

Staphylococcus aureus superantigens and their role in eosinophilic nasal polyp disease

Pongsakorn Tantilipikorn,¹ Chaweewan Bunnag,¹ Zhang Nan,² and Claus Bachert²

Summary

Nasal polyposis is a chronic disease of the upper airways which adversely affects the quality of life of patients. Its pathophysiology is still unclear. Recently, several studies have shown different inflammatory pathways which relate to both innate and adaptive immune responses. Moreover, different phenotypes may exist in different ethnic groups of patients. This article will review recent data regarding the type of inflammation, cytokine profiles, involvement of macrophages and dendritic cells, and the impact of various organisms (especially *Staphylococcus aureus* and its superantigens) and their association with lower airway disease (especially asthma). (*Asian Pac J Allergy Immunol* 2012;30: 171-6)

Key words: superantigen, *staphylococcus aureus*, nasal polyp, rhinosinusitis, airway inflammation

Introduction

Nasal polyps (NP) are whitish-gray mass-like lesions in the nose. They are almost always associated with chronic inflammation of the nose and paranasal sinuses, so called rhinosinusitis. Histologically, nasal polyps show interstitial tissue edema, pseudocyst formation and collagen matrix disruption with abundant inflammatory cell infiltration. They usually protrude from the middle and superior meatus into the nasal cavity, causing

rhinologic symptoms such as nasal blockage, anterior/posterior rhinorrhea, and smell disturbance.

The etiology of nasal polyps is still unknown. Several hypotheses have been proposed, for instance the fungal hypothesis, the role of biofilm formation and the specific impact of superantigens of *Staphylococcus aureus* enterotoxins (SEs) as disease modifiers. The fungal biofilm can be considered as one possibility but the role of *S.aureus* is supported by more evidence and is more clinically relevant; biofilms may serve as reservoir for *Staphylococcus aureus* under specific circumstances. As NPs represent a chronic mostly eosinophilic inflammation, the first line therapy is topical glucocorticosteroid application, either as spray or drops. However, in cases of steroid unresponsiveness or recurrence of disease, surgery is the next choice. Still, polyps may recur and up to ten surgeries per lifetime is not exceptional for the most severe cases (often aspirin sensitive patients). Furthermore, inflammation may expand to the lower airways and induce asthma; about one third of severe asthma sufferers have nasal polyps. Thus, there is an unmet need in terms of treatment options for severe cases of polyp disease with or without comorbidity. Recent trials propose new options such as doxycycline, anti-interleukin (IL)-5 and anti-immunoglobulin E (anti-IgE).

This article is a review of our up to date knowledge concerning nasal polyps and their associated diseases, especially asthma. The article will also focus on the superantigen hypothesis, biofilm formation and the involvement of innate and adaptive immunity in nasal polyp disease.

Nasal Polyps: Different types of inflammation

According to the European Position Paper on Polyposis and Sinusitis (EPOS), rhinosinusitis can be categorized as acute, subacute, recurrent and chronic rhinosinusitis (CRS).¹ The CRS can be subclassified as CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSSNP). CRS with and without nasal polyps differ from each other by

From the 1. Division of allergy and rhinology, Department of Otolaryngology, Faculty of Medicine Siriraj, Bangkok, Thailand.

2. Upper Airways Research Laboratory (URL), Department of Oto-Rhino-Laryngology, Ghent University Hospital, Belgium.

Corresponding author: Pongsakorn Tantilipikorn

E-mail: pongsakorn.tan@mahidol.ac.th

Submitted date: 9/4/2012



several markers related to inflammation and tissue remodeling; the foundation of NPs is a chronic inflammatory and remodeling process of the nasal mucosa.²

European and US publications have reported that the histology of NPs reveal prominent edema formation and tissue eosinophilia. The eosinophilic infiltration is more pronounced in patients with concomitant asthma and/or aspirin sensitivity. Eosinophilia in CRSwNP is caused by the increased inflammatory mediators and chemokines, especially interleukin-5 (IL5) and eotaxin. Thus, nasal polyposis is a distinct disease entity from CRSsNP based not only on the gross finding inside the nose but also the profile of immunopathogenesis. While CRS is characterized by a T helper(Th)1 polarization with high levels of IFN- γ and TGF- β , NPs mainly show a Th2 polarization with high IL-5 and IgE concentrations.³

The IgE in NPs is not due to an inhalant allergy, as allergic sensitization does not change the degree of eosinophilia or mediators within polyp tissue, but is mainly caused by enterotoxins released from *Staphylococcus aureus*, which act as superantigens. The superantigens induce a polyclonal T-cell and B-cell activation, and amplify the eosinophilic inflammation and edema formation.⁴ The pathological mechanism of superantigens involves nonspecific binding to the T cell receptor via the variable V beta region and the major histocompatibility complex (MHC) class II complex on antigen-presenting cells (APC). By this mechanism, a large number of APCs and T cells (as high as 30% of the T cell population, compared to 0.01% by normal antigen-responding T cell) is activated.

While the studies from European countries show 65-90% of NPs to be eosinophilic, studies from Thailand, Korea and Southern China present different immunopathologic features (Figure 1). A study of 145 patients with NP from Thailand in 2002 found that only 11.7% of the polyps demonstrated eosinophilia.⁵ A study of thirty patients from Korea in 2007 showed 33.3% of polyps to be eosinophilic,⁶ and a further study of 151 NPs from China revealed that less than half of specimens are characterized by eosinophilic inflammation.⁷ In 2008, Zhang et al. compared 29 NPs from South Chinese with 26 NPs from Belgium and demonstrated a clear difference between them, with samples from Asian patients being biased towards neutrophilic inflammation (eosinophilic

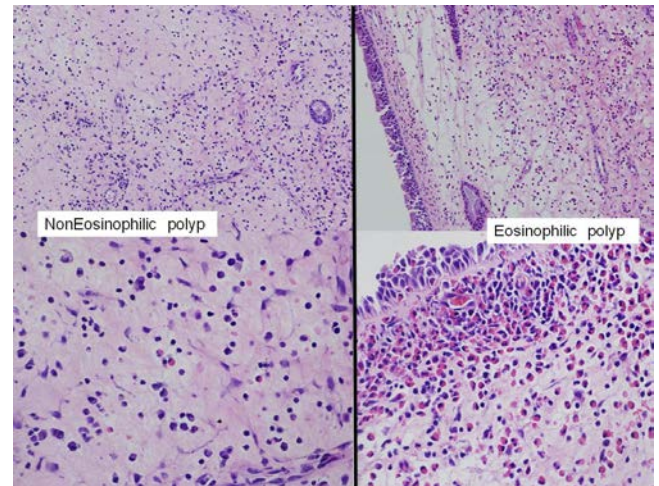


Figure 1. Eosinophilic and Non-eosinophilic polyps.

cationic protein/myeloperoxidase ratio = 0.25) with a significant down-regulation of T-regulatory cells.⁸

In both phenotypes, and in both eosinophilic or non-eosinophilic polyps, the principle finding is an impairment of regulatory T-cell function and the activation of T- and B-cells.⁹ The nasal mucosa is heavily exposed to the environment, including bacteria present in the nose and their products. Staphylococcal enterotoxins SAEs are potent activators of T cells and further induce the synthesis of immunoglobulins, including IgE, by B cells. The prevalence of IgE antibodies to *S. aureus* enterotoxin in European NPs patients is about fifty percent.¹⁰ In 2009, Corriveau et al. studied 21 NPs and found that T-helper2 (Th2) markers were increased related to the SE-IgE status, but not to the presence of *Staphylococcus aureus* in a smear, and this inflammatory reaction was dependent on the formation of SE-IgE within mucosal tissue.¹¹

Bacteriology and biofilms: the link to nasal polyps

The term biofilm describes the formation of a matrix of extracellular polymeric substances which can be found in many chronic diseases such as otitis media with effusion, chronic tonsillitis, cholesteatoma and chronic rhinosinusitis. Several species of bacteria and fungi can produce biofilms and are thereby able to 'hide' themselves from the activity of antibiotics. When inside the biofilm, germs can communicate and acquire multiple genetic alterations and also change their phenotype, which may lead to bacterial resistance.

There are two main methods of identifying biofilms in CRS. The first one is non-invasive using

an *ex vivo* biofilm-forming assay including culturing. By using a swab through the nostrils, which is protected by a speculum, to the sinus cavity, a physician is able to sample the suspected discharge for culturing. However, the sample may not be representative. The second method is the FISH (Fluorescence in situ hybridization) method with the confocal scanning laser microscope. Either the staining of bacteria according to their life cycle by BacLight LIVE DEAD or species-specific by FISH, the confocal scanning laser microscopy technique has proved to be more sensitive and specific for biofilm determination than both scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

S. aureus is the most common organism within the biofilms of CRS patients in a study by Foreman et al. 2009. In particular, *S. aureus* biofilms appear to be a predictor of severe disease recalcitrant to current treatment paradigms. However direct causal links between biofilms and host immune activation are currently lacking. A recent study aimed to document both the adaptive immune responses that characterize *S. aureus* biofilm associated CRS and the relative contributions of staphylococcal superantigens and *S. aureus* biofilms in the inflammatory make-up of this disease. 53 disease subjects and 15 controls were recruited. *S. aureus* biofilms and superantigens were significantly associated in CRS patients suggesting the biofilm may be a nidus for superantigen-eluting bacteria. The presence of *S. aureus* biofilms was associated with eosinophilic inflammation, across the spectrum of CRS, on the back of a T-helper₂ skewing of the host's adaptive immune response (elevated ECP and IL-5). This effect could be distinguished from the superantigenic effect resulting in the induction of IgE.¹²

Summarizing the current evidence, biofilms are associated with more severe disease clinically. Biofilms from *S. aureus* are related to more severe disease than biofilms from other species such as *Pseudomonas* or *Haemophilus*; biofilms carrying *S. aureus* are more common than other biofilms in nasal polyps and are related to more severe inflammation. They may prepare the planktonic invasion of the mucosa by *Staphylococcus aureus* and also may bias the immune system of the local mucosa into the Th2 direction, which then increases the survival of the germs.(Figure 2) In fact, *S. aureus* has been found intramucosally and intracellularly, most probably due to an impairment

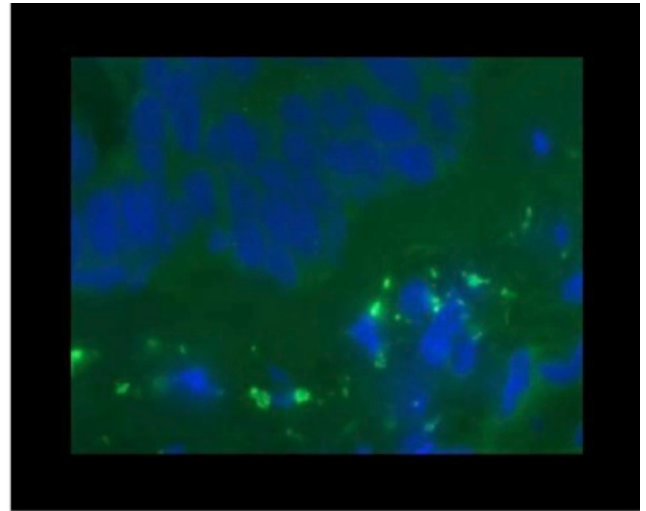


Figure 2. Intramucosal *Staphylococcus aureus* in a SE-IgE positive eosinophilic polyps, stained by the PNA-FISH technique.

of the phagocytosing and killing activity of macrophages.¹³

Innate immunity and macrophage deficit

Nasal polyps, rhinosinusitis, allergic rhinitis and asthma share a common pathology of mucosal inflammation. There is marked activation of epithelial cells in both the upper and lower airways. Epithelial cell functions include both mediation and regulation of innate immune responses and adaptive immune responses.¹⁴ Regarding the innate immunity role of mucosal epithelial cells, they act as a barrier to a variety of environmental agents. Sinonasal epithelial cells express pattern-recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) of those various microorganisms.^{15,16} One of the most important of PRRs is the Toll-like receptor (TLR) family, consisting of 10 members. TLRs will trigger a pro-inflammatory response by the activation of transcription factors in the nucleus, especially nuclear factor- κ B (NF- κ B).¹⁷

Macrophages are phagocytic cells which act along with the mucosal barrier as an innate immunity defense mechanism. Macrophages can be classified into pro-inflammatory M1 or an immunosuppressive M2 types.^{18,19} M1 macrophages support a Th1 response and prevent pathogen persistence by expression pro-inflammatory cytokines, such as IL1-beta, IL-12, IL-23, nitric oxide (NO) and tumor necrosis factor (TNF).²⁰ M2 macrophages, by contrast, show a reduced phagocytosis and support the intracellular survival

of bacteria and viruses by expression of non-opsonic receptors, such as the mannose receptor, scavenger receptor-1, and CD-163.²¹ Krysko et al. in 2010 studied 28 polyps by flow cytometry and immunohistochemical staining and found impaired phagocytosis function of macrophages to *S. aureus* in NPs, which allowed Staphylococcus to survive intracellularly (Figure 2).²² The activated M2 phenotype could thus contribute to persistent chronic inflammation in NPs and chronic rhinosinusitis.²³ These observations would explain the high rate of *S. aureus* colonization in NPs compared to controls (63.6% vs 33.3%), which points to a possible defect of the mucosal defense system.¹⁰

Impact of *S. aureus* on severity of inflammation

S. aureus can secrete a range of enterotoxins such as *Staphylococcus aureus* enterotoxin A (SEA), *Staphylococcus aureus* enterotoxin B (SEB), and Toxic shock syndrome toxin-1 (TSST-1).²⁴ IgE to these toxins can be found in several allergic disease, such as atopic eczema/dermatitis syndrome (AEDS)²⁵, allergic rhinitis (AR)^{26, 27}, and NPs²⁸. Particularly in NPs, levels of *S. aureus* enterotoxin – specific IgE correlate with markers of eosinophil activation and recruitment.⁴ By using freshly isolated and purified human nasal epithelial cells, Huvenne et al. in 2010 studied the effect of the classical superantigen SEB on chemokine production. Their data supported the pro-inflammatory effect of SEB, demonstrating chemotaxis and effects on the survival of granulocytes.²⁴ Patou et al. also studied the role of SEB and the surface proteins of *S. aureus* such as lipoteichoic acid (LTA) and protein A (SpA) in the release of cytokines and mediators from mast cells and T cells. They showed that SpA could induce mast cell degranulation and that SEB induced the release of cytokines with a Th2-skewed pattern in NPs, not favoring T regulatory cells.²⁹

Support from animal studies

From animal studies, SE has effects on both innate and adaptive immunity.³⁰ SE induces the migration and maturation of the dendritic cell (DC) population in vivo, and induces polyclonal activation of T-cells.³¹ In a mouse model, *S. aureus* enterotoxin B (SEB), furthermore, could facilitate the sensitization of CD4+ cells to nasally applied allergens, resulting in the activation of B cells and the production of IgE.²⁴ To confirm the contribution of DCs in this activation process, nasal application of SEB was combined with FITC-labeled ovalbumin

(OVA) which was administered intratracheally in mice. CD86 was expressed in a higher level in OVA/SEB-treated mice compared to OVA/saline-treated mice.

Impact of nasal polyp inflammation on asthma comorbidity and severity of asthma

Asthma is present in 45% of all CRSwNP patients according to a recent cohort study by the Global Allergy and Asthma European Network (GA2LEN) consortium. CRSwNP and asthma are linked by a common inflammatory pathway which includes eosinophils and the mucosal airway epithelium as major players. A study in an adolescent, mostly allergic population, showed that the serum expression of IgE against SE was significantly associated with an increased risk for asthma.³² This finding has now been confirmed in a larger European study in adults, confirming SE-IgE as a risk factor (unpublished data). Of interest, allergic rhinitis increases the risk for early onset asthma, whereas CRS has an impact on late-onset asthma.³³

NPs and asthma also clearly co-exist in aspirin-exacerbated respiratory disease (AERD), which is a clinical syndrome related to severe inflammation of the upper and lower airways. It consists of severe/recurrent nasal polyposis, aspirin-sensitivity to NSAIDs leading to acute exacerbations of the disease, and often severe asthma. This syndrome is caused by abnormalities in the arachidonic acid biosynthetic pathway; a key feature of this condition is an intense chronic eosinophilic inflammation, also including the overexpression of IgE. A study by Perez-Novo in 2004 showed that the concentration of IgE antibodies to SAEs were significantly increased in CRSwNP and AERD compared to controls and CRSsNP.³⁴

In a study of CRSwNP and co-morbid asthma patients, 34% suffered from both diseases.³⁵ SE-IgE was detected in 37.3% of patients in nasal tissues; this finding was associated with a Th2-biased inflammation and high concentrations of ECP and total IgE. The expression of SE-IgE in nasal polyps predicted the presence of asthma with an odds ratio (OR) of 5.8 (95% CI, 1.8-29.6); total IgE values above 1400 kU/l, as frequently found as a consequence of SE impact, increased the risk of asthma even further.

Consequently, when Kowalski et al. did a 12 month observational study in 109 patients with severe refractory asthma and 101 patients with non-severe asthma, these authors found SE-IgE in the

majority of severe asthma patients, together with an increase of total IgE and ECP concentrations in serum.³⁶ The mean level of enterotoxin-specific IgE was threefold higher in patients with severe asthma compared with patients with non-severe asthma ($p=0.01$). Furthermore, concentrations of SE-IgE were significantly associated with low respiratory function test results (forced expiratory volume in 1 second (FEV1)). These findings support a role for *Staphylococcus aureus* enterotoxins also in lower airway disease.

Conclusion

Nasal polyps (NPs) are characterized by a chronic inflammation of the nose and paranasal sinuses. Although its pathogenesis still remains obscure, tremendous progress in the understanding of this disease has been made. In the past few years, our focus has been directed to *Staphylococcus aureus* enterotoxin (SEs) which act as T- and B-cell superantigens. From in-vitro and in-vivo studies, SEs induce an intense eosinophilic inflammatory process of the upper and lower airways with polyclonal IgE production unrelated to atopy.

Acknowledgement

The study related to this manuscript is supported by the Siriraj Research Funds and the National Research University (NRU) funds to Mahidol University.

References

1. Fokkens WJ, Lund VJ, Mullol J. European position paper on nasal polyps. *Rhinology*. 2007;45(Suppl 20):1-139.
2. van Crombruggen K, Zhang N, Gevaert P, Tomassen P, Bachert C. Pathogenesis of chronic rhinosinusitis: Inflammation. *Current Perspectives*. *J Allergy Clin Immunol*. 2011;128:728-32.
3. van Zele T, Claeys S, Gevaert P, van Maele G, Holtappels G, van Cauwenberge P, et al. Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy*. 2006;61:1280-9.
4. Bachert C, Gevaert P, Holtappels G, Johansson S, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol*. 2001;107:607-14.
5. Jareonchasri P, Bunnag C, Muangsomboon S, Tunsuriyawong P, Assanasane P. Clinical and Histopathological Classification of Nasal Polyps in Thais. *Siriraj Hosp Gaz*. 2002;54:689-97.
6. Kim JW, Hong SL, Kim YK, Lee CH, Min YG, Rhee CS. Histological and immunological features of non-eosinophilic nasal polyps. *Otolaryngol Head Neck Surg*. 2007;137:925-30.
7. Cao PP, Li HB, Wang BF, Wang SB, You XJ, Cui YH, et al. Distinct immunopathologic characteristics of various types of chronic rhinosinusitis in adult Chinese. *J Allergy Clin Immunol*. 2009;124:478-84.
8. Zhang N, van Zele T, Perez-Novo C, van Bruaene N, Holtappels G, DeRuyck N, et al. Different types of T-effector cells orchestrate mucosal inflammation in chronic sinus disease. *J Allergy Clin Immunol*. 2008;122:961-8.
9. Zhang N, Holtappels G, Claeys C, Huang G, van Cauwenberge P, Bachert C. Pattern of inflammation and impact of *Staphylococcus aureus* enterotoxins in nasal polyps from southern China. *Am J Rhinol*. 2006;20:445-50.
10. van Zele T, Gevaert P, Watelet J. *Staphylococcus aureus* colonization and IgE antibody formation to enterotoxin is increased in nasal polyposis. *J Allergy Clin Immunol*. 2004;114:981-3.
11. Corriveau M, Zhang N, Holtappels G, Van Roy N, Bachert C. Detection of *Staphylococcus aureus* in nasal tissue with peptide nucleic acid-fluorescence *in situ* hybridization. *Am J Rhinol Allergy*. 2009;23:461-5.
12. Foreman A, Holtappels G, Psaltis AJ, Jervis-Bardy J, Field J, Wormald PJ, et al. Adaptive immune responses in *Staphylococcus aureus* biofilm associated chronic rhinosinusitis. *Allergy*. 2011;66:1449-56.
13. Krysko O, Holtappels G, Zhang N, Kubica M, Deswarte K, Derycke L, et al. Alternatively activated macrophages and impaired phagocytosis of *S.aureus* in chronic rhinosinusitis. *Allergy*. 2011;66:396-403.
14. Schleimer RP, Kato A, Kern RC, Kuperman D, Avila PC. Epithelium: At the interface of innate and adaptive immune responses. *J Allergy Clin Immunol*. 2007;120:1279-84.
15. Claeys C, de Belder T, Holtappels G, Gevaert P, Verhasselt B, van Cauwenberge P, et al. Human beta-defensins and toll-like receptors in the upper airway. *Allergy*. 2003;58:748-53.
16. Tan BK, Schleimer RP, Kern RC. Perspectives on the etiology of chronic rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg*. 2010;18:21-6.
17. Vroeling AB, Fokkens WJ, van Drunen CM. How epithelial cells detect danger: aiding the immune response. *Allergy*. 2008;63:1110-23.
18. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol*. 2009;27:451-83.
19. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol*. 2004;25:677-86.
20. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol*. 2003;3:23-35.
21. Fairweather D, Cihakova D. Alternatively activated macrophages in infection and autoimmunity. *J Autoimmun*. 2009;2009(4):222-30.
22. Krysko O, Vandenabeele P, Krysko DV, Bachert C. Impairment of phagocytosis of apoptotic cells and its role in chronic airway disease. *Apoptosis*. 2010;15:1137-46.
23. Krysko O, Holtappels G, Zhang N, Deswarte K, Derycke L, Claeys S, et al. Alternatively activated macrophages and impaired

- phagocytosis of *S. aureus* in chronic rhinosinusitis. *Allergy*. 2011;66:396-403.
24. Huvenne W, Callebaut I, Reekmans K, Hens G, Bobic S, Jorissen M, et al. *Staphylococcus aureus* enterotoxin B augments granulocyte migration and survival via airway epithelial cell activation. *Allergy*. 2010;65:1013-20.
25. Leung DY, Harbeck R, Bina P, Reiser RF, Yang E, Norris DA, et al. Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. *J Clin Invest*. 1993;92:1374-80.
26. Rossi RE, Monasterolo G. Prevalence of serum IgE antibodies to the *Staphylococcus aureus* enterotoxin (SAE, SEB, SEC, SED, TSST-1) in patients with persistent allergic rhinitis. *Int Arch Allergy Immunol*. 2004;133:261-6.
27. Okano M, Takishita T, Yamamoto T, Hattori H, Yamashita Y, Nishioka S, et al. Presence and characterization of sensitization to staphylococcal enterotoxins in patients with allergic rhinitis. *Am J Rhinol*. 2001;15:417-21.
28. Carayol N, Crampette L, Mainprice B, Ben-Soussen P, Verrecchia M, Bousquet J, et al. Inhibition of mediator and cytokine release from dispersed nasal polyp cells by mizolastine. *Allergy*. 2002;57:1067-70.
29. Patou J, Gevaert P, van Zele T, Holtappels G, van Cauwenberge P, Bachert C. *Staphylococcus aureus* enterotoxin B, protein A, and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol*. 2008;121:110-5.
30. Derycke L, Perez-Novo CA, Van Crombruggen K, Corriveau M, Bachert C. *Staphylococcus aureus* and Chronic Airway Disease. *WAO Journal*. 2010;3:223-8.
31. Muraille E, De Trez C, Pajak B, Brait M, Urbain J, Leo O. T cell-dependent maturation of dendritic cells in response to bacterial superantigens. *J Immunol*. 2002;168:4352-60.
32. Hollams E, Hales B, Bachert C, Huvenne W, Parsons F, de Klerk NK, et al. Th2-associated immunity to bacteria in asthma in teenagers and susceptibility to asthma. *Eur Respir J*. 2010.
33. Jarvis D, Newson R, Lotval J, Hastan D, Tomassen P, Bousquet PJ, et al. Asthma in adults and its association with chronic rhinosinusitis: The GA2LEN survey in Europe. *Allergy*. 2012;67:91-8.
34. Perez-Novo CA, Kowalski ML, Kuna P, Ptasinska A, Holtappels G, van Cauwenberge P, et al. Aspirin sensitivity and IgE antibodies to *Staphylococcus aureus* enterotoxins in nasal polyposis: Studies on the relationship. *Int Arch Allergy Immunol*. 2004;133:255-60.
35. Bachert C, Zhang N, Holtappels G, De Lobel L, van Cauwenberge P, Liu S, et al. Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. *J Allergy Clin Immunol*. 2010;126:962-8.
36. Kowalski ML, Cieslak M, Perez-Novo CA, Makowska JS, Bachert C. Clinical and immunological determinants of severe/refractory asthma (SRS): association with *Staphylococcal* superantigen-specific IgE antibodies. *Allergy*. 2011;66:32-8.