

***In vitro* diagnosis of chronic nasal inflammation**

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Summary

Background Differential diagnosis of chronic nasal inflammation is insufficient when based solely on clinical examination and radiography of paranasal sinuses. Patients complain about more or less similar symptoms. Activation of mast cells and eosinophils is pivotal in nasal inflammation.

Objective To compare tryptase and eosinophilic cationic protein (ECP) in nasal secretions in different forms of chronic nasal inflammation and to establish norm values.

Methods The study included 1710 patients presenting with nasal complaints. Nasal secretions were gained by the cotton wool method and analysed for tryptase, as a marker of mast cell activation, and for ECP, as a marker of tissue eosinophilia and activation. Patients were grouped according to their diagnosis: chronic, non-allergic rhinosinusitis (sinusitis, $n = 194$), non-allergic nasal polyposis (polyposis, $n = 138$), non-allergic rhinitis with eosinophilia syndrome (NARES, $n = 198$), isolated perennial allergic rhinitis (AR) ($n = 126$), isolated seasonal AR ($n = 132$), and patients allergic to both, seasonal and perennial allergens ($n = 193$). Seven hundred and twenty-nine patients with nasal complaints due to a deviated septum and without any nasal inflammation served as controls.

Results Nasal tryptase was highly significantly ($P < 0.001$) elevated in polyposis, NARES, and in AR. ECP was highly significantly ($P < 0.001$) elevated in all groups of patients suffering from chronic nasal inflammation. Based on our data and method we established norm values (95% confidence interval of mean value) for nasal tryptase in healthy adults, ranging from 12.0 to 18.7 ng/mL and for ECP ranging from 84.4 to 102.6 ng/mL.

Conclusion Mast cells and eosinophils are involved in non-allergic and allergic forms of chronic nasal inflammation. We established an *in vitro* assay for tryptase and ECP in nasal secretions and defined norm values based on our data and method. *In vitro* measurement of biological markers in nasal secretions provides important information for differential diagnosis and therapeutic strategies of chronic nasal inflammation.

Keywords allergic rhinitis, ECP, NARES, nasal inflammation, nasal secretions, norm values, polyposis, sinusitis, tryptase

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Introduction

Chronic diseases of the upper airways are extremely common. Besides impairment of quality of life, they are of enormous socio-economic impact [1–3]. Differential diagnosis of chronic nasal inflammation is often difficult. Patients suffering from chronic nasal inflammation complain about more or less similar symptoms, such as headaches, nasal obstruction and rhinorrhea. Clinical examination alone is insufficient for determining an accurate diagnosis, even when adding radiography of paranasal sinuses.

Common and chronic forms of nasal inflammation include rhinosinusitis, nasal polyposis, non-allergic rhinitis with eosinophilia syndrome (NARES), and allergic rhinitis (AR).

Various definitions exist for chronic rhinosinusitis. In the definition proposed by the International Conference on Sinus Disease in 1993, the criteria for chronic rhinosinusitis in adults were persistent symptoms and signs for 8 weeks or four episodes per year of recurrent acute sinusitis, each lasting at least 10 days, in association with persistent changes on the computed tomography (CT) scan 4 weeks after medical treatment without intervening acute infection [4]. Grading systems based on alterations in CT scans of paranasal sinuses were provided by many authors emphasizing the importance of radiography in the diagnosis of chronic rhinosinusitis, but they involve problems of recurrent exposition to radiation and socio-economic aspects. Complaints of chronic rhinosinusitis constitute rhinorrhea, headache and nasal obstruction [5]. Prevalence rates are estimated to be around 14% of the global US population, with increasing numbers [1, 3].

The pathophysiology of nasal polyposis is far from being understood. Nasal polyps appear as glistening, pale-grey, smooth, and semi-translucency masses, prolapsing into the

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nasal cavity [6]. They arise typically from the ostiomeatal complex and affect bilaterally all paranasal sinuses. Various grading systems exist, from which we prefer the one provided by our group [7]. Tissue eosinophilia is a general characteristic of nasal polyps and is found in 80–90% of all cases [8] reflecting the pivotal role of eosinophils in this disease [6]. Nasal polyps in adults frequently point to a manifestation of other systemic eosinophilic diseases, such as intrinsic asthma or aspirin idiosyncrasy (Samter's triad). Clinical symptoms of nasal polyposis are largely caused by the diminished airflow through the nose and include headaches, nasal obstruction and rhinorrhea. Hosemann et al. [9] suggested a prevalence of this disease of 1–2% of the adult population in Europe, with a mean age of manifestation after the age of 20 years.

NARES is less well described. It constitutes a chronic, eosinophilic form of nasal inflammation. Patients complain about typical symptoms of perennial AR, mainly nasal congestion and rhinorrhea, but all *in vitro* and *in vivo* allergy tests fail to detect a sensitization [10]. A chronic, unspecific liberation of histamine and a self-perpetuating, chronic and eosinophilic nasal inflammation are suggested as the pathogenic factors of the disease. Excessive eosinophilia in nasal secretions is pathognomonic for this disease. There are only very few data about prevalence rates of NARES, ranging from 2% to 14% of patients with chronic rhinitis [11, 12].

Pathophysiology of AR is probably best described: AR constitutes an IgE-mediated, eosinophilic and Th2-type immune disease. Allergens can be described as seasonal or perennial. Patients suffer from rhinorrhea, nasal obstruction, and nasal pruritus. Impairment of quality of life, including loss of concentration, headaches, sleep disturbances and much more is well documented [13]. AR constitutes the most frequent manifestation (45%) of atopic diseases. Prevalence rates are increasing by about 3.5% each decade and are estimated to be 15–25% in Europe [14].

Various inflammatory cell types are involved in chronic nasal inflammation. Among these pivotal cell types are mast cells and eosinophils. Activation of resident mast cells is well described in the pathophysiology of AR, but is reported to play a relevant role in nasal polyposis as well [15]. In the early 1960s, mast cells were reported to have trypsin-like activity [16]. In 1981, this activity was shown to belong to a tryptic protease and was called tryptase [17]. Tryptase, as a major protein component of human mast cells, was found in both connective tissue and mucosal mast cells [18]. It was found in negligible amounts in basophils, but not in other human cells types [19]. Tryptase is a neutral serine protease, located in the secretory granules of mast cells and it is released together with histamine during degranulation [20]. Tryptase has a longer plasma half-life (1.5–2 h) compared with histamine (1–3 min) [21]. For this biological characteristic, tryptase is considered superior to histamine in order to study mast cell activation. The physiologic role of nasal tryptase in not yet clear. Among others, it is involved in cleaving C3 into the anaphylatoxin C3a, increasing vascular permeability and causing bronchospasm [22].

Eosinophils are pivotal in the pathophysiology of all studied forms of chronic nasal inflammation. There is strong evidence for the crucial role of eosinophils in ongoing inflammation and tissue damage [23]. Granules of eosinophils contain toxic basic protein. The major part of these protein

constitutes eosinophilic cationic protein (ECP) [24]. Experimental studies on eosinophil function have shown cytotoxic effects on airway epithelium [25] resulting in ciliostasis and lysis of epithelium [6]. ECP constitutes a well-described and standardized marker for tissue eosinophilia and activation [26]. In AR, ECP levels in nasal secretions are significantly higher than serum levels, are better related to disease activity and clinical symptoms, and should therefore be preferred over ECP serum measurements [27]. ECP levels in nasal secretions can be used to monitor the activity of eosinophilic inflammation in different kinds of rhinitis with eosinophilic involvement and they constitute an indicator for the efficacy of treatment [26].

Studies on nasal secretions are widely used to determine mediators involved in the disease. In contrast to biopsies they are easy, non-invasive, repeatedly and reproducibly accessible and reflect local pathophysiology much better than systemic mediators [27, 28]. Different methods of gaining nasal secretions are described [26, 29]. We choose the cotton wool method, because it is easily accessible without unpleasant sensations for the patient. Further it is very well described for several mediators in several studies [26–32] and one need not deal with hardly calculable effects of pre-dilution like in nasal lavage [29].

Objective

The aim of our study was to compare tryptase and ECP in nasal secretions between different forms of chronic nasal inflammation. Measuring *in vitro* parameter in nasal secretions should give insights in pathogenic pathways involved and should provide important data for differential diagnosis. Therefore we established norm values based on our data and method. In order to exclude effects of comorbidity we studied large numbers of patients suffering from one form of chronic nasal inflammation solely.

In order to determine possible effects of actual pollen exposure, we subdivided those patients who are solely allergic to seasonal allergens in co- and extra-seasonal.

Materials and methods

Patients

The study was performed in the Department of Oto-Rhino-Laryngology, Head and Neck Surgery of Ludwig-Maximilians-University, Munich, Germany. We included 1710 patients (965 males:745 females; mean age 38.8 years $\pm$ 16.5) presenting themselves with nasal complaints in the years between 1997 and 2002. All patients were tested for an underlying nasal disease by patient's history and nasal endoscopy. Patients without any history of sinusitis and with normal endoscopic status underwent plain X-rays of paranasal sinuses (occipito-mental and occipito-frontal) for evaluation of sinus status. Patients with mucosal swellings in plain X-rays, a history of chronic sinusitis or endoscopically visible nasal polyposis were examined by CT scan to evaluate paranasal sinus state.

In vitro tests

We obtained nasal secretions by the cotton wool method: small, cone-shaped cotton wool strips (absorbent cotton,

Hartmann Inc., Heidenheim/Brenz, Germany) with a length of about 4 cm and a diameter of about 6 mm were placed into the middle meatus of the nose with bayonet pincers under anterior rhinoscopic view and were left for 10 min; followed by centrifugation of the cotton wool strips at 2000g for 10 min. The samples were stored at -20°C and pre-diluted 1:5 before testing.

Patient's sera were tested for total IgE (UniCAP, Pharmacia Diagnostics, Freiburg, Germany), total eosinophil counts and the widely used allergy screening test SX1 (Phadiatop) [33–37] (UniCAP, Pharmacia Diagnostics). In case of a suspected allergy (positive SX1 and/or total IgE in serum above norm value (100 U/mL) and/or very typical symptoms) patients underwent further *in vivo* and *in vitro* allergy tests, such as skin prick test, intranasal allergen provocation, and allergen-specific IgE determination [CAP-class] in serum (UniCAP, Pharmacia Diagnostics).

Nasal secretions of all patients were analysed for tryptase (UniCAP, Pharmacia Diagnostics) as a marker of mast cell activation. Thresholds of detection, according to the producer, of the used test kit range from 5 to 1000 ng/mL. Further, nasal secretions of all patients were analysed for ECP (UniCAP, Pharmacia Diagnostics) as a marker of tissue eosinophilia and activation. Thresholds of detection, according to the producer, of the used test kit range from 10 to 1000 ng/mL.

Disease groups

Patients were grouped according to the underlying form of chronic nasal inflammation:

- Chronic, non-allergic rhinosinusitis (sinusitis) with typical symptoms according to the above-mentioned criteria including pathological mucosal swelling in paranasal sinuses (CT scans). An AR was excluded by patient's history, a negative allergy screening test SX1, and normal total IgE. Nasal polyposis was excluded by endoscopy.
- Non-allergic nasal polyposis (polyposis) with endoscopically visible bilateral nasal polyposis grade II and above [7]. An AR was excluded by patient's history, a negative allergy screening test SX1, and normal total IgE.
- NARES with typical nasal symptoms, excessive nasal ECP and not diseased X-rays of paranasal sinuses. An AR was excluded by patient's history, a negative allergy screening test SX1, and normal total IgE.

- Patients suffering from AR were grouped according to their specific allergen into: isolated perennial AR, isolated seasonal AR, and in patients allergic to both, seasonal and perennial allergens. Those 132 patients, who were allergic to seasonal allergens solely, were subdivided according to their natural pollen season into co-seasonal ($n = 43$) and extra-seasonal ($n = 89$) in order to determine differences due to actual allergen exposure. Patients with a history of chronic rhinosinusitis or pathologic X-rays of paranasal sinuses or visible nasal polyps were excluded from this group.
- Healthy adults with nasal complaints due to a deviated septum and without any nasal inflammation (no history of chronic sinusitis, normal endoscopically findings, no elevated ECP in nasal secretions, negative allergy screening tests, and no mucosal swelling in X-rays of paranasal sinuses) served as controls. For epidemiologic data refer to Table 1.

Symptoms

In order to evaluate main nasal symptoms, we provided a very short questionnaire, asking the patient to rate nasal obstruction and rhinorrhea within the last 24 h with a given symptom score ranging from 0 (not troubled) to 3 (extremely troubled).

Statistics

A standard PC with SPSS 11.5 software was used for the statistical evaluation. Significance was evaluated by the independent samples *t*-test. *P*-values <0.05 were regarded as significant. Mean values $\pm$ standard deviation were chosen for the graphical presentation of the results. Correlations were determined by Pearson's two-tailed, bivariate correlation test.

Results

Except for sinusitis we found a predominance of male individuals affected by chronic nasal inflammation. Patients suffering from polyposis were significantly ($P < 0.001$) older and patients allergic to both seasonal and perennial allergens were significantly ($P < 0.001$) younger than the controls.

Nasal tryptase (ng/mL) was highly significantly ($P < 0.001$) elevated in polyposis, NARES, and in AR compared with

Table 1. Epidemiologic data total population

	Sinusitis	Polyposis	NARES	AR			Controls
				Perennial	Seasonal	Both	
<i>n</i>	194	138	198	126	132	193	729
% of studied population	11.3	8.1	11.6	7.4	7.7	11.3	42.6
% male	42.2	64.5	63.1	65.9	59.1	60.6	53.6
Mean age $\pm$ SD	41.8 $\pm$ 15.3	46.1 $\pm$ 15.8	40.2 $\pm$ 16.9	36.5 $\pm$ 14.9	37.3 $\pm$ 14.9	30.5 $\pm$ 14.7	39.2 $\pm$ 16.9

Sinusitis, chronic, non-allergic rhinosinusitis; polyposis, non-allergic nasal polyposis; NARES, non-allergic rhinitis with eosinophilia syndrome; AR, allergic rhinitis; perennial, isolated perennial allergic rhinitis (allergic against one or more perennial allergens of house dust mites = 70.6%; animals = 12.7%; molds = 5.6%; storage mites = 41.3%); seasonal, isolated seasonal allergic rhinitis (allergic against one or more seasonal allergens of grass = 72.7%; trees = 48.5%; herbs = 22.7%); both, patients allergic to both, seasonal and perennial allergens; controls, healthy adults without any nasal inflammation.

controls (Fig. 1). We found highest concentration in polyposis. Nasal ECP (ng/mL) was highly significantly ($P<0.001$) increased in all studied forms of chronic nasal inflammation with excessive amounts in NARES (Fig. 2).

Inter-group comparison revealed significantly elevated concentrations of nasal tryptase for polyposis ($P<0.001$), for NARES ($P<0.01$), and for AR (perennial AR $P<0.001$, seasonal AR $P<0.05$, and for patients allergic to both $P<0.001$) when compared with sinusitis. Significantly elevated concentrations were observed in polyposis ($P<0.05$), perennial AR ($P<0.05$), and perennial and seasonal AR ($P<0.05$) when compared with NARES. Comparison of nasal tryptase against isolated seasonal AR revealed significantly elevated levels in polyposis ($P<0.05$), perennial AR ($P<0.05$), and perennial and seasonal AR ($P<0.05$). There was no significant difference between polyposis, perennial AR or perennial and seasonal AR.

Inter-group comparison revealed highly significantly ($P<0.001$) elevated concentrations of nasal ECP for all studied diseases when compared with sinusitis. Compared against isolated perennial AR, we observed significantly

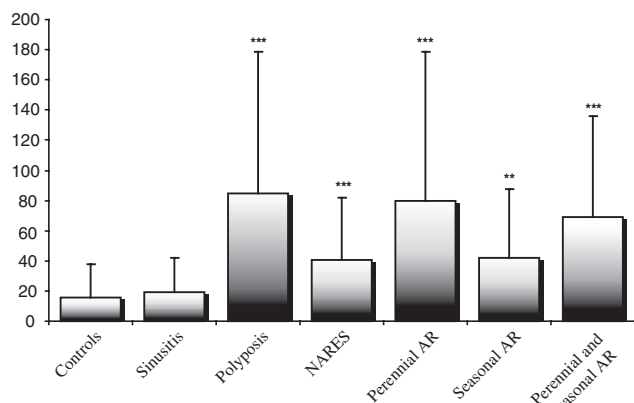


Fig. 1. Tryptase in nasal secretions (ng/mL). Controls, no nasal inflammation, $n = 729$; rhinosinusitis, chronic, non-allergic sinusitis, $n = 194$; polyposis, non-allergic nasal polyposis, $n = 138$; NARES, non-allergic rhinitis with eosinophilia syndrome, $n = 198$; perennial AR, isolated perennial allergic rhinitis, $n = 126$; seasonal AR, isolated seasonal allergic rhinitis, $n = 132$; perennial and seasonal AR, patients allergic to both, perennial and seasonal allergens, $n = 193$; graphic presentation, mean values $\pm$ standard deviation, significance (t -test): * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with controls.

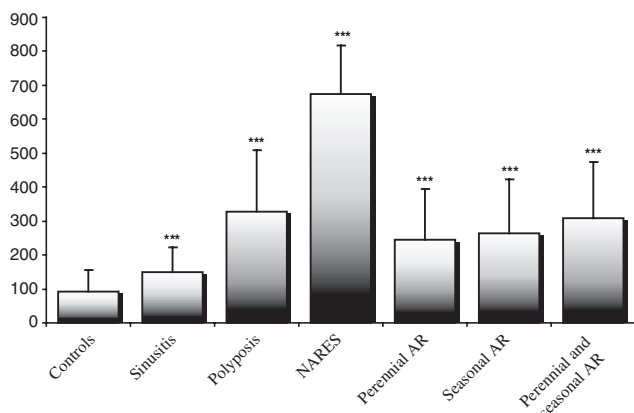


Fig. 2. ECP in nasal secretions (ng/mL). Legend as in Fig. 1.

elevated levels for NARES ($P<0.001$) and polyposis ($P<0.05$). There was no significant difference of nasal ECP in between the AR group. ECP concentrations in NARES were still highly significantly ($P<0.001$) elevated when compared with polyposis.

Based on our data and method, we could establish a norm value (95% confidence interval of mean value) for nasal tryptase in healthy adults ranging from 12.0 to 18.7 ng/mL and for ECP ranging from 84.4 to 102.6 ng/mL.

Total IgE and eosinophil in serum were significantly elevated in polyposis and AR when compared with controls (Figs 3 and 4).

There was no significant difference in nasal tryptase or nasal ECP comparing co- vs. extra-seasonal in isolated seasonal AR. Total IgE was not significantly altered by pollen season as well, but blood eosinophils increased significantly ($P<0.05$) in season (Table 2).

In total, our patients were significantly ($P<0.001$) more impaired by nasal obstruction (mean symptom score: 1.82 ± 1.1) than by rhinorrhea (mean symptom score: 1.31 ± 1.0).

Nasal obstruction was most troublesome for patients suffering from perennial AR (mean symptom score 2.20 ± 1.1 , $P<0.01$), followed by polyposis (mean symptom score 2.16 ± 1.1 , $P<0.01$) and seasonal and perennial AR (mean symptom score 1.99 ± 1.0 , $P<0.05$), compared with controls (mean symptom score 1.78 ± 1.2). The remaining diseases showed no significant increase in nasal obstruction.

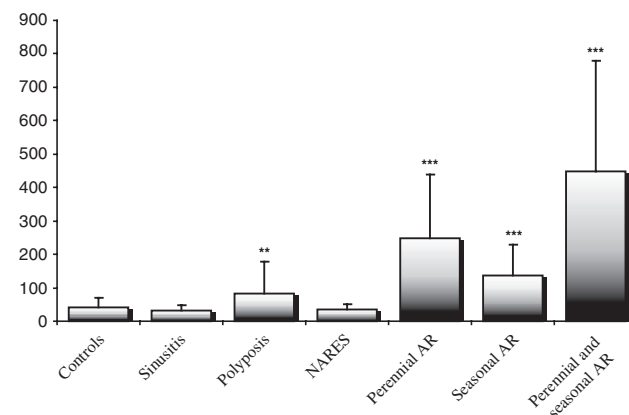


Fig. 3. Total IgE in serum (U/mL). Legend as in Fig. 1.

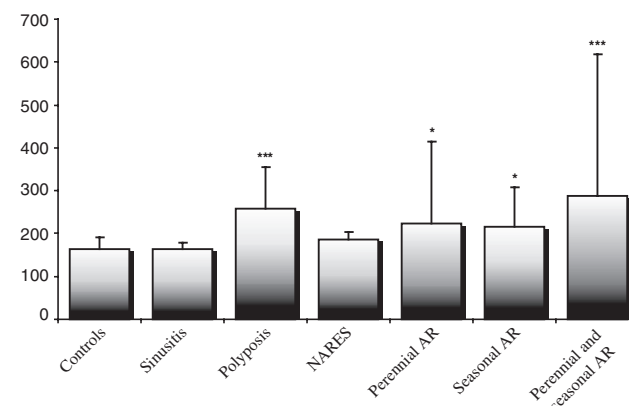


Fig. 4. Eosinophils in serum (/mm³). Legend as in Fig. 1.

Table 2. Comparison of co- vs. extra-seasonal in patients suffering from isolated seasonal allergic rhinitis

	Tryptase (ng/mL)	ECP (ng/mL)	Total IgE (U/mL)	Eosinophils (/mm ³)
Co-seasonal (<i>n</i> = 43)	41.98	238.66	141.87	341.36
Standard deviation	73.6	297.5	176.2	318.2
Extra-seasonal (<i>n</i> = 89)	41.48	277.78	133.26	157.96
Standard deviation	101.0	324.8	189.7	78.0
<i>P</i> -value	0.975	0.494	0.804	0.014

Patients suffering from allergic rhinitis solely to seasonal allergens (*n* = 132) were subdivided according to their natural pollen season into co-seasonal (*n* = 43) and extra-seasonal (*n* = 89) in order to determine the impact of actual pollen exposure. Tryptase and ECP were measured in nasal secretions; total IgE and eosinophilia count in serum. ECP, eosinophilic cationic protein.

Rhinorrhea was rated most troubling in seasonal and perennial AR (mean symptom score 1.87 ± 0.9 , $P < 0.001$), followed by perennial AR (mean symptom score 1.73 ± 0.9 , $P < 0.001$) and polyposis (mean symptom score 1.52 ± 1.1 , $P < 0.01$), compared with controls (mean symptom score 1.14 ± 1.0). Remaining diseases showed no significant increase in rhinorrhea.

We found a weak, but significant ($P < 0.01$) correlation between nasal tryptase and nasal obstruction ($r = 0.118$) and rhinorrhea ($r = 0.145$).

Discussion

Differential diagnosis of chronic nasal inflammation is insufficient when based solely on clinical examination and radiography of paranasal sinuses. Patients complain about more or less similar symptoms. Mucosal swelling in paranasal sinuses, as visible in plain X-rays or CT scans, is not disease specific either. *In vitro* parameters are established and essential for diagnosis of various forms of chronic nasal inflammation such as AR (total and allergen-specific IgE in serum) or NARES (ECP in nasal secretions).

We studied the release of established mediators in nasal secretions of cells pivotal in the pathophysiology of chronic nasal inflammation. For mast cell activation, we choose tryptase and for tissue eosinophilia and activation, we choose ECP.

Gaining nasal secretions by the cotton wool method constitutes an easy, non-invasive, reproducible and non-bothering method. There are no effects of pre-dilution, possibly influencing the data like in nasal lavage [29]. It reflects local inflammation much better than systemic mediators, as demonstrated in our results and in previously published studies [27, 28]. Further, it is widely used and well described for various mediators and diseases [26–32]. Therefore, gaining nasal secretions by the cotton wool method is our preferred and recommended method and should be performed in every patient with nasal complaints.

We compared large numbers of patients suffering from various forms of nasal inflammation, such as chronic, non-allergic rhinosinusitis (sinusitis), non-allergic, nasal polyposis (polyposis), NARES and AR. The latter group was divided according to the nature of allergens, into seasonal and perennial. Seasonal AR was further subdivided into co- and extra-seasonal, in order to determine possible effects of the

actual pollen exposure. For definition of the groups we refer to the corresponding parts above. In order to exclude effects of comorbidity we studied patients suffering from one form of chronic nasal inflammation solely. This is easy to achieve, when excluding allergic individuals in the sinusitis, polyposis, NARES or control groups. But we cannot exclude for sure mild or asymptomatic forms of chronic rhinosinusitis in every patient suffering from NARES, AR or in the controls. Every individual would have to be CT scanned for this purpose, which is unethical due to the exposure to radiation. However, we judge this as not relevant due to the large numbers of patients studied and reflected by the clear-cut differences in mediators measured between the groups.

Except for sinusitis, we found a predominance of male individuals affected by chronic nasal inflammation. This is in accordance with epidemiologic data for polyposis and AR [9, 14, 38], but our data do not support those authors describing a predominance of females in NARES [39]. Patients suffering from polyposis were significantly ($P < 0.001$) older and patients allergic to both, seasonal and perennial allergens were significantly ($P < 0.001$) younger than the controls. This is in accordance to published data as well [6, 14, 38]. In these regards, our patients are representative. When looking at prevalence rates, one has to take into consideration that we studied individuals presenting themselves in our Oto-Rhino-Laryngology Department with nasal complaints. This might help to interpret the relatively high prevalence rates of polyposis or NARES (both around 10%) in our patients.

When asking our patients to rate the main nasal symptoms, generally accepted to be found in each of the studied diseases, we found that nasal obstruction was significantly ($P < 0.001$) more troubling than rhinorrhea. Both correlated weakly, but significantly to nasal tryptase, pointing to a relevant role of mast cell activation in nasal obstruction and rhinorrhea. Interestingly, we found significantly more nasal obstruction and rhinorrhea only in the AR and polyposis groups. When interpreting these data, one has to take into consideration that all patients presented themselves with nasal complaints. Controls included mainly patients with septal deviation. Thus, our data might be interpreted that nasal obstruction caused by NARES or sinusitis is not worse than nasal obstruction caused by septal deviation.

Mast cells and eosinophils are pivotal cell types in chronic nasal inflammation. For mast cell activation, we choose tryptase as an established marker. We could demonstrate resident mast cell activation in all studied forms of chronic nasal inflammation except for sinusitis, which is in accordance with a previous publication [40]. Our findings support those authors not listing antihistamines as a medication of choice for chronic rhinosinusitis [41].

Surprisingly, we found highest concentrations of nasal tryptase in non-allergic nasal polyposis. This is in accordance with biopsy data (showing increased numbers of mast cells in nasal polyps [42, 43]), with increased tryptase in nasal lavage of 32 polyposis patients [15], with *in vitro* data (showing inhibition of mediator and cytokine release from dispersed nasal polyp cells by mizolastine [44]), and with symptom reduction in patients suffering from nasal polyposis by cetirizine [45]. Thus, although not established as first line therapy yet [46], antihistamines might be established in future therapeutic strategies.

Our data point to promising effects of antihistamines in NARES as well and support previously published positive therapeutic effects of antihistamines, when combined with steroids [10, 47].

Increased numbers of mast cells in nasal mucosa [48] and increased concentration of tryptase in nasal lavage of 50 patients with AR [15] are in accordance with our findings. Therapeutic effects of antihistamines in the treatment of AR are beyond doubt and well documented [49].

For eosinophils, we found a more or less similar picture. Highest amounts of nasal ECP was found in NARES, which is not surprising since the diagnosis of NARES is based on nasal eosinophilia. Our data underline the importance of measuring nasal ECP in the process of differential diagnosis, because otherwise one would simply overlook these patients. Further, excessive ECP but no increase in serum eosinophils demonstrates that NARES is a localized form of nasal eosinophilic inflammation and not an epiphenomenon of systemic eosinophilia.

In polyposis and AR, we found systemic eosinophilia, certainly influencing levels of nasal ECP. Surprisingly, we found highest levels of ECP in non-allergic conditions.

Our data support frequently published positive therapeutic effects of drugs interfering with eosinophilia migration and activation (such as nasal steroids) in non-allergic conditions like sinusitis [50], polyposis [46] or NARES [10] and, of course, in AR [49].

To summarize, our data reflect the involvement of mast cells and eosinophils in the pathophysiology of chronic nasal inflammation and help to explain positive therapeutic effects of antihistamines or nasal steroids in non-allergic and allergic diseases.

When comparing co- vs. extra-seasonal in isolated seasonal AR we found surprisingly no significant effect of the actual pollen exposure, neither for nasal tryptase or ECP, nor for systemic total IgE. Only serum eosinophils increased significantly ($P < 0.05$). This is in dissonance to authors, who found increased levels of nasal tryptase and ECP during natural pollen season [51]. This might be caused by inter-individual differences when comparing different patients, as we did. A longitudinal study design, studying same individuals twice (co- and extra-seasonally) is needed to finally answer this question.

We modified a commercially available serum tryptase and ECP assay for nasal secretions. Automatic measurement by the UniCAP System, provided by Pharmacia Diagnostics, allows an easy determination of tryptase and ECP levels in nasal secretions, helpful for differential diagnosis of chronic nasal inflammation. Costs for tryptase or ECP measurement account for less than 10% of the costs for a CT scan of paranasal sinuses in our hospital, which is of economic interest as well, not to mention avoidance of (repeated) exposure to radiation.

Based on our data and method, we could establish a norm value (95% confidence interval of mean value) for nasal tryptase in healthy adults ranging from 12.0 to 18.7 ng/mL and for ECP ranging from 84.4 to 102.6 ng/mL. The latter is higher compared with a previously published norm value based on 156 individuals [26]. This difference might be explained by different numbers of patients (729 in our study vs. 156 in the 1999 study) and different method of ECP

measurement used (UniCAP in our study vs. competitive RIA in the study from 1999, both Pharmacia Diagnostics).

Concentrations of tryptase and ECP in nasal secretions were higher than previously published concentrations in serum (tryptase: 4.4 ng/mL [52]; ECP: 3.8 ng/mL [53]), again emphasizing the fact that local inflammation is much better reflected in nasal secretions than in serum [27].

Conclusions

Mast cell activation, reflected by tryptase release, and tissue eosinophilia and activation, reflected by ECP release, are involved in non-allergic and allergic forms of chronic nasal inflammation. We established an *in vitro* assay for tryptase and ECP in nasal secretions and defined norm values based on our data and method.

In vitro measurement of biological markers in nasal secretions provide important information for differential diagnosis and therapeutic strategies of chronic nasal inflammation.

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