Calgranulin C (S100A12) Is Differentially Expressed in Subtypes of Chronic Rhinosinusitis

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Abstract

Background: Calgranulin C (S100A12) is an innate immune peptide at the air–mucosal interface associated with neutrophil involvement, which when overexpressed has been implicated as a biomarker of inflammatory diseases. Decreased epithelial expression of certain innate immune peptides has been reported in chronic rhinosinusitis (CRS). We hypothesized that S100A12 is differentially expressed in the sinonasal mucosa of patients with CRS compared to controls and that S100A12 is a potential biomarker of CRS-specific quality of life (QOL) and disease severity.

Methods: A prospective observational study was conducted which included 70 patients: 17 controls, 28 having CRS without (CRSsNP), and 25 with (CRSwNP) nasal polyps. The expression of S100A12 and human neutrophil elastase (HNE) was assessed in the anterior ethmoid tissues from all patients using enzyme-linked immunosorbent assay (ELISA) and immunohistochemical (IHC) analyses. Disease-specific QOL (Rhinosinusitis Disability Index) and disease severity (computed tomography [CT] and endoscopy) were evaluated and correlated to the expression levels of S100A12.

Results: S100A12 and HNE were significantly elevated in patients with CRSsNP compared to normal controls ($P < .05$ and $P < .001$, respectively) and patients with CRSwNP ($P < .05$ and $P < .001$, respectively), as measured by ELISA and IHC analyses. Patients with CRS exhibited worse CRS-specific disease severity compared to normal controls ($P < .05$), and the increased protein levels of S100A12 were significantly correlated to disease severity, represented by CT scores ($P < .05$).

Conclusions: S100A12 is differentially expressed in CRS subtypes and is significantly elevated in patients with CRSsNP and associated with CRS-specific disease severity.

Keywords

sinusitis, rhinosinusitis, chronic disease, innate immune peptide, quality of life, calgranulin C, biomarker, human neutrophil elastase

Introduction

There is a growing interest in characterizing innate immune mediators in chronic rhinosinusitis (CRS) to understand its underlying pathophysiology.^{1–4} Chronic mucosal inflammation in CRS may be associated with a maladaptive innate immune response in which danger-associated molecular patterns (DAMPs) or other mediators acting as DAMPs cause pro-inflammatory signaling⁵ through the activation of pattern recognition receptors (PRRs) such as the Toll-like receptors (TLRs)⁶ and the receptor for advanced glycation end products

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(RAGE).^{7,8} Increasing evidence suggests that PRRs serve pivotal roles in CRS,^{9–11} as PRRs expressed at the mucosal surface act as immune rheostats, controlling the input–output responses at the mucosal–air interface. Therefore, defining which molecules activate PRRs and contribute to the inflammation in CRS would help further classify the disease with its associated risk factors and provide insight to its pathogenesis.

Calgranulins are calcium-binding S100 proteins that are secreted from neutrophils, macrophages, and damaged cells^{12–15} and known ligands of RAGE.^{16,17} Binding to RAGE by S100 proteins leads to numerous cellular outcomes that include the activation of TLR-4-mediated signaling in human inflammatory diseases such as asthma and cystic fibrosis.^{18–21} The S100 proteins have been investigated for their expression and antimicrobial effects and roles in sinonasal epithelial barrier function within the context of CRS.²² Increased levels of S100A8/9 have been observed in the nasal polyp tissues from patients with CRS with nasal polyps (CRSwNP) compared to control patients²³ and to those measured in inferior turbinate and uncinate tissues from patients with CRS without nasal polyps (CRSSNP).²² The upregulation of S100A8/9 in nasal polyp tissue was significantly correlated to elevated human neutrophil elastase (HNE) expression,²² as well as in response to interleukin 6 (IL-6) stimulation,²⁴ both important biomarkers involved in Th1-driven inflammation. Contrastingly, the increased expression of S100A7 in the uncinate tissues from patients with CRSSNP compared to those from CRSwNP has been reported.²² S100A12 is an important stimulus for PRRs, is a biomarker in chronic inflammatory diseases,^{12,25,26} has been shown to induce edema, and is a potent chemoattractant for neutrophils, mast cells, and monocytes.^{25,27–31} These data suggest that S100 proteins are differentially expressed in subtypes of CRS and that their expression and function are dependent on the local tissue environment; however, the role of S100A12 in CRS has yet to be evaluated.

Our objectives were to quantitate the expression of S100A12 in patients with CRSSNP and CRSwNP and to determine whether differences in S100A12 expression associate with CRS-specific quality of life (QOL) and disease severity. We hypothesized that S100A12 is differentially expressed in the sinonasal mucosa of patients with CRS compared to controls and that S100A12 is a biomarker of CRS-specific QOL and disease severity.

Materials and Methods

Patients and Tissue Samples

Patients were recruited from the Rhinology-Sinus and Skull Base Program at the University of Utah

(Salt Lake City, UT) with approval from the institutional review board. Inclusion criteria included adult patients (>18 years) diagnosed with symptomatic refractory CRSSNP or CRSwNP who had elected to undergo endoscopic sinus surgery (ESS).³² Patients diagnosed with recurrent acute rhinosinusitis (RARS), autoimmune disease, Wegener's granulomatosis, and those on oral steroid therapy within 2 weeks of surgery were excluded from the analysis. Patients without a history of CRS, RARS, and previous ESS electing to undergo endoscopic transsphenoidal approaches to the pituitary gland or nasal obstruction surgery were enrolled as normal control patients. After obtaining an informed consent, each patient provided demographic, social, and medical history data including age, gender, race, asthma, allergies (test confirmed), acetyl-salicylic acid (ASA) intolerance, current tobacco use, and previous sinus surgeries. All study patients underwent standard clinical examinations consisting of a medical history, head and neck physical evaluation, computed tomography (CT) imaging, and sinonasal endoscopy.

Sinonasal tissue specimens (~2–4.0 mm²) were removed from the polypoid mucosa in the anterior ethmoid sinus cavity during ESS. A portion of tissue was fixed in 4% formalin for histology, and a second portion of tissue was flash frozen or immersed in RNAlater® (Life Technologies, Carlsbad, CA) to stabilize the tissue for biochemistry.

Reagents and General Supplies

All reagents, general supplies, and protein standards used for histochemical and biochemical analyses were obtained from Fisher Scientific (Hampton, NH) unless specified.

Sinus Tissue Preparation for Immunohistochemical Analysis

Sinonasal tissues were fixed with 4% formalin (Ted Pella, Redding, CA) for 24 h and dehydrated through immersion in ethanol (70%, 95%, and 100%, 2 × 5 min) and xylene (2 × 10 min). Tissues were embedded into paraffin, cut into 4-μm-thick sections, and fixed to microscope slides for further examination.

Immunohistochemical Analysis of S100A12 and HNE

Sinonasal tissues were deparaffinized in xylene (3 × 10 min) and rehydrated in ethanol (100%, 95%, and 70%; 2 × 5 min) and ddH₂O (2 × 5 min). Unless stated, all reagents were obtained from and used as recommended by Vector Laboratories (Burlingame, CA). Heated antigen retrieval was performed in citrate buffer (pH 6.0), and tissues were blocked in BLOXALL and first incubated with rabbit antihuman S100A12 (1:200; 2 h at room temperature [RT])

(Abcam, Cambridge, MA), followed by ImmPRESSTM HRP antirabbit IgG and ImmPACT diaminobenzidine peroxidase chromogen. Tissues were then incubated with mouse antihuman HNE (1:200; 1 h at RT) (R&D Systems, Minneapolis, MN), followed by ImmPRESSTM HRP antimouse IgG, ImmPACT SG peroxidase, and hematoxylin. Microscopy was performed using a BX43 light microscope (Olympus, Tokyo, Japan) with Digital Photo Professional 4 software (Canon Inc., Tokyo, Japan).

Sinus Tissue Homogenate Preparation for Enzyme-linked Immunosorbent Assays

Frozen anterior ethmoid tissues were slowly thawed on ice, added to bead homogenizing tubes, weighed, and diluted with 200 to 500 μ L of phosphate-buffered saline containing 20% glycerol and a protease inhibitor cocktail (Promega; Madison, WI) depending on the weight of the tissue. The tissues were homogenized for 6 $\times$ 30 s using a BeadBug homogenizer (Benchmark Scientific, Edison, NJ), followed by centrifugation (10 000 rpm, 10 min) at 4°C. The supernatants were collected and subjected to total protein determination via the Bradford Protein Assay (Bio-Rad, Hercules, CA) using a Tecan Infinite[®]200 PRO plate reader (Männedorf, Switzerland).

S100A12 and HNE Protein Quantification by Enzyme-linked Immunosorbent Assays

The protein levels of S100A12 and HNE in the anterior ethmoid homogenates of control patients and patients with CRSsNP and CRSwNP were assayed using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Abcam). A Tecan Infinite[®]200 PRO plate reader (Männedorf) was employed to detect colorimetric changes, and S100A12 and HNE concentrations were normalized to total protein concentration for each sample. Statistical outliers were eliminated.

Disease-specific QOL Measures

Patients completed the Rhinosinusitis Disability Index (RSDI), which is a 30-item questionnaire used to assess the impact of CRS on a patient's physical, functional, and emotional status.³³ Total RSDI scores ranged from 0 to 120, with scores on each domain ranging from 0 to 44 (physical), 0 to 36 (functional), and 0 to 40 (emotional). Lower total and subdomain scores indicate a better QOL.

Objective Measures Assessment of Disease Severity

The Lund–Mackay bilateral scoring system was used to evaluate and stage CT images, with higher scores

indicating a higher severity of disease (range: 0–24).³⁴ Endoscopic examinations were scored using the Lund–Kennedy endoscopy staging system, which quantifies the bilateral severity of nasal polyps, crusting, scarring, edema, and discharge.³⁵ Total endoscopic scores range from 0 to 20, with higher scores representing worse disease severity.

Statistical Analysis

Categorical variables were summarized by count (%) and continuous variables by mean (standard deviation [SD]) or median (interquartile range) and stratified by diagnostic group (CRSsNP, CRSwNP, and control). Analysis of variance and Tukey's post hoc tests were employed to compare continuous variables across groups due to the distributions appearing approximately normal and exhibiting homogeneous variances according to the Bartlett's test ($P > .05$). Spearman's correlation was used to compare S100A12 and HNE levels with QOL, and disease severity measures in the CRSsNP, CRSwNP, and control groups, and 95% confidence intervals were reported. Significance was evaluated at the .05 level, and all tests were 2-tailed. Statistical analyses were conducted using StrataCorp Strata Statistics software (Version 14.1; College Station, TX).

Results

Cohort Characteristics

Seventy patients were prospectively enrolled and categorized into 3 study groups: CRSsNP ($n = 28$), CRSwNP ($n = 25$), and normal control patients ($n = 17$). Baseline characteristics of each group are presented in Table 1. No significant differences were found between groups for the following demographics: gender, race, ethnicity, asthma, allergy, and previous sinus surgeries, whereas a significant difference was found between patients with CRS and control patients for ASA sensitivity ($P = .011$).

Evaluation of S100A12 and HNE in Sinonasal Homogenates

The protein expression of S100A12 and HNE was, respectively, evaluated in 68 and 58 sinonasal tissue homogenates using ELISAs. A significant increase in S100A12 levels was demonstrated in CRSsNP tissue homogenates (344.27 ± 283.03 pg/mg protein; $P < .05$) when compared to normal controls (181.57 ± 126.95 pg/mg protein) and the CRSwNP group (178.33 ± 147.70 pg/mg protein; $P < .05$) (Figure 1(a)). Similarly, a significant increase in HNE levels was measured in the tissue homogenates from patients with CRSsNP (58.25 ± 53.00 ng/mg protein; $P < .01$)

Table 1. Demographic Comparisons of Normal Control Patients and Patients With CRSsNP and CRSwNP.

Demographics	CRSsNP (n = 28) n (%)	CRSwNP (n = 24) n (%)	Control (n = 17) n (%)	P
Gender				
Male	14 (50%)	15 (62.5%)	12 (70.6%)	.939
Female	14 (50%)	9 (37.5%)	5 (29.4%)	
Race (White/Caucasian)	96.4%	100%	88.2%	.247
Ethnicity (Non-Hispanic/Latino)	96.4%	100%	100%	.262
Asthma	9 (32.1%)	16 (66.7%)	4 (23.5%)	.910
Allergies	14 (50%)	20 (83.3%)	2 (11.8%)	.130
ASA sensitivity	2 (7.1%)	6 (25%)	0 (0%)	.011*
Previous surgery	15 (53.6%)	16 (66.7%)	2 (11.8%)	.187

Abbreviations: ASA, acetyl-salicylic acid; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps.

* $P < .05$.

Figure 1. Quantitative comparisons of S100A12 and HNE protein levels in the anterior ethmoid tissue homogenates collected from normal control patients and from patients having CRSsNP and CRSwNP. Enzyme-linked immunosorbent assays demonstrate significant increases in (a) S100A12 (normal vs CRSsNP: $P < .05$; CRSsNP vs CRSwNP: $P < .05$) and (b) HNE (normal vs CRSsNP: $P < .01$; CRSsNP vs CRSwNP: $P < .01$) expression in patients with CRSsNP compared to controls and patients with CRSwNP. (c), Regression analysis shows a significant negative correlation between the levels of S100A12 and HNE in CRSwNP ($r^2 = -.83$, $P < .01$). CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; HNE, human neutrophil elastase; NS, not significant.

compared to normal controls (8.98 ± 8.38 ng/mg protein) and the CRSwNP group (6.61 ± 9.35 ng/mg protein; $P < .01$) (Figure 1(b)). Regression analysis demonstrated a positive correlation between the levels of S100A12 and HNE in CRSsNP ($r^2 = .34$, not significant, data not shown) and a significant negative correlation in CRSwNP ($r^2 = -.83$, $P < .01$) (Figure 1(c)).

Examination of S100A12 and HNE in Sinonasal Tissues

We performed immunohistochemical (IHC) analysis of anterior ethmoid biopsies from normal control patients and patients with CRSsNP and CRSwNP. Consistent with the ELISA data, increased S100A12 (brown) and HNE (dark gray) staining were observed within the mucosa and Bowman's glands of patients with CRSsNP compared to those from normal controls and

patients with CRSwNP, which qualitatively showed similar S100A12 and HNE levels (Figure 2).

S100A12 and Chronic Rhinosinusitis QOL and Disease Severity

Mean differences in disease-specific QOL (measured by the RSDI) and disease severity (measured by CT and endoscopy) across the 3 study groups are shown in Table 2. Differences were found for the RSDI physical domain and RSDI total score, with post hoc tests showing CRS patients scoring higher than control patients, although not significant. Regression analysis demonstrated a significant correlation between the levels of S100A12 and disease severity, represented by CT scores ($r^2 = .51$, $P < .05$) with no significant correlations between S100A12 and CRS disease-specific QOL measures (Table 3).

Figure 2. Immunohistochemical analyses of S100A12 (brown) and HNE (dark gray) illustrate increased signal in the mucosa and Bowman's glands of anterior ethmoid tissues from patients with CRSsNP compared to normal controls and patients with CRSwNP. CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; HNE, human neutrophil elastase.

Table 2. Disease-specific Quality of Life and Severity Comparisons of Normal Control Patients and Patients With CRSsNP and CRSwNP Expressed as the Mean (M) and SD.

	CRSsNP (n = 17–28) M (SD)	CRSwNP (n = 12–23) M (SD)	Normal (n = 9–11) M (SD)	P
RSDI total	50.3 (15.7)	55.5 (24.8)	22.4 (16.9)	.225
RSDI physical	19.2 (7.5)	22.6 (8.4)	7.9 (7.1)	.855
RSDI functional	16.3 (6.9)	15.9 (8.4)	6.2 (5.8)	.521
RSDI emotional	14.8 (6.8)	16.8 (10.3)	8.3 (6.4)	.217
Endoscopy score	3.1 (2.0)	7.5 (3.0)	1.8 (1.3)	.046*
CT Score	8.9 (4.6)	15.5 (6.0)	3.1 (3.6)	.184

Abbreviations: CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; CT, computed tomography; RSDI, Rhinosinusitis Disability Index; SD, standard deviation.

*P < .05.

Table 3. Spearman Correlations Between S100A12 Protein Levels and Disease-specific Quality of Life and Severity Measures for Normal Control Patients and Patients With CRSsNP and CRSwNP.

	CRSsNP (n = 17–28) r_s (CI)	CRSwNP (n = 12–24) r_s (CI)	Normal (n = 7–15) r_s (CI)
RSDI total	−0.27 (−0.79, 0.24)	0.11 (−0.57, 0.78)	0.61 (0.04, 1.19)
RSDI physical	−0.25 (−0.70, 0.20)	−0.03 (−0.76, 0.71)	0.62 (0.07, 1.17)
RSDI functional	−0.16 (−0.65, 0.34)	0.09 (−0.60, 0.79)	0.54 (−0.09, 1.17)
RSDI emotional	−0.24 (−0.71, 0.24)	0.09 (−0.61, 0.80)	0.54 (−0.07, 1.15)
Endoscopy score	−0.06 (−0.56, 0.43)	−0.15 (−0.69, 0.40)	0.28 (−0.25, 0.82)
CT score	0.51* (0.22, 0.81)	0.17 (−0.30, 0.64)	0.27 (−0.70, 1.24)

Abbreviations: CI, confidence interval at 95%; CRSsNP, chronic rhinosinusitis without nasal polyps; CRSwNP, chronic rhinosinusitis with nasal polyps; CT, computed tomography; r_s , Spearman correlation coefficient; RSDI, Rhinosinusitis Disability Index;

*P < .05.

Discussion

The sinonasal mucosa of patients with CRSsNP is predominantly characterized by neutrophil infiltration and Th1-associated cytokines, whereas CRSwNP pathology is marked by eosinophil infiltration and a Th2-biased

cytokine profile.^{36,37} Differences in inflammatory mediators between CRSsNP and CRSwNP suggest that these conditions may have differing underlying etiologies. The current literature suggests that S100 proteins have pleiotropic activity and balance the Th1/Th2 response at the transcription level.³⁸ Calprotectin (S100A8) levels have

been correlated with increased expression of interferon gamma,³⁹ whereas S100A9 enhances IL-1 β secretion, which is abrogated by priming with Th2 cytokines.⁴⁰ Contrastingly, S100A8/A9 is upregulated in the lung and skin of respective patients with asthma⁴¹ and chronic atopic dermatitis, both of these conditions are associated with Th2-type cytokines including IL-5, IL-13, and IL-4.³⁸

Our biochemical and IHC studies demonstrate that S100A12 and HNE are significantly increased in patients with CRSsNP compared to controls and that patients with CRSwNP had similar S100A12 levels to those of controls. A possible mechanism that supports a role for S100A12 in CRSsNP is its activation of RAGE by ligation.⁴² RAGE-S100A12 interactions (EN-RAGE) produce a host of pro-inflammatory responses, such as those coordinated by mitogen-activated protein kinases, nuclear factor kappa-light-chain-enhancer of activated B cells, and reactive oxygen species, and may thus be important for the inflammation seen in patients with CRS.^{7,43,44} RAGE also stimulates tumor necrosis factor- α (TNF α) and IL-1 β production in monocytes and endothelial cells²¹ but does not activate cytokines that favor a Th2-type response.³¹ TNF α upregulates S100A12 in neutrophils and monocytes/macrophages, representing an important feedback loop in chronic inflammation. These data suggest that S100 proteins may have a propensity toward a Th1-type response, which would explain the elevated levels of both S100A12 and HNE observed in patients with CRSsNP compared to patients with CRSwNP.

Previous studies by Tieu et al. and Van Crombruggen et al. have reported that S100A8/A9 is significantly elevated in the nasal polyp tissues of patients with CRSwNP compared to controls and patients with CRSsNP. The observed differential expression of S100A8/A9 in CRSwNP was suggested to result from enhanced binding affinity to negatively charged proteoglycans and other extracellular matrix molecules associated with increased tissue remodeling, commonly seen in polyp tissue.²³ These authors also postulate that the upregulation of S100A8/A9 in polyp tissue may be in response to combating *Staphylococcus aureus* infection, a large contributor to the pathophysiology of CRSwNP. In this study, we compare the levels of S100A12 in anterior ethmoid tissues of patients with CRSsNP and CRSwNP, wherein the pathophysiology is different from that in polyp tissue. Further studies are warranted to understand how local tissue environment affects the differential release and accumulation of S100A12 as well as to define the association of S100A12 to specific Th1- or Th2-type phenotypes in CRS.

There has been a growing interest in the innate immune system in the context of CRS, as it is uniquely associated with the air-mucosal interface in the sinuses. A delicate balance of this system is needed, as

uncontrolled signaling can result in chronic inflammation.³⁷ Innate immune system activation triggers the release of S100A12 from monocytes and granulocytes,⁴⁵ and in the case of autoinflammatory diseases, familial Mediterranean fever, and systemic-onset juvenile idiopathic arthritis, elevated serum and tissue levels of S100A12 correlate to disease activity.⁴⁶ In this study, we found a significant correlation between S100A12 levels in patients with CRSsNP and disease severity, supporting the potential role of S100A12 in the pathology and immune signaling of patients with CRSsNP. Further investigations are warranted to understand the role of S100A12 in sinonasal inflammation and how it mechanistically relates to disease severity, such as evaluating the expression and colocalization (EN-RAGE) of S100A12's known innate immune receptors, TLR-4 and RAGE. To explore the role of S100A12 signaling at the cellular level, functional response assays measuring the secretion of Th1-associated cytokines, IL-6, IL-8, IL-1 β , and TNF α , from the anterior ethmoid tissues or air-liquid interface cultures of control patients and patients with CRSsNP and CRSwNP upon S100A12 stimulation will be undertaken.

Limitations of this study include the sample size and the ability to obtain true normal control tissues. Even control surgical patients without a history of CRS are most likely to have a decreased QOL at the time of surgery, potentially attributing to the high RSDI scores and SD observed in this study.

Conclusion

This is the first translational study to examine the expression of S100A12 and its associations between HNE levels, QOL measures, and disease severity indices in patients with CRS. Significant increases in S100A12 and HNE expression were demonstrated in the sinonasal tissues from patients with CRSsNP compared to normal controls and patients with CRSwNP. Interestingly, there was a significant negative correlation between S100A12 and HNE levels in patients with CRSwNP, possibly due to the dominance of Th2-mediated pathology generally observed in these patients. Studies to quantify the levels of S100A12 in nasal polyp tissue and other sinus regions that may be directed by different pathways are underway, as previous studies have reported that the release and accumulation of S100 proteins S100A7, S100A8, and S100A9 are dependent on the local tissue environment.²²

Author(s) Note

The first two authors contributed equally to this work.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr Alt is a consultant for Medtronic ENT (Jacksonville, FL), Spirox (Menlo Park, CA), and GlycoMira Therapeutics, Inc. (Salt Lake City, UT), all of which are not affiliated with this research. Dr Pulsipher declares financial interests with GlycoMira Therapeutics, Inc. (Salt Lake City, UT).

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References

1. Hariri BM, Cohen NA. New insights into upper airway innate immunity. *Am J Rhinol Allergy*. 2016;30:319–323.
2. Schleimer RP. Immunopathogenesis of chronic rhinosinusitis and nasal polyposis. *Annu Rev Pathol*. 2017;12:331–357.
3. Lane AP, Truong-Tran QA, Schleimer RP. Altered expression of genes associated with innate immunity and inflammation in recalcitrant rhinosinusitis with polyps. *Am J Rhinol*. 2006;20:138–144.
4. Detwiller KY, Smith TL, Alt JA, Trune DR, Mace JC, Sautter NB. Differential expression of innate immunity genes in chronic rhinosinusitis. *Am J Rhinol Allergy*. 2014;28:374–377.
5. Van Crombruggen K, Jacob F, Zhang N, Bachert C. Damage-associated molecular patterns and their receptors in upper airway pathologies. *Cell Mol Life Sci*. 2013;70:4307–4321.
6. Vogl T, Tenbrock K, Ludwig S, et al. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. *Nat Med*. 2007;13:1042–1049.
7. Van Crombruggen K, Holtappels G, De Ruyck N, Derycke L, Tomassen P, Bachert C. RAGE processing in chronic airway conditions: involvement of *Staphylococcus aureus* and ECP. *J Allergy Clin Immunol*. 2012;129:1515–1521.e1518.
8. Bryborn M, Adner M, Cardell LO. Psoriasin, one of several new proteins identified in nasal lavage fluid from allergic and non-allergic individuals using 2-dimensional gel electrophoresis and mass spectrometry. *Respir Res*. 2005;6:118.
9. Hirschberg A, Kiss M, Kadocs E, et al. Different activations of toll-like receptors and antimicrobial peptides in chronic rhinosinusitis with or without nasal polyposis. *Eur Arch Otorhinolaryngol*. 2016;273:1779–1788.
10. Shin SH, Ye MK, Kim YH, Kim JK. Role of TLRs in the production of chemical mediators in nasal polyp fibroblasts by fungi. *Auris Nasus Larynx*. 2016;43:166–170.
11. Wang X, Ji W, Xu Y, Guo H, Zhao C. [Expression and significance of Toll like receptor 2 and Toll like receptor 4 in chronic rhinosinusitis]. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*. 2014;28:1285–1288.
12. Foell D, Frosch M, Sorg C, Roth J. Phagocyte-specific calcium-binding S100 proteins as clinical laboratory markers of inflammation. *Clin Chim Acta*. 2004;344:37–51.
13. Kessel C, Holzinger D, Foell D. Phagocyte-derived S100 proteins in autoinflammation: putative role in pathogenesis and usefulness as biomarkers. *Clin Immunol*. 2013;147:229–241.
14. Rammes A, Roth J, Goebeler M, Klempt M, Hartmann M, Sorg C. Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. *J Biol Chem*. 1997;272:9496–9502.
15. Urban CF, Ermert D, Schmid M, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathog*. 2009;5:e1000639.
16. Buhimschi CS, Baumbusch MA, Dulay AT, et al. Characterization of RAGE, HMGB1, and S100beta in inflammation-induced preterm birth and fetal tissue injury. *Am J Pathol*. 2009;175:958–975.
17. Jung ES, Chung W, Kim AJ, et al. Associations between soluble receptor for advanced glycation end products (sRAGE) and S100A12 (EN-RAGE) with mortality in long-term hemodialysis patients. *J Korean Med Sci*. 2017;32:54–59.
18. Cheng Z, Dai LL, Cao DF, et al. [Changes of HMGB1 and RAGE in induced sputum from patients with bronchial asthma]. *Zhonghua Yi Xue Za Zhi*. 2011;91:1538–1542.
19. Rauvala H, Rouhiainen A. RAGE as a receptor of HMGB1 (Amphoterin): roles in health and disease. *Curr Mol Med*. 2007;7:725–734.
20. Rouhiainen A, Kuja-Panula J, Tumova S, Rauvala H. RAGE-mediated cell signaling. *Methods Mol Biol*. 2013;963:239–263.
21. Hofmann MA, Drury S, Fu C, et al. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell*. 1999;97:889–901.
22. Tieu DD, Peters AT, Carter RG, et al. Evidence for diminished levels of epithelial psoriasin and calprotectin in chronic rhinosinusitis. *J Allergy Clin Immunol*. 2010;125:667–675.
23. Van Crombruggen K, Vogl T, Perez-Novo C, Holtappels G, Bachert C. Differential release and deposition of S100A8/A9 proteins in inflamed upper airway tissue. *Eur Respir J*. 2016;47:264–274.
24. Cho SH, Hong SJ, Han B, et al. Age-related differences in the pathogenesis of chronic rhinosinusitis. *J Allergy Clin Immunol*. 2012;129:858–860.e852.
25. Oesterle A, Bowman MA. S100A12 and the S100/calgranulins: emerging biomarkers for atherosclerosis and possibly therapeutic targets. *Arterioscler Thromb Vasc Biol*. 2015;35:2496–2507.

26. Yamaoka M, Maeda N, Nakamura S, et al. Gene expression levels of S100 protein family in blood cells are associated with insulin resistance and inflammation (peripheral blood S100 mRNAs and metabolic syndrome). *Biochem Biophys Res Commun*. 2013;433:450–455.
27. Leach ST, Yang Z, Messina I, et al. Serum and mucosal S100 proteins, calprotectin (S100A8/S100A9) and S100A12, are elevated at diagnosis in children with inflammatory bowel disease. *Scand J Gastroenterol*. 2007;42:1321–1331.
28. Sidler MA, Leach ST, Day AS. Fecal S100A12 and fecal calprotectin as noninvasive markers for inflammatory bowel disease in children. *Inflamm Bowel Dis*. 2008;14:359–366.
29. Yan WX, Armishaw C, Goyette J, et al. Mast cell and monocyte recruitment by S100A12 and its hinge domain. *J Biol Chem*. 2008;283:13035–13043.
30. Yang Z, Tao T, Raftery MJ, Youssef P, Di Girolamo N, Geczy CL. Proinflammatory properties of the human S100 protein S100A12. *J Leukoc Biol*. 2001;69:986–994.
31. Yang Z, Yan WX, Cai H, et al. S100A12 provokes mast cell activation: a potential amplification pathway in asthma and innate immunity. *J Allergy Clin Immunol*. 2007;119:106–114.
32. Rosenfeld RM, Andes D, Bhattacharyya N, et al. Clinical practice guideline: adult sinusitis. *Otolaryngol Head Neck Surg*. 2007;137:S1–S31.
33. Benninger MS, Senior BA. The development of the Rhinosinusitis Disability Index. *Arch Otolaryngol Head Neck Surg*. 1997;123:1175–1179.
34. Lund VJ, Mackay IS. Staging in rhinosinusitis. *Rhinology*. 1993;31:183–184.
35. Lund VJ, Kennedy DW. Staging for rhinosinusitis. *Otolaryngol Head Neck Surg*. 1997;117:S35–S40.
36. Van Zele T, Claeys S, Gevaert P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy*. 2006;61:1280–1289.
37. Tomassen P, Vandeplas G, Van Zele T, et al. Inflammatory endotypes of chronic rhinosinusitis based on cluster analysis of biomarkers. *J Allergy Clin Immunol*. 2016;137:1449–1456.e1444.
38. Gittler JK, Shemer A, Suarez-Farinas M, et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol*. 2012;130:1344–1354.
39. Wang K, Xuan X, Wang L, et al. [Expression and correlation analysis between inflammatory cytokines and calprotectin in the rat model of ulcerative colitis]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*. 2014;30:278–280, 283.
40. Koy M, Hambruch N, Hussen J, Pfarrer C, Seyfert HM, Schuberth HJ. Recombinant bovine S100A8 and A9 enhance IL-1beta secretion of interferon-gamma primed monocytes. *Vet Immunol Immunopathol*. 2013;155:162–170.
41. Pham DL, Yoon MG, Ban GY, et al. Serum S100A8 and S100A9 enhance innate immune responses in the pathogenesis of Baker's asthma. *Int Arch Allergy Immunol*. 2015;168:138–146.
42. Ramasamy R, Yan SF, Schmidt AM. RAGE: therapeutic target and biomarker of the inflammatory response—the evidence mounts. *J Leukoc Biol*. 2009;86:505–512.
43. Bellussi LM, Chen L, Chen D, Passali FM, Passali D. The role of High Mobility Group Box 1 chromosomal protein in the pathogenesis of chronic sinusitis and nasal polyposis. *Acta Otorhinolaryngolog Ital*. 2012;32:386–392.
44. Tardif MR, Chapeton-Montes JA, Posvandzic A, Page N, Gilbert C, Tessier PA. Secretion of S100A8, S100A9, and S100A12 by neutrophils involves reactive oxygen species and potassium efflux. *J Immunol Res*. 2015;2015:296149.
45. Wen W, Liu W, Zhang L, et al. Increased neutrophilia in nasal polyps reduces the response to oral corticosteroid therapy. *J Allergy Clin Immunol*. 2012;129:1522–1528.e1525.
46. Gohar F, Orak B, Kallinich T, et al. Correlation of secretory activity of neutrophils with genotype in patients with familial Mediterranean fever. *Arthritis Rheumatol*. 2016;68:3010–3022.