Published in final edited form as:

Int Forum Allergy Rhinol. 2017 November ; 7(11): 1058–1064. doi:10.1002/alr.22005.

A prospective analysis evaluating tissue biopsy location and its clinical relevance in chronic rhinosinusitis with nasal polyps

Ava R. Weibman, BA¹, Julia He Huang, DDS¹, Whitney W. Stevens, MD, PhD², Lydia A. Suh, BS², Caroline P.E. Price, BA¹, Alcina K. Lidder, BA^{1,3}, David B. Conley, MD¹, Kevin C. Welch, MD¹, Stephanie Shintani-Smith, MD¹, Anju T. Peters, MD, MS², Leslie C. Grammer, MD², Atsushi Kato, PhD², Robert C. Kern, MD¹, Robert P. Schleimer, PhD^{1,2}, and Bruce K. Tan, MD, MS¹

¹Department of Otolaryngology-Head and Neck Surgery, Northwestern University Feinberg School of Medicine, Chicago, IL

²Division of Allergy-Immunology, Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL

³University of Rochester School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY

Abstract

Background—Chronic rhinosinusitis with nasal polyps (CRSwNP) has a high propensity for recurrence. Studies suggest that eosinophilia influences disease severity and surgical outcomes, but the selection of sinonasal site for measuring eosinophilia has not been examined. The aim of this study was to investigate how region-specific tissue eosinophilia affects radiographic severity, comorbidity prevalence, and polyp recurrence risk following sinus surgery.

Methods—Eosinophil cationic protein (ECP) levels in uncinate tissue (UT) and nasal polyp (NP) homogenates from 116 CRSwNP patients were measured using enzyme-linked immunosorbent assay (ELISA). Clinical history, radiographic severity, and time to polyp recurrence were obtained from electronic health records. The correlations between baseline Lund-Mackay scores and comorbidities were compared between UT and NP ECP levels. Cox regression and Kaplan-Meier analysis were then performed to assess whether UT or NP ECP better predicted recurrence. Censoring occurred at 4 years or at last follow-up if there was no endoscopic diagnosis of recurrent polyps.

Results—Lund-Mackay scores were significantly correlated with UT and NP ECP ($r = 0.46$ and 0.26 respectively, $p < 0.05$). UT but not NP ECP was significantly higher in patients with asthma ($p < 0.01$) and aspirin-exacerbated respiratory disease (AERD) ($p < 0.05$). Polyp recurrence risk was only significantly higher for patients with eosinophilic UT tissue (hazard ratio [HR] = 2.84, $p = 0.025$). When measured in NP, eosinophilia did not predict recurrence.

Correspondence to: Bruce K. Tan, MD, Department of Otolaryngology, Northwestern University Feinberg School of Medicine, 676 North St Clair, Suite 1325, Chicago, IL 60611; b-tan@northwestern.edu.

Potential conflict of interest: None provided.

Presented at the ARS Meeting at the annual Combined Otolaryngology Spring Meetings (COSM) on April 26–30, 2017, in San Diego, CA.

Conclusion—Although ECP in NP was higher than in UT tissue, eosinophilia in UT tissue was a more clinically coherent biomarker of baseline radiographic severity, comorbid asthma and AERD, and prospective polyp recurrence risk than NP eosinophilia.

Keywords

Chronic sinusitis; outcomes; biomarkers; disease severity

Chronic rhinosinusitis (CRS) is 1 of the most common chronic conditions, with a symptomatic prevalence of 10.9% to 11.9% in the general population.^{1, 2} CRS is also typically classified into 2 major clinical subtypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). Increased tissue eosinophilia is reported in most (80–90%) of CRSwNP but is also found in a substantial minority (30–50%) of patients with CRSsNP who undergo endoscopic sinus surgery (ESS).^{3, 4} Together, it is thus likely that the majority of ESS in Western countries is performed for eosinophilic CRS. In addition to its effects on CRS, eosinophilic forms of CRS are also more often comorbid with asthma and atopy potentially because of their shared pathological feature of eosinophilia.^{5–7}

Although CRSwNP or eosinophilic CRS is not associated with worse overall quality of life,⁸ studies have found that tissue eosinophilia correlates with worse baseline radiographic severity and olfactory loss.^{9–13} It is additionally recognized that the presence of NP and eosinophilia at the time of sinus surgery are also associated with increased rates of postsurgical medication use, recurrence, and revision sinus surgery.^{14–20} Thus, accurately identifying patients at risk for recurrence is important for tailoring individualized treatment plans. Given the increasing availability of biological agents specifically targeting aspects of eosinophilic inflammation, accurately identifying the patients most at risk for pathogenic eosinophilic inflammation is also becoming increasingly important.^{21–23}

To our knowledge, few studies have explored the extent to which eosinophilia varies across regions of the sinonasal anatomy and even fewer have evaluated their relationship with cross-sectional and longitudinal outcomes in CRS. Our laboratory has traditionally used eosinophilic cationic protein (ECP) as a surrogate for eosinophilia. ECP is an eosinophil granule protein that correlates well with cytokines (eg, interleukin 5 [IL-5] and IL-13) and chemokines (eg, eotaxin-2/CCL24 and eotaxin-3/CCL26) as well as blood and tissue eosinophilia.^{9, 24, 25} In prior studies, we found that NP has higher levels of ECP than uncinate tissue (UT) even among patients with CRSwNP. More recently, we found that although ECP levels measured in NP were higher than in UT, UT ECP levels were moderately to strongly correlated with radiographic severity and other type-2 mediators.⁹ In contrast, when examining the relationship of NP with those factors, only weak correlations were found. Thus, the objective of this study was to compare the value of ECP measured in UT vs NP to serve as a biomarker of severity and recurrence in CRSwNP patients.

Patients and methods

Study population and data collection

We performed a prospective longitudinal study of 116 adult CRSwNP patients who had ESS at Northwestern Memorial Hospital between 2008 and 2014. All patients provided informed consent for the potential use of their surgically resected tissue for research and Institutional Review Board approval was obtained from Northwestern University Feinberg School of Medicine. All patients satisfied CRSwNP diagnostic criteria as recommended by the guidelines for diagnosis of rhinosinusitis and nasal polyps using symptoms, endoscopic findings, and radiographic findings.^{26, 27} Only patients from whom both UT and NP tissue were available for protein analysis were included. Exclusion criteria included patients with cystic fibrosis, antrochoanal polyps, and human immunodeficiency virus infection. The electronic medical records of patients who had undergone ESS were reviewed for relevant demographic and medical history including age on the date of surgery, sex, prior sinus surgery, asthma status, atopic status, aspirin allergy at the time of ESS, and preoperative oral steroid use. Patients were designated as asthmatic if they were diagnosed with asthma and had symptoms as an adult (ie, excluding patients with only childhood asthma). Atopy was defined as a positive skin-prick test to at least 1 aeroallergen or a documented history of atopy. Four patients had an unknown atopic status and as a result were not included in the analysis involving this variable. Patients were diagnosed with aspirin-exacerbated respiratory disease (AERD) if they were previously diagnosed with asthma and had a documented history of respiratory systems that were exacerbated by aspirin or other nonsteroidal anti-inflammatory drugs. Preoperative oral steroid use was defined if patients were treated with oral steroids within 2 weeks of their surgery. Baseline radiographic severity was quantified by preoperative computed tomography (CT) score using the Lund-Mackay staging system. Two patients did not have an accessible baseline CT scan so these patients were excluded from analyses involving this variable. After ESS, all records of postoperative office visits where a nasal endoscopy was performed were reviewed. Postoperative steroid use was defined in 3 groups if patients were prescribed greater than 1 month of: (1) oral corticosteroids; (2) topical nasal steroids used in an off-label manner (eg, budesonide or mometasone nasal rinses/drops/nebulization); and (3) nasal steroid sprays (eg, fluticasone, mometasone, triamcinolone). All postoperative steroid groups were considered as independent variables in longitudinal analyses. Nasal polyps were considered to have recurred at the first endoscopic instance when recurrent nasal polyps within or beyond the middle meatus were identified. The study follow-up length was restricted to a 4-year maximum. Patients who recurred during the study period were followed from the date of surgery until the date of recurrence detection. If patients did not recur during the study period, follow-up ceased on the date of the last office visit. Patients with last office visits greater than or equal to 4 years after surgery were censored 4 years after their surgery date.

ECP measurement

ECP levels in UT and NP tissue homogenates were measured by enzyme-linked immunosorbent assay (ELISA) using Mesacup ECP test kit (MBL, Woburn, MA). The ECP concentration was expressed as ng/mL and normalized to total protein concentration using the BCA assay (Thermo Scientific Waltham, MA). A previously established ECP threshold

value of 290 ng/mg total protein was used to define tissue eosinophilia.⁸ This threshold was the 95th percentile of ECP values measured in 82 control patients who did not have CRS.

Statistical analysis

Statistical analyses were conducted using SPSS software package (IBM Corp., Armonk, NY). The relationship between categorical factors (asthma, AERD, atopy, sex, preoperative oral steroid use, prior sinus surgery) and ECP levels in UT and NP were individually assessed by chi square or Mann-Whitney *U* test. The association between continuous factors (age, CT score, follow-up length) and ECP levels in UT and NP was measured using Spearman's rank correlation, Mann-Whitney *U*, or the 2-sample *t* test where appropriate. Univariate Cox regression models were used to compute the relative effect of postoperative steroid prescription and UT and NP ECP levels on the polyp recurrence risk. The comparative risk was expressed as the hazard ratio (HR) and the associated 95% confidence interval (CI). Multivariate Cox regression analyses were performed to control for postoperative steroid prescription whenever univariate analyses had a $p < 0.2$. Kaplan-Meier curves were constructed to illustrate differences in recurrence-free survival. A $p < 0.05$ in 2-tailed tests was considered statistically significant.

Results

Baseline clinical and demographic characteristics associated with tissue eosinophilia

The characteristics of the study population are summarized in Table 1. Preoperative oral steroid use was not associated with tissue ECP levels. ECP levels from UT were increased in patients with asthma (Fig. 1A) or AERD (Fig. 1B) compared to patients without asthma or AERD ($p = 0.006$ and $p = 0.016$, respectively). Atopy was not significantly associated with higher UT ECP levels. Unlike ECP values in UT, there were no significant differences in NP ECP levels when stratified by any of the 3 investigated comorbidities. Increased UT ECP levels were also observed in female patients compared to male patients ($p = 0.025$) (Fig. 1C), whereas NP ECP levels were only trending toward significance ($p = 0.066$). There were no age-related differences in ECP levels when measured in UT or NP. We further correlated radiographic severity with ECP levels in each region and found that even though computerized tomography score was significantly associated with both UT and NP ECP, the correlation was stronger with UT ECP ($r = 0.46$, $p < 0.001$) than NP ECP ($r = 0.26$, $p = 0.005$) (Fig. 2A,B).

Tissue eosinophilia as a predictor of recurrence

We next examined the prognostic value of ECP in UT compared to NP. Postoperative polyp recurrence was detected in 30.9% of patients with eosinophilic UT and 12.5% of patients with non-eosinophilic UT. By contrast, recurrent NP were found in 27.4% of patients with eosinophilic NP and 16.3% of patients with non-eosinophilic NP. The univariate Cox regression analysis of UT and NP ECP showed that ECP ≥ 290 ng/mg total protein in UT was a significant predictive factor of recurrence after ESS (HR = 2.84, $p = 0.025$) (Table 2). While polyp recurrence risk was also greater for patients with eosinophilic NP compared to patients with non-eosinophilic NP, this difference was not statistically significant (HR = 1.74, $p = 0.207$). Postoperative nasal or oral steroid use did not confound the relationship

between eosinophilia and recurrence risk as determined by multivariate Cox regression analyses (Table 3). The Kaplan-Meier survival curves showed how CRSwNP patients with ECP levels ≥ 290 ng/mg protein in UT (Fig. 3A) and NP (Fig. 3B) exhibit shorter recurrence-free survival compared to patients with ECP levels < 290 ng/mg protein, but this difference in recurrence-free survival is only significant when based on UT ECP levels. The number of patients at risk for recurrence at each yearly milestone is outlined in Figure 3C.

Discussion

In prior studies, patients with CRSwNP have been reported to have increased recurrence rates, worse radiographic severity, and suffer a higher prevalence of comorbid asthma and allergy compared to patients with CRSsNP.^{6, 14, 28} Identifying specific molecular characteristics of CRSwNP that drive these pathogenic features is key to understanding the etiological mechanisms underlying this disease, but no prior analysis, to our knowledge, has examined the anatomical location to best assess the nature of inflammation in CRSwNP. In the present study, we found that while ECP levels in both UT and NP were significantly correlated with radiographic severity as determined by CT scores, the correlation was consistently stronger for UT compared to NP. Similarly, UT ECP levels were significantly elevated in patient populations previously identified as having more severe disease, including patients with asthma or AERD, as well as women.^{5, 29, 30} Furthermore, we showed that ECP levels greater than 290 ng/mg protein in UT, a threshold selected based on the 95th percentile of non-CRS tissue, was a significant predictor of polyp recurrence. Although recurrence rate was also higher for patients with eosinophilic NP compared to patients with non-eosinophilic NP, this difference did not reach statistical significance. Together, these data suggest the degree of eosinophilia in UT might be more representative of disease burden in CRSwNP at baseline and a better predictor of outcomes after ESS. An association between tissue eosinophilia and baseline disease severity has been previously reported. There are multiple different markers of severity in CRS, including symptom severity, patient reported outcome measures, radiographic, and endoscopic severity. According to prior studies, tissue eosinophilia is correlated with olfactory dysfunction in CRS.^{8, 10, 31} Other studies concluded that there is a relationship between tissue eosinophilia and worse disease severity as defined by preoperative CT and endoscopy scores; however, these study cohorts included both CRSsNP and CRSwNP patients and the analyses were not always stratified by subtype.^{10, 11} We note that in these studies Kountakis et al.¹¹ did not specify the exact biopsy location and Soler et al.¹⁰ evaluated eosinophilia in ethmoid mucosa. Therefore, the conclusions made from these studies have relatively limited specificity regarding relevant subtypes or sinonasal regions. Our findings that UT ECP is more strongly correlated with CT scores than NP ECP are in line with the results reported by Min et al.⁹ ($r = 0.58$ and $r = 0.37$). Together, these results suggest that eosinophilic inflammation in UT may have stronger associations with radiographic severity in patients with CRSwNP.

The frequent coexistence of asthma, atopy, and aspirin sensitivity in CRS is well established. While the etiology of the link between these conditions is still debated, there is strong evidence that these conditions have similar inflammatory pathways.^{32, 33} Furthermore, patients with CRS who also have these comorbidities tend to have worse disease severity and prognosis than the general CRS population.^{6, 28} Identifying mucosal markers associated

with these comorbid illnesses helps better establish the concept of a unified airway in which upper and lower airway inflammation are linked. Multiple studies have investigated the relationship between eosinophilic inflammation and comorbid asthma, but the histopathologic definitions of eosinophilia used in these studies are quite variable.^{11, 17, 20, 31} Zuo et al.³¹ and Kountakis et al.¹¹ both reported an increased frequency of asthma in CRS patients with higher eosinophil densities (>5 eosinophils/high-power field [HPF]) in sinus mucosal or NP tissue. Similarly, Tokunaga et al.²⁰ found that asthma was more prevalent in CRS patients with higher eosinophil densities (>70 eosinophils/HPF) in NP or polypoid tissue from the ethmoid cavity. These analyses indicate a positive relationship between tissue eosinophilia and comorbid asthma, which is consistent with our findings. The anatomic site from which mucosal eosinophilia was measured in these prior studies was unclear because “sinonasal tissue” is frequently used for assessment, and study populations included both CRSsNP and CRSwNP patients. Our data shows that although ECP levels in UT and NP are elevated in asthmatic CRSwNP patients, this only reaches statistical significance in UT. This suggests that eosinophilia in UT is a more coherent biomarker for comorbid asthma compared to eosinophilia in NP. Similar results were found when we analyzed the AERD comorbidity. Stevens et al.²⁹ in our group had previously compared ECP levels in NP from AERD patients with patients with CRSwNP and found ECP levels significantly elevated in AERD patients compared to CRSwNP patients without AERD. In contrast, our data shows that ECP levels only significantly increased in UT from AERD patients. Since the number of AERD patients included in the analysis by Stevens et al.²⁹ (n = 15) was twice as large as the number of AERD patients included in our analysis (n = 8), we suspect that the discrepancy in our findings reflects the fact that UT ECP provides a larger effect size for distinguishing patients with these comorbidities than NP ECP. Prior studies in China have reported greater eosinophil density in sinus tissue among atopic patients,³¹ but we did not find associations between UT or NP ECP levels and atopic status. Eosinophilic sinusitis in China has a significantly different clinical presentation to that of Western countries, with a large number of patients with CRSwNP exhibiting non-eosinophilic inflammation.³⁴ Likewise, a smaller study we carried out on patients of East Asian extraction in our Chicago-based population found lower levels of eosinophilia among second-generation East Asian patients, suggesting the determinants of eosinophilic CRS in East Asian populations may be different.

Several studies have demonstrated that tissue eosinophilia is associated with increased recurrence rate, although most prior analyses did not consider time to recurrence in their analysis and used very different eosinophil counting techniques and thresholds.^{14–20} The study closest in design to our study was a survival analysis of recurrence in 114 CRSwNP patients by Nakayama et al.¹⁵ They found that CRSwNP patients with 70 or higher eosinophils/HPF in the submucosa of the ethmoid cavity or NP had a significantly lower recurrence-free survival rate (HR = 3.65). While the relative recurrence risk observed in patients with eosinophilic UT in our study was not as large (HR = 2.84), we note that the Nakayama et al.¹⁵ study had longer average and minimum follow-up of 18.35 and 6 months, respectively, greater power, and had an eosinophil threshold that was selected to optimize differences in recurrence rates. Finally, the Nakayama et al.¹⁵ study mixed both NP and ethmoid tissue for determining eosinophil counts and did not perform analyses comparing biopsy location. It is therefore possible that eosinophilia in ethmoid mucosa, like UT

eosinophilia, similarly provides a larger effect size for predicting recurrence risk compared to NP eosinophilia.

Our study had some limitations. Due to the observational nature of the study, follow-up depended on the patient choosing to schedule a postoperative office visit. Therefore, length of follow-up was variable. While Cox regression analyses were utilized to account for this, many patients had limited follow-up, with a median follow up time of 9.4 months. It is likely that some patients who developed recurrent polyps did not visit the clinic for treatment. This likely decreased the statistical power of our study and increased the chances of type II error occurring. Additionally, in this study, not all NP recurrence may be symptomatically or clinically significant and we are underpowered to detect differences in revision surgery rates in the 2 groups. Finally, in this study, we were only able to compare UT and NP but did not assess other mucosal sites (eg, ethmoid mucosa) to comprehensively evaluate the effect of measuring eosinophilia in different regions of the sinonasal cavity. Possible explanations for why UT provides more coherent biomarkers include its anatomic location within the osteomeatal complex, which affects drainage of the frontal, anterior ethmoid, and maxillary sinuses, and that it fuses into mucosa of the lamina papyracea and skull base and thus may better reflect residual eosinophilia after sinus surgery. However, given the widespread use of NP tissue in prior studies we believe our results are relevant to the ongoing development of adjunctive treatments such as biologic medications targeting eosinophilic inflammation. Given the expense associated with these medications, they will likely be reserved for patients at risk for failing existing treatments. Our data would suggest that biopsy location affects the clinical value of biomarkers measured from tissue.

Conclusion

This is the first study to investigate and confirm that biopsy location is important to consider when evaluating the utility of tissue eosinophilia for predicting disease burden and clinical course in CRSwNP. Our findings indicate that ECP in UT is a more coherent source of biomarkers in CRSwNP compared to ECP in NP tissue.

Acknowledgments

Funding sources for the study: NIH grants (K23 DC012067 to B.K.T.), (Chronic Rhinosinusitis Integrative Studies Program [CRISP] U19 AI106683 to B.K.T., R.C.K., K.E.H., A.K., R.P.S.), R01 HL078860, R37 HL068546, R01 HL068546, R01 AI072570, R01 AI104733 (to R.P.S.); Triological Society/American College of Surgeons (to B.K.T.); Ernest S. Bazley Foundation.

References

1. Hastan D, Fokkens WJ, Bachert C, et al. Chronic rhinosinusitis in Europe—an underestimated disease. A GA(2)LEN study. *Allergy*. 2011; 66:1216–1223. [PubMed: 21605125]
2. Hirsch AG, Stewart WF, Sundaresan AS, et al. Nasal and sinus symptoms and chronic rhinosinusitis in a population-based sample. *Allergy*. 2017; 72:274–281. [PubMed: 27590749]
3. Tan BK, Klingler AI, Poposki JA, et al. Heterogeneous inflammatory patterns in chronic rhinosinusitis without nasal polyps in Chicago, Illinois. *J Allergy Clin Immunol*. 2017; 139:699–703. e7. [PubMed: 27639939]
4. Huvenne W, van Bruaene N, Zhang N, et al. Chronic rhinosinusitis with and without nasal polyps: what is the difference? *Curr Allergy Asthma Rep*. 2009; 9:213–220. [PubMed: 19348721]

5. Bachert C, Patou J, Van Cauwenberge P. The role of sinus disease in asthma. *Curr Opin Allergy Clinical Immunol.* 2006; 6:29–36. [PubMed: 16505609]
6. Pearlman AN, Chandra RK, Chang D, et al. Relationships between severity of chronic rhinosinusitis and nasal polyposis, asthma, and atopy. *Am J Rhinol Allergy.* 2009; 23:145–148. [PubMed: 19401038]
7. Baroody FM, Mucha SM, Detineo M, Naclerio RM. Nasal challenge with allergen leads to maxillary sinus inflammation. *J Allergy Clin Immunol.* 2008; 121:1126–1132. e7. [PubMed: 18367240]
8. Thompson CF, Price CP, Huang JH, et al. A pilot study of symptom profiles from a polyp vs an eosinophilic-based classification of chronic rhinosinusitis. *Int Forum Allergy Rhinol.* 2016; 6:500–507. [PubMed: 26683389]
9. Min JY, Ocampo CJ, Stevens WW, et al. Proton pump inhibitors decrease eotaxin-3/CCL26 expression in patients with chronic rhinosinusitis with nasal polyps: possible role of the nongastric H,K-ATPase. *J Allergy Clin Immunol.* 2017; 139:130–141. e11. [PubMed: 27717558]
10. Soler ZM, Sauer DA, Mace J, Smith TL. Relationship between clinical measures and histopathologic findings in chronic rhinosinusitis. *Otolaryngol Head Neck Surg.* 2009; 141:454–461. [PubMed: 19786212]
11. Kountakis SE, Arango P, Bradley D, Wade ZK, Borish L. Molecular and cellular staging for the severity of chronic rhinosinusitis. *Laryngoscope.* 2004; 114:1895–1905. [PubMed: 15510011]
12. Soler ZM, Sauer D, Mace J, Smith TL. Impact of mucosal eosinophilia and nasal polyposis on quality-of-life outcomes after sinus surgery. *Otolaryngol Head Neck Surg.* 2010; 142:64–71. [PubMed: 20096225]
13. Lavin J, Min JY, Lidder AK, et al. Superior turbinate eosinophilia correlates with olfactory deficit in chronic rhinosinusitis patients. *Laryngoscope.* (in press). Epub 2017 Mar 21. <https://doi.org/10.1002/lary.26555>
14. Vlamincck S, Vauterin T, Hellings PW, et al. The importance of local eosinophilia in the surgical outcome of chronic rhinosinusitis: a 3-year prospective observational study. *Am J Rhinol Allergy.* 2014; 28:260–264. [PubMed: 24980239]
15. Nakayama T, Yoshikawa M, Asaka D, et al. Mucosal eosinophilia and recurrence of nasal polyps - new classification of chronic rhinosinusitis. *Rhinology.* 2011; 49:392–396. [PubMed: 21991563]
16. Grgic MV, Cupic H, Kalogjera L, Baudoin T. Surgical treatment for nasal polyposis: predictors of outcome. *Eur Arch Otorhinolaryngol.* 2015; 272:3735–3743. [PubMed: 25634061]
17. Van Zele T, Holtappels G, Gevaert P, Bachert C. Differences in initial immunoprofiles between recurrent and nonrecurrent chronic rhinosinusitis with nasal polyps. *Am J Rhinol Allergy.* 2014; 28:192–198. [PubMed: 24980230]
18. Matsuwaki Y, Ookushi T, Asaka D, et al. Chronic rhinosinusitis: risk factors for the recurrence of chronic rhinosinusitis based on 5-year follow-up after endoscopic sinus surgery. *Int Arch Allergy Immunol.* 2008; 146(Suppl 1):77–81. [PubMed: 18504412]
19. Tosun F, Arslan HH, Karslioglu Y, Deveci MS, Durmaz A. Relationship between postoperative recurrence rate and eosinophil density of nasal polyps. *Ann Otol Rhinol Laryngol.* 2010; 119:455–459. [PubMed: 20734966]
20. Tokunaga T, Sakashita M, Haruna T, et al. Novel scoring system and algorithm for classifying chronic rhinosinusitis: the JESREC Study. *Allergy.* 2015; 70:995–1003. [PubMed: 25945591]
21. Gevaert P, Van Bruaene N, Cattaert T, et al. Mepolizumab, a humanized anti-IL-5 mAb, as a treatment option for severe nasal polyposis. *J Allergy Clin Immunol.* 2011; 128:989–995. e8. [PubMed: 21958585]
22. Bachert C, Zhang L, Gevaert P. Current and future treatment options for adult chronic rhinosinusitis: focus on nasal polyposis. *J Allergy Clin Immunol.* 2015; 136:1431–1440. quiz 1441. [PubMed: 26654192]
23. Bachert C, Mannent L, Naclerio RM, et al. Effect of subcutaneous dupilumab on nasal polyp burden in patients with chronic sinusitis and nasal polyposis: a randomized clinical trial. *JAMA.* 2016; 315:469–479. [PubMed: 26836729]

24. Ochkur SI, Kim JD, Protheroe CA, et al. A sensitive high throughput ELISA for human eosinophil peroxidase: a specific assay to quantify eosinophil de-granulation from patient-derived sources. *J Immunol Methods*. 2012; 384(1–2):10–20. [PubMed: 22750539]
25. Min SB, Nylund CM, Baker TP, et al. Longitudinal evaluation of noninvasive biomarkers for eosinophilic esophagitis. *J Clin Gastroenterol*. 2017; 51:127–135. [PubMed: 27479142]
26. Fokkens WJ, Lund VJ, Mullol J, et al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology*. 2012; 50:1–12. [PubMed: 22469599]
27. Orlandi RR, Kingdom TT, Hwang PH, et al. International Consensus Statement on Allergy and Rhinology: Rhinosinusitis. *Int Forum Allergy Rhinol*. 2016; 6(Suppl 1):S22–S209. [PubMed: 26889651]
28. Batra PS, Tong L, Citardi MJ. Analysis of comorbidities and objective parameters in refractory chronic rhinosinusitis. *Laryngoscope*. 2013; 123(Suppl 7):S1–S11.
29. Stevens WW, Ocampo CJ, Berdnikovs S, et al. Cytokines in chronic rhinosinusitis. Role in eosinophilia and aspirin-exacerbated respiratory disease. *Am J Respir Crit Care Med*. 2015; 192:682–694. [PubMed: 26067893]
30. Stevens WW, Peters AT, Suh L, et al. A retrospective, cross-sectional study reveals that women with CRSwNP have more severe disease than men. *Immun Inflamm Dis*. 2015; 3:14–22. [PubMed: 25866636]
31. Zuo K, Guo J, Chen F, et al. Clinical characteristics and surrogate markers of eosinophilic chronic rhinosinusitis in Southern China. *Eur Arch Otorhinolaryngol*. 2014; 271:2461–2468. [PubMed: 24493563]
32. Feng CH, Miller MD, Simon RA. The united allergic airway: connections between allergic rhinitis, asthma, and chronic sinusitis. *Am J Rhinol Allergy*. 2012; 26:187–190. [PubMed: 22643942]
33. Stevens WW, Schleimer RP. Aspirin-exacerbated respiratory disease as an endotype of chronic rhinosinusitis. *Immunol Allergy Clin North Am*. 2016; 36:669–680. [PubMed: 27712762]
34. Wang X, Zhang N, Bo M, et al. Diversity of TH cytokine profiles in patients with chronic rhinosinusitis: a multicenter study in Europe, Asia, and Oceania. *J Allergy Clin Immunol*. 2016; 138:1344–1353. [PubMed: 27544740]

FIGURE 1.

ECP levels in UT and NP compared between patient groups. (A) UT ECP levels were elevated in patients with asthma and (B) AERD. (C) Women had higher UT ECP levels compared to men. Boxes represent medians with 25th and 75th percentiles, whiskers represent maximum and minimum values. * $p < 0.05$, ** $p < 0.01$ by Mann-Whitney U test. AERD = aspirin-exacerbated respiratory disease; ECP = eosinophil cationic protein; NP = nasal polyp; UT = uncinat tissue.

FIGURE 2.

CT scores were more strongly correlated with (A) ECP levels in UT than (B) in NP.

Correlation coefficient calculated using Spearman's rank correlation test. ** $p < 0.01$, *** $p < 0.001$. CT = computed tomography; ECP = eosinophil cationic protein; NP = nasal polyp; UT = uncinat tissue.

FIGURE 3.

Kaplan-Meier curves of the recurrence-free rate in patients stratified by ECP (ng/mg total protein) levels in UT and NP. Significantly worse recurrence-free survival in patients with increased ECP levels in UT (A) but not in NP (B). * $p < 0.05$. (C) Number of patients at risk for recurrence at the end of each time point. ECP = eosinophil cationic protein; ESS = endoscopic sinus surgery; NP = nasal polyp; UT = uncinat tissue.

TABLE 1

Study cohort characteristics

Characteristic	UT (n = 116)		p	NP (n = 116)		p
	Eosinophilic (n = 68)	Non-eosinophilic (n = 48)		Eosinophilic (n = 73)	Non-eosinophilic (n = 43)	
Asthmatic, n (%)	41 (60.3)	19 (39.6)	0.028 *	40 (54.8)	20 (46.5)	0.389
AERD, n (%)	7 (10.3)	1 (2.1)	0.086	7 (9.6)	1 (2.3)	0.136
Atopic, n (%) ^a	46 (70.8)	26 (55.3)	0.092	43 (61.4)	29 (69.0)	0.415
Female, n (%)	29 (42.6)	10 (20.8)	0.014 *	25 (34.2)	14 (32.6)	0.853
Prior surgery, n (%)	16 (23.5)	5 (10.4)	0.071	14 (19.2)	7 (16.3)	0.695
Recurred within 4 years, n (%)	21 (30.9)	6 (12.5)	0.021 *	20 (27.4)	7 (16.3)	0.171
Preoperation oral steroid use, n (%) ^b	18 (26.9)	12 (26.1)	0.927	19 (26.7)	11 (26.1)	0.947
Age (years), median (IQR)	43.0 (33.3–52.0)	47.5 (37.3–54.8)	0.064	45.0 (33.0–52.5)	47.0 (38.0–53.0)	0.243
Lund-Mackay score, median (IQR) ^c	15.5 (13.0–19.0)	11.0 (9.0–13.3)	<0.001 *	15.0 (11.0–18.0)	12.0 (10.0–15.0)	0.032 *
Follow-up (months), median (IQR)	8.2 (1.6–19.7)	10.4 (2.8–26.5)	0.514	8.6 (1.6–19.8)	10.5 (2.5–24.4)	0.544

^a n = 112.^b n = 113.^c n = 114.* $p < 0.05$.

AERD = aspirin-exacerbated respiratory disease; IQR = interquartile range; NP = nasal polyp; UT = uncinate tissue.

TABLE 2

Cox proportional hazard model for polyp recurrence within 4 years comparing UT and NP ECP

Factor	Recurrence rate (%)	Hazard ratio	95% CI	<i>p</i>
UT ECP (ng/mg total protein)				
290	30.9	2.84	1.14–7.03	0.025 *
<290	12.5	Reference		
NP ECP (ng/mg total protein)				
290	27.4	1.74	0.74–4.13	0.207
<290	16.3	Reference		

*
 $p < 0.05$.

CI = confidence interval; ECP = eosinophil cationic protein; NP = nasal polyp; UT = uncinate tissue.

TABLE 3

Multivariate Cox proportional hazard model for polyp recurrence within 4 years comparing UT and NP ECP

Factor	Hazard ratio	95% CI	<i>p</i>
UT ECP (ng/mg total protein)			
290	2.80	1.02–7.65	0.045 *
<290	Reference		
Postoperative nasal steroid spray use	0.55	0.23–1.32	0.179
Postoperative oral steroid use	1.42	0.57–3.55	0.453

*
 $p < 0.05$.

CI = confidence interval; ECP = eosinophil cationic protein; NP = nasal polyp; UT = uncinat tissue.