ORIGINAL STUDY

The role of HMGB1 protein in chronic rhinosinusitis with nasal polyposis – is it a real proinflammatory mediator?

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ABSTRACT

OBJECTIVE. The aim of the present study was to investigate if the expression and localization (nuclear, cytoplasmic and extracellular) of the High Mobility Group Box 1 (HMGB1) protein-cytokine is, in some way, related to the severity and complexity of the histological and clinical picture.

MATERIAL AND METHODS. We performed a retrospective clinical study on 49 patients diagnosed with chronic rhinosinusitis with or without nasal polyps and 3 controls. Patients' assessment was based on age range, clinical history, symptoms and their severity evaluation, presence or absence of allergy, nonsteroidal anti-inflammatory drugs (NSAIDs) - intolerance or other allergic manifestations, previous surgery, recurrences. Patients were evaluated for nasal obstruction, smell impairment, rhinorrhea, sneezing and headache, using a Visual Analogue Scale (VAS). Tissue samples from polyps or nasal mucosa were prelevated and histopathologically examined; the cellular HMGB1 distribution, nuclear, cytoplasmic and extracellular staining were also determined.

RESULTS. Analyzing the data we found a significant statistical correlation between nuclear HMGB1 and eosinophils infiltrate (p-value= 0.050) and between nuclear HMGB1 and inflammatory infiltrate (p-value = 0.050). More significant was the correlation between extracellular HMGB1 expression (p-value=0.0192) and the presence of allergic-hyperreactive conditions, such as asthma, allergic rhinitis, NSAIDs intolerance (p-value = 0.0006), antibiotic allergy, in comparison with non-allergic subjects. HMGB1 was found predominantly extracellular in patients with recurrent chronic rhinosinusitis (p-value=0.050). Patients with chronic rhinosinusitis presented increased nuclear HMGB1 levels of staining, in comparison to controls (p=0.0014).

CONCLUSION. Taking into consideration the inflammatory and anti-inflammatory mechanisms exposed in this paper, a higher extracellular expression of HMGB1 in patients with more severe clinical pathology was observed. The therapeutic research could address to finding new molecules able to reduce the extracellular levels of this protein by a scavenger mechanism. By decreasing the extracellular positivity, we can reduce the inflammation in chronic rhinosinusitis, without interfering with the nuclear transcriptional messengers; in the same time, new ways of blocking the HMGB1 feedback loop has to be found.

KEYWORDS: HMGB1 protein, upper airways inflammation, allergy, chronic rhinosinusitis, nasal polyposis

INTRODUCTION

Rhinosinusitis is an important chronic health problem, characterized by long-term inflammation of the nasal mucosa and paranasal sinuses[1]. Chronic rhinosinusitis (CRS) with or without nasal polyposis is a multifactorial disease with different elements (mechanical, viral, bacterial, fungal infection, immunological disorders, hyperreactivity, pollution) acting on the nasal cavities mucosa, that are able to determine epithelial damage and chronic inflammation.

Meltzer and colleagues divided patients into chronic rhinosinusitis with and without nasal polyps[2], suggesting the fact that patients without nasal polyps have mostly neutrophils as inflammatory cells. The most common histopathologic difference between CRS with and without nasal polyposis is the eosinophilic infiltrate which is mostly associated with nasal polyps.

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Inflammatory infiltrate (frequent lymphoid and monocytic elements), eosinophils presence, intramucosal edema, neoangiogenesis and fibrosis are the histological findings associated, from a clinical point of view, with nasal congestion, discharge and facial pain or headache[3]. In order to identify the mechanisms of chronic rhinosinusitis, there is a permanent interest for investigating other inflammatory components, besides the well-known processes.

The recent discovery of HMGB1, as a main protein in the pathogenesis of several inflammatory diseases, has stimulated the research in the field of chronic nasal inflammation. The final purpose of these studies is to identify new treatment methods for bacterial or viral infection, as well as other inflammatory pathologies of the upper airways[4].

HMGB1, also called amphoterin, is an ancient evolutionary protein with a DNA-binding protein role, expressed in the nucleus of almost all eukaryotic cells. However, unlike the more tightly bound histones, this protein is only loosely bound to chromatin[5]. In the same time, translational factors could play a role in maintaining nuclear homeostasis, as well as cytokine function[6].

HMGB1 is secreted in an active manner by monocytes or macrophages and it is released in a passive manner by necrotic or damaged cells. Due to its reduced ability to promote inflammation, HMGB1 has the property of signalling the necrosis of a cell to its neighbours[7]. Apoptotic cells do not release HMGB1 this protein having a strong bound with chromatin.

As HMGB1 is released into the extracellular milieu, it acts as a proinflammatory cytokine[8]. HMGB1 has a structure composed of three different domains: two homologous DNA-binding sequences (box A and box B) and a negatively charged C sequence. The B box domain represents the proinflammatory cytokine of the molecule, while the A box domain has a therapeutic function due to its anti-inflammatory effect[9].

As a cytokine, HMGB1 plays a central role in mediating the local and systemic responses to pathogenic microorganisms, traumas, necrotic cells, cancer or sepsis. There are two time- and dose-dependent mechanisms of extracellular release of HMGB: passive release by necrotic cells[8] or active release by immune activated cells[9]. After the interaction with its specific receptors, HMGB1 induces endothelial–cell activation (with increased expression of adhesion-molecules – VCAM-1, ICAM-1) and subsequent recruitment of inflammatory cells, increasing in the same time their survival[10-12]. HMGB1 is released in the extracellular milieu as a result of membrane integrity loss after necrosis of nucleated cells (including neutrophils) and after leukocytes activation.

HMGB1 is involved, due to its alarmin function, in the development of both acute and chronic inflammation, but also in the immune response activation. The levels of neutrophil-derived alarmins are high in many inflammatory conditions, the blockade of these mediators being able to reduce the manifestations of acute inflammatory reactions. There is increased evidence that HMGB1 contributes to the pathogenesis of chronic inflammatory and autoimmune disease, due to its pro-inflammatory and immune-stimulatory properties.

HMGB1 plays an immunostimulatory role, being able to promote inflammatory responses and to eliminate the causal agent. When the immunity response becomes too strong, even pathogenic, HMGB1 can determine tissue damage[13]. HMGB1 is also involved in several pathologies and diseases, such as sepsis[14], rheumatoid arthritis[15] or systemic lupus erithematosus[16]. HMGB1 overexpression can alter genes transcription which play a key role in neoplastic diseases[17].

As it has multiple effects on different organs and apparatuses (pleitropic), it is necessary to investigate this protein role in upper airways chronic inflammation pathogenesis. Recently, higher levels of HMGB1 were found in induced sputum from asthmatic and chronic obstructive pulmonary disease patients, in comparison with healthy controls[18,19]; the elevated levels can be correlated with asthma severity[20].

In previous researches, it was postulated a role of HMGB1 in the pathogenesis of chronic rhinosinusitis (CRS) with or without nasal polyposis (NP)[21] and it was found increased expression of the protein in the nucleus of nasal mucosa epithelial cells. Also, HMGB1 was overexpressed in the focal subepithelial infiltration and in the inflammatory cells of patients with CRS with NP in comparison with controls.

These results suggested us a possible pathogenic role of HMGB1 in CRS with NP[22]. The aims of the present study were:

1) To confirm the role of the new HMGB1cytokine in the pathogenesis of CRS with nasal polyposis;
2) To investigate if the expression and localization (nuclear, cytoplasmic and extracellular) of the HMGB1 protein-cytokine is, in some way, related to the severity and complexity of the histological and clinical aspect.

MATERIAL AND METHODS

14 biopsies of nasal mucosa from patients with CRS without NP and 35 from patients with CRS with NP (II degree according to Lund-McKay grading system[19]) were randomly selected from tissue archives of Department of Anatomopathology and ENT Department, "Sfanta Maria" Hospital, Bucharest, Romania. As controls, we prelevated biopsies from 3 healthy patients, with no symptoms of CRS and no diagnosed atopy.

Histopathologic examination of the normal tissue

Table 1
Histopathologic Score in Chronic Rhinosinusitis

Density and distribution of the inflammatory infiltrate	Histopathologic score
Rare, spread lymphoid and monocytic elements	1
Frequent, spread lymphoid elements	2
Frequent lymphoid elements, subepithelial +/- perivascular densification or aggregate presence	3
Eosinophils presence	**Histopathologic score**
Absence of eosinophils	0
Rare eosinophils	1
Frequent eosinophils	2
High frequency of eosinophils > 30/40X	3

samples revealed the absence of eosinophils, lymphoid or monocytic cells.

We included in our study patients diagnosed with CRS after clinical and paraclinical evaluation (CT scans, nasal endoscopy, histopathological confirmation).

Study exclusion criteria were: hematologic diseases, pregnancy.

Patients' assessment was based on age range, clinical history, symptoms and their severity evaluation, presence or absence of allergy, NSAIDs intolerance or other allergic manifestations, previous surgery, as well as recurrences.

Patients were evaluated for nasal obstruction, smell impairment, rhinorrhea, sneezing and headache, using a Visual Analogue Scale (VAS), graded from 0 to 10, where 0 was absence of symptom and 10 the highest intensity.

The tissue samples were fixed with 10% neutral formalin, paraffin embedded. Histopathologic re-examination and immunohistochemistry tests were performed according to the standard protocol by two pathologists at "Victor Babes" National Institute of Pathology from Bucharest.

Immunohistochemistry tests (IHC) were done by using polyclonal antibody HMGB1 (ab18256) 1:1000 (Abcam, USA). Heat mediated antigen retrieval step was performed using citrate buffer pH 6.2. Samples were then blocked and incubated with HMGB1 polyclonal antibody (ab18256) at a 1:1000 dilution for 1 hour.

To determine the cellular distribution of HMGB1 (nuclear, cytoplasmic and extracellular) staining was estimated as a percentage of the total staining in the examined area, with the summarized total staining in the area equal to 100%. This scoring was done manually by 2 observers, in a blinded manner; the results were expressed as the mean values of the evaluations from the 2 observers. We classified the intensity of HMGB1 positivity, according to the distribution percentage.

The IHC method was an indirect, bistadial technique performed with a polymer based detection system (EnVision FLEX System-HRP, Dako, Carpinteria, CA, code K8010) according to the manufacturer's instructions.

All specimens were counterstained with Meyer's Hematoxylin, examined and photographed with a Nikon Eclipse 600 microscope.

To ensure the reliability of the experimental study, internal quality control of IHC techniques was performed.

Also, we performed an evaluation of the histopathologic score[3], according to the staging method described in Table 1, staging that we recently proposed and introduced as quantitative measuring method of the upper airways inflammation.

Statistical analysis

Statistical analysis was performed by using the statistical package SPSS version 15.0. A Student *t* or a Mann-Whitney test was used for comparison of two different groups and an ANOVA test or Kruskall-Wallis test for more than two groups. Kendall's tau-b correlation was used to assess correlation. A p-value < 0.05 was considered statistically significant. Data are presented as mean ± SD and median.

Kendall tau-b correlation coefficient interpretation is the following: if the agreement between the two rankings is perfect and the two rankings are the same, the coefficient has value 1. If the disagreement between the two rankings is perfect and one ranking is the reverse of the other, the coefficient value is -1. For all other arrangements the value lies between -1 and 1, and increasing values imply increasing agreement between the rankings. If the rankings are independent, the coefficient value is 0.

In the present study we made a correlation between the histological classification (taking into consideration the degree of density and distribution of the in-

Table 2
Distribution of patients according to allergic comorbidities

Allergy Type	Patients	
	No.	%
Asthma	5	10.20%
Allergic Rhinitis (AR)	3	6.12%
NSAID Intolerance	4	8.16%
Asthma + Allergic Rhinitis	3	6.12%
Asthma + NSAID Intolerance	3	6.12%
Asthma + Allergic Rhinitis + NSAID Intolerance	2	4.08%
Asthma + Antibiotic Allergy	2	4.08%
Asthma + NSAID Intolerance + Antibiotic Allergy	2	4.08%
Allergic Rhinitis + Antibiotic Allergy	1	2.04%
Allergic Rhinitis + NSAID Intolerance	1	2.04%
No Allergy	23	46.96%

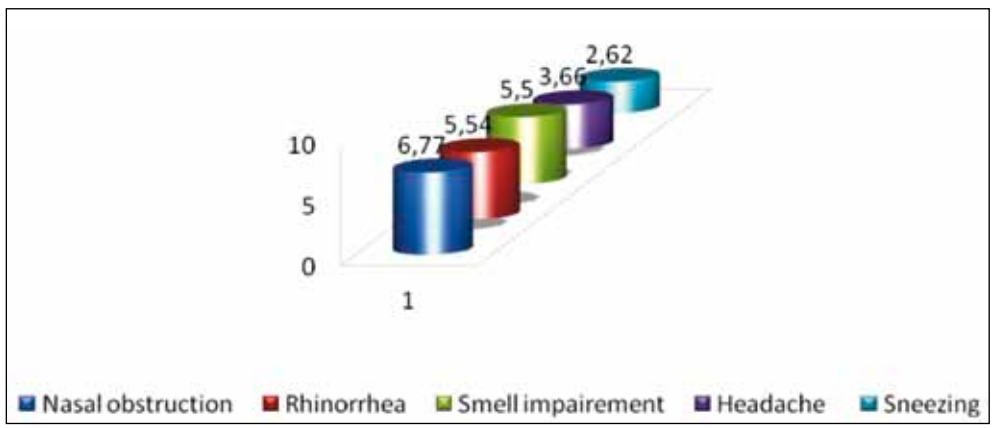

Chart 1 Average VAS score for the main symptoms in chronic rhinosinusitis

flammatory infiltrate and eosinophils presence) and the HMGB1 protein positivity and localization.

RESULTS

Patients included in our study (n=49) were aged between 15 and 77 years, mean age for the entire study group being 46.30 years (male mean age = 45.39 years; female mean age = 47 years). According to our results, men (31 patients, 63.26%) were more affected by this disease, comparing to women (18 patients, 36.73%) (male:female=1.72).

Regarding the potential risk factors, 28 of the patients were smokers (57.14%), with 24 male and 4 female.

In order to identify the importance of allergic conditions, patients were evaluated for asthma, allergic rhinitis and drug allergy - 15 patients had asthma (30.61%), 9 had allergic rhinitis (18.36%; 5 males, 4 females) with positive prick skin tests (the most frequent aeroallergens were dust, pollens, dust mites and moulds) and 14 patients (28.57%) had drug allergy – 10 patients with NSAIDs intolerance and 4 with antibiotic allergy (Table 2).

Symptom score

Main presenting symptoms (Chart 1) for the patients evaluated by Visual Analogue Scale were, in order: nasal obstruction, nasal discharge, smell disorders (hyposmia, anosmia), headache (predominantly located in the frontal region) and sneezing (with elevated scores found in allergic patients).

Regarding "nasal obstruction" parameter most patients had a score between 6 and 9 (35 patients), with a mean subjective score of 6.77. According to our results, nasal obstruction was the most annoying symptom in chronic rhinosinusitis with/without NP.

Mean VAS score for rhinorrhea was 5.54, none of the patients relating a score above 8.

Smell disorders represented the third most im-

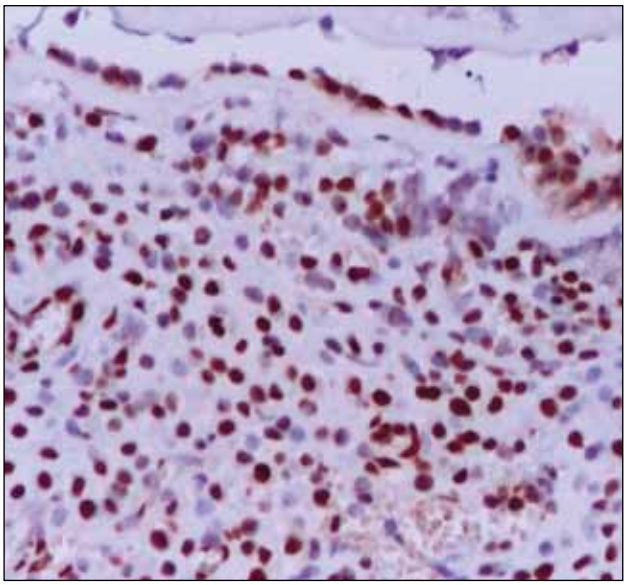

Figure 1 Citokine expression evaluation: up to 95% positive for inflammatory cells and 60% positive for endothelial cells.

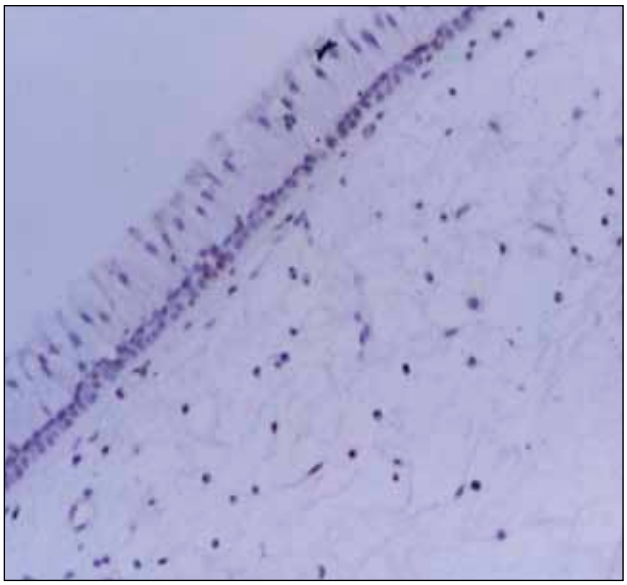

Figure 2 Normal nasal and sinus mucosa with weak positive, scattered cells

portant symptom, after nasal obstruction, with a 5.5 mean score.

Mean score for "headache" parameter was 3.66, with the most frequent localization in the frontal region.

For sneezing the mean score was 2.62, the higher score being found in a 65-year-old male patient diagnosed with both allergic rhinitis and asthma – 7.8.

Immunohistochemistry evaluation

The stained slides were evaluated independently by 2 individuals in a blind manner using a semi quantitative method. The entire section of the biopsy samples was analyzed in 2 different ways:

1) To evaluate the cytokine expression, the amount of positively stained cells was scored on masked sections by the 2 independent observers. Sinuses tissue samples (n=14) had between 30 and 90% positive cells - epithelial, inflammatory and endothelial cells (Figure 1).

Nasal polyps from 35 biopsy samples presented between 20 and 95% cells, according to the density of the inflammatory infiltrate (Figure 2).

The average score of density and distribution of the inflammatory infiltrate was 2.51 (29 scores of 3, 16 scores of 2 and 4 scores of 1).

When analyzing the patients with a score of 3 (frequent lymphoid elements, subepithelial +/- perivascular densification or aggregate presence), we found 4 patients with asthma, 3 patients with asthma and allergic rhinitis, 1 patient with allergic rhinitis and 7 patients

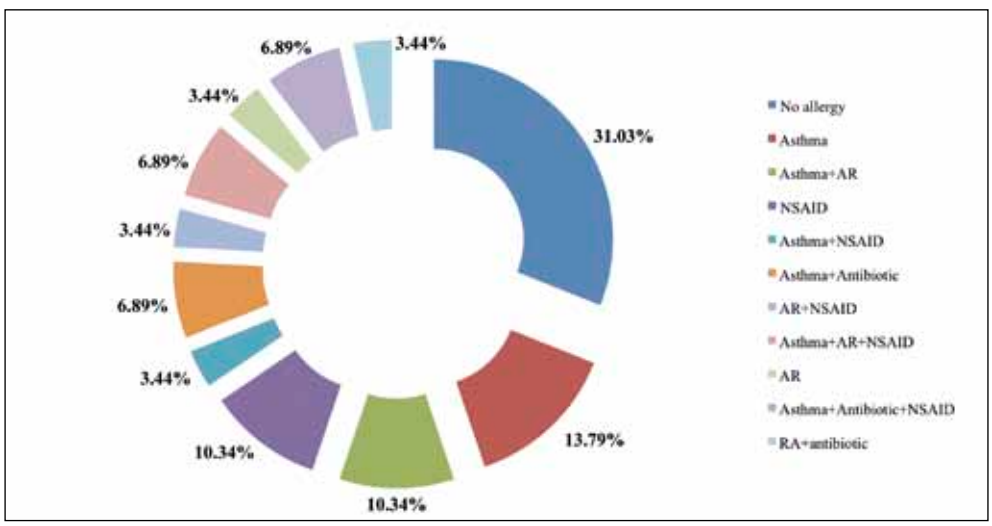

Chart 2 Distribution according to allergic conditions of patients with high inflammatory infiltrate (score 3)

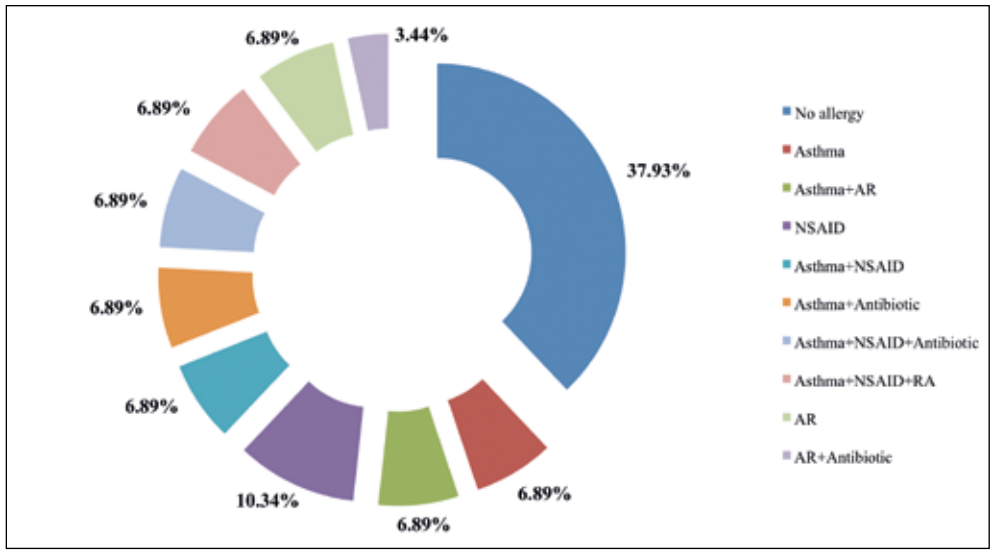

Chart 3 Distribution according to allergic conditions of patients with high frequency of eosinophils (score 3)

with NSAIDs intolerance (1 associated asthma, 1 allergic rhinitis, 2 both diseases and 3 were diagnosed only with NSAIDs intolerance). In 5 patients included in this group we identified an antibiotic allergy - 2 of them associated asthma, 2 asthma and NSAIDs and 1 allergic rhinitis (Chart 2). 9 patients were non-atopic.

As the results of this study show, patients with a high inflammatory infiltrate and eosinophils presence can have an allergic condition, which may induce and sustain nasal mucosa inflammation. It is known that chronic rhinosinusitis and atopy often coexist and tend to have common symptoms and also to worsen the clinical and histopathological picture of these patients.

Among patients with histopathologic score of 2 (16 patients) we found: 2 patients with allergic rhinitis; 1 patient with NSAIDs intolerance; 2 patients with asthma and NSAIDs intolerance; 1 patient with asthma, NSAIDs intolerance and antibiotic allergy. In 10 patients, no allergic conditions were found.

4 patients scored 1: in 3 of them no atopy was identified and 1 was diagnosed with asthma.

The mean score for eosinophils presence was=2.35: 29 patients scored 3, 9 scored 2 and 11 scored 1.

Among patients scored 3 for eosinophils' presence (high frequency) 2 patients were reported with allergic rhinitis, 2 with asthma, 3 patients with NSAIDs intolerance, 2 patients with asthma and allergic rhinitis, 2 with asthma and NSAIDs intolerance, and 2 patients associated asthma with allergic rhinitis and NSAIDs intolerance (Chart 3). In 5 patients an antibiotic allergy was identified - 2 of them associated an allergic rhinitis, 2 patients asthma and 2 asthma and NSAIDs intolerance. In 13 patients no allergic conditions were found.

In 9 patients scoring 2, considering eosinophil infiltration, the following findings were reported: 1 patient was diagnosed with allergic rhinitis, 2 with asthma, 3 with NSAIDs intolerance (in 1 patient it was associated with asthma and in 1 patient with allergic rhinitis) and 3 with no allergic conditions.

11 patients scored 1: in 9 of them no allergic conditions were found, asthma was reported in 1 patient, whereas a patient was diagnosed with both asthma and allergic rhinitis.

2) To determine the cellular distribution of nuclear, cytoplasmic and extracellular **HMGB1**, staining was estimated as a percentage of the total staining in the examined area, with the summarized total staining in the area equal to 100%.

This scoring was done manually by the 2 observers in a blinded manner. The results were expressed as the mean values of the evaluations from the 2 observers. Both evaluators reported an almost diffuse nuclear positivity of epithelial, inflammatory and endothelial cells

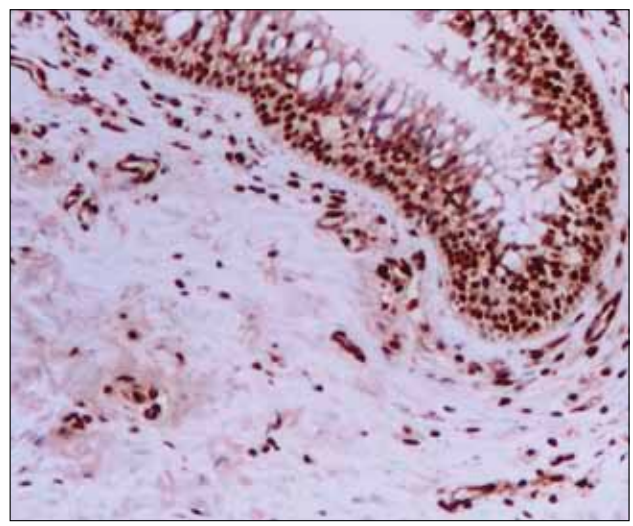

Figure 3 IHC positive nasal polyps – 95% positive cells (4x)

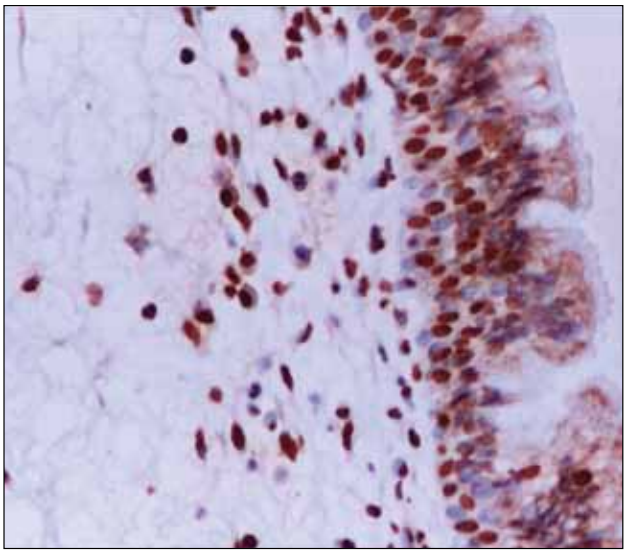

Figure 4 Nasal polyp with basal cell hyperplasia and IHC staining for HMGB1 - 90% nuclear positive cells and 10% positive cytoplasmatic cells

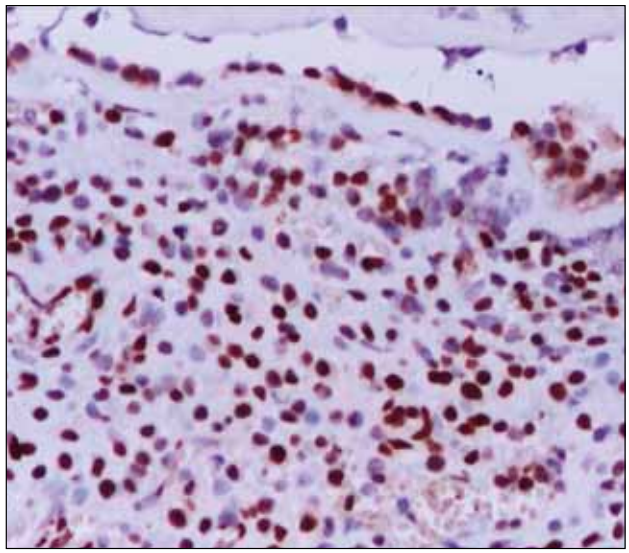

Figure 5 Inflammatory cells staining: up to 95% positive for inflammatory cells and 60% positive for endothelial cells.

within a range of 20 up to 95% (Figure 3). There were no significant differences between the 2 observers.

In case of normal pseudostratification preservation a basal nuclear positivity of respiratory epithelium (90%) and focal cytoplasmatic staining (10%) were described (Figure 4). In those samples with epithelium squamous metaplasia observed at the histopathologic examination (6 out of 49, with 80% positivity), the nuclear positivity of the epithelial cells was up to the surface.

The staining for inflammatory cells was diffusely distributed with nuclear positivity of 95%. A degree up to 60% positivity in the nucleus of endothelial cells was also expressed (Figure 5) and the background staining, significant for the extracellular distribution, was almost absent.

Normal nasal mucosa samples had few (less than 10%), isolated positive cells (epithelial and endothelial). There was a nuclear HMGB1 positivity between 20 and 30% among control patients. It is important to note the absence of HMGB1 from other location sites, comparing with biopsies prelevated from nasal mucosa or nasal polyps in patients with chronic rhinosinusitis.

In our study group, 17 patients had recurrences (between 1 and 11) correlated with high cytoplasmic HMGB1 positivity (60-95%, p-value=0.027); 5 patients had extracellular HMGB1 positivity (between 60 and 95%), with a relatively strong correlation between this location and recurrences (p-value=0.050).

Correlations between HMGB1 expression, location sites and histopathologic score

We investigated the correlation between histopathologic score (presence of eosinophils, inflammatory infiltrate) and HMGB1 location sites (nuclear, cytoplasmatic, extracellular) using Kendall's tau-b correlation and mean ± SD, median (Table 3).

Analyzing the data from the total group of patients,

Table 3
HMGB1 and Histopathologic Score Correlation

HMGB1	Eosinophils				Inflammatory Infiltrate		
	0	1	2	3	1	2	3
Nuclear	25.00±5.00* 25.00**	83.75±20.35 90.00	88.75±3.53 90.0	79.14±20.31 90.0	87.50±5.00 90.00	86.87±10.14 90.00	78.27±22.60 90.0
Cytoplasmatic	0 0	21.81±36.82 0	16.25±30.67 5.00	21.81±36.82 0	1.66±43.89 0	20.57±29.67 0	20.57±29.67 5.00
Extracellular	0 0	16.25±32.96 0	21.87±40.70 0	15.20±31.64 0	20.00±40.00 0	1.66±6.45 0	24.80±36.63 0

**M±SD, **Median*

we didn't observe a significant statistical correlation between eosinophils and the percent of positive nuclear HMGB1 (Kendall = -0.171), cytoplasmatic HMGB1 (Kendall = 0.032), extracellular HMGB1 (Kendall = 0.031); the same results in case of inflammatory infiltrate and nuclear HMGB1 (Kendall = -0.218), cytoplasmatic HMGB1 (Kendall = 0.119) and extracellular HMGB1 (Kendall = 0.228).

Nuclear HMGB1 positivity percent has lower values in case of spread lymphoid and monocytic elements – score 1 (mean = 87.50, median = 90), comparing to frequent, spread lymphoid elements – score 2 (mean = 86.75, median = 90) or frequent lymphoid elements, subepithelial +/- perivascular densification or aggregate presence – score 3 (mean = 78.27, median = 90.00).

Therefore, investigating the correlation between eosinophils presence and nuclear HMGB1 values, we noticed decreased nuclear levels in patients with eosinophils absence – score 0 (mean = 83.75, median = 90.00) and increased levels in patients with higher eosinophils scores (2 or 3).

We also investigated if there can be an association between histopathologic scores (eosinophils, inflammatory infiltrate) and HMGB1 positivity percent using ANOVA/Kruskall-Wallis tests. Analyzing the data we found a statistical significant association between nuclear HMGB1 and eosinophils (p-value = 0.050); also between nuclear HMGB1 and inflammatory infiltrate (p-value = 0.050).

Allergic-hyperactive conditions (asthma, allergic rhinitis, NSAIDs intolerance, antibiotic allergy) influence was additionally assessed on HMGB1 levels. Due to HMGB1 involvement in inflammation pathology, we decided to investigate both allergic and non-allergic patients. We found statistical significant correlation between extracellular HMGB1 and allergic comorbidities (p-vlue=0.0192). In the same time, our results revealed a statistically significant association between extracellular HMGB1 and NSAIDs intolerance (p-value = 0.0006); also between antibiotic allergies and nuclear HMGB1 (p-value=0.0432).

Patients with chronic rhinosinusitis presented increased nuclear HMGB1 levels of staining, in comparison to controls (p-value=0.0014).

DISCUSSIONS

The first European Position Paper on Rhinosinusitis and Nasal Polyps (EP3OS) published in 2004, as well as the following updates from 2007 and 2012, defined and classified the inflammatory pathologies of the nose and the paranasal sinuses based on strict criteria, according to the scientific information available in literature. EPOS 2012 made a precise differentiation between CRS with and CRS without nasal polyps[23,24]. Despite the fact that clinical aspects, inflammatory processes and treatment effects often superpose in CRS with and without nasal polyps, there are some differences regarding their inflammatory profile and treatment response.

The prevalence and the incidence of chronic rhinosinusitis with or without nasal polyposis, continues to be high, affecting approximately 10% of the general population, both in Europe and in USA, with a prevalence variability range between 6.9 and 27.1%[25]. CRS onset mean age is almost 42 years, 7 years later than the average age for asthma onset.

From the etiopathogenic point of view, chronic sinonasal inflammatory pathologies can be considered multifactorial diseases: anatomic variations of the nasal fossa (nasal septum deviations, concha bullosa, paradoxal bending of the middle turbinate, etc.), viral and bacterial infections, systemic diseases that affect the mucociliary clearance (cystic fibrosis, primary ciliary dyskinesia, etc.), immunodeficiency system or hyperreactivity (allergies or presence of fungal antigens). This pathogenic hypothesis is explained and sustained by the Evidence Based Medicine and it can prove the primary or the secondary role of some associated comorbidities in inflammatory processes.

Chronic rhinosinusitis can be described as a dysfunctional host-environment interaction at the site of interface of the nose and paranasal sinuses mucosa. Therefore, a successful therapeutic approach should address to the modulation of these inflammatory processes.

In the last decades, for better understanding the immunological mechanisms, there have been studied molecular patterns recognized by the immune system of the superior organisms which have been classified as "Danger-Associated Molecular Patterns" (DAMPs). From DAMPs, the Pathogen-Associated Molecular Patterns (PAMPs) and the so called "alarmins" should be highlighted. The PAMP2 are molecules expressed only by pathogens agents, as bacteria or viruses, while the alarmins are released from the damaged and necrotic cells of the host.

The HMGB1 protein is an alarmin which acts by interacting with specific membrane receptors (RAGE, TLR2 and TLR4) from the surface of macrophages and monocytic elements, and by activating intra-cellular mechanism of signalling (for example the transcriptional factor NF-kB). NF-KB has a major function in the inflammatory processes[26] being essential for the transcription of cytokines, chemokines and adhesion molecules.

Glucocorticoisteroids are currently considered the first line therapy in the chronic inflammatory diseases of the nose and paranasal sinuses. They interact directly with NF-kB blocking its binding to DNA, the result being the inhibition of transcriptional activity of the inflammatory mediators[27].

Recently, a substance called glycyrrhizin, found in the roots of "*Glycyrrhiza glabra*" and used in the food industry as sweetener, was associated with the ability to bind to HMGB1[28]. The studies performed on an experimental model on mice with viral hepatitis sustained the role of glycyrrhizin in reducing liver inflammation by reducing HMGB1 pro-inflammatory effect[29,30].

Further studies need to be done in order to evaluate the efficacy of glycyrrhizin in treating inflammatory diseases of the upper and lower respiratory tract[27]. Increased quality of life is a final purpose for searching new therapeutic perspectives in chronic rhinosinusitis with or without nasal polyposis.

CONCLUSIONS

Chronic rhinosinusitis with or without nasal polyps is an important health problem, characterized by the persistent inflammation of the nasal and the paranasal sinuses mucosa. As the results show, it mostly affects males, with a ratio of 1.72, the medium age of appearance being the fourth decade for both men and women. Smoking was proposed as a risk factor in appearance of chronic rhinosinusitis, hypothesis also confirmed by our study (57.14% were smokers).

The prevalence of positive skin prick tests in patients with CRS varies from 50 to 84%, most of them (60%) having multiple sensitization. Epidemiologic studies show a high prevalence of allergic rhinitis among patients diagnosed with CRS, but the role of allergy in CRS still remains unclear. Nose allergic inflammation predisposes the atopic individual to the development of CRS, both conditions sharing the same increasing prevalence and being frequently associated.

High inflammatory infiltrate of lymphoid, eosinophils and monocytic elements is associated with the presence of different allergic comorbidities, that may induce and sustain chronic nasal inflammatory response.

It seems that HMGB1 has an elevated extracellular distribution in patients with severe clinical and inflammatory disease and in those with associated comorbidities. Analyzing HMGB1 distribution in control patients, they presented a lower nuclear HMGB1 positivity (20-30%), even being absent in some location sites, comparing to the patients with chronic rhinosinusitis.

As HMGB1 might be a marker of certain disease activity, we advice that both allergic and non-allergic patients should be investigated. We found a significant increase in extracellular and nuclear HMGB1 distribution in allergic patients. If we take into consideration the inflammatory and anti-inflammatory mechanisms exposed in our paper, a higher extracellular expression of HMGB1 in patients with more severe clinical pathology was observed.

In conclusion, we think that any therapeutic research should address to finding new molecules able to reduce the extracellular HMGB1 levels by a scavenger mechanism. By decreasing the extracellular positivity, we can reduce the inflammation in chronic rhinosinusitis, without interfering with the nuclear transcriptional messengers; in the same time, new ways of blocking the HMGB1 feedback loop has to be found.

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