ORIGINAL ARTICLE

CONTENTS

Assessment of local immunity markers in patients with chronic rhinosinusitis and biofilms in the upper airway mucosa

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ABSTRACT

Aim: To comprehensively evaluate local immune markers in patients with chronic rhinosinusitis (CRS) with and without biofilms in the upper airway mucosa, and to determine pathogenetically significant immune alterations associated with biofilm presence that may contribute to chronic inflammation and reduced treatment effectiveness.

Materials and Methods: Oropharyngeal secretion was analyzed in 20 CRS patients (with and without biofilms) and 8 healthy controls. Levels of interleukin-1β, α-interferon, secretory IgA, immune complexes, and cellular composition were evaluated. Biofilms were detected via SYTO9/propidium iodide fluorescent staining.

Results: CRS patients with biofilms showed significantly reduced α-interferon levels and increased concentrations of immune complexes. IL-1β and sIgA levels did not differ between CRS subgroups. All CRS patients exhibited reduced epithelial cell counts and increased neutrophil percentages.

Conclusions: The study demonstrates that low α-interferon levels and high immune complex concentrations in patients with CRS and biofilms represent pathogenetically significant immune alterations. These findings highlight the need for further investigation of local and systemic immune mechanisms that support biofilm persistence and may open perspectives for more effective therapeutic strategies.

KEY WORDS: mucosal immunity, rhinosinusitis, biofilms, chronic, interferon-alpha, immune complexes

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INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common inflammatory diseases of the upper respiratory tract and significantly affects patients' quality of life, causing persistent symptoms such as nasal congestion, facial pain, hyposmia, and nasal discharge. Despite extensive research efforts, the pathogenesis of CRS remains complex and multifactorial, which limits the effectiveness of standard treatment approaches [1]. One of the key factors believed to contribute to CRS pathogenesis is local immune deficiency, which promotes biofilm formation and the chronicity of inflammation in the mucosal lining.

Biofilms are structured microbial communities in which bacteria adhere to surfaces—such as the epithelium of the nasal cavity or paranasal sinuses—and are embedded in an extracellular matrix. This matrix, composed of polysaccharides, proteins, and nucleic acids, serves a protective function, making the microorganisms significantly less susceptible to antibiotics and immune responses. Biofilm formation is a stepwise process involving initial bacterial adhesion, active proliferation, the development of a stable polysaccharide matrix, and the potential dispersion of individual cells or clusters capable of colonizing new mucosal areas. The complex composition of the biofilm matrix provides unique properties, including a high degree of intra- and interspecies communication, metabolic flexibility, and the ability to adapt to environmental stressors [2, 3]

The presence of biofilms in the nasal cavity and paranasal sinuses is associated with persistent or recurrent forms of CRS and often explains the poor efficacy of antibiotic therapy. The protective matrix of biofilms hinders the penetration of medications into bacterial colonies and reduces their susceptibility to phagocytosis and other immune mechanisms. Chronic inflammation is sustained by ongoing interactions between biofilm-associated cells and the immune system, leading to epithelial barrier damage, activation of pro-inflammatory cytokines (IL-6, IL-8, TNF-α), and suppression of regenerative processes [1].

Biofilms are capable of disrupting the function of the ciliated epithelium, which plays a key role in mucociliary clearance, and they promote the accumulation of pathogenic and opportunistic microorganisms, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [2] In CRS with biofilms, host defense mechanisms are frequently impaired. For example, biofilms can alter the expression of antimicrobial peptides such as lysozyme, lactoferrin, and β-defensins, thereby reducing the mucosa's ability to neutralize pathogens [4, 5]. In addition, the production of interferons—key mediators of antiviral defense and regulators of the inflammatory response—is suppressed. Reduced levels of these molecules in saliva may weaken antiviral immunity and increase susceptibility to secondary infections [6, 7]. Although some studies have addressed the role and mechanisms of biofilm influence on local and systemic immunity, further investigation is warranted.

AIM

To comprehensively evaluate local immune markers in patients with chronic rhinosinusitis (CRS) with and without biofilms in the upper airway mucosa, and to determine pathogenetically significant immune alterations associated with biofilm presence that may contribute to chronic inflammation and reduced treatment effectiveness.

MATERIALS AND METHODS

The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Kolomiychenko Otolaryngology Institute of National Academy of Medical Sciences of Ukraine (Approval No. 0412/2023, Date: 04/12/2023). Written informed consent was obtained from all participants prior to inclusion in the study.

A total of 20 patients with chronic rhinosinusitis (with and without biofilms) and 8 practically healthy individuals (control group) were immunologically examined. The material used for the study was oropharyngeal secretion (OPS), which is considered an indicator not only of local but also of general mucosal immunity. Samples of OPS were collected at a fixed time in the morning, prior to tooth brushing. The samples were then centrifuged using a refrigerated centrifuge (800R, Turkey) at 4°C and 100 g for 15 minutes. The liquid phase was separated, transferred into Eppendorf tubes, and stored at -25°C for up to 2 months.

The presence of biofilms in mucosal samples of the upper respiratory tract was assessed by fluorescent staining using dyes specific to components of the extracellular matrix [8]. A combination of SYTO9 and propidium iodide stains (LIVE/DEAD™ BacLight™ Bacterial Viability Kit, Thermo Fisher Scientific, USA) was used to differentiate between viable and non-viable bacteria within the biofilm [8].

Tissue samples were initially fixed in 4% paraformaldehyde for 30 minutes, then rinsed with phosphate-buffered saline (PBS, pH 7.4), and incubated with the working solution of dyes in the dark for 15 minutes at room temperature. The stained specimens were then analyzed using a fluorescence microscope (Axio Imager.A2, Zeiss, Germany) equipped with appropriate filters for green (SYTO9) and red (propidium iodide) fluorescence channels. Images were captured with a digital camera, and quantitative assessment of biofilm density was performed using ImageJ software (NIH, USA). The presence of a biofilm was defined by the visualization of structured bacterial microcolonies embedded in a dense matrix, with both viable and damaged cells visible in distinct fluorescence spectra.

The levels of the pro-inflammatory cytokine interleukin-1β (IL-1β) (LABOR Diagnostik Nord, Germany), early interferon-α (FineTest, China), and secretory immunoglobulin A in two forms—secretory (sIgA) and monomeric (mIgA) (Hema Medica, Ukraine)—were measured in the oropharyngeal secretion.

In addition, the content of immune complexes in the oropharyngeal secretion was determined using a precipitation assay with 3.75% polyethylene glycol. Cellular components of various histogenetic origins were quantified in the sediment of the OPS using smear preparation and hematoxylin-eosin staining, in accordance with standard recommendations.

The analyses were performed using the enzyme-linked immunosorbent assay (ELISA) method with a StatFax 2100 reader (USA). Statistical analysis of the obtained data was carried out using the one-sided non-parametric Mann–Whitney U test. Calculations were performed using open-access software packages WINPEPI and Biostat, following established guidelines from the literature [9]. Results were presented as arithmetic means (M) and interquartile ranges (Q25–Q75). In cases of limited data volume, ranges (min–max) were used instead of quartiles. The number of observations was denoted as *n*. Differences were considered statistically significant at $p < 0.05$.

RESULTS

A comparative assessment of inflammatory, antiviral, humoral, and cellular parameters in oropharyngeal secretion was performed across three groups: CRS

Table 1. Levels of interleukin-1β (pg/mL) in oropharyngeal secretion of patients with chronic rhinosinusitis with biofilms (BF+) and without biofilms (BF−) compared to control group (C)

Parameter:	BF+	BF-	C (Control)
n	10	10	8
Mean (M)	58.1	128.4	36.5
Median	40.7	135.5	22.4
Interquartile range (Q25—Q75)	17.0—85.0	80-155.2	17.8-71.2
P-value (vs. control)	0.06	< 0.05	Reference level

Source: compiled by the authors of this study

Table 2. Levels of α-interferon (pg/mL) in oropharyngeal secretion of patients with chronic rhinosinusitis with biofilms (BF+), without biofilms (BF−), and in the control group (C)

Parameter:	BF+	BF-	C (Control)
n	10	10	8
Mean (M)	0.125	7.5	5.3
Median	0	3,8	3,9
Interquartile range (Q25—Q75)	0—0.4	2.25—14.8	1.4—6.8
P-value (vs. control)	<0.02	>0.05	Reference level

Source: compiled by the authors of this study

Table 3. Levels of secretory immunoglobulin A (g/L) in oropharyngeal secretion of patients with chronic rhinosinusitis with biofilms (BF+), without biofilms (BF−), and in the control group (C)

Parameter:	BF+	BF-	C (Control)
N	10	10	8
Mean (M)	0.40	0.25	0.45
Median	0.30	0.28	0.35
Interquartile range (Q25—Q75)	0.24—0.49	0.22—0.40	0.25—0.55
P-value (vs. control)	>0.05	>0.05	Reference level

Source: compiled by the authors of this study

Table 4. Levels of immune complexes (arbitrary optical density units) in oropharyngeal secretion of patients with chronic rhinosinusitis with biofilms (BF+), without biofilms (BF−), and in the control group (C)

Parameter:	BF+	BF-	C (Control)
N	10	10	8
Mean (M)	63.6	39.2	24.0
Median	60.0	20.0	36.6
Interquatile range (Q25—Q75)	48.5—70.9	12.5—75.5	19.0—43.0
P-value (vs. control)	= 0.0048	>0.05	Reference level

Source: compiled by the authors of this study

patients with biofilms (BF+), CRS patients without biofilms (BF−), and healthy controls (C). The analysis focused on differences in median values, variability within groups, and distributional patterns of the examined indicators.

The concentration of IL-1β was elevated in both CRS groups compared with healthy individuals (Table 1). The BF− group demonstrated the highest values, with a median of 135.5 pg/mL and a broad interquartile range (80.0–155.2 pg/mL), indicating marked heterogeneity of inflammatory activation. The BF+ group showed a more moderate increase (median 40.7 pg/mL, IQR 17.0–85.0 pg/mL), yet most individual values remained above those of controls. Although the BF+ group did not reach the threshold of statistical significance when compared with the control group ($p = 0.06$), the consistent upward trend suggests a clinically relevant elevation. Direct comparison of BF+ and BF− groups revealed that IL-1β levels were generally higher in CRS patients without biofilms.

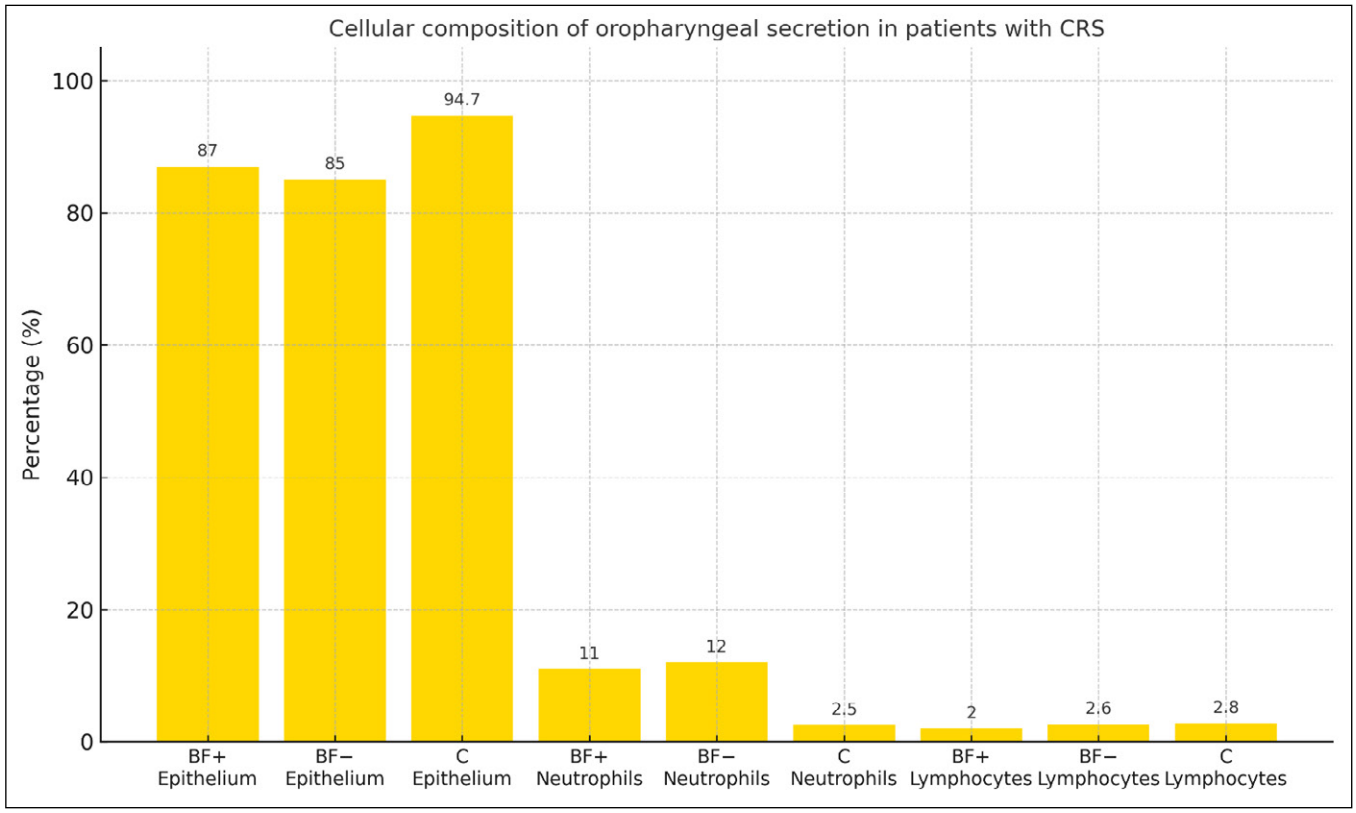

Fig. 1. Cellular composition (in percentage) of oropharyngeal secretion in patients with chronic rhinosinusitis with (BF+) and without (BF–) biofilms.
Note: Statistically significant differences in segmented neutrophils for both BF+ and BF– groups ($p < 0.05$).
Picture taken by the authors

A pronounced difference in α-interferon levels was observed across the groups (Table 2). Nearly all BF+ patients exhibited extremely low or undetectable concentrations (median 0 pg/mL, IQR 0–0.4 pg/mL), with minimal variability within the group. In contrast, the BF– group showed substantially higher and more dispersed values (median 3.8 pg/mL; IQR 2.25–14.8 pg/mL), including several measurements comparable to the control range. Healthy individuals demonstrated expected physiological levels of α-interferon (median 3.9 pg/mL, IQR 1.4–6.8 pg/mL). The suppression of α-interferon in the BF+ group was statistically significant ($p < 0.02$), forming a distinct pattern not observed in BF– patients.

No statistically significant differences in sIgA were recorded among the three groups (Table 3). Nevertheless, a tendency toward reduced sIgA was observed in the BF– group (median 0.28 g/L), while the BF+ group demonstrated values closer to those of healthy individuals (median 0.30 g/L vs 0.35 g/L in controls). The distributions of sIgA overlapped broadly between all groups, and the interquartile ranges were comparable. Thus, despite a downward trend in some CRS patients, sIgA levels did not show diagnostic or discriminatory value in the present cohort.

The analysis of circulating immune complexes (ICs) demonstrated clearer group differentiation (Table 4). The BF+ group displayed consistently elevated IC concentrations, with median values (60.0 optical units) more than twice as high as in the control group (36.6 optical units). The interquartile range in BF+ was narrow (48.5–70.9), reflecting a stable pattern of elevated ICs among all participants in this group. In contrast, the BF– group showed substantial variability (IQR 12.5–75.5), with individual values spanning both below and above the ranges of BF+ and control subjects. This heterogeneity prevented statistical significance. The BF+ group differed from controls at $p = 0.0048$, indicating a robust upward shift in IC levels in the presence of biofilms.

Analysis of the cellular profiles revealed characteristic shifts in the CRS groups (Fig. 1). Both BF+ and BF– patients demonstrated a marked increase in segmented neutrophils compared with healthy controls, and this elevation reached statistical significance ($p < 0.05$). The proportion of epithelial cells showed a downward trend in both CRS groups, though the variability within the control samples limited statistical separation. Lymphocyte counts remained largely comparable across all groups and showed no significant trends. Importantly, the cellular distribution in BF+ and BF– patients was similar, indicating that biofilm presence had no distinct impact on the cytological composition of oropharyngeal secretion.

DISCUSSION

Despite intensive research efforts, the pathogenesis of chronic rhinosinusitis (CRS) remains complex and multifactorial, which limits the effectiveness of standard treatment approaches. One of the important pathogenic factors in CRS is believed to be the presence of biofilms in the mucosa of the upper respiratory tract, which may be associated with the development of local immune deficiency. Numerous studies of oropharyngeal mucosal immunity have indicated that the main components involved in protection against microbial and viral agents include immunoglobulins, interferons, phagocytes, and various groups of defensins [10]. Among the tested parameters, the most pronounced deviations from healthy controls were observed in the levels of interferon-α. The reduced concentration of this cytokine in the oropharyngeal secretion of CRS patients with biofilms may indicate a weakened antiviral defense, particularly during the early stages of infection.

Another test that showed statistically significant deviations from the control group was the level of immune complexes, which was 2.5 times higher in the BF+ group. Elevated levels of immune complexes in saliva may, on the one hand, indicate activation of humoral immune responses, but on the other hand, immune complexes can act as immunopathological factors capable of causing tissue damage under certain conditions.

Regarding the level of the pro-inflammatory cytokine IL-1 in oropharyngeal secretion, it was found to be elevated in both groups—BF+ and BF−—with very similar values. This suggests that the increased IL-1 level is likely due to the prolonged inflammatory process present in both patient groups, making it impossible to determine the specific role of biofilms in this context. A similar conclusion applies to the cellular composition of the secretion: the relative proportion of inflammatory cells—segmented neutrophils—was nearly identical in both study groups and exceeded that of the control group. The relative number of epithelial cells showed a clear downward trend, but the difference compared to the control was not statistically significant. The level of secretory immunoglobulin A was reduced only in the BF− group and did not demonstrate meaningful diagnostic value.

Thus, the conducted study indicates that in cases of CRS associated with biofilm formation, certain factors may be considered pathogenetically significant—namely, a low level of α-interferon and a high concentration of immune complexes in oropharyngeal secretion. Further research into the local and systemic immune mechanisms that contribute to the formation and persistence of biofilms in CRS appears to be warranted.

CONCLUSIONS

A low level of α-interferon is a pathogenetically significant factor in chronic rhinosinusitis with biofilm presence. A high concentration of immune complexes in patients with CRS and biofilms represents an immunopathological mechanism contributing to the persistence of inflammation. The levels of interleukin-1β, the cellular composition of oropharyngeal secretion, and the concentration of secretory immunoglobulin A are characteristic of CRS without biofilm structures in the upper respiratory mucosa.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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