



PREDICTIVE SIGNIFICANCE OF HISTOPATHOLOGIC PARAMETERS IN THE OUTCOME OF CHRONIC RHINOSINUSITIS WITH NASAL POLYPOSIS

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Abstract. Introduction. Chronic rhinosinusitis with nasal polyposis is an important and complex disease characterized by a persistent inflammatory process involving the sinonasal mucosa, representing a serious health problem and economic challenge, with pathogenic mechanisms still debated. Researchers in this area are currently concerned with finding new molecular pathways that coordinate this complex chronic inflammatory process of the sinonasal mucosa, thus aiming to identify both clear pathogenic mechanisms and a potential value of predictive and prognostic markers. **OBJECTIVE.** The aim of this study was to estimate the predictive value of the ultrastructural changes in the nasal mucosa in patients with chronic rhinosinusitis with nasal polyps, in order to establish the best therapeutic option. **MATERIAL AND METHODS.** We performed a prospective study between January 2012- February 2014 on 82 patients with Chronic Rhinosinusitis with Nasal Polyps (CRSwNP) assessed by histopathologic and immunohistochemical methods to determine the presence of inflammatory markers such as eosinophilic infiltrate and proinflammatory protein HMGB1. The patients underwent endoscopic sinus surgery and the specimens taken during the surgery were examined. Histopathological evaluation of biopsy fragments taken from nasal polyps was performed to quantify the eosinophilic infiltrate from a much complex score containing also fibrosis, angiogenesis and oedema of the mucosa. Immunohistochemistry determined the distribution of the proinflammatory protein HMGB1 extracellular. Based on these parameters, the patients were categorized in four groups. To evaluate the outcomes a visual analogue scale (VAS) for each symptom (nasal obstruction, rhinorrhea, anosmia/hyposmia) was filled by the patients and the nasal endoscopic score Lund-Kennedy was assessed by the physician. The predictive value of the histopathological and immunohistochemical parameters in clinical evolution and recurrence of nasal polyposis was evaluated by comparing these results at 1 month, 3,6 months and 1 year). **RESULTS.** Our results demonstrated that the extracellular HMGB1 protein and eosinophilic infiltrate show a great importance as predictive factors for both evolution and recurrences in patients with chronic rhinosinusitis with nasal polyps. Analyzing all four groups, we observed that group 4 (HMGB1 extracellular positive and eosinophilic infiltrate) had the worst evolution and the highest number of recurrences (19,23%), while group 1 (HMGB1 extracellular negative with infiltrate non-eosinophilic), exhibited the best improvement and a recurrence of only 3%. **CONCLUSION.** The results of our study showed that HP and IHC parameters could be considered significant predictive factors in post-therapeutic clinical evolution and recurrence of nasal polyposis.

Keywords: nasal polyps, HMGB1, immunohistochemistry, histopathologic score

INTRODUCTION

Chronic rhinosinusitis with nasal polyposis is an important and complex disease charac-

terized by a persistent inflammatory process involving the sinonasal mucosa, representing at the same time a serious health problem and economic challenge [1]. Although latest research have revealed an impairment in the regulation of multiple inflammatory markers and cell types, the etiology of nasal polyposis

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is still debated [1,2]. The increased inflammatory cell infiltration and persistent inflammation are able to initiate epithelial damage leading to morphological changes of the respiratory epithelium [2]. Researchers in this area are currently concerned with finding new molecular pathways that coordinate this complex chronic inflammatory process of the sinonasal mucosa, thus aiming to identify both clear pathogenic mechanisms and a potential value of predictive and prognostic markers [2,3]. The diversity of molecular markers in relation to nasal mucosa morphostructural changes often complicate the evolution and response to therapy [3]. A relatively small number of studies, attempting to analyze the impact of histological features of nasal polyps as a relevant long-term outcome predictive factors, are published in literature. Some studies demonstrated that the presence of mucosal eosinophilia (>10 eosinophils/high-power field) is an important severity predictor, patients experiencing lower improvement in the quality of life and specific symptoms of the disease and a higher recurrence rate [4]. Other studies found a positive correlation between increased local eosinophilia and IL-5 activity and long-term outcome, indicating poorer prognosis in patients with asthma and allergies [5]. The attention of the latest studies was oriented towards the observation of new molecules that play a role in the pathogenesis of nasal polyposis. A novel protein, which is able to trigger inflammatory response, called HMGB1, has been identified in patients affected with nasal polyposis, which suggested a possible role in the pathogenesis of these disease. HMGB1 (high mobility group box-1), recently included in the alarmin family, is a nuclear high-mobility, multifunctional protein, highly expressed by all human body cells, that is known to be part of the group of molecules called DAMP (damage-associated molecular pattern) [6]. These molecules represent endogenous signals released by necrotic cells, having the capacity to induce an inflammatory or immune response [7]. Besides signaling danger, they have extra roles, depending on its localization (nuclear, cytoplasm or extracellular). The intracellular functions are not part of our study, but it seems that necrotic cells release the protein passively, through a rupture in the plasmalemma and carirolema [8] and it may also be actively secreted specially by immune system cells [9].

Extracellular HMGB1 induces diffuse endothelial activation and systemic activation of

inflammation effector cells [10]. Recent studies have shown that extracellular HMGB1 has a proinflammatory function, involved in the pathogenesis of multiple inflammatory or autoimmune pathologies (rheumatoid arthritis, systemic lupus erithematosus, sepsis). The research that concentrates on its proinflammatory function has found an obvious connection between extracellular HMGB1 levels in different tissues and immune system activity [11]. Extracellular HMGB1 protein can be a very useful instrument in the diagnosis, follow-up and treatment of different diseases. Based on current information relating to histopathology, molecular and cellular biology of nasal polyposis, the present work is focused on the evaluation of immunohistochemical and histopathological prognostic factors in the development of this pathology. Since there are few data that suggest the importance of histological and immunohistochemical prognostic factors in long-term outcome, with this study we tried to evaluate, if they are useful for the therapeutic approach. The aim of this study was to assess the predictive value of the ultrastructural changes in the nasal mucosa, in patients with chronic rhinosinusitis with nasal polyps, in order to establish the best therapeutic option for them.

MATERIAL AND METHODS

We performed a prospective study on 82 patients with CRSwNP between January 2012 and February 2014 who underwent endoscopic sinus surgery in the ENT department of "Sfanta Maria" Hospital, Bucharest. Selection of patients was made from a number of 217 patients that have been admitted in our ambulatory care service diagnosed with CRSwNP. The diagnosis was based on clinical history, clinical and paraclinical investigation (nasal endoscopy). History of asthma, allergy in terms of NSAIDs intolerance, allergic rhinitis was noted. Only patients with primary CRSwNP with a minimum post-therapeutic follow up of 1 year were enrolled. Patients with cystic fibrosis, primary ciliary dyskinesia, hematologic diseases, pregnancy or previous nasal surgery were excluded from the study. Informed consent for study participation was obtained from all patients. All patients received long-term medical therapy (continuous daily treatment with topical corticosteroids mometasone furoate 50 ug/puf, 2 puff x2/day) for 3 months followed a flash-therapy with systemic corticosteroids-Dexametosone (im, 8 mg/day, 5 days). After the end of treatment, the patients

have been reevaluated and the indication for surgery was suggested on those who fulfill the following conditions: 1. VAS score minimum 5 for 2 out of 3 parameters (nasal obstruction, rhinorrhea, smell disorders), 2. nasal polyposis degree 2/3 bilaterally, according to the Lindholdt scale. All patients were operated by the same surgeon. Biopsy from polyps tissue was sampled during surgery, the next step being post-surgery histopathological and immunohistochemical evaluation. Postoperative treatment was similar for all patients: local aspirations for the first 10 postsurgical days, nasal irrigations with normal saline for 1 month, and local corticosteroid therapy with mometasone furoate for 3 months, beginning at the end of the first month after the surgery (2 puffs/day, 400 ug/day).

All patients were assessed pre- and postoperatively using subjective and objective methods, in order to compare the disease evolution and to detect possible recurrences. Postoperative clinical follow-up was performed at 1 month, 3, 6 and 12 months. The subjective assessment of disease severity was performed using a visual analogue scale (VAS) of three symptoms: nasal obstruction, rhinorrhea and anosmia/hyposmia. Endoscopic findings were quantified using the Lund Kennedy scoring system (score range 0-20). This endoscopy scoring system grades visual pathologic states of nasosinus mucosa, and include the following parameters: polyps, edema, discharge, scarring and crusting.

Recurrence of polyps was defined on patients that required surgical revision, based on the presence of nasal polyps on endoscopic evaluation during follow up visits using grading system proposed by Lindholt (**grade 2 and 3** were labeled as recurrence) and on **VAS score** minimum 5 for 2 out of 3 parameters (nasal obstruction, rhinoreea and smell disorders).

Histopathology and immunohistochemistry

The bioptic samples from the nasal polyps were evaluated in the Histopathology Laboratory of "Sfanta Maria" Hospital Bucharest as it follows:

Histopathological evaluation was performed in order to identify eosinophilic infiltrate in the bioptic specimens. Considering the histopathological changes of nasal mucosa the ones that highlights most eloquently the severity of disease it was achieved a score that gathers a set of five parameters, namely inflammatory

infiltrate, eosinophilia, submucosal edema, angiogenesis, fibrosis. Not all changes in this score are assigned defining for nasal polyposis, which is why in this study we used the parameter eosinophils, being considered the most important element for the onset and persistence of the nasal mucosa inflammatory process. Eosinophils presence was quantified as follows: 0 points (absent), 1 point (rare, <10/40x), 2 points frequent (10-30/40x), 3 points very frequent (>30/40x). In order to simplify, we considered patients with eosinophilic infiltrate of 0 and 1 as being non-eosinophilic (Eo-) and the ones having an infiltrate of 2 and 3 as being eosinophilic (Eo+).

Immunohistochemical measurement was performed in order to identify the presence of extracellular HMGB1 protein in the bioptic material. This was quantified as follows: 0 points (absent), 1 point (0-30%), 2 points (30-60%), 3 points (>60%). In order to simplify, we considered patients with HMGB1 of 0 as being HMGB1- and the ones having an infiltrate of 1, 2 and 3 as being HMGB1+.

Following histopathological and immunohistochemical measurements, we divided the patients in four groups, as it follows:

- Group 1 (15 patients)- Eo- and HMGB1-
- Group 2 (32 patients)- Eo+- and HMGB1-
- Group 3 (9 patients)- Eo- and HMGB1+
- Group 4 (26 patients)- Eo+ and HMGB1 +

Statistical analysis

Correlations between the different variables were analyzed using linear regression while the Spearman coefficient was calculated to evaluate the degree of correlation in a non-parametric population. A correlation was considered to be significant if $p < 0.05$ (two-tailed, CI of 95%). Comparisons between two variables were calculated using a Mann-Whitney test.

RESULTS

A number of 82 patients with CRSwNP, who underwent endoscopic sinus surgery followed by local corticosteroid therapy with mometasone furoate for 3 months was studied. Patients enrolled in the study were aged between 18 and 82 (mean age=47.23 years). The sex ratio was male 68.29%, females 31,71%.

In order to identify the predictive value of HP and IHC parameters in evaluating the disease prognosis and severity in patients with nasal polyposis we compared the evolution of the four groups using VAS scores as mean for nasal obstruction, rhinorrhea and anosmia/hyposmia and nasal endoscopic scores.

VAS scores for **nasal obstruction** shows significant changes between groups. In group 1 (HMGB1 extracellular negative with infiltrate non-eosinophilic) VAS score shows a 51% lower value in the first month compared to preoperative values (from 5.73 to 2.8), 61% at 3 months (from 5.73 to 2.20). In groups 2, 3 and 4 the evolution is mild positive, the smallest improvement being identified in group 4 (HMGB1 extracellular positive and eosinophilic infiltrate). In the latter evolution has a specific timeline, reaching a 44% lower values during the first month, but then slowly raising when approaching 12 months. The minimal reached value in group 4 is significantly lower than other groups ($p < 0.05$) (**Table I**).

| Nasal obstruction | Preop | 1M | 3M | 6M | 12M |
|-------------------|-------|------|------|------|------|
| Group 1 | 5.73 | 2.80 | 2.20 | 2.01 | 2.07 |
| Group 2 | 6.28 | 3.72 | 3.32 | 3.00 | 2.45 |
| Group 3 | 6.85 | 3.63 | 2.92 | 3.03 | 3.22 |
| Group 4 | 7.18 | 3.99 | 3.85 | 4.12 | 4.19 |

Table I. Pre-and postoperative evaluation of patients with CRSwNP using VAS scale for nasal obstruction

VAS score decreases progressively in group 1 while in groups 2, 3 and 4 it decreases at slower pace; the response in group 4 is the lowest, as it tends to grow again after 12 months; the minimum reached value is highest in group 4 ($p < 0.05$).

Regarding the evaluation of patients using VAS scale for **rhinorrhea** the results show that all groups have a similar evolution and there are no significant differences between them. (**Table II**)

| Rhinorrhea | Preop | 1M | 3M | 6M | 12M |
|----------------|-------|------|------|------|------|
| Group 1 | 3.83 | 2.03 | 1.75 | 2.00 | 2.22 |
| Group 2 | 4.75 | 2.89 | 2.26 | 2.15 | 2.46 |
| Group 3 | 3.82 | 2.16 | 2.05 | 2.10 | 2.21 |
| Group 4 | 5.15 | 3.21 | 2.98 | 2.53 | 2.38 |

Table II. Pre-and postoperative evaluation of patients with CRSwNP using VAS scale for rhinorrhea - shows no significant differences between groups at 12 months follow-up

Olfaction evaluation shows that patients have different evolutions Postoperatively, group 1 (HMGB1 extracellular negative with infiltrate non-eosinophilic) exhibits a significant change starting with the first visit (45%), while patients in group 4 show a less important decrease (37%) at 1 month. All groups register a better VAS value at 12 months, but group 4 shows a smaller degree of improvement (35% in group 4, 43% in group 3, 53% in group 2 and 57% in group 1). (**Table III**)

Nasal endoscopic score reveals a rapid improvement in group 1 at 12 months from surgery and registers a much lower value than the preoperative one (66%). Groups 2 and 3 have a similar evolution at 12 month- the registered endoscopic score values being 60% lower in group 2, respectively 56% smaller in group 3. Group 4 has the lowest improvement compared to the rest (54%) (**Table IV**).

Recurrences. Nine patients (11%) developed recurrent nasal polyposis during the follow up period. The number of recurrences per

group was different at 12 months. The highest rate of recurrence was found in group 4 (5/26; 19.23%) ($p < 0.05$), followed progressively by the other groups: 1/9 in group 3, 2/32 in group 2 and 1/15 in group 1 (**Table V**).

Statistical analysis by ANOVA variance test revealed that extracellular HMGB1 is a very important factor for recurrence prediction ($p < 0.0001$) but eosinophilic infiltrate is not ($p = 0.57$).

| Smell disorder | Preop | 1M | 3M | 6M | 12M |
|----------------|-------|------|------|------|------|
| Group1 | 5.13 | 2.80 | 2.10 | 2.00 | 2.20 |
| Group2 | 6.05 | 3.80 | 3.04 | 2.58 | 2.81 |
| Group3 | 6.12 | 3.72 | 3.04 | 3.14 | 3.47 |
| Group4 | 7.45 | 4.68 | 3.84 | 4.59 | 4.85 |

Table III. Pre-and postoperative evaluation of patients with CRSwNP using VAS scale for olfaction- all groups register a better VAS value at 12 months, but group 4 shows a lower degree of improvement

| Nasal endoscopic score | Preop | 1M | 3M | 6M | 12M |
|------------------------|-------|------|------|------|------|
| Group 1 | 14.33 | 5.69 | 4.73 | 4.3 | 4.84 |
| Group 2 | 14.9 | 6.81 | 5.6 | 5.12 | 5.87 |
| Group 3 | 14.44 | 6.54 | 6.03 | 6.18 | 6.42 |
| Group 4 | 15.84 | 7.5 | 6.47 | 6.05 | 7.20 |

Table IV. Pre and postoperative evaluation of patients using nasal endoscopic score evolution. The most significant improvement was identified in groups 1, 2 and 3, with slightly progressive values. The lowest improvement was in group 4, registering the highest post-operative endoscopic score (7.20)

| Group | Patients at risk | Number of recurrence at 12 months | Percent of recurrence |
|-------|------------------|-----------------------------------|-----------------------|
| 1 | 15 | 1 | 3% |
| 2 | 32 | 2 | 6.25% |
| 3 | 9 | 1 | 11% |
| 4 | 26 | 5 | 19.23% |

Table V. Recurrence rate in patients with CRSwNP was higher in groups 3 and 4 but highest in group 4 (19,23%)

DISCUSSION

Several prognostic factors may play a role in the development of disease and recurrence, therefore in the recent years, one of the most discussed topics on the management of nasal polyposis is the discovery of significant markers for the evolution of this pathology[12].

Many attempts were made in view of predicting the evolution and response to treatment in nasal polyposis. In the latest years attention was concentrated on histopathological, immunohistochemical and immunological studies on nasal polyposis in search of parameters that could play an important predictive role [13]. Studies focusing on the development of nasal polyposis analyzed the relationship between the inflammatory disease severity and prognosis, using different parameters in an

attempt to describe or quantify the degree of inflammation of the mucosa. These parameters include the number of eosinophils in the peripheral blood and eosinophilic infiltrate of the nasal mucosa. Through these parameters, it was found that the degree of inflammation positively correlated with the severity of the disease, using different subjective and objective criteria [13,14, 15].

Despite progress in pharmacology, increased number of trials and improvement in surgical techniques, no etiological treatment has been found up to date. New therapeutic regimens are proposed along with the understanding of the complexity of pathophysiological mechanism. With all the medical and surgically available resources relapse is still significant [16]. The goal of our study was to identify pa-

rameters which will allow achieving objective associations between ultrastructural changes and evolution of nasal polyposis.

Patients with HMGB1 extracellular negative and non-eosinophilic infiltrate presented a rapid improvement for all symptoms, and endoscopic score after one month, with a positive evolution until the end of the follow-up. In contrast, patients with HMGB1 extracellular positive and eosinophilic infiltrate showed the lowest improvement, with unfavorable outcome for nasal obstruction and impaired smell until the end of the study. Patients with non-eosinophilic infiltrate associated with HMGB1 extracellular positive and those with eosinophilic infiltrate and extracellular HMGB1 negative had an intermediate evolving, being less favorable in patients with protein HMGB1 extracellular positive. The objective information provided by nasal endoscopic score highlights the influence of the two parameters in the development of nasal polyposis. The fact that patients in group 4 had the highest values, thus demonstrating the aggravating role of HMGB1 extracellular positive associated with eosinophilic infiltrate on the development of nasal polyposis.

The presence of extracellular HMGB1 is an important predictive factor for recurrence of nasal polyposis with strong statistical significance ($p < 0.0001$), but also for unfavorable evolution of the disease. The result obtained by our study regarding the influence of the eosinophilic infiltrate in the course of the disease supports its prognostic value in the development of rhinosinusitis with nasal polyps.

Our results demonstrated that HMGB1 extracellular and eosinophilic infiltrate show a great importance as predictive factors for both evolution and recurrences in patients with rhinosinusitis with nasal polyps. By analyzing the study groups, we observed that patients with HMGB1 extracellular positive and eosinophilic infiltrate had the worst evolution and the highest number of recurrences (19, 23%), while patients with HMGB1 extracellular negative and non-eosinophilic infiltrate displayed the best improvement and a recurrence of only 3%.

Given the results of our study, we believe that the combination of the two main parameters (HMGB1 extracellular and eosinophilic infiltrate) can be used as a model in anticipation of development of this pathology.

So far, literature has no available studies concentrating on the association of HMGB1 extracellular and eosinophilic infiltrate, in order to predict the evolution of chronic rhinosinusitis with nasal polyps. This study may represent a starting point for the development of a predictive score using histopathological and immunohistochemical parameters.

Future research might focus on inhibiting the proinflammatory action of HMGB1 and/or eosinophils action, at the level of the nasal mucosa. Hence, evolution may be improved and the necessity for reintervention reduced.

CONCLUSION

The results obtained in our study stand as a basis for future studies regarding the predictive value of histopathological parameters, in order to better understand the evolution of the disease and to choose the optimal treatment. Differences between pre and post therapeutic evolution allowed stratification of patients according to the predictive value of the two parameters, as follows: 1. favorable prognosis- patients who associated HMGB1 extracellular negative and non-eosinophilic infiltrate; 2. intermediate prognosis - patients that have experienced the following associations- eosinophilic infiltrate with HMGB1 extracellular negative and non-eosinophilic infiltration with HMGB1 extracellular positive; 3. unfavorable prognosis-patients who presented the association between HMGB1 extracellular positive and eosinophilic infiltrate.

As showed, HP and IHC parameters could be considered significant predictive factors in post-therapeutic clinical evolution and recurrence of nasal polyposis.

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