

Original article

Differentiation of chronic sinus diseases by measurement of inflammatory mediators

Background: Chronic rhinosinusitis (CRS) clinically is a heterogeneous group of sinus diseases, which may cover different disease entities, or may represent a disease continuum. Studying inflammatory cells and mediators in clearly defined disease subgroups may lead to a better differentiation of chronic sinus diseases.

Methods: Sinonasal mucosal tissue from 10 nasal polyp (NP) patients, 13 cystic fibrosis patients (CF-NP), eight CRS subjects without polyps, and nine control patients were stained for CD3, CD25, CD68, CD20, myeloperoxidase (MPO), CD138 and tissue homogenates were assayed for eotaxin, interleukin (IL)-1 β , IL-2sR α , IL-5, interferon (IFN)- γ , IL-8, transforming growth factor (TGF)- β 1, tumor necrosis factor- α , and MPO by enzyme-linked immunosorbent assay or UNICAP system.

Results: Nasal polyp and CF-NP showed increased numbers and activation of T cells, while only NP displayed an increase in plasma cells. Nasal polyp had significantly higher levels of eosinophilic markers [eosinophils, eotaxin, and eosinophil cationic protein (ECP)] compared with CRS, controls and CF-NP. Chronic rhinosinusitis was characterized by a Th1 polarization with high levels of IFN- γ and TGF- β , while NP showed a Th2 polarization with high IL-5 and immunoglobulin (Ig) E concentrations. Nasal polyp and CF-NP were discriminated by edema from CRS and controls, with CF-NP displaying a very prominent neutrophilic inflammation.

Conclusion: Based on cellular and mediator profiles, we suggest that CRS, NP, and CF-NP are distinct disease entities within the group of chronic sinus diseases.

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Key words: chronic rhinosinusitis; cystic fibrosis; cytokine profile; inflammation; nasal polyps; remodeling; Th1/Th2 polarization.

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Rhinosinusitis remains a significant health problem with a considerable socio-economic burden and is still increasing in prevalence and incidence. US data of 1997 indicate a prevalence of apparently 15% of chronic rhinosinusitis (CRS) patients (defined as having 'sinus trouble' for more than 3 months in the year before the interview) in the general population (1, 2). Chronic rhinosinusitis is a heterogeneous group of sinus disease that may represent an umbrella covering different disease entities. Often, nasal polyposis and CRS are taken together as one disease entity since there is only limited and contradictory

data available to clearly differentiate between them. Until now it is not clear whether CRS is a result of recurrent acute rhinosinusitis which ultimately will lead to polyp growth or whether these entities are distinct diseases with a different pathophysiology. In order to summarize the current knowledge in pathophysiology and the therapeutic and diagnostic management of sinus disease, two position papers have recently been developed in the USA (3) and in Europe (4). In these consensus documents rhinosinusitis is diagnosed based on symptoms and duration of symptoms, clinical examination, nasal endoscopy, and CT scan. However, the pattern of symptoms and signs is overlapping in all patients with chronic sinus inflammation, whether they have formation of nasal polyps (NP) or not (CRS). In the EP³OS position paper differentiation between CRS and NP must be based on endoscopy in which NP represents a subgroup of CRS.

So far, NP formation in specific conditions, such as cystic fibrosis (CF) and allergic fungal sinusitis (AFS) can be differentiated as disease entities, based on genetic defects in CF and a specific immunoglobulin (Ig)

Abbreviations: CRS, chronic rhinosinusitis; NP, nasal polyps; CF-NP, cystic fibrosis-nasal polyps; Th, T helper cell; IL, interleukin; TNF- α , tumor necrosis factor alpha; TGF- β 1, transforming growth factor beta; ECP, eosinophil cationic protein; MPO, myeloperoxidase; IgE, immunoglobulin E; IFN- γ , interferon gamma; IL-2sR α , interleukin 2 soluble receptor alpha; IL-2R, interleukin 2 receptor; ROC, receiver operating characteristic; COPD, chronic obstructive pulmonary disease; SEM, standard error of the mean; HE, hematoxylin and eosin.

E-mediated immune response to fungi in allergic fungus rhinosinusitis (AFRS) respectively (5, 6).

For the majority of chronic sinusitis cases; however, classification awaits further insights into pathomechanisms and the introduction of appropriate disease markers. Such markers could possibly be derived from inflammatory cells, the Th1/Th2 polarization, remodeling processes linked to fibrosis or edema formation, or from innate or adaptive immunity products like as Toll-like receptors or immunoglobulins. Differences in some of these markers in sinus disease *vs* nasal control tissue have been described, but these have not proven useful to differentiate disease entities of CRS. (7) Several studies have also attempted to classify NPs and CRS based on several inflammatory and immunohistochemical markers. Most of the studies describe a pronounced eosinophilic inflammation in nasal polyposis (8, 9). For example, interleukin (IL)-5, an eosinophil survival and differentiation factor, and eosinophil cationic protein (ECP), an indicator for eosinophil activation, have been found to be significantly increased in NP *vs* controls (10). In contrast with these findings other studies show that there is no significant higher mRNA expression of IL-4, IL-5, and interferon (IFN)-gamma in NP tissue compared with controls (11, 12). Nevertheless, a recent publication by Riechelmann et al. (13), using a principal component analysis, confirmed that IgE and IL-5 were specific biomarkers for NPs. Recently, differences in the expression of metalloproteinases and their inhibitors could also be demonstrated in CRS *vs* NP mucosal tissue (14).

In our study, we investigated the cytokine and mediator pattern in different subgroups of chronic sinusitis: CRS without NP formation, patients with NP, CF patients with nasal polyps (CF-NP), and controls (no sinus disease; CO). Based on previous studies in CRS with and without NP (15) and comparative studies of NP and CF-NP (7), the following groups of mediators were carefully selected: proinflammatory cytokines [IL-1 β , tumor necrosis factor (TNF)- α], eosinophil-related mediators (ECP, eotaxin), neutrophil-related mediators [IL-8, myeloperoxidase (MPO)], T cell and subset markers [sIL-2R α , IFN- γ , IL-5], a T-regulatory marker [transforming growth factor (TGF)- β 1], and IgE. Secondly, an immunohistochemical analysis for several key inflammatory cells (T lymphocytes, eosinophils, neutrophils, macrophages, and B lymphocytes) was performed. The aim of this study was to possibly identify differences in the profile of inflammatory mediators and cellular characteristics for each of the selected disease subgroups.

Materials and methods

Patients

Nasal tissue was obtained from 10 polyp patients, 13 CF patients, eight CRS subjects, and nine control patients at the department of Oto-Rhino-Laryngology of the University Hospital of Ghent,

Belgium. Nasal polyp, CRS, and CF samples were obtained during routine endonasal sinus surgery. Patients undergoing septoplasty because of anatomical variations were considered as controls, samples were obtained from the inferior turbinate during septal surgery. The diagnosis of sinus disease was based on history, clinical examination, nasal endoscopy, and computed tomography (CT) scan of the sinuses according to the EP³OS guidelines (4). Computed tomography scans were graded as per Lund-Mackay (16). Individual rhinosinusitis symptoms were evaluated by a physician on a scale of 0–3 (no symptoms, mild, moderate, severe) before surgery. Polyps were graded by size and extent in both the left and right nasal fossa on a scale of 0–3, according to the Davos classification (16). Cystic fibrosis patients were diagnosed and referred to our clinic by the pediatric department. Only CF patients with endoscopically visible polyps were included in the CF group. The reasons for surgical procedure were unrelated to the study in all patients.

The atopic status was evaluated by skin prick tests to common inhalant allergens. The diagnosis of asthma was performed by a pneumologist. None of the subjects used oral or nasal corticosteroids 3 weeks before surgery. All patients gave their written informed consent and the ethics committee of the Ghent University Hospital approved the study.

Measurement of cytokines and IgE in tissue homogenates

Freshly obtained tissue specimens were weighed, and 1 ml of 0.9% NaCl solution was added per every 0.1 g tissue. The tissue was then homogenized with a mechanical homogenizer (B.Braun, Melsungen, Germany) at 1811 g for 5 min on ice as described previously (17). After homogenization, the suspension was centrifuged at 3000 rpm for 10 min at 4°C and the supernatants separated and stored at –80°C until analysis. All samples were assayed for eotaxin, IL-1 β , IL-2sR α , IL-5, IFN- γ , IL-8, TGF- β 1, TNF- α , and MPO using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Quantikine ELISA, Minneapolis, Portland, MN, USA; MPO: Oxis International, OR, USA). Immunoglobulin E and ECP were measured by the UNICAP system (Pharmacia, Uppsala, Sweden).

Immunohistochemistry

Specimens were snap-frozen in liquid nitrogen cooled methyl butane and stored at –80°C. Cryostat sections were prepared (6 μ m) and mounted on SuperFrost Plus glass slides (Menzel Glaeser, Braunschweig, Germany), packed in aluminum paper and stored at –30°C until staining. Sections were stained with hematoxylin–eosin (HE) and immunohistochemically stained with the following mouse monoclonal antibodies: CD3 (clone UCHT1; Dako, Glostrup, Denmark), CD25 (clone ACT-1; Dako), CD68 (clone EBM11; Dako), CD20 (clone L26; Dako), MPO (clone 2C7; Serotec, Oxford, UK), and CD138 (clone MI15; Dako). For immunohistochemical staining specimens were fixed in acetone. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in tris-buffered saline containing 0.1% sodium azide for 20 min. Primary antibodies or negative controls, consisting of the corresponding isotype control, were incubated for 1 h and detected using the LSAB+ technique conjugated with peroxidase according to the manufacturer's instructions (labeled streptavidin-biotin; Dako). The peroxidase activity was detected using AEC Substrate chromogen (Dako), which results in a red-stained precipitate. Finally, sections were counterstained with hematoxylin and mounted.

Edema and eosinophil counts were evaluated on HE-stained sections. Edema was semiquantitatively scored on a three-point

scale, 0 represented the lowest and 2 the highest score. The scoring system was calibrated by examining a representative number of samples.

The number of positive cells was analyzed using a magnification of 400× and scored by two independent observers who did not know the diagnosis and clinical data as previously described (18). Briefly, a grading scale from 0 to 3 was applied, ranging from absent to numerous stained cells. For all cellular markers a score 0: represents no positive cells, score 1: <10 positive cells/field, score 2: 10–100 positive cells/field, and score 3: >100 positive cells/field. The analysis included all areas of the biopsy and for each sample 10 fields were scored.

Statistical analysis

Statistical analysis was performed with MEDCALC software (F. Schoonjans, Belgium; <http://www.medcalc.be>; accessed 7 September 2006). Data are expressed in Box-and-Whisker plots. Statistical analysis was performed by the Kruskal–Wallis and Mann–Whitney *U* two-tailed tests for unpaired comparisons. When comparisons were made between groups, the Kruskal–Wallis test was used to establish the significant intergroup variability. The Mann–Whitney *U*-test was then used for between-group comparison. Baseline variables were analyzed by an one-way ANOVA test or Fishers' exact test. The significance level was set at $\alpha = 0.05$.

To determine the best cytokine markers we generated receiver operating characteristic (ROC) analysis for all cytokines and mediators. This is a plot of the true positive rate (sensitivity) against the false-positive rate (100% specificity) for the different possible cut points of a diagnostic analyte. Each point in a ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold or cutoff value. For each cytokine marker a cutoff value was determined with the highest accuracy (minimal false-negative and false-positive results). This cutoff value reflects the concentration of a cytokine with the highest specificity and sensitivity for the discrimination of two disease entities.

Results

Patient characteristics

Forty-eight patients were enrolled in this study. The mean age for the control, CRS, and NP groups was 33.8–49.3 years, while in CF-NP the mean age was 13.2 years. Three controls, three CRS patients, five NP, and two CF-NP patients were positive for at least one of the most common aeroallergens (Table 1). A significant higher frequency of asthma was reported in NP patients, all other subjects were free of asthma symptoms. Only one patient in the NP group had a history of aspirin intolerance. One-way ANOVA showed that the total symptom score was the highest in NP patients of which nasal congestion and loss of smell were the most predominant symptoms. The scores for nasal congestion and loss of smell were significantly higher in NP vs CRS patients, whereas CRS patients reported higher symptom scores for headache and postnasal drip compared with the other disease groups (Table 1). Finally, both poly-poid diseases (NP and CF-NP) showed the highest CT score and had a mean polyp score of 4.8 and 2.9 respectively.

Immunohistochemistry

The cryostat section of NP and sinus mucosa were scored semiquantitatively by two independent evaluators. Staining with HE identified the eosinophils by a characteristic staining of granules and typical configuration of the eosinophilic nucleus. Eosinophil numbers were significantly higher in NP tissue (Fig. 1 and Table 2) compared

Table 1. Patient characteristics and symptom scores

	Controls	Chronic sinusitis	Nasal polyposis	Cystic fibrosis-nasal polyps	One-way ANOVA (Fishers' exact test)
<i>N</i>	9	8	10	13	
Age (years; range)	33.8 (18–57)	36.2 (22–70)	35.6 (21–70)	12.4 (3–26)	0.28
Female/male	3/6	3/5	2/8	3/10	0.210*
Asthma in history	0/9	0/8	4/10	0/13	0.004*
Skin prick test-positive	2/9	3/8	4/10	2/11	0.519*
Aspirin intolerance	0/9	0/8	1/10	0/13	0.380*
Smoking	0/9	2/8	1/10	0/13	0.144*
Duration of disease (years; range)	6.8 (1–21)	4.2 (0.1–10.3)	6.7 (1.2–16.8)	5.7 (0.2–12.45)	0.795
CT score (Lund-Mackay)	0 (0–1)	6.0 (2–11)	17.0 (7–24)	14.0 (5–20)	<0.001
Polyp score (Davos)	0	0	5.1 (2–6)	3.2 (2–6)	<0.001
Total symptom score	4 (3–5)	6.1 (4–10)	10.4 (4–14)	3.9 (0–9)	<0.001
Nasal congestion	3.0 (2–3)	1.0 (0–3)	3.0 (2–3)	2.0 (0–3)	<0.001
Sneezing	0	0.1 (0–1)	0.2 (0–2)	0.6 (0–2)	0.520
Rhinorrhea	0.3 (0–2)	1.6 (0–3)	1.6 (0–3)	1.0 (0–3)	0.066
Loss of smell	0	0	2.3 (0–3)	1.0 (0–3)	<0.001
Postnasal drip	0	1.4 (0–2)	1.3 (0–3)	0.6 (0–2)	0.002
Headache	0.9 (0–2)	2.5 (1–3)	1.6 (0–3)	1.2 (0–3)	0.001

N, number of included patients.

The total symptom scores is the sum of the score for nasal congestion, rhinorrhea, headache, postnasal drip, loss of smell, and sneezing. The level of significance (*P*) was obtained by ANOVA and *Fishers' exact test. The CT score was obtained using the Lund-Mackay classification. The polyp size was scored according to the Davos classification.

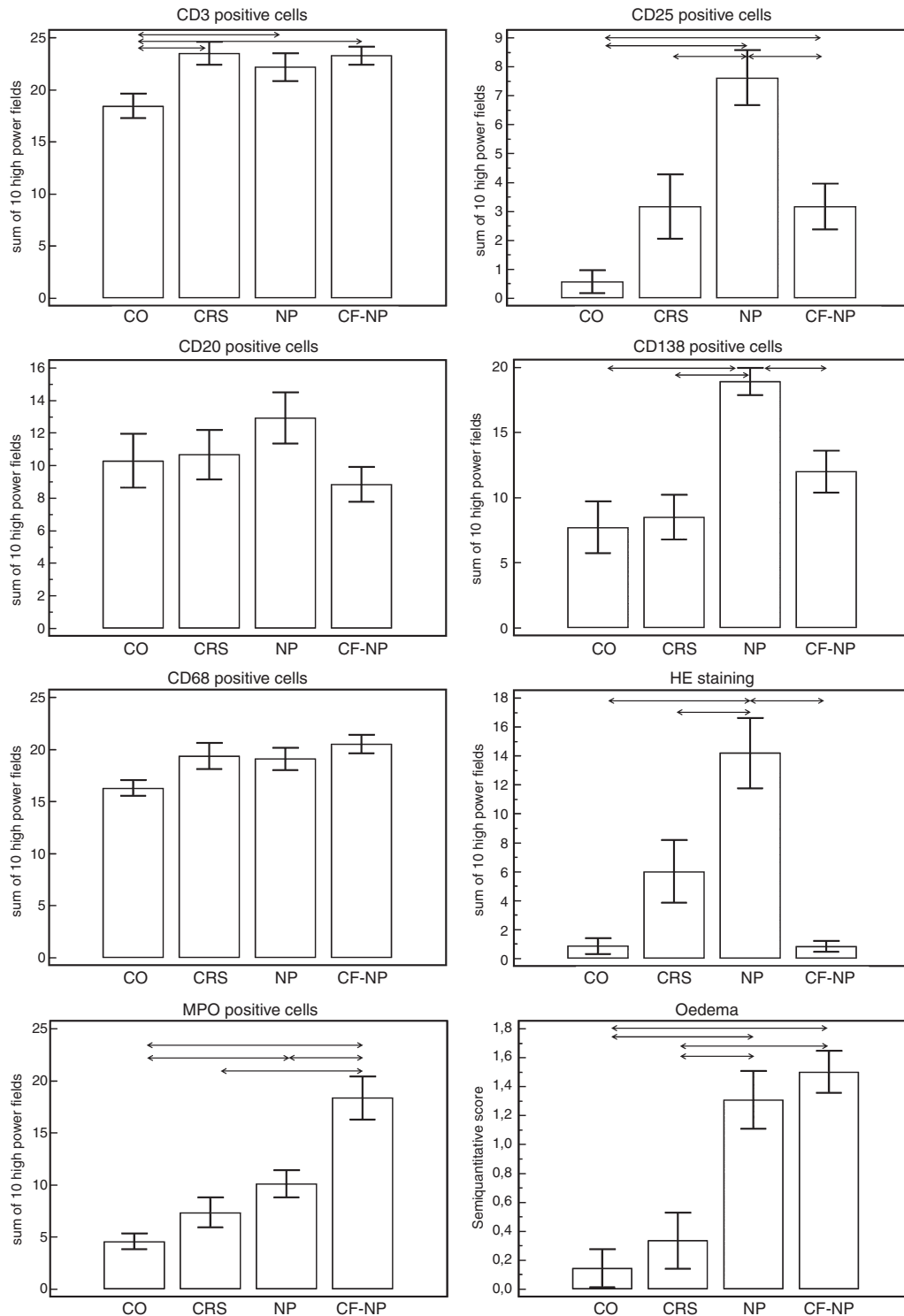


Figure 1. Semiquantitative evaluation of positive cells for CD3 (T cells), CD20 (naive B cells), CD25, CD68 (macrophages), CD138 (plasma cells), and myeloperoxidase (neutrophils) eosinophils, and edema were evaluated using a hematoxylin and eosin staining. The sum of 10 high-power fields was calculated for each subject and data are expressed as mean values of this sum with SEM for each group. Statistical analysis was performed using the Kruskal–Wallis test followed by a Mann–Whitney *U*-test. The significance level was set at $\alpha = 0.05$ and significant differences between groups are indicated with an arrow. The *P*-values are summarized in Table 2.

Table 2. Overview of significant differences for the semiquantitative evaluation of positive cells for CD3 (T cells), CD20 (naive B cells), CD25, CD68(macrophages), CD138 (plasma cells), and MPO (neutrophils)

	Kruskal–Wallis	CO vs NP*	CO vs CRS*	CO vs CF-NP*	NP vs CRS*	NP vs CF-NP*	CRS vs CF-NP
CD3	0.0235	0.0144	0.0221	0.0041	0.5686	0.8066	0.7431
CD25	0.0007	0.0006	0.1375	0.0384	0.0353	0.006	0.8514
CD20	0.4313						
CD138	0.0005	0.002	0.7308	0.1179	0.0014	0.0047	0.937
HE	0.0013	0.0004	0.1375	0.8357	0.0202	0.0019	0.1797
MPO	0.0011	0.006	0.2343	0.0012	0.3029	0.0114	0.0087
CD68	0.09						
Edema	0.0003	0.0055	0.6282	0.001	0.0226	0.6245	0.005

Eosinophils and edema were evaluated using HE staining. Statistical analysis was performed using the Kruskal–Wallis test followed by a Mann–Whitney *U*-test. The significance level was set at $\alpha = 0.05$ (see also Fig. 1).

MPO, myeloperoxidase; HE, hematoxylin and eosin.

with other sinus diseases and controls. All chronic sinus diseases had a significantly higher number of T lymphocytes (CD3) and CF-NP, and NP had significant more activated T lymphocytes (CD25) compared with control patients and CRS. Regarding B lymphocytes there were almost no naive B lymphocytes (CD20) present in all patient groups, although a significantly higher number of plasma cells (CD138) was present in NP vs all other groups. CF-NP showed the highest number of MPO-positive cells (neutrophils) compared with controls and CRS controls. Although a trend was observed toward a higher number of macrophages (CD68) in all chronic sinus diseases a significant difference was not reached. Finally, an overall score for edema revealed that edema formation was only present in NP and CF-NP.

Cytokine determination

In order to investigate the cytokine and mediator pattern in different subgroups of chronic sinusitis, a broad spectrum of cytokines and mediators covering cytokines, proinflammatory, eosinophilic, and neutrophil-related mediators were measured in homogenized tissue. In NP tissue homogenates the levels of the Th2 cytokine IL-5 reached significantly higher concentrations compared with other groups in which almost all concentrations were below detection level. On the other hand, in CRS an abundant expression of the Th1 cytokine IFN- γ was observed, whereas IFN- γ was mostly below detection level in NP and CF-NP (Fig. 3). Nasal polyp and CF-NP had a significantly higher expression of IL-2sR α in tissue homogenates compared with controls (Fig. 3). Strikingly, the level of TGF- β 1 was the highest in CRS compared with controls (Fig. 3). The highest concentrations of the neutrophilic markers MPO and IL-8 were found in CF-NP. In both CRS and CF-NP, the levels of the proinflammatory mediators IL-1 β and TNF- α were significantly higher compared with controls (Fig. 2).

The eosinophilic markers, ECP and eotaxin, showed significantly higher concentrations in NP tissue compared with all other groups, with NP, CRS, and CF-NP levels

being significantly higher compared with controls (Fig. 2). Finally, in NP tissue homogenates, total IgE concentrations were significantly higher compared with all other groups (Fig. 3).

To determine the best cytokine markers which could discriminate the subgroups CRS, NP or CF-NP, we calculated the sensitivities and specificities for all cytokines and mediators using ROC analysis. Table 3 lists the derived sensitivities and specificities for the comparison of CRS vs NP, NP vs CF-NP, and CRS vs CF-NP. Nasal polyp can be differentiated from CRS using the markers IL-5, ECP, and IgE with sensitivities and specificities above 75%. Vice versa, CRS can be differentiated from NP using IFN- γ with similar power. CF-NP and NP can be differentiated using ECP, IL-5, IgE, IL-8, and MPO. Moreover, MPO and IL-8 also discriminates CF-NP from CRS with sensitivities and specificities above 75%.

Discussion

Several studies have attempted to classify NPs and CRS based on symptoms inflammatory markers. Recent guidelines have attempted to classify the disease subgroups and unify the current pathophysiologic concepts (3, 4). There is a growing consensus that NP and CRS show distinct pathophysiology, but it is not clear whether these two disease entities are subsequent stages of disease.

Our results indicate that there is an apparent profile of symptoms in NP and CRS. Although most of the symptoms occur in both diseases, NP have a more pronounced nasal obstruction and loss of smell, while in CRS patients complain more of headache and postnasal drip. Especially the partial or complete loss of smell with a concomitant effect on taste and the loss to discriminate subtleties of flavor – is a characteristic feature of NPs (19). Despite the obstructed sinuses, facial pain or headaches are rare symptoms in NP patients. As described before by Deal and Kountakis (20), scoring of sinus CT scan with the Lund-Mackay score clearly

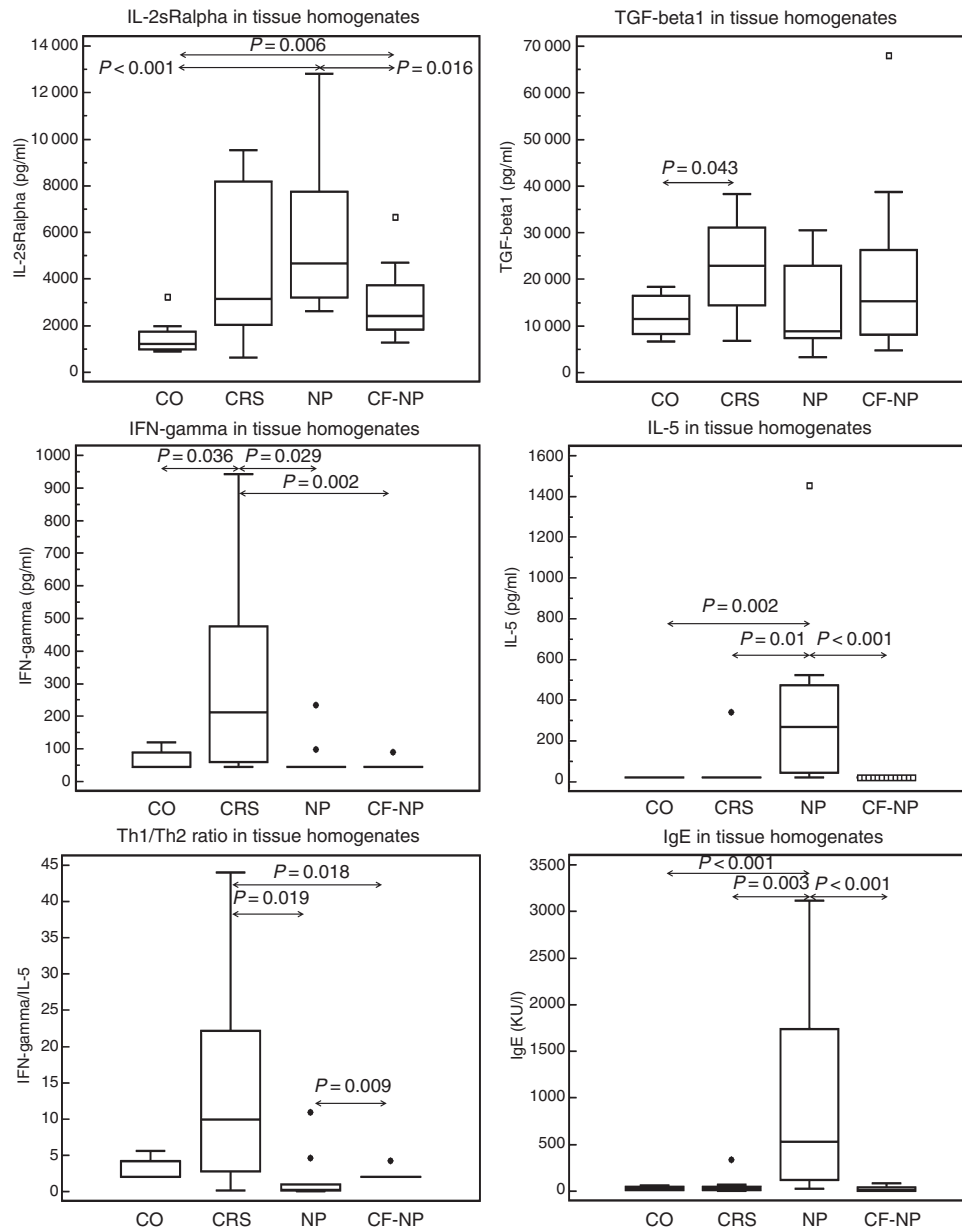


Figure 2. Measurement of interleukin (IL)-2sR α , transforming growth factor- β 1, IL-5, interferon (IFN)- γ , IL-5/IFN- γ ratio, and immunoglobulin E by enzyme-linked immunosorbent assay in 10 controls (CO), 10 chronic rhinosinusitis patients (CRS), 14 nasal polyposis patients (NP), and 14 patients with cystic fibrosis-nasal polyps (CF-NP). Data are expressed in Box-and-Whisker plots that represent the median, the lower and upper quartile and the minimum to the maximum value. Statistical analysis was performed using the Kruskal–Wallis test followed by a Mann–Whitney *U*-test. The significance level was set at $\alpha = 0.05$.

revealed that NP patients have more extensive opacifications compared with CRS. The considerable nasal congestion in the control patients included in this study reflects the fact that these patients underwent surgery for anatomical deviations of the nose and/or turbinate hypertrophy unrelated to sinus disease.

Sharper than the clinical profiles, CRS without polyps, NP, and nasal polyps in cystic fibrosis patients (CF-NP) can be differentiated by cytokine, mediator and cellular profiles.

Immunohistochemical analysis for T lymphocytes demonstrated a significant increase of CD3 (T lymphocytes) and CD25 (IL-2R)-positive cells in all chronic sinus diseases but especially in NP. The notable upregulation of IL-2sR α CF-NP and NP vs controls illustrates that there is a T-cell-mediated immune response in the disease groups with polyps. Increased serum levels of sIL-2R α have also been shown to be associated with the severity of acute asthma exacerbations, indicating T-cell activation in acute lower airway disease (21), as well as in flaring

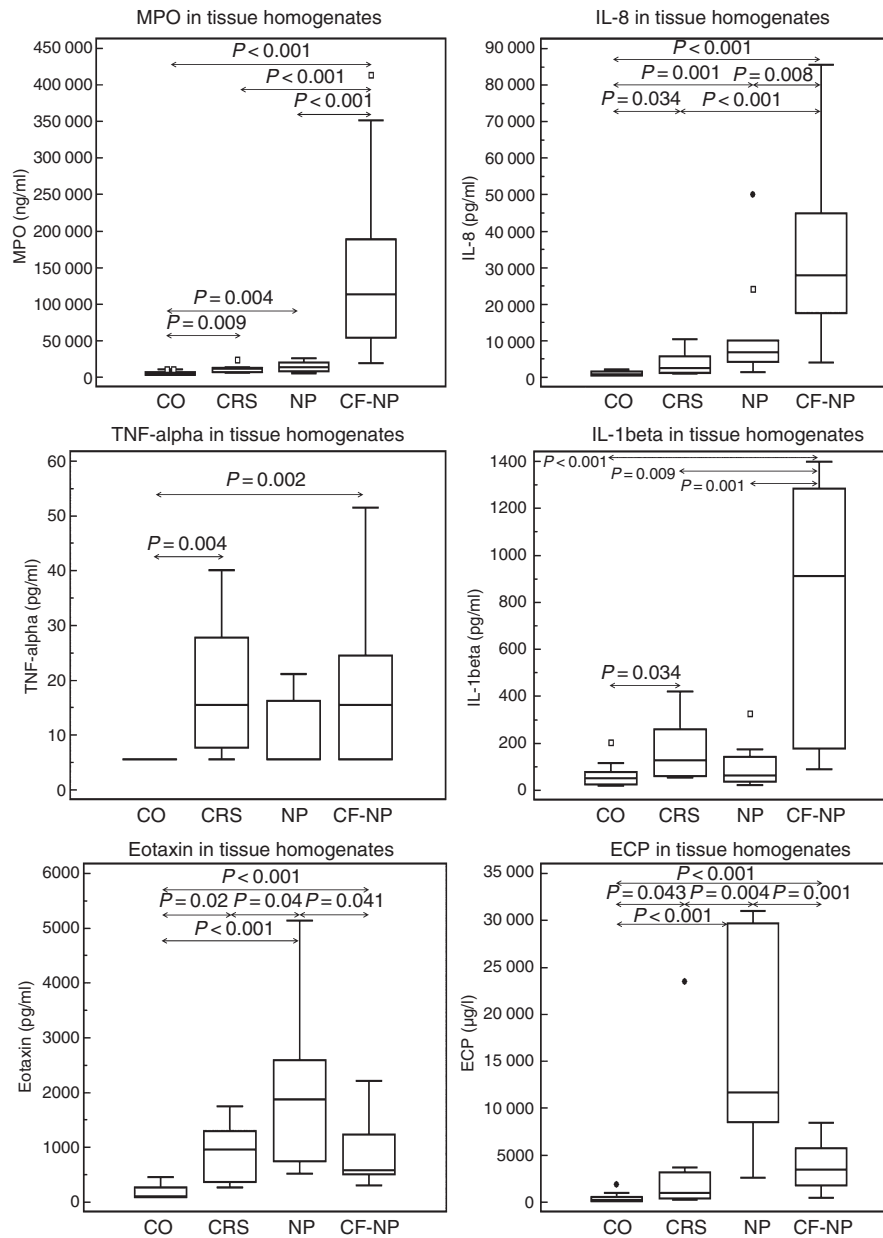


Figure 3. Measurement of eosinophil cationic protein, eotaxin, myeloperoxidase, interleukin (IL)-8, IL-1 β , and tumor necrosis factor- α by enzyme-linked immunosorbent assay in 10 controls (CO), 10 chronic rhinosinusitis patients (CRS), 14 nasal polyposis patients (NP), and 14 patients with cystic fibrosis-nasal polyps (CF-NP). Data are expressed in Box-and-Whisker plots that represent the median, the lower and upper quartile and the minimum to the maximum value. Statistical analysis was performed using the Kruskal–Wallis test followed by a Mann–Whitney *U*-test. The significance level was set at $\alpha = 0.05$.

chronic disease (22), namely allergic bronchopulmonary aspergillosis (15). Especially in NPs we found a significant increase in CD25-positive cells indicating a considerable activation of T lymphocytes. It is known that enterotoxins from *Staphylococcus aureus* acting as superantigens are able to induce a polyclonal activation of T cells and cause an exacerbation of the ongoing inflammation (23, 24). Recent insights have linked the inflammation in NP to an increased prevalence of colonization with *S. aureus* and the release of their cell products (25).

Specific IgE to *S. aureus* enterotoxins and consecutive polyclonal IgE formation has been demonstrated in NP tissue, which correlates with markers of eosinophilic inflammation, pointing to a possible modifying role of bacterial superantigens in the pathophysiology of NP (26, 27). High levels of total IgE were, again in this study, only found in the NP group, which was characterized by a diffuse presence of plasma cells suggesting that IgE synthesis could occur locally in the mucosa. B-cell foci and diffuse lymphoid accumulations have been described

Table 3. Sensitivities and specificities for inflammatory markers in chronic rhinosinusitis vs nasal polyposis, nasal polyposis vs cystic fibrosis-nasal polyps, and chronic rhinosinusitis vs cystic fibrosis-nasal polyps obtained by ROC analysis

	Nasal polyposis vs chronic rhinosinusitis	Nasal polyposis vs cystic fibrosis nasal polyps	Chronic rhinosinusitis vs cystic fibrosis nasal polyps
ECP (%)			
Sensitivity	90.0	80.0	84.6
Specificity	87.5	100.0	62.5
IgE (%)			
Sensitivity	90.0	90.0	61.5
Specificity	87.5	92.3	62.5
Eotaxin (%)			
Sensitivity	66	60.0	61.5
Specificity	100.0	84.6	62.5
IFN- γ (%)			
Sensitivity	90.0	20.0	71.4
Specificity	71.4	100.0	100.0
IL-1 β (%)			
Sensitivity	40.0	70.0	100.0
Specificity	100.0	100.0	69.2
IL-2sR α (%)			
Sensitivity	100.0	100.0	92.3
Specificity	42.9	53.8	42.9
IL-5 (%)			
Sensitivity	80.0	80.0	100.0
Specificity	87.5	100.0	12.5
IL-8 (%)			
Sensitivity	80.0	80.0	100.0
Specificity	75.0	92.3	92.3
MPO (%)			
Sensitivity	50.0	100.0	87.5
Specificity	87.5	84.6	100.0
TNF- α (%)			
Sensitivity	100.0	70.0	76.9
Specificity	42.9	69.2	42.9
TGF- β 1 (%)			
Sensitivity	60.0	60.0	46.2
Specificity	87.5	69.2	87.5

MPO, myeloperoxidase; TGF, transforming growth factor; IFN, interferon; IgE, immunoglobulin; ECP, eosinophil cationic protein; TNF, tumor necrosis factor; ROC, receiver operating characteristic.

in NP, endorsing this hypothesis (25). In contrast, specific IgE to enterotoxins and polyclonal IgE formation in tissue is a rare finding in CRS and also CF-NP (7), although *S. aureus* belongs to the usual germ flora especially in upper airway manifestations of CF (28).

The increase in and activation of T cells is similar in all sinus diseases; however, different T-lymphocyte subsets contribute to it. As indicated by different cytokine signals, namely IFN- γ (Th1-related) and IL-5 (a Th2 cytokine), the Th1/Th2 ratio characterized CRS as a Th1 polarized disease, whereas NP reveals a Th2 polarization, accompanied by abundant eosinophils and IgE formation. A similar pattern of Th1–Th2 polarization has also been described in lower airway inflammation, with IFN- γ characterizing chronic obstructive pulmonary disease (COPD) and IL-5 being predominant in asthma (29). These findings are in contrast with earlier studies, which

did show a significantly higher expression of IL-4, IL-5, and IFN-gamma mRNA in NP tissue compared with controls and allergic rhinitis patients (9, 11, 12). However, the differences in cytokine profiles just may reflect the difference in selection criteria, differentiating or not between CRS and NP.

Furthermore, TGF- β 1 is upregulated in CRS, but not in NP, which could point to either an increased T-regulatory cell activity (30) or an increased fibrogenic potential (31, 32) in CRS. An overexpression of TGF- β 1 has been demonstrated before in CRS vs NP, confirming our current results (33). Specifically, it has been shown that TGF- β 1 (34) is able to abrogate the prolonging effects of hematopoietins-like IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor on eosinophil survival and to induce eosinophil apoptosis, which may explain the association of low TGF- β 1 concentrations in a predominantly eosinophilic disease, such as NP. Transforming growth factor- β 1 is also a potent fibrogenic cytokine that stimulates extracellular matrix formation, with increased levels contributing to fibrosis in CRS, and a relative deficit in TGF- β 1 explaining edema formation in NP.

Nasal polyp has repeatedly been characterized as eosinophilic inflammation, with highly increased concentrations of ECP as marker of eosinophil activation, and of eotaxin, a CC chemokine, which co-operates with IL-5 to recruit and activate eosinophils (35). Both, ECP and eotaxin have been described to be significantly increased in NP vs controls, and here we show that this also holds true in comparison with CRS (35).

Although nothing has been published on the variation of cytokines in nasal tissue across different age groups, we should consider the age of CF-NP patient group as a potential confounding factor for our results. On the other hand, CF patients develop NPs at a younger age, and biopsies were taken from the same place and the same tissue type. Therefore, we may consider these patients as a second control group within the NP population. Here, we describe a neutrophilic predominance in CF-NP, with IL-8 (a CXC chemokine), MPO (an enzyme released by neutrophil granulocytes), and the proinflammatory mediator IL-1 β surpassing the tissue concentrations measured in any other disease group (36). Immunohistochemistry also confirmed the neutrophilic response showing increased numbers of MPO-positive cells in CF-NP. Similarly, in the lower respiratory tract a predominant neutrophilic infiltration is present – with increased MPO and IL-8 levels – in sputum of CF patients compared with chronic bronchitis patients (37). In contrast, no difference between CRS and NP was found for markers of neutrophilic inflammation, such as IL-8 and MPO.

As a result of these observations, we attempted to use certain cytokines to distinguish CRS, NP, and CF-NP through ROC analysis (Table 3). Determination of sensitivity and specificity for all cytokines and inflammatory mediators used in this study revealed that ECP, IgE, and

IL-5 contribute to differentiate NP from CRS and CF-NP with sensitivities and specificities above 80%. These findings confirm that eosinophils and related inflammatory products are the hallmark of NP-associated inflammation compared with CRS or CF-NP, confirming former studies (13, 15, 35). Finally, CF-NP can be differentiated from CRS using the neutrophil-related markers IL-8 and MPO which are overexpressed in CF-NP.

According to most recent position statements in chronic sinus disease, CRS is considered a disease continuum with 'extremes', such as CRS with and without NPs. However, here we show that chronic sinus disease rather represents distinguishable disease entities with specific cytokine and mediator profiles, which enable the differentiation of diseases based on pathomechanisms. The differentiation possibilities of disease entities based on such small numbers is intriguing; however, considerably greater number of samples are needed with the potential of reaching higher sensitivity and specificity by combining specific markers using multiple regression analysis. An intense clinical phenotyping of patients with chronic sinus disease with the measurement of inflammatory and T-cell markers could lead to the recognition of several sinus disease entities which will allow to finally introduce new definitions for studies on the epidemiology,

diagnostic management, and lower airway co-morbidities, and eventually will open differentiated and new therapeutic approaches.

So far, our study provides evidence that different pathomechanisms are involved in chronic sinus disease, which need to be differentiated, comparable with asthma and COPD, in order to ameliorate disease management. In crude terms, CRS may reflect COPD in terms of cytokine regulation, whereas NP mirrors the cytokine pattern of asthma. As obstruction, like in COPD, is a hallmark of CRS (i.e. the ostiomeatal complex), it would be appropriate to rename this disease as Chronic Obstructive Sinus Disease (COSD). To conclude, based on cytokines, inflammatory mediators and cellular characteristics, we suggest that NP, CRS, and CF-NP are distinct disease entities within the group of chronic sinus diseases.

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