagnosis of allergic rhinitis was based on the clinical history, on the physical examination and on the allergen-specific intracutaneous (Prick) test. To confirm these findings, serum concentrations of inhalative allergen-specific IgE were also measured by ELISA in all cases. The diagnosis of bronchial asthma was based on the clinical history and on the respiratory functional test. Aspirin intolerance was based on the presence of aspirin-induced hypersensitive reaction in the clinical history of patients. Repeated ESS was performed in 39.2 % ($n = 22$) of patients, which is an important predictive factor of NP recurrence. In this group, number of previous surgeries varied from 1 to 7 with an average number of 4.1. Preoperative, systemic antibiotic treatment (moxifloxacin, 400 mg/day for 7 days in 1 case and cefuroxime 1,000 mg/day for 5 days in 2 cases) was applied in 3 (5.3 %) patients due to recurring acute rhinosinusitis. Patients were not treated by other anti-inflammatory drugs. Short courses of oral corticosteroid administration or medication by anti-leukotrienes were excluded in patients with CRSwNP.

Ten patients ($n = 10$, 9 men, 1 women, mean age 32.58 years, range 22–46 years) with nasal obstruction, who underwent septoplasty were recruited into the control group. Controls did not have allergic rhinitis or CRSwNP and were not treated by INCS. Control tissue specimens of

approximately 0.2 cm³ were obtained from the anterior mucosal surface of the inferior turbinate.

All patients and control subjects gave their informed written consent before donating their tissue samples for the study. The Institutional Ethical Committee approved our study. The study was carried out according to the Declaration of Helsinki.

Study design

A case-controlled retrospective experimental study was performed on nasal polyp specimens obtained from patients with CRSwNP, who underwent ESS at the Department of Otorhinolaryngology and Head and Neck Surgery, University of Debrecen. Nasal polyps were collected between May 2011 and November 2012. Surgically removed nasal polyp samples were processed to histopathologic analysis. Only nasal polyps larger than 2 cm were analyzed, which could be removed by a straight ESS forceps without any surface injury or disruption of biofilm layers. The removal was gently performed at the root of nasal polyps. Only superficial nasal polyps were analyzed that could be affected by INCS treatment. Skin Prick test negativity was the main inclusion criteria for the study. According to the clinical history of INCS treatment, patients were recruited into three study groups. In the first group of patients (group A, $n = 21$) all subjects were treated by topical mometasone-furoate monohydrate (200 µg/day) therapy before surgery. The average period of INCS treatment was 28.9 months that varied from 15 to 60 months. In the second group (group B, $n = 17$), patients with CRSwNP were never treated by INCS. Finally, the third study group (group C, $n = 18$) consisted of patients with CRSwNP, who were treated by intranasal mometasone-furoate monohydrate (200 µg/day) therapy that was discontinued 6 months before ESS and tissue sampling. In this group, the average period of INCS treatment was 18.1 months that varied from

6 to 26 months. Table 1 summarizes the clinical history of the three patient groups with CRSwNP.

Outcome measurements

To investigate the presence of bacterial biofilms and predominant subepithelial inflammatory cell populations in nasal polyps obtained from different patient groups with CRSwNP and without allergic rhinitis according to the clinical history of intranasal steroid treatment.

Statistical analysis

Statistical analysis was performed by Mann–Whitney's U probe with a 95 % confidence interval (SPSS 9.0 for Windows).

Hematoxylin–eosin (H.E.) and Gram staining

A total of 66 nasal mucosa specimens were fixed in 10 % (w/v) formaldehyde. Specimens were embedded in 15 % (w/v) purified gelatin (24 h, 56 °C) and refixed in 4 % (w/v) paraformaldehyde (24 h, 20 °C). Blocks were cryoprotected in 20 % (w/v) sucrose solution (2 h, 4 °C) and sectioned into 5 µm slides at −40 °C (MNT-200, Slee, Mainz, Germany). Slides were stored in 0.1 M PBS containing 0.03 % (w/v) sodium azide at 4 °C. Two consecutive 5 µm frozen cut sections were examined as follows: (1) *conventional staining with hematoxylin and eosin (H.E.)* and (2) *conventional Gram staining*. Histologic pretreatment protocol (fixation, cryoprotection, embedding and sectioning) was performed by an independent laboratory assistant. Two independent researchers evaluated the histopathologic results: *P.C.s.* analyzed the sections stained by H.E., while *T.K.* assessed biofilm characteristics according to Gram staining. Investigators were blinded to the clinical histories of INCS treatment in each subject.

Table 1 Clinically relevant data of controls and the three patient groups with CRSwNP

	Group A	Group B	Group C	Controls
Number of patients (n)	21	17	18	10
Female–male ratio (n , ratio)	12/9 (1.33)	6/11 (0.54)	11/7 (1.57)	1/9 (0.11)
Average age (years, range)	51.2 (31–62)	47.8 (29–67)	53.4 (33–69)	32.58 (22–46)
Allergic rhinitis (n , %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Skin Prick test positivity	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Bronchial asthma (n , %)	2 (9.5 %)	3 (17.6 %)	2 (11.1 %)	0 (0 %)
Aspirin intolerance (n , %)	1 (4.7 %)	2 (11.7 %)	0 (0 %)	0 (0 %)
Average Lund-Mackay score (score, range)	18 (12–24)	20 (10–24)	18 (11–24)	0
Previous ESS (n , %)	7 (33.3 %)	5 (29.4 %)	10 (55.5 %)	0 (0 %)
Systemic antibiotic treatment (n , %)	0 (0 %)	1 (5.8 %)	2 (11.1 %)	0 (0 %)
Topical steroid treatment (n , %)	21 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)

The criteria for the histopathologic detection of bacterial biofilms were the presence of characteristic morphology, Gram-positive/negative microcolonies and the presence of surrounding polysaccharide layer on the surface of nasal epithelium [13, 18]. Structure and cellular infiltration of the epithelial and also the subepithelial layers were correlated to the presence or absence of bacterial biofilms. Average thickness of biofilm layers was individually calculated via a 4,000 μm long line segment on the surface of nasal mucosa with 10 measuring points located into the center of each 400 μm segment, respectively (Fig. 1). Mean count of inflammatory cells located in the subepithelial layer was calculated in the average of 10 large magnification microscopic fields (200 $\mu\text{m} \times 200 \mu\text{m}$) (Fig. 1).

Results

Altogether 56 patients with CRSwNP, who underwent ESS were included in this study. Histopathologic examination revealed inflammatory nasal polyps with various patterns and amounts of polymorphonuclear (neutrophil, eosinophil and basophil granulocytes), lymphocyte and plasmocyte infiltration of the subepithelial layer in all cases. Bacterial biofilms were detected in 47 (83.9 %) of 56 patients with CRSwNP (Figs. 2, 3, 4; Table 2). In the biofilm-negative cases ($n = 9$, 16.1 %), histopathologic analysis revealed normal ciliated columnar epithelium with goblet cells and with predominant plasmocyte infiltration of the subepithelial layer (Table 2). No biofilm-like polysaccharide layers were detected in mucosal specimens obtained from the inferior turbinate of patients ($n = 10$) applied as controls (Fig. 5). According to previous observations, the average number of previous ESS procedures due to recurring nasal polyposis were significantly higher ($p < 0.001$) in the biofilm-positive patient group ($n = 20$, 42.5 %, average = 5.2) compared to biofilm-negative cases ($n = 2$, 22.2 %, average = 1) (Table 1).

In our series, H.E. staining represented a direct correlation with the results of Gram staining and was found to be a reliable predictor of the presence or absence of bacterial biofilms (Figs. 2, 3, 4; Table 2) [13]. In biofilm-positive cases of group A ($n = 18$, 85.7 %), average thickness of biofilms varied from 10.12 to 25.31 μm with the average of 18.95 μm (Table 2). In biofilm-positive cases of group B ($n = 13$, 76.4 %), average thickness of biofilm-like polysaccharide layers varied from 11.05 to 23.29 μm with the average of 16.72 μm (Table 2). In group C, 16 biofilm-positive nasal polyps were detected (88.8 %), where the average thickness of biofilms was 16.49 μm (8.92–22.41 μm) (Table 2). Disintegrated architecture or squamous cell metaplasia of the respiratory epithelium were strong predictors for biofilm detection (Figs. 2, 3, 4; Table 2). It was found that normal respiratory

mucosa with columnar ciliated epithelium was not associated with the presence of different types of biofilms (Fig. 5; Table 2). No statistically significant associations were found with biofilm positivity, biofilm thickness or microscopic architecture of the respiratory mucosa according to the INCS treatment in the medical history of three patient groups ($p > 0.05$) (Table 2).

In biofilm-positive nasal polyps, the subepithelial layer displayed a serious inflammatory cell infiltration of various patterns of different cell types, which was significantly associated with the clinical history of INCS usage ($p < 0.001$) (Figs. 2, 3, 4; Table 2). According to H.E. staining, predominant cell populations such as mononuclear

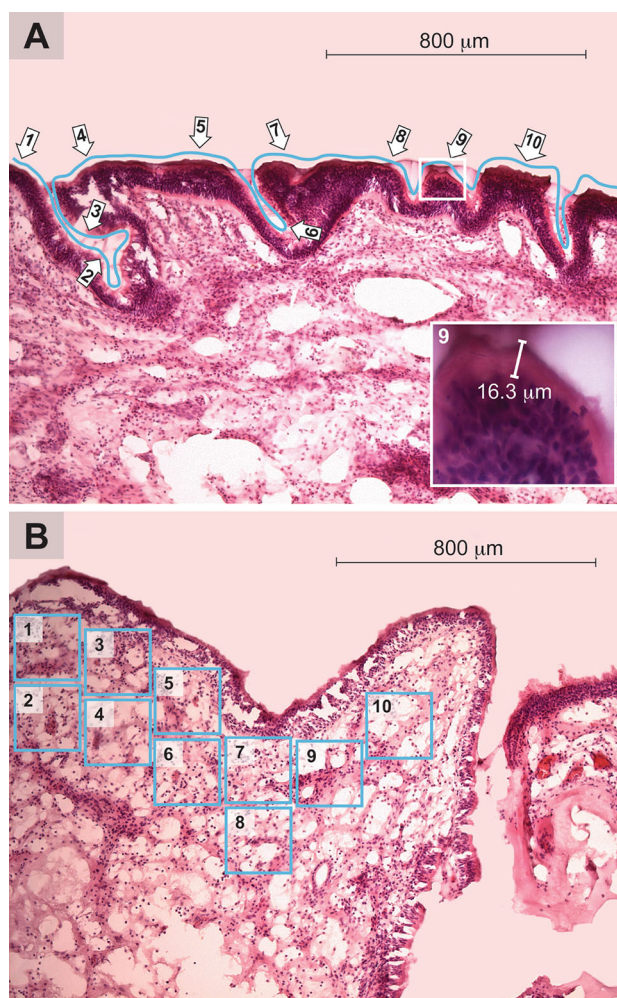


Fig. 1 Histopathologic representation of the methods of biofilm thickness measurement and establishment of mean counts of inflammatory cell populations in the subepithelial layers of nasal polyps. **a** Average thickness of biofilm layers was individually calculated via a 4,000 μm long line segment (blue fitted line) on the surface of nasal mucosa with 10 measuring points located into the center of each 400 μm segment (white, numbered arrows). **b** Mean count of inflammatory cells located in the subepithelial layer was calculated in the average of ten 200 $\mu\text{m} \times 200 \mu\text{m}$ -sized areas (blue, numbered rectangles)

Fig. 2 Histopathologic representation of a biofilm-positive nasal polyp originated from group A of patients. **a** At low magnification, a destroyed respiratory epithelium can be detected that is covered by a continuous basophilic layer of microbial biofilm (H.E., *black arrow*). **b** Higher magnification of the previous section (H.E.). The epithelial layer is seriously disintegrated and covered by a complete biofilm showing homogeneous basophilic staining (*black arrow*). **c** The subepithelial layer is massively infiltrated by small mononuclear cells showing a homogeneous gland-like pattern (*white arrows*). **d** Gram staining reveals a bacterial biofilm consisted of individual colonies of Gram-positive cocci (*small insert*)

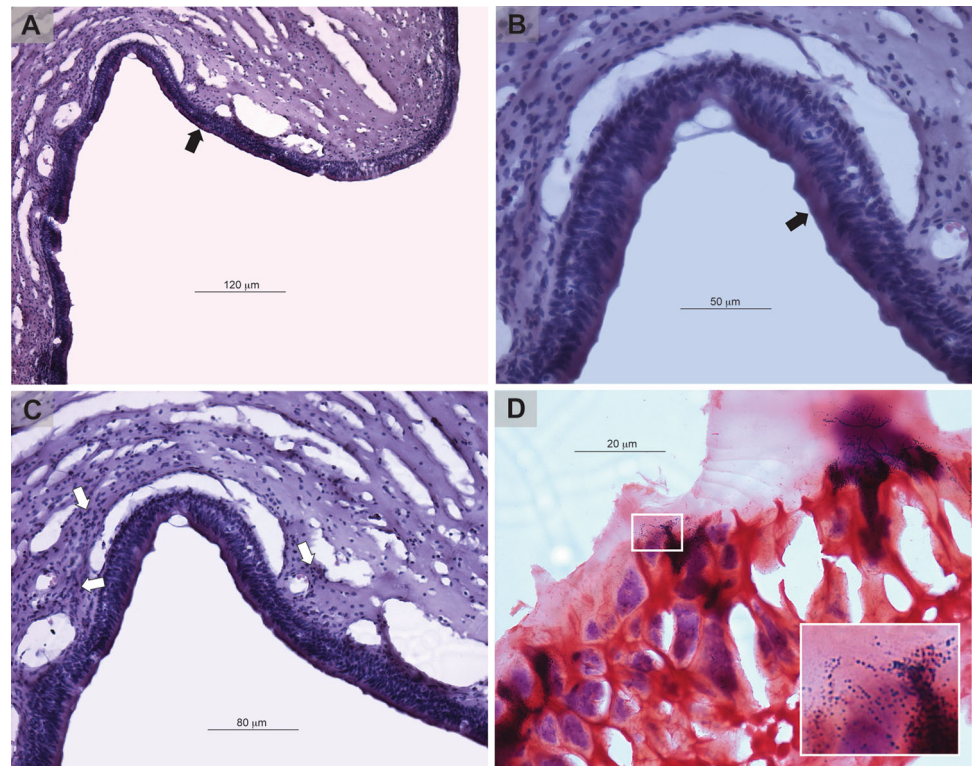
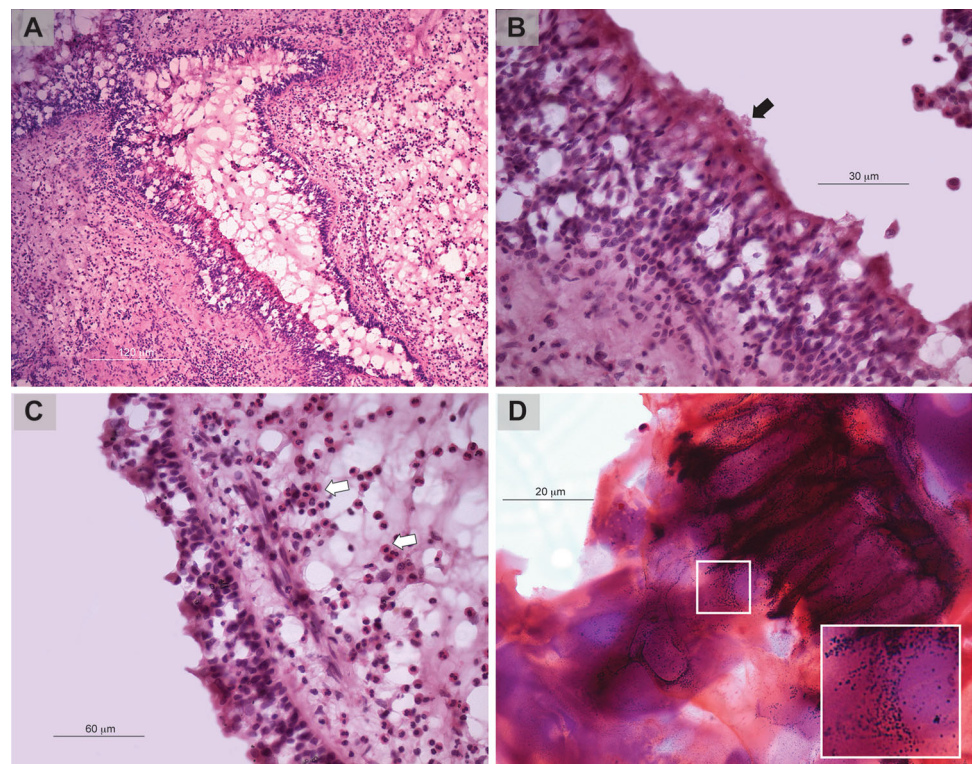


Fig. 3 Histopathologic representation of a biofilm-positive nasal polyp originated from group B of patients. **a** At low magnification, the respiratory epithelium shows serious disintegration. The subepithelial layer is infiltrated by heterogeneous subpopulations of polymorphonuclear cells (H.E.). **b** Higher magnification of the previous section (H.E.). The epithelial layer is covered by a complete biofilm layer showing homogeneous basophilic staining (*black arrow*). **c** The subepithelial layer is predominantly infiltrated by eosinophil granulocytes (*white arrows*). **d** Gram staining reveals a bacterial biofilm consisted of individual colonies of Gram-positive cocci (*small insert*)



cells, plasmocytes and different types of granulocytes could be well identified in all cases (Figs. 2, 3, 4; Table 2). However, it should be established that mononuclear cells are small and monomorph immune cell types with round nuclei

and narrow basophilic cytoplasm that are difficult to characterize by conventional staining protocols [9]. They also can be evaluated as lymphocytes or dendritic cells that require antigen-specific immunoreactions [9].

Fig. 4 Histopathologic representation of a biofilm-positive nasal polyp originated from group C of patients. **a** At low magnification, moderately injured columnar epithelium and empty goblet cells can be detected (H.E.). A bulky and continuous biofilm layer can be identified on the surface of the epithelial layer (*black arrow*). **b** Higher magnification of the previous section (H.E.). The epithelial layer is covered by a complete biofilm layer showing homogeneous basophilic staining (*black arrow*). **c** The subepithelial layer is predominantly infiltrated by eosinophil- and neutrophil granulocytes (*white arrows*). **d** Gram staining reveals a bacterial biofilm consisted of individual colonies of Gram-positive cocci (*small insert*)

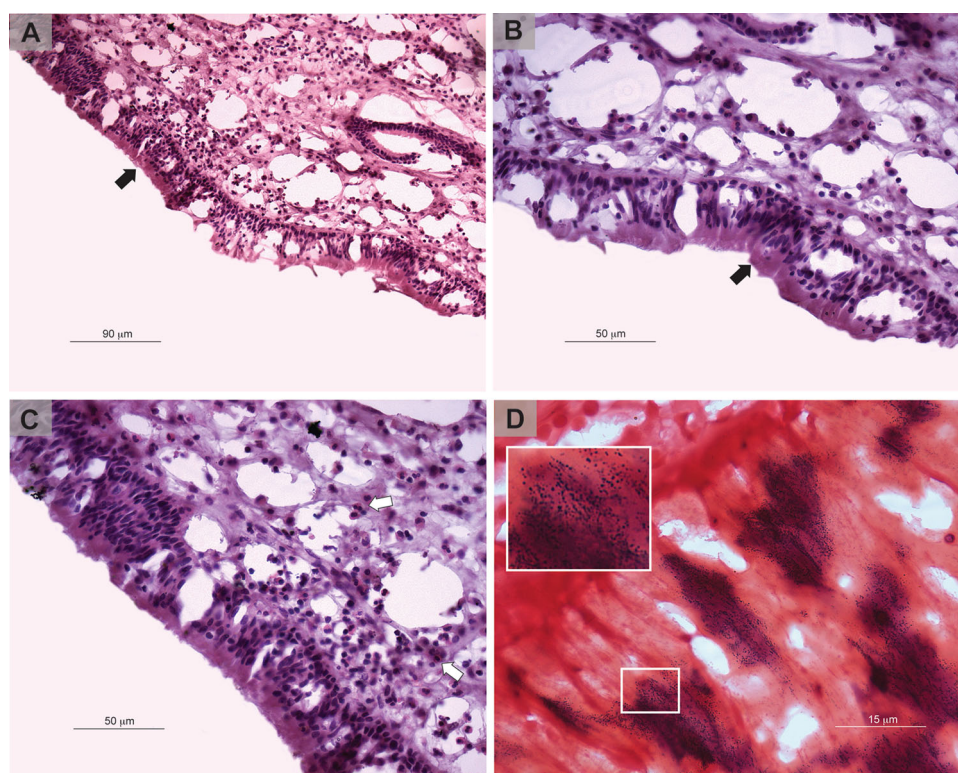


Table 2 Histologic characteristics of biofilms, epithelial and subepithelial layers in the three patient groups with CRSwNP

	Group A (<i>n</i> = 21)	Group B (<i>n</i> = 17)	Group C (<i>n</i> = 18)	<i>p</i> value ¹
Biofilm positivity (<i>n</i> , %)	18 (85.7 %)	13 (76.4 %)	16 (88.8 %)	<i>p</i> > 0.05 (n.s.)
Mean biofilm thickness (μm, range) ^a	18.95 (10.12–25.31)	16.72 (11.05–23.29)	16.49 (8.92–22.41)	<i>p</i> > 0.05 (n.s.)
Histologic findings in the epithelial layer				
Normal respiratory epithelium (<i>n</i> , %)	3 (14.2 %)	4 (23.5 %)	2 (11.1 %)	<i>p</i> > 0.05 (n.s.)
Destructed epithelium (<i>n</i> , %)	16 (88.8 %)	10 (58.8 %)	13 (72.2 %)	<i>p</i> > 0.05 (n.s.)
Squamous cell metaplasia (<i>n</i> , %)	2 (9.5 %)	3 (17.6 %)	3 (16.6 %)	<i>p</i> > 0.05 (n.s.)
Histologic findings in the stromal layer ^b				
Eosinophil cells (mean, range)	18.1 (12.3–34.6)	112.4 (87.6–123.9)	102.3 (78.7–132.5)	<i>p</i> < 0.001
Neutrophil cells (mean, range)	21.3 (14.5–37.6)	136.7 (115.8–198.6)	156.5 (123.2–189.3)	<i>p</i> < 0.001
Basophil cells (mean, range)	8.9 (5.1–18.9)	36.2 (29.1–45.5)	33.3 (25.4–53.2)	<i>p</i> < 0.001
Lymphocytes-plasmocytes (mean, range)	231.6 (187.8–301.9)	55.2 (38.9–65.3)	42.1 (28.2–51.8)	<i>p</i> < 0.001

¹ *p* value at 95 % confidence interval (Mann–Whitney's *U* probe) comparing the data of groups A and B; and A and C

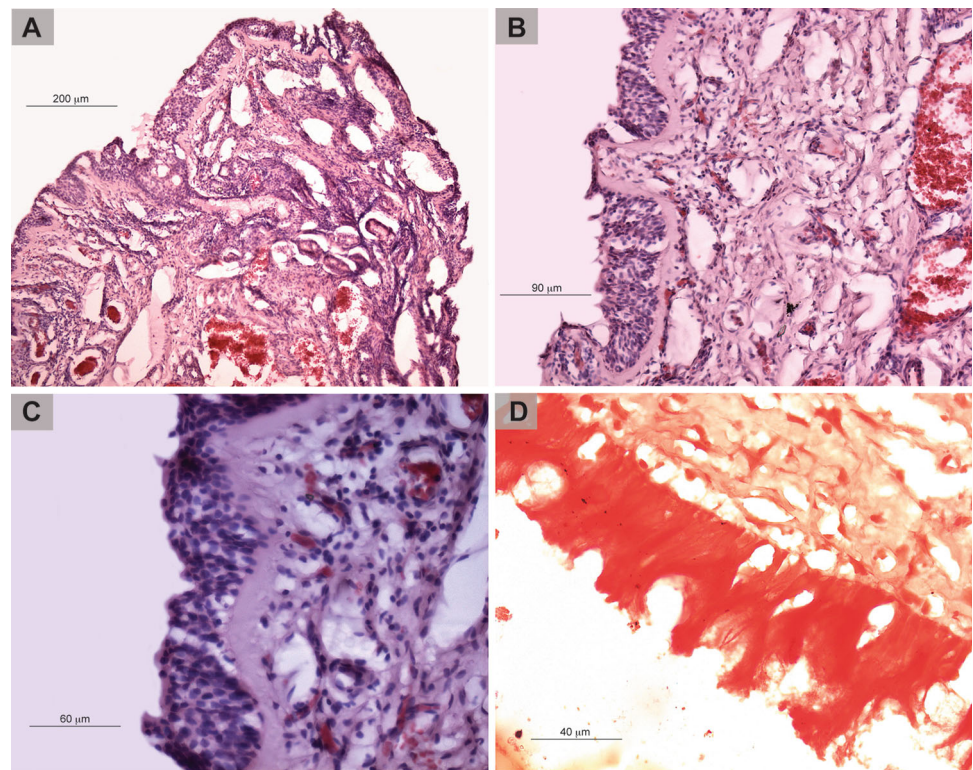
^a Mean values of biofilm thickness in the average of 10 measurement points in a 4,000 μm long epithelial surface (range)

^b Mean values of inflammatory cell number in the average of 10 200 × 200 μm-sized microscopic fields (range)

In group A, lymphocyte and plasmocyte predominance was found in the subepithelial layer (Fig. 2; Table 2). In this group of patients, the subepithelial layer of biofilm-positive nasal polyps displayed a statistically significant (*p* < 0.001) increase in the average numbers of mononuclear cells compared to other patient groups (B and C) (Figs. 2, 3, 4; Table 2). In biofilm-positive nasal polyps from group A, mononuclear cell count varied from 187.8 to 301.9 with the average of 231.6 cells (Fig. 2; Table 2). In

biofilm-positive cases of group B and C, an obvious polymorphonuclear predominance was found in the stromal substance of nasal polyps (Figs. 3, 4; Table 2). In these groups of patients, the average counts of polymorphonuclear cells showed a statistically significant increase compared to group A (*p* < 0.001) that was varied between different subpopulations of neutrophil, eosinophil and basophil granulocytes (Figs. 3, 4; Table 2). Interestingly, INCS treatment-associated pattern of inflammatory cell

Fig. 5 Histopathologic representation of the normal nasal mucosa. **a** Low-power magnification of an inferior turbinate specimen applied as control (H.E.). **b** The respiratory epithelium is intact and shows regular columnar pattern. The subepithelial layer represents several blood vessels and heterogeneous populations of mononuclear cells. **c** Biofilm structures cannot be detected on the surface of nasal epithelium. **d** Gram staining is negative for bacterial or fungal elements; the polysaccharide matrix is absent



populations in the subepithelial layer of nasal polyps was found to be an independent factor of biofilm presence (Figs. 2, 3, 4; Table 2). In biofilm-negative cases of the three patient groups and in controls, the subepithelial layer represented a moderate inflammatory cell infiltration that was predominantly formed by plasmocytes and small mononuclear cells (Table 2). This phenomenon of biofilm-negative nasal polyps and control mucosal specimens was found to be independent from INCS application. Age ranges and gender ratios were not found to be significant predictors for biofilm positivity or subepithelial changes in different patient groups (Table 1).

Discussion

In the present study, we demonstrated the presence of bacterial biofilms in 47 (83.95 %) non-allergic patients with CRSwNP by the combined application of H.E. and Gram staining. According to the strong correlation between the two staining protocols, absence of biofilms was not associated to the single application of H.E. or Gram staining [13, 18]. Detection of biofilms displayed statistically significant correlation with epithelial destruction or metaplasia, which was independent from INCS treatment. It was found that INCS medication has a significant effect on the pattern and average numbers of inflammatory cells infiltrating the subepithelial layer of nasal polyps. In the

INCS treated group of patients, a predominant lymphocyte and plasmocyte infiltration was confirmed. In contrast, patients groups without INCS treatment were characterized by increased numbers of polymorphonuclear cells. These differences were statistically significant and showed an INCS-dependent pattern. The main limitations of our study are the relatively low number of subjects and the employment of a biofilm detection protocol that is not widely accepted [13, 18]. Exact microbiological identification of bacterial biofilms still requires scanning electron microscopy (SEM), confocal laser scanning microscopy (CLSM) or fluorescent in situ hybridization (FISH) analysis with species-specific oligonucleotide primers [19, 20].

There are increasing evidences that persisting microbial biofilms correlates with the intensity of subepithelial inflammatory reaction and consecutive extracellular matrix production in CRSwNP [5, 8, 10, 12]. In general, inflammatory cells such as plasmocytes, lymphocytes, polymorphonuclear cells and antigen presenting dendritic cells accumulating in the subepithelial layer are responsible for the increased expression of several proinflammatory and inflammatory cytokines and growth factors [4, 8, 9]. Pathologically increased release of these peptides is responsible for disintegration of respiratory and olfactory epithelium and also for the increased production of extracellular matrix in the subepithelial layer [6, 8, 12].

Corticosteroids have beneficial effects on the symptoms of CRS/NP and significantly decrease nasal obstruction and

olfactory dysfunction [1–3, 14]. Intranasal and oral administration of corticosteroids (prednisolone, mometasone-furoate) significantly decreases the expression levels of IL-1, IL-6 and TNF- α in the subepithelial layer of nasal polyps [21–23]. These findings may support the hypothesis that inflammatory reactions in CRSwNP correlate to the elevated levels of proinflammatory cytokines [2, 4, 10, 24]. It has been confirmed that long-term systemic antibiotic treatment is not as effective as higher doses of INCS in CRSwNP [3, 14]. The complex immunologic basis of the pathogenesis of CRS also reveals a cellular infiltrate theoretically suppressed by INCS [3, 7, 14]. It has been confirmed in recent clinical studies of CRS with or without nasal polyposis [14, 15]. Vento et al. [16] have confirmed that intranasal application of triamcinolone-acetonide prevents the recurrence of nasal polyps after ESS in acetylsalicylic acid (ASA) tolerant patients, but not in ASA intolerant cases. Based on the systemic review of different clinical studies, Aouad et al. [2] have reported that combination of FESS and postoperative INCS treatment still remains the mainstay of therapy in CRSwNP. In the postoperative care, INCS medication is effective in reducing the formation of adhesions and may prevent nasal polyp reformation [2, 3, 16]. These observations have been confirmed by several molecular biological studies investigating the effects of INCS treatment on the expression of cytokines and growth factors [8, 21–23]. Li et al. [25] have confirmed that long-term application of INCS increases the expressions of Foxp3 and IL-10 that attenuates the chronic inflammatory reactions in CRSwNP. Yariktas et al. [23] have reported that INCS treatment decreases the expression level of basic fibroblast growth factor (bFGF) in nasal polyps resulting in fibroblast inactivation and diminished extracellular matrix production. Molet et al. [26] have reported that intranasal administration of fluticasone propionate significantly decrease the production of type I, III and V collagens and the expression of profibrotic cytokines IL-11 and IL-17. In a double-blind, placebo-controlled trial, Hamilos et al. [27] have observed that intranasal application of fluticasone propionate decreased the expression levels of IL-4, IL-13, TNF- α and IL-1 β , however, it has no effects on the expression of endothelial vascular cell adhesion molecule (VCAM)-1 and P-selectin.

It has been suspected that INCS treatment may have adverse effects on the pathogen bacterial and fungal flora of the nasal cavity in CRS [8, 13]. In contrast, Ho and Chan [17] have confirmed that application of INCS did not influence the rate of bacterial culture, recurring acute infections or antibiotic sensitivity in patients with CRSwNP. On the other hand, INCS treatment may have conflicting effects on the biofilm presence and thickness in CRSwNP via the inhibition of local immune response [4, 5, 8, 13, 14, 21, 22]. Steroid and systemic antibiotic treatment

might take a severe influence on the flora of nasal mucosa and might affect the structure and thickness of microbial biofilms [4, 5, 8, 13]. In our previous studies, patients with CRSwNP were all treated by INCS; therefore this association seemed to be strong [8, 13]. In contrast, the great majority of biofilm-negative specimens were obtained from patients with allergic rhinitis who were regular topical steroid users [8, 13]. According to current observations, this contradiction can be resolved, since long-term INCS medication has no effects on the presence and thickness of bacterial biofilms in non-allergic patients with CRSwNP compared to non-steroid users.

This study indicates a strong correlation between INCS treatment and predominant inflammatory cell populations in the subepithelial layer of nasal polyps obtained from patients with CRSwNP. According to the steroid-mediated selection of inflammatory cells showing anti-fibroblast activity, INCS treatment is supposed to have inhibitory effects on the extracellular matrix production in CRSwNP. It can be established that preoperative administration INCS is a non-specific, effective and well-tolerated supportive therapeutic option in CRSwNP that does not influence the presence and thickness of bacterial biofilms. Although specific treatments are not yet available to target biofilm, its detection is an important requirement, since it is strongly associated with treatment failures and persisting symptoms of CRSwNP.

This study was carried out according to the declaration of Helsinki. All subjects gave their informed written consents to our study.

Acknowledgments This work was supported by the grants of Hungarian Scientific Research Fund (OTKA PD75371 and OTKA K108292).

Conflict of Interest Authors have no conflicts of interest.

Ethical Standard This study was approved by the Institutional Ethical Committee.

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