ORIGINAL ARTICLE

Distinct eicosanoid patterns in severe recalcitrant nasal polyposis

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Abstract

Background: Although altered eicosanoid levels are related to disease severity in chronic rhinosinusitis with nasal polyps (CRSwNP), identifying patients prone to recurrent nasal polyps (NPs) is still difficult. We investigated levels of nasally secreted eicosanoids before and after NP surgery in patients with or without NP recurrence (NPR) and explored potential endotypes based on pre-surgical eicosanoid levels.

Methods: Levels of leukotriene (LT) E_4, LTB_4, prostaglandin (PG) D_2, PGE_2 and 15(S) hydroxyeicosatetraenoic acid (15[S]-HETE) were measured in nasal secretions with specific immunoassays at pre-surgery ($n = 38$) and 6 and 12 months post-surgery ($n = 35$), with NPR identified endoscopically. Pre- and post-surgical levels were compared between patients with and without NPR. Eicosanoid patterns among patients were explored with cluster analysis and evaluated with clinical parameters.

Results: Patients with recurrent NPs had pronounced pre-surgical levels of nasal 15(S)-HETE, PGD_2 and LTE_4. From pre-surgery to 12 months post-surgery, NPR was associated with significant decreases of 15(S)-HETE and PGD_2 relative to non-recurrence, whereas levels of LTE_4 decreased at 6 months but increased again at 12 months. Clustering revealed three potential endotypes. Clusters 1 and 3 featured high and low eicosanoid levels, respectively. Cluster 2 had higher levels of LTE_4 and PGD_2, lower levels of PGE_2 and LTB_4, and more cases of recurrent NPs and previous NP surgeries.

Conclusion: Elevated nasal LTE_4 12 months post-surgery in NP recurrent subjects suggests that postoperative LTE_4 measurements may indicate rapid NP regrowth. A distinct nasal eicosanoid profile may be used for the identification of the most severe recalcitrant patients in need of targeted immunomodulatory therapies.

KEYWORDS
biomarker, chronic rhinosinusitis, disease severity, eosinophilic rhinitis and nasal polyposis, post-operative

1 | INTRODUCTION

Chronic rhinosinusitis with nasal polyps (NPs; CRSwNP) is a persistent inflammatory condition of the paranasal sinuses that causes long-term nasal discharge, obstructions, facial pain and loss of smell.[1] Progression of CRSwNP impacts the quality of life and poses a socioeconomic burden to the health care system.[2] When conventional pharmacotherapy fails to control the disease progression, functional endoscopic sinus surgery (FESS) is performed.[3] However, multiple surgical interventions are often needed due to NP regrowth.[4,5] Comorbid asthma affects about half of the patients with CRSwNP and some of these patients develop intolerance to cyclooxygenase-1 inhibitors such as aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs). The combination of NSAID-intolerant asthma and CRSwNP is currently diagnosed as aspirin exacerbated respiratory disease (AERD) characterized by high dependency on oral corticosteroids (OCS) and revision surgery.[6]

CRSwNP is predominantly a type 2 T-helper cell (Th2)-driven inflammation characterized by interleukin (IL)-4, IL-5, IL-13, as well as infiltrating eosinophils, mast cells, and type 2 innate lymphoid cells (ILC2s).[7–9] In recent years, monoclonal antibodies (mAbs) that target IL-5 or IL-4/IL-13 signaling pathways have been introduced as add-on treatment.[10] High tissue infiltration of eosinophils is commonly regarded as a hallmark of difficult-to-treat CRSwNP. However, studies have shown that pharmacological depletion of eosinophils in peripheral blood or NP tissue may not improve symptoms or affect disease severity.[11] Furthermore, immuno-targeted treatment against IL-5 or the receptor for IL-4 and IL-13 did not affect the number of tissue eosinophils, whereas they significantly lowered the release of eicosanoids.[12,13]

Bioactive lipids derived from arachidonic acid, that is, eicosanoids, are implicated in CRS.[14] We have previously shown that changes in immunoreactive eicosanoid levels in nasal secretions are evident in higher NP severity and AERD.[15] Cysteinyl-leukotrienes (CysLTs; LTC_4, LTD_4, LTE_4) and prostaglandin (PG) D_2 are especially pronounced in AERD patients in parallel with a diminished release of anti-inflammatory PGE_2.[16] Mast cell-derived PGD_2 and CysLTs are implicated in Th2 inflammatory reactions with bronchoconstriction and eosinophil recruitment in severe asthma[17,18] and AERD.[19] Mast cells and other leukocytes may therefore be of significance in NP pathogenesis through a dysregulated eicosanoid metabolism.[12] In contrast, PGE_2 may have a protective role as broncho- and vasodilator[20] and suppressor of type 2 cytokine production by ILC2s.[21] Also, alterations of the mucosal epithelia are evident in CRSwNP, as 15-lipoxygenase (15-LO) expression was exclusively enhanced in epithelial cells from NPs and correlated with sinus disease severity.[22,23] Although the role of 15-LO-derived metabolites in NP pathogenesis is currently unclear, a lack of 15-LO expression by a loss-of-function mutation protects against NP formation.[24]

Our previous findings[15] suggest that NP severity is important to take into consideration during inflammatory characterization of CRSwNP patients. Current guidelines recommend treatment with biological therapies for recalcitrant and severe cases of CRSwNP.[3,25] However, to identify severe cases by predicting NP recurrence (NPR) is still difficult. Improved characterization of the underlying inflammatory processes in those with rapidly regrowing NPs is therefore needed. The clinical role of nasally secreted eicosanoids in severe recalcitrant cases remains to be elucidated. Thus, in the current study we aimed to assess eicosanoid levels in nasal secretions prior to surgery and 12 months post-surgery, comparing patients with and without NPR. Given the inflammatory heterogeneity of the nasal mucosa,[9] we also strived to explore potential eicosanoid-based endotypes in CRSwNP by cluster analysis.

2 | METHODS

2.1 | Follow-up enrollment of patients and assessment of clinical parameters

The present exploratory study is a follow-up study on patients recruited in a consequent manner at the Department of Otorhinolaryngology, Sophiahemmet Hospital, Sweden. CRSwNP was diagnosed in accordance with the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS).[26] The patients had previously undergone FESS which included bilateral dissection of the maxillary sinuses and complete ethmoidectomy (both anterior and posterior) performed in a similar manner on all patients. They were subsequently asked to participate in a follow-up study at their regular clinic appointments at 6 and 12 months post-surgery. Ethical approval was obtained from the Swedish Ethical Review Authority (2017/1461-31, 2019-06,185) and written informed consent was obtained from all participants. In line with the EPOS all participants were prescribed nasal steroids and saline rinses during the study. None of the patients were under OCS treatment ≤1 month prior to sinus surgery or follow-up visits. Furthermore, no patient was treated with an immune-targeted mAb at any time.

Thirty-eight patients with CRSwNP were included in the follow-up measurements. Pre-surgical values on nasal eicosanoids and other clinical parameters for the whole group were previously reported.[15] In the present study,

TABLE 1 Pre-surgical characteristics of patients with or without nasal polyp recurrence identified 12 months following functional endoscopic sinus surgery.

	Non-NPR (*n* = 23)	NPR (*n* = 15)	*p*-value (non-NPR versus NPR)
Age, years	49 (37–60)	43 (31–49)	0.07
Sex, F/M: *n* (% M)	4/19 (82.6)	6/9 (60)	0.15
Allergy, *n* (%)	7 (30.4)	6 (40.0)	0.73
Asthma, *n* (%)	10 (43.5)	4 (26.7)	0.33
AERD, *n* (%)	3 (13.0)	5 (33.3)	0.22
NP score	6 (4–6)	6 (6–8)	0.072
NP-high, *n* (%)	14 (60.9)	14 (93.3)	0.056
CT score	14 (14–18)	19 (18–22)	**0.006**
Prior surgery, *n* (%)	9 (39.1)	10 (66.7)	0.18
Self-reported anosmia, *n* (%)	10 (43.5)	13 (86.7)	**0.016**
B-eosinophil count/mL	400 (200–600)	600 (400–800)	0.085
CRP, mg/mL	<5	<5	–
Rescue OCS, *n* (%)[a]	2 (8.7)	4 (26.7)	0.19
Clinical variables with missing data			
	n = 20	*n* = 12	
SNOT-22	45 (34–62)	56.5 (39.3–72.0)	0.19
	n = 14	*n* = 10	
Smell test score	14.9 (1.00–26.8)	4.5 (0.0–8.5)	0.068
	n = 14	*n* = 9	
FeNO	37.5 (17.8–77.0)	37.0 (22.5–89.0)	0.59

Note: Differences between NPR and non-NPR were tested with Fisher's exact test and the Kruskal-Wallis test. Asthma indicates aspirin-tolerant asthma.
Bold values signifies a *p*-values below 0.05, i.e., highlighting a statistical significance between groups.
Abbreviations: AERD, aspirin exacerbated respiratory disease; CRP, C-reactive protein; CT, Lund-Mackay computed tomography; FeNO, fractional exhaled nitric oxide; NPR, NP recurrence; NP, nasal polyp; OCS, oral corticosteroids; SNOT-22, Sino-Nasal Outcome Test.
[a]Additional dose of OCS required after initial surgery.

data for the included CRSwNP patients were re-analyzed to compare patients with and without recurrence of NPs (Table 1).

Asthma and allergy were identified by confirmed diagnoses retrieved from medical records and questionnaires. AERD was defined as previous incidence of airway hypersensitive reactions to intake of aspirin or NSAIDs. Results of a smell test (Burghart Sniffin' Sticks Extended Test n-butanol, Medisense), the Sino-Nasal Outcome Test (SNOT-22), the occurrence of previous FESS, bilateral endoscopic NP scores (ranging from 0–8), and Lund-Mackay computed tomography (CT) scores (ranging from 0–24) were also obtained from our previous study.[15] Blood eosinophil count (HemoCue WBC DIFF System, Ängelholm, Sweden), fractional exhaled nitric oxide (FeNO; NIOX VERO, Aerocrine, Solna, Sweden), and C-reactive protein (Aidian Oy, Espoo, Finland) were included to indicate eosinophilia, type 2 airway inflammation, and ongoing infections, respectively.[15] Patients were further dichotomized into NP-low and NP-high with an NP score of ≤4 or ≥5, respectively. Lastly, cytokine levels in nasal secretions, analyzed with Luminex Bio-Plex 200 systems

(Hu 17-Plex cytokine panel, Bio-Rad, Hercules, CA, USA) were also retrieved from our previous study.[15]

Additional clinical data at pre-surgery, not previously shown, are self-reported sense of smell as anosmia, hyposmia, or normosmia, obtained from questionnaires. Subscores from the Burghart smell test are reported here to show different aspects of smell loss. As a routine at the clinic, all CRS patients were prescribed OCS directly after their FESS for 21 days; 30 mg once daily (OD) for 1 week, 20 mg OD for 1 week, and lastly 10 mg OD for 1 week. Additional needs for OCS after initial surgery were noted from medical records and/or questionnaires given at revisits. NPR was identified by endoscopy performed during the return visit to the clinic 12 months after surgery, defined as having an NP score of ≥1.

2.2 | Collection of samples

Nasal secretions and urine spot samples were collected at 6 and 12 months after surgery as previously described.[15] In brief, two swabs were pre-conditioned in 800 μL saline

each and placed into the bottom of the nasal cavities for 10 min. The swabs were subsequently transferred to a swab filter tube (Swab Storage tube, Salimetrics) and centrifuged at 10,000 g for 15 min for extraction of secretions. All samples were stored in −80°C prior to biomarker analyses.

2.3 | Biomarker measurements

Nasal secretions were serially diluted and analyzed as previously described [15] for the content of LTE_4, PGD_2, and 15[S] hydroxyeicosatetraenoic acid (15[S]-HETE) using specific and commercially available enzyme-linked immunosorbent assay kits (Cayman Chemical, Ann, MI, USA, kits: 501060, 512011, 534721) according to manufacturer's protocol. Likewise, urine was serially diluted and assayed for the content of LTE_4 and 11β-$PGF_{2\alpha}$ (Cayman Chemical, kit: 501060, 516521), adjusted for the level of urinary creatinine (Cayman Chemical, kit: 500701), to be presented as ng/mmol creatinine. Limit of detection ranged from 5.0 to 58.9 pg/mL for the above mentioned kits.

2.4 | Statistical analysis

Generalized estimating equations (GEE) were used to assess the difference in the mean change in eicosanoid levels over time between patients with NPR and non-recurrence. Time of measurements, NPR, and an interaction term were included as independent variables. Although normality is a common concern with smaller sample sizes, GEE was deemed appropriate as it can handle non-normal and skewed data.[27] Models included a robust estimator with autoregressive lag 1 working correlation matrix and a gamma identity link function. Estimated marginal means with standard deviation (SD) from each model are presented which account for within-subject correlation over time.

Prior to principal component analysis (PCA) and clustering, input eicosanoid variables were log-transformed followed by standardization (z-score) to account for positive skewness and different scales. PCA with orthogonal rotation and kaiser normalization was initially conducted to simplify the data into fewer uncorrelated dimensions while retaining trends and patterns in the data. This procedure was performed as correlations between measured eicosanoids were apparent and such variables tend to get weighted unproportionally during clustering.[28] The number of PCs retained was based on the eigenvalue scree plot; PCs with eigenvalues of >1 were retained. Agglomerative hierarchical cluster analysis using Ward's method on squared Euclidean distance with PC scores from each patient was employed as input. Large changes in the agglomerative coefficient were used to indicate the number of clusters. The cluster solution was further assessed visually with a PC plot and a dendrogram. One-way analysis of variance with multiple comparisons with Bonferroni adjustment was used lastly to check cluster quality in terms of within- and between-cluster variability.

Differences in clinical characteristics and mediator levels between groups or clusters were analyzed with Kruskal-Wallis or Fishers exact test with multiple comparisons test with Benjamini-Hochberg adjustment. R software v4.0.3. was used for Kruskal-Wallis tests with multiple comparisons test whereas all other analyses were conducted with IBM SPSS 27.0.1.0 (IBM Corp, Armonk, NY USA). Significance was considered at $p < 0.05$.

3 | RESULTS

Fifteen out of thirty-eight patients had NPR, identified endoscopically. Clinical characteristics for non-NPR and NPR patients at pre-surgery are shown in Table 1. These groups were comparable in terms of allergy, asthma, AERD, sex, and age as well as the incidence of previous sinus surgery. Furthermore, NPR patients tended to have a more severe NP score at pre-surgery. Similarly, CT scores and self-reported anosmia were significantly higher in patients with NPR. Although there were missing data, FeNO and SNOT-22 appeared to be similar between the groups, but patients with NPR tended to score lower in smell tests.

3.1 | Levels of nasally secreted eicosanoids

Mean values of nasally secreted 15(S)-HETE, PGD_2, and LTE_4 at pre-surgery, and at 6 and 12 months after surgery are illustrated in Figure 1 (upper panel). NPR patients were significantly associated with a larger drop in nasal 15(S)-HETE as compared to non-NPR (NPR mean change −402 ± 150 ng/mL vs. non-NPR mean change −3.86 ± 66.3 ng/mL, $p = 0.015$), as well as for PGD_2 (NPR mean change −0.64 ±0.20 ng/mL vs. non-NPR mean change 0.05 ± 0.11 ng/mL, $p = 0.002$), from pre-surgery to 12 months post-surgery. In addition, in NPR patients, levels of 15(S)-HETE tended to decrease to a larger extent than in non-NPR patients between pre-surgery and 6 months post-surgery (NPR mean change −352 ± 135 ng/mL vs. non-NPR mean change −83.1 ± 67.0 ng/mL, $p = 0.073$), whereas levels of PGD_2 appeared unchanged for both NPR and non-NPR. From 6 months to 12 months post-surgery, 15(S)-HETE levels tended to decrease in NPR patients
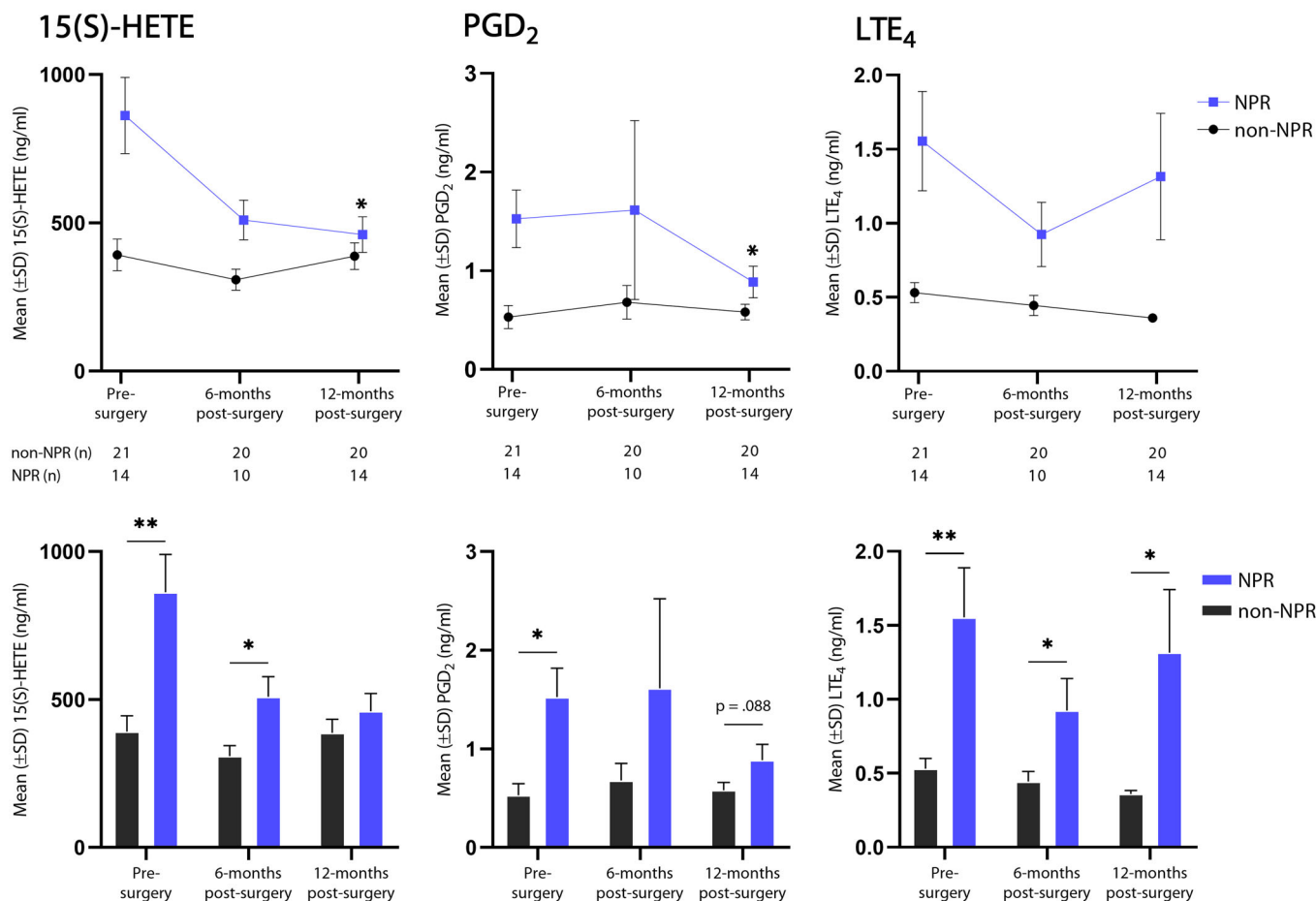
FIGURE 1 Upper panel: Mean change of nasal 15(S)-HETE, PGD$_2$, and LTE4 over time from pre-surgery to 6 and 12 months post-surgery, in patients with nasal polyp recurrence (NPR) as compared to patients with non-recurrence (non-NPR). Lower panel: Comparisons of nasal eicosanoid levels between NPR and non-NPR patients at each time point

(NPR mean change -49.2 ± 58.7 ng/mL vs. non-NPR mean change 79.3 ± 32.4 ng/mL, $p = 0.056$), whereas the mean change of PGD$_2$ did not significantly differ between NPR and non-NPR.

The mean change of LTE$_4$ from pre-surgery to 12 months post-surgery did not differ between NPR and non-NPR patients. There was a trend for levels in patients with NPR to be lowered to a larger extent between pre-surgery and 6 months post-surgery (NPR mean change -0.63 ± 0.32 ng/mL vs. non-NPR mean change -0.09 ± 0.09 ng/mL, $p = 0.09$). NPR patients showed an elevation of LTE$_4$ between 6 months and 12 months post-surgery, whereas non-NPR had a slight decrease, but without statistical significance (NPR mean change 0.40 ± 0.35 ng/mL vs. non-NPR mean change -0.08 ± 0.06 ng/mL, $p = 0.18$).

Post-hoc analyses (lower panel of Figure 1) showed that NPR patients were associated with significantly higher LTE$_4$ at pre-surgery ($p = 0.003$), and at 6 months ($p = 0.035$) and 12 months post-surgery ($p = 0.026$). For nasal levels of 15(S)-HETE, higher levels in NPR patients were observed at pre-surgery ($p < 0.001$) and 6 months

post-surgery ($p = 0.008$), whereas levels of PGD$_2$ in NPR patients were only significantly higher at pre-surgery ($p = 0.002$).

3.2 | Urinary 11β-PGF$_{2\alpha}$ and LTE$_4$

Urinary levels of 11β-PGF$_{2\alpha}$ and LTE$_4$ did not significantly change over time, from pre-surgery to 6 months or up to 12 months post-surgery in NPR or non-NPR patients (upper panel Figure 2). Statistically significant difference between NPR and non-NPR patients regarding urinary levels was observed of LTE$_4$ at 6 months post-surgery (lower panel Figure 2, $p = 0.036$).

3.3 | Emerging eicosanoid-based clusters in CRSwNP

The first two components from the PCA were retained which explained 76.4% of the variance in the eicosanoid
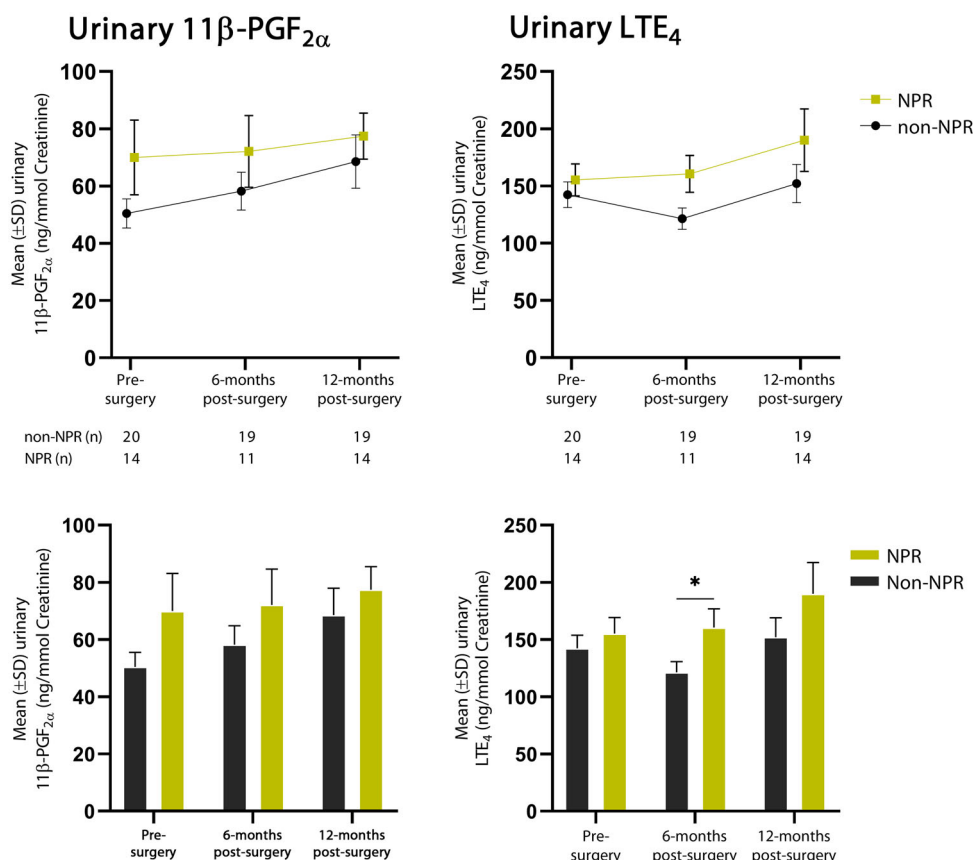
FIGURE 2 Upper panel: Mean change of urinary 11β-PGF2α and LTE4 over time from pre-surgery to 6 and 12 months post-surgery, in patients with nasal polyp recurrence (NPR) as compared to non-recurrence (non-NPR). Lower panel: Comparisons of urinary 11β-PGF2α and LTE4 between NPR and non-NPR patients at each time point

data. Figure 3A describes how each biomarker loads onto the retained PCs. Each eicosanoid marker was represented at least once in a PC. The patterns of eicosanoid loadings onto each PC reflected reasonable biological relationships. For instance, anti-inflammatory PGE_2 (represented in PC2) may be viewed as counterpart to the actions of LTE_4 and PGD_2 (represented in PC1) (Figure 3A).

Agglomerative hierarchical cluster analysis, utilizing PC values as input, revealed three potential underlying clusters. The changes in the mean agglomerative coefficient were minimal in the first steps of cluster formation, whereas large increases occur in the latter steps. Specifically, a pronounced increase in the agglomerative coefficient is apparent between the formation of the three- and two-cluster solutions (Figure 3B), indicating that the change in agglomerative coefficient is negligible between the fourth and the 37th cluster solution. Obtained clusters were separated when plotted according to PC1 and PC2 (Figure 3C), and visual evaluation of the following dendrogram further supported the three-cluster solution (Figure 3D). Furthermore, the mean score from each PC was significantly different between clusters, which further

support the appropriate discrimination of patients based on previous clustering (Table 2). Comparing the mean standardized level of each utilized biomarker revealed distinct eicosanoid patterns across the clusters (Figure 4). Cluster 2 was characterized by higher LTE_4 and PGD_2 and differed from cluster 1 by lowered PGE_2 and LTB_4. The values for 15(S)-HETE differed between clusters 1, 2, and 3, while cluster 3 had low levels of all measured nasal eicosanoids.

3.4 | Inflammatory and clinical differences between clusters

Eicosanoids illustrated as raw values instead of standardized scores showed similar inflammatory patterns across clusters (Figure 5). Both clusters 1 and 2 had significantly higher PGD_2 and LTE_4 relative to cluster 3, whereas cluster 2 was differentiated from cluster 1 by significantly lower levels of PGE_2 and LTB_4. Cluster 3 had significantly lower 15(S)-HETE relative to all other clusters. Levels of LTB_4 and PGE_2 were highest in cluster 1 and significantly different from the other clusters.
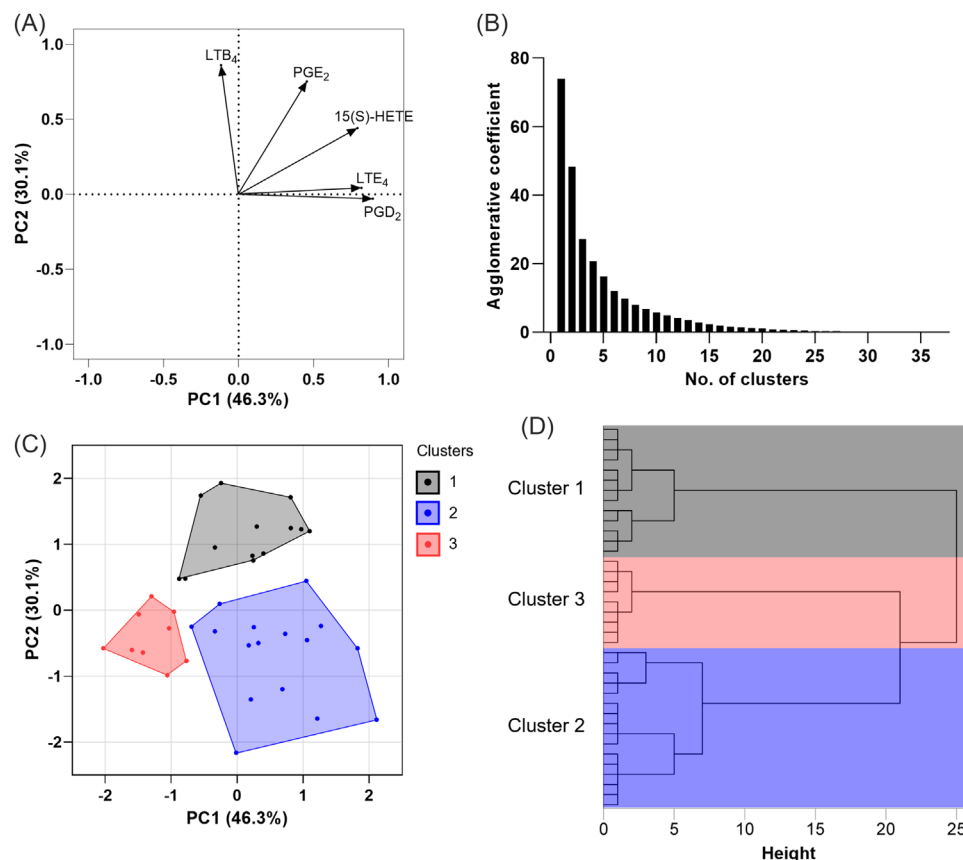
FIGURE 3 Clustering based on principal component (PC) 1 and 2. (A) Vectors indicating the relation of each eicosanoid to the transformed PC1 and PC2. (B) Agglomeration coefficient values, which indicate changes in within-cluster variation at each clustering step. (C) Patient clusters visualized with a principal component analysis (PCA) plot based on their PC scores. (D) Dendrogram showing which order the clusters were joined with hierarchical clustering based on Ward's method. The length of the horizontal lines indicates dissimilarity between clusters

TABLE 2 Mean principal component (PC) scores (log-transformed and standardized) compared across clusters.

	Cluster 1	Cluster 2	Cluster 3	*p*-value
PC1	0.16 ± 0.67***	0.60 ± 0.78***	−1.30 ± 0.39* **	**<0.01**
PC2	1.13 ± 0.46**,***	−0.68 ± 0,71*	−0.41 ± 0.40*	**<0.01**

Note: Comparison between clusters with one-way analysis of variance followed by multiple comparison tests adjusted with Bonferroni.
Bold values signifies a *p*-values below 0.05, i.e., highlighting a statistical significance between groups.
*$p < 0.05$ compared with Cluster 1.
**$p < 0.05$ compared with Cluster 2.
***$p < 0.05$ compared with Cluster 3.

Significantly elevated levels of IL-6, IL-7, and IL-8 were apparent in cluster 1 as compared to cluster 3, while IL-6 levels tended to be higher in cluster 1 relative to cluster 2 ($p = 0.077$). Levels of chemokines MIP-1β ($p = 0.051$) and G-CSF ($p = 0.064$) tended to be higher in cluster 1 relative to cluster 3, whereas MIP-1β was the only chemokine significantly elevated in cluster 2 as compared to cluster 3. Cluster 1 tended to have higher levels of IL1β as compared to cluster 2 ($p = 0.053$) and cluster 3 ($p = 0.078$). The detection of IL-4, IL-13, and IFN-γ were generally low in the study population, with the highest appearance in patients in cluster 2.

Clinical characteristics between clusters showed no differences in terms of coexisting comorbidities or in sinonasal mucosal severity assessed with NP score and CT score (Table 3). However, the frequency of NPR, identified 12 months after surgery, was significantly higher in cluster 2 as compared to cluster 3 and tended to be higher in cluster 1 relative to cluster 3 ($p = 0.069$). The proportion of patients with previous sinus surgery tended to be higher in cluster 2 than in cluster 1 ($p = 0.081$) and cluster

TABLE 3 Differences in clinical characteristics across clusters.

	Cluster 1 (n = 13)	Cluster 2 (n = 16)	Cluster 3 (n = 9)	p-value across clusters
Allergy, n (%)	3 (23.1)	6 (37.5)	4 (44.4)	0.57
Asthma, n (%)	6 (46.2)	5 (31.3)	3 (33.3)	0.69
AERD, n (%)	2 (15.4)	5 (31.3)	1 (11.1)	0.47
NP score, n (%)	6 (4–7)	6 (5.75–6.25)	6 (4–6)	0.42
NP-high, n (%)	9 (69.2)	14 (87.5)	5 (55.6)	0.22
CT score	18 (14–19)	18.5 (14.0–20.5)	15.0 (13.5–18.0)	0.39
NP recurrence, n (%)	6 (46.2)	9 (56.3)	0 (0)**	**0.015**
Previous surgery, n (%)	4 (30.8)	12 (75)	3 (33.3)	**0.04**
Previous surgeries, n (%)				
1	2 (15.4)	6 (37.5)	3 (33.3)	0.49
2	1 (7.7)	3 (18.8)	0 (0)	0.43
3	0 (0)	3 (18.8)	0 (0)	0.16
4	1 (7.7)	0 (0)	0 (0)	0.58
Reported anosmia, n (%)	9 (69.2)	11 (68.8)	3 (33.3)	0.19
Eosinophil count/μl	500 (400–600)	600 (400–825)	400 (200–500)	0.08
Rescue OCS[a]	2 (15.4)	3 (18.8)	1 (11.1)	1.0
Clinical variables with missing data				
	n = 9	n = 8	n = 6	
FeNO	37 (32–50)	78 (62–88)	21.5 (17.25–25.75)	0.12
	n = 9	n = 9	n = 6	
Smell test score	8.0 (3.0–19.5)	4.0 (0.0–10.0)	20.3 (4.2–27.1)	0.2
T score	0.0 (0.0–1.8)	0.0 (0.0–0.0)	2.3 (0.4–4.6)**	**0.04**
D score	4.0 (3.0–9.0)	2.0 (0.0–4.0)	5.5 (0.8–8.0)	0.47
I score	4 (1–12)	2 (0–3)	11 (3–13)	0.24
	n = 12	n = 12	n = 8	
SNOT-22 score	38.5 (27.8–63.8)	55.5 (45.8–67.5)	52.0 (43.3–62.8)	0.29

Note: Data are presented as medians with interquartile range or count (percentage). Differences between clusters were tested with Fisher's exact test or Kruskal-Wallis test and multiple comparisons with Dunn's tests adjusted with Benjamini-Hochberg. Asthma indicates aspirin-tolerant asthma.

Bold values signifies a p-values below 0.05, i.e., highlighting a statistical significance between groups.

Abbreviations: AERD, aspirin exacerbated respiratory disease; CT, Lund-Mackay computed tomography; D score, discrimination score from Sniffin' Sticks smell test; FeNO, fractional exhaled nitric oxide; I score, identification score from Sniffin' Sticks smell test; NP, nasal polyp; NP-high, patients with an NP score of >4; OCS, oral corticosteroids; SNOT-22, Sino-Nasal Outcome Test; T score, threshold score from Sniffin' Sticks smell test.

[a]Additional dose of OCS required after initial surgery.

*p < 0.05 compared to Cluster 1.

**p < 0.05 compared to Cluster 2.

3 (p = 0.131). Self-reported anosmia, FeNO, Sniffin' sticks total score, and SNOT-22 scores appeared higher in cluster 2 although the differences were not significant. Lastly, blood eosinophil count tended to be higher in cluster 2 relative to cluster 3 (Table 3, p = 0.083). Additional need for prescribing OCS post-FESS was rare and not significantly different between the clusters.

4 | DISCUSSION

We report the differences in pre-surgical levels of nasal eicosanoids and the changes in these at 6 and 12 months after surgery in patients with and without endoscopically identified NPs. The results indicate that eicosanoid release in nasal mucosa is dysregulated in NP recurrent individuals evidenced by elevated pre-surgical levels of LTE_4, PGD_2 and 15(S)-HETE. Surgical removal of sinonasal mucosa effectively reduced the PGD_2 and 15(S)-HETE release, while generation of LTE_4 was rapidly restored 12 months post-surgery in NP recurrent subjects. Variation of nasal levels was apparent; therefore cluster analysis was applied to explore potential endotypes. To the best of our knowledge, this is the first time clustering based on nasal eicosanoids in CRSwNP has been reported. Our data indicated three potential nasal eicosanoid endotypes, one of
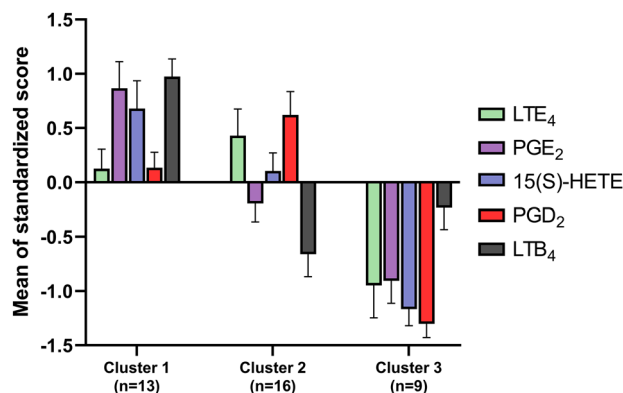
FIGURE 4 Means of log-transformed and standardized values of each mediator compared across clusters

them primarily signified by elevated LTE_4 and PGD_2 and a lack of PGE_2 together with higher incidence of NPR and previous surgical removal of NPs. Eicosanoid patterns presented here might predict NPR and potentially identify severe recalcitrant patients in need of immunotarget therapy to prevent revision surgery.

In CRSwNP patients with high eosinophil infiltration, evidence suggests that these cells are the main producer of CysLTs in CRSwNP.[14,29] Hence, the increase in nasal LTE_4 12 months after surgery among NP recurrent patients may reflect a recruitment of eosinophils into the mucosa. This finding also suggests that early polyp formation is characterized by a restored capacity of the sinonasal mucosa to produce LTE_4 which potentially drives the NP regrowth. Direct effects on the sinonasal mucosa by CysLTs include hypersecretion and edema,[30] which are implicated in the development of CRSwNP. In addition, CysLTs strongly promote the production of IL-4, IL-5, and IL-13 in ILC2s.[31,32] An overexpression of IL4/IL13 inducible genes alters epithelial cells into a state of aberrant differentiation,[23] which may lead to a mucosa that is more prone to reform NPs.

Our results suggest that recalcitrant CRSwNP is characterized by pronounced nasal secretions of 15(S)-HETE, PGD_2, and LTE_4. In line with these findings, CysLTs and the corresponding receptor, CysLT1, were elevated in patients with high occurrence of refractory polyps and IL-5 positive mucosa.[30] In contrast, levels of tissue PGD_2 between recurrent and non-recurrent cases were not different, whereas the PGD_2 receptor CRTH2 was shown to be overexpressed in NP recurrent cases.[33] The heterogeneously distributed nasal PGD_2 as suggested by our clustering results may be a reason for these contrasting findings. Moreover, in contrast to nasal secretions, no change in urinary LTE_4 or 11β-PGF_{2a} was observed after surgery. Thus, eicosanoids measured in urine are more likely to reflect the severity of comorbid asthma as previously suggested by us and others.[15,34]

To explain the potential heterogeneity of the pre-surgical eicosanoid data, cluster analysis was performed. The analysis revealed three clusters with clinically important differences. An increase in LTE_4 and PGD_2 with a diminished PGE_2 release is implicated in AERD[14] and interestingly our results showed a similar lipid mediator profile in patients of cluster 2. However, only one-third in this subgroup of patients had AERD, which suggests that recalcitrant CRSwNP is characterized by this eicosanoid profile regardless of co-existing AERD. Consistent with our results, a similar lipid mediator profile was associated with higher disease severity in AERD.[35] Cluster 2 showed clinically important differences relative to other clusters with higher occurrences of previous FESS and NPR as well as a lower sense of smell, higher eosinophil blood count, and higher FeNO levels. All of these are relevant factors that characterize severe CRSwNP.[3] It may therefore be of great value to pharmacologically target signaling pathways of CysLTs or PGD_2 in CRS patients with distinct eicosanoid signatures, as presented in cluster 2. Previous clinical trials with CRTH2[36] and CysLT1[37] antagonists have revealed only modest effects, which may be explained by prominent LTE_4 and PGD_2 differences among patients as reported here. In a non-controlled study, clinical benefits from CysLT1 antagonist treatment in recalcitrant patients with higher levels of CysLT and CysLT1 expression was reported[30] and provided circumstantial evidence that certain patients are more responsive than others. Further studies comparing therapeutic responses between patients with low versus high CysLTs levels and CysLT1 expression would be most interesting.

A lack of PGE_2 signaling may contribute to T2 inflammation and NP formation due to its regulatory actions on eosinophil recruitment,[38] mast cell lipid mediator release,[17,39] fibroblast proliferation,[40] and cytokine production by ILC2s.[21] Hence, the low PGE_2 secretion may skew the mucosal inflammation into more aggressive forms of recalcitrant NPs, as shown here in cluster 2.

Extensive research in past years has revealed that CRS can manifest through Th1, Th2, or Th17 inflammatory immune responses, referred as T1, T2, and T3 endotypes, respectively.[9,41] Although eosinophils are dominantly observed in T2 endotypes, neutrophils also affect the severity and prognosis in CRSwNP.[42] Increased disease burden is observed in cases with mixed eosinophilic and neutrophilic involvement.[9,43] The presentation of raw values of eicosanoids and cytokines revealed that clusters 1 and 2 were mainly discriminated by LTB_4 and PGE_2. Elevated levels of LTB_4 and IL-8, which both are neutrophil chemoattractants, strongly suggest higher neutrophilic involvement in cluster 1. NPR was also apparent in cluster 1, but to a lesser extent than in cluster 2 (46% vs. 56%) and

	Cluster 1 (n=13)	Cluster 2 (n=16)	Cluster 3 (n=9)
LTE_4 (ng/ml)	0.70 (0.48-0.97)	0.91 (0.62-1.26)	0.26 (0.24-0.49)
PGD_2 (ng/ml)	0.53 (0.51-0.91)	0.97 (0.57-2.43)	0.13 (0.11-0.16)
15(S)-HETE (ng/ml)	923.6 (399.7-1099)	565.0 (399.2-687.8)	178.9 (154.7-226.7)
PGE_2 (ng/ml)	9.12 (5.98-10.6)	3.41 (2.15-4.76)	1.81 (1.55-2.37)
LTB_4 (ng/ml)	2.06 (1.73-2.44)	0.81 (0.60-1.07)	1.10 (0.90-1.28)
IL-6 (pg/ml)	15.1 (8.42-41.8)	5.98 (3.96-15.6)	0.77 (0.03-7.64)
IL-7 (pg/ml)	24.2 (15.7-29.9)	15.0 (10.4-26.1)	8.91 (6.55-14.1)
IL-8 (pg/ml)	502.8 (347.6-785.7)	190.5 (134.8-1033)	167.4 (73.7-282.9)
MIP-1β (pg/ml)	27.6 (12.6-53.3)	18.1 (6.17-74.1)	2.71 (0.16-8.88)
G-CSF (pg/ml)	240 (84.1-377)	145 (30.2-778)	20.7 (0.58-146)
IL-1β (pg/ml)	5.68 (3.38-12.18)	2.47 (1.03-5.33)	2.09 (1.41-4.83)
MCP-1 (pg/ml)	21.68 (12.31-29.73)	13.95 (8.87-20.82)	9.55 (6.37-11.22)
IFN-γ +, n (%)	3 (23.1)	7 (43.8)	2 (22)
IL-4 +, n (%)	2 (15.4)	5 (31.3)	0 (0)
IL-13 +, n (%)	4 (30.8)	7 (43.8)	1 (11.1)
IL17 +, n (%)	2 (15.4)	2 (12.5)	0 (0)
TNF-α +, n (%)	10 (76.9)	7 (43.8)	4 (44.4)

Significantly higher than two other clusters
Significantly higher than one other cluster
No significant differences
Significantly lower than one other cluster
Significantly lower than two other clusters

FIGURE 5 Modified heatmap based on differences in raw eicosanoid and cytokine levels across clusters, excluding cytokines with no detectability, analyzed with Kruskal-Wallis test or Fisher's exact test with Benjamini-Hochberg adjustment for multiple comparisons to compare between clusters

levels of IL-1β and G-CSF tended to be higher in cluster 1. In line with our findings, Poposki et al. showed that NPR was associated with a higher neutrophil infiltration and an IL-1β neutrophil derived production.[43] Furthermore, IL-8, G-CSF, IL-1β, and tissue neutrophils clustered together, and G-CSF was further linked to neutrophil survival.[44] Cluster 1 also featured higher LTE_4 and PGD_2 as compared to cluster 3, which is indicative of T2 inflammation as well as eosinophil or mast cell activation.[16,19] Taken together, a more pro-inflammatory milieu (IL-1β and IL-6) as well as a mixed eosinophilic and neutrophilic involvement was apparent in cluster 1.

The level of 15(S)-HETE is reflected by 15-LO activity and has previously been associated with asthma severity and tissue eosinophilia.[45] As the key enzyme for 15(S)-HETE biosynthesis, 15-LO is predominantly expressed by epithelial cells in CRS and is transcriptionally elevated in AERD.[22] Despite enhanced expression of 15-LO, a corresponding significant increase in tissue 15(S)-HETE could not be reported.[22] However, a novel metabolite, 15-oxo-ETE, was found to be formed by the conversion of 15(S)-HETE by hydroxyprostaglandin dehydrogenase. The converting enzyme was excessively expressed by mast cells that colocalized with epithelial cells.[22] Hence,

differences in 15(S)-HETE between clusters as reported here may reflect discrepancies of further transcellular metabolism.

There are some limitations to this study that need to be acknowledged. NPR was defined by a NP score ≥ 1 present at post-surgical revisits. Hence, this was not a direct measure of future need of revision surgery, but still a strong indicator of recalcitrant CRS. In support of this indication, most cases of NPR and prior sinus surgery were found in the same cluster. In addition, involved surgeons in the current study were not responsible for asthma medication. This information was dependent on patient-reported usage, for example, CysLT1 antagonist treatment was reported for one of the included patients. Differences in other medical treatments for asthma and AERD or other clinical variables may have influenced our findings. The sample size was most likely underpowered to assess differences across clusters, which also limited the number of included mediators used for clustering. Future research focusing on gene expression for enzymes and receptors involved in eicosanoid signaling may reveal more insights into pathophysiological mechanisms responsible for recalcitrant CRSwNP.

5 | CONCLUSIONS

The present study showed that pre-surgical levels of eicosanoids in nasal secretions may serve as prognostic markers for polyp regrowth. In addition, postoperative measurements of nasal LTE_4 may be of clinical importance for indicating early recurrence of NPs. Based on eicosanoid endotypes from nasal secretions, we also provide new insights into severe CRSwNP. Our findings suggest that distinct eicosanoid dysregulations, signified by excessive release of LTE_4 and PGD_2 along with a lack of PGE_2 and LTB_4, may be used for the identification of the most severe recalcitrant cases. Hence, endotypes based on eicosanoid signatures may improve ways to decide personalized treatment strategies for recalcitrant CRSwNP.

CONFLICT OF INTEREST STATEMENT
None of the authors have any conflict of interest to declare.

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