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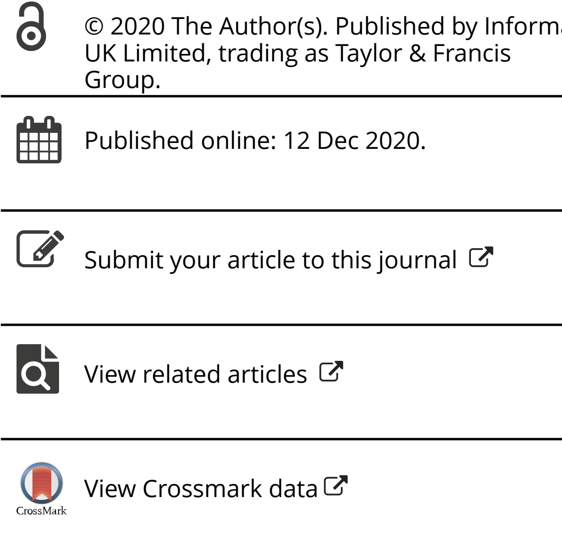

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RESEARCH ARTICLE

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Local inflammatory marker production in Lithuanian patients with chronic rhinosinusitis with nasal polyps

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ABSTRACT

There are two clinical subtypes of chronic rhinosinusitis (CRS): chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP). The aim of the study here was to determine the levels of invasive inflammatory markers in nasal mucosa samples taken from CRSwNP patients during the surgery and to identify markers that could serve as targets for potential clinical and therapeutic interventions. The study was carried out in 59 patients with proven CRSwNP and a control group consisting of 52 healthy individuals. Concentrations of the inflammatory markers of interest were determined using a LuminexR Assay multiplex kit. The data obtained indicated that levels of inflammatory cytokines interleukin (IL)-2, -4, -5, -7, -12, -17 and -22 were all significantly higher in the nasal polyps (NP) than those in the mucosa of control participants. No differences were seen between the study groups for IL -6, -10, -13, -21 and interferon (IFN)-γ. OR (Odds Ratio) analyses confirmed that elevations in mucosal levels of IL-2, -4, -5, -7, -12, -17, and -22 were likely immune markers of CRSwNP. In conclusion, the present study demonstrated that IL-2, -4, -12 and -22 may be important in the etiopathogenesis of CRSwNP; as markers, each show moderate sensitivity, but high specificity in the Lithuanian population. IL-17 had good sensitivity, but low specificity in the CRSwNP patients.

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Introduction

Chronic rhinosinusitis (CRS) is a heterogeneous, multi-factorial disease affecting 10.9% of the European population (Hastan et al. 2011) and 13.4% of the American population (Pleis et al. 2008). Currently described clinical CRS subtypes are based on phenotypes and dependent on subjective clinical parameters that are both time and observer dependent, such as the presence or absence of nasal polyps. These subtypes do not reflect the molecular mechanisms underlying the disease; thus, this classification system is not sufficiently specific for many clinical and research applications (Anderson 2008; Bachert and Akdis 2016).

CRS is divided into chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP). This classification is referred to as phenotype-driven classification and is defined as an inflammatory process of the nose and paranasal sinus mucosa that have been activated by the colonization of microorganisms that causes an excessive host response. The sinonasal epithelium-pathogen interactions involve a complex set of innate and adaptive immune pathways at the mucosal surface that drive inflammatory responses to protect the host from infection. Approximately one-third of the tissue obtained from nasal polyps shows an inflammatory response and the local production of mucosal polyclonal IgE. The type of inflammation, changes in T-cell subsets, interactions with the bacterial environment in the nose and immunological consequences of these interactions, such as IgE formation, must be monitored to make predictions and personalized treatment (Bachert et al. 2012). Defects in the sinonasal epithelial cell barrier, increased exposure to pathogenic and colonizing bacteria, and dysregulation of the host immune system are all thought to play important roles in pathogenesis of this disease (Stevens et al. 2016).

In the context of CRS, biomarkers indicating tissue inflammation are currently used in only research and clinical trials, but may be used in everyday practice to diagnose CRSwNP and evaluate treatment responses (Dennis et al. 2016). There is no single validated biomarker that can reliably determine whether a patient has CRSwNP, CRSsNP, acute sinusitis or no sinus disease at all. There are no biomarkers that can predict the outcome of medical or surgical treatment. Biomarker investigations in the peripheral blood, which is easy to obtain, may not reflect local inflammatory changes in nasal polyps. Stevens et al. (2015) examined whether there is a direct correlation between levels of inflammatory mediators measured in nasal lavage fluid and nasal polyp tissue from the same individuals. Of the 20 mediators evaluated, only IL-10 levels in the nasal lavage fluid and nasal polyp tissue were found to have a significant and positive correlation.

Clearly, additional studies are needed to further explore the clinical and pathophysiological features of CRSwNP so that

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biomarkers can be identified and novel advancements to improve disease treatment and management can be made. A new view on the type and role of inflammation in airway disease needs to be elaborated, which might lead to distinct therapeutic approaches and outcome parameters for specific types of inflammation (Zhang et al. 2008, 2014). Accordingly, the aim of the study undertaken here was to determine the concentrations of invasive inflammatory markers in nasal mucosa samples taken from CRSwNP patients during the surgery and to identify potential markers that could serve as targets for potential clinical and therapeutic interventions.

Materials and methods

Patient characteristics

The study was carried out in 59 patients with proven CRSwNP who underwent endonasal sinus surgery and 52 controls who underwent other rhinologic surgeries (such as those involving the skull base or lacrimal duct, orbital decompression surgery, or septoplasty) and had no history of any sinonasal diseases or recent trauma. All surgeries for the study subjects had taken place in the Department of Otorhinolaryngology at the Lithuanian University of Health Sciences Hospital Kauno Klinikos in the period from 2017 to 2019. All subjects provided informed written consent to participate in the study and before any the collection of tissue specimens. Diagnosis of CRS was made according to a 2012 European position paper on rhinosinusitis and nasal polyps.

Demographic data, medical comorbidities, and smoking history were obtained using a standard questionnaire. Patients who had undergone prior treatment(s) with antibiotics, systemic or topical corticosteroids, or other immunomodulating drugs in the 4-week period prior to surgery, who were $\leq$ 18-years-old, pregnant, presented with any autoimmune disease, diabetes, had cystic fibrosis, inverted papillomae, or sinonasal granulomatous disease were excluded from the study. This study was conducted with the approval of the Institutional Ethics Committee (#P1-86-2004) at the Lithuanian University of Health Sciences.

Determination of inflammatory marker concentrations

Tissue biopsies were collected from nasal polyps and the middle turbinate during endonasal sinus surgery on the CRSwNP subjects. From the non-CRS subjects, biopsies were taken from the middle turbinate during rhinologic surgeries (such as those involving the skull base or lacrimal duct, orbital decompression surgery, or septoplasty) and had no history of any sinonasal diseases or recent trauma. In all cases, samples were collected using standard endonasal equipment. Upon removal, each sample was immediately frozen and stored at −80 °C until use. For each analysis, 50 mg tissue sample was transferred to a gentle-MACS C tube containing Buffer AL extraction reagent (Quiagen, Hilden, Germany). The sample was then processed using a gentle-MACS homogenizer for 1–2 min until all materials were evenly suspended. Homogenized sample was then transferred to a 1.5-ml polypropylene tube and centrifuged (room temperature, 5 min, 10 000 × *g*). The resulting supernatant was collected and an aliquot removed and diluted twice with kit-provided buffer.

This material was then assessed for levels of interleukin (IL)-2, -4, -5, -6, -7, -10, -12, -13, -17, -21, and -22, as well as interferon (IFN)-γ using a LuminexR multiplex kit (Human Premixed Multi-Analyte, R&D Systems, Minneapolis, MN) according to manufacturer protocols. Specific antibodies in the kit had been pre-coated onto magnetic microparticles embedded with fluorophores at set ratios for each unique microparticle region. Microparticles, standards, and samples were pipetted into wells, and the immobilized antibodies were bound to the tested analytes. After any unbound substances were washed away, a biotinylated antibody cocktail specific to the tested analytes was added to each well. Following washes to remove unbound biotinylated antibody, streptavidin-phycoerythrin (PE)-conjugate was added to each well and the plates incubated at room temperature for 30 min. Final washes with kit buffer were then performed to remove unbound streptavidin-PE. The plates were then analyzed using a LuminexR 100 analyzer. Each sample was analyzed in triplicate.

Statistical analysis

Statistical data analysis was performed using the SPSS v.23.0 statistical package (IBM, Armonk, NY). A Kolmogorov–Smirnov test was employed to determine distribution of quantitative data. All data were found to be non-normally distributed and compared using a Mann–Whitney *U*-test. A Kruskal–Wallis test was used to compare three independent groups. A Chi-square and Fisher's exact test (for small sample size) were used to determine whether qualitative data were related. To assess minimally false negative and minimally false positive results with the greatest accuracy, a ROC (receiver operating characteristics) curve method was used. Binary logistic regression analysis was performed to determine the predictive values of cytokine levels for CRSwNP. Differences between groups were considered statistically significant at $p < 0.05$.

Results

Characteristics of the patients and controls in the study are shown in Table 1. Mean ages and ratios of females:males in both groups were similar. All patients were examined, treated, and followed up by a single surgeon. The frequency of allergy or asthma was significantly higher in the CRSwNP group than in the controls (Table 1). A total of 18.6% of patients in the CRSwNP group and only 7.7% of patients in the control group reported they were past or current smokers, but this difference was not significant.

The analyses of the inflammatory marker concentrations found that all of the investigated samples were within the upper and lower limits of the Luminex assay (provided by manufacturer). Theses analyses showed that in the nasal mucosa biosamples from the CRSwNP and control groups indicated that levels

Table 1. Characteristics of patients included in the study.

	CRSwNP	Control	*p* Value
Population	59 (53.2)	52 (46.8)	
Age (years ± SD)	50.3 ± 14.7	45.8 ± 19.5	0.177*
Male	31 (52.5)	28 (53.8)	0.891
Female	28 (47.5)	24 (46.2)	
Yes allergy	15 (25.4)	0 (0)	< 0.001
No allergy	44 (74.6)	52 (100)	
Yes asthma	18 (30.5)	0	< 0.001
No asthma	41 (69.5)	52 (100)	
Yes – smoking	11 (18.6)	4 (7.7)	0.092
No – smoking	48 (81.4)	48 (92.3)	

Except for age, all data reported as [N (%)], with N = Number of participants. *p* Values determined using a chi-square test, **p* value determined using a Student's *t*-test.

of inflammatory IL-2, -4, -5, -7, -12, -17, and -22 cytokines were significantly ($p < 0.001$) higher in the nasal polyp and middle turbinate tissues groups combined than in the mucosa of control subjects. Median IL-2 and IL-4 concentrations in the CRSwNP group were 485.8 and 208.3 (25th–75th percentile, 450.2–511.3 [IL-2] and 141.7–237.9 [IL-4]) pg/ml, respectively; corresponding control group values were 143.7 and 61.0 (25th–75th, 77.0–185.7 and 21.8–73.4) pg/ml.

Median IL-5 and IL-17 levels in the CRSwNP group samples were 29.9 and 101.7 (25th–75th, 24.1–97.7 [IL-5] and 70.0–113.5 [IL-17]) pg/ml, respectively; corresponding control values were 28.2 and 69.0 (25th–75th, 19.9–30.2 and 44.5–116.4) pg/ml ($p = 0.002$). The median IL-7 level in the CRSwNP group was 29.9 (25th–75th, 22.8–49.4) pg/ml, while that in the control group it was 26.3 (25th–75th, 22.8–31.3) pg/ml ($p = 0.022$). The median IL-12 level in the CRSwNP group was 677 (25th–75th, 290.5–711.9) pg/ml, and 136.9 (25th–75th, 110.9–226.33) pg/ml in the controls ($p < 0.001$). The median IL-22 concentration in the CRSwNP group was 109.7 (25th–75th, 75.1–136.7) pg/ml, but 44.3 (25th–75th, 32.7–52.0) pg/ml in the controls ($p < 0.001$). No differences in IL-6, IL-10, IL-13 and IFNγ levels between the study groups were noted (Table 2).

The data were also stratified and inflammatory marker concentrations then compared between the control group and CRSwNP subjects who had biosamples taken from the middle turbinate and polyps. Data from each of the latter groups were compared against one another as well as vs. controls. Even with these analyses, comparisons between the middle turbinate and polyp groups revealed only one statistically significant difference, i.e. in IL-22 levels (Table 3).

A comparison of all cytokines was also made in the context of host asthma and allergy status. Members of the CRSwNP group were sorted into four different groups, i.e. (1) without asthma or allergies, (2) with asthma and allergies, (3) with asthma but without allergies, and (4) with allergies but without asthma. Data from each group were then compared against one another as well as vs. controls. The comparisons of cytokine concentrations between the groups revealed there was a significant ($p < 0.001$) increase in IL-5 concentration associated with the asthma and allergy group (Group 2) vs. controls, same as in Group 3 (with asthma but without allergies) and Group 4 (with allergies but without asthma). The median value for IL-5 was 176.1 (25th–75th, 125.5–223.4) pg/ml in the Group 2 subjects; 78.2 (25th–75th, 24.1–129.8) pg/ml in the Group3 subjects; 30.6 (25th–75th, 29.9–219.9) pg/ml in the Group 4 subjects; the values was 28.2 (25th–75th, 19.9–30.2) pg/ml in the controls. Significant ($p < 0.001$) increases in IL-22 concentrations associated with Group 2 (with asthma and allergies) and Group 3 (with asthma but without allergies) vs Controls, Group 1(without asthma or allergies) and Group 4 (with allergies but without asthma) were also noted. The median value for IL-22 was 146.9 (25th–75th, 115.5–208.3) pg/ml in Group 2 subjects and 162.1 (25th–75th, 95.3–177.0) pg/ml in Group 3 subjects; in the controls, Group 1, and Group 4, the values were 44.3 (25th–75th, 32.7–52.0), 109.7 (25th–75th, 71.2–132.8), and 90.5 (25th–75th, 71.2–133.6) pg/ml, respectively (Table 4).

This study also determined the cutoff values for each cytokine using ROC analysis. IL-2, IL-4, IL-12, IL-17 and IL-22 levels were increased in the tissues from CRSwNP patients. ROC analyses indicated an 87.2% sensitivity and a 94.2% specificity for IL-22, 83.8% sensitivity and 96.2% specificity for IL-2, 83.8% sensitivity and 94.2% specificity for IL-4, 74.4% sensitivity and 92.3% specificity for IL-12, and 94.9% sensitivity and 40.4% specificity for IL-17. The results showed there were moderate values for the areas under the curve (Table 5).

Table 2. Levels of inflammatory markers in CRSwNP patients (pooled) and in controls.

	Control ($n = 52$)	CRSwNP ($n = 59$)	
Cytokine	Median [25th–75th percentile]		p Value
IL-6	229.8 [149.7–234.6]	208.3 [154.7–233.5]	0.838
IL-10	214.7 [135.1–271.3]	228.3 [128.9–345.3]	0.308
IL-13	153.3 [129.3–166.7]	156.6 [132.2–169.7]	0.478
IL-21	103.6 [71.0–111.1]	10.7 [77.9–117.5]	0.239
IFNγ	1490.5 [1440.1–1719.9]	1477.0 [1427–1730]	0.982
IL-2	143.7 [77.0–185.7]	485.8 [450.2–511.3]	< 0.001
IL-4	61 [21.8–73.4]	208.3 [141.7–237.9]	< 0.001
IL-5	28.2 [19.9–30.2]	29.9 [24.1–97.7]	0.002
IL-7	26.3 [22.8–31.3]	29.9 [22.8–49.4]	0.022
IL-12	136.9 [110.9–226.33]	677.0 [290.5–711.9]	< 0.001
IL-17	69.0 [44.5–116.4]	101.7 [70.0–113.5]	0.002
IL-22	44.3 [32.7–52.0]	109.7 [75.1–136.7]	< 0.001

All data as pg/ml.

Discussion

In this study, a wide spectrum of cytokines was assessed to identify potential indicators of (possible) local inflammation in the nasal mucosa and in polyps isolated from CRSwNP patients. Tomassen et al. (2016) identified distinct inflammatory endotypes of CRS within patients that largely correlated with phenotypes and further differentiated them. Classification by endotypes clearly provides a more accurate description of the inflammatory mechanisms involved than classification by phenotype only, and the types of inflammation in patients with CRS might be more diverse than previously assumed. Any CRS-related inflammation should be considered multi-dimensionally heterogeneous on the T-helper-cell (T_H) Type 1, T_H2, T_H17, eosinophilic/neutrophilic,

Table 3. Levels of inflammatory markers associated with samples from the control, middle turbinate, and polyp groups.

	Group			
Cytokine	Control ($n = 52$)	Middle turbinate ($n = 59$)	Polyp ($n = 59$)	
IL-2	*,‡143.7 [77.0–185.7]	*485.8 [453.8–514.9]	‡477.8 [448.0–509.1]	$\chi2 = 68.84$, df = 2, $p < 0.001$
IL-4	*,‡61.0 [21.8–73.4]	*189.0 [141.7–228.3]	‡212.7 [141.7–238.6]	$\chi2 = 86.72$, df = 2, $p < 0.001$
IL-5	*,‡28.2 [19.9–30.2]	*30.1 [24.1–97.2]	‡29.9 [24.1–98.1]	$\chi2 = 9.62$, df = 2, $p = 0.008$
IL-7	*,‡26.3 [22.8–31.3]	*29.9 [22.8–50.4]	‡29.9 [22.8–49.1]	$\chi2 = 5.69$, df = 2, $p = 0.058$
IL-12	*,‡136.9 [110.9–226.3]	*677.1 [234.4–714.2]	‡677.1 [444.4–707.2]	$\chi2 = 46.08$, df = 2, $p = 0.001$
IL-17	*,‡69.0 [44.5–116.4]	*101.7 [70.0–113.3]	‡97.7 [66.1–113.5]	$\chi2 = 9.64$, df = 2, $p = 0.008$
IL-22	*,‡44.3 [32.7–52.0]	*, #136.1 [109.7–167.5]	‡,#90.5 [66.4–132.8]	$\chi2 = 92.45$, df = 2, $p < 0.001$

All data are reported in pg/ml as Median [25th–75th percentile]. The p value was determined using a Kruskal–Wallis test; multiple comparisons p values were obtained using a Mann–Whitney test: $^{*}p < 0.05$ between Control and Middle turbinate groups; $^{‡}p < 0.05$ between Control and Polyp groups; $^{\#}p < 0.001$ between Middle turbinate and Polyp groups.

Table 4. Levels of inflammatory markers associated with samples from control, middle turbinate, and polyp groups – in the context of host asthma and/or allergy status.

	Group					
Cytokine	Control ($n = 52$)	1 ($n = 71$)	2 ($n = 20$)	3 ($n = 16$)	4 ($n = 10$)	
IL-2	143.7 [77.0–185.7]	*485.8 [453.8–514.9]	*469.8 [109.0–510.5]	*474.2 [449.8–499.6]	*506.2 [466.2–520.0]	$\chi^2 = 71$, df = 4, $p < 0.001$
IL-4	61.0 [21.8–73.4]	*211.2 [154.9–237.9]	*,#134.7 [82.3–172.2]	*,+217.9 [168.8–246.5]	*,+223.6 [159.2–248.3]	$\chi^2 = 94.117$, df = 4, $p < 0.001$
IL-5	28.2 [19.9–30.2]	28.2 [19.9–30.1]	*,#176.1 [125.5–223.4]	*,#,+78.2 [24.1–129.8]	*,#30.6 [29.9–219.9]	$\chi^2 = 54.666$, df = 4, $p < 0.001$
IL-7	26.3 [22.8–31.3]	*30.2 [26.3–50.4]	#26.3 [21.5–29.9]	*,#,+21.0 [10.9–23.7]	*,#,+,&138.9 [35.2–169.4]	$\chi^2 = 33.519$, df = 4, $p < 0.001$
IL-12	136.9 [110.9–226.3]	*681.7 [621.5–723.4]	#413.8 [106.2–684.0]	*670.1 [179.8–751.3]	*672.5 [505.3–707.2]	$\chi^2 = 54.537$, df = 4, $p < 0.001$
IL-17	69.0 [44.5–116.4]	*105.6 [70.0–113.3]	75.5 [70.0–105.6]	*105.7 [68.5–158.7]	107.7 [61.1–130.0]	$\chi^2 = 11.817$, df = 4, $p = 0.019$
IL-22	44.3 [32.7–52.0]	*109.7 [71.2–132.8]	*,#146.9 [115.5–208.3]	*,#162.1 [95.3–177.0]	*,+,&90.5 [71.2–133.6]	$\chi^2 = 91.194$, df = 4, $p < 0.001$

All data are reported in pg/ml as Median [2^{5th}–75th percentile].
Middle turbinate/Polyp groups: (1) neither asthma nor allergy; (2) asthma and allergy; (3) asthma but no allergy; (4) allergy but no asthma.
Value significantly different from: *control, #Group 1, +Group 2, &Group 3. At the 0.05 level, by Mann–Whitney test.

Table 5. Cytokine ROC distributions to test predictive values and characteristics across groups.

Cytokine Cutoff	Area under ROC curve (%)	Sensitivity, Specificity (%)	Control and CRSwNP [n (%)]	p Value	CRSwNP group OR [95% CI]
IL-2 (> 330.0)	90.0	83.8, 96.2	2 (3.8) 98 (93.8)	< 0.001	128.95 [28.88–575.77]
IL-4 (> 110.0)	94.8	83.8, 94.2	3 (5.8) 98 (93.8)	< 0.001	84.25 [23.78–298.50]
IL-5 (> 40.0)	64.8	29.9, 100	0 35 (29.9)		
IL-7 (> 36.0)	61.0	35.9, 87.0	7 (13.5) 42 (35.9)	0.003	3.60 [1.49–8.69]
IL-12 (> 360.0)	82.7	74.4, 92.3	4 (7.7) 87 (74.4)	< 0.001	34.80 [11.57–104.67]
IL-17 (> 62.0)	65.0	94.9, 40.4	31 (59.6) 111 (94.9)	< 0.001	12.53 [4.65–33.75]
IL-22 (> 62.0)	93.6	87.2, 94.2	3 (5.8) 102 (87.2)	< 0.001	111.07 [30.71–401.69]

CI: confidence interval; OR: odds ratio.

proinflammatory, superantigen, and possibly T_H22 axes (Tomassen et al. 2016).

The current study found that increased levels of IL-2, -4, -5, -7, -12, -17, and -22 in nasal tissue samples significantly differentiated CRSwNP patients from healthy individuals. CRSwNP in both Caucasian and Asian patients has previously been characterized by T-cell activation and impaired regulatory T-cell function (Zhang et al. 2008, 2014). However, T-effector cells in the samples from Caucasian patients were found to be T_H2-biased, whereas samples from their Asian counterparts demonstrated T_H1/T_H17 polarization. In general, T-cells are the major source of cytokines in nasal polyps. Among these, the T_H1-type cytokines like TNFα, IFNγ, IL-2, and IL-12, can induce cell-mediated immunity and tumor suppression, i.e. they have proinflammatory roles in local immune responses.

In contrast, T_H2-type cytokines inhibit T-cell-mediated immunity and primarily induce humoral immunity. The best known T_H2 cytokines are IL-4, IL-5, IL-6 (which plays dual roles), IL-8, and IL-10. Shi et al. (2009) analyzed T-cell cytokine profiles in nasal polyps from a Chinese patient population and found there were increased levels of IL-4, IL-5, and IFNγ, but decreases in IL-10 and transforming growth factor (TGF)-β production. The data from the current study showed similar results for IL-4 and IL-5, but different results for IFNγ and IL-10. The differences could potentially be ascribed to a fact that the frequency of allergy or asthma in the present study population was significantly higher in the CRSwNP group than in the controls. No differences in IFNγ and IL-10 tissue levels between the study groups were observed here. Thus, the current findings may provide new data specific to nasal polyp inflammatory mechanisms in Lithuanian patients.

The findings here regarding IL-5 appear to be of note. IL-5 is a key driver of eosinophilia, chemotaxis, differentiation, activation, and eosinophil survival (Kim et al. 2017). IL-5 has been shown to be prominent in 80% of nasal polyps in certain populations (Kartush et al. 2019). In addition, other studies have reported higher IL-5 concentrations in nasal polyps from CRSwNP patients than in control tissues. One study showed that IL-5 expression was significantly higher in nasal polyp tissue than in the inferior concha (Zhao et al. 2014). Data in a study by Kubota et al. (2017) demonstrated that high IL-5 expression might be closely associated with formation and/or development of nasal polyps. Thus, it would seem logical then that preventing IL-5 from binding to an IL-5R subunit (IL-5Ra) on an eosinophil would be one promising therapeutic strategy, Ekinci and Ozcan (2019) reported similar results as here regarding IL-17 and IL-10 levels in CRSwNP subjects, but different results regarding IL-4 and IL-5 levels. Those investigators found increased IL-17 levels in polyps isolated from CRSwNP patients, with no concurrent change in IL-10 levels. Oddly, in that study, while no significant changes to tissue IL-4 levels were present, levels of IL-5 were actually found to be lower in the afflicted group.

Ekinci and Ozcan (2019) also found that IFNγ concentrations and other T_H2 cytokines (i.e. IL-4, IL-5, IL-17, IL-10) were also lower in tissue samples from CRSwNP patients. From those results, it may seem that low IFNγ levels and increases in expression of regulatory IL-7 in the nasal mucosa may be important in the etiopathogenesis of nasal polyps (NP). Bikker et al. (2012) stated that IL-7 primarily acts on T-cells that richly express IL-7 receptors and that are increased at the site of inflammation, and mainly induces T_H17-associated cytokine secretion. Further, Saitoh et al. (2010) proved there was an IL-17 contribution to

CRS pathology by reporting a significant increase in IL-17 protein and mRNA levels in the nasal polyps associated with asthma compared with control sinus mucosa. In the current study, only 1/3 of CRS patients were presented with asthma; this clearly may have affected the present results. In addition, it is likely that the small difference in levels of IL-5 seen between the control and patient groups was likely impacted by the low number of allergic people involved in a study. The present study also noted no differences in IL-6, IL-10, IL-13 and IFNγ levels were found between the study groups. This contrasts with results of another study wherein levels of anti-inflammatory IL-10 were increased in the nasal tissues and peripheral blood of patients with allergic rhinitis (Moore et al. 2001). However, that outcome was only seen among individuals who had been successfully treated with long-term immunotherapies Thus, immunotherapy itself might have been a key factor for that particular induction of IL-10.

Although high levels of IL-17 were found in the tissues recovered from the CRSwNP patient group here, the ROC analyses revealed that the specificity of IL-17 as a potential marker was low. Similarly, the present study found that IL-22 levels were increased in the tissues from CRSwNP patients; ROC analyses indicated an 87.2% sensitivity and a 94.2% specificity. This outcome was along the lines of that from Noyama et al. (2017) who showed that IL-22 was mainly expressed in inflammatory cells infiltrating the nasal polyps and that IL-22 played a regulatory role in the pathogenesis of CRSwNP by enhancing MUC1 expression.

Conclusions

The present study demonstrated that increases in local invasive inflammatory markers (i.e. nasal turbinate and polyps) levels of IL-2, IL-4, IL-12 and IL-22 may be important in the etiopathogenesis of CRSwNP. The study also showed using ROC analyses that these cytokines were potential biomarkers of moderate sensitivity and high specificity in this Lithuanian population. In comparison, IL-17 as a marker showed good sensitivity, but low specificity in the CRSwNP patients. The utility of IL-6, IL-10, IL-13 and IFNγ levels as biomarkers was diminished as there were no significant differences in tissue levels of each between the tested study groups. At this point, it remains unclear if these potential biomarkers for use in evaluating CRSwNP have any utility beyond the Lithuanian populations evaluated here. Clearly, further collaborative studies are warranted to ascertain if these are good generic markers of CRSwNP or not.

Disclosure statement

The authors declare no conflicts of interest. The authors alone are responsible for the content of this manuscript.

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