Prostaglandins, Leukotrienes and Essential Fatty Acids 184 (2022) 102474

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Original research article

Levels of eicosanoids in nasal secretions associated with nasal polyp severity in chronic rhinosinusitis

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ARTICLE INFO

Keywords:
CRS
Nasal polyp score
Nasal secretions
Leukotriene
Prostaglandin
AERD

ABSTRACT

Severe nasal polyposis and mucosal inflammation, in patients with chronic rhinosinusitis (CRS) may include a dysregulated eicosanoid profile, but a clinical role for eicosanoids in CRS with nasal polyps (NP; CRSwNP) remains to be elucidated. This study focused on assessing levels and clinical implications of inflammatory mediators in nasal secretions and urine from patients with different NP severity or Aspirin Exacerbated Respiratory Disease (AERD). Levels of leukotrienes E_4 and B_4, prostaglandins D_2 and E_2 as well as 15(*S*)-hydroxyeicosatetraenoic acid were measured with enzyme immunoassays and cytokines with magnetic bead immunoassays. Patients with CRSwNP were subdivided based on NP score; CRSwNP-low (NP score ≤ 4, $n = 11$) or CRSwNP-high (NP score ≥ 5, $n = 32$) and compared to CRS without polyps (CRSsNP, $n = 12$), CRSwNP-AERD ($n = 11$) and individuals without CRS ($n = 25$). Smell test score, fractional exhaled nitric oxide (FeNO), blood eosinophils and Sinonasal outcome test-22 were assessed as clinical markers. Leukotriene E_4, prostaglandin D_2 and 15(*S*)-hydroxyeicosatetraenoic acid in nasal secretions correlated with NP score. Nasal leukotriene E_4 also correlated with FeNO and smell test score, with highest levels found in CRSwNP-AERD. Levels of prostaglandin D_2 in nasal secretion as well as urinary levels of the prostaglandin D_2 metabolite 11β-prostaglandin $F_{2\alpha}$ differed between CRSNP-high and CRSwNP-low. Urinary 11β-prostaglandin $F_{2\alpha}$ was associated with asthma comorbidity whereas a similar association with prostaglandin D_2 in nasal secretions was not observed. In conclusion, subdividing patients based on NP severity in combination with analysis of eicosanoids in non-invasively collected nasal secretions, may have clinical implications when assessing CRS disease severity.

Abbreviations

CRSw/sNP	Chronic rhinosinusitis with/without nasal polyps
NP score	Nasal polyp score
CRSwNP-AERD	CRSwNP with aspirin exacerbated respiratory disease
FeNO	Fractional exhaled nitric oxide
SNOT-22	Sinonasal outcome test-22
OCS	Oral corticosteroids
HrQoL	Health related quality of life
IL	Interleukin
LT	Leukotriene
PG	Prostaglandin
FESS	Functional endoscopic sinus surgery

1. Introduction

Chronic rhinosinusitis (CRS) is a common inflammatory condition of the sinonasal mucosa. Guidelines provided by European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS 2020) defines CRS as an inflammation of the nose and paranasal sinuses, followed by two or more rhinologic symptoms, such as nasal obstruction or nasal discharge, for at least 12 consecutive weeks [1]. Other symptoms may be facial pain, reduced sleep quality and a loss of smell which all have a significant impact on Health related Quality of Life (HrQoL) [2]. The diagnosis of CRS is mainly based on the EPOS guidelines as well as a distinction between CRS with nasal polyps (NP), (CRSwNP) or without (CRSsNP). The ultimate current treatment is functional endoscopic sinus surgery (FESS) for removal of inflamed tissue as well as nasal and oral corticosteroids (OCS). The assessment of disease severity can be measured with Sinonasal Outcome Test 22 (SNOT-22) to estimate disease specific

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https://doi.org/10.1016/j.plefa.2022.102474
Received 25 April 2022; Received in revised form 8 July 2022; Accepted 21 July 2022
Available online 23 July 2022

HrQoL or evaluation with endoscopy and computer tomography (CT) scans. Nasal polyp score is a physician-reported tool to grade the extent and severity of NP growth in the paranasal sinuses [1]. Increased NP score is related to reduced HrQoL [3].

The association between asthma and CRS is especially pronounced in patients with CRSwNP. Moreover, Aspirin Exacerbated Respiratory Disease (AERD) is characterized by the combination of CRSwNP, asthma, and acute hypersensitivity to aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) [4]. Individuals diagnosed with AERD usually feature the most severe manifestations of NP and asthmatic burden relative to other CRS subtypes. The exact pathogenesis of NP growth is currently unclear, although a type 2 inflammation seems dominant for CRSwNP; Elevated levels of Th2 cells expressing interleukin-4 (IL-4), IL-5, and IL-13 in NP [5, 6], and eosinophil infiltration is significantly high [7]. Furthermore, the magnitude of type 2 inflammation is associated with an increased likelihood of comorbid asthma or AERD [8] as well as postsurgical relapse of NP [9].

A dysregulated arachidonic acid metabolism, i.e., altered biosynthesis and release of eicosanoids [10–14], have been observed in CRSwNP and AERD which appears related to elevated IL-5 and tissue eosinophils [15]. We and others have previously reported on increased levels of urinary leukotriene E_4 (LTE_4) in patients with AERD as compared to patients with asthma without hypersensitivity to aspirin or NSAIDs [16–19]. Urinary LTE_4 has also been studied in patients with CRS and has been suggested as a diagnostic marker or used to distinguish between CRSwNP with or without aspirin hypersensitivity [17, 19, 20]. Elevated urinary LTE_4 was also demonstrated to relate to several severity markers of disease in patients with CRSwNP [21], and may reflect the tissue eosinophilia in CRS [22].

Biological therapy, such as antibodies targeting Th2 specific cytokines and receptors, are promising and soon available for treatment of CRSwNP [23, 24]. The advent of biological therapies warrants easily accessible and clinically relevant biomarkers to identify the more severe cases of CRS with type 2 inflammation [6, 24]. More recently, in a study of effects of anti-IL-5 treatment in AERD patients, decreased levels of several eicosanoids were reported in nasal lining fluid [25]. Moreover, anti-IL-4/IL-13 treatment significantly diminished urinary LTE_4 as well as mucosal type 2 cytokines in CRSwNP patients [26]. In the present study, we focused on eicosanoid release into nasal secretions in association with disease severity as evaluated by NP score in patients with CRS with or without concomitant asthma or AERD. Moreover, we sought to evaluate the correlation of urinary and locally secreted eicosanoids to clinical disease markers such as loss of smell, fractional exhaled nitric oxide (FeNO) and SNOT-22 in CRSwNP patients.

2. Materials and methods

2.1. Study population

Patients scheduled for sinus surgery at the department of Otolaryngology, Sophiahemmet Hospital due to bilateral CRSsNP, CRSwNP or CRSwNP-AERD, were consecutively recruited. Included patients had persistent disease burden despite of prevalent medical treatment; daily nasal CS and at least one previous course of OCS. Diagnosis was based on criteria provided by EPOS [27]; persistent sinonasal symptomatic burden for >12 weeks and signs of paranasal inflammation and obstructions evaluated through endoscopy and CT. CRSwNP-AERD was defined by episodes of intolerance reaction to aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) noted in medical records and further supported by the patient reporting incidence of airway hypersensitivity. Asthma and allergy (i.e., allergic rhinitis, food allergy or atopic dermatitis) is presented for all participants based on physicians' diagnosis retrieved from medical records. Patients serving as controls underwent nasal septoplasty surgery, had no previous clinical history of sinus disease or asthma and had no signs of mucosal inflammation assessed endoscopically. The exclusion criteria for all participants were a completed treatment of OCS less than one month before sample collection, autoimmune disease or ongoing treatment with immuno-targeted mAbs (i.e., biologics). As a routine at the clinic, all patients are asked to avoid any NSAIDs one week prior to their surgery. All included patients were also specifically asked on the day of surgery about NSAID intake the last two weeks.

2.2. Clinical parameters

Smell test was performed essentially as described [28]. Burghart Sniffin' Sticks (Extended Test n-Butanol, MediSense) is a validated tool to assess the degree of smell loss and consists of three individual subtests, the threeshold (T), discrimination (D) and identification (I) test. Patients were exposed to odors with felt-tip pens brought under their nose to identify or discriminate between different odors. The odor threshold is established from 16 available triplet pens with decreasing level of odor from the first to the 16th. Each triplet of pens contains one odorous and two blank pens for the subject to identify the odorous pen. In the odor discrimination test, 16 triplets of pens are presented to the subject with the difference that one of the pens has a unique odor and the other two has the same odor. Here the subject is asked to identify the unique pen from the other two. Lastly, in the identification test with 16 single pens, the subject is asked to choose one out of four given odorant alternatives for each pen. The three subtest scores (0–16) are summarized to a total TDI score (0–48) to determine normal (TDI = 30–48), partial loss (TDI = 16–30) and complete loss of olfactory function (TDI = 0–16) [28].

FeNO (NIOX VERO) [29], eosinophil blood count (HemoCue WBC DIFF System) and CT findings less than three months prior to surgery shown as Lund-Mackay score [30] were also assessed. Moreover, NP severity was investigated endoscopically. Each nostril was scored on a scale of 0 (no polyps) to 4 (large polyps blocking inferior meatus), with the total score being the sum of left and right nostril scores (range 0–8) [31].

2.3. Collection of nasal secretion and urine samples

One SalivaBio oral swab (10 × 30 mm, Salimetrics) repurposed for collection of nasal secretion, was inserted into each nostril extending into the bottom of the nasal cavity. The swabs were pre-soaked with 800 μl saline solution and left in the nasal cavity for 10 min, after which the swabs were put in a swab filter tube (Swab storage tube, Salimetrics) and centrifugated at 10,000 g for 15 min. The fluid was aliquoted and stored at −80 °C prior to analysis.

Spot urine samples were collected, aliquoted and stored at −80 °C before analysis.

2.4. Analyses of eicosanoids

Nasal secretions were analysed in serially diluted samples for content of LTB_4, LTE_4 Prostaglandin (PG) D_2, PGE_2 and 15(*S*)-hydroxy eicosatetraenoic acid (15-(S)HETE), using specific ELISA kits (Cayman Chemical, Ann Arbor, MI, USA). All analyses were performed according to manufactures instructions and measured spectrophotometrically at 410 nm with the infinite M200 Pro-microplate reader (Tecan, Switzerland). The lower detection limits range from 5.0 to 58.9 pg/ml for the different ELISA assays.

Analysis of urinary LTE_4 was performed in serially diluted samples with an enzyme linked immunosorbent Leukotriene E_4 ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) essentially as previously described [32]. The specificity of the rabbit polyclonal antiserum was LTE_4 (100%) and for LTD_4 and LTC_4 (<0.01%). Analysis of 11β-$PGF_{2\alpha}$ was performed with a similar protocol as previously described [33] using a commercially available 11β-$PGF_{2\alpha}$ ELISA kit (Cayman Chemical Company, Ann Arbor, MI, USA). The antibody cross-reacted with 11β-$PGF_{2\alpha}$ (100%), 2,3-dinor-9α,11β-PGF_2 (10%), 11β−13,14

dihydro-15-keto-$PGF_{2\alpha}$ (0.5%). All urine samples were subjected to creatinine analysis using a colorimetric assay (Cayman Chemical, Ann Arbor, MI, USA) and results are expressed as ng/mmol of creatinine for correction of diuresis variations.

2.5. Analyses of cytokines

A magnetic bead multiplex immunoassay (Hu 17-plex cytokine panel; BioRad, Hercules, CA, USA) was utilized for the quantification of 17 cytokines/chemokines according to the manufacturer's instructions. In brief, nasal secretions were diluted (1:1.25) using kit-provided sample buffer with addition of bovine serum albumin to a final concentration of 0.5%. Fifty μl of each sample/standard were mixed with 50 μl bead solution and incubated for 30 min, after which 25 μl Biotinylated detection antibody was added followed by a 30 min incubation. The final addition of 50 μl Streptavidin-phycoerhrin and subsequent incubation for 10 min forms a bead detection complex where phycoeryhrin serves as the fluorescent reporter. Each incubation step was performed in RT on a plate-shaker (850 rpm), followed by washing with the HydroFlex™ microplate washer (Tecan, Switzerland). The formed bead detection complex in each sample/standard was then resuspended with kit provided assay buffer and analysed with Bio-Plex 200 system provided with Bio-Plex manager software v6.2 (Bio-Rad, Hercules, CA, USA).

2.6. Statistical analysis

Clinical characteristics of included patients as well as levels of inflammatory mediators were presented as median with interquartile range (25th-75th percentiles) for continues variables and frequency with percentage for dichotomous variables. As normality of data could not be assumed, non-parametric Kruskal-Wallis tests and Dunn's tests adjusted with Benjamini-Hochberg method for multiple comparison testing between subgroups. The difference of dichotomous variables was tested with Fishers exact test and the pairwise fishers exact test adjusted for Benjamini-Hochberg method. Correlation between levels of inflammatory mediators and clinical parameters was tested with Spearman's Rho test. Cytokine values outside of assay range was given a value 1/10 of lower detection limit, as previously described [34]. In cases were a mediator featured large proportion of undetectability (>33%), i.e., below the detection limit, that cytokine/chemokine was dichotomized and expressed in terms of 0 (not detected) or 1 (detected) [6]. Two-way aligned rank transformed analysis of variance (ART ANOVA) was performed to investigate the influence of comorbid asthma on lipid mediator level in those with CRSwNP-low or -high. The presence of CRSwNP-low or -high and asthma or non-asthma were treated as independent variables in the analysis and included an interaction term between the two as well. R software v4.0.3 was utilized for inferential statistics, GraphPad Prism V.8.4.2 for graphical presentation, and significant difference was considered at $p < 0.05$.

2.7. Ethical considerations

This study was approved by the Swedish Ethical Review Authority (2017/1461–31, 2019–06,185). All subjects gave written informed consent for participation in the study, prior to sample collection and surgery. All subjects were informed that participation was voluntary, and they had the right to withdraw at any time without giving a reason and without impact on future care.

3. Results

A total of 25 individuals with no CRS and 72 patients diagnosed with CRS were recruited, whereas six CRS patients were excluded due to completed OCS treatment less than one month before enrollment. Disease severity was primarily identified by NP score and CRSwNP patients were subgrouped accordingly; NP score of ≤ 4 was defined as low NP severity (CRSwNP-low), whereas a NP score of ≥ 5 indicated high NP severity (CRSwNP-high). Urine samples were collected from 23 controls, 12 patients with CRSsNP, 10 CRSwNP-low patients, 30 CRSwNP-high patients and 11 CRSwNP-AERD patients. Nasal secretions were collected from 16 controls, nine CRSwNP-low patients, 19 CRSwNP-high patients and 11 with CRSwNP-AERD.

Characteristics of the study population are shown in Table 1. Patients included did not differ in gender, age, allergy, Hb, B-glucose or CRP (<5) between groups. CRSwNP-high and CRSwNP-AERD patients had significantly higher asthma prevalence, blood eosinophil count, and Lund-Mackay score as compared to CRSsNP. CRSwNP also had significantly higher FeNO as compared to controls. Moreover, patients with CRSwNP-AERD featured significantly higher Lund-Mackay score and FeNO than CRSwNP-low and CRSsNP as well, and they scored significantly lower in the smell test as compared to CRSwNP patients. The SNOT-22 score (i.e., HrQoL) were comparable between CRSwNP and CRSwNP-AERD patients.

3.1. Urinary levels of eicosanoids

Urinary levels of LTE_4 and the PGD_2 metabolite 11β-$PGF_{2\alpha}$ in the different groups of participants were compared between the subgroups; CRSsNP, CRSwNP-low, CRSwNP-high and CRSwNP-AERD. We were not able to show any significant differences in urinary LTE_4 between the groups; e.g., CRSwNP-AERD (median (IQR): 167 (121 - 227) ng/mmol creatinine)) as compared to controls (139 (113 - 163)). Levels of urinary 11β-$PGF_{2\alpha}$ were significantly higher in CRSwNP-AERD (50.0 (42.7 - 68.3)) as compared to both CRSsNP (35.3 (21.7 - 44.1) and controls (38.0 (29.5 - 47.6)). Significantly higher levels of 11β-$PGF_{2\alpha}$ were also observed in CRSwNP-high (46.3 (35.7 - 68.6)) versus CRSsNP. Levels of 11β-$PGF_{2\alpha}$ tended to be higher in CRSwNP-high as opposed to control ($p = 0.08$).

3.2. Levels of eicosanoids in nasal secretions

Results from analyses of eicosanoids in nasal secretions from controls, as well as patients with CRSwNP-low, CRSwNP-high and AERD are illustrated in Fig. 1.

Levels of LTE_4 in nasal secretions from patients with CRSwNP-AERD were significantly higher than controls, CRSwNP-low and a tendency towards CRSwNP-high ($p = 0.07$). A tendency of elevated levels of LTE_4 in patients with CRSwNP-high was observed when compared to controls ($p = 0.06$). In addition, LTE_4 levels tended to increase from controls to low polyp grade, to high polyp grade, and with the highest levels seen in CRSwNP-AERD.

Significantly higher levels of PGD_2 was found in nasal secretions from patients with CRSwNP-high as compared to controls and CRSwNP-low. In addition, the PGD_2 level in nasal secretions from patients with CRSwNP-AERD was of the same high magnitude as in CRSwNP-high although no significant differences could be shown as compared to other groups.

All subgroups of CRS had somewhat lowered nasal PGE_2 levels than control patients, but statistically significant differences could not be reached. Moreover, nor could any significant differences in levels of LTB_4 be observed. However, LTB_4 tended to be decreased from low to high CRSwNP, and with lowest values observed in those with CRSwNP-AERD (Fig. 1).

3.3. Cytokine levels in nasal secretions

Cytokine levels in nasal secretions from patients with CRSwNP-low, CRSwNP-high, CRSwNP-AERD as well as controls are shown in Table 2. A total of 10 cytokines and chemokines could not be detected in a large proportion (>33%) of the collected nasal secretions. Therefore, patients with undetectable levels were denoted as a cytokine negative (-) and

Table 1
Clinical characteristics between Controls, CRSsNP, CRSwNP-low, CRSwNP-high and CRSwNP-AERD. Continues variables are presented as median with interquartile ranges and categorical variables are presented as count with percentage.

	Control	N	CRSsNP	N	CRSwNP-low	N	CRSwNP-high	N	CRSwNP-AERD	N	P-value
Total patients (n)	25	-	12	-	11	-	32	-	11	-	-
Males	68%	17	50%	6	72.7%	8	68.8%	22	72.7%	8	.78
Age, yr	40 (28-56)	25	46 (38-52)	12	37 (34-45)	11	51 (46-60)	32	47 (34-57)	11	.07
Hb	-	-	140 (135-155)	12	150 (139-155)	11	147 (131-152)	32	144 (137-155)	11	.83
b-glucose	-	-	5.6 (4.5-5.9)	12	4.7 (4.3-5.0)	11	5.1 (4.6-5.5)	32	5.0 (4.9-5.6)	11	.20
CRP, mg/ml	-	-	<5	12	<5	11	<5	32	<5	11	-
Asthma	NA	-	16.7%	2	36.4%	4	59.4% [S]	19	100% [S L H]	11	**<.001**
Allergy	NA	-	16.7%	2	36.4%	4	21.9%	7	18.2%	2	.73
B-EOS, count/ul	-	-	200 (100-350)	11	450 (325-500)	10	400 (300-625) [S]	28	550 (400-750) [S]	10	**.016**
Previous FESS	NA	-	25%	3	9%	1	46.9%	15	72.7% [L]	8	**.012**
LMS	-	-	3.5 (2.0- 13.5)	10	14.0 (13.3-16.5)	10	18.0 (14.8-21.3) [S]	32	21.0 (19.0-22.0) [S L]	11	**<.001**
Last OCS, days	-	-	84 (74-244)	5	132 (82-241)	6	90 (50-201)	29	108 (60-169)	11	.86
Never used OCS	-	-	58.3%	7	45.5%	5	9.4% [S L]	3	0% [S L]	0	**<.001**
SNOT-22	-	-	-	-	47 (33-63)	8	59 (41-67)	15	48 (38-60)	7	.73
FeNO, ppb	13 (10-19)	13	-	-	30 (16-41)	6	37 (20-76) [C]	11	84 (44-90) [C]	6	**<.001**
Smell test score	-	-	-	-	22.3 (5.6-26.3)	6	13.0 (7.0-16.5)	11	1.0 (0.0-2.0) [L H]	7	**.014**
NP score	NA	-	NA	-	4 (4-4)	11	6 (6-8) [L]	32	6 (5.5-6.5) [L]	11	**<.001**

Abbreviations: Hb = Haemoglobin; CRP = C-reactive protein; B-EOS = Blood Eosinophils; FESS = functional endoscopic sinus surgery; LMS = Lund-Mackey score ranging from 0 to 24 (higher score indicate more opacification); OCS = oral corticosteroid; SNOT-22 = Sinonasal outcome test-22 ranging from 0 to 110 (higher score indicate poorer outcome; FeNO = fractional exhaled nitric oxide; NP score = nasal polyp score. Boldface indicates significant difference and p-value are from Kruskal-Wallis test or Fisher's exact test. Post-hoc multiple comparisons tests between subgroups were subsequently performed adjusted with Benjamin-Hochberg (see footnotes below).

[C] $P < 0.05$ compared with control.
[S] $P < 0.05$ compared with CRSsNP.
[L] $P < 0.05$ compared with CRSwNP-low.
[H] $P < 0.05$ compared with CRSwNP-high.

were compared to those with detectable levels; cytokine positive (+) patient.

Elevated levels of the pro-inflammatory IL-6, and chemokine MIP-1β were predominantly observed in patients with CRSwNP-high as well as CRSwNP-AERD, with significantly different levels to both CRSwNP-low and controls. Cytokine (+) patients for the type 2 cytokines (IL-4 and IL-13) were solely observed in CRSwNP-AERD and CRSwNP-high. The proportion of IL-13 (+) patients in CRSwNP-AERD was significantly higher than CRSsNP-low and controls. Moreover, the proportions of IFN-γ (+) patients tended to be higher in CRSwNP-high and CRSwNP-AERD (Table 2).

3.4. Eicosanoid levels in association with disease severity

Correlations between levels of eicosanoids with clinical severity markers in patients with CRSwNP and CRSwNP-AERD are shown (Fig. 2, Table 3). Moderate correlation of nasal LTE_4 to a decreasing smell test score ($r = -0.52$; $p = 0.01$) as well as an increasing FeNO ($r = 0.63$; $p = 0.002$) was seen (Fig. 2A and 2B), whereas a low but significant correlation to eosinophil blood count was observed ($r = 0.39$; $p = 0.02$). Lastly, NP score was found to correlate with nasal LTE_4 ($r = 0.35$; $p = 0.036$), PGD_2 ($r = 0.40$; $p = 0.015$) as well as 15(*S*)-HETE ($r = 0.43$; $p = 0.009$).

Moreover, urinary LTE_4 levels moderately correlated to increasing NP score ($r = 0.43$; $p = 0.002$, Table 3). Likewise, a moderate correlation between urinary 11β-$PGF_2\alpha$ and a decreasing smell test score was detected ($r = -0.41$; $p = 0.045$, Table 3). We could not demonstrate any significant correlation between eicosanoids and degree of SNOT-22 score in our study population.

3.5. Comparison of urinary and nasal eicosanoids

The levels of nasal eicosanoids were further correlated to mediators in urine, from CRSwNP and CRSwNP-AERD, to investigate potential relatability between these two biological fluids. No correlation between urinary and nasal LTE_4 ($r = 0.27$; $p = 0.12$, Fig. 3A), or between nasal PGD_2 and urinary 11β-$PGF_2\alpha$ could be seen ($r = 0.21$; $p = 0.24$, Fig. 3B).

3.6. The effects of comorbid asthma on mediator levels in patients with low and high NP score

A potential influence of comorbid asthma on lipid mediator level in patients with CRSwNP-high and CRSwNP-low was investigated using ART ANOVA. Nasal levels of PGD_2 were significantly higher in CRSwNP-high (median (IQR): 0.71(0.46–1.27)) versus CRSwNP-low (0.30 (0.13–0.51)) (ART ANOVA: $F = 10.6$, df = 1,24, $p = 0.003$), though comorbid asthma had no significant effect on PGD_2 level ($F = 0.28$, df = 1, 24, $p = 0.60$) nor was the difference between CRSwNP-low and CRSwNP-high dependent on comorbid asthma ($F = 0.01$, df = 1, 24, $p = 0.93$). For all other nasal lipid mediators, no significant differences between CRSwNP-low and CRSwNP-high, presence of or absence of comorbid asthma nor any interaction effects on nasal lipid mediator levels was observed in the ART ANOVA analyses.

The ART ANOVA model revealed significantly higher urinary 11β-PGF2α levels in CRSwNP-high (46.3 (35.7 - 68.6)) versus CRSwNP-low (38.9 (32.6 - 45.7)) ($F = 4.22$, df = 1, 36, $p = 0.047$, Fig. 4A). Also, patients with asthma had significantly higher levels of urinary 11β-PGF2α (50.8 (38.8–81.0)) as opposed to those without asthma (38.6 (34.7–46.5)) ($F = 6.20$, df = 1, 36, $p = 0.017$, Fig. 4B). The effect of asthma seemed to affect urinary 11β-PGF2α in those with CRSwNP-high to a larger extent as opposed to CRSwNP-low though such dependency was not statistically significant ($F = 2.68$, df = 1, 36, $p = 0.11$, Fig. 4C).

Urinary LTE_4 was not significantly different between CRSwNP-high versus CRSwNP-low ($F = 0.39$, df = 1, 36, $p = 0.54$), presence versus absence of asthma ($F = 0.40$, df = 1, 36, $p = 0.53$), nor did the ART ANOVA analysis show any interaction effects between the two ($F = 2.76$, df = 1, 36, $p = 0.10$).

3.7. Potential effects of oral corticosteroid

To investigate the potential effect of OCS on measured lipid mediators, patients with CRSwNP were divided into three groups based on time since their last completed course of OCS; 1–2, 2–4 and > 4 months or never used OCS (Table 5). None of the measured lipid mediators in nasal secretions or urine showed significantly difference between the allocated groups although 15(*S*)-HETE tended to be different between

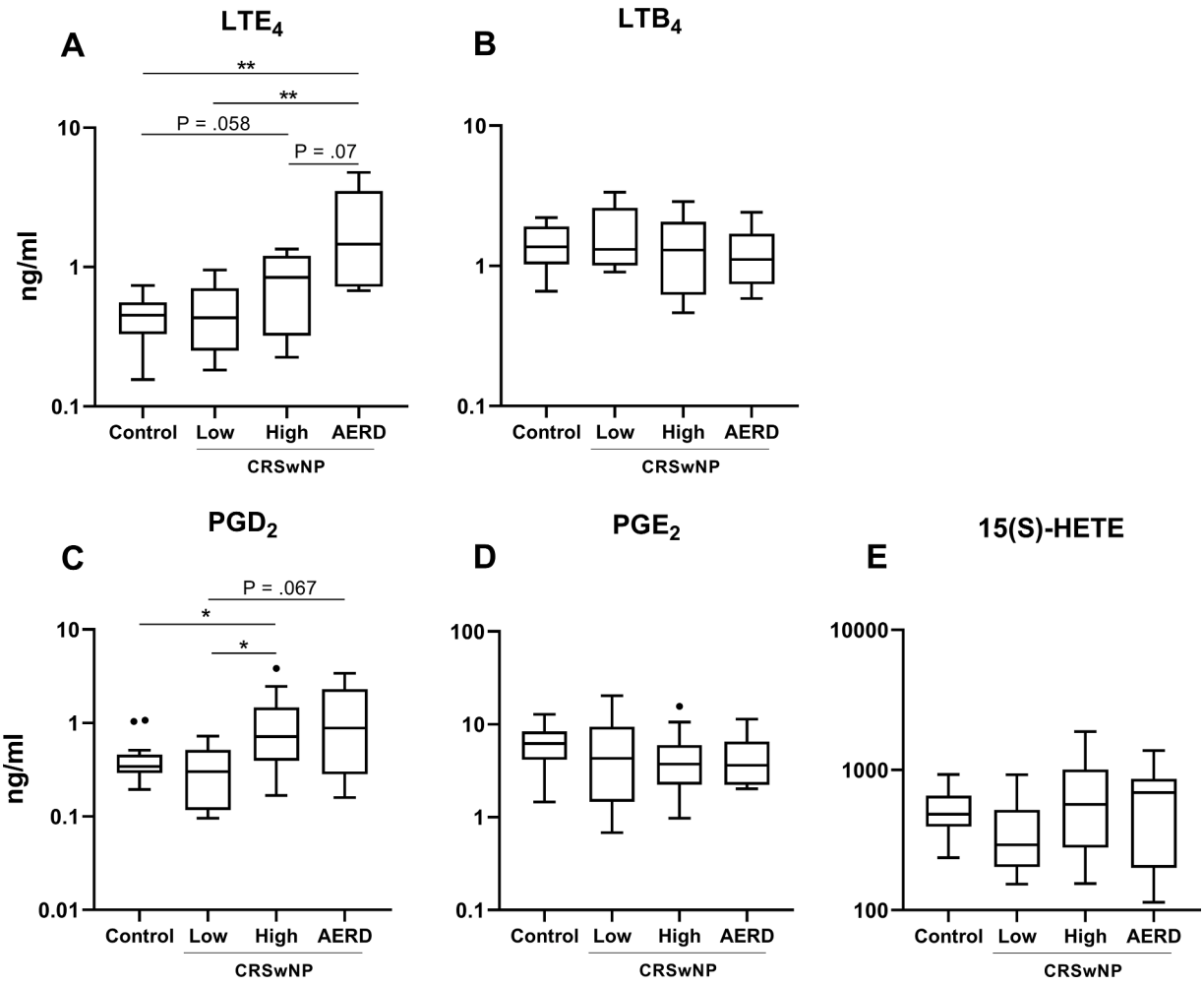

Fig. 1. Levels of lipid mediators in nasal secretions differ in terms of low or high NP severity or AERD. Measured levels of (A) LTE_4, (B) LTB_4, (C) PGD_2, (D) PGE_2 and (E) 15(*S*)-HETE (ng/ml) in nasal secretions from controls ($n = 16$), CRSwNP-Low ($n = 9$), CRSwNP-High ($n = 19$), CRSwNP-AERD ($n = 8$). Mediator levels are presented with median and interquartile range, in a log scale. Significance levels *$p < 0.05$; **$p < 0.01$.

Table 2
Difference of cytokines levels (pg/ml) in nasal secretions from controls, CRSwNP-Low, CRSwNP-high, and CRSwNP-AERD. Dichotomous cytokine values are presented as count number of analyte positive (+), whereas cytokines with numerous values are presented as median with interquartile ranges.

	Controls ($n = 16$)	CRSwNP-low ($n = 9$)	CRSwNP-high ($n = 19$)	AERD ($n = 8$)	P value
GCS-F, pg/ml	76.7 (9.1–173.3)	43.6 (0.6–84.1)	140.3 (29.6–928.4)	265.3 (130.6–402.0)	0.08
IL1b, pg/ml	4.8 (2.1–6.2)	3.2 (1.5–8.1)	4.6 (2.3–9.1)	2.7 (1.4–3.7)	0.72
IL6, pg/ml	2.4 (1.1–4.3)	0.9 (0.03–15.1)	6.5 (4.3–20.0) *	14.5 (9.8–32.6) * §	**0.003**
IL7, pg/ml	11.3 (8.3–14.2)	13.6 (8.4–24.2)	15.9 (10.7–27.04)	16.8 (10.4–19.9)	0.11
IL8, pg/ml	311.7 (224.5–440.5)	183.1 (86.5–355.4)	449.6 (161.0–1054.2	212.9 (137.5–410.2)	0.38
MCP-1, pg/ml	9.7 (5.9–13.9)	9.8 (7.9–12.3)	15.5 (8.5–29.2)	19.9 (14.9–62.5)	0.08
MIP-1b, pg/ml	4.6 (2.5–6.6)	4.1 (2.6–12.6)	20.9 (8.9–75.3) * §	21.1 (7.6–60.7) * §	**0.001**
Dichotomized cytokine					
GM-CSF+, n (%)	0 (0%)	0 (0%)	1 (5.3%)	0 (0)	1.00
IFN-γ +, n (%)	2 (12.5%)	1 (11.1%)	7 (36.8%)	4 (50%)	0.133
IL-2+, n (%)	0 (0%)	0 (0%)	1 (5.3%)	0 (0%)	1.00
IL-4+, n (%)	0 (0%)	0 (0%)	5 (26.3%)	2 (25%)	**0.043**
IL-5+, n (%)	0 (0%)	0 (0%)	1 (2.7%)	0 (0%)	1.00
IL-10+, n (%)	0 (0%)	0 (0%)	2 (10.5%)	0 (0%)	0.771
IL-12p70+, n (%)	0 (0%)	1 (11.1%)	3 (15.8%)	0 (0%)	0.346
IL-13+, n (%)	0 (0%)	0 (0%)	7 (36.8%) *	5 (62.5%) * §	**<0.001**
IL-17+, n (%)	0 (0%)	0 (0%)	3 (15.8%)	1 (12.5%)	0.250
TNF-α+, n (%)	6 (37.5%)	6 (66.7%)	13 (68.4%)	2 (25%)	0.095

P-values are from Kruskal-Wallis tests (continuous variables) or Fishers exact tests (dichotomous variables) and boldface indicate significant difference. Post-hoc multiple comparisons tests between subgroups were subsequently performed adjusted with Benjamin-Hochberg (see footnotes below).

* $P < 0.05$ compared with control.

§ $P < 0.05$ compared with CRSwNP-Low.

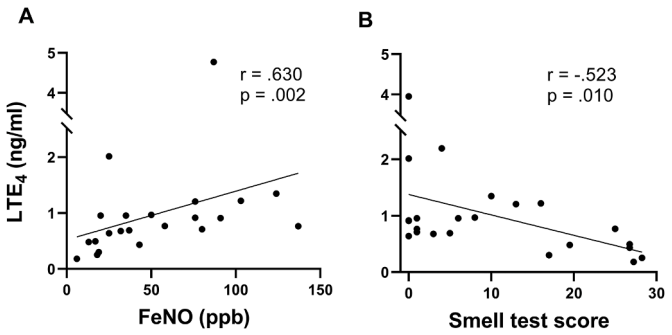

Fig. 2. Increasing FeNO and worsened smell correlates with LTE_4 levels in nasal secretions. Correlated levels of LTE_4 in nasal secretions from CRSwNP-low, CRSwNP-high and CRSwNP-AERD patients, to clinical parameters; (A) FENO ($n = 23$) and (B) smell test score ($n = 24$).

the groups ($p = 0.052$, Table 5). Additionally, days since last completed course of OCS (denoted as a numerical variable) was correlated to lipid mediator levels in nasal secretions and urine (Table 4), but further showed no correlation.

4. Discussion

The results of the present study indicate that altered levels of LTE_4, PGD_2 as well as 15(*S*)-HETE, in easy and non-invasively collected nasal secretions is associated with nasal polyp severity in patients with CRS. Nasal LTE_4 was also associated to FeNO levels and smell test score. Urinary levels of the PGD_2 metabolite, 11β-$PGF_{2}\alpha$, were increased in patients with higher NP severity or AERD and associated with concomitant asthma. IL-6, IL-4 and IL-13 were more abundant in nasal secretions from patients with CRSwNP-high and CRSwNP-AERD.

The demographics with regards to age and gender were similar in the different groups of patients diagnosed with CRS. Since the recruitment of the CRSwNP patients were consecutive the spread in age and gender may very well mimic the population of patients as a middle aged, and potentially male dominant population, also reported by others [35]. The non-CRS individuals were adequately matched regarding age and gender.

The allergy prevalence was similar in all patients with CRS. However, comorbid asthma was present in the groups of patients with CRS, with an incidence of approximately 17%, 36%, 59% and 100%, in CRSsNP, CRSwNP-low, CRSwNP-high and CRSwNP-AERD, respectively. Concomitant asthma significantly altered the urinary levels of 11β-$PGF_{2}\alpha$ to a larger extent in CRSwNP-high versus -low (Fig. 4). In contrast, a similar influence on the nasal PGD_2 could not be observed. Thus, our data suggest that nasal PGD_2 may reflect the NP severity, independently of concomitant asthma, whereas urinary 11β-$PGF_{2}\alpha$ levels may be more influenced by the presence of asthma. Previous reports have shown that several eicosanoids including PGD_2 metabolites increases in urine with regard to asthma severity (mild-to-moderate versus severe asthma) [18]. Their results are in agreement with our findings of urinary mediators, reflecting the lower airway inflammation to a larger extent than sinonasal inflammation. Thus, our results showing a lack of correlation between nasal and urinary eicosanoids (Fig. 3), may be due to this discrepancy.

In the present study, the alterations in the basal urinary levels of LTE_4, between the groups, could however not be shown to be related to concomitant asthma. A difference in urinary eicosanoid levels between individuals without asthma and patients with mild-to-moderate asthma has not been generally reported [36]. Previous investigations, however, show increased urinary levels of LTE_4 as well as 11β-$PGF_{2}\alpha$ in association with provoked asthma attacks towards allergen, aspirin or exercise [33, 37, 38]. Furthermore, elevated basal levels of urinary LTE_4 have predominantly been found in patients with a diagnosed AERD [16] and an ongoing aspirin sensitivity demonstrated through provocation with aspirin via an oral [39], inhaled [40], intravenous [41] or nasal route [42]. The sensitivity to aspirin has been suggested to fluctuate [43] and no confirmation of an active sensitivity with an accurate provocation was performed in the relatively small group of AERD patients in the present study. Hence, the large variation in urinary LTE_4 levels in CRS patients allocated to this group may be explained by this uncertainty.

The results clearly show that the collection of nasal secretions, with swabs inserted into the nostrils, is an easy and rapid method for access to upper airway fluids which is tolerable and not very uncomfortable for the patients. For reproducibility and minimum variation between samples, the swabs were soaked in a specific amount of saline prior to insertion into to the nostrils for a specific time. In previous studies on asthma, induced sputum has been used for eosinophil count, isolation of inflammatory cells and analyses of soluble inflammatory markers [44]. However, in the case of sinonasal inflammation as in CRS it may be more accurate to investigate nasal secretions in order to appreciate disease severity in the upper airways. The results show that immunoreactive eicosanoids were easily detectable and notable differences between the groups could be shown. We believe that nasal secretions are preferable

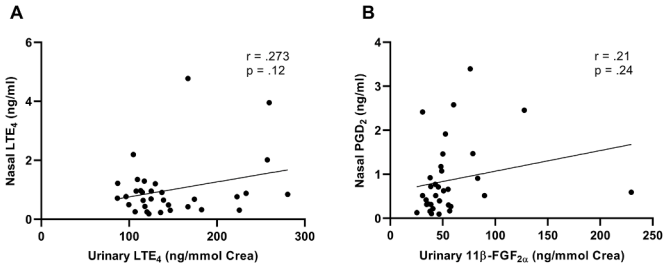

Fig. 3. Correlations between eicosanoids in urine and nasal secretions. (A) Correlation between nasal and urinary LTE_4 ($n = 33$) and (B) nasal PGD_2 and urinary 11β-$FGF_{2}\alpha$ ($n = 33$). Analysed samples are from patients with CRSwNP-low, CRSwNP-high and CRSwNP-AERD.

Table 3
Correlation between lipid mediators in nasal secretion (ng/ml) or urine (ng/mmol creatinine) and clinical variables; FENO, Smell test score or NP score in patients with CRSwNP-low, CRSwNP-high and AERD.

	FeNO			Smell test score			NP score		
	Spearmans r	n	P value	Spearmans r	n	P value	Spearmans r	n	P value
Nasal secretion									
LTE_4	0.63	22	**0.002**	−0.52	23	**0.01**	0.35	36	**0.036**
LTB_4	0.06	22	0.78	0.17	23	0.44	−0.15	36	0.39
PGD_2	0.23	22	0.30	−0.23	23	0.29	0.40	36	**0.015**
PGE_2	0.12	22	0.60	−0.01	23	0.95	0.22	36	0.20
15(*S*)-HETE	0.22	22	0.32	−0.26	23	0.24	0.43	36	**0.009**
Urine									
LTE_4	−0.08	23	0.70	−0.33	24	0.12	0.43	51	**0.002**
11b-PGF_{2a}	0.16	23	0.46	−0.41	24	**0.045**	0.27	51	0.056

Boldface indicates significant correlation after Spearmans Rho test at $p < 0.05$.

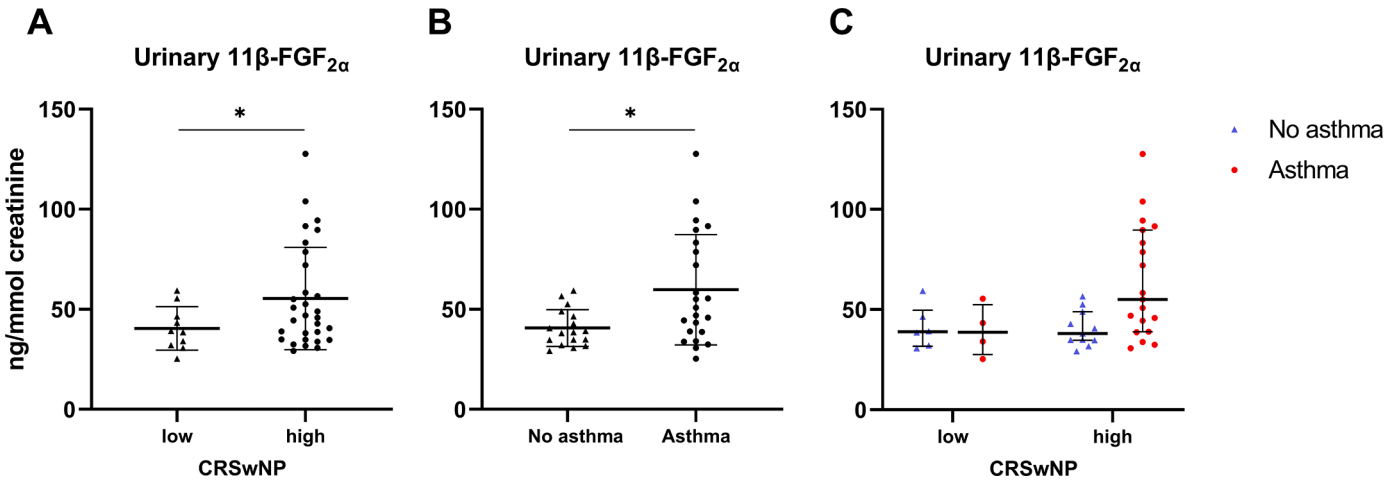

Fig. 4. Effect of comorbid asthma on urinary 11β-$FGF_{2α}$ level in patients with low and high NP severity. Difference in urinary levels of 11β-$FGF_{2α}$ in aspirin-tolerant CRSwNP patients in terms of (A) CRSwNP-low (NP score ≤ 4, $n = 10$) versus CRSwNP-high (NP score ≥ 5, $n = 30$), (B) presence ($n = 23$) or absence ($n = 17$) of comorbid asthma (C) and the presence or absence of asthma in patients with low or high NP score. Analysis comprised of an aligned rank ANOVA with urinary levels of 11β-FGF2α as dependent variable, CRSwNP-low or -high and presence or absence of asthma as categorical independent variables as well as an interaction term with both. Data presented as median with interquartile range. Significance levels $*p < 0.05$.

Table 4
Correlation between lipid mediator and number of days since last completed course of oral corticosteroid before surgery, i.e. the day of sample collection, in patients with CRSwNP-low, CRSwNP-high and CRSwNP-AERD.

	Days since completed OCS		
	Spearmans r	n	P-value
Nasal secretions			
LTE_4	−0.02	29	0.91
LTB_4	0.11	29	0.59
PGD_2	−0.05	29	0.82
PGE_2	−0.08	29	0.67
15(*S*)-HETE	−0.08	29	0.67
Urine			
LTE_4	−0.04	50	0.76
11b-PGF_{2a}	0.11	50	0.44

Boldface indicates significant correlation after Spearmans Rho test at $p < 0.05$.

to urine, to appreciate disease-related release of mediators in the upper airways of patients with CRS. A decrease in multiple type 2 related mediators was reported after anti-IL-5 [25] or anti-IL-4/IL-13 treatment [26] using similar nasal secretion sample techniques. Mediator profile in nasal mucus samples also strongly correlated with mediators in tissue samples [45].

We could also show that locally secreted nasal LTE_4 correlated well with FeNO which suggests that nasal LTE_4 may indeed be closely linked to type 2 airway inflammation. Increased FeNO level is mainly driven by the presence type 2 airway inflammation [46] and FeNO has been postulated as a prognostic marker for asthma severity [47]. Evidence suggests that the response to anti-IL-4/IL-13, in terms of reduction of exacerbation rates, are predominantly seen in those with higher FeNO and/or higher blood eosinophil count [48]. Previously, LTE_4 production has been linked to type 2 inflammation in CRSwNP [15] and AERD [49].

To analyze cytokines with the employed method in nasal secretions was however somewhat difficult. The levels for several cytokines were dichomatized, in an attempt to disclose differences between the subgroups of patients. It may be speculated that one reason for levels below detection was due to high dilution of the nasal secretions during sampling. Previous studies have either analysed undiluted nasal secretory samples [45] or with less dilution [25]. Nonetheless, our results point to increased pro-inflammatory nasal IL-6 and type 2 IL-4 and IL-13 in CRSwNP-high and CRSwNP-AERD which are in accordance with previous studies reporting on nasal secretion cytokine levels in AERD [45, 50] or CRSwNP [51, 52]. However, as opposed to our results, none of these studies considered the NP severity differences. In addition, these two groups featured higher levels of FeNO. blood eosinophil counts as well as nasal PGD_2 and LTE_4 levels, indicating a potential exacerbated type 2 inflammation. It is interesting that one of the currently investigated biologics (*i.e.*, Dupilumab) is designed to diminish the IL-4/IL-13 signaling pathway, and CRSwNP patients with elevated Th2 levels are believed to benefit the most from such biologics [53]. Thus, the recruited patients with CRSwNP-high and CRSwNP-AERD in our study, most likely represent such severe cases that may benefit from biologics.

Eicosanoid release may be affected by concomitant medications. Intake of Cyclooxygenase inhibitors such as aspirin and NSAIDs may influence levels of prostaglandins. However, due to the preoperative routine at the clinic, with NSAIDs not taken before surgery and of course never for the patients with AERD, this was not a confounder that we needed to take into consideration. Glucocorticosteroids could also, at least theoretically, influence mediator release. The last day of completed course of OCS was recorded for each patient and the orally administered steroid treatment did not seem to influence our data (Tables 4 and 5). Other investigators have shown that at least urinary eicosanoid levels are independent of OCS [18]. Nasal steroids, locally administrated in the nostrils were used by the patients up until surgery and could potentially have an effect on the production and/or release of inflammatory mediators into the nasal secretions. This concomitant medication was however the same in all patients with CRS.

Smell test score is an important marker of CRS disease and HrQoL, whereas severity of comorbid asthma, reflected by elevated FeNO, further exacerbates the sinonasal inflammation [4]. In our findings, nasal LTE4 appeared to be related to the degree of smell loss and also FeNO in CRSwNP and AERD patients (Fig. 2), which further suggests the relevance to measure eicosanoids for indication of disease severity. However, it should be noted that the degree of loss of smell may be affected by other factors, including previous repeated FESS, leading to varying tissue damage and scarring. In addition, a well-treated patient with asthma may lower the FeNO value significantly, limiting the discriminatory benefit of FeNO. Regardless of such potential bias, measurements of FeNO and smell test scores may be of esteemed value to be sided together with biomarker analysis in order to accurately follow disease progression and treatment effects.

Another potential limiting factor in our study can be that analyses of eicosanoids were performed with enzyme immunoassays and not with liquid chromatography tandem mass spectrometry (LC-MS/MS), which

Table 5
Difference of lipid mediator levels in nasal secretions (ng/ml) and urine (ng/mmol creatinine) between CRSwNP patients that have completed their last course of oral corticosteroid 1–2, >2–4 and >4 months or never used, prior to sample collection, i.e., before their surgery.

	Months since completed OCS course						
	1–2 months	n	>2–4 months	n	>4 months or never used	n	P value
Nasal secretions							
LTE_4	0.84 (0.71–0.91)	9	0.78 (0.60–1.15)	8	0.48 (0.28–1.1)	19	0.41
LTB_4	0.88 (0.66–1.46)	9	1.53 (1.30–1.85)	8	1.28 (0.91–1.88)	19	0.26
PGD_2	0.63 (0.33–1.46)	9	0.91 (0.52–1.28)	8	0.52 (0.23–0.75)	19	0.46
PGE_2	3.10 (2.17–5.56)	9	8.55 (3.90–10.76)	8	3.63 (2.05–5.20)	19	0.18
15(*S*)-HETE	539.8 (290.0–640.6)	9	1064.3 (652.6–1441.1)	8	399.7 (217.2–637.3)	19	0.052
Urine							
LTE_4	127.5 (113.3–180.5)	14	160.2 (124.1–233.2)	13	138.9 (116.9–167.6)	24	0.34
11b-PGF_{2a}	45.3 (34.6–57.0)	14	48.9 (38.7–83.3)	13	45.4 (37.1–57.2)	24	0.60

Boldface indicates significant difference and p-value are from Kruskal-Wallis.

has been considered the most reliable method for quantification of eicosanoids in the literature (18). We are aware that the absolute amounts of the specific mediators may not be given with immunoassay. However, the purpose was to explore clinically relevant differences between the allocated patient groups. The cross-reactivities of the antibodies used in the ELISAs were negligible to other metabolites of concern for the interpretation of our findings. Thus, in our opinion the relative quantification with ELISA serves the aim of the current study.

To the best of our knowledge, NP score is usually not taken into account during inflammatory characterization using mucosal lipid mediators and cytokines in patients. Our study show that an abnormal eicosanoid release is apparent in regard of lower versus higher NP severity which furthermore associates with disease severity markers such as FeNO, blood eosinophil count and smell loss. There is a growing body of evidence that CRSwNP is not a singular disease entity but rather a heterogenous disease comprised of underlying inflammatory endotypes and different clinical characteristics [1, 6, 24]. Although the nasal milieu is notably high of type 2 cytokines in CRSwNP [15] other reports indicate that CRSwNP is more complex and characterized by a multitude of different inflammatory mediators, also including non-type 2 [6, 45, 54]. Thus, our findings shed lights on the importance of assessing NP score and suggest that the inflammatory heterogeneity present in CRSwNP, may be explained, at least partially, by differences in NP severity, and may improve the establishment of endotypes in the future.

In conclusion, our findings suggest that levels of eicosanoids in readily available nasal secretions may distinguish between patients with different NP severity and AERD and seem related to the degree of loss of smell and increased FeNO. Dividing patients with CRSwNP into groups of CRSwNP-low and CRSwNP-high, in combination with biomarker analyses, may have clinical implications in the future when biologics become an accessible treatment of CRSwNP where disease progression and treatment effects are to be followed. Whether similar eicosanoid inflammatory signatures are related to progression and refractory nasal polyp growth remains to be elucidated in further studies.

Funding sources

This work was funded by Sophiahemmet Research Foundation [2021–2022] and Swedish Asthma and Allergy research foundation [F2019–0029]. The funders were not involved in study design, collection, analysis or interpretation of data nor in the writing of the article or in the decision to submit our work for publication.

CRediT authorship contribution statement

Mattias Jangard: Patient recruitment, clinical assessments, receiving informed consent, manuscript editing. **Michael Ryott:** Patient recruitment, clinical assessments, receiving informed consent and manuscript editing. **Axel Nordström:** Execution of sample collection, analysis, and data interpretation, writing and manuscript editing. **Maria Kumlin:** Study design, data collection, writing and manuscript editing. **Marie Svedberg:** Study design, analysis, manuscript editing.

Declaration of Competing Interest

None of the authors have any conflict of interest to declare.

Acknowledgments

We sincerely thank staff, nurses, and physicians, at the ENT clinic for help with patient recruitment and blood sample collection.

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