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Eosinophil peroxidase: a biomarker for eosinophilic chronic rhinosinusitis agnostic of polyp status

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Abstract

Objective: To evaluate eosinophil peroxidase (EPX) as a biomarker for tissue levels of eosinophilia, cytokines, and chemokines within chronic rhinosinusitis (CRS).

Methods: Twenty-eight subjects undergoing sinonasal surgery were prospectively enrolled. Ethmoid tissue was analyzed with an in-house EPX immunoassay and a 48-plex cytokine-chemokine array. Clinical severity was assessed using SNOT-22 and Lund-Mackay scores. Subjects were grouped as follows: controls, polyp status (CRS with [CRSwNP] and without nasal polyps [CRSsNP]), tissue eosinophilia (eosinophilic CRS [eCRS], non-eosinophilic CRS [neCRS]), or combinations thereof (eCRSwNP, eCRSsNP, neCRSsNP). eCRS was defined as >10 eosinophils per high power field (HPF). Subjects without CRS or asthma were enrolled as controls.

Results: EPX was elevated in CRSwNP compared to control (p=0.007), in eCRS compared to neCRS (p=0.002), and in eCRSwNP along with eCRSsNP compared to neCRSsNP (p=0.023, p=0.015, respectively). eCRS displayed elevated IL-5 compared to neCRS (p=0.005). No significant differences in EPX or IL-5 were observed between eCRSwNP and eCRSsNP. IL-5 was elevated in eCRSwNP (p=0.019) compared to neCRSsNP. Area under the receiver operator characteristic curve was 0.938 (95% CI, 0.835-1.00) for EPX and tissue eosinophilia, with an optimal cut-point of 470 ng/mL being 100% specific and 81.25% sensitive for tissue eosinophilia. Linear regression revealed a strong correlation between EPX and IL-5 (R^2=0.64, p<0.001). Comparing EPX and IL-5, only EPX displayed significant correlation with SNOT-22 (p=0.04) and Lund-Mackay score (p=0.004).

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Level of Evidence: 3

Conclusion: EPX is associated with tissue eosinophilia in CRS patients regardless of polyp status. EPX correlates with IL-5 and could be potentially considered a biomarker for anti-IL-5 therapies.

Lay Summary:

Eosinophil peroxidase may prove a valuable biomarker to identify patients with eosinophilic chronic rhinosinusitis.

Keywords

biomarker; sinusitis; chronic rhinosinusitis; nasal polyps; eosinophil peroxidase; interleukin 5; IL-5; eosinophilia

Introduction

The classic scheme of chronic rhinosinusitis (CRS) classification based on nasal polyp status has provided a long-standing valuable management paradigm for CRS,[1,2] but is now recognized as inadequate in capturing the molecular diversity within the CRS population and the individual patient.[3,4] Substantial molecular heterogeneity is observed within the CRS population,[5-7] and polyp-based categorizations oversimplify underlying pathophysiologic processes. However, biomarkers for assessing endotypes are not clearly defined yet.[8] In addition, CRS patients with polyps (CRSwNP) remain the primary population assessed in most clinical trials for biomarkers.[8]

Classification by tissue eosinophilia status (eosinophilic CRS, eCRS) has been proposed to more accurately identify CRS endotype.[4] eCRS has been shown to be associated with greater prevalence of polyps[9] and increased recurrence rate in CRS.[10] However, most studies on eCRS have focused on polyp-positive patients with eosinophilia, with a paucity of molecular data on non-polyp patients with eosinophilia.[11] In practice, there is a tendency to associate CRSwNP with eosinophils and CRSsNP with non-eosinophilic disease. However, eCRSwNP and non-eosinophilic CRSwNP (neCRSwNP) may have distinct mRNA and mi-RNA profiles,[12-14] suggesting immunological heterogeneity among CRSwNP patients. While higher in CRSwNP,[15] tissue eosinophilia is also common in CRS without nasal polyps (CRSsNP).[7] We previously demonstrated that over one-third of CRS patients undergoing surgery had tissue eosinophilia greater than 10 per high power field (HPF), and tissue eosinophilia correlated with higher patient reported 22-item sinonasal outcome test (SNOT-22) scores.[16] CRSwNP have higher revision surgery rates,[17] but eCRSsNP patients have been shown to experience the least improvement in quality of life after sinus surgery and standard of care.[18]

The gold standard for definition of eCRS is histopathological profiling,[19] but there is lack of widespread adoption of standardized histopathology in clinical practice, as well as a lack of consensus regarding the most appropriate cutoff value to denote “significant” tissue eosinophilia. A widely used cutoff is >10 eosinophils/HPF.[19,20] However, quantifying tissue eosinophilia with histopathology may be imperfect, as eosinophil death with release of extracellular traps is common in eCRS,[21] and degranulated eosinophils are difficult to

measure with standard histology. Biomarkers have the potential to overcome this limitation, and those explored in research settings have included serum levels of eosinophil cationic protein (ECP), eosinophil derived neurotoxin (EDN), and eosinophil peroxidase (EPX) and tissue levels of IL-5.[22] The qualities of useful biomarkers may include ease of acquisition, high degree of specificity and sensitivity, and actionable results. EPX holds advantages over other assessed biomarkers. ECP, EDN, EPX, and major basic protein-1 (MBP-1) are all eosinophil cationic granule proteins, though ECP and EDN have been shown to be present in neutrophils, while MBP-1 is present in mast cells and basophils.[23-25] MBP-1 has been shown to be elevated in nasal mucus from CRS patients though it had lower presence in the tissues from CRS patients.[26] EPX is a biomarker that is highly sensitive in assaying both degranulated and non-degranulated eosinophils. The ability to measure degranulated eosinophils is particularly important as histology is poor for measuring degranulated eosinophils and may under-estimate the burden of CRS. Additionally, EPX is the only marker among these not shown to be associated with other leukocytes.

Additionally, studies of eCRS have largely used patients with polyps, making it difficult to discern whether these biomarkers can be applied to eCRS in general or only to those with polyps. Previously, we found EPX histopathology staining was significantly higher in CRS tissue compared with controls.[27] Prior work in our laboratory developed a high throughput assay for EPX quantification and demonstrated increased EPX in nasal lavage from CRS over control subjects as well as CRSwNP over CRS.[28]

In this study, to investigate and evaluate the utility of tissue levels of EPX to identify patients with eCRS, we analyzed the tissue levels of EPX, along with an unsupervised array of chemokines and cytokines measured through a 48-plex commercial kit. Additionally, we assessed the predictive ability of EPX for eCRS, with a specific focus on comparing patients based on combined eosinophilia and polyp status (e.g., eCRSsNP versus eosinophilic CRSwNP [eCRSwNP]). To accomplish these goals, we utilized prospectively collected sinonasal tissue samples and analyzed them through an in-house EPX and multiplex immunoassay.

Materials and Methods

This study was approved by the Mayo Clinic Institutional Review Board (IRB #16-008609). Twenty-eight subjects were prospectively enrolled in the study. Written consent was obtained from all subjects. Symptoms, nasal endoscopy and paranasal sinus CT scans were obtained in all subjects to classify them into CRSwNP, CRSsNP, and non-CRS (i.e., control) using 2015 American Academy of Otolaryngology-Head and Neck Surgery consensus guidelines.[29] Exclusion criteria included history of immunodeficiency disorders, oral corticosteroid therapy in the last 6 months, oral antimicrobial therapy in the last one month, or current use of biologic therapy.

Demographics, clinical diagnoses, asthma status, SNOT-22 scores, serum IgE levels, peripheral blood eosinophil counts, and sinus CT scan scores (Lund-Mackay [LM] stage) information was prospectively collected. Asthma and allergic rhinitis status was confirmed by an Allergy-Asthma specialist. Tissue specimens were obtained from ethmoid sinuses of

patients with CRS undergoing endoscopic sinus surgery and control subjects undergoing surgery for non-CRS indications (e.g., endoscopic skull base surgery). One half of each CRS sample was biobanked through rapid freezing in liquid nitrogen with no preservative added. Specimens were assigned unique identification numbers on each specimen container. Specimens were stored at −80°C until retrieval for analysis. The other half was sent for structured histopathological analysis in the format described by Snidvongs et al.[19] Eosinophilic CRS was classified as >10 eosinophils per HPF. Patients were sub-classified based on tissue eosinophilia (i.e., >10 eosinophils per HPF) into CRSsNP with or without eosinophilia (i.e., eCRSsNP, neCRSsNP) and CRSwNP (i.e., eCRSwNP as all had eosinophilia)

Tissue specimens were processed for immunoassays as described previously.[30] Briefly, frozen specimens were weighed, thawed, and mixed with an equal volume of phosphate-buffered saline (PBS) with a cocktail of protease inhibitors (Millipore Sigma, Burlington, MA) then homogenized with microcentrifuge pestles. After vortexing vigorously for 30 seconds, the samples were centrifuged, and the supernatants were collected for analyses. The samples were analyzed for the levels of cytokines and chemokines (48-plex) using manufacturer protocol with a Millipore multiplex kit (Billerica, MA) on a Bio-Rad MAGPIX multiplex reader (Hercules, CA). Samples below the minimum detectable concentration (MinDC) were assigned half the value of the MinDC, while values above the standard curve limit were assigned the highest value obtained from the respective standards. Cytokines and chemokines detected in less than 10% of samples (17 cytokines/chemokines) were excluded from analysis. Median values for all cytokines analyzed can be found in Table 1. Levels of EPX from cell lysates were assessed using an in-house sandwich enzyme-linked immunosorbent assay (ELISA) similar to that described previously by Ochkur et al.[28]

Statistical comparisons between continuous variables were performed using Wilcoxon-rank sum test, Kruskal-Wallis with Dunn’s test adjusted with Bonferroni correction for multiple comparisons, or Kruskal-Wallis with Benjamini Hochberg adjustment within the cytokine array data for the most commonly used CRSwNP, CRSsNP, and control groupings. Comparisons between categorical variables were performed utilizing Chi Squared or Fisher’s exact test when applicable. Correlation between EPX and other cytokines was calculated using Pearson correlation. Optimal cut point from Receiver Operator Curve (ROC) was calculated using Youden’s J statistic. P-values <0.05 were considered significant. Statistical analysis was performed in RStudio Team (2022, Boston, MA) and GraphPad Prism (Version 9.2.0 for Windows, GraphPad Software, San Diego, CA).

Results

Of the 28 samples analyzed 32.1% (9/28) were from men (Table 2). Three CRSwNP patients had a diagnosis of aspirin exacerbated respiratory disease (AERD). All CRSwNP patients had eCRS, while 50% of CRSsNP had eCRS. Prevalence of asthma and allergic rhinitis was not significantly different between both CRSwNP and CRSsNP groups. Serum IgE non-significantly trended higher in the CRSsNP group. Clinical characteristics of groups inclusive of eosinophilia are detailed in Table 3. The eCRSsNP group tended to have increased proportion of allergic rhinitis than neCRSsNP, though non-significantly. Asthma

and serum IgE levels displayed no significant differences across groups. Peripheral blood eosinophils tended to be elevated in the eCRSsNP and eCRSwNP groups as compared to neCRSsNP, though again non-significantly.

Comparison of the tissue levels of EPX revealed elevated EPX in CRSwNP over control samples, in eCRS over CRS samples, and in eCRS (both CRSsNP and eCRSwNP) over neCRSsNP samples (Fig 1A). Also noted from these comparisons was that there existed no significant difference between CRSwNP and CRSsNP or eCRSwNP and eCRSsNP. No significant differences were found in any other cytokines analyzed between CRSwNP and CRSsNP groups (Table 4). Tissue IL-5 was significantly increased in eCRS as compared to neCRS (Fig 1B). Tissue IL-5 was also significantly elevated in eCRSwNP compared to neCRSsNP (Fig 1C). Additionally, MDC (CCL22), a chemokine for lymphocytes, was significantly elevated in eCRSsNP as compared to neCRSsNP (Fig 1C). Significant differences were not found for other cytokines utilizing this combined phenotype-histotype categorization (Table 5).

ROC analysis of predictive ability of EPX for tissue eosinophilia status revealed area under the curve (AUC) of 0.938 (Figure 2A, $p=0.002$) with high sensitivity and specificity at an optimal cut-point of 470 ng/ml. ROC analysis of predictive ability of IL-5 for tissue eosinophilia status was significant with AUC of 0.875 (Figure 2B, $p=0.008$), though with lower sensitivity at the optimal cut-point. Linear regression analysis revealed a significant correlation between EPX and IL-5 with R of 0.81 and R^2 of 0.64 (Figure 2C, $p<0.001$). With the exception of IL-5, no other strong correlations were found between EPX and all other cytokines and chemokines that were assayed (Supplemental Table 1).

Finally, as expected, Lund-Mackay scores were significantly elevated in the CRSwNP patients over neCRSsNP but not over eCRSsNP (Figure 3A). No significant differences were observed in the Lund-Mackay scores between eCRSsNP and neCRSsNP. There were no significant differences in SNOT-22 scores among these three groups (Figure 3B). Correlation of EPX and IL-5 with SNOT-22 and Lund-Mackay scores revealed stronger correlation between EPX with these measures, with R of 0.55 vs. R of 0.40 for Lund-Mackay scores (Figure 3C) and R of 0.38 vs. R of 0.14 for SNOT-22 (Figure 3D). While these correlations are not very strong, they were also significant for EPX and insignificant for IL-5 (SNOT-22 $p=0.041$, Lund-Mackay $p=0.004$).

Discussion

Use of phenotypic classifications must be supplanted by use of biomarkers that subtype inflammation so as to personalize therapy in CRS. We found EPX to be consistently elevated in eCRS regardless of grouping by nasal polyp status. As expected, IL-5 was found to be significantly higher in eCRS over neCRS. EPX was also shown to correlate closely with IL-5. Importantly, separating into eCRSsNP and neCRSsNP revealed that eCRSsNP had elevated EPX similar to eCRSwNP. However, no significant difference in the IL-5 levels was observed between neCRSsNP and eCRSsNP, suggesting that EPX may be more useful than IL-5 as a biomarker to identify patients with eCRS irrespectively of polyp status. We speculate that the lack of difference in IL-5 levels between eCRS groups with or

without polyps may be due to the smaller sample size or that IL-5 levels may contribute disproportionately to nasal polyp formation in eCRS. Indeed, both IL-5 and chemokines are necessary for effective recruitment of eosinophils to the inflamed tissues.[31] Interestingly, EPX uniquely has been shown to modify mucous “density” forming plugs in sputum and may have similar functions in CRS in promoting features of CRS disease.[32]

Elevation of macrophage derived chemokine (MDC/CCL22) was noted in eCRSsNP. MDC is a potent chemoattractant for immature dendritic cells and Th2 cells,[33-35] and has been proposed as a stimulus of eosinophil degranulation.[36] It is constitutively expressed in macrophages and dendritic cells, with variable expression in T cells and NK cells.[33] mRNA levels of MDC have previously been shown to be elevated in CRSwNP,[37] and we previously identified MDC protein as part of a cytokine cluster associated with non-polyp patients and controls.[30] Elevated protein levels of MDC have been found in allergic rhinitis[38] and atopic dermatitis[39] patients, with levels corresponding to disease severity in the latter patients. Murine studies have also shown that MDC-deficient mice had increased susceptibility to inflammatory diseases along with excessive T cell responses.[40] The discovery of elevated MDC within solely the eCRSsNP group raises some intriguing possibilities regarding the roles of this chemokine in immunity and chronic airway inflammation.

Other investigators have shown that serum EPX levels lacked correlation with eCRS and CRS.[41] Given the current findings and in our prior studies, we posit that EPX may be confined to the local mucosa. Currently the most widespread biomarkers for eCRS are blood or tissue eosinophilia and serum IgE.[42] Serum IgE has been shown to correlate with tissue IL-5 levels but only in CRSwNP patients.[43] While blood eosinophil count remains the most accessible and useful of these markers,[22] sensitivity and specificity for predicting eCRS is below 80%.[9] Additionally, diurnal variation in eosinophil counts are observed[44], making accurate enumeration of eosinophils challenging. Serum levels of IL-5, a key cytokine in CRSwNP,[5] has been shown to correlate with serum EDN in eCRS patients,[41] though it has not been demonstrated in nasal tissue directly.

In this study, we found that the levels of EPX strongly correlate specifically with those of IL-5 and not any other cytokines or chemokines immunoassayed by the 48-plex kit. Given that IL-5 is a primary cytokine involved in type 2 airway inflammation, the strong relationship between IL-5 and EPX points to the potential role of EPX in Th2-type inflammation. Furthermore, we found that EPX levels were demonstrably higher if patients had eCRS regardless of the polyp status. We also observed that EPX was significantly correlated with SNOT-22 and Lund-Mackay scores. Therefore, EPX may potentially be used to inform medical decision making in the use of biological agents targeting IL-5 or eosinophils. Of note however, we did not demonstrate significantly elevated IL-5 levels within eCRSsNP patients, so EPX analysis within this group may potentially be superior to assess eosinophilic inflammation. To elaborate on this point, the disconnect between IL-5 and EPX in eCRSsNP patients indicates that EPX may not be simply an indicator of Th2-type inflammation. This alludes to the complexity of classifying CRS subtypes, as Th2-type inflammation, as well as eosinophilia alone, may be too simplistic a viewpoint for classification. The most useful classification scheme and biomarker for CRS would be the one that allows prediction of patient outcomes and guidance for optimal treatment

approaches. Current tools based on Th2 and eosinophilia classification may be convenient but may not reflect the heterogeneity and complexity in the CRS populations. While the current study utilized tissue samples for cytokine analysis, our results are consistent with prior studies demonstrating elevated EPX in nasal lavage of CRS patients[28] or nasal swabs from poorly controlled asthma patients.[45] Exploration of these non-invasive techniques in CRS patients with a focus on eCRS could establish an accessible method for assessment of eCRS that might be potentially superior to use of structured histopathology. Given the disease burden and high recurrence rates within eCRS patients,[10] EPX could assist significantly in treatment paradigms.

Limitations of this study include the small sample size. No CRSwNP patients in this study had neCRS, though this is reflective of the type 2 inflammation in CRSwNP in the United States. This limits the generalizability and comparability between subgroups, making it difficult to dissect the effect of nasal polyp versus eosinophilia status in CRSwNP patients. Additionally, AERD was not excluded from this study, and the presence of three AERD patients within the CRSwNP patients may overrepresent this disorder within CRSwNP populations as a whole.[46] In terms of defining tissue eosinophilia, we used a cutoff score of 10/HPF, and this is not universally accepted. Prior studies have proposed that most predictive of eCRS recurrence is >55 eosinophils per HPF.[10] In addition, other methods for endotyping CRS, such as urinary leukotriene E4,[47] IgE,[9] and peripheral blood eosinophil count,[9] may be considered and used in conjunction with EPX.

Finally, we acknowledge that this current study utilizes tissue sampling, an invasive methodology. Our future directions include measuring EPX in nasal passages through non-invasive methodology, such as nasal swab and washes, and validation studies in a wide spectrum of neCRS and eCRS subjects. Further work to assess if this relationship holds true with the use of topical nasal swabs or nasal lavage could create the basis for a clinical tool allowing non-invasive assessment of tissue eosinophilia. This study also lacked investigation of EPX levels following surgical intervention and correlation of EPX levels with outcomes. Future studies should include multiple timepoints to address this and to clarify alterations of EPX in response to interventions. EPX needs also to be assessed for its use in guiding clinical decision-making for anti-eosinophil or IL-5 therapies. Although several anti-IL-5 biologics are currently approved for use in nasal polyps, not all patients respond to these therapies; biomarkers that might predict responsiveness is a critical need.[48,49,50] Additionally, the chemokine MDC (CCL22) was identified as a potential molecular marker specific to eCRSsNP that warrants further investigation, as eCRSsNP patients remain an understudied population that suffers from poor outcomes.

Strengths of this study include the use of both nasal polyp and tissue eosinophilia characteristics, which allowed for analysis of molecular factors driving what could be two distinct processes: eCRS and nasal polyp formation. The use of a commercially available cytokine array allows for an unsupervised non-biased selection of chemokines and cytokines for study, as well as ease of access, reproducibility and verification. Despite the substantial number of cytokines assessed by the 48-plex assay, EPX specifically correlated strongly with only IL-5. In addition, in contrast to other cytokines such as ECP which is also produced by neutrophils, EPX is eosinophil specific.[23] Finally, EPX captures granulated

as well as degranulated eosinophils, and may be superior to classifying purely based on histopathology which might under-estimate disease by not accounting for degranulated eosinophils. Our study illustrates the potential utilization of EPX as a biomarker to identify eCRS agnostic of polyp status.

Conclusions

EPX from tissue serves as a marker for tissue eosinophilia regardless of patient polyp status and has a strong relationship with IL-5. No significant differences in EPX or IL-5 were observed between eCRSwNP and eCRSsNP. In addition, in contrast to other biomarkers investigated for eCRS, EPX is specific to eosinophils. Finally, in contrast to histopathology, which might under-estimate disease by not accounting for degranulated eosinophils, EPX sensitively assays both granulated and degranulated eosinophils. Our study illustrates the potential utilization of EPX as a biomarker to identify eCRS agnostic of polyp status and could have additional use in guiding clinical decision-making for anti-eosinophil or IL-5 therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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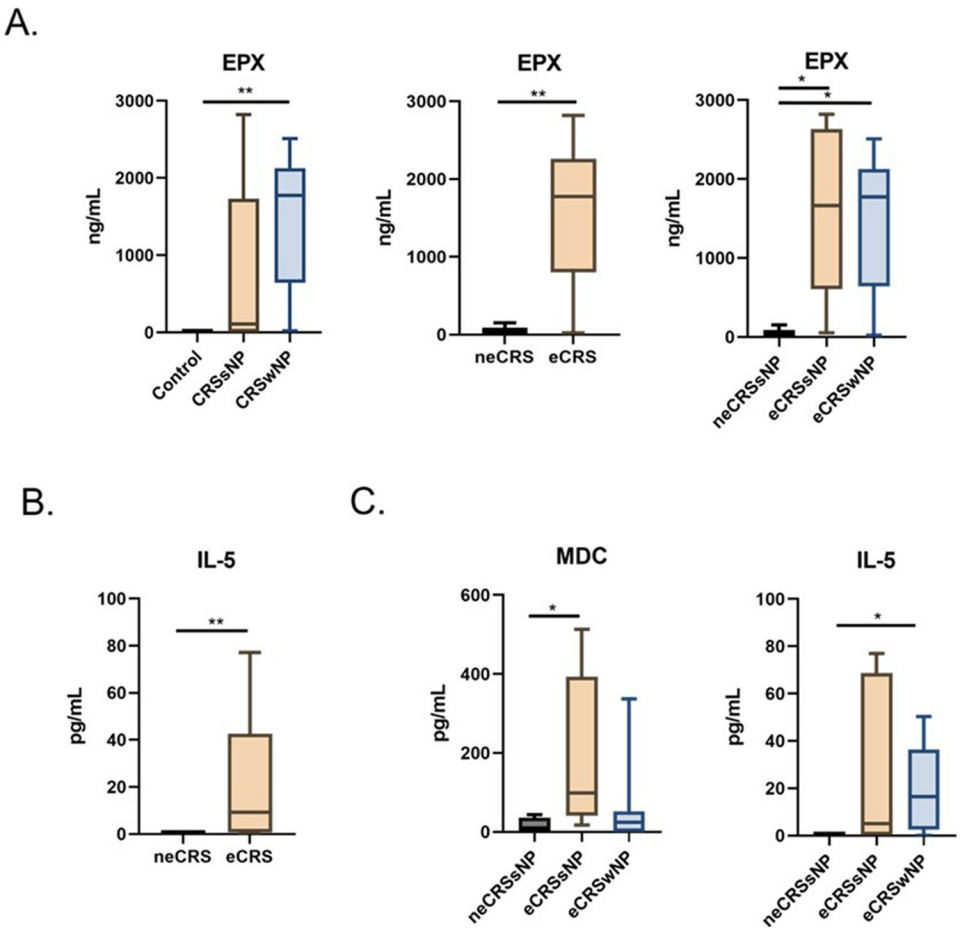
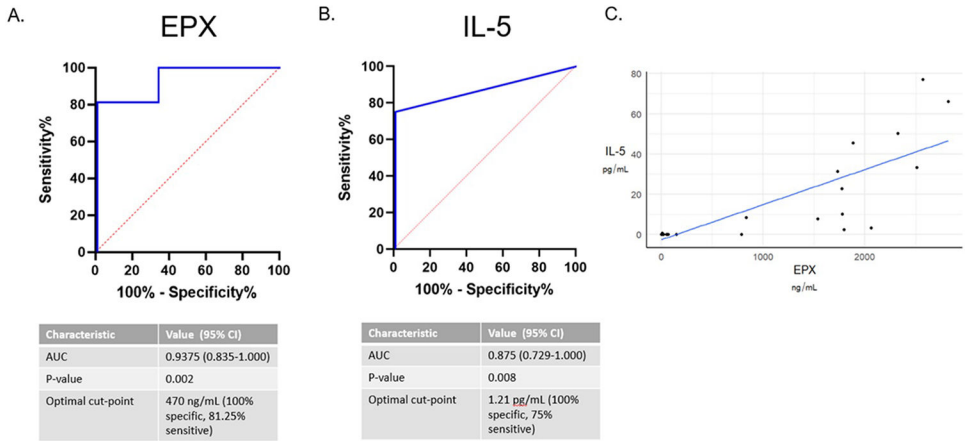


Figure 1.

A. Box and whisker plots of comparing EPX values across the three groupings of CRS patients. **B.** Box and whisker plots comparing IL-5 in eCRS with neCRS. **C.** Box and whisker plots comparing neCRSsNP, eCRSsNP, and eCRSwNP. * indicates $p<0.05$, ** indicates $p<0.01$



Characteristic	Value (95% CI)
AUC	0.9375 (0.835-1.000)
P-value	0.002
Optimal cut-point	470 ng/mL (100% specific, 81.25% sensitive)

Characteristic	Value (95% CI)
AUC	0.875 (0.729-1.000)
P-value	0.008
Optimal cut-point	1.21 pg/mL (100% specific, 75% sensitive)

Figure 2.

A. Receiver operating characteristic (ROC) curve assessing predictive ability of EPX for classification into eCRS category (tissue eosinophilia >10 per high power field). **B.** ROC curve assessing predictive ability of IL-5 for classification into eCRS category. **C.** Linear regression curve for IL-5 as a function of EPX.

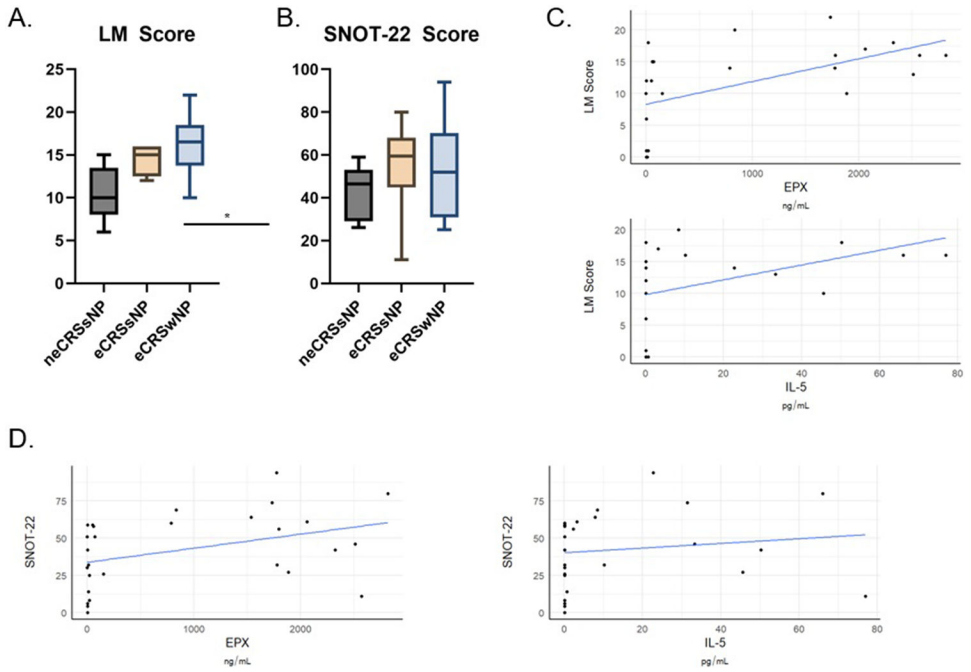


Figure 3.

A. Box and whisker plot comparing Lund-Mackay CT score between neCRSsNP, eCRSsNP, and eCRSwNP. **B.** Box and whisker plot comparing SNOT-22 score between neCRSsNP, eCRSsNP, and eCRSwNP.**C**. Linear regression curve for Lund-Mackay score as a function of EPX and IL-5. **D**. Linear regression curve for SNOT-22 score as a function of EPX and IL-5.

Table 1.

Median values of cytokines or chemokines in control, CRSsNP, and CRSwNP patients. All values in pg/mL except for EPX (ng/mL).

Cytokine/chemokine	Control	CRSsNP	CRSwNP
EGF	32.91	13.10	52.26
FGF2	12112.05	8996.76	5091.98
FLT3L	3.94	6.05	5.56
CX3CL1(Fractalkine)	34.07	32.16	96.46
CXCL1(GROα)	496.47	348.46	244.28
IFNγ	4.63	5.72	2.52
IL-1RA	47.46	365.56	76.40
IL-6	0.07	11.90	2.91
CXCL8(IL-8)	34.02	60.81	117.13
IL-12p40	55.39	60.05	46.08
IL-18	164.78	52.41	41.80
CXCL10(IP10)	304.62	130.24	104.82
CCL2(MCP1)	48.14	121.80	198.38
CCL7(MCP3)	11.80	6.76	9.97
MCSF	171.92	428.74	467.90
CCL22(MDC)	9.61	38.15	24.99
CXCL9(MIG)	15403.54	13466.40	7011.71
PDGF-AA	267.71	129.12	122.92
CCL5(RANTES)	1957.83	1479.02	828.94
TGFα	2.47	3.24	1.37
VEGF-A	1662.55	743.20	717.17
IL-5	0.09	0.09	16.45
EPX	7.00	111.00	1776.50
sCD40L	295.99	2.83	2.83
CCL11(Eotaxin-1)	1.54	19.87	18.00
GCSF	1.88	4.82	6.19
IL-4	0.10	0.10	0.10
IL-10	0.46	1.81	0.46
IL-13	1.29	1.29	1.29
IL-15	0.37	0.37	0.37
CCL3(MIP1α)	1.91	3.66	1.91
CCL4(MIP1β)	0.19	12.80	5.65

EGF: epidermal growth factor; FGF2: fibroblast growth factor 2; FLT3L: FMS-like tyrosine kinase 3 ligand; IFNγ: interferon gamma; IL-1RA: interleukin-1 receptor antagonist; IL-12p40: interleukin 12 subunit beta; CXCL10(IP10): interferon gamma-induced protein 10; CCL2(MCP1): monocyte chemoattractant protein 1; CCL7(MCP3): monocyte chemoattractant protein 3; MCSF: macrophage colony-stimulating factor; CCL22(MDC): macrophage-derived chemokine; CXCL9(MIG): monokine induced by gamma interferon; PDGF-AA: platelet derived growth factor AA; CCL5(RANTES): regulated on activation, normal T cell expressed and secreted; TGFα: transforming growth factor alpha; VEGF-A:

vascular endothelial growth factor A; EPX: eosinophil peroxidase; sCD40L: soluble CD40 ligand; GCSF: granulocyte colony-stimulating factor; CCL3(MIP1α): macrophage inflammatory protein-1 alpha; CCL4(MIP1β): macrophage inflammatory protein-1 beta

Table 2.

Clinical characteristics of subjects grouped by control and polyp status

Characteristic	n	Male, n (%)	AERD, n (%)	Tissue eosinophil count >10 per HPF, n (%)	Allergic rhinitis	Asthma	Serum IgE, median [SD], IU/mL	Blood eosinophil counts, median [SD], count x 10^9/L
Overall	28 (100%)	9 (32%)	3 (11%)	16 (27%)	17 (60%)	14 (50%)	103 [795] (n=14)	0.25 [0.17] (n=17)
Control, n (%)	6 (21%)	3 (50%)	0 (0%)	NA	1 (17%)	0 (0%)	NA	0.25 [0.08] (n=3)
CRSsNP, n (%)	12 (43%)	2 (17%)	0 (0%)	6 (50%)	9 (75%)	8 (67%)	500 [936] (n=8)	0.24 [0.21] (n=9)
CRSwNP, n (%)	10 (36%)	4 (40%)	3 (30%)	10 (100%)	7 (70%)	6 (60%)	37 [323] (n=6)	0.31 [0.14] (n=5)

SD: standard deviation

NA: not assessed

Table 3.

Clinical characteristics of subjects grouped inclusive of eosinophilia and polyp status

Characteristic	n	Male, n (%)	AERD, n (%)	Allergic rhinitis	Asthma	Serum IgE, median [SD], IU/mL	Blood eosinophil counts, median [SD], count x 10^9/L
neCRSsNP, n (%)	6 (27.3%)	1 (16.7%)	0 (0%)	3 (50%)	4 (67%)	99 [1481] (n=3)	0.12 [0.07] (n=4)
eCRSsNP, n (%)	6 (27.3%)	1 (16.7%)	0 (0%)	6 (100%)	4 (67%)	824 [655] (n=5)	0.36 [0.21] (n=5)
eCRSwNP, n (%)	10 (45.5%)	4 (40%)	3 (30%)	7 (70%)	6 (60%)	37 [324] (n=6)	0.31 [0.14] (n=5)

SD: standard deviation

Table 4.

Statistical comparisons within cytokines or chemokines between control, CRSsNP, and CRSwNP patients. Dunn's test was performed for final p-value <0.05.

Cytokine/chemokine	Kruskal-Wallis Test (*p*-value)	Benjamini Hochberg adjusted *p*-value	Dunn's Test (*p*-value)		
			CRSwNP vs CRSsNP	CRSwNP vs Controls	CRSsNP vs Controls
EPX	0.0089	NA	0.240	0.007	0.299
EGF	0.0348	0.4798			
FGF2	0.1851	0.5112			
FLT3L	0.5845	0.6591			
CX3CL1(Fractalkine)	0.0739	0.4798			
CXCL1(GROα)	0.9709	0.9709			
IFNγ	0.4073	0.6013			
IL-1RA	0.0935	0.4798			
IL-6	0.2128	0.5112			
CXCL8(IL-8)	0.1157	0.4798			
IL-12p40	0.6881	0.711			
IL-18	0.2183	0.5112			
CXCL10(IP10)	0.1077	0.4798			
CCL2(MCP1)	0.4546	0.6406			
CCL7(MCP3)	0.6263	0.6695			
MCSF	0.1167	0.4798			
CCL22(MDC)	0.1827	0.5112			
CXCL9(MIG)	0.2480	0.5112			
PDGF-AA	0.1393	0.4798			
CCL5(RANTES)	0.3062	0.5112			
TGFα	0.5703	0.6591			
VEGF-A	0.2855	0.5112			
IL-5	0.0215	0.4798			
sCD40L	0.2957	0.5112			
CCL11(Eotaxin-1)	0.3791	0.5876			
GCSF	0.2533	0.5112			
IL-4	0.5410	0.6591			
IL-10	0.3133	0.5112			
IL-13	0.5432	0.6591			
IL-15	0.5953	0.6591			
CCL3(MIP1α)	0.5402	0.6591			

Cytokine/chemokine	Kruskal-Wallis Test (*p*-value)	Benjamini Hochberg adjusted *p*-value	Dunn's Test (*p*-value)		
			CRSwNP vs CRSsNP	CRSwNP vs Controls	CRSsNP vs Controls
CCL4(MIP1β)	0.1391	0.4798			

EPX: eosinophil peroxidase; EGF: epidermal growth factor; FGF2: fibroblast growth factor 2; FLT3L: FMS-like tyrosine kinase 3 ligand; IFNγ: interferon gamma; IL-1RA: interleukin-1 receptor antagonist; IL-12p40: interleukin 12 subunit beta; CXCL10(IP10): interferon gamma-induced protein 10; CCL2(MCP1): monocyte chemoattractant protein 1; CCL7(MCP3): monocyte chemoattractant protein 3; MCSF: macrophage colony-stimulating factor; CCL22(MDC): macrophage-derived chemokine; CXCL9(MIG): monokine induced by gamma interferon; PDGF-AA: platelet derived growth factor AA; CCL5(RANTES): regulated on activation, normal T cell expressed and secreted; TGFα: transforming growth factor alpha; VEGF-A: vascular endothelial growth factor A; sCD40L: soluble CD40 ligand; GCSF: granulocyte colony-stimulating factor; CCL3(MIP1α): macrophage inflammatory protein-1 alpha; CCL4(MIP1β): macrophage inflammatory protein-1 beta

Table 5.

Statistical comparisons within cytokines or chemokines between neCRSsNP, eCRSsNP, and CRSwNP patients. Dunn's test was performed for p-value <0.05 from Kruskal-Wallis Test.

Cytokine/chemokine	Kruskal-Wallis Test (*p*-value)	Dunn's Test (*p*-value)		
		neCRSsNP vs eCRSsNP	neCRSsNP vs eCRSwNP	eCRSsNP vs eCRSwNP
EPX	0.0081	0.023	0.015	1.000
EGF	0.0392	1.000	0.060	0.202
FGF2	0.2533			
FLT3L	0.4046			
CX3CL1(Fractalkine)	0.1290			
CXCL1(GROα)	0.7370			
IFNγ	0.1139			
IL-1RA	0.1895			
IL-6	0.7184			
CXCL8(IL-8)	0.5330			
IL-12p40	0.1046			
IL-18	0.3010			
CXCL10(IP10)	0.5678			
CCL2(MCP1)	0.3939			
CCL7(MCP3)	0.5290			
MCSF	0.4109			
CCL22(MDC)	0.0328	0.034	1.000	0.156
CXCL9(MIG)	0.1015			
PDGF-AA	0.6979			
CCL5(RANTES)	0.1114			
TGFα	0.3849			
VEGF-A	0.2923			
IL-5	0.0192	0.107	0.019	1.000
sCD40L	0.5768			
CCL11(Eotaxin-1)	0.4123			
GCSF	0.7704			
IL-4	0.1980			
IL-10	0.2013			
IL-13	0.2845			
IL-15	0.9439			
CCL3(MIP1α)	0.4618			
CCL4(MIP1β)	0.0313	0.050	1.000	0.074

EPX: eosinophil peroxidase; EGF: epidermal growth factor; FGF2: fibroblast growth factor 2; FLT3L: FMS-like tyrosine kinase 3 ligand; IFNγ: interferon gamma; IL-1RA: interleukin-1 receptor antagonist; IL-12p40: interleukin 12 subunit beta; CXCL10(IP10): interferon gamma-induced protein 10; CCL2(MCP1): monocyte chemoattractant protein 1; CCL7(MCP3): monocyte chemoattractant protein 3; MCSF: macrophage colony-stimulating factor; CCL22(MDC): macrophage-derived chemokine; CXCL9(MIG): monokine induced by gamma interferon; PDGF-AA: platelet derived growth factor AA; CCL5(RANTES): regulated on activation, normal T cell expressed and secreted; TGFα: transforming growth factor alpha; VEGF-A: vascular endothelial growth factor A; sCD40L: soluble CD40 ligand; GCSF: granulocyte colony-stimulating factor; CCL3(MIP1α): macrophage inflammatory protein-1 alpha; CCL4(MIP1β): macrophage inflammatory protein-1 beta