

Blood Eosinophilic and Basophilic Trends in Recurring and Non-Recurring Eosinophilic Rhinosinusitis With Nasal Polyps

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Abstract

Background: Very few studies have compared blood eosinophil and basophil levels before and after surgery in patients with eosinophilic CRSwNP (eCRSwNP). No investigations seem to have repeatedly measured them pre- and postoperatively in eCRSwNP patients with recurring versus non-recurring disease to examine how their levels evolved.

Objective: Hence this study to analyze blood eosinophil and basophil levels in patients with eCRSwNP immediately before surgery and afterwards, at 4 months, 1 year, and then yearly up to 5 years.

Methods: Fifty-one eCRSwNP patients were enrolled, who all had preoperative laboratory data and the results of at least 4 of the 6 scheduled postoperative follow-up tests.

Results: Seventeen patients had recurrent disease. Blood eosinophil counts ($p = 0.005$) and percentages ($p = 0.002$) were both higher in these patients than in those whose eCRSwNPs did not recur after surgery. Blood basophil counts ($p = 0.04$) and percentages ($p < 0.05$) were also significantly higher in patients whose eCRSwNPs relapsed. The time by relapse interaction was not significant for either counts or percentages, though an effect of time was detected for basophil counts ($p = 0.01$).

Conclusions: In eCRSwNP, the statistical analysis of repeated quantitative laboratory data can shed light on the evolution of a patient's systemic inflammatory picture in response to previous treatments, and above all to long-term therapies. Long-term monitoring of blood eosinophil and basophil levels could be of significant value when monoclonal antibodies that inhibit IL signaling will widely enter in clinical practice for eCRSwNP treatment to follow the effectiveness of therapy over time.

Keywords

basophils, blood, eosinophils, values, inflammatory cells, chronic rhinosinusitis, eosinophilic-type CRSwNP, postoperative repeated measures, recurrence, prognosis

Introduction

Patients suffering from chronic polypoid sinusitis (CRSwNP) need personalized care based on their CRSwNP endotype to monitor their evolution, and prescribe adequate medical or surgical treatment to control the disease and reduce its recurrence rate. To date, despite dedicated medical and surgical therapies, relapses remain a problem.

Attention has recently turned to the value of blood inflammatory cell assay in shedding light on the pathophysiology of CRSwNP and predicting the course of the disease.^{1,2} In particular, blood levels of eosinophils and basophils have attracted increasing interest.³ In 2017, in

a study on 280 patients with CRSwNP, our clinical research group examined the prognostic role of these blood levels, establishing that preoperative eosinophil

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and basophil levels had been significantly higher in patients whose CRSwNPs recurred.⁴ The ratios of eosinophils or basophils to lymphocytes,^{5,6} and the ratio of eosinophils to basophils⁷ in the patients' blood also seem to have a clinically prognostic role. These values could help rhinologists to identify CRSwNP phenotypes at high risk of recurrence.^{6,7} Generally speaking, these conventional cytological parameters – obtainable using such a minimally-invasive method as blood sampling – could make it easier to: provide patients with appropriate information pre- and postoperatively; adopt rational follow-up protocols; and administer dedicated postoperative medical treatments to patients at high risk of recurrence.

In eosinophilic-type CRSwNP (eCRSwNP), it has been reported that tissue and blood eosinophilia are closely related.⁸ In 2018, our group compared blood eosinophil and basophil levels in eCRSwNP patients before and after endoscopic sinus surgery (ESS).⁹ Our preliminary results showed a significant drop in mean blood eosinophil counts and percentages occurring after surgery. This seems to support the hypothesis that ESS enables the clearance of polyps, the removal of inflammatory tissue, and a reduction in the inflammatory cytokines generated. Consistently with such a biological mechanism, ESS could bring about a reduction in blood eosinophil levels in eCRSwNP patients. This raises the interesting question of whether blood levels of eosinophils remain stably lower after surgery than preoperatively in patients whose eCRSwNPs show no endoscopic signs of recurrence in the long term.

The main aim of the present study was thus to examine blood eosinophil and basophil counts and percentages immediately before surgery, and then postoperatively at 4 months, 1 year, and yearly thereafter up to 5 years in patients with histologically-confirmed eCRSwNP whose disease did or did not recur.

Materials and Methods

This retrospective study was conducted in accordance with the principles of the Helsinki Declaration. All patients signed a detailed informed consent form, and gave their written permission for clinical case publication. Data were examined in agreement with the Italian privacy and sensitive data laws. Furthermore, all enrolled patients signed a form in which they consented “to the use of their clinical data for scientific research purposes in the medical, biomedical and epidemiological fields, also in order to be recalled in the future for follow-up needs”.

A series of adult patients treated with ESS for eCRSwNP at the Otolaryngology Section of Padova University was retrospectively assessed. Their diagnosis of eCRSwNP was confirmed by histological examination

of surgical samples. After staining surgical tissue with hematoxylin and eosin, the pathologist considered the eosinophil count, examining 5 high-power fields (HPF) (400x) selected from each sample and recording the average number of eosinophils. The eosinophilic histotype corresponded to a mean score of >10 eosinophils/HPF. Patients who had eosinophilic granulomatosis with polyangiitis, or inflammatory, autoimmune, acute or chronic infectious diseases other than sinusitis, or malignancies, hematological disorders, a history of systemic corticosteroid therapy, or chronic renal insufficiency, or underwent treatment with biologics monoclonal antibodies were ruled out for the purposes of the present study.

Blood counts and percentages of eosinophils and basophils were obtained immediately preoperatively, then 4 months and 1 year after ESS, and yearly up to 5 years. All samples were obtained after suspending local and systemic steroid therapy⁴ for at least three months and one month, respectively. All tests were conducted at the same laboratory (Padova University Hospital's Laboratory Medicine Service), which is certified according to ISO 15189, and the data for the present study were collected from the Laboratory's electronic archives [Galileo]. All patients were treated postoperatively with isotonic saline solution irrigations twice a day (20 ml per irrigation), nasal steroids (mometasone furoate 200 µg daily [100 µg per nostril], or fluticasone furoate 110 µg daily [55 µg per nostril]), and two cycles a year of oral steroids (methylprednisolone 32 mg daily on days 1–5, then 16 mg daily on days 6–10, then 8 mg daily on days 11–20¹⁰) as our group previously described.¹¹ Adequate therapy was prescribed for asthmatic and allergic patients.

Follow-up with rigid 0° or 30° endoscopes was scheduled 3, 6 and 12 months after ESS, and yearly thereafter. Patients were classified as cases of recurrence if they had endoscopic evidence of at least grade I polyposis.¹² Recurrent eCRSwNP with evidence of polyps protruding below the middle turbinate (grade II), or with massive polyposis (grade III)¹² underwent ESS revision.

Statistical Methods

A mixed model, repeated measures analysis was run to test the effect of relapse and time, and the relapse by time interaction, considering an undetermined correlation matrix. Statistical significance was set at the 5% level for the main effects, and at the 10% level for the interaction. Means were estimated with the least squares method, along with 95% confidence intervals (CI).

The statistical analysis was performed with SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA), and the STATA version 10 statistical package (Stata Corp LP, College Station, TX, USA).

Results

Out of a series of 159 adult patients consecutively treated with ESS for eCRSwNP by our group, 51 cases (35 males, 16 females; mean age 52.0 ± 13.1 years, median 52 years) meeting our inclusion criteria were considered for the present study. They all had laboratory data obtained preoperatively and at 4 or more of the 6 scheduled postoperative follow-ups. Seventeen of these 51 patients developed recurrent disease after a median disease-free interval of 20 months (MIN 5 months; MAX 50 months). None of the patients reported significant adverse effects that could be attributable to the steroids administered.

Comparison of Long-Term Laboratory Data for eCRSwNP Patients With and Without Recurrent Disease

Figure 1 summarizes the distributions of the pre- and postoperative laboratory data by sub-cohort of eCRSwNP patients with and without relapsing disease after ESS. Figure 2 shows the corresponding least squares means. Blood eosinophil counts ($p = 0.005$)

and percentages ($p = 0.002$) were both higher in eCRSwNP patients whose disease relapsed. Blood eosinophil levels remained persistently elevated compared with the nonrecurrent group in the repeated measures model. Blood basophil counts ($p = 0.04$) and percentages ($p < 0.05$) were also significantly higher in the sub-cohort of eCRSwNP patients with recurrent disease after ESS. The time by relapse interaction was not significant for either counts or percentages, though an effect of time was detected for basophil counts ($p = 0.01$).

Discussion

Eosinophilic inflammation has been recognized as a major phenotype associated with poorly-controlled disease and recurrence in patients with CRSwNP. As tissue and blood eosinophilia are closely related in eCRSwNP, it has been suggested that patients' circulating eosinophils were in a pre-activated state before their extravasation and migration to polyps.² Tissue basophilia in CRSwNP has only occasionally been investigated. Recent studies found significant direct associations between recurrent CRSwNP and blood basophil counts and percentages.² Basophils release interleukin

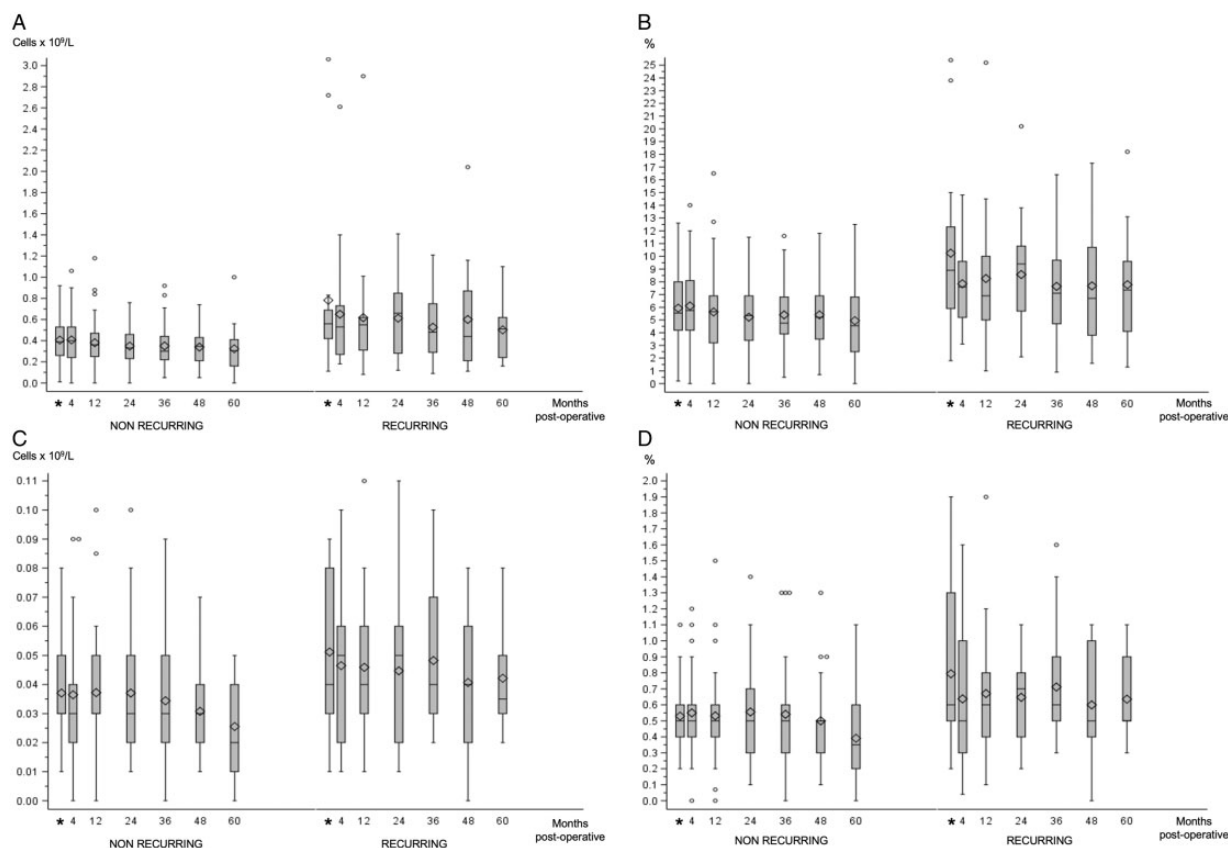


Figure 1. Box plots of eosinophil blood counts (A) and percentages (B), and basophil blood counts (C) and percentages (D) obtained immediately before surgery (*), and at 4 and 12 months after surgery, then yearly up to 5 years in sub-cohorts of eCRSwNP patients with and without recurrences. o = outlier.

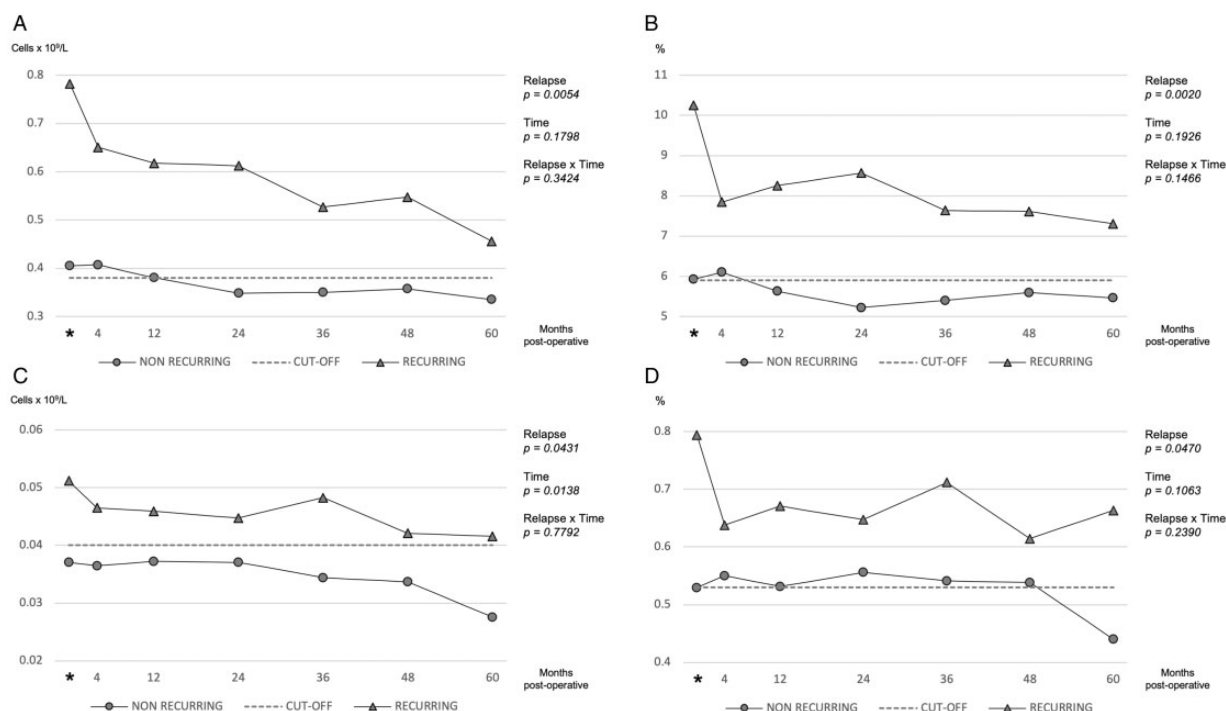


Figure 2. Graphic representation of least squares estimates for mean eosinophil blood counts (A) and percentages (B), and mean basophil blood counts (C) and percentages (D) obtained immediately before surgery (*), and at 4 and 12 months after surgery, then yearly up to 5 years in sub-cohorts of eCRSwNP patients with and without recurrences. In the sub-cohort with recurrent disease, 7 relapses occurred within the 1st postoperative year, then 3 in the 2nd, 5 in the 3rd, 1 in the 4th, and 1 in the 5th year. The dotted line shows the previously-calculated cutoff⁴ for higher risk of disease relapse.

(IL) 4 in response to a variety of stimuli. It is widely accepted that IL-4 plays a major part in promoting the differentiation of naive CD4⁺ T cells into T helper 2 cells.¹³ Basophil-derived IL-4 enhances the expression of the chemokine CCL11 (better known as eosinophil chemotactic protein, or eotaxin-1), as well as IL-5, IL-9, and IL-13 in type 2 innate lymphoid cells, leading to an accumulation of eosinophils.¹⁴ In 2017, considering quite a large series of consecutive eCRSwNPs (127 cases), our group calculated the best cutoffs for binarizing patients' preoperative blood variables (eosinophil and basophil counts and percentages) for the purpose of prognosticating eCRSwNP recurrence after ESS.⁴ For blood eosinophil counts, eosinophil percentages, basophil counts, and basophil percentages, these cutoffs were 0.38 cells $\times 10^9/L$, 5.9%, 0.04 cells $\times 10^9/L$, and 0.5%, respectively⁴ (see Figure 2(A) to (D)).

Our understanding of CRSwNP pathophysiology has gradually evolved¹⁵ and the identification of various inflammatory biomarkers (including cell lines) has led to the development of monoclonal antibodies that target the underlying mechanisms of inflammation.¹⁶ Dupilumab is a fully human monoclonal antibody that inhibits the signaling of IL-4 and IL-13, which are key drivers of type 2 inflammation. In patients with severe CRSwNP, dupilumab reduced polyp size, sinus

opacification, and severity of symptoms, and it was well tolerated.¹⁷ In June 2019, the U.S. Food and Drug Administration (FDA) approved dupilumab for treating adults with inadequately-controlled CRSwNP.¹⁸ Mepolizumab and benralizumab, are currently being investigated as a treatment for severe CRSwNP.^{18,19}

Very few studies have compared blood eosinophil and basophil counts in eCRSwNP patients before and after surgery. In 2016, Honma et al.²⁰ measured the blood eosinophil counts of 22 eCRSwNP patients before and 6 months after ESS. Despite the limited number of cases with postoperative data ($n = 13$), they found that blood eosinophil counts decreased significantly after surgery. More recently, our clinical research group considered a larger series of 79 consecutive patients with eCRSwNP treated with ESS, finding a significant drop in mean blood eosinophil levels from before to after ESS in the cohort as a whole.⁹ A statistically significant reduction in blood basophil levels after surgery only emerged in the sub-cohort of non-allergic eCRSwNP patients. The laboratory data were only available for a single postoperative test, however, so they could not reveal the trends of circulating inflammatory cellularity over time. The present study was based instead on repeated measures. Although single measures are more accessible, the

repeated measure design: (i) reduces the variance of estimates of treatment-effects, allowing statistical inference to be made with limited number of participants; (ii) allows researchers to monitor how participants change over time, both long- and short-term situations. Clinical medical investigations often rely on such an approach, as it can have conceptual and pragmatic advantages. The return to health or the progression of disease can often be discerned best by assessing the same individual several times. The adoption of this approach in the field of CRSwNP is still very uncommon, but repeatedly collecting data prospectively or retrospectively (as we did) can reveal changes over time and their variability within individuals. As suggested by Everitt²¹ the first step in our analysis of our laboratory data from repeated measures involved plotting the means by prognosis-based eCRSwNP sub-cohort for each time point. For all the eCRSwNP patients considered in our analysis, laboratory findings had been obtained preoperatively and at 4 or more of the 6 scheduled postoperative follow-ups. The main strengths of the present study lie in the homogeneity of the series of patients considered since: (i) only cases of eCRSwNP were considered; (ii) histopathological analyses were all done by a dedicated head and neck pathologist; (iii) ESS was performed by the same team of surgeons; (iii) the endoscopic follow-up after surgery was conducted by the same team; (iv) recurrent eCRSwNP was always confirmed endoscopically; and, last but not least, (v) all blood tests were performed over a long-term postoperative period at the same laboratory. One surely interesting aspect is that our sub-cohorts have maintained their characteristics over time in terms of circulating eosinophil and basophil values. This is a consideration not obtainable from single measurements. The main weaknesses of the study concern the retrospective setting and the limited number of patients.

Very recently, Leung et al.²² developed a probabilistic risks-based decision analysis which was constructed from literature reported incidences and impacts of adverse events of oral steroids and ESS. They concluded that, given reported oral steroids effectiveness in the range of 34–71% in CRSsNP and 46–63% in CRSwNP, the analysis suggested that the inclusion of oral steroids in appropriate medical therapy may be the lower risk strategy. Considering that the use of systemic and not only nasal steroids is envisaged in specific contexts of CRSwNP²³ but also that the modality and number of cycles may vary, it is possible that in series treated with dosages and number of oral steroid cycles different from that described in our study, the long-term trend of circulating eosinophil and basophil values may differ. It is desirable for a correct comparison that other studies based on CRSwNP series treated differently with postoperative oral steroids apply a repeated measures approach on circulating inflammatory cells.

Several authors have reported a positive association between preoperative blood eosinophilia and CRSwNP recurrence rates after surgery.^{4,24,25} To the best of our knowledge, the present study is the first to adopt a repeated measures approach to the issue. We found blood eosinophil and basophil counts and percentages significantly higher in patients with eCRSwNPs whose disease relapsed than in those remaining disease-free after surgery.

Analyzing most of the laboratory variables considered, the regression slopes of the same features over the same time intervals did not differ significantly between the recurring vs non-recurring eCRSwNP sub-cohorts. This (albeit very preliminary) intriguing evidence would seem to support the theory that, whatever the medical and surgical treatment administered, the characteristics of patients distinguished by their prognosis (disease relapse occurrence) remained unchanged in the long term as regards their circulating eosinophils. This might plausibly be because the two sub-cohorts with the same phenotype (a rich eosinophilic component at histology) differ in pathophysiological terms. Interestingly, an effect of time was detected for basophil counts. This supports the conclusions of recent studies that, although basophils are the rarest granulocytes, they have a crucial and largely non-redundant role in inflammation.²

The present results could be related to our clinical and therapeutic setting. However, we hope that these laboratory variables can be investigated through repeated measures by other groups. In eCRSwNP, the statistical analysis of repeated quantitative laboratory data can shed light on the evolution of a patient's systemic inflammatory picture in response to previous treatments, and above all, to long term therapies. Long-term monitoring of blood eosinophil and basophil values could be of significant value when monoclonal antibodies that inhibit IL signaling will widely enter clinical practice for eCRSwNP treatment to follow the effectiveness of therapy over time.

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