Chapter 14

The upper airways in severe asthma

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Summary

Although reiterated frequently, the relationship between upper and lower airways is not yet appreciated from a clinical standpoint and not sufficiently understood in terms of pathomechanisms. Here, we summarise the evidence for the impact of acute episodes of rhinitis, allergic, nonallergic or viral, on asthma severity and exacerbations, and differentiate subgroups of chronic rhinosinusitis in terms of their likelihood to interfere with lower airway disease and severity in the long term. We also review recent findings on the effect of staphylococcal enterotoxins, also referred to as superantigens, on mucosal inflammation and how they could serve as a link between nasal polyp disease and late-onset partially severe asthma. Finally, we provide evidence for a possible impact of superantigens on severe refractory asthma independent of sinus disease, *via* an amplification of the eosinophilic inflammation and polyclonal immunoglobulin E formation. The superantigen concept needs to be taken into consideration in epidemiological approaches to the problem of severe asthma, and if confirmed could eventually lead to new therapeutic targets.

Keywords: **Asthma, chronic rhinosinusitis, nasal polyps, polyclonal immunoglobulin E, rhinitis, staphylococcal superantigens**

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The "united airways" concept is seemingly well accepted by rhinologists and allergists nowadays; the coexistence of upper and lower airway disease has been described repeatedly, and is highlighted by a World Health Organization (WHO) initiative called Allergic Rhinitis and its Impact on Asthma (ARIA) [1, 2]. This guideline describes an important role for the upper airways in the initiation, maintenance and exacerbation of respiratory diseases; however, this view does not seem to be appreciated by recent official standards for the diagnosis and treatment of asthma of either the American Thoracic Society (ATS) or the European Respiratory Society [3]. With respect to the question as to whether upper airway disease may be related to severe asthma, none of these documents gives a conclusive answer today. In contrast, single reports seem to clearly imply a consistent association between nasal polyps and asthma comorbidity as well as asthma severity [4–6], and describe patients with aspirin-exacerbated respiratory disease (AERD)

with the highest rates of exacerbations and hospital admissions [7]. AERD develops according to a sequence of symptoms, with persistent rhinitis, appearing at a mean age of 29.7 ± 12.5 yrs, followed by asthma, aspirin intolerance and nasal polyposis. In about half of these patients, asthma is severe and steroid dependent.

There is a great lack of epidemiological, clinical, pathophysiological and therapeutic knowledge and understanding of the link between upper and lower airway disease, especially with respect to severe disease. A prerequisite for the recognition of meaningful pathophysiological links between airway diseases is the feasibility to depict disease phenotypes in both the upper and the lower airways. Such approaches are new for chronic rhinosinusitis (CRS), as this term has been used as a basket for different disease entities [8]. There is, however, increasing evidence that several subtypes of rhinosinusitis can be differentiated nowadays, or do not represent one disease: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP) [9–13]. Specific markers may allow a further differentiation even within a clinical subgroup such as CRSwNP [11, 14]. However, clinical signs and symptoms may not be sufficient to allow for such differentiation, and biomarkers have possibly to be introduced. This may hinder the translation of recent knowledge into practice, and may even make it impossible for epidemiological approaches to differentiate sinus disease.

Recent attempts in severe asthma patients, partially using unsupervised modelling methods, have provided a new view on asthma subtypes, also including severe asthma. Carefully reading such papers, one can find a small amount of information on the upper airways. In the framework of the Severe Asthma Research Program (SARP) 438 subjects with asthma were studied; 204 had severe asthma [15]. 54% of those severe asthmatics reported sinus disease (67% *versus* 45% in late *versus* early onset disease), and 27% even reported former surgery for sinusitis. Those findings were confirmed recently when the group was increased to 726 asthmatics who could be differentiated into clusters [16]. One cluster was characterised by severe airflow obstruction with bronchodilator responsiveness, and smaller numbers of positive skin prick tests (SPT). 80% of those patients were severe asthmatics according to ATS criteria, again, more than half of the patients suffered from sinus disease, and nearly half of those patients had previous surgery for sinusitis. However, the upper airway findings were based on questionnaires but not on clinical investigations; no conclusions can therefore be drawn with respect to the sinus disease subgroups, as mentioned previously. There is an urgent need for a large multicentre study investigating both the lower and the upper airways, to be performed by the respective specialists to extend our current knowledge on airway comorbidity in general and in severe airway disease specifically.

According to the new WHO definition of asthma severity [17], patients with difficult-to-treat severe asthma represent an asthma category in which partial or poor response to treatment may reflect the presence of factors other than asthma, which may impact on the disease. This could include patients with rhinitis and rhinosinusitis, among many other factors, however, a group of treatment-resistant severe asthma, either poorly or just controlled by high-dose inhaled corticosteroids, may also suffer from uncontrolled nose or sinus disease. In fact, all of the severe asthma patient groups could potentially suffer from upper airway disease, which may contribute to disease severity and unresponsiveness to therapy.

Taking all the limitations discussed previously into account, herein we summarise the current knowledge on the role of allergic rhinitis and viral or chronic rhinosinusitis in severe asthma, and introduce a new hypothesis on the role of Staphylococcal enterotoxins (SEs) for severe upper and lower airway disease [18–20]. SEs may act as superantigens and have the potential to amplify airway disease; recent evidence suggests that this principle not only applies to the upper but also to the lower airways.

Allergic and nonallergic rhinitis

Epidemiological studies have consistently shown that asthma and rhinitis often co-exist [1]. The prevalence of asthma in subjects without rhinitis is usually $<2\%$, but between 10–40% in patients

with rhinitis. In contrast, the majority of patients with asthma present with rhinitis symptoms. The impact of rhinitis on asthma severity is less clear, although adults and children with asthma and documented concomitant allergic rhinitis experience more asthma-related hospitalisations and general practitioners (GPs) visits, and incur higher asthma drug costs than adults with asthma alone in most, but not all studies [1].

In a study with French general GPs, >14,000 asthma patients were analysed based on questionnaires; 4% suffered from severe asthma [21]. The frequency of allergic rhinitis in asthmatic patients in this study was 55%; frequency and severity of rhinitis increased with the severity of asthma, and allergic rhinitis was associated with a worse asthma control independent of the severity of asthma. In addition, prescription of anti-asthma treatments significantly increased with the severity of rhinitis. In a cross-sectional, observational survey conducted among 1,173 asthmatic patients (aged 12–45 yrs) who were recruited by GPs and chest physicians in Belgium, self-reported allergic rhinitis was present in 74% of the asthmatic population and nonallergic rhinitis in 14% [22]. Both allergic and nonallergic rhinitis were associated with an increased risk of uncontrolled asthma, as evaluated by the Asthma Control Questionnaire (ACQ score >1.5). Multivariate linear regression analysis showed that both forms of rhinitis had a modest, though significant, negative impact on the global asthma-specific quality of life evaluated through the Mini Asthma Quality of Life Questionnaire, even after adjustment for the level of asthma control and demographic characteristics. Thus, this survey provided direct evidence that rhinitis is associated with an incremental adverse impact on the disease-specific quality of life and the level of asthma control. However, severe asthma patients were not specifically addressed in these studies.

There is a recent prospective study in 557 patients with severe asthma according to the Global Initiative for Asthma (GINA) criteria followed for 1 year with a record of emergency room visits and supply of topical corticosteroids for asthma and rhinitis, which confirms these observations [23]. 31% of the patients with severe asthma also had moderate/severe rhinitis, according to ARIA guidelines [1]. Moderate/severe rhinitis was a predictor for any emergency room visit in the follow-up period and for the presence of uncontrolled asthma after 1 year of follow-up. Again, the severity of rhinitis was positively correlated with asthma severity. Thus, in a population with severe asthma, moderate/severe rhinitis is a strong predictor for greater severity of asthma. This study, however, does not allow us to predict the impact of rhinitis on asthma severity by clinical or laboratory signs, or offer an explanation for the pathomechanical link. Furthermore, the findings were not supported by a SARP study in 438 subjects with asthma, 204 with severe asthma [15]. The severe group had less atopy by skin tests, although 71% of patients still had at least one positive SPT. Actually, among other criteria, fewer positive skin tests increased the risk for severe disease in this study.

Acute viral rhinosinusitis

Although there is no evidence that viral respiratory infections may lead to severe asthma, they are the most common cause of an acute asthma exacerbation in both children and adults. Obviously, these infections cause a greater degree of morbidity in asthmatic subjects than in the healthy population, emphasising a defect in the antiviral response of asthmatic patients [24]. Mechanisms of virus-induced asthma exacerbations have extensively been reviewed recently [25]. Rhinovirus infections of the upper airway obviously spread into the lower airways, as demonstrated by reverse transcription-polymerase chain reaction (RT-PCR) and Southern blotting of experimentally infected volunteers [26].

Upper respiratory viral infections were associated with 80–85% of asthma exacerbations in school-age children in a community study, using PCR to detect the viruses; the most commonly identified virus type was rhinovirus [27]. In children of this age, two distinct peaks of asthma exacerbation were found in spring and autumn; rhinoviruses were detected in 27% of the cases in autumn and in 24.3% in spring [28]. Colds were also reported in 80% of episodes with symptoms of wheeze, chest tightness or breathlessness in adults and 89% of colds were associated with asthma

symptoms in a longitudinal study [29]. Nearly half of the episodes with mean decreases in flow rate $\geqslant 50$ L·min^{-1} were associated with laboratory confirmed infections, such as rhinoviruses, coronaviruses, influenza B, respiratory syncytial virus, parainfluenza virus or Chlamydia. It also has been proposed that air pollution plays a role in cold-associated exacerbations; of 130 asthma exacerbations in a 1-year study in Vancouver (Canada), 47% were associated with cold symptoms [30]. Asthma exacerbations with colds were associated with higher levels of sulfur dioxide and nitric oxide during March to November in comparison with asthma exacerbations without cold symptoms. Patients with asthma are not at greater risk of rhinovirus infections than healthy individuals but have more severe and longer lasting symptoms upon infection [31].

Chronic rhinosinusitis: CRSwNP and CRSsNP

The term CRS describes an inflammatory disease manifesting in the nose and sinuses for >12 weeks; typical symptoms are nasal obstruction, nasal secretion, post-nasal drip, headache/facial pain or pressure and loss of smell. Furthermore, the disease impairs quality of life, sleep and work performance of patients. However, these clinical signs are not sufficient to make the diagnosis of CRS, either a computed tomography (CT) scan of the sinuses and/or a nasal endoscopy needs to support the diagnosis [8]. However, a CT scan may also be "positive" in patients without any complaints, especially when taken during an acute viral cold episode, and a nasal endoscopy might be negative especially in CRSsNP. Furthermore, these clinical signs are not valid in differentiating between CRSsNP and CRSwNP, only the clear visualisation of nasal polyps by rigid endoscopy, best after extensive decongestion of the nasal cavity, would allow diagnosing polyps with sufficient accuracy.

CRSsNP and CRSwNP are separate diseases in terms of inflammatory patterns, remodelling patterns and asthma comorbidity. Whereas CRSsNP resembles a low predominantly neutrophilic T-helper cell (Th) type 1-biased inflammation profile, CRSwNP is mostly characterised by a strong eosinophilic Th2-biased inflammation [9]. In CRSsNP, there is a normal or increased transforming growth factor (TGF)-β expression in the tissue, and a normal number of T-regulatory cells, whereas TGF-β, its receptors and the number of T-regulatory cells are down-regulated in CRSwNP [10, 12]. As a consequence, CRSsNP shows an increased activation of the TGF-β pathway and a strong upregulation of collagen synthesis, whereas CRSsNP lacks adequate collagen synthesis and is characterised by tissue oedema. We recently could demonstrate that these completely different remodelling patterns in chronic sinus diseases are fairly identical in Caucasian and Asian populations [13], in contrast to the involved T-cell populations. Here we could demonstrate that Chinese CRSwNP patients clearly differ in terms of T-cell bias from their European counterparts: whereas in Caucasian CRSwNP patients >80% express a Th2 profile with interleukin (IL)-5 secretion, this profile is found in <20% of Chinese CRSwNP patients, who rather show a Th17 bias [11]. Of interest, asthma comorbidity in Caucasians was 14 out of 26 polyp patients, whereas only two out of 29 patients in the Chinese group suffered from asthma.

Thus, there is a difference in asthma comorbidity between CRSsNP and CRSwNP patients, but also within the group of nasal polyp patients, which might be related to the inflammatory profile found within the polyp tissue. In a recent pan-European sinusitis cohort study within the Global Allergy and Asthma European Network (GA^{2}LEN) research programme, we collected clinical data and nasal tissue from 825 patients with CRS; whereas asthma comorbidity was significantly higher in nasal polyp patients (45%), it was not different from the control population in the CRSsNP group (13–18%). In parallel, the prevalence of AERD was 8.2% in the polyp group, and significantly higher than in controls or CRSsNP (1.4–1.5%). As indicated before, these diseases are characterised by different inflammatory profiles. Can we further define the markers within the tissue which predict asthma comorbidity, in order to understand the pathomechanism behind this observation?

Markers associated with asthma comorbidity in nasal polyp patients

In an attempt to identify the mucosal factors associated with asthma comorbidity in nasal polyp disease, we analysed the type of inflammation, the presence of T-cell cytokines and SE-immunoglobulin (Ig)E antibodies in CRSwNP mucosal samples [14]. These studies were performed on 70 Belgian (Caucasian) patients, 34% of them also suffering from asthma, and in Chinese polyp patients, a group with a low asthma comorbidity rate (9%). We demonstrated earlier that nasal polyps from Caucasian *versus* Chinese patients showed a clearly different cytokine profile, providing us with a unique chance to confirm findings in Caucasians in an independent, but also different population.

To differentiate eosinophilic from neutrophilic polyps, we used the eosinophil cationic protein (ECP)/myeloperoxidase (MPO) ratio established previously [11]; 54% of the Belgian, but only 7.5% of the Chinese polyp samples showed a predominantly eosinophilic inflammation, whereas the neutrophilic compound prevailed in all other samples. A cluster analysis approach identified IL-5 as the main positive determinant for the eosinophilic type of inflammation, which could be expected from former studies in nasal polyps [32, 33]. Of note, 83% of Belgian, but only 16% of Chinese polyp samples were IL-5 positive, confirming the remarkable difference between the populations.

Within the Caucasian polyp group, 37% of mucosal tissue samples contained IgE antibodies to SEs *versus* 17% in the Chinese group; however, SE-IgE in tissue was associated with significantly increased total IgE and ECP concentrations in both groups, indicating an amplification of the mucosal immune response. The vast majority of SE-IgE positive samples also were IL-5 positive, suggesting that enterotoxins may need a Th2 background to unfold its activities optimally.

The cluster analysis further identified SE-IgE and increased IgE and ECP concentrations as main positive predictors of comorbid asthma (odds ratios (OR) of 13 and 8, respectively). The prevalence of asthma comorbidity in the Belgian nasal polyp group was 34% overall; however, the prevalence of comorbid asthma was significantly increased among those individuals who showed SE-IgE positivity in nasal polyp tissue (57%) *versus* SE-IgE negative patients (20%, $p<0.01$). These results were confirmed in the Chinese polyp samples.

To summarise, mucosal inflammation in nasal polyps orchestrated by Th2 cytokines and amplified by *Staphylococcus aureus* enterotoxins (SAE) is characterised by an upregulated eosinophilic inflammation and the formation of IgE antibodies. This phenotype is associated with comorbid asthma in Caucasian and Asian patients and is unique for nasal polyps. It is unclear today, if and how this phenotype is related to asthma severity; it is also unclear whether this pathomechanism is limited to the upper airways, or would be valid in the lower airways without any upper airway manifestation; larger studies need to be performed to answer these questions.

SAEs and their effects on mucosal inflammation

S. aureus is a frequent coloniser of the nose, with up to a third of humans in Europe being lifelong carriers of coagulase-positive *S. aureus*, and is specifically frequent in patients with nasal polyps. In studies on the colonisation rate of the middle nasal meatus, which forms the key region at the entrance to the sinuses, we found modest rates for controls and CRSsNP subjects, whereas colonisation in CRSwNP patients was twice as high and reached 64% ($p<0.05$ *versus* CRS) [34]. Colonisation rates were 67% and 87% for polyp patients with asthma and aspirin sensitivity ($p<0.05$ *versus* CRS for both), respectively.

S. aureus may not only colonise the mucosa, but also may reside intramucosally or may form biofilms adherent to the mucosa; current studies focus on the possible role of biofilms in the persistence of *S. aureus*, serving as a reservoir for planktonic germs, which may invade the mucosa

repeatedly [35]. Our group, using peptide nucleic acid-fluorescence *in situ* hybridisation (PNA-FISH), recently discovered intramucosal *S. aureus* especially in polyp tissues from patients with AERD; these germs were shown to even reside intracellularly [36]. With the same technique, others recently added to those findings by showing that *S. aureus* were found in the epithelium of CRSwNP more often than of CRSsNP or control subjects [37]. This group also demonstrated intracellular survival and replication of *S. aureus* within nasal polyp epithelial cells. *S. aureus* recovered from the nose is able to synthesise and release a wide range of enterotoxins, among them the well-studied classical variants A, C, E and toxic shock syndrome toxin (TSST)-1 [38]. SEs are known as strong immune modifiers, and heavily activate T- and B-cells, as well as structural cells.

In animal models it has been demonstrated that SEs are able to aggravate airway inflammation in sensitised mice [39, 40]. Both nasal and bronchial SAE B (SEB) application enhanced the allergen-induced bronchial inflammation and increased the eosinophilic airway inflammation in ovalbumin (OVA)-sensitised mice; the aggravation of experimental asthma correlated with the expression of Th2 cytokines in the bronchi, and Th2 cytokines and OVA-specific and total IgE in the serum. These data illustrate the potential of both nasal as well as bronchial SEB to aggravate features of allergic asthma in a mouse model. These observations were recently extended by studies on the potential of SEB to facilitate sensitisation [41]. In fact, concomitant endonasal application of OVA and SEB resulted in OVA-specific IgE production in the mice, which normally develop tolerance when exposed to endonasal OVA alone. This effect was probably due to augmentation of dendritic cell migration and maturation, as well as the allergen-specific T-cell proliferation upon concomitant OVA and SEB application. The data suggest that SEB not only may aggravate airway inflammation, but also may facilitate IgE formation in animals.

There is a body of evidence that such effects can also be found in human nasal mucosa. We made use of an *ex vivo* human mucosal model to study the effects of *S. aureus*-derived enterotoxin B, protein A and lipoteichoic acid in nasal polyp and control inferior turbinate tissue [42]. Protein A stimulation resulted in a significant increase of histamine, leukotrienes and prostaglandin D_2 (PGD_2), indicating mast cell activation. Enterotoxin B stimulation over a period of 24 hours induced a significant increase of pro-inflammatory and Th2-cytokines, including IL-4, IL-5 and IL-13 in both groups, and also induced the release of IL-2, which activates further T-effector cells. The expression of Th2 cytokines was more rigidly increased than the expression of regulatory cytokines in polyp tissue; this shift skews the T-cell pattern even more into the Th2 direction and at the same time disfavours T-regulatory cell activity, possibly contributing to the persistent severe predominantly Th2-biased inflammation in polyp disease.

Many other effects have been demonstrated for SEs; SEB in a dose-dependent fashion induces chemokine release from epithelial cells, and also may enhance the survival of granulocytes, exerting a direct pro-inflammatory effect on human nasal epithelial cells [43]. However, among the most important activities of SEs is the induction of IgE formation, which has been described in the skin first [44, 45]. However, such activation of B-cells and their transformation into plasma cells can also be shown in nasal polyp tissue [46]. Naïve B-cell and plasma cell counts were significantly higher in polyp tissue compared to controls, and IgA, IgG and IgE concentrations were significantly higher in tissue homogenates, but not in serum of polyp *versus* control subjects. Especially in polyps with SE-IgE, reflecting the impact of enterotoxins on the mucosal inflammation, significantly higher concentrations of total IgE were demonstrated compared to SE-IgE negative samples. These findings support a local production of immunoglobulins including IgE in nasal polyps in response to the chronic microbial trigger, SEs. Further studies need to prove the local switch to IgE synthesis within the polyp tissue, and the role of SEs in this switch; studies in allergic nasal mucosa and also bronchial mucosa from allergic and nonallergic asthmatics have clearly shown that local IgE synthesis occurs, but these studies did not link IgE switching to SEs [47, 48].

Polyclonal IgE in tissue is functional

SEs induce IgE formation and high mucosal IgE concentrations are associated with asthma comorbidity in polyp patients. However, is this polyclonal tissue IgE functional? Would it contribute to the persistence and severity of disease?

As demonstrated in a recent publication, specific IgE antibodies in polyp tissue only constitute a fraction of the total mucosal tissue IgE compared to allergic rhinitis mucosal samples (3% *versus* 15%, $p<0.005$), which is also reflected in the serum of those patients (0.6% *versus* 7%, $p=0.03$) [49]. This is in line with a former publication [50], in which we detailed specific IgE antibodies to four allergen mixtures (grass pollen, house dust mites, moulds and trees, comprising 20 allergens) and additionally to six SAE allergens in a group of 16 nasal polyp patients; we here calculated the specific IgE fraction (to 26 allergens) of the total IgE to be 4% in tissue and 2% in serum. This means that in order to explain 100% of the total IgE, one would need several hundred allergen specific IgE antibodies. We therefore suggest that *S. aureus*-derived superantigens induce a polyclonal IgE formation to multiple inhalant and non-inhalant allergens, driven by the abundant polyclonal activation of T- and B-cells. SEs such as SEB themselves also may serve as allergens in this case and thus may contribute to the continuous mast cell degranulation in nasal polyps.

We also investigated the *ex vivo* degranulation of tissue mast cells upon allergen exposure in nasal polyp tissue from polyp subjects in comparison to inferior turbinates from allergic rhinitis patients to study the functionality of IgE antibodies [49]. The mucosal tissues were stimulated with anti-IgE, and the allergens house dust mite, grass pollen and SEB; mast cell activation was measured as PGD_2 release, as described previously [51]. We furthermore tested the transferability of this IgE reactivity using RBL SX38 cells.

In allergic rhinitis patients, total IgE in serum (101 $kU \cdot L^{-1}$ range (66.2–173.5)) and inferior turbinate tissue homogenates (151 $kU \cdot L^{-1}$ range (43.7–332.8)) highly correlated, as expected ($r=0.91$, $p<0.0001$). Tissue total IgE also significantly correlated to the release of PGD_2 upon anti-IgE exposure; a tissue total IgE of 200 $kU \cdot L^{-1}$ corresponded to a PGD_2 release of 1,095 $pg \cdot mL^{-1}$ upon challenge with 10 $\mu g \cdot mL^{-1}$ anti-IgE. In contrast, in polyp patients, total IgE in serum (141 $kU \cdot L^{-1}$ range (111–210)) and tissue (420 $kU \cdot L^{-1}$ range (264–1311)) did not correlate. Tissue total IgE again significantly correlated to the release of PGD_2 upon anti-IgE exposure; however, a tissue total IgE of 200 $kU \cdot L^{-1}$ corresponded to a PGD_2 release of only 656 $pg \cdot mL^{-1}$ upon challenge, which tended to be lower compared to inferior turbinate release.

Reactivity of tissue mast cells upon allergen exposure and presence of specific IgE antibodies to the allergens corresponded in almost all cases for tissue specific and serum IgE antibodies in the allergic rhinitis patients. However, in nasal polyp patients, such reactivity was dependent on the presence of tissue-specific IgE antibodies, but was dissociated from serum IgE antibodies. Allergens such as grass pollen and house dust mite efficiently degranulated tissue mast cells in polyp patients who did not have specific serum IgE antibodies, clearly demonstrating the functionality of local IgE antibodies and the dissociation from serum IgE findings. Furthermore, tissue homogenates containing specific IgE to grass pollen mediated rat basophilic leukaemia (RBL) cell degranulation after stimulation with grass pollen irrespective of the patient's SPT result. Tissue homogenates with increased IgE but without IgE to grass pollen could not induce any RBL cell degranulation after stimulation with grass pollen. Thus, local tissue IgE to grass pollen and other allergens is clearly functional, although it is polyclonal and locally formed.

We therefore assume that mucosal polyclonal IgE antibodies may induce mast cell degranulation to numerous inhalant and non-inhalant allergens, and postulate that polyclonal IgE antibodies in airway disease contribute to severe persistent inflammation by continuously activating mast cells; this phenomenon may be independent of the serum IgE findings or SPT results of an individual patient. Similar mechanisms may also be relevant in severe lower airway disease with or without upper airway disease [14, 52]. A proof-of-concept study on the role of polyclonal IgE in nasal polyp disease with comorbid asthma has been undertaken to support our concept; the results of

this anti-IgE (omalizumab) treatment study will unravel the usefulness of this concept in the near future [53].

SE-IgE and severe asthma

It is tempting to speculate that the same pathomechanisms may also apply for lower airway disease, with or without comorbid nasal polyposis, as the inflammatory pattern [2, 3] and the findings of local IgE formation [48] resemble those found in polyp disease. In a previous observation the presence of IgE antibodies to SAE, SAE C (SEC) and TSST-1 was found significantly more often in serum from asthmatics compared to controls, especially when comparing severe asthmatics to controls (62% *versus* 13%, $p=0.01$) [52]. Severe asthma was defined by the need for regular high-dose inhaled corticosteroid therapy and, despite this treatment, persistently impaired lung function. These findings for the first time suggested a possible relationship between the presence of IgE antibodies to SEs in serum and the severity of asthma. Independent from our work, COHEN *et al.* [54] recently described a group of 31 severe asthmatics according to ATS criteria in a SARP study, and found serum IgE concentrations of 473 ± 794 IU·mL^{-1} without further comment.

In a collaborative study with M. KOWALSKI *et al.* [55], the specific IgE to SEs, total IgE and ECP concentrations in the serum of asthmatic subjects were measured; this study included 109 patients with severe refractory asthma and 101 patients with non severe asthma according to ATS criteria [34]. A significant risk for severe asthma was associated with known factors such as female sex, history of wheezing in childhood, presence of hypersensitivity to aspirin and body mass index (BMI). However, the mean level of enterotoxin specific IgE was three-fold higher in patients with severe refractory asthma as compared with patients with nonsevere asthma or controls ($p=0.01$). The presence of specific IgE to enterotoxins carried a significant risk for patients to have serum total IgE levels above 100 kU·L^{-1} (OR 7.84), the mean serum total IgE levels were significantly higher in SE-IgE positive as compared to negative patients (187 kU·L^{-1} *versus* 50 kU·L^{-1}, ($p=0.001$)). Of interest, concentrations of SE-IgE antibodies were significantly associated with respiratory function parameters (forced expiratory volume in 1 s (FEV_1), FEV_1/forced vital capacity (FVC), and mid-expiratory flow at 25–75% of FVC ($MEF_{25–75\%}$)) and increased airway reversibility in response to albuterol. Total IgE levels were significantly higher in severe asthmatics as compared to nonsevere asthmatics despite the fact that the prevalence of allergic sensitisation was not different between groups (72–80%). Aspirin hypersensitivity was also significantly more frequent in the severe asthma patients compared to the nonsevere asthmatics (34% *versus* 21%). Interestingly, the number of SE-IgE positive patients was not different in the polyp from the non-polyp group (64–71%), and total serum IgE was even significantly higher in the non-polyp group; this argues for the possibility that the presence of SE-IgE is a phenomenon of severe asthma independent of the comorbidity of nasal polyps.

To summarise, these findings strongly suggest that specific immune responses to enterotoxins are associated with clinical and immunological parameters of asthma severity, and thus suggest a role for SEs in the pathogenesis of severe asthma [20]. The effect of staphylococcal superantigens on the development and severity of asthma could already start in early childhood, as serum SE-IgE can be detected significantly more often in persistent wheezers as early as age 5 years [56], and was highest amongst atopics and associated with asthma risk at the age of 16 years [57]. These findings were supported by recent evidence that *Staphlococcus* species were present in excess in the airways of children with difficult-to-treat asthma [58].

Conclusions

There are different ways of how upper airway diseases could impact on asthma and its severity. Rhinitis, atopic or non-atopic, if moderate-to-severe, is associated with an increased risk of uncontrolled asthma and, in a severe asthma population, an increased number of emergency visits.

Viral infections of the nose are the most common cause of an acute asthma exacerbation in both children and adults, and may lead to inadequately long and symptomatic asthma episodes during common cold periods of the year. The impact of chronic sinus inflammation on asthma has recently been partly unravelled based on a biomarker supported phenotyping of CRS.

CRSwNP in Caucasians carries a high risk of comorbid asthma, with asthma, aspirin intolerance and nasal polyps as the most severe form of airway disease. However, aspirin-tolerant nasal polyposis, in atopic or non-atopic patients, also shares the strongly eosinophilic inflammation and the high local mucosal IgE concentrations when associated with asthma comorbidity. SAEs, acting as superantigens, have been identified as the causal agents, derived from germs which colonise and invade the polyp mucosa, and heavily amplify the local inflammation. Patients with nasal polyps and SE-IgE antibodies in the polyp tissue are at great risk to have high serum total IgE concentrations, blood eosinophilia and high ECP levels, and finally to develop asthma comorbidity of the late-onset non-atopic type. However, atopic asthma may also be amplified by superantigens already in childhood.

There are several consequences from these findings. The upper airways should be routinely included in the clinical investigation for asthma, especially in asthma exacerbations and severe asthma. The serum of severe asthma patients should be tested for total IgE, but also for specific IgE antibodies to SEs. Epidemiological studies need to be performed in severe asthmatics to evaluate the impact of upper airway disease and superantigens in this population. Finally, new therapeutic options should be tested for patients with high serum IgE based on a polyclonal activation by superantigens, which may include antibiotic or anti-IgE strategies.

Statement of Interest

None declared.

References

1. Bousquet J, van Cauwenberge P, Khaltaev N, *et al.* Management of allergic rhinitis and its impact on asthma (ARIA). *J Allergy Clin Immunol* 2001; 108: S147–S334.
2. Bousquet J, Khaltaev N, Cruz AA, *et al.* Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA2LEN and AllerGen). *Allergy* 2008; 63: Suppl. 86, 8–160.
3. Reddel HK, Taylor DR, Bateman ED, *et al.* An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009; 180: 59–99.
4. Bresciani M, Paradis L, Des RA, *et al.* Rhinosinusitis in severe asthma. *J Allergy Clin Immunol* 2001; 107: 73–80.
5. ten Brinke A, Grootendorst DC, Schmidt JT, *et al.* Chronic sinusitis in severe asthma is related to sputum eosinophilia. *J Allergy Clin Immunol* 2002; 109: 621–626.
6. Ceylan E, Gencer M, San I. Nasal polyps and the severity of asthma. *Respirology* 2007; 12: 272–276.
7. Szczeklik A, Nizankowska E, Duplaga M. Natural history of aspirin-induced asthma. AIANE Investigators. European Network on Aspirin-Induced Asthma. *Eur Respir J* 2000; 16: 432–436.
8. Fokkens W, Lund V, Mullol J, *et al.* European position paper on rhinosinusitis and nasal polyps. *Rhinology* 2007; 20: 1–136.
9. Van Zele T, Claeys S, Gevaert P, *et al.* Differentiation of chronic sinus diseases by measurement of inflammatory mediators. *Allergy* 2006; 61: 1280–1289.
10. Van Bruaene N, Perez-Novo C, Basinski T, *et al.* T-cell regulation in chronic paranasal sinus disease. *J Allergy Clin Immunol* 2008; 121: 1435–1441.
11. Zhang Nan., Van Zele T., Perez-Novo C., et al. Different types of T effector cells orchestrate mucosal inflammation in chronic sinus disease. *JACI* 2008; 122: 961–968.
12. Van Bruaene N, Derycke L, Perez-Novo CA, *et al.* TGF-β signaling and collagen disposition in chronic rhinosinusitis. *JACI* 2009; 124: 253–259.
13. Li X, Meng J, Qiao X, *et al.* Expression of TGF, matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in Chinese chronic rhinosinusitis. *J Allergy Clin Immunol* 2010; 125: 1061–1068.
14. Bachert C, Zhang N, Holtappels G, *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: 962–968.
15. Moore WC, Bleecker ER, Curran-Everett D, *et al.* Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol.* 2007; 119: 405–413.

16. Moore WC, Meyers DA, Wenzel SE, *et al.* Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010; 181: 315–323.
17. Bousquet J, Mantzouranis E, Cruz AA, *et al.* Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma. *J Allergy Clin Immunol* 2010; 126: 939–940.
18. Bachert C, Gevaert P, Holtappels G, *et al.* Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001; 107: 607–614.
19. Zhang N, Gevaert P, van Zele T, *et al.* An update on the impact of *Staphylococcus aureus* enterotoxins in chronic sinusitis with nasal polyposis. *Rhinology* 2005; 43: 162–168.
20. Bachert C, Claeys SE, Tomassen P, *et al.* Rhinosinusitis and asthma: a link for asthma severity. *Curr Allergy Asthma Rep* 2010; 10: 194–201.
21. Magnan A, Meunier JP, Saugnac C, *et al.* Frequency and impact of allergic rhinitis in asthma patients in everyday general medical practice: a French observational cross-sectional study. *Allergy* 2008; 63: 292–298.
22. Vandenplas O, Dramaix M, Joos G, *et al.* The impact of concomitant rhinitis on asthma-related quality of life and asthma control. *Allergy* 2010; 65: 1290–1297.
23. Ponte EV, Franco R, Nascimento HF, *et al.* Lack of control of severe asthma is associated with coexistence of moderate-to-severe rhinitis. *Allergy* 2008; 63: 564–569.
24. Jackson DJ, Johnston SL. The role of viruses in acute exacerbations of asthma. *J Allergy Clin Immunol* 2010; 125: 1178–1187.
25. Papadopoulos NG, Xepapadaki P, Mallia P, *et al.* Mechanisms of virus-induced asthma exacerbations: state-of-the-art. A GA^{2}LEN and InterAirways document. *Allergy* 2007; 62: 457–470.
26. Gern JE, Galagan DM, Jarjour NN, *et al.* Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. *Am J Respir Crit Care Med* 1997; 155: 1159–1161.
27. Johnston SL, Pattemore PK, Sanderson G, *et al.* Community study of role of viral infections in exacerbations of asthma in 9–11 year old children. *BMJ* 1995; 310: 1225–1228.
28. João Silva M, Ferraz C, Pissarra S, *et al.* Role of viruses and atypical bacteria in asthma exacerbations among children in Oporto (Portugal). *Allergol Immunopathol (Madr)* 2007; 35: 4–9.
29. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993; 307: 982–986.
30. Tarlo SM, Broder I, Corey P, *et al.* The role of symptomatic colds in asthma exacerbations: influence of outdoor allergens and air pollutants. *J Allergy Clin Immunol* 2001; 108: 52–58.
31. Corne JM, Marshall C, Smith S, *et al.* Frequency, severity, and duration of rhinovirus infections in asthmatic and nonasthmatic individuals: a longitudinal cohort study. *Lancet* 2002; 359: 831–834.
32. Bachert C, Wagenmann M, Hauser U, *et al.* IL-5 is upregulated in human nasal polyp tissue. *J Allergy Clin Immunol* 1997; 99: 837–842.
33. Simon HU, Yousefi S, Schranz C, *et al.* Direct demonstration of delayed eosinophil apoptosis as a mechanism causing tissue eosinophilia. *J Immunol* 1997; 158: 3902–3908.
34. Van Zele T, Gevaert P, Claeys G, *et al. Staphylococcus aureus* colonization and IgE antibody formation to enterotoxins is increased in nasal polyposis. *J Allergy Clin Immunol* 2004; 114: 981–983.
35. Foreman A, Psaltis AJ, Tan LW, *et al.* Characterization of bacterial and fungal biofilms in chronic rhinosinusitis. *Am J Rhinol Allergy* 2009; 23: 556–561.
36. Corriveau MN, Zhang N, Holtappels G, *et al.* Detection of *Staphylococcus aureus* in nasal tissue with peptide nucleic acid – fluorescence *in situ* hybridization. *Am J Rhinol Allergy* 2009; 23: 461–465.
37. Sachse F, Becker K, von Eiff C, *et al. Staphylococcus aureus* invades the epithelium in nasal polyposis and induces IL-6 in nasal epithelial cells *in vitro*. *Allergy* 2010; 65: 1430–1437.
38. Van Zele T, Vaneechoutte M, Holtappels G, *et al.* Detection of enterotoxin DNA in *S. aureus* strains obtained from the middle meatus in controls and nasal polyp patients. *Am J Rhinol* 2008; 22: 223–227.
39. Hellings PW, Hens G, Meyts I, *et al.* Aggravation of bronchial eosinophilia in mice by nasal and bronchial exposure to *Staphylococcus aureus* enterotoxin B. *Clin Exp Allergy* 2006; 36: 1063–1071.
40. Herz U, Rückert R, Wollenhaupt K, *et al.* Airway exposure to bacterial superantigen (SEB) induces lymphocyte-dependent airway inflammation associated with increased airway responsiveness-a model for nonallergic asthma. *Eur J Immunol* 1999; 29: 1021–1031.
41. Huvenne W, Callebaut I, Plantinga M, *et al. Staphylococcus aureus* enterotoxin B facilitates allergic sensitization in experimental asthma. *Clin Exp Allergy* 2010; 40: 1079–1090.
42. Patou J, Van Zele T, Gevaert P, *et al. Staphylococcus aureus* enterotoxin B, protein A and lipoteichoic acid stimulations in nasal polyps. *J Allergy Clin Immunol* 2008; 121: 110–115.
43. Huvenne W, Callebaut I, Reekmans K, *et al. Staphylococcus aureus* enterotoxin B augments granulocyte migration and survival *via* airway epithelial cell activation. *Allergy* 2010; 65: 1013–1020.
44. Hofer MF, Lester MR, Schlievert PM, *et al.* Upregulation of IgE synthesis by staphylococcal toxic shock syndrome toxin-1 in peripheral blood mononuclear cells from patients with atopic dermatitis. *Clin Exp Allergy* 1995; 25: 1218–1227.
45. Hofer MF, Harbeck RJ, Schlievert PM, *et al.* Staphylococcal toxins augment specific IgE responses by atopic patients exposed to allergen. *J Invest Dermatol* 1999; 112: 171–176.

46. Van Zele T, Gevaert P, Holtappels G, *et al.* Local immunoglobulin production in nasal polyposis is modulated by superantigens. *Clin Exp Allergy* 2007; 37: 1840–1847.
47. Durham SR, Gould HJ, Thienes CP, *et al.* Expression of epsilon germ-line gene transcripts and mRNA for the epsilon heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. *Eur J Immunol* 1997; 27: 2899–2906.
48. Takhar P, Corrigan CJ, Smurthwaite L, *et al.* Class switch recombination to IgE in the bronchial mucosa of atopic and nonatopic patients with asthma. *J Allergy Clin Immunol* 2007; 119: 213–218.
49. Zhang N, G Holtappels., P Gevaert., et al. Mucosal tissue polyclonal IgE is functional in response to allergen and SEB. *Allergy* 2010; 166: 141–148.
50. Gevaert P, Holtappels G, Johansson SGO, *et al.* Organisation of secondary lymphoid tissue and local IgE formation to *Staphylococcus aureus* enterotoxins in nasal polyp tissue. *Allergy* 2005; 60: 71–79.
51. Patou J, Holtappels G, Affleck K, *et al.* Enhanced release of IgE-dependent early phase mediators from nasal polyp tissue. *J Inflamm* 2009; 6: 11.
52. Bachert C, Gevaert P, Howarth P, *et al.* IgE to *Staphylococcus aureus* enterotoxins in serum is related to severity of asthma. *J Allergy Clin Immunol* 2003; 111: 1131–1132.
53. Verbruggen K, Van Cauwenberge P, Bachert C. Anti-IgE for the treatment of allergic rhinitis – and eventually nasal polyps? *Int Arch Allergy Immunol* 2008; 148: 87–98.
54. Cohen L, Xueping E, Tarsi J, *et al.* Epithelial cell proliferation contributes to airway remodeling in severe asthma. *Am J Respir Crit Care Med* 2007; 176: 138–145.
55. Kowalski ML, Cie●lak M, Pérez-Novo CA, *et al.* Clinical and immunological determinants of severe/refractory asthma (SRA): association with Staphylococcal superantigen-specific IgE antibodies. *Allergy* 2011; 66: 32–38.
56. Semic-Jusufagic A, Bachert C, Gevaert P, *et al. Staphylococcus aureus* sensitization and allergic disease in early childhood: population-based birth cohort study. *J Allergy Clin Immunol* 2007; 119: 930–936.
57. Hollams EM, Hales BJ, Bachert C, *et al.* Th2-associated immunity to bacteria in teenagers and susceptibility to asthma. *Eur Respir J* 2010; 36: 509–516.
58. Hilty M, Burke C, Pedro H, *et al.* Disordered microbial communities in asthmatic airways. *PLoS One* 2010; 5: e8578.