

Local and systemic proteolytic responses in chronic rhinosinusitis with nasal polyposis and asthma

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Background: Chronic rhinosinusitis with nasal polyposis (CRSwNP) and asthma coexist frequently and share similar features of inflammation and remodeling. Remodeling has become an important concept in the pathophysiology of asthma and CRSwNP. It happens early in the development of these diseases and is relatively resistant to treatments. The key enzymes responsible for remodeling are matrix metalloproteinases (MMPs). In this study we examined whether asthma and CRSwNP share similar MMP profiles.

Methods: Nasal secretion and serum specimens of controls (19 subjects) and patients with asthma (12), CRSwNP (39), or both (16) were collected between December 2007 and May 2009. Groups were divided into 2 subgroups according to atopy. MMP-7, MMP-9, MMP-13, tissue inhibitors of metalloproteinases (TIMPs), TIMP-1 and TIMP-2, myeloperoxidase (MPO), and human neutrophil elastase (HNE) were measured using enzyme-linked immunosorbent assay (ELISA), and MMP-8 was determined using immunofluorometric assay. High-sensitivity C-reactive protein (hs-CRP) was measured to estimate systemic involvement.

Results: Patients with asthma, CRSwNP, or both exhibited lower MMP-9, MMP-9/TIMP-1, MMP-9/TIMP-2, and MPO in

nasal secretions ($p < 0.05$ in CRSwNP) and higher MMP-9, MMP-9/TIMP-1, MMP-9/TIMP-2, and HNE in serum ($p < 0.05$ in all groups) compared to controls, whereas no difference in MMP-7, MMP-13, TIMP-1, and TIMP-2 were detected. Atopy increased nasal MMP-9 and MPO expression. hs-CRP was higher in patients with CRSwNP and asthma compared to controls.

Conclusion: Our findings suggest shared pathomechanisms behind asthma and CRSwNP. Contrasting local vs systemic results reflect a different ability of healthy mucosa to react to exogenous stimuli, possibly indicating a protective function of MMP-9 and possibly also MMP-8 in the airways. © 2015 ARS-AAOA, LLC.

Key Words:

asthma; atopy; chronic rhinosinusitis; metalloproteinases; nasal polyps

How to Cite this Article:

Katainen E, Kostamo K, Virkkula P, et al. Local and systemic proteolytic responses in chronic rhinosinusitis with nasal polyposis and asthma. *Int Forum Allergy Rhinol.* 2015;5:294-302.

Chronic rhinosinusitis (CRS) is one of the most common chronic diseases. It manifests as 2 different

phenotypes, CRS with nasal polyposis (CRSwNP) and CRS without nasal polyps (CRSsNP). In a recent postal questionnaire survey conducted in 12 European countries the overall prevalence of CRS was 10.9%.¹ High disease prevalence combined with costly diagnostic and treatment methods lead to substantial direct economical burden. The impact is further highlighted by the strong link between chronic upper and lower airway diseases, the concept known as unified airways.²

CRSwNP differs from CRSsNP in terms of inflammatory profile, structural changes (remodeling) in the nasal mucosa and lower airway comorbidities even in the early stages

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Funding sources for the study: Academy of Finland (TS), Helsinki University Central Hospital Research Foundation (EK, PV), Finnish Society of Allergy and Immunology (EK), Otolaryngological Research Foundation (EK). Potential conflict of interest: None provided.

Received: 17 September 2014; Revised: 7 December 2014; Accepted: 16 December 2014
DOI: 10.1002/alr.21486
View this article online at wileyonlinelibrary.com.

of the disease.³ It is noteworthy that asthma comorbidity is significantly higher in CRSwNP than in CRSsNP.^{2,3} Moreover, the severity of asthma correlates with the degree of radiological findings in the sinuses.^{4,5} In both asthma and CRSwNP the inflammation can be either eosinophilic or non-eosinophilic. However, in CRSwNP and pediatric asthma similar remodeling patterns are present both in patients with eosinophilic disease and non-eosinophilic inflammation.^{3,6} Remodeling seems to be a more versatile phenomenon in adult asthmatic subjects, shown to be less profound in milder forms of the disease as well as in non-eosinophilic asthma.^{7,8} Within both CRSwNP and asthma there is significant variation in inflammatory and remodeling patterns, disease severity, associated comorbidities, and response to treatment, reflecting the existence of multiple subgroups, or endotypes, with different pathophysiologic mechanisms.^{3,6,9} Thus, instead of a single disease, CRS and asthma can be regarded as manifestations of an inflammatory airway syndrome in 2 different compartments of the airways. Considering the multiple similarities between these diseases, there are CRSwNP and asthma endotypes with common pathophysiology, possibly mediated by systemic processes involving bone marrow and constitutional repair mechanisms.¹⁰

Remodeling is a complex dynamic process that consists of cycles of extracellular matrix (ECM) production and degradation leading to normal tissue repair or pathologic structural alterations.¹¹ The normal daily ECM turnover in the airways is estimated to be over 10%, making it easy to understand that even slight imbalances in the system may lead to serious consequences.¹² Traditionally, pathologic airway remodeling has been viewed as a secondary process occurring as a consequence of persistent inflammation. However, during the last few years studies in asthma and CRS have revealed that remodeling appears to be independent of and occurs parallel to or even precedes inflammation.^{6,13–15} The characteristic remodeling patterns shared by CRSwNP and asthma include epithelial disruption, reticular basement membrane thickening, goblet cell hyperplasia, subepithelial edema, increased number of myofibroblasts, and alterations in ECM components.^{11,13,15} Modern asthma medication has only a limited effect on remodeling.¹⁶ Studies concerning the effect of medication and surgery on structural changes in CRSwNP are scarce, but they also imply that purely anti-inflammatory treatments or minimally invasive surgery are not sufficient to reverse pathologic remodeling.¹⁷

The key enzymes responsible for ECM degradation are matrix metalloproteinases (MMPs).¹⁸ By this proteolytic activity MMPs affect the adherence of the cells to ECM and release bioactive ECM molecules and mediators stored in ECM (eg, transforming growth factor- β [TGF- β]), altering the microenvironment of the cells and allowing them to react to stimuli.¹⁹ MMPs degrade and process also non-matrix bioactive substrates, thereby further modifying cell behavior and defensive immune responses.¹⁸ Tissue inhibitors of metalloproteinases (TIMPs) regulate

MMP activities in a 1:1 stoichiometric ratio.¹⁸ Failures in MMP functions are caused by either excessive MMPs or deregulated MMP/TIMP ratios.¹⁹ Controversially, certain MMPs have also been shown to exhibit anti-inflammatory properties in mouse models of respiratory diseases.^{20–22}

MMPs have been studied extensively in respiratory diseases but much less is known about their functions in the upper airway diseases. MMP-9 is the predominant MMP in asthma. Elevated levels of MMP-9 have been detected in bronchoalveolar lavage fluid, induced sputum, and serum in asthmatic patients, being higher in more severe disease with nonreversible obstruction than in milder asthma.¹⁹ MMP-9 is also the most studied MMP in CRS. The elevated expression of MMP-9 in the stroma of nasal polyp tissue is the most congruent finding, but increased MMP-9 staining has also been detected in the epithelium, endothelium, glands, and inflammatory cells.^{23–27} The higher MMP-9 expression is associated with recurrence of nasal polyps as well as with poorer healing quality after endoscopic surgery.^{23,24} However, no correlation between MMP-9 gene expression and extension of CRSwNP evaluated by the Lund-Mackay score has been detected in another study.²⁶

The fact that CRSwNP and asthma share similar histopathologic findings of airway remodeling led to the hypothesis that the proteolytic profiles of these 2 diseases are also similar. We compared patients with CRSwNP to healthy controls with respect to their history of asthma and atopy to get a robust endotyping of CRSwNP. Similar subclassification of CRS has been proposed by Han²⁸ in his recent thesis. We evaluated 4 MMPs, their inhibitors TIMP-1 and TIMP-2, as well as their molar ratios. Since neutrophils are the main source of MMP-8 and MMP-9, we also studied biomarkers of neutrophil activity, myeloperoxidase (MPO) and human neutrophil elastase (HNE). To get an estimation of systemic involvement, we measured their levels both in nasal secretions and in serum, as well as high-sensitivity C-reactive protein (hs-CRP). To our knowledge, this is the first study estimating both local and systemic involvement in CRSwNP and asthma by simultaneously comparing proteolytic profiles in nasal secretions and serum.

Patients and methods

Patients

Eighty-six patients were recruited to the study at the Department of Otorhinolaryngology–Head and Neck Surgery, in Helsinki University Central Hospital between December 2007 and May 2009. Fifty-five were male and 31 female. Mean age was 41 years (range, 18 to 60 years). Fifty-five patients were diagnosed with CRSwNP according to the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) guidelines.²⁹ Endoscopic sinus surgery was performed on all CRSwNP patients. Thirty-one patients, who were operated on because of a deviated septum, served as controls. Sixteen patients in the CRSwNP group and

TABLE 1. Number of subjects in groups and atopic/non-atopic subgroups

Group	Atopic	Non-atopic	Total
Controls	7	12	19
Asthma	8	4	12
CRSwNP	21	18	39
CRSwNP+asthma	10	6	16
All	46	40	86

CRSwNP = chronic rhinosinusitis with nasal polypsis.

12 patients in the control group had a history of asthma (Table 1). Five patients had a history of aspirin intolerance, and of these, 2 patients had asthma but no CRSwNP, 1 patient had CRSwNP but no asthma, and 2 patients had both asthma and CRSwNP.

A skin-prick test (SPT) was performed using 10 common aeroallergens, including house dust mites (*Dermatophagoides pteronyssinus*), animal danders (cat, dog, horse), molds (*Cladosporium*, *Alternaria alternata*), and pollens (meadow fescue, Timothy grass, ragweed, alder, birch). Histamine and isotonic saline were used as positive and negative controls. A positive SPT (SPT+) was defined as a wheal of at least 3 mm because 0.9% saline control solution gave negative results. Atopy was defined as the presence of at least one positive SPT (46 subjects).

The study was approved by the Ethical Committee of Helsinki University Central Hospital. Written consent was obtained from all patients. This work was performed in the Department of Otorhinolaryngology, Helsinki University Central Hospital and the Institute of Dentistry, University of Helsinki.

Nasal secretions and serum samples

Nasal secretions were collected directly before the operation, as described by Watelet et al.³⁰ Nasal secretions were obtained by inserting a Merocel nasal swab (Ivalon® 3.5 cm × 0.6 cm × 1.2 cm) bilaterally in the nasal cavity for 5 minutes. Three milliliters (3 mL) of 0.9% NaCl solution were added to all freshly obtained nasal swabs. After 2 hours at 4°C the suspensions were extracted from the swabs and centrifuged at 1500g for 15 minutes at +4°C. The supernatants were separated and stored in aliquots at –70°C until analyzed.

Serum samples were collected from patients on the same day as nasal secretion samples and were stored at –20°C until analyzed.

Measurement of MMPs, TIMPs, MPO, and HNE

MMP-7, MMP-9, MMP-13, HNE, MPO, TIMP-1, and TIMP-2 analyses were carried out by enzyme-linked immunosorbent assays (ELISA) as described.³¹ The levels of

the studied MMPs, TIMP-1, TIMP-2, HNE, and MPO were expressed as nanograms per milliliter (ng/mL).

MMP-8 concentrations were determined by a time-resolved immunofluorometric assay (IFMA).³²

The values were analyzed from the nasal secretions and serum samples. The molar ratios of MMP-8/TIMP-1, MMP-8/TIMP-2, MMP-9/TIMP-1, and MMP-9/TIMP-2 were calculated.

hs-CRP

The concentration of hs-CRP was measured according to the standard criteria used in the Helsinki University Central Hospital. The levels of hs-CRP were expressed as milligrams per liter (mg/L).

Statistical methods

For statistical analysis, we divided the controls and patients into 4 groups: controls without asthma (control group); controls with asthma (asthma group); patients with CRSwNP but no asthma (CRSwNP group); and patients with CRSwNP and asthma (CRSwNP+A group) (Table 1). To estimate the effect of atopy, these 4 groups were further classified as SPT+ and SPT– subgroups.

Statistical analysis was performed using the statistical software package PASW Statistics 18.0.0 (SPSS, Chicago, IL). Values were described as median with 95% confidence interval. When comparisons were made between groups, significance in between-group variability was established by using the Kruskal-Wallis test. The Spearman's rho test was used to estimate correlation coefficients (ρ) between values. A statistical difference of $p < 0.05$ was considered significant.

Results

Nasal secretions

MMP-8, MMP-9, MPO, and HNE concentrations were the highest in the control group with statistically significant difference between controls and CRSwNP patients in MMP-9 and MPO levels (Fig. 1). There were no differences in the concentrations of TIMP-1 and TIMP-2.

When we compared MMP-8/TIMP-1, MMP-8/TIMP-2, MMP-9/TIMP-1, and MMP-9/TIMP-2 molar ratios, the highest values were again detected in controls (Fig. 2). The differences in MMP-9/TIMP-1 and MMP-9/TIMP-2 ratios were significant between controls and CRSwNP patients.

In the subgroup analysis, SPT+ subjects in each group had higher levels of MMP-8, MMP-9, MPO, and HNE than SPT– subjects, the highest values were seen in SPT+ controls (Fig. 1). MMP-9 was significantly higher in the SPT+ control group and MPO in the SPT– control group compared with the SPT– CRSwNP group. Also MMP-9/TIMP-1 and MMP-9/TIMP-2 ratios were significantly elevated in both SPT+ and SPT– controls when compared to SPT– CRSwNP patients (Fig. 2). The difference in MMP-8 or

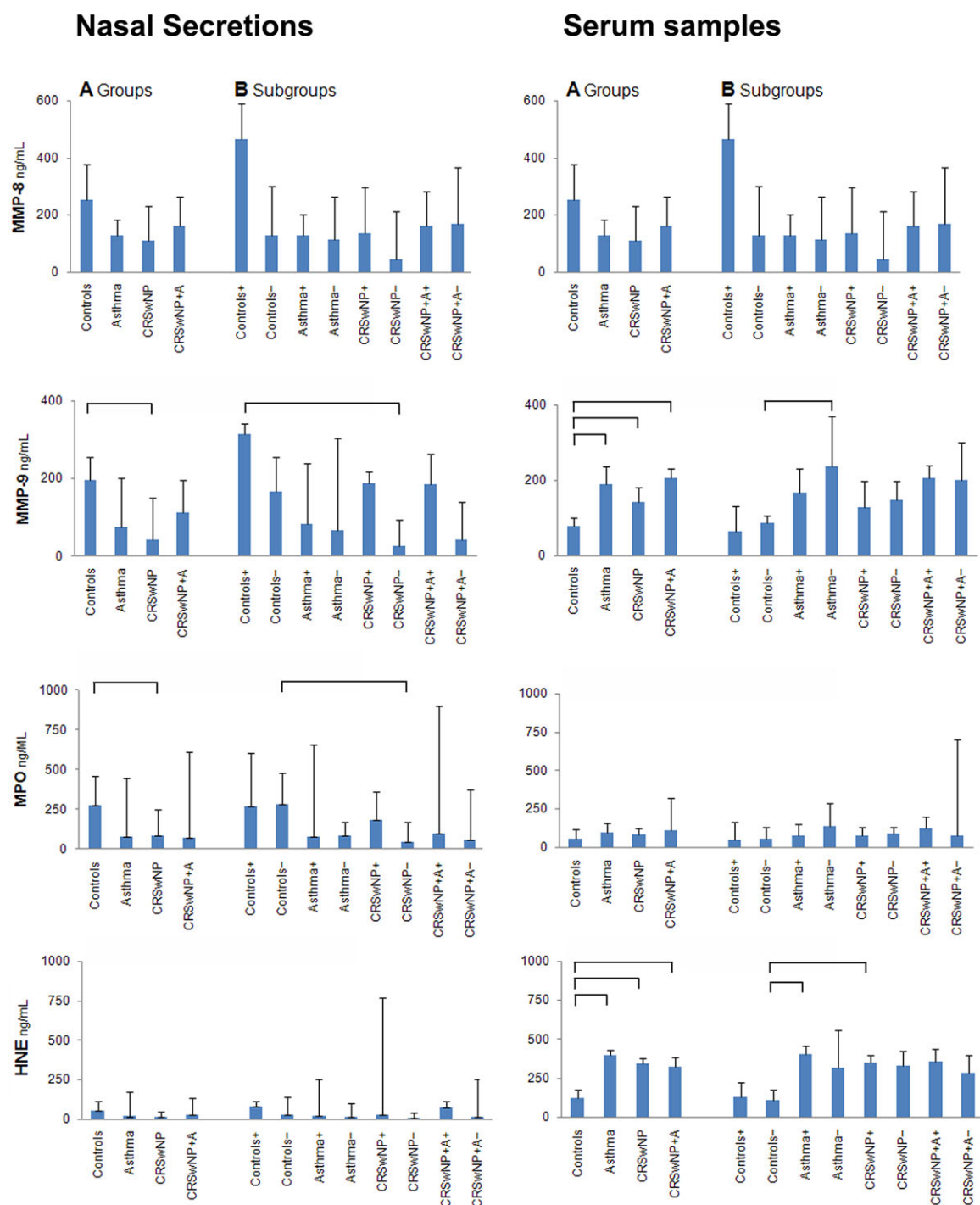


FIGURE 1. The median concentrations with 95% confidence interval of MMP-8, MMP-9, MPO, and HNE in controls and in patients with asthma, CRSwNP, or CRSwNP+A. Column A shows the values in group level and column B shows the values in atopic (+) and non-atopic (–) subgroups. The horizontal bars indicate statistically significant difference between groups/subgroups ($p < 0.05$, Kruskal-Wallis test). CRSwNP = chronic rhinosinusitis with nasal polyposis; CRSwNP+A = CRSwNP plus asthma; HNE = neutrophil elastase; MMP = matrix metalloproteinase; MPO = myeloperoxidase.

its ratios to TIMP-1 and TIMP-2 did not reach statistical significance between the subgroups, but a tendency in the same direction was seen ($p = 0.089$, $p = 0.054$, and $p = 0.060$, respectively).

MMP-7 and MMP-13 were barely detectable in nasal secretions, thus we did not calculate their ratios to TIMPs.

Serum

In contrast to the nasal secretions findings, healthy controls had significantly lower levels of MMP-9 and HNE than any of the other groups, but in the levels of MPO the difference was no longer evident (Fig. 1). In the subgroup analysis, significant difference was again seen in MMP-9 (SPT– controls vs SPT– asthma) and HNE (SPT– controls

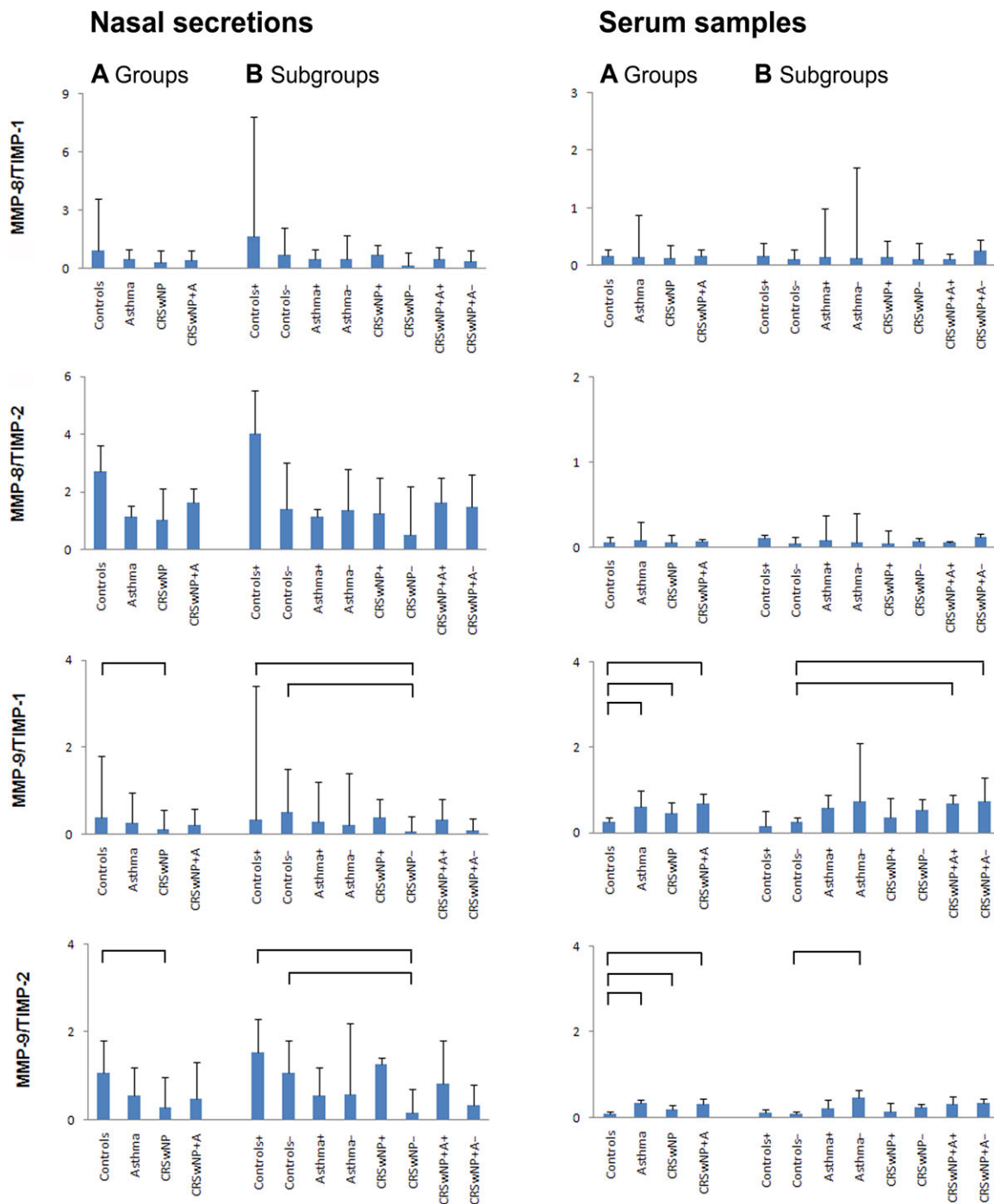


FIGURE 2. The median levels with 95% confidence interval of MMP-8/TIMP-1, MMP-8/TIMP-2, MMP-9/TIMP-1, and MMP-9/TIMP-2 molar ratios in controls and in patients with asthma, CRSwNP, or CRSwNP+A. Column A shows the values in group level and column B shows the values in atopic (+) and non-atopic (–) subgroups. The horizontal bars indicate statistically significant difference between groups/subgroups ($p < 0.05$, Kruskal-Wallis test). CRSwNP = chronic rhinosinusitis with nasal polypsis; CRSwNP+A = CRSwNP plus asthma; MMP = matrix metalloproteinase; MPO = myeloperoxidase; TIMP = tissue inhibitor of metalloproteinase.

vs SPT+ asthma and SPT+ CRSwNP). There were no differences in TIMP-1 or TIMP-2 concentrations.

The molar ratios of MMP-9/TIMP-1 and MMP-9/TIMP-2 in controls were significantly lower when compared with asthma, CRSwNP, and CRSwNP+A (Fig. 2). There were no differences in MMP-8/TIMP-1 and MMP-8/TIMP-2. In the subgroup analysis, MMP-9/TIMP-1 was significantly

lower in SPT– controls when compared to SPT– and SPT+ CRSwNP+A patients. There was also a statistically significant difference in MMP-9/TIMP-2 between SPT– controls and SPT– asthma, being lower in the controls.

As in nasal secretions, MMP-7 and MMP-13 concentrations were minimal also in serum and molar ratios were not calculated.

Correlations

In the nasal secretions both MPO and HNE showed strong positive correlations with MMP-8, MMP-9, and their molar ratio to TIMP-1 and TIMP-2 (ρ 0.78 to 0.88). MPO and HNE also correlated strongly ($\rho = 0.81$), as well as MMP-8 and MMP-9 ($\rho = 0.83$). A weaker correlation was observed between TIMP-2, but not TIMP-1, and MMP-8, MMP-9, MPO, and HNE (ρ 0.28 to 0.34).

In the serum samples similar but weaker correlation was seen between MMP-8, MMP-9, their molar ratios to TIMP-1 and TIMP-2, and MPO and HNE. A weak negative correlation was detected between TIMP-1 and MMP-8, MMP-9, MPO, and HNE ($\rho -0.32$ to -0.45), whereas TIMP-2 did not show any correlations.

The significant inverse correlation between nasal secretion and serum samples was detected in MMP-9 ($\rho = -0.228$) and MMP-9/TIMP-2 ($\rho = -0.225$). However, the correlation coefficients were low and thus the results were of minor importance.

hs-CRP

hs-CRP was found to be significantly higher in individuals who had both CRSwNP and asthma than in healthy controls (median 2.8 vs 0.5 mg/L, $p = 0.001$), but the levels of hs-CRP did not correlate with MMP-8, MMP-9, MPO, HNE, TIMP-1, TIMP-2, or the molar ratios of MMP-8 and MMP-9.

Discussion

In this study, we investigated the connection of asthma and CRSwNP in terms of proteolytic mechanisms in nasal secretions and serum. Moreover, we estimated the effect of atopy, a factor possibly dividing asthma and CRSwNP to different endotypes. The main findings were as follows: (1) patients with asthma and CRSwNP patients with or without asthma exhibited similar alterations in the measured values; (2) the proteolytic profile locally in nasal secretions was markedly different from the systemic one in serum; and (3) atopy affected MMP, MPO, and HNE levels in nasal secretions and in serum, but did not alter TIMP-1 and TIMP-2 levels.

Because the interactions between asthma and CRSwNP are thought to involve systemic mechanisms, serum samples were expected to reflect the findings in nasal secretions. Instead, almost the opposite was seen. Patients with asthma and patients with CRSwNP with or without asthma had similarly low MMP-8 (nonsignificant) and MMP-9 levels in nasal secretions but higher MMP-9, but not MMP-8, concentrations in serum compared with healthy controls. Also, alterations in MMP-9/TIMP-1 and MMP-9/TIMP-2 molar ratios followed the same pattern: lower in nasal secretions and higher in serum in asthma and CRSwNP. When atopy was taken into account, the lowest values in nasal secretions were always detected in the SPT– CRSwNP subgroup and highest values in either SPT+ or SPT–

controls in all but TIMP-1 and TIMP-2 levels. In serum samples there was more variation mainly within the subgroup with highest levels. Still the lowest values were found in either SPT+ or SPT– controls in MMP-9, its ratios to TIMP-1 and TIMP-2, MPO, and HNE. This discrepancy between nasal secretion and serum samples was further highlighted by the finding that significant correlations between nasal secretions and serum levels was detected only in MMP-9 and MMP-9/TIMP-2 levels, showing very weak inverse correlation.

There are no studies comparing local and systemic MMP levels in CRS. Studies in asthma have also shown a poor correlation in the MMP-9 levels between bronchoalveolar lavage (BAL) fluid and serum regardless of the results from multiple separate studies showing that increased level of MMP-9 in BAL fluid or sputum but also in serum is a constant finding in adult patients and correlates with disease severity.^{19,33} In the only published study comparing serum MMP levels in CRS patients with healthy controls Bugdayci et al.³⁴ found that the plasma MMP-9 concentrations were significantly higher in allergic CRSwNP patients relative to nonallergic CRSwNP patients and controls, whereas in nonallergic patients they were on the same level with controls. In our study serum MMP-9 levels were higher in both SPT+ and SPT– CRSwNP compared to SPT+ and SPT– controls, but the differences were not significant, nor did SPT+ CRSwNP differ from SPT– CRSwNP. The differences in study design might explain this finding: we had SPT+ controls, a SPT+/- asthma group, and a SPT +/- CRSwNP+asthma group in the statistical comparison. The assessment of MMPs and TIMPs in serum reflects perhaps too generally the MMP/TIMP balance of the whole body, not just the situation in the airways. Moreover, MMPs and TIMPs are released or synthesized locally in the airways and either do not seep into the circulation or are diluted to lower concentrations. Still, it is intriguing that all groups with active disease showed significant upregulation of serum MMP-9, MMP-9/TIMP-1, MMP-9/TIMP-2, and HNE to equal levels, together with a strong correlation between HNE and MMP-9 and its ratios to TIMP-1 and TIMP-2. Both MMP-9 and HNE are secreted from activated neutrophils, even though there are also other cellular sources of MMP-9.^{25,35} In contrast to the intracellular HNE, the main function of extracellular HNE is ECM protein degradation.³⁶ It also activates MMP-9 and TGF- β , another key effector molecule in tissue remodeling.³⁶ Thus, elevated MMP-9 and HNE concentrations in the serum may reflect ongoing chronic repair processes in these diseases.

In nasal secretions MMP-9, MMP-9/TIMP-1, MMP-9/TIMP-2, and MPO levels were significantly higher in controls and when atopy was taken into account, a borderline significance in MMP-8, MMP-8/TIMP-1, and MMP-8/TIMP-2 also appeared. It is interesting, that asthma patients exhibited alterations locally in the nose similar to patients with CRSwNP. Unfortunately we were not able to collect BAL or sputum samples to see if comparable results

would also be found in the lower airways. When interpreting the results, it is important to notice that we evaluated secreted forms of MMPs and TIMPs, whereas most studies in CRS have either analyzed tissue homogenates or used immunohistochemistry, making direct comparisons difficult. Even though MMP-9 levels in nasal secretions have been shown to reflect MMP-9 levels in tissue at least moderately, there are no studies concerning MMP-8 and TIMPs on this matter.^{23,37} Polymorphonuclear leukocytes are an important source of MMP-8 and MMP-9, thus the higher leukocyte count in the inflamed mucosa may explain the increased MMP levels in tissue homogenates in CRSwNP. Immunohistochemistry on the other hand is semiquantitative as its best and may show inactive as well as active forms of MMPs. Secreted forms might also have different functions than tissue-bound MMPs. In vitro study in a human breast carcinoma cell line has demonstrated that secreted MMP-9 promotes tumor cell invasion, tumor growth, and angiogenic switch more efficiently than membrane-bound MMP-9.³⁸ This difference in efficacy might also exist in other diseases. Moreover, diverse assays may create disparity between results. Neutrophils do not synthesize MMP-8 and MMP-9 outside the bone marrow, thus transcript assays will not reflect MMP-8 and MMP-9 secreted by these cells in the airways.³⁵

Immunohistochemical studies have detected increased expression of MMP-8 and MMP-9 in the epithelium, endothelium, and glands in nasal polyps as well as in the inflammatory cells within polyp tissue in relation to healthy nasal mucosa.^{23–27} Moreover, MMP-9 expression is also enhanced in the polyp stroma.^{23–25} In keeping with immunohistochemical results, immunoassays from tissue homogenates have shown increased MMP-9 levels in CRSwNP, whereas MMP-8 is not studied.^{27,39} In our earlier work, CRSwNP patients had elevated MMP-8 concentration in nasal secretions compared to healthy controls.⁴⁰ The patient group was quite heterogeneous, which might explain the difference with current results. In another study, we demonstrated that CRSwNP patients with more active disease requiring re-operation had significantly lower levels of MMP-8, MMP-9, and their molar ratios to TIMP-1 in nasal secretions, raising the question about a possible anti-inflammatory or protective effect of MMP-8 and MMP-9 in CRSwNP.⁴¹ The null mouse models of allergic asthma have shown that both MMP-8 and MMP-9 are able to reduce the accumulation of inflammatory cells in the airways as well as accelerate the resolution of inflammation.^{20–22} Studies on mice have been followed by a later report on humans; a group of patients with diisocyanate-induced asthma were followed for 3 years.⁴² At baseline examination, MMP-9 levels in BAL fluid were lower in patients than in controls, and an increase of MMP-9 was seen after treating this group with inhaled corticosteroids, which are known to diminish inflammation in the airways. Our patients showed decrease in nasal MMP-9 levels in relation to controls, possible reflecting this protective function. There was a strong positive correlation with MMP-8 and MMP-9, thus they might act

synergistically in controlling inflammation and remodeling in the airways.

Along with MMP expression, their molar ratio to TIMPs is considered to be a critical factor in the regulation of tissue remodeling. TIMPs also exhibit MMP-independent cytokine-like functions and can affect cell differentiation, growth, migration, invasion, angiogenesis, and apoptosis.⁴³ We did not find any differences in TIMP-1 or TIMP-2 concentrations in nasal secretion or in serum, and the molar ratios with MMP-8 and MMP-9 reflected more the alterations in MMP than TIMP levels. This is in accordance with the previous studies demonstrating elevated levels of TIMP-1 and TIMP-2 in CRSsNP, whereas in CRSwNP the values were on the same level than in controls.^{25,26}

HNE (significant difference in serum) and MPO (significant difference in nasal secretions) results also showed alterations similar to MMP levels: higher in nasal secretion and lower in serum in controls relative to patients. HNE and MPO are synthesized during promyelocytic differentiation, are subsequently stored as active enzymes in the azurophilic granules and released either into the phagosome or extracellularly by activated neutrophils.^{36,44,45} When released extracellularly, MPO may damage cell membranes, whereas HNE alters ECM remodeling and local inflammatory responses.^{36,44} The high HNE levels we observed in the serum of patients suffering from CRSwNP and asthma are in line with preceding works. Elevated HNE levels in blood have been found in neutrophilic asthma and may act as a marker of disease activity.⁴⁶ However, increase in nasal MPO and HNE concentrations in controls was an unexpected finding, because the expression of these mediators is known to elevate also locally in inflammatory conditions.^{36,44} The most plausible explanation is that these results reflect the irritation caused by nasal secretion collection method. The nasal swabs, which cause discomfort and mechanical irritation, were placed into the nasal cavity for 5 minutes. This is likely long enough to cause the activation of neutrophils in nasal secretion, leading to neutrophil degranulation and release of MPO and HNE. Both MPO and HNE showed a strong positive correlation with MMP-8 and MMP-9, implying that the major source of MMP-8 and MMP-9 were neutrophils. Thus, the collection method might also have influenced MMP levels, because MMP-8 and MMP-9 are stored as latent enzymes within specific granules in neutrophils and are released upon neutrophil activation.⁴⁵ However, the observed differences demonstrated that healthy nasal mucosa, or the neutrophils on it, has a different capacity to react to exogenous stimuli compared to the diseased nasal mucosa.

The influence of atopy must be assessed cautiously, because only some values showed statistical significance. Moreover, within each group there was no significant difference between SPT+ and SPT– subjects in any of the measured parameters. In our data, atopy seemed to result to higher MMP-8, MMP-9, and MPO concentrations locally in nasal secretions, with the lowest variation in the asthma group. This is consistent with the earlier results

Conclusion

Patients with asthma, CRSwNP, or both exhibited similarly lower MMP-9, MMP-9/TIMP-1, MMP-9/TIMP-2, and MPO levels in nasal secretions and higher MMP-9, MMP-9/TIMP-1, MMP-9/TIMP-2, and HNE levels in serum compared to controls, whereas no change in TIMP-1 and TIMP-2 levels were detected. These similarities in active diseases support the earlier results implicating common pathomechanisms behind asthma and CRSwNP. Contrasting local vs systemic results reflected probably a different ability of healthy mucosa to react to exogenous stimuli, possibly indicating a protective function of MMP-9 and maybe also MMP-8 in the airways. Antiprotease therapy is also an interesting field in the airway diseases, but it might also affect the protective functions and lead to unexpected side effects. Atopy seemed to increase local MMP-9 and MPO expression, but a lack of this enhancement might also exist in patient groups concerning MMP-8 expression in particular. Thus when the effect of atopy is studied in the context of respiratory tract diseases, it seems necessary to include an atopic control group in the analysis, not just healthy controls, to get a proper general view. 

in CRSwNP and asthma.^{34,47–49} Atopic patients have been shown to exhibit faster and stronger neutrophil activation in the nasal cavity.⁵⁰ Could atopy then be the sole explanation for the elevated nasal MMP and MPO levels detected in the control group; ie, when SPT+ and SPT– subjects were evaluated as one group? When looking at the histograms, the only column standing out in MMP-8, MMP-8/TIMP-1, and MMP-8/TIMP-2 graphs is the column for SPT+ controls. In contrast, MMP-9, its ratios to TIMP-1 and TIMP-2, and MPO levels exhibited more variation with significant differences between SPT– controls and subgroups with active disease as well. Thus, it is unlikely that atopy would be the only cause in the latter cases. Interestingly, this enhancement of MMP-8 and its ratios seen in controls was abolished in patient groups. Repeated neutrophil stimulation is known to lead to a desensitization and diminished ability to respond not only to the same stimulus but in some cases also to different stimuli.⁴⁵ Perhaps constant neutrophil activation in CRSwNP and asthma could result in this cross-desensitization, which in turn might alter the MMP-8 balance. It is also noteworthy, that this lack of enhancement would not have been detected if we had had only a SPT– control group.

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