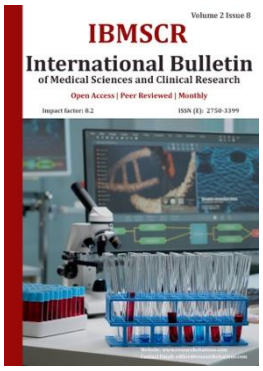

IMMUNOHISTOCHEMICAL ANALYSIS OF AGE-RELATED FACTORS IN POST-TRAUMATIC FORMS OF RHINOSINUS POLYPS

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Abstract

This study investigates the age-related immunohistochemical characteristics of post-traumatic rhinosinus polyps. Morphological and immunohistochemical analyses of polyp tissues were performed, including the assessment of T- and B-lymphocytes, macrophages, and cytokine expression, with comparisons across different age groups. The results demonstrate that age significantly influences the immunological profile of polyp tissue, with notable differences in inflammatory cell activity and cytokine expression observed in younger patients. This research provides insights into the pathogenesis of post-traumatic rhinosinus polyps and the mechanisms of age-dependent immune responses, offering valuable data for the development of individualized clinical management strategies.

Keywords: *rhinosinus polyps, post-traumatic, immunohistochemistry, age-related changes, morphology.*

Relevance of the problem

Immunohistochemical investigation of post-traumatic rhinosinus polyps represents a relevant scientific topic in otorhinolaryngology and immunology, as it contributes to a deeper understanding of the pathogenetic mechanisms of nasal polyposis and local immune responses. Chronic rhinosinusitis with nasal polyps (CRSwNP) is a disease associated with persistent inflammation, and numerous studies have demonstrated that the expression of interleukins and other inflammatory markers varies across different age groups and correlates with disease severity and clinical presentation [1].

Analyses of immunopathogenesis indicate that CRSwNP tissue is predominantly characterized by a type 2 (T2) immune response, with cytokines such as interleukin-5 (IL-5) and interleukin-13 (IL-13) playing a key role. These mediators reflect the involvement of eosinophils in sustaining local inflammatory processes within polyp tissue [2]. In addition, variations in immune cell populations and cytokine profiles contribute to the formation of distinct clinical phenotypes and determine specific inflammatory patterns of the disease [3].

Age-related alterations of the immune system also significantly influence the development and progression of rhinosinus polyps. In particular, differences in the expression levels of IL-2, IL-4, IL-5 and IL-22 among various age groups highlight the importance of age-stratified immunological analysis in CRSwNP [4]. The immune response in nasal polyposis is not limited solely to T2-driven mechanisms but is also associated with epithelial barrier dysfunction and dysregulation of innate immune pathways, further emphasizing the diagnostic value of immunohistochemical studies [5,6]. Evaluation of cellular composition and inflammatory mediators demonstrates pronounced inflammatory activity within polyp tissue, providing a basis for the development of targeted therapeutic strategies aimed at modulating immune remodeling in nasal polyps [5,6]. Moreover, nasal polyps are frequently observed in

both younger and elderly patients, and studies suggest that age-dependent molecular pathways, including altered IL-6 trans-signaling and changes in genes related to epithelial barrier protection, may play a significant role in disease progression, thereby opening new perspectives for therapeutic research [7].

Furthermore, the role of anti-inflammatory cytokines such as interleukin-10 (IL-10) in nasal polyp tissue has been investigated, indicating their potential importance in immune regulation and improvement of prognostic assessment in patients with CRSwNP [8].

Overall, age-oriented analysis of immunohistochemical markers and cytokine expression is of considerable importance for elucidating the molecular pathogenesis of post-traumatic rhinosinus polyps and for advancing personalized treatment approaches. These studies not only enhance understanding of the underlying mechanisms of the disease but also provide fundamental data necessary for the development of effective therapeutic strategies in clinical practice [9,14].

Research Aim

The aim of this study is to investigate age-related immunohistochemical characteristics of rhinosinus polyps developing after nasal trauma by analyzing the expression patterns of immune cells and cytokines, with the purpose of elucidating the key immunopathogenetic mechanisms underlying this pathology.

Materials and Methods

The study was conducted at the Republican Center of Pathological Anatomy between 2019 and 2024. A total of 68 patients were included in the investigation. The main group consisted of patients diagnosed with rhinosinus polyps that developed following nasal trauma. The control group included 16 individuals who showed no signs of inflammation or polypoid changes in the rhinosinus region during clinical, endoscopic, and instrumental examinations.

For immunohistochemical analysis, tissue samples were obtained from rhinosinus polyps during functional endoscopic sinus surgery. The collected specimens were processed using standard histological techniques and subsequently subjected to immunohistochemical staining. During analysis, the characteristics of immune cell populations and cytokine expression were compared across different age groups.

Results

In this study, the immunomorphological state of the nasal mucosa in patients with chronic rhinosinusitis with polyps (CRSwNP) developing after nasal trauma was comprehensively evaluated using immunohistochemical methods. The results were analyzed according to age groups, allowing a comparative assessment of inflammatory activity, cellular and humoral immune responses, and the degree of proliferative processes in the mucosal tissue.

Immunohistochemical analyses focused on the expression levels of T-lymphocytes (CD3), B-lymphocytes (CD20), and the proliferation marker Ki-67. The findings provided insights into age-related changes in the nasal mucosa, the remodeling of immune-competent cells, and the balance between regenerative and atrophic processes. Detailed immunohistochemical results were recorded for each age group.

Group 1 (18–30 years). In this age group, patients with post-traumatic CRSwNP exhibited a predominance of active inflammatory processes within the mucosal layer. Ki-67 expression was generally low to moderate, with 58.5% of cases showing low and 41.5% moderate positivity, resulting in a mean proliferative index of 11.01 ± 0.89. The expression was primarily

localized to fibroblasts, histiocytes, sparse macrophages, and lymphocytes within the stromal regions of the polyp tissue. High Ki-67 expression was not observed in the epithelial layer, indicating the absence of uncontrolled cellular proliferation.

Regarding T-lymphocytes, CD3 expression was strongly positive in 71.11% of cases, predominantly distributed around mucosa-associated lymphoid tissue (MALT), perivascular zones, and the stromal compartment of the mucosa. This pattern reflects an active local cellular immune response and indicates that the inflammatory process is in an active chronic phase.

CD20 expression was also notably high in this group, with 80.01% of cases demonstrating strong positivity. The dense localization of B-lymphocytes within MALT structures suggests a highly active humoral immune component contributing to the ongoing inflammation (see Figure 1).

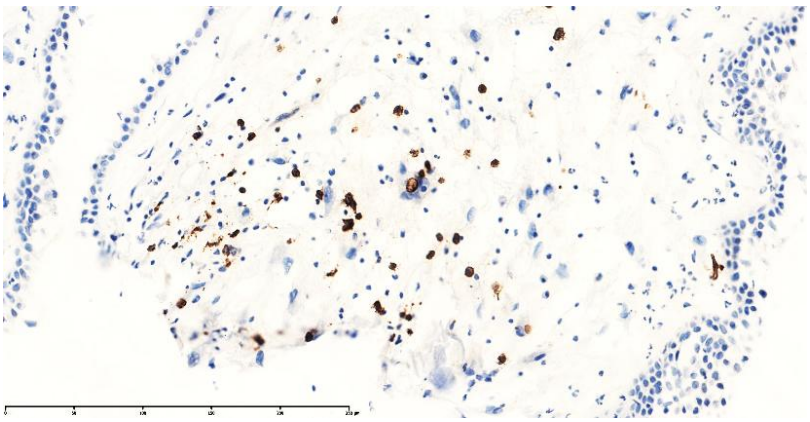

Figure 1. Chronic rhinosinusitis with nasal polyps, Group 1 (1-year duration). Patient aged 18 years. Low-level Ki-67 expression is observed. The sample was scanned and analyzed using QuPath 0.4.0 software to determine the degree of marker expression. Positively stained cells are depicted in dark brown. DAB chromogen was used for staining. Magnification: ×40.

Group 2 (31–45 years)

In patients of this age group, immunohistochemical findings indicated that the inflammatory process had progressed to a relatively stable phase. Ki-67 expression was predominantly moderate, observed in 63.12% of cases, while low-level expression was seen in 31.14%, and negative staining in 5.74% of samples. The mean proliferative index was 13.07 ± 1.02, with active mitotic foci mainly located within the stromal compartment. Although epithelial cells showed signs of metaplasia, dysplasia was minimal or absent.

CD3 expression remained high in Group 2 (65.15%), although a slight decrease compared to Group 1 was noted. CD20 positivity was observed in 74.69% of cases, indicating that B-lymphocyte activity and humoral immune responses remained prominent (see Figure 2).

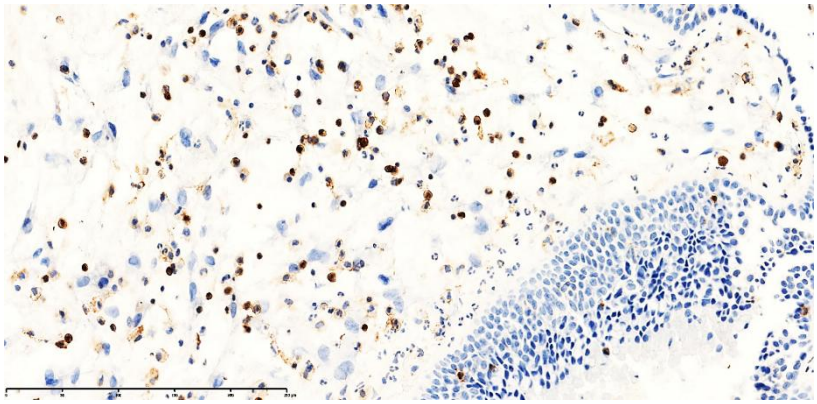

Figure 2. Chronic rhinosinusitis in a 38-year-old patient. Low-level Ki-67 expression is observed. The sample was scanned and analyzed using QuPath 0.4.0 software to determine the degree of marker expression. Positively stained cells appear in dark brown. DAB chromogen was used for staining. Magnification: ×40.

Group 3 (46–60 years)

In patients with a disease duration of 3–5 years, morphological alterations in the nasal mucosa became more pronounced. Atrophic and sclerotic processes were more extensive, accompanied by increased foci of squamous cell metaplasia and the formation of fibrotic polyps. Ki-67 expression significantly decreased in this group, with low-level positivity observed in 73.13% of cases and moderate expression in 19.21%. The mean proliferative index was 10.21 ± 1.03, indicating a reduction in reparative regeneration and decreased mitotic activity within the epithelial layer.

CD3 expression also exhibited a declining trend. The total number of T-lymphocytes decreased, reflecting a shift of the immune response toward a predominantly humoral orientation. This observation is likely related to reduced activity of T-cell receptors containing the CD3G gene. Despite persistence of both high and moderate CD20 positivity, the overall expression was noticeably lower compared to Groups 1 and 2, indicating a gradual attenuation of the humoral immune response (see Figure 3).

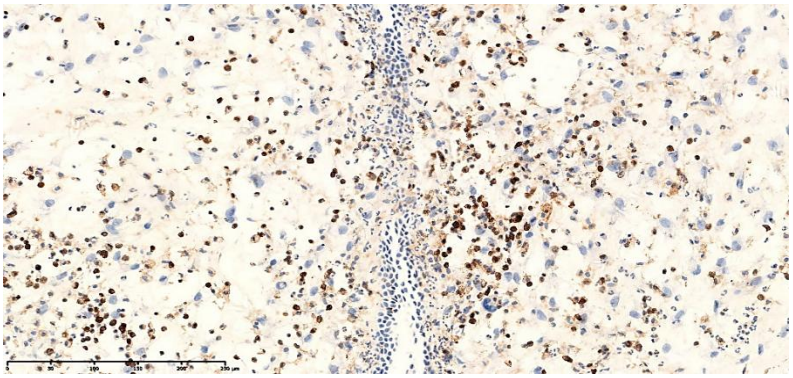

Figure 3. Chronic rhinosinusitis with nasal polyps showing high-level CD3 expression. The tissue sample was scanned and analyzed using QuPath 0.4.0 software to quantify the degree of marker expression. Positively stained cells appear dark brown. DAB chromogen was used for staining. Magnification: ×40.

Group 4 (over 60 years)

In patients over 60 years of age with a disease duration of 5 years or more, chronic rhinosinusitis with nasal polyps was characterized by pronounced atrophic and sclerotic

changes in the mucosal layer. Epidermization, foci of squamous cell metaplasia, and atrophy of the secretory glands predominated, reflecting long-standing tissue remodeling. Ki-67 expression markedly decreased in this age group, with low-level positivity observed in 91.01% of cases and negative staining in 8.9%. The proliferative index was 6.59 ± 0.52 in epithelial cells and 8.39 ± 0.72 in mesenchymal cells, indicating near-complete attenuation of reparative regeneration and the absence of active proliferative processes.

CD3 expression was negative in 40.24% of cases, confirming a significant decline in local cellular immunity. These findings suggest that in elderly patients, both cellular and proliferative components of the immune response are substantially reduced, and the chronic inflammatory process is largely stabilized, with minimal regenerative activity (see Figure 4).

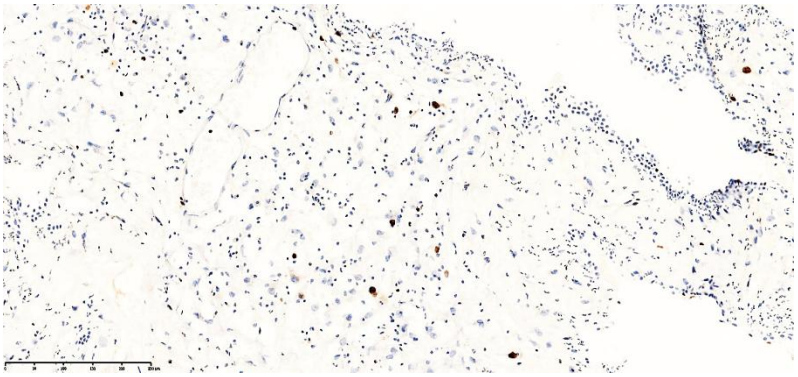

Figure 4. Chronic rhinosinusitis demonstrating low-level CD3 expression. The tissue sample was scanned and analyzed using QuPath 0.4.0 software to quantify the degree of marker expression. Positively stained cells appear dark brown. DAB chromogen was used for visualization. Magnification: ×40.

The mucosa-associated lymphoid tissue (MALT) structures exhibited significant atrophy, and the number of T-lymphocytes was markedly reduced. CD20 expression also declined considerably, with strong positivity observed in only **19.5% of cases.** These findings indicate a progressive weakening of the humoral immune response and suggest that the mucosal layer has transitioned to an immunologically "impoverished" state.

Conclusion

The results of this study demonstrate that the morpho-functional state of the nasal mucosa in chronic rhinosinusitis with nasal polyps developing after nasal trauma undergoes significant age-related changes. In younger patients (18–30 years), proliferative activity remains relatively high, with Ki-67 expression predominantly low to moderate, reflecting a predominance of regenerative processes. This is accompanied by high activity of T-lymphocytes (CD3) and B-lymphocytes (CD20), indicating that immune-competent cells remain active and the consequences of chronic inflammation are associated with minimal atrophic changes.

In middle-aged and older patients (31–60 years), Ki-67 expression decreases, and the reduced proliferative index correlates with a decline in regenerative processes, accompanied by more pronounced atrophic and sclerotic alterations. A reduction in CD3 expression reflects diminished T-lymphocyte-mediated immune responses, with a relative predominance of humoral components. Similarly, CD20 expression declines with age, indicating an overall weakening of mucosal immune reactivity. In elderly patients (over 60 years), both the number and activity of immune-competent cells are further reduced, while atrophic, sclerotic, and

metaplastic changes dominate the mucosal architecture. These findings indicate that immunohistochemical alterations and proliferative activity in post-traumatic chronic rhinosinusitis are strongly age-dependent, which has important implications for clinical management, patient treatment strategies, and the assessment of tissue regenerative potential.

Overall, the study suggests that evaluating Ki-67, CD3, and CD20 markers provides a reliable method to assess cell proliferation and immune cell activity in the nasal mucosa in an age-dependent manner. This approach may serve as a crucial tool for predicting disease progression and optimizing therapeutic strategies for post-traumatic chronic rhinosinusitis with nasal polyps.

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