



Blood eosinophil-to-basophil ratio in patients with sinonasal polyps Does it have a clinical role?

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ABSTRACT

Background: In a recent preliminary study, eosinophil and basophil counts were calculated in chronic rhinosinusitis with nasal polyps (CRSwNP) using conventional histologic and immunohistochemical methods. The tissue eosinophil-to-basophil ratio differed in the CRSwNP endotypes considered.

Objective: To compare the blood eosinophil-to-basophil ratio (bEBR) in a large series of patients with CRSwNP with that in a control group of consecutive rhinological patients with no evidence of nasal, paranasal, or systemic inflammatory disorders.

Methods: A retrospective study was performed on 334 patients with CRSwNP to compare the preoperative bEBR among different endotypes and with controls (69 cases).

Results: The mean bEBR was significantly higher in the CRSwNP group than in the control group ($P = .0006$). The eosinophil and basophil counts were significantly and directly correlated in the CRSwNP cases ($P = .0000$). The mean bEBR was significantly higher in the sub-cohorts of CRSwNP with allergy ($P = .0007$), asthma ($P = .0000$), and aspirin-exacerbated respiratory disease ($P = .0153$). The mean bEBR was significantly higher in the sub-cohort with eosinophilic CRSwNP than in the sub-cohort with noneosinophilic CRSwNP ($P = .0000$).

Conclusion: This study confirms the increasingly interesting role emerging for blood eosinophils and basophils in different CRSwNP endotypes. The bEBR seems to be a parameter worth investigating in different CRSwNP endotypes, because it is significantly higher in patients with allergy, asthma, and aspirin-exacerbated respiratory disease.

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Introduction

A better understanding of the diverse pathophysiologic mechanisms behind chronic rhinosinusitis has led to the identification of disease variants as different endotypes.¹ Current attempts to endotype chronic rhinosinusitis with nasal polyps (CRSwNP) based on distinctive pathophysiologic pathways have increased the precision of diagnostic descriptors for CRSwNP.^{2,3}

Taking an endotype-based approach, Mahdavinia et al⁴ used conventional histologic methods and immunohistochemistry to obtain eosinophil and basophil counts in chronic rhinosinusitis with and without nasal polyps. Basophils and eosinophils use similar recruitment pathways and might be expected to be recruited at an inflammatory site in parallel, but Mahdavinia et al⁴

found mean eosinophil-to-basophil ratios of 3.0 in normal unciliated tissue, 13.1 in chronic rhinosinusitis without nasal polyps, and 49.0 in CRSwNP. The eosinophil-to-basophil ratio was highest in nasal polyps, with a mean of 89.2 in polyps unassociated with aspirin-exacerbated respiratory disease (AERD) and a mean of 287.3 in polyps associated with AERD.

The emerging role of blood eosinophils and basophils in different CRSwNP endotypes is attracting increasing interest. When investigating a series of 280 patients who underwent surgery for CRSwNP, Brescia et al⁵ found significantly higher mean preoperative blood eosinophil and basophil levels in patients whose CRSwNP recurred than in those who remained free of disease after treatment.

The aim of the present study was to compare the blood eosinophil-to-basophil ratio (bEBR) in a large series of patients with CRSwNP ($n = 334$) with that in a control group of consecutive rhinological patients with no evidence of nasal, paranasal, or systemic inflammatory disorders. Appropriate statistical methods

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were used to assess the relation between the bEBR and patients' demographic details and CRSwNP endotypes, comorbidities, and histopathologic features.

Methods

Patients

Study group

The study was approved by the otolaryngology section's in-house committee and conducted in accordance with the principles of the Declaration of Helsinki. All patients signed a detailed informed consent form before undergoing surgery.

All patients older than 18 years with nasal polyps were treated medically according to the European Position Paper on Rhinosinusitis and Nasal Polyps guidelines⁶ with oral and nasal steroids. Patients with endoscopic evidence of polyps protruding below the middle turbinate (grade 2) or massive polyposis (grade 3) according to Mackay and Lund⁷ underwent endoscopic sinus surgery (ESS) under general anesthesia, with septoplasty and turbinoplasty as necessary. Patients with polyps restricted to the middle meatus (grade 1) were not treated surgically and excluded from the present study. Other exclusion criteria were a postoperative follow-up shorter than 12 months or a diagnosis of eosinophilic granulomatosis with polyangiitis. Patients with inflammatory, autoimmune, acute, or chronic infectious diseases other than sinusitis or with malignancies, hematologic disorders, a history of systemic corticosteroid therapy, or chronic renal insufficiency also were excluded. The final study sample included 334 patients (mean age 49.5 ± 14.7 years; 213 men, 121 women) with CRSwNP who underwent ESS.

All laboratory tests were performed before surgery, at least 3 months after withdrawing oral steroids, and at least 1 month after stopping nasal steroid treatment. Patients had a blood sample taken to obtain their eosinophil and basophil counts, and their bEBR was calculated. Serum total and specific immunoglobulin E (IgE) for *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, birch pollen, pellitory, grass mix, cat and dog dander, *Alternaria alternata*, *Aspergillus fumigatus*, and common ragweed also were determined. At histopathology, 3 high-power fields (magnification $\times 400$) from each surgical specimen were examined to quantify the eosinophil component and distinguish between 2 patterns of polyposis, one containing at least 10 eosinophils per field (eosinophilic) and the other containing fewer than 10 eosinophils per field (noneosinophilic). Then, all cases of CRSwNP were classified as eosinophilic or noneosinophilic. Postoperatively, patients underwent nasal irrigations of isotonic saline solution twice a day (20 mL per irrigation) and local nasal therapy with 200 $\mu\text{g/d}$ of mometasone furoate (100 μg per nostril)⁸ or 110 $\mu\text{g/d}$ of fluticasone furoate (55 μg per nostril). The patient with eosinophilic-type sinonasal polyposis associated with asthma also received 2 cycles a year of oral steroids⁹ with methylprednisolone (32 mg/d on days 1–5, then 16 mg/d on days 6–10, and then 8 mg/d on days 11–20). During periods of pollination, patients with a known pollen allergy were treated with antihistamines. Patients with asthma received appropriate therapy.

Endoscopic follow-ups after ESS were performed using rigid 0° and 30° (4-mm-diameter) endoscopes and were scheduled 3, 6, and 12 months after surgery and yearly thereafter. Patients with endoscopic evidence of at least grade 1 polyposis were classified as having recurrent CRSwNP.

Control group

The control group consisted of 69 consecutive patients (mean age 41.4 ± 14.2 years; 56 men, 13 women) with no chronic rhinosinusitis and no nasal or systemic inflammatory diseases who underwent septoplasty and/or turbinoplasty and whose eosinophil

and basophil counts had been ascertained in a preoperative blood sample. The bEBR was calculated for each patient in the control group.

All assays for the 2 groups (CRSwNP cases and controls) were performed at the same laboratory (EIA Unit, Laboratory Medicine Service, Padova General Hospital, Padova, Italy; certified in accordance with ISO 15189).

Statistical Analysis

The statistical significance of the differences between the mean bEBR findings was ascertained using Student *t* test with Welch correction for unequal population variances. The odds ratios for CRSwNP recurrence (1/0) vs variables (blood eosinophil and basophil counts) with 95% confidence intervals also were calculated. Where appropriate, Spearman rank correlation also was applied.

The statistical significance of the *P* value was set at less than .05. The STATA 8 statistical package (StataCorp LP, College Station, Texas) was used for all analyses.

Results

Clinical Features of Study Group

Table 1 lists details of the distribution of CRSwNP cases for associated allergy, asthma, and AERD (CRSwNP and asthma with intolerance to medications that inhibit the cyclooxygenase-1 enzyme).

A final histologic diagnosis of eosinophilic CRSwNP was established for 156 patients. Recurrent CRSwNP was diagnosed endoscopically in 58 of 334 cases.

bEBR in Study vs Control Group

Student *t* test showed that the mean bEBR was significantly higher in the CRSwNP group than in the control group (11.8 ± 10.2 vs 7.3 ± 6.5 , *P* = .0006; Fig 1).

Association Between bEBR and Clinical, Pathologic, and Prognostic Variables in CRSwNP Group

When analyzing the series of CRSwNP as a whole, the eosinophil and basophil counts were significantly and directly correlated (Spearman rank correlation test, $\rho = 0.40$, *P* = .0000). For the associated clinical features, Student *t* test disclosed that the mean bEBR was significantly higher in the sub-cohorts of patients with allergy (*P* = .0007), asthma (*P* = .0000), and AERD (*P* = .0153; see Table 1 for details). Fourteen patients with AERD also had allergy and 9 patients with AERD did not have allergy; Student *t* test ruled out any significant difference in mean bEBR between these 2 sub-cohorts (16.4 ± 12.2 vs 17.2 ± 11.5 , *P* = .88).

Table 1

Laboratory Data for Entire Sample of Patients With Chronic Rhinosinusitis With Nasal Polyps (n = 334) Stratified by Clinical, Pathologic, and Prognostic Features

Sub-cohorts	Cases, n	bEBR, mean $\pm$ SD	<i>P</i> value ^a
Allergy	110	14.5 ± 12.3	.0007
No allergy	224	10.5 ± 8.7	
Asthma	85	15.7 ± 12.5	.0000
No asthma	248	10.4 ± 8.9	
AERD	23	16.8 ± 12.0	.0153
No AERD	311	11.4 ± 10.0	
Eosinophilic histology	156	15.2 ± 12.4	.0000
Noneosinophilic histology	178	8.8 ± 6.5	
With disease recurrence	58	13.4 ± 11.8	.1844
Without disease recurrence	276	11.4 ± 9.8	

Abbreviations: AERD, aspirin-exacerbated respiratory disease; bEBR, blood eosinophil-to-basophil ratio.

^aBy Student *t* test with Welch correction.

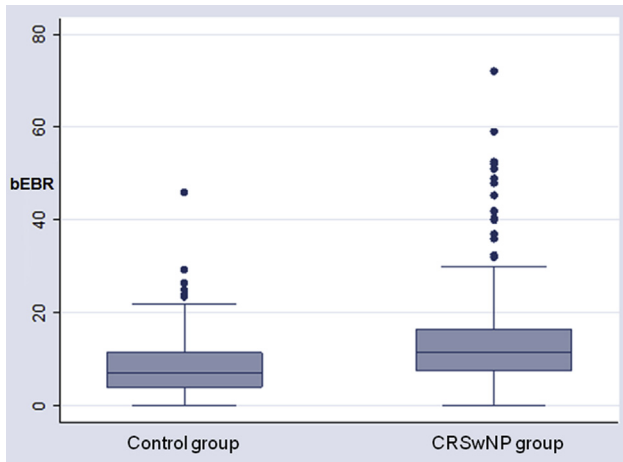


Figure 1. Boxplot of the blood eosinophil-to-basophil ratio (bEBR) in cases of chronic rhinosinusitis with nasal polyps (CRSwNP) and controls.

At histology, the mean bEBR was significantly higher in the sub-cohort of eosinophilic CRSwNP cases than in the sub-cohort of noneosinophilic cases ($P = .0000$ by Student *t* test).

Student *t* test showed that the mean eosinophil (0.47 ± 0.51 vs 0.32 ± 0.31 cells $\times 10^9/L$) and basophil (0.04 ± 0.02 vs 0.03 ± 0.02 cells $\times 10^9/L$) counts were significantly higher in the sub-cohort with recurrent CRSwNP than in the sub-cohort without disease after surgery ($P = .03$ and $P = .03$, respectively). However, statistical analysis ruled out any significant difference in mean bEBR between the 2 sub-cohorts ($P = .1844$ by Student *t* test).

Discussion

A limited number of studies have investigated the role of blood sampling in CRSwNP.¹⁰ High levels of eosinophils in peripheral blood have been investigated as a potential predictor of the histologic diagnosis of eosinophilic CRSwNP.^{11,12} Zadeh et al¹³ conducted a retrospective chart review of 620 patients who had undergone ESS for chronic rhinosinusitis and concluded that patients with blood eosinophilia had a worse prognosis. A direct association between CRSwNP recurrence rates and blood hypereosinophilia also was reported more recently by Ikeda et al¹⁴ and by Ottaviano et al.¹⁵ In the Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis of 1,716 patients (including chronic rhinosinusitis with and without nasal polyps) treated with ESS, the investigators concluded that patients with chronic rhinosinusitis who had more than 10% eosinophils in their peripheral blood had a significantly higher disease recurrence rate than patients with smaller eosinophil counts.¹⁶

There is definitely an emerging interest in investigating the pathophysiologic role of basophils in CRSwNP. Basophils are the rarest granulocytes but recent studies have demonstrated that they have a crucial, nonredundant role in the immune system. Basophils release interleukin (IL)-4 in an IgE-dependent and an IgE-independent manner in response to different stimuli. It is widely accepted that IL-4 plays a major part in promoting the differentiation of naïve CD4⁺ T cells into T-helper type 2 cells. Basophil-derived IL-4 enhances the expression of IL-5, IL-13, IL-33, and interferon- γ in type 2 innate lymphoid cells, leading to the accumulation of eosinophils.^{2,4,5} A recent study of ours found significant direct associations between recurrent CRSwNP and blood basophil counts and percentages.¹⁷ In a larger retrospective series (280 cases), our group recently calculated that the sub-cohort of patients with CRSwNP and preoperative blood basophil counts of at least 0.03 cells $\times 10^9/L$ and percentages of at least 0.5% had odds

ratios for recurrence of 2.19 and 2.24, respectively.⁵ In the same investigation, blood eosinophils and basophils (counts and percentages) were found to be significantly and directly correlated in the sample as a whole.

The present study is the first to have examined the role of bEBR in a large series of patients with CRSwNP and to have investigated how it differed in various different sub-cohorts of patients with CRSwNP stratified by their clinical, histologic, and prognostic features. This approach is consistent with emerging evidence of CRSwNP being remarkably heterogeneous in its biology and behavior. The main strengths of this study are the homogeneity of the series of patients for the following aspects: (1) the same team of surgeons decided whether ESS was indicated; (2) the histologic diagnosis was established by the same pathologist; (3) the same team conducted the endoscopic follow-up for at least 1 year; (4) recurrent CRSwNP was always confirmed endoscopically; and—probably most importantly—(5) all laboratory tests were performed at the same laboratory. The bEBRs in the CRSwNP cohort were compared with those in a control group of 69 consecutive rhinological patients with no evidence of nasal, paranasal, or systemic inflammatory disorders. The main weakness of our study concerns its retrospective setting.

A significantly higher mean bEBR emerged in our group of 334 cases of CRSwNP than in the control group. After analyzing the cases of CRSwNP by endotype-based sub-cohorts, the mean bEBR was significantly higher in patients with allergy, asthma, and AERD. We hypothesize that the difference in bEBR between patients with and without these associated conditions is due to a disproportionate increase in blood eosinophils vis-à-vis the basophil count.

It has been reported that eosinophilic and noneosinophilic polyps are histopathologically 2 entities, with distinct genetic and protein expression patterns.¹⁸ After stratifying the CRSwNP cases by polyp histopathology, the mean bEBR was significantly higher in the eosinophilic than in the noneosinophilic type. Evidence of a high eosinophil count in polyp tissue associated with a high bEBR supports the hypothesis that an eosinophilic CRSwNP histology is part of a systemic phenomenon and not merely the expression of local inflammation.

Focusing on prognosis, in our sample of CRSwNP cases (1) the mean eosinophil and basophil counts were significantly higher in the sub-cohort of patients whose disease recurred after surgery and (2) the blood eosinophil and basophil counts (the numerator and denominator, respectively, in the bEBR) were strongly and directly correlated. These 2 features can reasonably explain why the mean bEBR in patients whose CRSwNP recurred after ESS was not significantly higher than the mean bEBR in patients who experienced no recurrences. It is noteworthy that the odds ratio for disease recurrence was 1.19 (95% confidence interval 1.04–1.36) for the basophil count and 1.02 (95% confidence interval 1.01–1.04) for the eosinophil count. This supports the increasing interest in the role of basophils (and in blood) in the prognosis for CRSwNP as we previously reported.⁵

In conclusion, although bEBR was significantly associated with comorbidities (allergy, asthma, and AERD) in the present study, the bEBR did not emerge as a reliable tool for pinpointing patients at higher risk of CRSwNP recurrence after ESS. However, it is noteworthy that this study confirmed significantly higher preoperative mean eosinophil and basophil counts in the sub-cohort of patients with recurrent CRSwNP after surgery, supporting the potential prognostic role of the 2 variables (eosinophil and basophil counts).

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