

Nasal biomarker profiles in acute and chronic rhinosinusitis

H. Riechelmann, T. Deutschle, A. Rozsasi, T. Keck, D. Polzehl and H. Bürner

Department of Otorhinolaryngology – Head and Neck Surgery, University Hospital of Ulm, Ulm, Germany

Summary

Background Clinical manifestations of rhinosinusitis include acute rhinosinusitis, chronic rhinosinusitis (CRS) with nasal polyps and CRS without polyps.

Objective Possible mechanisms defining these three forms of rhinosinusitis should be investigated assessing biomarker profiles in nasal secretions.

Methods Fifteen cytokines, three cellular activation markers and total IgE were determined in nasal secretions of seven patients with acute rhinosinusitis, 12 patients with CRS without polyps, 13 patients with CRS with polyps and six healthy controls. Principal component analysis was used to extract relevant factors.

Results Irrespective of the clinical manifestation, all biomarkers assessed were increased in patients with rhinosinusitis when compared with controls ($P < 0.001$). Principal component analysis allowed the extraction of three factors explaining 83% of data variance. The general inflammatory activation was mainly reflected by the first factor. The second factor differentiated acute from CRS. This factor correlated with IL-12, which is involved in pathogen-related immune activation by antigen-presenting cells. It was also positively correlated with IL-4, IL-10 and IL-13, which play an important role in the resolution of infections. The third factor differentiated CRS with polyps from CRS without polyps ($P = 0.001$). It represented IL-5 and nasal IgE (nIgE), whereas eosinophil cationic protein and tryptase were not specific for CRS with polyps.

Conclusion In mucosal infection, numerous inflammatory mediators are activated. Simple correlations of few biomarkers with a specific disease process bear the risk of overestimating a possibly unspecific effect. To assess biomarker profiles, more complex analytic tools may be more appropriate to delineate mechanisms underlying mucosal disease. Using principal component analysis, it was found that high nIgE and IL-5 levels are specific for CRS with nasal polyps.

Keywords biological markers, chemokines, cytokines, nasal lavage, nasal mucosa, nasal polyps, physiopathology, principal component analysis, rhinitis, sinusitis

Submitted 27 January 2004; revised 3 June 2005; accepted 22 June 2005

Introduction

Rhinosinusitis defines a group of disorders characterized by inflammation of the mucosa of the nose and paranasal sinuses. The diagnosis is based upon the presence of typical symptoms and clinical examination findings. If symptoms last 12 weeks or longer, the disease is categorized as chronic rhinosinusitis (CRS) [1]. Although this condition affects more than 10% of the population of western countries, the alterations of nasal and paranasal sinus mucosa are not yet sufficiently defined [2]. Depending on the presence of inflammatory polyps on endoscopic examination, CRS can be grouped into CRS with or without nasal polyps. It is unclear, whether these conditions represent different aetiological and pathophysiologic entities, or rather different stages and courses of one underlying disease [1, 3].

To characterize inflammatory changes of the upper respiratory mucosa, cellular secretory products in nasal secretions may be determined [4]. Inflammatory cell granule-derived proteins, such as mast cell tryptase (TRY), neutrophil elastase (ELA) or eosinophil cationic protein (ECP), provide effector functions and have been identified in nasal secretions of patients with nasal allergy, acute and CRS [5]. Igs are the chief components of humoral antigen recognition, and high IgE levels, possibly as a consequence of polyclonal IgE activation, have been found in nasal polyp fluids [6]. Cytokines serve various regulatory functions and can be readily detected in nasal secretions [4]. The cytokine profile in nasal secretions may help to recognize mechanisms underlying mucosal disease.

In this cross-sectional study, a panel of cellular secretory products were assessed in nasal secretions of patients with acute rhinosinusitis, CRS with polyps, CRS without polyps, and healthy controls. The attempt was made to identify possible characteristics of nasal biomarker profiles between these groups. In particular, we were interested in gaining some additional information on the question of whether nasal

Correspondence: Herbert Riechelmann, Department of Otorhinolaryngology – Head and Neck Surgery, University Hospital Ulm, Prittwitzstr. 43, 89075, Ulm, Germany.

E-mail: herbert.riechelmann@medizin.uni-ulm.de

polyps show specific alterations suggesting pathomechanisms different from CRS without polyps.

Methods

Patients with acute or CRS referred to the Department of Otorhinolaryngology – Head and Neck Surgery of the University Hospital of Ulm between June and December 2003 and healthy controls volunteered in the study. Study aims were detailed to each participant and informed consent was obtained complying with the Guidelines for Tissue Sampling and Handling of the Ethics Committee of the University of Ulm.

Study population, clinical examination, scores and inclusion criteria

For diagnosis of rhinosinusitis, the multidisciplinary Rhinosinusitis Task Force criteria were employed [7]. If the duration of symptoms was less than 4 weeks, acute rhinosinusitis was diagnosed, if it was 12 weeks or longer, the diagnosis of CRS was made. Patients with subacute rhinosinusitis (4–12 weeks), recurrent acute rhinosinusitis or acute exacerbations of CRS were not included. Patients considered atopic, based on the results of skin prick tests (SPTs) for common Central European inhalant allergens, were excluded [8]. The sensitivity of SPTs was considered sufficient for the purpose of this study. However, additional in vitro parameters such as serum-specific IgE determinations would have been desirable to exclude atopy more reliably. To rule out aspirin intolerance, only patients who had consumed aspirin or other relevant analgesics without adverse effects within the last 5 years were included. Moreover, patients with unilateral disease or allergic fungal rhinosinusitis, according to the clinical criteria established by Bent and Kuhn [9], were not included.

Nasal endoscopy was performed in all participants after topical anaesthesia and decongestion. The endoscopic examination followed a standard sequence as described by Stammberger and Hawke [10]. Particular importance was attached to the presence of secretions or polyps lateral to the middle turbinate. For each nasal cavity, polyps were categorized according to Malm in 0 (no polyps), 1 (small polyps lateral to the middle turbinate), 2 (nasal polyps extending below the inferior border of the middle turbinate) and 3 (massive polyps reaching the nasal floor) [11]. Patients with CRS and a Malm score of 0 were categorized as CRS without nasal polyps, and patients with a Malm score greater than 2 were categorized as CRS with nasal polyps. Patients with Malm scores of 1 or 2 were not included.

In all patients with CRS, coronal computed tomography (CT) scans in bone window settings were available. The radiographic extent of disease was assessed with the Lund and MacKay score, which, according to the extent of opacification, assigns a value between 0 and 2 to the frontal, maxillary, anterior ethmoid and posterior ethmoid sinuses, and 0 or 2 to the ethmoidal infundibulum of each side [12]. Only patients with CRS and a CT score greater than 6 were included.

Sampling of nasal secretions

Patients with CRS discontinued nasal or systemic steroid or antibiotic treatment at least 2 weeks prior to sampling of nasal secretions. Open cell flexible polyurethane foam samplers of 28 × 18 × 6 mm were placed into each nasal cavity posterior to the mucocutaneous junction under direct visualization and left in place for 10 min. Following removal, the fluid was extracted from the sampler by centrifugation [4].

Cell activation markers and total immunoglobulin E in nasal secretions

ECP, TRY and nasal IgE (nIgE) levels in nasal secretions were measured using a fluorescence immunoassay (UniCAP 100 Diagnostic System, Pharmacia, Freiburg, Germany) according to the manufacturer's recommendations. Sample dilutions were fivefold for ECP and TRY; total IgE was measured undiluted. The detection limit was 2 ng/mL for ECP, 1 ng/mL for TRY and 2 U/mL for total IgE. Human polymorphonuclear leucocyte (PMN) ELA was measured using a quantitative enzyme-linked immunosorbent assay (Bender MedSystems, Vienna, Austria). Samples were diluted 100-fold. The limit of detection for PMN ELA was 3 ng/mL.

Cytokine determination in nasal secretions

Nasal secretions were diluted 1:10 and cytokine concentrations were analysed on a Bio-Plex™ Suspension Array System (Bio-Rad Laboratories, Munich, Germany) for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, TNF-α, IFN-γ, granulocyte-macrophage colony-stimulating factor (GMC), granulocyte colony-stimulating factor (GCS), monocyte chemoattractant protein-1 (MCP), and macrophage inflammatory protein-1β (MIP) employing the Multiplex Cytokine Assay for 17 cytokines (Bio-Rad Laboratories) according to the manufacturer's instructions. Data were evaluated with the Bio-Plex Manager™ Software 3.0 (Bio-Rad Laboratories). The limit of detection was less than 0.5 pg/mL.

Analysis

Biomarker concentrations in nasal secretions were transformed to their logarithm (base 10) and subjected to a principal component analysis with varimax rotation. Each resulting factor was compared with a one-way ANOVA by the four disease categories, i.e. acute rhinosinusitis, CRS with polyps, CRS without polyps and controls. Specific comparisons of the obtained principal components with the four disease categories were then added.

Results

Thirty-eight participants met the selection criteria and volunteered for the study (Table 1). Overall, more men than women took part in the study (2.2:1). The youngest participants were the patients with acute rhinosinusitis, and the eldest group were the patients with CRS with polyps. In all patients with acute rhinosinusitis, the clinical symptoms and pathologic findings resolved completely within 2 weeks with appropriate antibiotic treatment.

Table 1. Diagnosis, gender and age of study participants

	Acute rhinosinusitis	CRS without polyps	CRS with polyps	Controls	Total
<i>n</i>	7	12	13	6	38
Male	5	8	9	4	26
Female	2	4	4	2	12
Age (mean $\pm$ SD)	29 $\pm$ 13	38 $\pm$ 14	44 $\pm$ 11	31 $\pm$ 9	37 $\pm$ 13

CRS, chronic rhinosinusitis.

Analyte concentrations in nasal secretions

IL-17 was below the detection limit of 0.5 pg/mL in 31/39 patients and not evaluable. GMC levels did not vary among groups, were not correlated with any other biomarker and considered unreliable. The concentrations of the remaining 19 cellular secretory products investigated followed a log-normal distribution. In healthy controls, comparatively high levels of GCS, IL-6, IL-8, and ELA were found. Irrespective of the specific clinical diagnosis (acute rhinosinusitis, CRS with polyps or CRS without polyps), biomarker concentrations in nasal secretions were 1–2 log levels higher in patients with rhinosinusitis than in controls (Fig. 1). On top of this

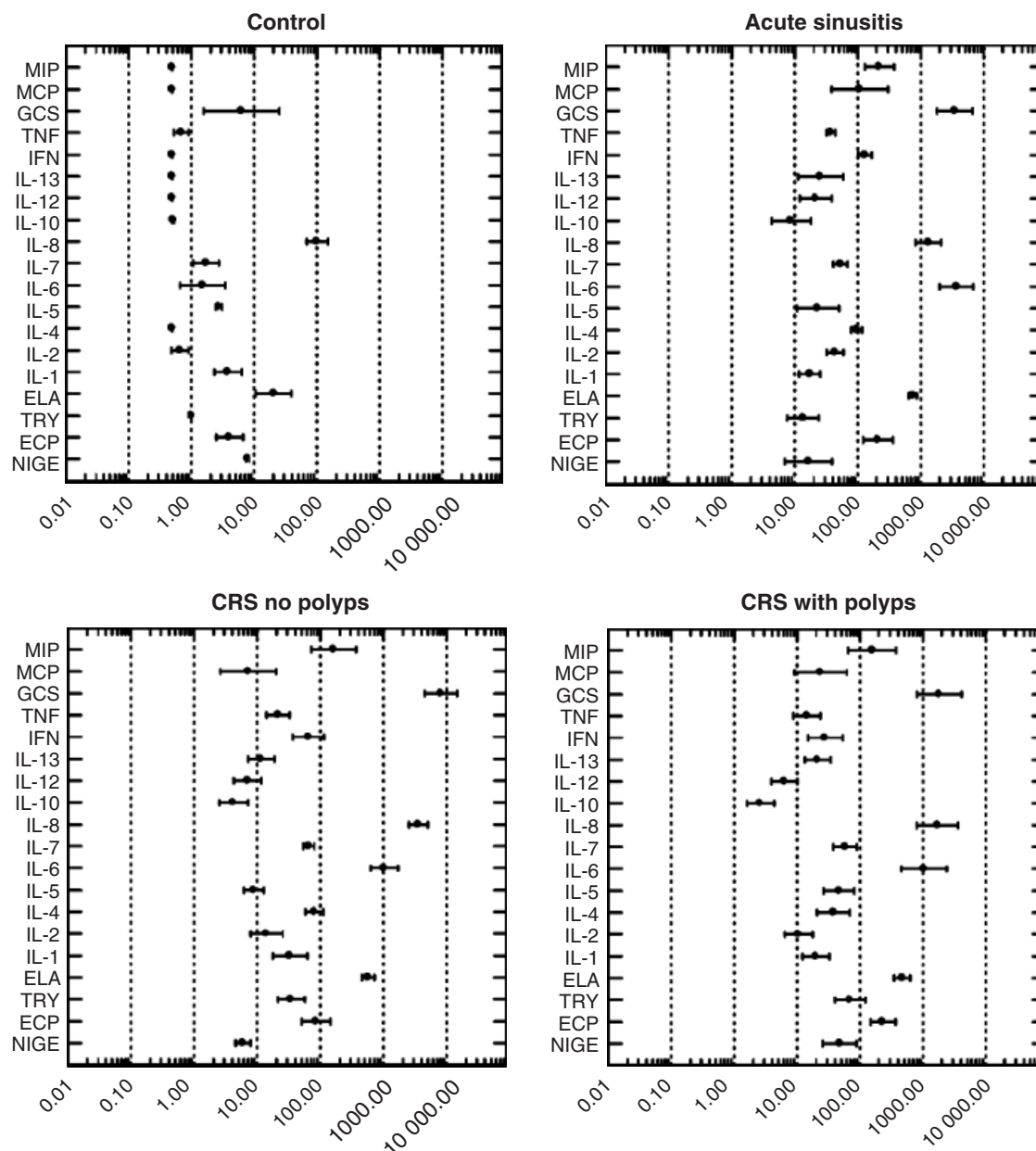


Fig. 1. Concentrations of cellular secretory products (mean $\pm$ SEM) in nasal secretions in healthy controls ($n = 6$), patients with acute rhinosinusitis ($n = 7$), chronic rhinosinusitis (CRS) without polyps ($n = 12$) and CRS with polyps ($n = 13$). X-axis (log-scale) represents pg/mL for all analytes except elastase (ELA), Tryptase (TRY) and eosinophil cationic protein (ECP), which are measured in ng/mL, and nasal IgE (nIgE), which is measured in U/mL.

Table 2. Rotated component loadings (varimax rotation) representing the correlation of the observed biomarker concentrations with the scores of the three principal components [(FCTR(1)–FCTR(3))] extracted from the data

	FCTR		
	(1)	(2)	(3)
GCS	0.926	0.271	0.038
IL-1	0.834	0.198	0.027
MIP	0.827	0.274	0.373
IL-8	0.827	0.317	–0.002
IL-6	0.801	0.439	0.288
IL-7	0.726	0.442	0.164
ELA	0.724	0.322	0.268
TNF	0.687	0.668	0.136
IFN	0.663	0.677	0.045
IL-4	0.771	0.523	0.208
IL-10	0.535	0.559	0.194
IL-12	0.203	0.923	0.100
IL-2	0.335	0.901	0.036
MCP	0.264	0.794	0.257
IL-13	0.297	0.768	0.451
ECP	0.615	0.128	0.640
TRY	0.558	–0.134	0.537
NigE	–0.036	0.157	0.882
IL-5	0.162	0.489	0.772

Loadings >0.5 were considered relevant and set bold.

GCS, granulocyte colony-stimulating factor; MIP, macrophage inflammatory protein; ELA, elastase.

unspecific, presumably inflammation-related analyte elevation, modulations of biomarker concentrations specific for the respective rhinosinusitis group were assumed, but not quantifiable without more sophisticated statistical procedures.

Principal component analysis

The multitude of highly intercorrelated analytes made result interpretation complex. Employing a principal component analysis with varimax rotation, three weighted linear composites of the observed biomarkers with eigenvalues greater than 1 were extracted. The three factors explained 83% of the variability in the data: the first factor [FCTR(1)] explained 39% of data variance, the second factor [FCTR(2)] 29% and the third factor [FCTR(3)] 15%. The component loadings (Table 2) represent the correlation of the analyte concentrations with the scores of each factor. The concentrations of GCS, IL-1, MIP, IL-8, IL-6, IL-7 and ELA were highly correlated with, or loaded high on, the scores for FCTR(1) and poorly with the other two factors. FCTR(2) was, among others, correlated with IL-12, IL-2, IL-4, IL-10 and IL-13, whereas FCTR(3) mainly represented the concentrations of nIgE and IL-5. TNF, IFN, IL-4 and IL-10 loaded high on both, FCTR(1) and FCTR(2), whereas ECP and TRY loaded high on FCTR(1) and FCTR(3).

Factor scores by clinical diagnosis

In general, the factor scores were higher in patients with rhinosinusitis than in controls ($P < 0.001$, Fig. 2). In nasal secretions from patients with acute rhinosinusitis, factor 2 scores were particularly high, in CRS without polyps factor 1

Table 3. Analysis of variance results (P -values), separately for each of the three factors [FCTR(1)–FCTR(3)] extracted by principal component analysis (columns 2–4)

	FCTR		
	(1)	(2)	(3)
Overall significance	<0.0005	0.033	0.002
Control vs. acute rhinosinusitis	0.001	0.004	0.190
Control vs. CRS no polyps	<0.0005	0.095	0.758
Control vs. CRS with polyps	<0.0005	0.104	0.002
Acute rhinosinusitis vs. CRS no polyps	0.172	0.075	0.225
Acute rhinosinusitis vs. CRS with polyps	0.965	0.061	0.06
CRS no polyps vs. CRS with polyps	0.094	0.928	0.001

Clinical diagnosis (acute rhinosinusitis, chronic rhinosinusitis (CRS) without polyps CRS with polyps, and controls) served as the categorical variable. The overall significance (second row) reflects that the factor scores differ significantly for at least one of the four diagnostic categories. In the rows, specific comparisons (contrasts) for each combination of the categorical variables are presented. Because of the explorative character of the study, results were not adjusted for multiple comparisons.

scores were high and factor 3 scores were low, and in patients with nasal polyps factor 3 scores were high (Table 3). Particularly, CRS with polyps revealed significantly higher factor 3 scores than CRS without polyps ($P = 0.001$).

Discussion

Clinically, rhinosinusitis can take different courses and present with different symptoms and examination findings [1]. It is unclear what mechanisms drive the disease towards a particular manifestation. The aim of this study was to gain some insight into these mechanisms by the analysis of secretory cell products in nasal secretions of patients with different forms of rhinosinusitis. It was not the aim of this explorative study to gain representative data with sufficient numbers to infer on the general population. For this purpose, the number of study participants is considered too low. Instead, we intended to carefully identify and select patients presenting with typical clinical features of the rhinosinusitis subgroups studied.

Three distinctive forms of rhinosinusitis, i.e. acute community-acquired rhinosinusitis, CRS with nasal polyps and CRS without polyps, were investigated. Healthy volunteers without sinus complaints and normal endoscopy findings served as control. To objectify the diagnosis and clinical type of rhinosinusitis, generally accepted definitions and score systems were employed [1], including the Lund and Mackay CT score [12], and the Malm score for endoscopic examinations [11].

Acute rhinosinusitis was diagnosed in patients representing at our on-duty medical unit with acute midfacial pain and additional major and minor rhinosinusitis symptoms during the course of an upper respiratory infection. Four patients presented with early-stage orbital complications, four had purulent nasal secretions and *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Staphylococcus aureus* were cultured, three were feverish and all had elevated CRP values indicating acute bacterial sinusitis. Because CT scans are not regularly obtained in these patients and, for ethical

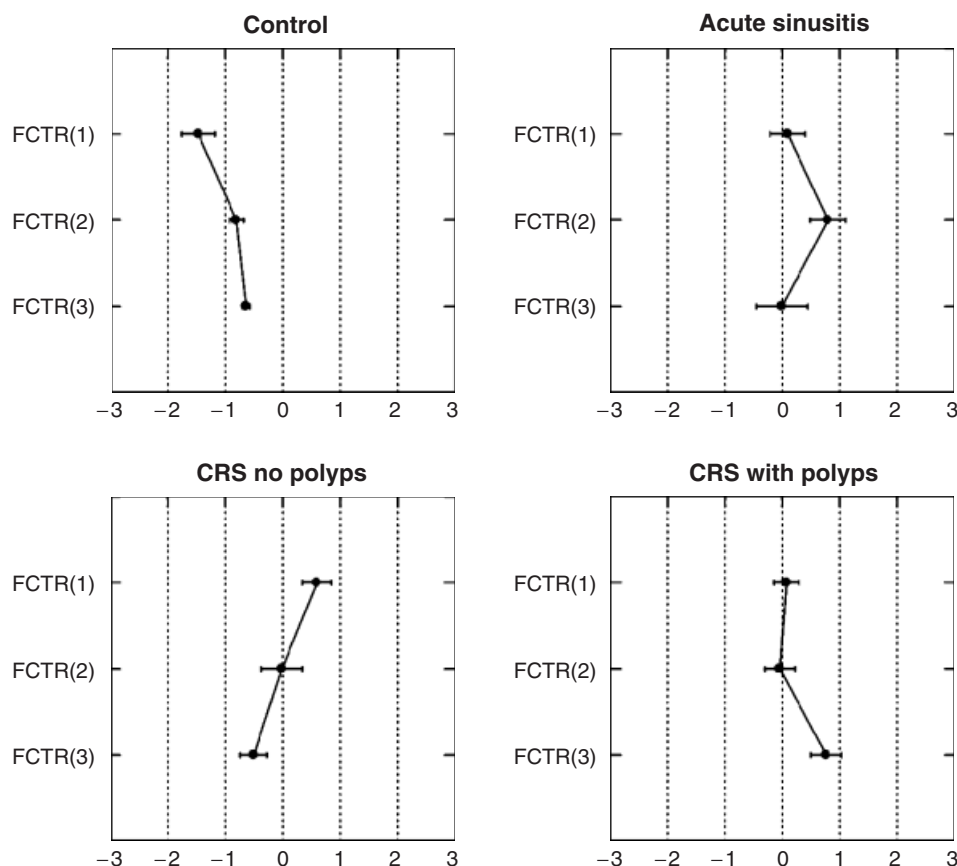


Fig. 2. Standardized scores (x-axis) for three factors [FCTR(1)–FCTR(3)] obtained by principal component analysis from the data presented in Fig. 1. The first factor primarily reflects unspecific mucosal inflammation and is highly correlated with granulocyte colony-stimulating factor, IL-1, IL-6, IL-8 and neutrophil elastase (Table 2). The second factor discriminates between acute and chronic rhinosinusitis. It is correlated with IL-12 involved in immune activation by antigen-presenting cells, and with IL-4, IL-10 and IL-13, which are involved in the resolution of acute inflammation. The third factor is the nasal polyp factor. It is specifically correlated with nasal total IgE and IL-5. Eosinophil cationic protein and tryptase were not specific for polyps.

considerations, not in healthy volunteers, they were only available in patients with CRS. In order to document relevant sinus disease in CRS patients, a minimum CT score of 7 was needed for inclusion. The presence or absence of nasal polyps was diagnosed by a thorough endoscopic examination by an experienced clinician [10]. To reduce uncertainty regarding the presence of polyps, patients with low Malm scores of 1 or 2 were not included. Patients with CRS and a Malm score of 0 were classified as CRS without polyps, and those with a score of 3 or more as CRS with polyps.

The course and clinical appearance of rhinosinusitis may be influenced by additional factors, such as atopy, aspirin intolerance or fungal disease. To achieve more homogenous groups, patients with a medical history or findings suspicious of one of these additional factors were not included. Also patients with unilateral disease or possible dental origin were not included. Based on this algorithm the clinical diagnosis in these selected patients is considered valid for the purpose of this study.

In all study participants, sufficient undiluted nasal secretions for the planned investigations were obtained with a polyurethane foam sampler. To adapt to the working range of the particular assays, the samples were diluted by appropriate factors [4]. Cytokine concentrations were assessed with a cytokine bead assay. It allows the determination of 17 cytokines in one run, avoiding the variability when

different assay systems are used. Approximately 40 duplicate samples can be tested simultaneously and unbiased by interassay variability. Although interesting mediators such as RANTES, eotaxin, TGF- β , IL-1 receptor antagonist and others could not be included, a reasonable profile of cytokines possibly involved in the pathophysiology of rhinosinusitis was obtained. This profile was supplemented with determinations of cellular activation markers including neutrophil ELA as an indicator of neutrophil activation [13], TRY as an indicator of mast cell activation, and ECP as an indicator of eosinophil activation [14]. Total IgE in nasal secretions, possibly involved in the aetiology of nasal polyps [6], was also assessed. Arachidonic acid metabolites, which may play an important role in the pathophysiology of rhinosinusitis [15], could not be included. Thus, a wide range of mediators, but by far not all considered relevant for rhinosinusitis, were investigated in this study.

Irrespective of the particular disease manifestation, almost all secretory cell products investigated were significantly elevated in patients with rhinosinusitis when compared with healthy controls. This presumably reflects an unspecific cell activation in consequence of mucosal inflammation. It might also explain why the vast majority of biomarkers in this study were positively correlated, even if no functional relation may be assumed. In order to identify factors that might influence the course of rhinosinusitis on top of this general inflamma-

tory activation, a principal component analysis was performed. This statistical standard procedure provides more concise delineation of multivariate effects. It is based on correlations of biomarker concentrations and does not necessarily reflect functional relations.

The first extracted factor was almost equally elevated in all three forms of rhinosinusitis investigated, and is assumed to mainly reflect unspecific inflammatory mucosal activation. Although the analytes loading high on the first factor derive from various cellular sources, it seems remarkable that cytokines expressed by airway epithelial cells such as GCS, IL-6, IL-8 and also ELA as a neutrophil secretory product load high on the first factor [13, 16]. These biomarkers have also been found in relatively high concentrations in controls, which goes with the fact that these cells are particularly common in normal nasal mucosa. Moreover, that these biomarkers were readily detectable in controls might have contributed to their merging into a common factor by the statistical algorithm, irrespective of a possible functional meaning.

Only in acute rhinosinusitis was the second factor significantly increased when compared with healthy controls ($P = 0.004$, Table 3). The biomarker loading highest on the second factor was heterodimeric IL-12, which is preferentially released by B cells and antigen-presenting cells such as dendritic cells and macrophages in response to, e.g. viral, bacterial or fungal stimuli [17]. Moreover, cytokines involved in the resolution of inflammation such as IL-4, IL-10 and IL-13 loaded high on factor 2 (Table 2) [18]. Although not statistically significant, there was a trend towards lower factor 2 scores in CRS without ($P = 0.075$) and CRS with nasal polyps ($P = 0.065$, Table 3), which might to some extent reflect their reduced propensity to heal. Bone marrow-derived mononuclear cells preponderate as possible cellular sources of factor 2 biomarkers.

The third factor is the nasal polyp factor. In secretions obtained from CRS patients with nasal polyps, this factor was relevantly higher when compared with controls and the two other forms of rhinosinusitis (Fig. 2). The polyp specificity of the third factor is underlined by the observation that controls, acute rhinosinusitis and CRS without polyps revealed no relevant factor 3 differences (Table 3). IL-5 and nIgE were the two biomarkers that exclusively loaded high on factor 3, whereas TRY and ECP also loaded high on factor 1. This reflects that the latter two biomarkers were also unspecifically elevated in acute rhinosinusitis and CRS without polyps. The variance of IL-5 is only 34% explained by ECP (adjusted $R^2 = 0.34$), pointing towards cellular IL-5-sources different from eosinophils in rhinosinusitis, preferentially T cells [19]. This goes with recent studies that put the predominant role of the eosinophil in CRS with polyps into perspective [20].

In conclusion, numerous mediators are activated in mucosal inflammation. Simple correlations of few biomarkers with a specific disease process or manifestation carry the risk of overestimating an unspecific effect. To evaluate biomarker profiles, more complex analytic tools may improve our understanding of mechanisms underlying mucosal disease. Using principal component analysis, it was in particular found that high nIgE and IL-5 levels are specific for CRS with nasal polyps. This suggests a specific, yet unknown, mechanism in the aetiology of this disease.

Acknowledgements

Supported by a grant of the State of Baden-Wuerttemberg, BWL 22010.

References

- 1 Meltzer EO, Hamilos DL, Hadley JA et al. Rhinosinusitis: establishing definitions for clinical research and patient care. *J Allergy Clin Immunol* 2004; 114:155–212.
- 2 Bachert C, Hormann K, Mosges R et al. An update on the diagnosis and treatment of sinusitis and nasal polyposis. *Allergy* 2003; 58:176–91.
- 3 Pawankar R, Nonaka M, Yamagishi S, Yagi T. Pathophysiologic mechanisms of chronic rhinosinusitis. *Immunol Allergy Clin North Am* 2004; 24:75–85.
- 4 Riechelmann H, Deutsche T, Friemel E, Gross H-J, Bachem M. Biological markers in nasal secretions. *Eur Respir J* 2003; 21:600–5.
- 5 Kramer MF, Burow G, Pfrogner E, Rasp G. In vitro diagnosis of chronic nasal inflammation. *Clin Exp Allergy* 2004; 34:1086–92.
- 6 Bachert C, Gevaert P, Holtappels G, Johansson SG, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001; 107:607–14.
- 7 Rhinosinusitis Task Force Report of the Rhinosinusitis Task Force Committee Meeting. Alexandria, VA, August 17, 1996. *Otolaryngol Head Neck Surg* 1997; 117:S1–68.
- 8 Dreborg S, Frew A. Allergen standardization and skin tests. The European academy of allergology and clinical immunology. Position paper. *Allergy* 1993; 48:48–82.
- 9 Bent JP, Kuhn FA. Allergic fungal sinusitis/polyposis. *Allergy Asthma Proc* 1996; 17:259–68.
- 10 Stammberger H, Hawke M. Essentials of functional endoscopic sinus surgery. Mosby Year Book Inc., St Louis, 1993; ix–212.
- 11 Malm L. Assessment and staging of nasal polyposis. *Acta Otolaryngol* 1997; 117:465–7.
- 12 Lund VJ, Mackay IS. Staging in rhinosinusitis. *Rhinology* 1993; 31:183–4.
- 13 Cohen AB, Rossi M. Neutrophils in normal lungs. *Am Rev Respir Dis* 1983; 127:S3–9.
- 14 Rasp G, Thomas PA, Bujia J. Eosinophil inflammation of the nasal mucosa in allergic and non-allergic rhinitis measured by eosinophil cationic protein levels in native nasal fluid and serum. *Clin Exp Allergy* 1994; 24:1151–6.
- 15 Steinke JW, Bradley D, Arango P et al. Cysteinyl leukotriene expression in chronic hyperplastic sinusitis-nasal polyposis: importance to eosinophilia and asthma. *J Allergy Clin Immunol* 2003; 111:342–9.
- 16 Bedard M, McClure CD, Schiller NL, Francoeur C, Cantin A, Denis M. Release of interleukin-8, interleukin-6, and colony-stimulating factors by upper airway epithelial cells: implications for cystic fibrosis. *Am J Respir Cell Mol Biol* 1993; 9:455–62.
- 17 Abdi K. IL-2: the role of p40 versus p75. *Scand J Immunol* 2002; 56:1–11.
- 18 de Waal Malefyt R. Role of interleukin-10, interleukin-4, and interleukin-13 in resolving inflammatory responses. In: Gallin JI, Snyderman R, eds. *Inflammation basic principles and clinical correlates*. Philadelphia, PA: Lippincott Williams & Wilkins, 1999; 837–49.
- 19 Hamilos DL, Leung DY, Huston DP, Kamil A, Wood R, Hamid Q. GM-CSF, IL-5 and RANTES immunoreactivity and mRNA expression in chronic hyperplastic sinusitis with nasal polyposis (NP). *Clin Exp Allergy* 1998; 28:1145–52.
- 20 Gerstner AO, Gutsche M, Bucheler M et al. Eosinophilia in nasal polyposis: its objective quantification and clinical relevance. *Clin Exp Allergy* 2004; 34:65–70.