Involvement of inflammatory cells in chronic rhinosinusitis with nasal polyps

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Abstract

Inflammation plays an important role in the pathogenesis of nasal polyposis. Understanding the biomolecular action mechanisms of inflammatory elements can contribute to improving the prognosis of these lesions. The study analyzed the distribution and immunohistochemically quantified eosinophils [eosinophil major basic protein (BMK-13)], lymphocytes [cluster of differentiation (CD) 4, CD8, CD20] and plasmocytes (CD138) in both the epithelial and stromal compartment in relation to composite scores, which included specific histopathological parameters for 50 sinonasal polyps. Inflammatory elements predominated at stromal level, the high histological composite scores being frequently associated with increased expression of inflammatory elements. Also, the numerical distribution of inflammatory elements indicated positive linear relations within the groups BMK-13/CD8 and CD4/CD20/CD138, and a negative linear relation between the two groups. This aspect can support the existence of alternative or sequential pathogenic mechanisms involved in the pathogenesis of sinonasal polyposis, and the results obtained can be used for a better stratification of patients in order to optimize the therapy.

Keywords: chronic rhinosinusitis, lymphocytes, plasmocytes, eosinophils.

Introduction

Chronic rhinosinusitis with nasal polyps is a subgroup of chronic rhinosinusitis [1], defined as chronic inflammatory disease of the paranasal sinuses and nasal mucosa [2]. The most severe form of chronic rhinosinusitis is nasal polyposis [3]. The incidence of the disease increases with age and is about 2–4% compared to the general population [4].

It is appreciated that inflammation from chronic rhinosinusitis is a secondary process due to local immune dysfunction by altering the integrity of the epithelial barrier and regulating the response of this process to foreign antigens [5].

Numerous inflammatory cells can be identified in polypous tissue, including lymphocytes, plasmocytes, macrophages, eosinophils, and basophils, and under certain conditions with proliferation of blood vessels, fibrosis, and tissue necrosis [6, 7]. The increased number of activated eosinophils, neutrophils and plasmocytes in the nasal polyps, as compared to the normal nasal mucosa, suggests that inflammatory processes play an important role in the pathophysiology of nasal polyposis [8]. The degree of inflammatory cell infiltration of the sinus mucosa can be a useful indicator of recurrence after surgery [9]. The use of immunohistochemistry allows detailed investigation of the heterogeneity and dynamics of inflammatory cells.

Aim

In this study, we followed the expression of markers for the dominant inflammatory cells in chronic rhinosinusitis with nasal polyposis.

Materials and Methods

The study included a number of 50 sinonasal polyps selected from patients admitted and operated in the Department of Ear, Nose and Throat (ENT), Emergency County Hospital of Craiova, Romania. The tissue specimens were fixed in 10% neutral buffered formalin, processed by classical paraffin embedding technique followed by Hematoxylin–Eosin (HE) staining. For the selected cases, we analyzed a series of histopathological (HP) parameters that received scores, according to similar literature studies [10–13] (Table 1): • epithelial compartment:
  – basal membrane thickening: 0 (<9 μm), 1 (10–19 μm), 2 (20–29 μm), 3 (≥30 μm);
  – goblet cell hyperplasia: 0 (<3 cells), 1 (3–10 cells), 2 (11–20 cells), 3 (>20 cells);
  – epithelial infiltration with eosinophils: 0 (0 cells), 1 (1–2 cells), 2 (3–10 cells), 3 (>10 cells);
  – basal layer hyperplasia: 0 (absent), 1 (focal), 2 (zonal), 3 (diffuse);
Results

The study of the 50 analyzed polyps followed the evaluation of the epithelial and stromal changes in relation to CHSs, obtaining 38 (76%) cases with low values (low CHS – LCHS) and 12 (24%) cases with high values (high CHS – HCHS). Analysis of the immunohistochemical (IHC) expression of markers used to highlight inflammatory cells involved in chronic rhinosinusitis with nasal polypos revealed different aspects in relation to the CHS values (Table 2).

Table 2 – Distribution of the immunomarkers’ expression in relation to the CHS values

| Immunomarker / CHS | Epithelial | Stromal |
|--------------------|------------|---------|
|                   | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 |
| BMK-13 LCHS        | 27 | 11 | 0 | 0 | 8 | 16 | 14 |
| HCHS               | 7  | 0  | 4 | 1 | 0 | 0  | 12 |
| CD4 LCHS           | 0  | 0  | 0 | 0 | 17| 17 | 4  |
| HCHS               | 0  | 0  | 0 | 0 | 7 | 2  | 2  |
| CD8 LCHS           | 33 | 5  | 0 | 0 | 3 | 20 | 15 |
| HCHS               | 3  | 7  | 1 | 1 | 2 | 3  | 6  |
| CD20 LCHS          | 34 | 4  | 0 | 0 | 27| 11 | 0  |
| HCHS               | 9  | 2  | 1 | 0 | 8 | 2  | 1  |
| CD138 LCHS         | 0  | 0  | 0 | 0 | 9 | 15 | 15 |
| HCHS               | 0  | 0  | 0 | 0 | 4 | 7  | 1  |

BMK-13: Eosinophil major basic protein; CD: Cluster of differentiation; CHS: Composite histological score; HCHS: High CHS; LCHS: Low CHS.

BMK-13 immunoeexpression

Analysis of BMK-13 immunoeexpression indicated cytoplasmic positivity in the stromal infiltrate with eosinophils in all analyzed cases (100%), while at epithelial level, the immunolabelling was identified in only 16 (32%) cases. In LCHS cases, the positivity in the epithelial compartment was identified in 11 cases, with an average of 1.6±0.5 cells/×100 and an average CHS value of 1 (Figure 1A). For HCHS cases, we found positivity in five cases, with an average of 9.6±3.2 cells/×100 and the mean CHS value of 2.2 (Figure 1B). In the stromal compartment, for LCHS we observed immunostaining in BMK-13 in all 38 investigated cases, with an average of 9.4±8 cells/×100 and the mean CHS value of 15.5 (Figure 1C). For the cases with HCHS, we also found positivity in all cases, with an average of 23.1±2 cells/×100 and a CHS mean of 3 (Figure 1D).

For the semi-quantitative assessment of analyzed markers, we used an adapted scoring system based on literature data [14–20], which followed the evaluation of immunolabeled cells number. The reactions were quantified as follows:

- squamous metaplasia: 0 (absent), 1 (focal), 2 (zonal), 3 (diffuse);
- stromal edema: 0 (absent), 1 (focal), 2 (zonal), 3 (diffuse);
- epithelial alteration: 0 (absent), 1 (partial denudation), 2 (complete denudation).

Finally, for each case we calculated the composite histological score (CHS) by summing the scores given to various parameters, whose values ranged from 0–32. We considered low CHS less than 11 and high CHS above this value.

Table 1 – Panel with the immunohistochemical study

| Antibody | Clone / Manufacturer | Dilution | Antigen retrieval buffers | External control |
|----------|----------------------|----------|---------------------------|-----------------|
| BMK-13   | NBP1-42140 / Bio-Rad | 1:100    | Pepsin                    | Bronchial mucosa (bronchial asthma) |
| CD4      | 4B12 / Dako          | 1:20     | EDTA, pH 8                | Spleen          |
| CD8      | 4B11 / Dako          | 1:200    | Tris-EDTA, pH 9           | Spleen          |
| CD20     | L26 / Dako           | 1:500    | Citrate, pH 6             | Tonsil          |
| CD138    | M15 / Thermo Fisher Scientific | 1:200 | Pepsin                   | Tonsil          |

BMK-13: Eosinophil major basic protein; CD: Cluster of differentiation; EDTA: Ethylenediaminetetraacetic acid.

For the semi-quantitative assessment of analyzed markers, we used an adapted scoring system based on literature data [14–20], which followed the evaluation of immunolabeled cells number. The reactions were quantified as follows:

- for eosinophils (BMK-13): epithelial 0 (0 cells), 1 (1–2 cells), 2 (3–10 cells), 3 (>10 cells), and stromal 0 (0 cells), 1 (1–3 cells), 2 (4–15 cells), 3 (>15 cells);
- for lymphocytes and plasmocytes [cluster of differentiation (CD) 4, CD8, CD20, CD138]: epithelial 0 (0 cells), 1 (1–2 cells), 2 (3–5 cells), 3 (>5 cells) and stromal 0 (0 cells), 1 (<10 cells), 2 (10–20 cells), 3 (>20 cells).

CD4 immunoexpression

Analysis of CD4 immunoexpression indicated cytoplasmic positivity in 26 (52%) of analyzed cases and for CD8 in 45 (90%) cases. For CD4, we identified positivity only in stromal cells, whereas CD8 positivity was present in both epithelial (14 cases) and stromal (45 cases) compartments.

For stromal CD4, in the cases with LCHS we observed positivity in 21 of the investigated cases, with an average
of 6±1.6 cells/×100 and a CHS mean of 1 (Figure 2A). For HCHS cases, we found positivity in five of the analyzed cases, with an average of 12.2±5.9 cells/×100 and a CHS mean of 1.8 (Figure 2B). Analysis of stromal CD4 values indicated differences at the limit of statistical significance depending on CHS, with increased CD4 scores being associated with HCHS (χ² test, \( p = 0.052 \)).

**CD8 immunoexpression**

In the epithelial compartment, CD8 positivity was identified in five LCHS cases, with an average of 1.6±0.5 cells/×100 (Figure 2C). We found positivity in nine cases of HCHS polyps, with an average of 2.7±1.9 cells/×100 and a CHS mean of 1.3 (Figure 2D). At stromal level, in LCHS polyp cases, we observed CD8 positivity in 35 of the investigated cases, with an average of 9.2±3.3 cells/×100, with a CHS mean of 1.4, and for HCHS cases, we found positivity in 10 of all analyzed cases, with an average of 12.1±4.4 cells/×100 and a CHS mean of 1.8 (Figure 2E and F).

**CD20 immunoexpression**

Analysis of CD20 immunoexpression indicated cytoplasmic positivity in 15 (30%) of the analyzed cases, both in epithelial (seven cases) and stromal (15 cases) cell infiltrate. We found that in epithelial compartment the CD20 positivity in LCHS cases was present in four of all investigated cases, with a mean of 1 cell/×100 and a CHS average of 1 (Figure 3A). For HCHS cases, we identified CD20 positivity in three cases, with a mean of 2.3 cells/×100 and a CHS average of 1.3 (Figure 3B). In the stromal compartment, in LCHS cases we observed positivity in 11 of analyzed cases, with an average 5.9±1.3 cells/×100 and a CHS mean of 1 (Figure 3C). For the HCHS cases, we found positivity in only four of the investigated cases, with an average of 13±7.6 cells/×100 and a CHS mean of 1.7 (Figure 3D). The analysis of CD20 reactions indicated that increased immunoscores were associated in a non-significantly statistical manner with HCHS in both epithelial (χ² test, \( p = 0.212 \)) and stromal (χ² test, \( p = 0.072 \)) compartments.

**CD138 immunoexpression**

Analysis of CD138 immunoexpression indicated cytoplasmic positivity in 41 (82%) of the analyzed cases, only at stromal level. In these LCHS cases, we observed positivity in 29 of the investigated cases, with an average of 9.6±3.5 cells/×100 and a CHS average of 1.5 (Figure 4A). For HCHS cases, we found positivity in all 12 analyzed cases, with an average of 12.9±7.5 cells/×100 and a CHS average of 1.75 (Figure 4B).

**Statistical analysis**

Stromal analysis of CD4 and CD8 values indicated a non-significant negative linear correlation (\( p = 0.421 \), Pearson’s test). The distribution of stromal values of BMK-13 and CD4 indicated a statistically significant negative linear correlation (\( p = 0.011 \), Pearson’s test), whereas in relation to CD8 the correlation was linear positive at the limit of statistical significance (\( p = 0.086 \), Pearson’s test) (Figure 4C). At the same time, the numerical values of the CD20 reactions indicated a linear statistically non-significant correlation related to BMK-13 (\( p = 0.562 \), Pearson’s test) and CD8 (\( p = 0.560 \), Pearson’s test), and a significantly positive linear correlation with CD4 (\( p = 0.003 \), Pearson’s test). Analysis of the distribution of the numerical values for CD138 reactions related to BMK-13 indicated a statistically non-significant negative linear correlation (\( p = 0.922 \), Pearson’s test), same aspect being also observed in relation to CD8 values (\( p = 0.157 \), Pearson’s test) (Figure 4D). Compared with CD4, the values CD138 indicated a positive linear correlation (\( p = 0.157 \), Pearson’s test) (Figure 4E). The obtained statistical correlations indicate a positive linear association between CD138/CD4/CD20 and BMK-13/CD8, groups that overall had a negative linear relation.
Figure 2 – (A, C and E) LCHS polyp; (B, D and F) HCHS polyp. CD4 stromal immunoexpression: (A and B) ×100. CD8 epithelial immunoexpression: (C and D) ×100. CD8 stromal immunoexpression: (E and F) ×100. CD: Cluster of differentiation; CHS: Composite histological score; HCHS: High CHS; LCHS: Low CHS.

Figure 3 – (A and C) LCHS polyp; (B and D) HCHS polyp. CD20 epithelial immunoexpression: (A and B) ×100. CD20 stromal immunoexpression: (C and D) ×100. CD: Cluster of differentiation; CHS: Composite histological score; HCHS: High CHS; LCHS: Low CHS.
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Figure 4 – (A) LCHS polyp; (B) HCHS polyp; (C) Distribution of CD4, CD8 and BMK-13 values at stromal level; (D) Distribution of CD20, CD4, CD8 and BMK-13 values at stromal level; (E) Distribution of BMK-13, CD138, CD4 and CD8 values at stromal level. CD138 stromal immunoexpression: (A and B) ×100. BMK-13: Eosinophil major basic protein; CD: Cluster of differentiation; CHS: Composite histological score; HCHS: High CHS; LCHS: Low CHS.

 Discussions

Chronic nasal polyposis is a common disease of the nasal cavity and sinuses that affects millions of people worldwide [21]. The pathogenesis of chronic rhinosinusitis, whether or not associated with polyps, is highly complex and involves, among other mechanisms, activation and migration of inflammatory cells [22].

Immune cell infiltration observed from nasal polyps includes almost constantly eosinophils, which account for over 10% of inflammatory cells [23]. Current counting of eosinophils can be carried out in the usual HE staining, with other possibilities being histochemical (Congo Red) or IHC [major basic protein (MBP) – BMK-13 and eosinophil cationic protein (EG1, EG2)] stainings [19, 24].

In this study, we observed the association of high BMK-13 scores with HCHS at both epithelial and stromal levels. In polyps with reduced eosinophilic infiltration, eosinophils are frequently MBP+ and rarely EG2+, indicating that most eosinophils that have infiltrated the polyp have not been activated [25].

Lymphocyte infiltration of the sinonasal and paranasal mucosa is one of the essential HP changes due to inflammation in chronic rhinosinusitis. Lymphocyte activation determines the type of lymphocyte cell to be recruited, as well as the type of inflammation that develops, a mechanism to which other inflammatory cells contribute through cytokine-mediated cellular communication [26].

T-lymphocytes play major roles in regulating the inflammatory process in the mucosa. Most lymphocytes infiltrated into the nasal polyps are T-cells, with a significantly increased frequency of CD4+ and CD8+ T-cells [20]. The results of several IHC studies indicated that CD8+ (suppressor/cytotoxic) T-cells are more numerous in chronic rhinosinusitis than CD4+ (helper/inducer) T-cells, compared to healthy subjects [27–29]. Baba et al. reported an average CD4+ cell count of 3.4 (2.3–7.3), and for CD8+ of 36.6 (16.2–56.1) for chronic rhinosinusitis with eosinophilic polyps [30]. In our study, CD4 immuno-reactions were identified only in stromal compartment, the high scores being associated with HCHS at the limit of statistical significance.

CD8 immunoexpression allowed quantification of suppressor T-lymphocytes and showed their presence at both epithelial (28%) and stromal (90%) levels. High CD8 scores were associated with HCHS, which were at the limit of statistical significance in the stromal compartment. Although the importance of CD4+ T-cells has been demonstrated in the pathogenesis of chronic rhinosinusitis, the function of CD8+ T-cells is not fully known [28].

In our study, we found a positive linear relation of BMK-13 values and CD8+ lymphocytes.

B-cells are a key component of the adaptive immune response and are known to play several important roles in a variety of inflammatory conditions, including at the level of mucosa [33, 34]. Several studies have reported that in the nasal polyps versus the control and chronic rhinosinusitis without polyps, almost no native B-lymphocytes (CD20+) were present, although a significantly higher number of plasmocytes (CD138+) was present [35, 36], aspect that was also identified in our study.

It is acknowledged that B-cells accumulate in the nasal polyps in patients with chronic rhinosinusitis. It is not
yet clear whether B-cells enter tissue as naive cells and get activated later or if they enter as primitive cells with memory to respond to tissue aggression [37].

In our study, we found the association of increased CD20/CD138 scores with HCHS, as well as a positive linear correlation of the number of CD20-, CD138- and CD4-positive cells.

Literature data indicate the key role of B-cells in sustaining chronic polyposis rhinosinusitis [38-40]. In addition to their ability to produce antibodies that contribute to the pathogenesis of the disease, B-cells can function as antigen-presenting or regulatory cells and produce a variety of cytokines and chemokines that can influence the evolution of inflammation. These studies suggest that polyps are a favorable environment for B-cell survival and antibody production that may play important roles in the pathogenesis of chronic rhinosinusitis with nasal polyps [18].

**Conclusions**

The frequency of the inflammatory elements analyzed from the stromal level was higher compared to the epithelial compartment. Analysis of the immunostaining values for the inflammatory elements analyzed indicated statistically significant association of BMK-13 with CD8+ lymphocytes. The negative linear relation between CD4 and CD8 values may indicate cellular competition for immune mechanisms or the predominant association of CD8 with allergic inflammatory status. In this study, we found positive linear associations CD138/CD4/CD20 and BMK-13/CD8, groups inflammatory status. In this study, we found positive linear correlation of the number of CD20-, CD138- and CD4-positive cells in sinonasal polyps [18].

**Conflict of interests**

The authors declare that they have no conflict of interests.

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