

Diverse phenotypes and endotypes of fungus balls caused by mixed bacterial colonization in chronic rhinosinusitis

Dong-Kyu Kim, MD, PhD^{1,*}, Young Chan Wi, MD^{2,*}, Su-Jin Shin, MD, PhD³, Kyung Rae Kim, MD, PhD⁴, Dae Woo Kim, MD, PhD⁵ and Seok Hyun Cho, MD, PhD⁴

Background: The pathogenic roles of fungus and bacteria in chronic rhinosinusitis (CRS) remain unclear. Recently, we described the bacterial ball, which is distinct from the fungus ball, as an unusual phenotype of bacterial infection. In this study, we investigated the clinical, histopathologic, and immunologic characteristics of sinonasal microorganic materials, including fungus ball and bacterial ball.

Methods: In this study, we enrolled 80 CRS patients with sinonasal microorganic materials and 10 control subjects who underwent skull base surgery or endoscopic dacryocystorhinostomy and had no signs or symptoms of nasal inflammation. All specimens were stained with hematoxylin-eosin, Gomori-methenamine-silver, and Gram stain to identify fungal organisms and Gram-positive/negative bacterial colonies. The expression of tumor necrosis factor (TNF)- α ; interleukin (IL)-1 β ; S100A7; S100A8/A9; and short, palate, lung, and nasal epithelial clone 1 (SPLUNC1) were evaluated by enzyme-linked immunosorbent assay using sinus lavage fluid.

Results: We histologically classified sinonasal microorganic materials into the following 4 groups: fungus ball (n = 45); bacterial ball (n = 6); mixed ball (formed by a mixture of fungus and bacteria, n = 27); and double ball (formed by separate fungal and bacterial balls, n = 2). Com-

pared with the fungus ball, the mixed ball was more frequently detected in immunocompromised patients ($p < 0.0001$). In addition, TNF- α expression was significantly higher in fungus and mixed balls than in control, whereas the mixed ball showed higher expression of IL-1 β compared with the fungus ball. Moreover, the expression of S100A7 and S100A8/A9 protein in the mixed ball was significantly decreased when compared with the fungus ball, whereas there was no significant difference in SPLUNC1 expression between fungus and mixed balls.

Conclusion: Our findings suggest that fungal and bacterial interactions are diverse in CRS. Specifically, the mixed ball is prevalent in CRS with an immunocompromised state and it may decrease epithelial barrier function. © 2019 ARS-AAOA, LLC.

Key Words:

rhinosinusitis; fungus; bacteria; immune response; barrier

How to Cite this Article:

Kim D-K, Wi YC, Shin S-J, Kim KR, Kim DW, Cho SH. Diverse phenotypes and endotypes of fungus balls caused by mixed bacterial colonization in chronic rhinosinusitis. *Int Forum Allergy Rhinol* 2019;00:1-7.

¹Department of Otorhinolaryngology–Head and Neck Surgery, Chuncheon Sacred Heart Hospital, Hallym University College of Medicine, Chuncheon, Republic of Korea; ²Department of Pathology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea; ³Department of Pathology, Hanyang University College of Medicine, Seoul, Republic of Korea; ⁴Department of Otorhinolaryngology–Head and Neck Surgery, Hanyang University College of Medicine, Seoul, Republic of Korea; ⁵Department of Otorhinolaryngology–Head and Neck Surgery, Boramae Medical Center, Seoul National University College of Medicine, Seoul, Republic of Korea

Correspondence to: Seok Hyun Cho, MD, PhD, Department of Otorhinolaryngology–Head and Neck Surgery, Hanyang University College of Medicine, 222-1 Wangsimni-ro, Seongdong-gu, Seoul 04763, Republic of Korea; e-mail: shcho@hanyang.ac.kr
Dae Woo Kim, MD, PhD, Department of Otorhinolaryngology–Head and Neck Surgery, Boramae Medical Center, Seoul National University College

Chronic rhinosinusitis (CRS) is characterized by inflammation of the mucosa of the nose and paranasal sinuses with a duration of at least 12 consecutive weeks, and is comprised of 2 subtypes: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).¹ To date, several heterogeneous factors, including host

of Medicine, 425 Shindaebang 2-dong, Dongjak-gu, Seoul 07061, Republic of Korea; e-mail: kicubi@daum.net

*The first 2 authors (D.K.-K. and Y.C.W.) contributed equally to this work. Funding sources for the study: Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT, and Future Planning (NRF-2018R1D1A3B07040862 to D.-K.K., NRF-2017R1A2B4003445 to D.W.K.). Potential conflicts of interest: None provided.

Received: 16 April 2019; Revised: 29 July 2019; Accepted: 29 July 2019
DOI: 10.1002/alr.22410
View this article online at wileyonlinelibrary.com.

factors (eg, allergy, immunodeficiency, ciliary immotility, and anatomic obstruction) and environmental factors (viral, bacterial, fungal, and pollutants), play a critical role in the pathogenesis of CRS.²⁻⁵ However, the condition is still not fully understood. In addition, there are complex host-environmental interactions, but failure of the fine-tuning or balancing mechanism may elicit a diverse spectrum of CRS.

Among the several etiologies of CRS, fungus and bacteria are well known to play major roles in its pathogenesis. Fungal infection is generally categorized as either invasive or noninvasive, according to the presence or absence of fungal hyphae within the mucosa, submucosa, bone, or blood vessels of the paranasal sinuses.⁶⁻⁹ Meanwhile, bacterial infection can cause biofilm formation, secretion of superantigens, and dysbiosis.¹⁰⁻¹⁴ The fungus ball is the most common subtype of fungal rhinosinusitis and has been regarded as a simple entity. However, its interaction with bacterial infection has never been investigated.⁹

Recently, we reported a new phenotype of the bacterial ball that has clinical and histologic features that differ from those seen with the fungus ball.¹⁵ Interestingly, it is very difficult to discriminate between a bacterial ball and fungus ball during endoscopic sinus surgery, because they share a very similar gross appearance. However, on histologic analysis, the bacterial ball shows negative Gomori-methenamine-silver (GMS) staining with positive Gram staining, and also demonstrates varying amounts of bacterial colonies in the acellular mucous matrix. Thus, further study is required, and other possible phenotypes and/or endotypes should be sought.

In this study, we investigated the clinical, histopathologic, and immunologic properties of sinonasal microorganic materials in suspected fungus ball to better understand the complex interactions of fungus and bacteria in CRS. Specifically, for evaluation of immunologic effect, we assessed the expression of tumor necrosis factor- α (TNF- α) and interleukin 1 β (IL-1 β), because TNF- α is one of the major cytokines involved in the inflammatory response and IL-1 β is crucial for host defense responses to infection/injury.¹⁶⁻¹⁹ Moreover, we evaluated the expression of S100A7, S100A8/A9, and SPLUNC1 proteins, which were secreted by respiratory epithelium as natural antibacterial or antifungal factors to determine their effects on epithelial barrier function.^{20,21} Our aims were to investigate suspected fungus balls (sinonasal microorganic materials, bioballs) and define the phenotypes of sinonasal bioballs, and also to evaluate their effect on mucosal inflammation and the epithelial barrier from an immunologic perspective.

Patients and methods

Study population and clinical assessment

From January 2016 to October 2018, we retrospectively reviewed clinical data and pathologic reports of adult patients (≥ 18 years of age) with CRS who had undergone

functional endoscopic sinus surgery. CRS was diagnosed based on the 2012 guidelines proposed in the European Position Paper on Rhinosinusitis and Nasal Polyps.¹ Patients were excluded if they were < 18 years of age or had antrochoanal polyp, inverted papilloma, hamartomatous lesions, or malignancy. Our study included a total of 80 cases of biomaterials (suspected fungal ball) among the 476 patients with CRSwNP and CRSsNP, and 10 cases of control subjects who underwent skull base surgery or endoscopic dacryocystorhinostomy with no signs or symptoms of nasal inflammation. Serum total immunoglobulin E (IgE) and percent blood eosinophils were measured, and atopic status was evaluated by skin-prick testing using a commercially available kit (Allergopharma, Reinbek, Germany) with 13 inhaled allergens, a negative control (0.9% saline), and a positive control (1% histamine). Skin test results were interpreted after 15 minutes by measuring the mean size of the flares and wheals for each allergen. A positive reaction on the skin-prick test was defined as an allergen-histamine wheal size ratio of ≥ 1 . Asthma was diagnosed by an allergist based on spirometry (change in forced expiratory volume in 1 second of $\geq 12\%$ after administration of a short-acting β -agonist) or methacholine challenge test. Disease severity was categorized based on the Lund-Mackay staging system using computed tomography (CT) images, and olfactory function was expressed by the TDI score (comprehensive score of threshold, discrimination, and identification abilities). The institutional review board of Hanyang University Medical Center (IRB No. 2016-12-008-004) approved this study and informed consent was obtained from the patients.

Histopathologic assessment

We stained all formalin-fixed, paraffin-embedded sections with hematoxylin and eosin (H&E) for pathologic examination and GMS/Gram stain to identify fungal organisms and Gram-positive/negative bacterial colonies. If fungal organisms and bacterial colonies were identified simultaneously, we also performed double-staining for GMS and Gram stain to verify the locations of the fungal organisms and bacterial colonies. All pathologic materials were reviewed by 2 pathologists (Y.C.W. and S.J.S.). According to the histopathologic findings, we classified sinonasal microorganic materials into the following 4 groups: fungus ball; bacterial ball; mixed ball; and double ball. Fungal balls consisted of fungal organisms without matrix, whereas bacterial balls consisted of Gram-positive bacterial colonies within the thick acellular mucous matrix. Mixed balls were defined when bacterial colonies were found in fungal ball without matrix, whereas double balls were defined when fungal and bacterial balls existed separately.

Immunologic assessment

In this study, we measured cytokine expression in the paranasal sinus lavage fluid obtained from the control ($n = 10$), fungus ball ($n = 20$), and mixed ball ($n = 12$)

TABLE 1. Demographics of chronic rhinosinusitis with sinonasal bioballs

	Control (n = 10)	Sinonasal bioballs (n = 80)
Gender male (%)	2 (20%)	25 (31.25%)
Mean age, years (range)	57 (50-61)	62 (24-88)
Smoking, n (%)	1 (10)	6 (7.5)
Atopic status, n (%)	2 (20)	22 (27.5)
Asthma, n (%)	0 (0)	1 (1.25)
Lund-Mackay CT score (mean $\pm$ SD)	5 $\pm$ 2	5.4 $\pm$ 2.9
Olfaction, TDI score (mean $\pm$ SD)	30 $\pm$ 11	29.8 $\pm$ 10.7

CT = computed tomography; TDI = threshold, discrimination, and identification.

samples for investigating altered immune responses. To obtain lavage fluids, we flushed with 5 mL phosphate-buffered saline into the maxillary sinus and the fluid was aspirated with the suction collector connected to a sterile container. The paranasal sinus lavage fluids were centrifuged in 15-mL conical tubes for 10 minutes at 1107 g, and then stored at -80°C . Until the sample was concentrated 2-fold, the resulting supernatants were repeatedly centrifuged at 1107 g for 10 minutes. The concentrated supernatants were aliquoted into tubes and stored at -20°C until use. We evaluated the samples to detect TNF- α ; IL-1 β ; S100A7; S100A8/9; and short, palate, lung, and nasal epithelial clone 1 (SPLUNC1) protein levels using commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) in accordance with the manufacturer's instructions. Each sample was measured in triplicate.

Statistical analysis

Because this was pilot study, we did not calculate the sample size. Data were analyzed by Mann-Whitney *U* test, Pearson chi-square test, and Fisher's exact test for comparison analysis. Statistical significance ($p < 0.05$) was analyzed using the same 3 testing procedures. Statistical analyses were performed using SPSS Statistics version 21.0 (IBM Corp, Armonk, NY) and GraphPad Prism version 6.0 (GraphPad, Inc, La Jolla, CA).

Results

Clinical characteristics of study population

We investigated a total of 80 patients with suspected fungus ball. Mean subject age was 62 (range, 24-88) years, and 55 participants (67.0%) were female, as shown in Table 1. They showed a mild degree of sinus inflammation (mean Lund-Mackay score = 5.4) and low comorbidity rate of atopy ($n = 22$, 27.5%) and asthma ($n = 1$, 1.25%). Olfactory function was slightly decreased in most cases (mean

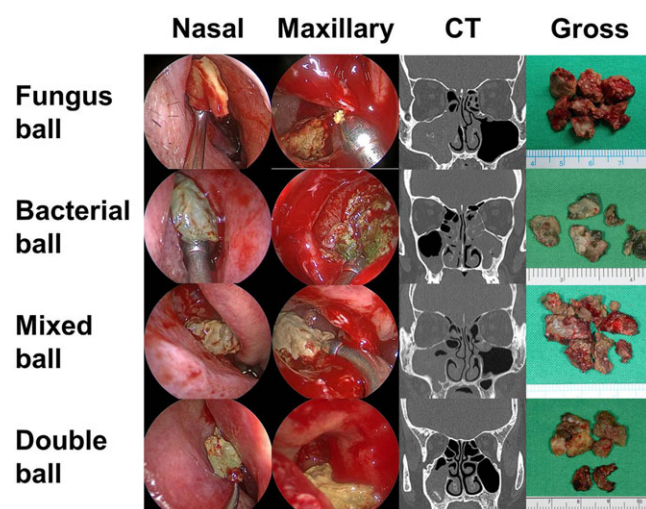


FIGURE 1. Clinical and radiologic findings of fungus ball, bacterial ball, mixed ball, and double ball during endoscopic sinus surgery. Various phenotypes of sinonasal microorganic materials (bioballs) exist, which are similar and thus difficult to distinguish. CT = computed tomography.

TDI score = 29.8). In addition, there was no significant difference in age, sex, atopic status, and asthma between control subjects and patients.

Different phenotypes of fungus and bacterial infection in CRS

All sinonasal microorganic materials, including fungus ball, bacterial ball, mixed ball, and double ball, were extramucosally located in the paranasal sinus. The clinical and radiologic findings were very similar (Fig. 1), but were easily differentiated by histopathologic assessment (Fig. 2). The incidence of each type was as follows: fungal ball ($n = 45$, 56.2%); mixed ball ($n = 27$, 33.8%); bacterial ball ($n = 6$, 7.5%); and double ball ($n = 2$, 2.5%). Thus, fungus and mixed balls were considered as major forms of sinonasal microorganic materials.

Clinically, fungus and mixed balls shared many similarities as well as some differences (Table 2). In most cases, fungus and mixed balls occurred in the maxillary sinus and were predominantly found in subjects with CRSsNP. In addition, subjects frequently showed calcification findings in CT images and histologic analyses. Interestingly, when we examined those with diabetes mellitus, autoimmune diseases such as rheumatic arthritis, and systemic malignancies as immunocompromised subjects, the mixed ball (40.7%) showed a significantly higher prevalence compared with the fungus ball (22.2%, $p < 0.001$). However, there were no significant differences in total IgE and percentage of eosinophils between fungus and mixed balls. In this study, we could not compare data of 2 minor bioballs (bacterial and double balls) due to the small sample size.

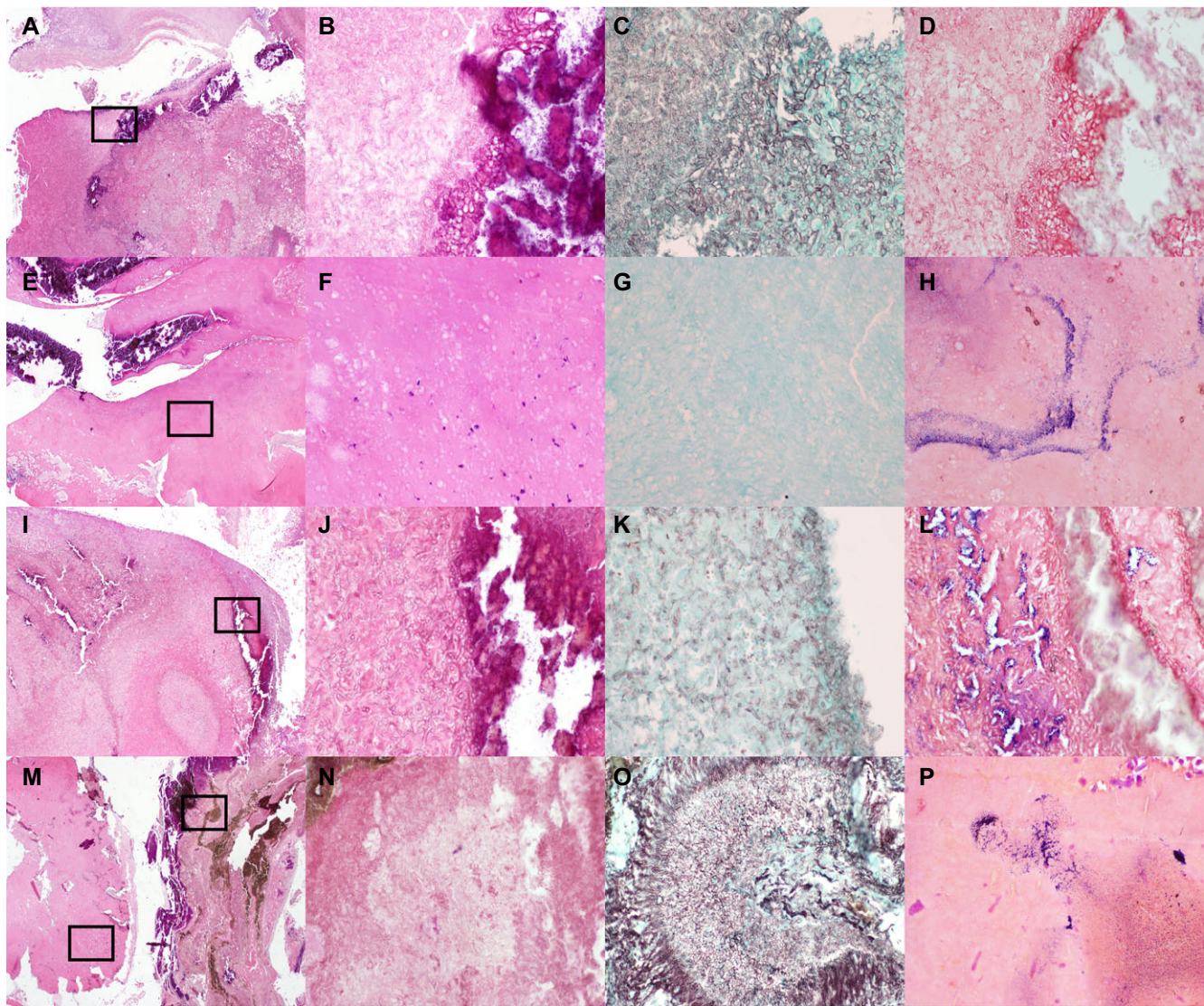


FIGURE 2. Pathologic findings of fungal balls, bacterial balls, mixed balls, and double balls in chronic rhinosinusitis. Fungal balls consisted of fungal organisms (morphologically *Aspergillus* species) without matrix [(A) H&E stain, $\times 40$; (B) box area of (A), H&E stain $\times 400$; (C) GMS stain, $\times 400$; (D) Gram stain, $\times 400$]. Bacterial balls consisted of the matrix with thick acellular mucous material [(E) H&E stain, $\times 40$; (F) box area of (E), H&E stain, $\times 400$] in which Gram-positive bacteria colonies were observed, but no fungal organisms were identified [(G) GMS stain, $\times 400$; (H) Gram stain, $\times 400$]. Mixed balls consisted of fungal organisms [(I) H&E stain, $\times 40$; (J) box area of (I), H&E stain, $\times 400$; (K) GMS stain, $\times 400$] in which some Gram-positive bacteria colonies [(L) Gram stain, $\times 400$] were detected without matrix. Double balls consisted of fungal ball (right) and bacterial ball (left) separately [(M) H&E stain, $\times 40$]. Fungal organisms were found in fungal balls [right box area of (M); (N) H&E stain, $\times 400$; (O) GMS stain, $\times 400$], and Gram-positive bacteria colonies were found in the acellular mucous matrix [(P) left box area of (M), Gram stain, $\times 400$]. GMS = Gomori-methenamine-silver; H&E = hematoxylin-eosin.

Different endotypes of fungus and bacterial infection in CRS

First, we measured TNF- α and IL-1 β protein expression in the paranasal sinus lavage fluid (Fig. 3). Expression of TNF- α proteins in fungus and mixed ball was significantly higher compared with controls ($p = 0.011$ and $p < 0.001$, respectively). IL-1 β levels in patients with mixed ball were elevated compared with control subjects and patients with fungus ball ($p < 0.001$ and $p = 0.009$, respectively). These findings indicate an increased proinflammatory response in the sinus mucosa caused by bioballs. Next, we analyzed S100A7, S100A8/A9, and SPLUNC1 protein expression to determine their effects on epithelial barrier function

(Fig. 4). S100A7 expression was significantly decreased in patients with mixed ball compared with control subjects and those with fungus ball ($p < 0.001$ and $p = 0.011$, respectively). In addition, S100A8/A9 expression was significantly decreased in patients with mixed ball compared with fungus ball ($p = 0.0235$). However, there was no significant difference in SPLUNC1 protein expression among the 3 groups.

Discussion

In this study we have investigated the clinical, histopathologic, and immunologic effects of sinonasal bioballs in CRS

TABLE 2. Characterization of different types of sinonasal bioballs in chronic rhinosinusitis

	Fungus ball (n = 45)	Bacterial ball (n = 6)	Mixed ball (n = 27)	Double ball (n = 2)
Phenotype of CRS	CRSsNP, 40; CRSwNP, 5	CRSsNP, 2; CRSwNP, 4	CRSsNP, 22; CRSwNP, 5	CRSsNP, 2; CRSwNP, 0
Sinus location	M, 42; S, 3	M, 3; E, 2; S, 1	M, 25; E, 1; S, 1	M, 2
Calcification on CT	23 (51.1%)	2 (33.3%)	16 (59.3%)	1 (50%)
Calcification on histology	30 (66.7%)	2 (33.3%)	21 (77.8%)	2 (100%)
Blood eosinophils	2.59%	2.0%	2.0%	1.2%
Serum total IgE (kU/L)	118.9	79.8	84.1	68.4
Immunocompromised patients	10 (22.2%)	1 (16.7%)	11 (40.7%)	1 (50%)

CRS = chronic rhinosinusitis; CRSsNP = chronic rhinosinusitis without nasal polyp; CRSwNP = chronic rhinosinusitis with nasal polyp; CT = computed tomography; E = ethmoid sinus; IgE = immunoglobulin E; M = maxillary sinus; S = sphenoid sinus.

patients to identify their complex interactions. To our best knowledge, this is the first study to describe the diverse phenotypes and endotypes of fungus balls caused by mixed bacterial colonization in CRS. Previously, we found bacterial ball to be an unusual finding characterized by different clinical spectra, including a lower rate of calcification on CT scans compared with fungus ball. However, the exact pathophysiologic mechanism of bacterial ball and its clinical relevance could not be determined. Thus, we aimed to demonstrate the characteristics of sinonasal bioballs in detail. Our findings show that bacterial infection was related to bacterial ball, mixed ball, and double ball, whereas fungal infection was associated with fungus ball, mixed ball, and double ball. For fungus ball, calcification is typically observed by CT imaging and is more frequently detected in immunocompetent hosts.²² Accordingly, we observed calcification in CT images not only with fungus ball, but also with other related fungal infections, such as mixed and double balls. However, the prevalence of immunocompromised

patients was significantly higher in mixed ball; these patients were characterized as having combined fungal- and bacterial-infection-related forms rather than fungus ball alone. This suggests that immunocompromised states may increase the susceptibility of patients to bacterial infections.

In addition, to identify the immunologic effect on the sinus mucosa, we evaluated TNF- α and IL-1 β expression using paranasal sinus lavage fluids. Several studies have reported enhanced expression of TNF- α and IL-1 β .^{23–26} Consistent with those studies, we observed increased expression of TNF- α in fungus and mixed balls. Interestingly, mixed ball patients also showed higher expression of IL-1 β protein than those with fungus ball. Similar to our work, one previous study showed higher IL-1 β expression in neutrophilic CRSwNP than in eosinophilic CRSwNP.²³ Therefore, we thought concomitant bacterial infection may induce upregulation of IL-1 β inflammation, which may be involved in increasing neutrophilic inflammatory processes in mixed ball. Moreover, the respiratory epithelium provides barrier function to pathogens through the use of mucociliary function, tight junctions, and natural antimicrobial factor production. Among the natural antimicrobial factors, S100A7 protein, which is present at the skin epidermis, is an attractant of CD4⁺ lymphocytes and neutrophils.²⁷ In addition to its chemotactic properties, S100A7 protein can kill bacteria directly.²⁸ S100A7 exerts its antimicrobial activity by disrupting microbial membranes at low pH.²⁹ Moreover, S100A8/A9 protein is produced by neutrophils, monocytes, and epithelial cells and acts as a potent proinflammatory chemoattractant for neutrophils and monocytes.^{30,31} Similar to S100A7 protein, S100A8/A9 shows antibacterial and antifungal properties.^{32,33} Furthermore, SPLUNC1 protein is expressed in the epithelium and submucosa of the nasopharynx and is secreted into the nasal cavity. It can bind to the lipid A portion of lipopolysaccharide and inhibit the growth of *Pseudomonas aeruginosa*, *Mycoplasma pneumonia*, and presumably other Gram-negative organisms.^{34–36} Thus, we examined the expression of S100A7, S100A8/A9, and SPLUNC1 proteins. In this analysis, we detected a clear reduction in S100A7 and S100A8/A9 protein levels

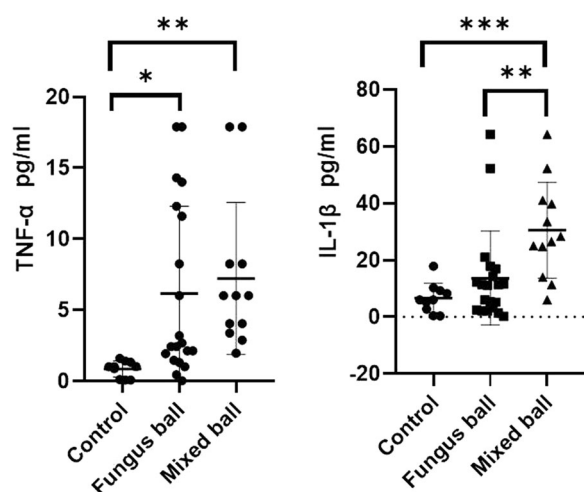


FIGURE 3. Evaluation of expression of TNF- α and IL-1 β proteins in sinonasal lavage fluids using ELISA (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$). ELISA = enzyme-linked immunoassay; TNF- α = tumor necrosis factor- α ; IL-1 β = interleukin-1 β .

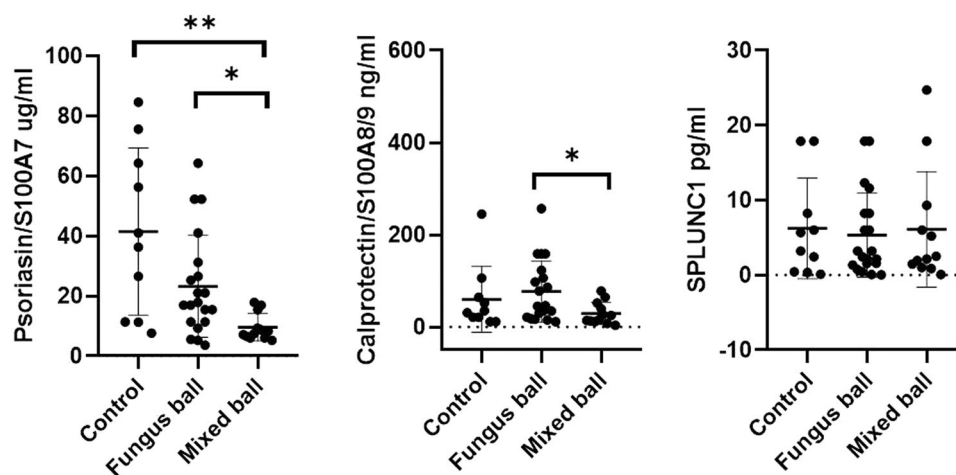


FIGURE 4. Evaluation of expression of human S100A7, S100A8/9, and SPLUNC1 proteins in sinonasal lavage fluids by ELISA (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$). ELISA = enzyme-linked immunoassay; SPLUNC1 = short, palate, lung, and nasal epithelial clone 1.

in mixed ball compared with fungus ball. Consistent with our findings, several previous studies demonstrated reduced expression of S100A7 and S100A8/A9 in CRS.^{37,38} However, SPLUNC1 protein expression did not differ among the 3 groups (control, fungus ball, mixed ball). Similar to our findings, one study also revealed that there was no significant difference in SPLUNC1 protein expression in the uncinate tissues of control subjects and patients with CRS.³⁹

Generally, fungal sinusitis is categorized as either invasive or noninvasive, based on presence or absence of fungal hyphae within the mucosa. The invasive form is subdivided into acute, chronic, and chronic granulomatous and, among these, the acute invasive form is the most fatal, usually occurring in immunocompromised patients. In contrast, the noninvasive form of fungal sinusitis is considered an indolent disease with a good prognosis. However, to date, there has been no definitive evidence of progressive development from fungus ball to invasive fungal sinusitis. Interestingly, we found that fungus ball is not a unique sinonasal bioball, but it shows diverse phenotypes and endotypes in CRS. Specifically, we observed decreased

expression of S100A7 and S100A8/A9 proteins in CRS patients with mixed ball. This implies that, when concomitant bacterial infection occurs, nasal respiratory epithelia may show the decreased epithelial barrier function. Thus, based on our findings, it is reasonable to hypothesize that invasive fungal sinusitis may develop from noninvasive fungus under certain conditions, such as mixed ball. In addition, consistent with our hypothesis, several studies have described the possibility of a progressive disease spectrum from noninvasive fungus to invasive fungal sinusitis.^{40–42}

Our study has several limitations. First, the sample size was relatively small and further study is needed to verify our findings. Second, we did not conduct a long-term cohort study; therefore, we could not determine the difference in prognosis according to the different type of sinonasal bioball. Third, although reduced levels of S100 proteins may compromise innate immunity, we could not determine the precise pathophysiologic mechanism. For this reason, future studies are required to identify the precise role of S100 proteins in immune barrier function and the possibility of progressive disease from fungus ball to the invasive form.

References

- Fokkens WJ, Lund VJ, Mullol J, et al. EPOS 2012–European Position Paper on Rhinosinusitis and Nasal Polyps 2012: a summary for otorhinolaryngologists. *Rhinology*. 2012;50:1-12.
- Kennedy DW. Pathogenesis of chronic rhinosinusitis. *Ann Otol Rhinol Laryngol Suppl*. 2004;193:6-9.
- Wilson MT, Hamilos DL. The nasal and sinus microbiome in health and disease. *Curr Allergy Asthma Rep*. 2014;14:485.
- Lee JT, Frank DN, Ramakrishnan V. Microbiome of the paranasal sinuses: update and literature review. *Am J Rhinol Allergy*. 2016;30:3-16.
- Ramakrishnan VR, Hauser LJ, Frank DN. The sinonasal bacterial microbiome in health and disease. *Curr Opin Otolaryngol Head Neck Surg*. 2016;24:20-25.
- Thompson GR 3rd, Patterson TF. Fungal disease of the nose and paranasal sinuses. *J Allergy Clin Immunol*. 2012;129:321-326.
- deShazo RD, O'Brien M, Chapin K, Soto-Aguilar M, Gardner L, Swain R. A new classification and diagnostic criteria for invasive fungal sinusitis. *Arch Otolaryngol Head Neck Surg*. 1997;123:1181-1188.
- Aribandi M, McCoy VA, Bazan C 3rd. Imaging features of invasive and noninvasive fungal sinusitis: a review. *Radiographics*. 2007;27:1283-1296.
- Grosjean P, Weber R. Fungus balls of the paranasal sinuses: a review. *Eur Arch Otorhinolaryngol*. 2007;264:461-470.
- Prince AA, Steiger JD, Khalid AN, et al. Prevalence of biofilm-forming bacteria in chronic rhinosinusitis. *Am J Rhinol*. 2008;22:239-245.
- Schubert MS. A superantigen hypothesis for the pathogenesis of chronic hypertrophic rhinosinusitis, allergic fungal sinusitis, and related disorders. *Ann Allergy Asthma Immunol*. 2001;87:181-188.
- Cleland EJ, Bassiouni A, Wormald PJ. The bacteriology of chronic rhinosinusitis and the pre-eminence of *Staphylococcus aureus* in revision patients. *Int Forum Allergy Rhinol*. 2013;3:642-646.
- Feazel LM, Robertson CE, Ramakrishnan VR, Frank DN. Microbiome complexity and *Staphylococcus aureus* in chronic rhinosinusitis. *Laryngoscope*. 2012;122:467-472.
- Bendouh Z, Barbeau J, Hamad WA, Desrosiers M. Biofilm formation by *Staphylococcus aureus* and *Pseudomonas aeruginosa* is associated with an unfavorable evolution after surgery for chronic sinusitis and nasal polyposis. *Otolaryngol Head Neck Surg*. 2006;134:991-996.
- Kim DK, Wi YC, Shin SJ, Jang YI, Kim KR, Cho SH. Bacterial ball as an unusual finding in patients with chronic rhinosinusitis. *Clin Exp Otorhinolaryngol*. 2018;11:40-45.
- Dayer JM. Interleukin 1 or tumor necrosis factor- α : which is the real target in rheumatoid arthritis? *J Rheumatol Suppl*. 2002;65:10-15.

17. Hamilos DL, Leung DY, Wood R, et al. Eosinophil infiltration in nonallergic chronic hyperplastic sinusitis with nasal polyposis (CHS/NP) is associated with endothelial VCAM-1 upregulation and expression of TNF-alpha. *Am J Respir Cell Mol Biol*. 1996;15:443-450.
18. Besnard AG, Togbe D, Couillin I, et al. Inflammasome-IL-1-Th17 response in allergic lung inflammation. *J Mol Cell Biol*. 2012;4:3-10.
19. Mills KH, Dungan LS, Jones SA, Harris J. The role of inflammasome-derived IL-1 in driving IL-17 responses. *J Leukoc Biol*. 2013;93:489-497.
20. Schleimer RP, Kato A, Kern R, Kuperman D, Avila PC. Epithelium: at the interface of innate and adaptive immune responses. *J Allergy Clin Immunol*. 2007;120:1279-1284.
21. Tieu DD, Kern RC, Schleimer RP. Alterations in epithelial barrier function and host defense responses in chronic rhinosinusitis. *J Allergy Clin Immunol*. 2009;124:37-42.
22. deShazo RD, O'Brien M, Chapin K, et al. Criteria for the diagnosis of sinus mycetoma. *J Allergy Clin Immunol*. 1997;99:475-485.
23. Zhang N, Van Zele T, Perez-Novo C, et al. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. *J Allergy Clin Immunol*. 2008;122:961-968.
24. Hori M, Kita M, Torihashi S, et al. Upregulation of iNOS by COX-2 in muscularis resident macrophage of rat intestine stimulated with LPS. *Am J Physiol Gastrointest Liver Physiol*. 2001;280:G930-G938.
25. Oyer SL, Mulligan JK, Psaltis AJ, Henriquez OA, Schlosser RJ. Cytokine correlation between sinus tissue and nasal secretions among chronic rhinosinusitis and controls. *Laryngoscope*. 2013;123:E72-E78.
26. Oyer SL, Nagel W, Mulligan JK. Differential expression of adhesion molecules by sinonasal fibroblasts among control and chronic rhinosinusitis patients. *Am J Rhinol Allergy*. 2013;27:381-386.
27. Jinquan T, Vorum H, Larsen CG, et al. Psoriasin: a novel chemotactic protein. *J Invest Dermatol*. 1996;107:5-10.
28. Glaser R, Harder J, Lange H, Bartels J, Christophers E, Schroder JM. Antimicrobial psoriasin (S100A7) protects human skin from *Escherichia coli* infection. *Nat Immunol*. 2005;6:57-64.
29. Michalek M, Gelhaus C, Hecht O, Podschun R, Schroder JM, Leippe M, et al. The human antimicrobial protein psoriasin acts by permeabilization of bacterial membranes. *Dev Comp Immunol*. 2009;33:740-746.
30. Ryckman C, Vandal K, Rouleau P, Talbot M, Tessier PA. Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. *J Immunol*. 2003;170:3233-3242.
31. Cornish CJ, Devery JM, Poronnik P, Lackmann M, Cook DI, Geczy CL. S100 protein CP-10 stimulates myeloid cell chemotaxis without activation. *J Cell Physiol*. 1996;166:427-437.
32. Nisapakultorn K, Ross KF, Herzberg MC. Calprotectin expression in vitro by oral epithelial cells confers resistance to infection by *porphyromonasgingivalis*. *Infect Immun*. 2001;69:4242-4247.
33. Sohnle PG, Hahn BL, Santhanagopalan V. Inhibition of *Candida albicans* growth by calprotectin in the absence of direct contact with the organisms. *J Infect Dis*. 1996;174:1369-1372.
34. Zhou HD, Li XL, Li GY, et al. Effect of SPLUNC1 protein on the *Pseudomonas aeruginosa* and Epstein-Barr virus. *Mol Cell Biochem*. 2008;309:191-197.
35. Gally F, Di YP, Smith SK, et al. SPLUNC1 promotes lung innate defense against *Mycoplasma pneumoniae* infection in mice. *Am J Pathol*. 2011;178:2159-2167.
36. Lukinskiene L, Liu Y, Reynolds SD, et al. Antimicrobial activity of PLUNC protects against *Pseudomonas aeruginosa* infection. *J Immunol*. 2011;187:382-390.
37. Richer SL, Truong-Tran AQ, Conley DB, et al. Epithelial genes in chronic rhinosinusitis with and without nasal polyps. *Am J Rhinol*. 2008;22:228-234.
38. 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.
39. Seshadri S, Lin DC, Rosati M, et al. Reduced expression of antimicrobial PLUNC proteins in nasal polyp tissues of patients with chronic rhinosinusitis. *Allergy*. 2012;67:920-928.
40. Kim YK, Kim HJ, Kim HY, et al. Extrasinonasal infiltrative process associated with a sinonasal fungus ball: does it mean invasive fungal sinusitis? *Diagn Interv Radiol*. 2016;22:347-353.
41. Ota R, Katada A, Bandoh N, et al. A case of invasive paranasal aspergillosis that developed from a non-invasive form during 5-year follow-up. *Auris Nasus Larynx*. 2010;37:250-254.
42. Rowe-Jones JM, Moore-Gillon V. Destructive non-invasive paranasal sinus aspergillosis: component of a spectrum of disease. *J Otolaryngol*. 1994;23:92-96.