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Allergy in Severe Asthma

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ABSTRACT

It is well recognized that atopic sensitisation is an important risk factor for asthma, both in adults and in children. However, the role of allergy in severe asthma is still under debate. The term "Severe Asthma" encompasses a highly heterogeneous group of patients who require treatment on steps 4–5 of GINA guidelines to prevent their asthma from becoming "uncontrolled", or whose disease remains "uncontrolled" despite this therapy. Epidemiological studies on emergency room visits and hospital admissions for asthma suggest the important role of allergy in asthma exacerbations. In addition, allergic asthma in childhood is often associated with severe asthma in adulthood. A strong association exists between asthma exacerbations and respiratory viral infections, and interaction between viruses and allergy further increases the risk of asthma exacerbations. Furthermore, fungal allergy has been shown to play an important role in severe asthma. Other contributing factors include smoking, pollution and work-related exposures. The "Allergy and Asthma Severity" EAACI Task Force examined the current evidence and produced this position document on the role of allergy in severe asthma.

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INTRODUCTION

Numerous epidemiological studies have demonstrated that atopic sensitisation is a strong risk factor for asthma in childhood(1, 2) and adulthood(3), both in the developed(3) and in the developing countries(1, 2, 4), supporting the notion that asthma is in part an allergic disease. However, the role of allergy in severe asthma remains the issue of considerable controversy. The term "severe asthma" encompasses a highly heterogeneous group of patients, which is defined in various ways in the literature(5). Recent international guidelines define "severe asthma" as asthma which requires treatment at GINA steps 4–5 during the previous year or systemic corticosteroids (CS) for ≥50% of the previous year to prevent it from becoming ''uncontrolled'', or asthma which remains ''uncontrolled'' despite this therapy, or controlled asthma that worsens on tapering high doses of inhaled corticosteroids (ICS), systemic CS or additional biologics(6).

Asthma exacerbations are one of the key features of severe asthma. Emergency room visits and hospital admissions due to acute asthma attacks are increased in children who are sensitised and exposed to high levels of inhalant allergens in their homes, emphasising the importance of "allergy" in asthma exacerbations(7). The phenotypes of childhood onset allergic asthma and early sensitisation are often associated with severe asthma in adulthood(8). However, some data indicated that the proportion of severe asthma cases attributable to allergy may be overestimated, and that aetiological mechanisms other than allergy may be important in the pathogenesis of severe asthma. For example, numerous studies have reported a strong association between asthma exacerbations and respiratory viral infections, suggestive of a viral-induced mechanism. Rather than being mutually exclusive, viruses and allergens may interact in increasing the risk of asthma development(9).

Furthermore, fungal sensitisation is strongly associated with severe asthma, hence, recently a new subtype of Severe Asthma with Fungal Sensitization (SAFS) has been proposed(10).

Finally, the role of several co-factors, such as smoking, pollution and work-related exposures must be considered when evaluating a patient with severe asthma.

The "Allergy and Asthma Severity" EAACI Task Force produced this position document on the role of allergy in severe asthma, searching the literature of the last 10 years in the main databases (MEDLINE, Scopus, ISI) and including milestone and important papers at the discretion of the different co-authors.

DEFINITION AND ROLE OF INHALANT ALLERGENS IN ASTHMA

Atopy, allergy and asthma

The association between atopy and asthma appears specific to inhalant allergens(4). In general, atopic sensitisation is defined either when allergen-specific serum IgE (sIgE) are detected, or a positive skin prick test (SPT) to extracts made from whole allergen sources(11, 12), often using arbitrary cut-off points of sIgE>0.35 KU/L, or a mean wheal diameter ≥3mm. These standard allergy tests have high sensitivity, but in themselves do not signify disease. For example, a considerable proportion of such defined sensitised individuals have no evidence of asthma(13), and a positive test in an asthmatic patient does not always result in clinical response upon allergen exposure. Thus, there is a difference between allergic asthma with asthma symptoms induced by exposure to a defined allergen, and asthma in a subject characterized as "sensitised" with no relation between allergen exposure and clinical reaction. It has been suggested that a positive allergy test (assessed either by sIgE or SPT) should not be considered as a sole diagnostic marker of atopic sensitisation(14).

Quantification of atopic sensitisation increases the specificity in relation to asthma presence and severity

The last decade has seen the shift in the way we interpret the results of IgE and SPTs. The sum of the levels of specific IgE antibodies (or the summative size of SPT wheals) to inhalant allergens is a better predictor of the onset, presence, persistence and severity of childhood asthma than the mere presence of a "positive allergy test"(15-17). The clinical importance of "quantitative atopic sensitisation" has been confirmed in subsequent studies in adult asthma(18). It is now recognized that quantification of atopic sensitisation in early life amongst young children with wheezing is one of the best discriminators to identify those who are at high risk of subsequent development of persistent asthma(19).

Additionally, a clear quantitative relationship between the level of sIgE and the size of SPT responses has been observed in relation to asthma severity, both in adults and in children(20, 21). For example, one of the phenotypic characteristics of severe treatment-resistant asthma (STRA) in childhood is the large size of SPT wheals to inhalant and food allergens. In patients with STRA, results of sIgE measurements and SPTs are not always concordant, indicating the need to carry out

both tests(17, 20). The level of sIgE is also associated with an increased risk of severe asthma exacerbations requiring hospitalization among both children(17, 22) and adults(23). Finally, it has been shown that there is a strong interaction between the levels of sIgE to inhalant allergens and respiratory virus infections in increasing the risk of severe asthma exacerbations requiring hospital admission(24), suggesting a synergism between quantitative sensitisation and respiratory virus infections. This synergism has been indirectly confirmed in a study showing that pre-seasonal anti-IgE- targeted therapy with omalizumab decreases seasonal exacerbations of asthma ("back-to-school asthma"), which are almost certainly (rhino)virus-induced(25). In contrast, a recent study showed that although impaired IFN-β and IFN-λ induction by rhinovirus was a feature of bronchial epithelial cells from highly sensitised children with STRA(26), there was no relationship between sensitisation and Th2-mediated inflammation with impaired interferon production, raising a possibility of two independent mechanisms (atopy-related and virus-related).

All of the above data indicate that in the assessment of patients with asthma (including severe asthma), the results of specific IgE measurement and SPT are not mutually exclusive but complementary, and should not be reported as being "positive" or "negative", but as the level of sIgE and the size of SPT wheal diameter (*i.e.,* quantified). For SPTs, the size of the positive and negative control should be taken into account. Recent data suggest that diagnostic accuracy of specific IgE antibody measurement in the context of asthma and the distinction between "benign" atopy (*i.e.,* sensitisation in the absence of allergic symptoms) and "pathologic" atopy (*i.e.,* sensitisation related to allergic symptoms), may be improved by the measurement of allergen-specific IgG antibody levels(27), although their measurement is not recommended routinely.

Heterogeneity of atopic sensitisation

It has recently been proposed that "atopic sensitisation" maybe an umbrella term for a collection of several different subgroups of sensitisation which differ in their association with asthma and other allergic diseases(14). Distinct subgroups (or classes) of sensitisation were described in one population-based birth cohort (Manchester Asthma and Allergy Study) by applying a machine learning approach with Bayesian inference to the SPTs and sIgE data collected longitudinally from early life to school age(28), and similar latent structure was subsequently described using comparable approach to longitudinal data on atopic sensitisation in another birth cohort (Isle of Wight study)(14). Children who would be considered sensitised using conventional definitions were clustered into four distinct subgroups characterised by a unique pattern of the responses to different allergens and the timing of onset of allergen-specific sensitisation(28) (Figure 1). Importantly, the

risk of asthma was increased more than 20-fold amongst children belonging to one of these subgroups (those sensitised to multiple allergens in early life - comprising less than one third of the sensitised children), but not amongst those in other classes(14, 28). Striking similarities were observed in the association between different subgroups of atopic sensitisation in these two cohorts in relation to asthma severity, with children in the subgroup of sensitisation characterised by IgE responses to multiple allergens in early life having higher FeNO levels, more hyperreactive airways, an increased risk of severe asthma exacerbations having significantly diminished lung function, compared to all other classes(14, 28, 29). It is of note however, that such subtypes (clusters/classes) of sensitisation can only be identified using statistical inference on longitudinal data(14, 28), and that differentiation between different clusters at any single cross-sectional point is not yet possible(30). Clinical translation of this important observation requires the development of specific and sensitive biomarkers which can be measured at the time of presentation to clinic and which aid differentiation between different sensitisation subgroups. Recent data indicate that IgE responses to individual allergenic molecules rather than whole allergen extracts may prove useful in differentiating the subtypes of sensitisation relevant to asthma onset and severity(31, 32).

Progression from Atopic Dermatitis to Allergic Asthma – fact or myth?

Although atopic dermatitis (AD) usually precedes allergic asthma or rhinitis, a clear causal relationship for the typical sequence in the development of these diseases – formerly termed as the 'atopic march' – remains to be confirmed. Recent analysis among 10,000 children followed from birth to school age, has demonstrated that, whilst point prevalence data for the whole population may show a profile consistent with the atopic march, modelling within individual data over the life course shows seven different patterns, with >94% of children with symptoms (AD, wheeze and rhinitis) during childhood not following the atopic march profile(33). Therefore, the atopic march may be just an epiphenomenon of different allergic subtypes occurring at similar time points of the individual development (co-manifestation), e.g. early-life wheeze and early-life sensitization. Evidence from longitudinal studies suggests that approximately one-third of patients with AD develop asthma and two-thirds develop allergic rhinitis support the hypothesis of an underlying common mechanism. A review of four population-based cohort studies with a minimum of 80% follow-up, confirmed that early-life AD (especially IgE-associated AD) is a significant risk factor for developing asthma later in life (pooled OR 2.14; 95% CI 1.76–2.75)(34). Interestingly, in two of these cohorts, the significant association of early-life eczema and asthma disappeared when adjusted for early-life wheeze and sensitization, but was still present when adjustment was confined to early-life wheeze, suggesting that sensitization is a major common factor. It also points to a

putative mechanism where AD may increase the risk of subsequent sensitization, which in turn increases the risk of asthma.

Filaggrin gene (FLG) mutations are associated both with atopic and nonatopic eczema starting in the first year of life. FLG mutations combined with eczema in the first year of life are associated with a later development of asthma and hay fever, and this may support the latter mechanism(35). This more modern view of the atopic march is furthermore strongly supported by recent data on the defective skin barrier function as the key factor for the pathogenesis of AD(36). Skin barrier dysfunction facilitates transdermal dehydration and infiltration of allergens, bacteria and bacterial toxins, thus inducing and enhancing allergen sensitization as a hallmark of the atopic march(37). Skin sensitization is followed by airway sensitization to the same allergen and is one of the most robust predictors for the development of childhood asthma(38). This is detailed further on in this review. In conclusion, there is evidence for the hypothesis linking AD as an initial (but probably not only) promoter of atopy/allergic sensitization with progression to asthma.

Component-resolved diagnostics in asthma

Recent advances in biochemistry and molecular biology have led to the isolation and characterisation of numerous allergenic proteins (components), facilitating the profiling of IgE reactivity to individual allergens at a molecular level. This new approach to allergy diagnosis has been termed molecular diagnosis or component-resolved diagnostics (CRD), and its commercialisation has facilitated the development of products in which sIgE to >100 allergen components can be measured simultaneously. Component resolved diagnostics may help in identifying patients at risk of developing more severe disease(31, 32). Sensitization to mite allergens Der p 2 and Der f 2 has been reported to be more common in severe asthma (39). Latex allergy and asthma is another example were sensitization to 3 out of 12 recombinant natural rubber antigens (5, 6.01/6.02), was strongly linked to those with latex sensitization and asthma (40)

The role of these novel tools in clinical practice and how best to interpret the complex data they generate is the subject of ongoing debate(41, 42). It has recently been reported that CRD may improve the assessment of asthma(31, 43), and help better understanding the role of allergy in severe asthma in childhood(44). However, it is likely that better interpretation algorithms are needed to capitalise fully on the potential of this exciting new technology(43).

SIMILARITIES AND DISTINCTIONS BETWEEN ADULT AND PAEDIATRIC SEVERE ASTHMA

A fundamental feature of severe asthma in both adults and children is its heterogeneity, with multiple clinical phenotypes(6, 45-50). When unsupervised cluster analyses are performed, whether in adults or children, several common clinical features provide phenotypic distinctions, including the age of onset of disease, presence of co-morbidities, differences in lung function and the degree of atopic sensitisation(50-52). Using this approach, it appeared that the role of atopic sensitisation might be more important in the pathogenesis of severe asthma in early life. Severe atopy, characterised by polysensitisation and high specific IgE levels, is integral to childhood severe disease, such that >85% of children with severe asthma are severely atopic(53). In concurrence, when phenotypic clusters are investigated in adults with severe asthma, the single most important factor that repeatedly distinguishes the importance of allergy is age of disease onset(45). The phenotype of childhood onset asthma is robust, is repeatedly identified in adult cluster analyses and is undoubtedly associated with very severe allergic disease(8). In contrast, severe adult onset asthma is a distinct phenotype that is usually not characterised by atopic sensitisation, but often associated with nasal polyposis and sputum eosinophilia(54).

Atopy and paediatric severe asthma

The importance of early atopic sensitisation contributing to childhood severe asthma is reflected in the evidence of early sensitisation in preschool children being the main predictor of asthma development by school age(19, 55). In addition, even though recurrent wheezing episodes caused by rhinovirus infections in the first 3 years of life strongly predict asthma development(56), early atopic sensitisation is the main risk factor determining progression to asthma(56). Moreover, the pattern of atopic sensitisation to inhalant allergens, in particular to perennial ones, and the level of specific IgE increase asthma risk(57).

The significant contribution of allergy to the pathogenesis of paediatric severe asthma is apparent from the clinical features that distinguish patients with difficult asthma (who have underlying modifiable factors) from those with genuine severe therapy resistant asthma (STRA)(58). Significantly, more patients with STRA are polysensitised, and have food allergy. Perhaps the most important distinctive feature of STRA becomes apparent when atopic sensitisation is quantified(18,

59). Patients with severe asthma have a much higher allergic burden(51, 60) suggesting that atopic sensitisation plays a critical role in the development, progression and persistence of paediatric severe disease.

Adult onset, severe asthma: an age-specific phenotype

Adult onset asthma is a recognised phenotype of severe asthma, presenting with several sub-phenotypes(61). Although it is considered predominantly non-allergic, a significant proportion of patients with adult onset disease are atopic (34%)(61). In those with severe disease, a worse prognosis is apparent in smokers and ex-smokers(62), and, as described later on, smoke exposure has a detrimental effect on severe asthma, resulting in reduced corticosteroid responsiveness, regardless of age(63). Distinguishing and specific features of adult onset asthma include association with co-morbidities, such as obesity, and a predominance in middle-aged women(64). The adult-onset obese, female predominant phenotype is characterised by the absence of inflammation and atopic sensitisation. Although this specific set of features is seen in adults, mechanisms resulting in obesity-associated asthma may not be dissimilar in children and adults. Children with severe asthma who have a higher BMI are less likely to have detectable inflammatory Th2 cytokines and have relatively higher lung function than those with lower BMI(53).

Another common adult-onset phenotype includes severe (non-allergic) eosinophilic phenotype, which is the most prevalent phenotype of severe asthma in adults, associated with aspirin sensitivity, nasal polyposis and eosinophilia, all persisting despite the treatment with high doses of inhaled corticosteroids(54). Innate immune mechanisms underlying this phenotype have recently been proposed since it has become apparent that patients respond to anti-IL-5 antibody therapies(65).

Contribution of allergy to mechanisms underlying severe asthma

The role of allergy in severe asthma needs to be understood to help identify underlying mechanisms of disease progression which will impact both on the choice of add-on therapies and on the discovery of novel therapeutics. Even though the majority of children and adults with early-onset severe asthma are sensitised, it is interesting that not all respond to treatment with omalizumab(66, 67)suggesting several different mechanisms contributing to the development of different allergic phenotypes.

Typically, the allergic asthma phenotype is associated with eosinophilia, elevated serum IgE and Th2 cytokines. However, in adult-onset asthma, eosinophilia may be present without overt evidence of allergy(65). The limited contribution of allergy to disease persistence is apparent in adults with severe asthma who show a non-allergic, inhaled corticosteroid "resistant" eosinophilic phenotype, which responds to systemic CS and targeted therapy with anti-IL-5 (mepolizumab)(68). Novel mechanisms that may contribute to this adult-onset phenotype include epithelial innate cytokines that directly induce the recruitment of innate lymphoid cells which secrete Th2/"allergic" cytokines without the generation of IgE or an adaptive immune response(69). Interestingly, even though it is thought that this is an innate, non-adaptive, non-allergic immune response, all murine experimental models investigating the role of innate cytokines in asthma pathogenesis used allergen exposure as the stimulus, suggesting allergy still plays a central mechanistic role in this phenotype(70). It is possible that allergy is a risk factor in the development of adult-onset "non-allergic" eosinophilic asthma, but the clinical manifestation of asthma changes with time and age, whereby it is less overtly "allergic", but remains eosinophilic.

In asthma, the effect of innate immunity eliciting Th2 responses seems to be strongly related to IL-33(71), and is especially associated with severe disease. IL-33 expression is increased in bronchial tissue from both adults(72, 73) and children(74)with severe asthma. Other important features of innate cytokines that may contribute to the pathogenesis of severe disease in both adults and children include their role in (relative) corticosteroid resistance(74) and their association with angiogenesis and airway remodelling, in particular as regards IL-25 (74-76).

An interesting distinction of adult asthma phenotypes based on gene expression of periostin by airway epithelial cells includes the separation in Th2 high and Th2 low phenotypes(77), and the utility of this biomarker to predict therapeutic response to antibodies that block Th2 cytokines(78). Although biomarkers that allow such distinctions have not yet been identified in children, and while in general children with severe asthma have low or undetectable Th2 cytokines in airway samples, there is a sub-group in whom Th2 cytokines can be detected(53), emphasising similarities between adult and childhood disease.

CROSS-TALK BETWEEN ENVIRONMENTAL FACTORS, ATOPIC SENSITISATION AND ASTHMA

The airway epithelial barrier

Environmental stimuli, such as viruses, bacteria and air pollutants, are known activators of innate immunity and may thus enhance the airway inflammation in asthmatic patients. Allergens, apart from being recognised by the adaptive immunity, may also play a crucial role in activating innate immunity through proteases, biologically active glycolipids and enzymes(79). The airway epithelial barrier, for long time perceived as only a mechanical barrier, is now also recognised as a gate to initiate atopic sensitization and allergic inflammation(80). Epithelial cells recognise the allergens with the help of pattern recognition receptors and produce an innate immune response. As apical junctional complexes between the airway epithelium cells are being disrupted by viral infections and inhaled airway irritants, they facilitate the entry of allergens from the lumen to be presented to the dendritic cells.

In bronchial biopsies and brushings especially from more severe asthmatic patients, airway epithelium cells showed structural and functional defects in apical junctional complexes compared to healthy controls(81). However, this reduced barrier function was found to be reversible by epidermal growth factor (EGF) treatment(81).

The role of microbiota

Early life airway and gut microbiota and influencing factors such as the delivery method, feeding practices, antibiotic use and living environment were shown to be related with allergic asthma development(82). Both the microbial burden and diversity within the lower airways were shown to be significantly higher in suboptimally controlled asthmatic patients compared to healthy individuals(83). Protobacteria species significantly predominated in asthmatic patients using inhaled corticosteroids and showed the strongest correlations with the degree of bronchial hyperresponsiveness(82). In addition, corticosteroid resistance in asthmatic patients was found to be related to airway microbiome diversity(84). In these patients, Haemophilus parainfluenza dominated the microbiome, and was shown to inhibit the response to corticosteroid treatment compared to corticosteroid responsive asthmatic patients. Microbial diversity was also shown to increase the risk of rhinovirus-induced asthma exacerbations in children(85). If rhinovirus existed concomitantly with Moraxella catarrhalis, Streptococcus pneumoniae, or Haemophilus influenzae within the airways, the risk of asthma exacerbations was found to be significantly increased as compared to children without these pathogens.

Viruses

The interaction between viral lower respiratory tract infections (LRTI) and atopic sensitization has been recognized as a major factor contributing to asthma development and exacerbation(86, 87). Birth cohort studies provide strong evidence for a synergistic effect of viral LRTI and atopic sensitization on asthma inception particularly in predisposed children(56, 88). Other factors reported to increase the risk of asthma development include the type of virus (more than 10-fold increased risk for asthma development with rhinovirus compared to 5-fold with respiratory syncytial virus), the severity of viral LRTI, the age during viral LRTI and the atopic predisposition(89). Very recently, the number of respiratory episodes in the first years of life, but not the particular viral trigger, was reported to be associated with later asthma development(90).

Respiratory viral infections in combination with atopic sensitisation and exposure to allergens increase the risk of hospital admission due to asthma exacerbation both in children(91) and adults(92). Rhinoviruses (RV), especially RV-C group, are the most frequent viruses detected during an asthma exacerbation(22)including severe asthma exacerbations with near fatal and fatal asthma(23). Also, allergic asthmatic individuals experience more severe and prolonged LRTI symptoms with RV infection compared to non-atopic healthy controls(93). Biological mechanisms including impaired innate or altered adaptive immune function, abnormal airway structure and function following prior infections, genetic influences and extrinsic factors, such as maternal smoking, air pollution and nutritional factors (vitamin D), may explain the altered immune response to viral infections in asthmatic/allergic patients(87). Recently, antibody titers to species specific RV infection in children during asthma exacerbation showed that antibody response to RV-C is low even when the virus was detected, pointing to a divergent and possibly less efficacious immune response to this subtype compared to RV-A and B(94). The association of susceptibility to RV infection in asthma was also investigated in human bronchial epithelial cells showing impaired interferon production to the virus in severe therapy resistant allergic asthmatic children(26) but normal responses in well controlled asthmatic adults who were mostly atopic(95). In contrast to RV data, interferon responses to influenza A virus and RSV in human bronchial epithelial cell cultures were preserved in adults with mild to severe asthma(96).

Outdoor, indoor and food allergens

Relationships between different types of allergens (outdoor, indoor, food) and the development and severity of allergic disease, including asthma, have been studied(97). For instance, pollen allergy has been found to be interrelated with various food allergies, digestive system Th2-inflammation and asthma(98, 99). Cross-reactivity between pollen and several plant-derived foods, nuts, and fruits has been well established(98). Food allergy without concomitant asthma has been found to be associated with increased nonspecific bronchial hyperresponsiveness(100, 101), while several studies report that children with asthma and concomitant food allergy have more severe disease, poorer control, greater morbidity, and require more anti-asthma medications(102, 103).

The most common indoor allergens associated with asthma include house dust mites, domestic animals (cats, dogs), and cockroaches(97, 104), while fungi can be found both indoor and outdoor. In a cohort of 300 asthmatic children (aged 4-12 y), higher *Der p* 1 and pet allergen levels were found to be associated with greater asthma severity(105).

Fungal exposure is universal and fungi can be linked to asthma in a variety of ways. Fungal allergy drives asthma severity and long-term or uncontrolled fungal infections are associated with a poor control of asthma, complications such as bronchiectasies and chronic allergic bronchopulmonary aspergillosis (ABPA)(106). In the general asthma population, sensitization to moulds ranges from 7 to 20%, in severe asthma patients from 35 to 75%, being 54-91% in life-threatening asthma population(107-111). The first evidence of the link between the severity of asthma and fungal sensitisation dates to 1978, when Schwartz et al. demonstrated a relationship between asthma severity and *Aspergillus spp* sensitisation(112). *Alternaria* or *Cladosporium spp* sensitisation was associated with asthma severity in the European Community Respiratory Health Survey. Furthermore, a recent paper has shown that fungal sensitisation in children with persistent asthma is associated with disease severity(113)and a 2014 review has shown increasing evidence that sensitized asthmatic children may be susceptible to asthma exacerbations when exposed to outdoor fungal spores and that the severity of exacerbation may vary with different fungi species(114).

The term “Severe Asthma with Fungal Sensitisation” (SAFS) was introduced by Denning et al. in 2006, to describe those patients who have persistent severe asthma (despite standard treatment) and evidence of fungal sensitisation, as defined by positive SPT, or fungus or fungal antigen-specific sIgE, and do not meet the criteria for ABPA(10). Proposed classification by an EAACI Task Force sets the total IgE cut-off at <1000 IU/ml for SAFS and >1000 IU/ml for ABPA. ABPA was accepted as an endotype(115), while SAFS remains a pragmatic definition(106). ABPA may develop in asthmatics with a genetic predisposition and therefore SAFS may have the same

background. Carefully genotyping patients with different forms of asthma may allow a better understanding of this disease.

"Trichophyton Asthma" is another clinical entity, where inhalation or the presence of cutaneous infection (athlete's foot, onychomycosis) in sensitised asthmatics is associated with disease severity(106, 116).

Smoking

Cigarette smoking itself may influence asthma, as it accelerates lung function decline(117), impairs the response to CS (both inhaled and oral)(118), increases airway oxidative stress(119), perpetuates symptoms despite of treatment(120) and induces the change of inflammatory phenotypes into more aggressive ones(121), thereby resulting in a more severe disease(122).

Smoking also increases serum IgE levels, especially in men(123). This may result in an increased risk of allergic sensitisation, at least for occupational allergens(124). However, the relationship between cigarette smoking and allergy in severe asthma is still debated: some studies identify smoking as a risk factor for allergic asthma(125), while others show a lower prevalence of atopic sensitisation in smoking patients with severe asthma(121). According to a large epidemiological survey (ECRHS II), smoking was more strongly associated with severe asthma in men than in women, particularly if they were sensitised to moulds (*Cladosporium*), house dust mites or cats(126). Even more conflicting data come from studies on the effect of passive smoking on the risk of development of atopic sensitisation(127).

Cigarette smoking usually results in a more neutrophilic airway inflammation, which is less responsive to ICS(121). Accordingly, alveolar macrophages from smokers have a reduced cellular CS responsiveness, which is associated with reduced histone deacetylase activity, an essential molecule for anti-inflammatory genes transcription(63, 128). In fact, they show an elevated glucocorticoid receptors (GR) ratio in PBMC which is in favour of GR-β (not able to induce any transcriptional activity) compared to GR-α (the active isoform with anti-inflammatory effects)(129). These molecular events make smoking asthmatics less responsive to CS, currently the standard controller therapy for asthma, leading them to a more probable evolution to severe asthma (figure 2).

Recently, a new distinct phenotype of severe asthma has been identified in frequent exacerbators, and history of smoking seems to be a risk factor for this phenotype(130). A novel risk score for

asthma exacerbations developed and validated by Bateman *et al.* supports the evidence that smoking status is a main predictor for uncontrolled asthma(131). Despite this well-known relationship, active smoking is still surprisingly common among asthmatics(132). More efficient smoking prevention programs and smoking cessation campaigns should be carried out to try to reduce the risk of developing severe asthma. Moreover, most clinical trials with new drugs aimed for severe asthma have been conducted in non-smoking patients, which results in incomplete knowledge on the efficacy of such therapeutic approaches in smokers. Large "real life" studies in severe asthma including smoking asthmatics should be encouraged. The complex relationship between cigarette smoking and atopic sensitisation increasing the risk of severe asthma should be better investigated as only few and conflicting data are presently available. However, this relationship remains difficult to address, particularly in cross-sectional studies, because of the potential selection bias (e.g. "healthy smoker effect")(133). Prospective studies in lifetime smokers with lifetime smoking are more appropriate to properly examine the relationships between smoking and severe asthma.

Pollution

The health effects caused by outdoor air pollution have been intensively studied during the last decades. The term "outdoor air pollution" involves particulate matter (PM), gaseous pollutions (nitrogen dioxide, sulphur dioxide and ozone) and traffic-related air pollution (elemental and carbon black, road dust)(134).

Increased exposure to ultrafine particles and carbon monoxide within the previous 4-7 days was associated with increased relative odds of a paediatric asthma visit(135). Other studies also indicate that sudden increase or decrease of exposure to air pollution may affect asthmatic symptoms or emergency department visits(136-138). Indeed, a decrease in the number of acute asthma events of over 40% was found after reduction of air pollution during summer Olympic games(138). So far, these studies were performed in children and included only a relatively low number of individuals.

Larger scale studies also demonstrated an adverse effect of outdoor air pollution on lung function(139-141). A multicenter birth cohort study (ESCAPE) showed an association between estimated levels of NO_2 and $PM_{2.5}$and decreases in FEV_1(139). In another birth cohort study (MAAS), lifetime exposure to PM_{10} and NO_2 was associated with significantly less growth in FEV_1 over time(140). In the same cohort, no association was found between long-term exposure to PM_{10} and NO_2 and the prevalence of asthma or wheeze(142). In adult asthmatics, exposure to NO_2 and

PM_{10} was associated with lower measures of FEV_1 and FVC(143)and exposure to ozone and PM_{10} increased the risk of uncontrolled asthma(144). Overall, these studies thus provide evidence of an inverse association between outdoor air pollution and lung function (Table 1). Whether asthma severity is directly affected by outdoor air pollution is unclear.

Several studies showed a positive association between exposure to air pollution during infancy and sensitisation to inhalant allergens(145-147). Although the mechanism underlying this association is not fully understood, some evidence suggests that ultrafine carbon black particles can directly induce maturation of dendritic cells *in vitro*(148), thereby facilitating sensitisation to inhalant allergens. Alternatively, airborne pollutants can induce the influx of inflammatory cells to the lungs, which might then lower the threshold for sensitisation. Indeed, it has recently been shown that allergen-specific Th2/Th17 cells accumulate in the lungs of mice exposed to both diesel exhaust particles and house dust mite extract(149). Diesel exhaust particles may also produce other immunological effects(150, 151) (Table 2). Furthermore, exposure to moderate air pollution during late pregnancy was found to cause increased cord blood IL-1β(152). A recent meta-analysis, however, showed no clear overall association between air pollution exposure and the development of sensitisation in children up to 10 years of age(153).

In summary, in multi-sensitised asthmatics, daily exposure to allergens in combination with other enhancing factors, including viral infections, environmental smoking, and/or pollution, will finally determine the asthma course and severity.

Occupational/Work-Related

Severe asthma may occur in patients affected by Work-Related Asthma (WRA).WRA encompasses both Occupational Asthma (OA), defined as "asthma caused by the workplace" and "Work-Exacerbated Asthma" (WEA), occurring in patients with pre-existing or concurrent asthma and exacerbated by different work-related factors (i.e. aeroallergens, exercise, irritants)(154). OA can be further divided into two subtypes: an allergic form (90% of all OA)(155), caused both by an IgE-mediated mechanism towards high (HMW) and low (LMW) molecular weight agents(106), and a non-IgE mediated form (Non-Allergic, Irritant-Induced [Occupational] Asthma (IIOA)), towards specific LMW agents in which the mechanism has not been characterized yet. The non-allergic IIOA can be further divided into the "Reactive Airway Dysfunction Syndrome" (RADS) and the "IIOA after multiple exposures". The first occurs after an acute, single exposure to very high

concentrations of irritating substances(156), while the second follows multiple exposure to irritants; in this subtype, onset of asthma can follow the exposures after some time(157, 158).

WRA should be suspected in patients whose asthma worsens while working or begin at work. Here a detailed occupational and medical history is fundamental(159, 160), while a clinical history only shows a low specificity in the diagnosis of OA(161). The investigation of WRA follows a well-defined protocol based on confirmation of bronchial asthma, work-related bronchoconstriction, sensitisation to occupational agents and on the confirmation of the causal role of occupational agents, being sensitisation *per se* not indicative of clinical symptoms(162) (Figure 3). Baseline spirometry is mandatory and it is strongly recommended that this should be complemented with non-specific bronchial hyperreactivity assessment with direct or indirect challenges. In individuals with suspected WRA, presenting with a normal respiratory function and/or negative methacholine challenge testing, serial lung function measurements and assessment of non-specific bronchial hyperreactivity are strongly recommended(162, 163). Additionally, spirometry can be performed during a work shift (Cross-shift spirometry). Furthermore, serial measurements of peak flow expiratory rate (serial PEFR) have been used to objectively confirm the link between the workplace and the asthmatic symptoms(164). Skin prick testing completes the diagnostic work-up, and the selection of specific allergens related to the individual's job is fundamental. Specific IgE evaluation is also of importance. The role of atopic mechanisms in severe occupational asthma has been confirmed by a recent study where treatment with omalizumab was successful in 90% of severe occupational asthma patients due to HMW and LMW agents, such as flour, animal dander, mites, moulds, isocyanate or acrylates(165). It is worth noting that, at least in OA, allergen exposure levels represent the major determinants both for the disease as such and for the severity of asthma(166, 167). Finally, specific inhalation challenges (SICs) or workplace inhalation challenges, complemented by the assessment of airway inflammation by induced sputum and FeNO may be considered.

Diagnosis of IIOA follows a well-defined protocol described in a recent EAACI Task Force document(158).

CONCLUSION

There is increasing evidence for the important, but not exclusive, role of allergy in severe asthma. Although some recent reports demonstrate that allergy may play only a limited role, this is likely not true for childhood disease, where early atopic sensitisation is critical in determining the severity of disease.

Mechanistic implications of co-factors interacting with allergy and asthma, such as virus infections, pollution, smoking, and work-related exposures, still need to be completely uncovered to allow the discovery of novel therapeutic targets.

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SRDG drafted the final version of this manuscript

All authors drafted different chapters and paragraphs of this work

All authors critically revised this work for important intellectual content

All authors approved the final version to be published

All authors agreed on accuracy and integrity of this work

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REFERENCES

1. Addo-Yobo EO, Custovic A, Taggart SC, Craven M, Bonnie B, Woodcock A. Risk factors for asthma in urban Ghana. *J Allergy Clin Immunol* 2001;**108**(3):363-368.
2. Al-Mousawi MS, Lovel H, Behbehani N, Arifhodzic N, Woodcock A, Custovic A. Asthma and sensitization in a community with low indoor allergen levels and low pet-keeping frequency. *J Allergy Clin Immunol* 2004;**114**(6):1389-1394.
3. Simpson BM, Custovic A, Simpson A, Hallam CL, Walsh D, Marolia H, et al. NAC Manchester Asthma and Allergy Study (NACMAAS): risk factors for asthma and allergic disorders in adults. *Clin Exp Allergy* 2001;**31**(3):391-399.
4. Stevens W, Addo-Yobo E, Roper J, Woodcock A, James H, Platts-Mills T, et al. Differences in both prevalence and titre of specific immunoglobulin E among children with asthma in affluent and poor communities within a large town in Ghana. *Clin Exp Allergy* 2011;**41**(11):1587-1594.
5. Custovic A, Johnston SL, Pavord I, Gaga M, Fabbri L, Bel EH, et al. EAACI position statement on asthma exacerbations and severe asthma. *Allergy* 2013;**68**(12):1520-1531.
6. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;**43**(2):343-373.
7. Sala KA, Carroll CL, Tang YS, Aglio T, Dressler AM, Schramm CM. Factors associated with the development of severe asthma exacerbations in children. *J Asthma* 2011;**48**(6):558-564.
8. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;**181**(4):315-323.
9. Holt PG, Strickland DH, Sly PD. Virus infection and allergy in the development of asthma: what is the connection? *Curr Opin Allergy Clin Immunol* 2012;**12**(2):151-157.
10. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006;**27**(3):615-626.
11. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy* 2009;**64**(10):1498-1506.
12. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**(9):813-824.
13. Custovic A, Arifhodzic N, Robinson A, Woodcock A. Exercise testing revisited. The response to exercise in normal and atopic children. *Chest* 1994;**105**(4):1127-1132.
14. Lazic N, Roberts G, Custovic A, Belgrave D, Bishop C, Winn J, et al. Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts. *Allergy* 2013.
15. Lodrup Carlsen KC, Soderstrom L, Mowinckel P, Haland G, Pettersen M, Munthe Kaas MC, et al. Asthma prediction in school children; the value of combined IgE-antibodies and obstructive airways disease severity score. *Allergy* 2010;**65**(9):1134-1140.
16. Simpson A, Soderstrom L, Ahlstedt S, Murray CS, Woodcock A, Custovic A. IgE antibody quantification and the probability of wheeze in preschool children. *J Allergy Clin Immunol* 2005;**116**(4):744-749.
17. Carroll WD, Lenney W, Child F, Strange RC, Jones PW, Whyte MK, et al. Asthma severity and atopy: how clear is the relationship? *Arch Dis Child* 2006;**91**(5):405-409.
18. Marinho S, Simpson A, Marsden P, Smith JA, Custovic A. Quantification of atopy, lung function and airway hypersensitivity in adults. *Clin Transl Allergy* 2011;**1**(1):16.
19. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, et al. Early identification of atopy in the prediction of persistent asthma in children. *Lancet* 2008;**372**(9643):1100-1106.

20. Frith J, Fleming L, Bossley C, Ullmann N, Bush A. The complexities of defining atopy in severe childhood asthma. *Clin Exp Allergy* 2011;**41**(7):948-953.
21. Just J, Gouvis-Echraghi R, Rouve S, Wanin S, Moreau D, Annesi-Maesano I. Two novel, severe asthma phenotypes identified during childhood using a clustering approach. *Eur Respir J* 2012;**40**(1):55-60.
22. Murray CS, Poletti G, Kebadze T, Morris J, Woodcock A, Johnston SL, et al. Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* 2006;**61**(5):376-382.
23. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study. *BMJ* 2002;**324**(7340):763.
24. Murray CS PG, Ahlstedt S, Soderstrom L, Johnston SL, Custovic A. Probability of hospital admission with acute asthma exacerbation increases with increasing specific IgE antibody levels. *Allergy Clin Immunol Int: J World Allergy Org* 2007;**Suppl 2**:270-273.
25. Teach SJ, Gill MA, Togias A, Sorkness CA, Arbes SJ, Jr., Calatroni A, et al. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. *J Allergy Clin Immunol* 2015;**136**(6):1476-1485.
26. Edwards MR, Regamey N, Vareille M, Kieninger E, Gupta A, Shoemark A, et al. Impaired innate interferon induction in severe therapy resistant atopic asthmatic children. *Mucosal Immunol* 2013;**6**(4):797-806.
27. Holt PG, Strickland D, Bosco A, Belgrave D, Hales B, Simpson A, et al. Distinguishing benign from pathologic TH2 immunity in atopic children. *J Allergy Clin Immunol* 2016;**137**(2):379-387.
28. Simpson A, Tan VY, Winn J, Svensen M, Bishop CM, Heckerman DE, et al. Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. *Am J Respir Crit Care Med* 2010;**181**(11):1200-1206.
29. Belgrave DC, Buchan I, Bishop C, Lowe L, Simpson A, Custovic A. Trajectories of lung function during childhood. *Am J Respir Crit Care Med* 2014;**189**(9):1101-1109.
30. Custovic A, Ainsworth J, Arshad H, Bishop C, Buchan I, Cullinan P, et al. The Study Team for Early Life Asthma Research (STELAR) consortium 'Asthma e-lab': team science bringing data, methods and investigators together. *Thorax* 2015;**70**(8):799-801.
31. Simpson A, Lazic N, Belgrave DC, Johnson P, Bishop C, Mills C, et al. Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years. *J Allergy Clin Immunol* 2015.
32. Custovic A, Sonntag HJ, Buchan IE, Belgrave D, Simpson A, Prosperi MC. Evolution pathways of IgE responses to grass and mite allergens throughout childhood. *J Allergy Clin Immunol* 2015;**136**(6):1645-1652 e1641-1648.
33. Belgrave DC, Granell R, Simpson A, Guiver J, Bishop C, Buchan I, et al. Developmental profiles of eczema, wheeze, and rhinitis: two population-based birth cohort studies. *PLoS Med* 2014;**11**(10):e1001748.
34. van der Hulst AE, Klip H, Brand PL. Risk of developing asthma in young children with atopic eczema: a systematic review. *J Allergy Clin Immunol* 2007;**120**(3):565-569.
35. Schuttelaar ML, Kerkhof M, Jonkman MF, Koppelman GH, Brunekreef B, de Jongste JC, et al. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. *Allergy* 2009;**64**(12):1758-1765.
36. Peng W, Novak N. Pathogenesis of atopic dermatitis. *Clin Exp Allergy* 2015;**45**(3):566-574.
37. Hogan MB, Peele K, Wilson NW. Skin barrier function and its importance at the start of the atopic march. *J Allergy (Cairo)* 2012;**2012**:901940.
38. Lodge CJ, Lowe AJ, Gurrin LC, Hill DJ, Hosking CS, Khalafzai RU, et al. House dust mite sensitization in toddlers predicts current wheeze at age 12 years. *J Allergy Clin Immunol* 2011;**128**(4):782-788 e789.

39. Sylvestre L, Jegu J, Metz-Favre C, Barnig C, Qi S, de Blay F. Component-Based Allergen-Microarray: Der p 2 and Der f 2 Dust Mite Sensitization Is More Common in Patients With Severe Asthma. *J Investig Allergol Clin Immunol* 2016;**26**(2):141-143.
40. Vandenplas O, Froidure A, Meurer U, Rihs HP, Rifflart C, Soetaert S, et al. The role of allergen components for the diagnosis of latex-induced occupational asthma. *Allergy* 2016;**71**(6):840-849.
41. Antonicelli L, Massaccesi C, Braschi MC, Cinti B, Bilo MB, Bonifazi F. Component resolved diagnosis in real life: the risk assessment of food allergy using microarray-based immunoassay. *Eur Ann Allergy Clin Immunol* 2014;**46**(1):30-34.
42. Nettis E, Bonifazi F, Bonini S, Di Leo E, Maggi E, Melioli G, et al. Molecular diagnosis and the Italian Board for ISAC. *Eur Ann Allergy Clin Immunol* 2014;**46**(2):68-73.
43. Prosperi MC, Belgrave D, Buchan I, Simpson A, Custovic A. Challenges in interpreting allergen microarrays in relation to clinical symptoms: A machine learning approach. *Pediatr Allergy Immunol* 2013.
44. Konradsen JR, Nordlund B, Onell A, Borres MP, Gronlund H, Hedlin G. Severe childhood asthma and allergy to furry animals: refined assessment using molecular-based allergy diagnostics. *Pediatr Allergy Immunol* 2014;**25**(2):187-192.
45. Haldar A, Gupta UD, Majumdar KK, Laskar K, Ghosh S, Sen S. Community perception of Dengue in slum areas of metropolitan city of West Bengal. *J Commun Dis* 2008;**40**(3):205-210.
46. Wu W, Bleecker E, Moore W, Busse WW, Castro M, Chung KF, et al. Unsupervised phenotyping of Severe Asthma Research Program participants using expanded lung data. *J Allergy Clin Immunol* 2014;**133**(5):1280-1288.
47. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;**18**(5):716-725.
48. Bell MC, Busse WW. Severe asthma: an expanding and mounting clinical challenge. *J Allergy Clin Immunol Pract* 2013;**1**(2):110-121; quiz 122.
49. Moore WC, Fitzpatrick AM, Li X, Hastie AT, Li H, Meyers DA, et al. Clinical heterogeneity in the severe asthma research program. *Ann Am Thorac Soc* 2013;**10 Suppl**:S118-124.
50. Fitzpatrick AM, Teague WG, Meyers DA, Peters SP, Li X, Li H, et al. Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. *J Allergy Clin Immunol* 2011;**127**(2):382-389 e381-313.
51. Just J, Deslandes-Boutmy E, Amat F, Desseaux K, Nemni A, Bourrat E, et al. Natural history of allergic sensitization in infants with early-onset atopic dermatitis: results from ORCA Study. *Pediatr Allergy Immunol* 2014;**25**(7):668-673.
52. Schatz M, Hsu JW, Zeiger RS, Chen W, Dorenbaum A, Chipps BE, et al. Phenotypes determined by cluster analysis in severe or difficult-to-treat asthma. *J Allergy Clin Immunol* 2014;**133**(6):1549-1556.
53. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, et al. Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. *J Allergy Clin Immunol* 2012;**129**(4):974-982 e913.
54. Amelink M, de Groot JC, de Nijs SB, Lutter R, Zwinderman AH, Sterk PJ, et al. Severe adult-onset asthma: A distinct phenotype. *J Allergy Clin Immunol* 2013;**132**(2):336-341.
55. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U, et al. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006;**368**(9537):763-770.
56. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;**178**(7):667-672.

57. Stoltz DJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Gern JE, et al. Specific patterns of allergic sensitization in early childhood and asthma & rhinitis risk. *Clin Exp Allergy* 2013;**43**(2):233-241.
58. Sharples J, Gupta A, Fleming L, Bossley CJ, Bracken-King M, Hall P, et al. Long-term effectiveness of a staged assessment for paediatric problematic severe asthma. *Eur Respir J* 2012;**40**(1):264-267.
59. Marinho S, Simpson A, Soderstrom L, Woodcock A, Ahlstedt S, Custovic A. Quantification of atopy and the probability of rhinitis in preschool children: a population-based birth cohort study. *Allergy* 2007;**62**(12):1379-1386.
60. Belgrave DC, Simpson A, Semic-Jusufagic A, Murray CS, Buchan I, Pickles A, et al. Joint modeling of parentally reported and physician-confirmed wheeze identifies children with persistent troublesome wheezing. *J Allergy Clin Immunol* 2013;**132**(3):575-583 e512.
61. Amelink M, de Nijs SB, de Groot JC, van Tilburg PM, van Spiegel PI, Krouwels FH, et al. Three phenotypes of adult-onset asthma. *Allergy* 2013;**68**(5):674-680.
62. Westerhof GA, Vollema EM, Weersink EJ, Reinartz SM, de Nijs SB, Bel EH. Predictors for the development of progressive severity in new-onset adult asthma. *J Allergy Clin Immunol* 2014;**134**(5):1051-1056 e1052.
63. Kobayashi Y, Bossley C, Gupta A, Akashi K, Tsartsali L, Mercado N, et al. Passive smoking impairs histone deacetylase-2 in children with severe asthma. *Chest* 2014;**145**(2):305-312.
64. de Nijs SB, Venekamp LN, Bel EH. Adult-onset asthma: is it really different? *Eur Respir Rev* 2013;**22**(127):44-52.
65. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: Eosinophilic airway inflammation in nonallergic asthma. *Nat Med* 2013;**19**(8):977-979.
66. Grimaldi-Bensouda L, Zureik M, Aubier M, Humbert M, Levy J, Benichou J, et al. Does omalizumab make a difference to the real-life treatment of asthma exacerbations?: Results from a large cohort of patients with severe uncontrolled asthma. *Chest* 2013;**143**(2):398-405.
67. Deschildre A, Marguet C, Salleron J, Pin I, Rittie JL, Derelle J, et al. Add-on omalizumab in children with severe allergic asthma: a 1-year real life survey. *Eur Respir J* 2013;**42**(5):1224-1233.
68. Bel EH, Ortega HG, Pavord ID. Glucocorticoids and mepolizumab in eosinophilic asthma. *N Engl J Med* 2014;**371**(25):2434.
69. Vercelli D, Gozdz J, von Mutius E. Innate lymphoid cells in asthma: when innate immunity comes in a Th2 flavor. *Curr Opin Allergy Clin Immunol* 2014;**14**(1):29-34.
70. Walker JA, McKenzie AN. Development and function of group 2 innate lymphoid cells. *Curr Opin Immunol* 2013;**25**(2):148-155.
71. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol* 2013;**132**(4):933-941.
72. Prefontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010;**125**(3):752-754.
73. Traister RS, Uvalle CE, Hawkins GA, Meyers DA, Bleecker ER, Wenzel SE. Phenotypic and genotypic association of epithelial IL1RL1 to human TH2-like asthma. *J Allergy Clin Immunol* 2015;**135**(1):92-99.
74. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol* 2013;**132**(3):676-685 e613.
75. Gregory LG, Jones CP, Walker SA, Sawant D, Gowers KH, Campbell GA, et al. IL-25 drives remodelling in allergic airways disease induced by house dust mite. *Thorax* 2013;**68**(1):82-90.
76. Corrigan CJ, Wang W, Meng Q, Fang C, Wu H, Reay V, et al. T-helper cell type 2 (Th2) memory T cell-potentiating cytokine IL-25 has the potential to promote angiogenesis in asthma. *Proc Natl Acad Sci U S A* 2011;**108**(4):1579-1584.

77. Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol* 2014;**133**(2):388-394.
78. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;**365**(12):1088-1098.
79. Triggiani M, De Feo G, Cardamone C, Parente R. The Emerging Role of Innate Immunity in Respiratory Allergy. *International Trends in Immunity* 2015;**3**(2):28-32.
80. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. *J Allergy Clin Immunol* 2014;**134**(3):509-520.
81. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, et al. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011;**128**(3):549-556 e541-512.
82. Panzer AR, Lynch SV. Influence and effect of the human microbiome in allergy and asthma. *Curr Opin Rheumatol* 2015;**27**(4):373-380.
83. Huang YJ, Nelson CE, Brodie EL, Desantis TZ, Baek MS, Liu J, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin Immunol* 2011;**127**(2):372-381 e371-373.
84. Goleva E, Jackson LP, Harris JK, Robertson CE, Sutherland ER, Hall CF, et al. The effects of airway microbiome on corticosteroid responsiveness in asthma. *Am J Respir Crit Care Med* 2013;**188**(10):1193-1201.
85. Kloepfer KM, Lee WM, Pappas TE, Kang TJ, Vrtis RF, Evans MD, et al. Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations. *J Allergy Clin Immunol* 2014;**133**(5):1301-1307, 1307 e1301-1303.
86. Gavala ML, Bertics PJ, Gern JE. Rhinoviruses, allergic inflammation, and asthma. *Immunol Rev* 2011;**242**(1):69-90.
87. James KM, Peebles RS, Jr., Hartert TV. Response to infections in patients with asthma and atopic disease: an epiphenomenon or reflection of host susceptibility? *J Allergy Clin Immunol* 2012;**130**(2):343-351.
88. Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007;**119**(5):1105-1110.
89. Mackenzie KJ, Anderton SM, Schwarze J. Viral respiratory tract infections and asthma in early life: cause and effect? *Clin Exp Allergy* 2014;**44**(1):9-19.
90. Bonnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin Immunol* 2015;**136**(1):81-86 e84.
91. Papadopoulos NG, Christodoulou I, Rohde G, Agache I, Almqvist C, Bruno A, et al. Viruses and bacteria in acute asthma exacerbations--a GA(2) LEN-DARE systematic review. *Allergy* 2011;**66**(4):458-468.
92. Sandrock CE, Norris A. Infection in severe asthma exacerbations and critical asthma syndrome. *Clin Rev Allergy Immunol* 2015;**48**(1):104-113.
93. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, et al. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. *Lancet* 2002;**359**(9309):831-834.
94. Iwasaki J, Smith WA, Khoo SK, Bizzintino J, Zhang G, Cox DW, et al. Comparison of rhinovirus antibody titers in children with asthma exacerbations and species-specific rhinovirus infection. *J Allergy Clin Immunol* 2014;**134**(1):25-32.
95. Sykes A, Macintyre J, Edwards MR, Del Rosario A, Haas J, Gielen V, et al. Rhinovirus-induced interferon production is not deficient in well controlled asthma. *Thorax* 2014;**69**(3):240-246.

96. Patel DA, You Y, Huang G, Byers DE, Kim HJ, Agapov E, et al. Interferon response and respiratory virus control are preserved in bronchial epithelial cells in asthma. *J Allergy Clin Immunol* 2014;**134**(6):1402-1412 e1407.
97. Baxi SN, Phipatanakul W. The role of allergen exposure and avoidance in asthma. *Adolesc Med State Art Rev* 2010;**21**(1):57-71, viii-ix.
98. Bartra J, Sastre J, del Cuvillo A, Montoro J, Jauregui I, Davila I, et al. From pollinosis to digestive allergy. *J Investig Allergol Clin Immunol* 2009;**19 Suppl 1**:3-10.
99. Bousquet J, Schunemann HJ, Samolinski B, Demoly P, Baena-Cagnani CE, Bachert C, et al. Allergic Rhinitis and its Impact on Asthma (ARIA): achievements in 10 years and future needs. *J Allergy Clin Immunol* 2012;**130**(5):1049-1062.
100. Thaminy A, Lamblin C, Perez T, Bergoin C, Tonnel AB, Wallaert B. Increased frequency of asymptomatic bronchial hyperresponsiveness in nonasthmatic patients with food allergy. *Eur Respir J* 2000;**16**(6):1091-1094.
101. Krogulska A, Dynowski J, Jedrzejczyk M, Sardecka I, Malachowska B, Wasowska-Krolikowska K. The impact of food allergens on airway responsiveness in schoolchildren with asthma: A DBPCFC study. *Pediatr Pulmonol* 2016.
102. Krogulska A, Dynowski J, Funkowicz M, Malachowska B, Wasowska-Krolikowska K. Prevalence and Clinical Impact of IgE-Mediated Food Allergy in School Children With Asthma: A Double-Blind Placebo-Controlled Food Challenge Study. *Allergy Asthma Immunol Res* 2015;**7**(6):547-556.
103. Wang J, Liu AH. Food allergies and asthma. *Curr Opin Allergy Clin Immunol* 2011;**11**(3):249-254.
104. Custovic A, Simpson A, Woodcock A. Importance of indoor allergens in the induction of allergy and elicitation of allergic disease. *Allergy* 1998;**53**(48 Suppl):115-120.
105. Gent JF, Belanger K, Triche EW, Bracken MB, Beckett WS, Leaderer BP. Association of pediatric asthma severity with exposure to common household dust allergens. *Environ Res* 2009;**109**(6):768-774.
106. Denning DW, Pashley C, Hartl D, Wardlaw A, Godet C, Del Giacco S, et al. Fungal allergy in asthma-state of the art and research needs. *Clin Transl Allergy* 2014;**4**:14.
107. Arbes SJ, Jr., Gergen PJ, Vaughn B, Zeldin DC. Asthma cases attributable to atopy: results from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2007;**120**(5):1139-1145.
108. Jaakkola MS, Ieromnimon A, Jaakkola JJ. Are atopy and specific IgE to mites and molds important for adult asthma? *J Allergy Clin Immunol* 2006;**117**(3):642-648.
109. O'Driscoll BR, Hopkinson LC, Denning DW. Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. *BMC Pulm Med* 2005;**5**:4.
110. Black PN, Udy AA, Brodie SM. Sensitivity to fungal allergens is a risk factor for life-threatening asthma. *Allergy* 2000;**55**(5):501-504.
111. O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, et al. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* 1991;**324**(6):359-363.
112. Schwartz HJ, Citron KM, Chester EH, Kaimal J, Barlow PB, Baum GL, et al. A comparison of the prevalence of sensitization to Aspergillus antigens among asthmatics in Cleveland and London. *J Allergy Clin Immunol* 1978;**62**(1):9-14.
113. Vicencio AG, Santiago MT, Tsirilakis K, Stone A, Worgall S, Foley EA, et al. Fungal sensitization in childhood persistent asthma is associated with disease severity. *Pediatr Pulmonol* 2014;**49**(1):8-14.
114. Tham R, Dharmage SC, Taylor PE, Katelaris CH, Vicendese D, Abramson MJ, et al. Outdoor fungi and child asthma health service attendances. *Pediatr Allergy Immunol* 2014;**25**(5):439-449.

115. Lotvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011;**127**(2):355-360.
116. Ward GW, Jr., Karlsson G, Rose G, Platts-Mills TA. Trichophyton asthma: sensitisation of bronchi and upper airways to dermatophyte antigen. *Lancet* 1989;**1**(8643):859-862.
117. James AL, Palmer LJ, Kicic E, Maxwell PS, Lagan SE, Ryan GF, et al. Decline in lung function in the Busselton Health Study: the effects of asthma and cigarette smoking. *Am J Respir Crit Care Med* 2005;**171**(2):109-114.
118. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC. Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J Respir Crit Care Med* 2003;**168**(11):1308-1311.
119. Barnes PJ, Ito K, Adcock IM. Corticosteroid resistance in chronic obstructive pulmonary disease: inactivation of histone deacetylase. *Lancet* 2004;**363**(9410):731-733.
120. Cerveri I, Cazzoletti L, Corsico AG, Marcon A, Niniano R, Grosso A, et al. The impact of cigarette smoking on asthma: a population-based international cohort study. *Int Arch Allergy Immunol* 2012;**158**(2):175-183.
121. Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, et al. Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe asthma. *J Allergy Clin Immunol* 2013;**131**(4):1008-1016.
122. Siroux V, Pin I, Oryszczyn MP, Le Moual N, Kauffmann F. Relationships of active smoking to asthma and asthma severity in the EGEA study. Epidemiological study on the Genetics and Environment of Asthma. *Eur Respir J* 2000;**15**(3):470-477.
123. Oryszczyn MP, Annesi-Maesano I, Charpin D, Paty E, Maccario J, Kauffmann F. Relationships of active and passive smoking to total IgE in adults of the Epidemiological Study of the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy (EGEA). *Am J Respir Crit Care Med* 2000;**161**(4 Pt 1):1241-1246.
124. Nielsen GD, Olsen O, Larsen ST, Lovik M, Poulsen LK, Glue C, et al. IgE-mediated sensitisation, rhinitis and asthma from occupational exposures. Smoking as a model for airborne adjuvants? *Toxicology* 2005;**216**(2-3):87-105.
125. Polosa R, Knoke JD, Russo C, Piccillo G, Caponnetto P, Sarva M, et al. Cigarette smoking is associated with a greater risk of incident asthma in allergic rhinitis. *J Allergy Clin Immunol* 2008;**121**(6):1428-1434.
126. Cazzoletti L, Marcon A, Corsico A, Janson C, Jarvis D, Pin I, et al. Asthma severity according to Global Initiative for Asthma and its determinants: an international study. *Int Arch Allergy Immunol* 2010;**151**(1):70-79.
127. Saulyte J, Regueira C, Montes-Martinez A, Khudyakov P, Takkouche B. Active or passive exposure to tobacco smoking and allergic rhinitis, allergic dermatitis, and food allergy in adults and children: a systematic review and meta-analysis. *PLoS Med* 2014;**11**(3):e1001611.
128. Adenuga D, Yao H, March TH, Seagrave J, Rahman I. Histone deacetylase 2 is phosphorylated, ubiquitinated, and degraded by cigarette smoke. *Am J Respir Cell Mol Biol* 2009;**40**(4):464-473.
129. Livingston E, Darroch CE, Chaudhuri R, McPhee I, McMahon AD, Mackenzie SJ, et al. Glucocorticoid receptor alpha:beta ratio in blood mononuclear cells is reduced in cigarette smokers. *J Allergy Clin Immunol* 2004;**114**(6):1475-1478.
130. Kupczyk M, ten Brinke A, Sterk PJ, Bel EH, Papi A, Chanez P, et al. Frequent exacerbators--a distinct phenotype of severe asthma. *Clin Exp Allergy* 2014;**44**(2):212-221.
131. Bateman ED, Buhl R, O'Byrne PM, Humbert M, Reddel HK, Sears MR, et al. Development and validation of a novel risk score for asthma exacerbations: The risk score for exacerbations. *J Allergy Clin Immunol* 2015;**135**(6):1457-1464 e1454.
132. Accordini S, Janson C, Svanes C, Jarvis D. The role of smoking in allergy and asthma: lessons from the ECRHS. *Curr Allergy Asthma Rep* 2012;**12**(3):185-191.

133. Becklake MR, Lalloo U. The 'healthy smoker': a phenomenon of health selection? *Respiration* 1990;**57**(3):137-144.
134. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. *Lancet* 2014;**383**(9928):1581-1592.
135. Evans KA, Halterman JS, Hopke PK, Fagnano M, Rich DQ. Increased ultrafine particles and carbon monoxide concentrations are associated with asthma exacerbation among urban children. *Environ Res* 2014;**129**:11-19.
136. Weinmayr G, Romeo E, De Sario M, Weiland SK, Forastiere F. Short-term effects of PM10 and NO2 on respiratory health among children with asthma or asthma-like symptoms: a systematic review and meta-analysis. *Environ Health Perspect* 2010;**118**(4):449-457.
137. Sarnat JA, Golan R, Greenwald R, Raysoni AU, Kewada P, Winquist A, et al. Exposure to traffic pollution, acute inflammation and autonomic response in a panel of car commuters. *Environ Res* 2014;**133**:66-76.
138. Friedman MS, Powell KE, Hutwagner L, Graham LM, Teague WG. Impact of changes in transportation and commuting behaviors during the 1996 Summer Olympic Games in Atlanta on air quality and childhood asthma. *JAMA* 2001;**285**(7):897-905.
139. Gehring U, Gruzieva O, Agius RM, Beelen R, Custovic A, Cyrys J, et al. Air pollution exposure and lung function in children: the ESCAPE project. *Environ Health Perspect* 2013;**121**(11-12):1357-1364.
140. Molter A, Agius RM, de Vocht F, Lindley S, Gerrard W, Lowe L, et al. Long-term exposure to PM10 and NO2 in association with lung volume and airway resistance in the MAAS birth cohort. *Environ Health Perspect* 2013;**121**(10):1232-1238.
141. Eeftens M, Hoek G, Gruzieva O, Molter A, Agius R, Beelen R, et al. Elemental composition of particulate matter and the association with lung function. *Epidemiology* 2014;**25**(5):648-657.
142. Molter A, Agius R, de Vocht F, Lindley S, Gerrard W, Custovic A, et al. Effects of long-term exposure to PM10 and NO2 on asthma and wheeze in a prospective birth cohort. *J Epidemiol Community Health* 2014;**68**(1):21-28.
143. Adam M, Schikowski T, Carsin AE, Cai Y, Jacquemin B, Sanchez M, et al. Adult lung function and long-term air pollution exposure. ESCAPE: a multicentre cohort study and meta-analysis. *Eur Respir J* 2015;**45**(1):38-50.
144. Jacquemin B, Kauffmann F, Pin I, Le Moual N, Bousquet J, Gormand F, et al. Air pollution and asthma control in the Epidemiological study on the Genetics and Environment of Asthma. *J Epidemiol Community Health* 2012;**66**(9):796-802.
145. Morgenstern V, Zutavern A, Cyrys J, Brockow I, Koletzko S, Kramer U, et al. Atopic diseases, allergic sensitization, and exposure to traffic-related air pollution in children. *Am J Respir Crit Care Med* 2008;**177**(12):1331-1337.
146. Gruzieva O, Bellander T, Eneroth K, Kull I, Melen E, Nordling E, et al. Traffic-related air pollution and development of allergic sensitization in children during the first 8 years of life. *J Allergy Clin Immunol* 2012;**129**(1):240-246.
147. Nordling E, Berglind N, Melen E, Emenius G, Hallberg J, Nyberg F, et al. Traffic-related air pollution and childhood respiratory symptoms, function and allergies. *Epidemiology* 2008;**19**(3):401-408.
148. de Haar C, Kool M, Hassing I, Bol M, Lambrecht BN, Pieters R. Lung dendritic cells are stimulated by ultrafine particles and play a key role in particle adjuvant activity. *J Allergy Clin Immunol* 2008;**121**(5):1246-1254.
149. Brandt EB, Biagini Myers JM, Acciani TH, Ryan PH, Sivaprasad U, Ruff B, et al. Exposure to allergen and diesel exhaust particles potentiates secondary allergen-specific memory responses, promoting asthma susceptibility. *J Allergy Clin Immunol* 2015;**136**(2):295-303 e297.
150. Acciani TH, Brandt EB, Khurana Hershey GK, Le Cras TD. Diesel exhaust particle exposure increases severity of allergic asthma in young mice. *Clin Exp Allergy* 2013;**43**(12):1406-1418.

151. De Grove KC, Provoost S, Hendriks RW, McKenzie AN, Seys LJ, Kumar S, et al. Dysregulation of type 2 innate lymphoid cells and TH2 cells impairs pollutant-induced allergic airway responses. *J Allergy Clin Immunol* 2016.
152. Latzin P, Frey U, Armann J, Kieninger E, Fuchs O, Roosli M, et al. Exposure to moderate air pollution during late pregnancy and cord blood cytokine secretion in healthy neonates. *PLoS One* 2011;**6**(8):e23130.
153. Gruzieva O, Gehring U, Aalberse R, Agius R, Beelen R, Behrendt H, et al. Meta-analysis of air pollution exposure association with allergic sensitization in European birth cohorts. *J Allergy Clin Immunol* 2014;**133**(3):767-776 e767.
154. Moscato G, Pala G, Barnig C, De Blay F, Del Giacco SR, Folletti I, et al. EAACI consensus statement for investigation of work-related asthma in non-specialized centres. *Allergy* 2012;**67**(4):491-501.
155. Mapp CE, Boschetto P, Maestrelli P, Fabbri LM. Occupational asthma. *Am J Respir Crit Care Med* 2005;**172**(3):280-305.
156. Brooks SM, Weiss MA, Bernstein IL. Reactive airways dysfunction syndrome (RADS). Persistent asthma syndrome after high level irritant exposures. *Chest* 1985;**88**(3):376-384.
157. Tarlo SM, Broder I. Irritant-induced occupational asthma. *Chest* 1989;**96**(2):297-300.
158. Vandenplas O, Wiszniewska M, Raulf M, de Blay F, Gerth van Wijk R, Moscato G, et al. EAACI position paper: irritant-induced asthma. *Allergy* 2014;**69**(9):1141-1153.
159. Cullinan P. Clinical aspects of occupational asthma. *Panminerva Med* 2004;**46**(2):111-120.
160. Vandenplas O, Ghezzo H, Munoz X, Moscato G, Perfetti L, Lemiere C, et al. What are the questionnaire items most useful in identifying subjects with occupational asthma? *Eur Respir J* 2005;**26**(6):1056-1063.
161. Malo JL, Ghezzo H, L'Archeveque J, Lagier F, Perrin B, Cartier A. Is the clinical history a satisfactory means of diagnosing occupational asthma? *Am Rev Respir Dis* 1991;**143**(3):528-532.
162. Vandenplas O, Toren K, Blanc PD. Health and socioeconomic impact of work-related asthma. *Eur Respir J* 2003;**22**(4):689-697.
163. Tarlo SM, Balmes J, Balkissoon R, Beach J, Beckett W, Bernstein D, et al. Diagnosis and management of work-related asthma: American College Of Chest Physicians Consensus Statement. *Chest* 2008;**134**(3 Suppl):1S-41S.
164. Moscato G, Godnic-Cvar J, Maestrelli P, Malo JL, Sherwood Burge P, Coifman R. Statement on self-monitoring of peak expiratory flow in the investigation of occupational asthma. Subcommittee on Occupational Allergy of the European Academy of Allergology and Clinical Immunology. *Allergy* 1995;**50**(9):711-717.
165. Lavaud F, Bonniaud P, Dalphin JC, Leroyer C, Muller D, Tannous R, et al. Usefulness of omalizumab in ten patients with severe occupational asthma. *Allergy* 2013;**68**(6):813-815.
166. Baur X, Chen Z, Liebers V. Exposure-response relationships of occupational inhalative allergens. *Clin Exp Allergy* 1998;**28**(5):537-544.
167. Jones MG. Exposure-response in occupational allergy. *Curr Opin Allergy Clin Immunol* 2008;**8**(2):110-114.

Figure 1. For those children who suffered a hospital admission with wheeze or asthma after age 3 years, a highly significant increase in the risk was seen only among children in the multiple early sensitisation subgroup (HR 9.2; 95% CI, 3.5–24; P < 0.001), but not other atopy classes.

From (28): Simpson A, Tan VY, Winn J, et al. Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. Am J Respir Crit Care Med 2010;181(11):1200-6, with permission

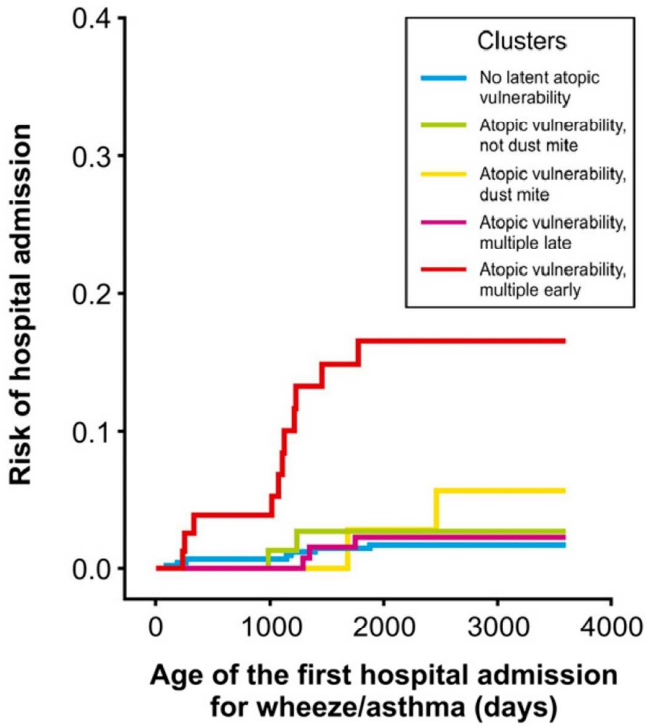


Table 1: Main pollutants and examples of their effects on respiratory function.

Pollutant	Outcome
Nitrogen dioxide (NO_2)	Decreased FEV_1(139)
	Less growth of FEV_1 over time(140)
	Lower measures of FEV_1(143)
	Lower measures of FVC(143)
$PM_{2.5}$	Decreased FEV_1(139)
PM_{10}	Less growth of FEV_1 over time(140)
	Lower measures of FEV_1(143)
	Lower measures of FVC(143)
	Increased risk of uncontrolled Asthma(144)
Ozone (O_3)	Increased risk of uncontrolled Asthma(144)

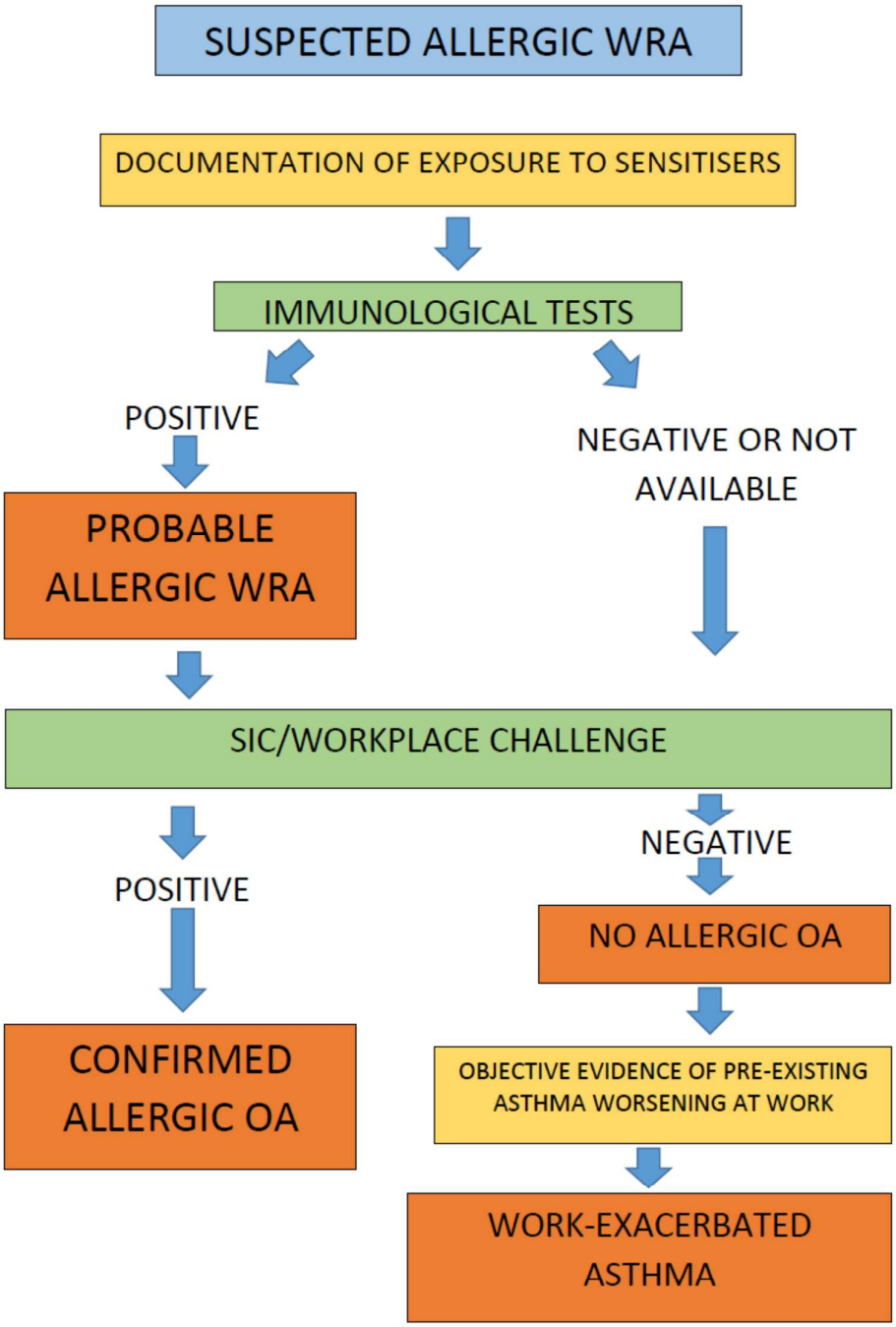
Table 2: Pollutants and examples of their effects on allergic inflammation

Pollutant	Outcome
Ultrafine Carbon Black Particles	Induced maturation of Dendritic Cells in vitro (148)
Diesel Exhaust Particles and House dust Mite Extract	Increased allergen-specific IgE and other cardinal features of asthma (150)
	Accumulation of allergen-specific Th2/Th17 cells in lungs (149)
	Both Th2 and ILC2 contribute to DEP-enhanced airway inflammation (151)

Figure 2: Influence of smoking and atopy in determining more severe asthma.

Figure 3: Allergic Occupational Asthma: diagnostic flow-chart.

From: Moscato G, Pala G, Barnig C, De Blay F, Del Giacco SR, Folletti I, et al. EAACI consensus statement for investigation of work-related asthma in non-specialized centres. Modified from: Allergy. 2012;67(4):491-501, with permission.



Allergy in Severe Asthma

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ABSTRACT

It is well recognized that atopic sensitisation is an important risk factor for asthma, both in adults and in children. However, the role of allergy in severe asthma is still under debate. The term “Severe Asthma” encompasses a highly heterogeneous group of patients who require treatment on steps 4–5 of GINA guidelines to prevent their asthma from becoming "uncontrolled", or whose disease remains "uncontrolled" despite this therapy. Epidemiological studies on emergency room visits and hospital admissions for asthma suggest the important role of allergy in asthma exacerbations. In addition, allergic asthma in childhood is often associated with severe asthma in adulthood. A strong association exists between asthma exacerbations and respiratory viral infections, and interaction between viruses and allergy further increases the risk of asthma exacerbations. Furthermore, fungal allergy has been shown to play an important role in severe asthma. Other contributing factors include smoking, pollution and work-related exposures. The “Allergy and Asthma Severity” EAACI Task Force examined the current evidence and produced this position document on the role of allergy in severe asthma.

ABSTRACT WORD COUNT: 174

INTRODUCTION

Numerous epidemiological studies have demonstrated that atopic sensitisation is a strong risk factor for asthma in childhood(1, 2) and adulthood(3), both in the developed(3) and in the developing countries(1, 2, 4), supporting the notion that asthma is in part an allergic disease. However, the role of allergy in severe asthma remains the issue of considerable controversy. The term "severe asthma" encompasses a highly heterogeneous group of patients, which is defined in various ways in the literature(5). Recent international guidelines define "severe asthma" as asthma which requires treatment at GINA steps 4–5 during the previous year or systemic corticosteroids (CS) for ≥50% of the previous year to prevent it from becoming ''uncontrolled'', or asthma which remains ''uncontrolled'' despite this therapy, or controlled asthma that worsens on tapering high doses of inhaled corticosteroids (ICS), systemic CS or additional biologics(6).

Asthma exacerbations are one of the key features of severe asthma. Emergency room visits and hospital admissions due to acute asthma attacks are increased in children who are sensitised and exposed to high levels of inhalant allergens in their homes, emphasising the importance of "allergy" in asthma exacerbations(7). The phenotypes of childhood onset allergic asthma and early sensitisation are often associated with severe asthma in adulthood(8). However, some data indicated that the proportion of severe asthma cases attributable to allergy may be overestimated, and that aetiological mechanisms other than allergy may be important in the pathogenesis of severe asthma. For example, numerous studies have reported a strong association between asthma exacerbations and respiratory viral infections, suggestive of a viral-induced mechanism. Rather than being mutually exclusive, viruses and allergens may interact in increasing the risk of asthma development(9).

Furthermore, fungal sensitisation is strongly associated with severe asthma, hence, recently a new subtype of Severe Asthma with Fungal Sensitization (SAFS) has been proposed(10).

Finally, the role of several co-factors, such as smoking, pollution and work-related exposures must be considered when evaluating a patient with severe asthma.

The "Allergy and Asthma Severity" EAACI Task Force produced this position document on the role of allergy in severe asthma, searching the literature of the last 10 years in the main databases (MEDLINE, Scopus, ISI) and including milestone and important papers at the discretion of the different co-authors.

DEFINITION AND ROLE OF INHALANT ALLERGENS IN ASTHMA

Atopy, allergy and asthma

The association between atopy and asthma appears specific to inhalant allergens(4). In general, atopic sensitisation is defined either when allergen-specific serum IgE (sIgE) are detected, or a positive skin prick test (SPT) to extracts made from whole allergen sources(11, 12), often using arbitrary cut-off points of sIgE>0.35 KU/L, or a mean wheal diameter ≥3mm. These standard allergy tests have high sensitivity, but in themselves do not signify disease. For example, a considerable proportion of such defined sensitised individuals have no evidence of asthma(13), and a positive test in an asthmatic patient does not always result in clinical response upon allergen exposure. Thus, there is a difference between allergic asthma with asthma symptoms induced by exposure to a defined allergen, and asthma in a subject characterized as "sensitised" with no relation between allergen exposure and clinical reaction. It has been suggested that a positive allergy test (assessed either by sIgE or SPT) should not be considered as a sole diagnostic marker of atopic sensitisation(14).

Quantification of atopic sensitisation increases the specificity in relation to asthma presence and severity

The last decade has seen the shift in the way we interpret the results of IgE and SPTs. The sum of the levels of specific IgE antibodies (or the summative size of SPT wheals) to inhalant allergens is a better predictor of the onset, presence, persistence and severity of childhood asthma than the mere presence of a "positive allergy test"(15-17). The clinical importance of "quantitative atopic sensitisation" has been confirmed in subsequent studies in adult asthma(18). It is now recognized that quantification of atopic sensitisation in early life amongst young children with wheezing is one of the best discriminators to identify those who are at high risk of subsequent development of persistent asthma(19).

Additionally, a clear quantitative relationship between the level of sIgE and the size of SPT responses has been observed in relation to asthma severity, both in adults and in children(20, 21). For example, one of the phenotypic characteristics of severe treatment-resistant asthma (STRA) in childhood is the large size of SPT wheals to inhalant and food allergens. In patients with STRA, results of sIgE measurements and SPTs are not always concordant, indicating the need to carry out

both tests(17, 20). The level of sIgE is also associated with an increased risk of severe asthma exacerbations requiring hospitalization among both children(17, 22) and adults(23). Finally, it has been shown that there is a strong interaction between the levels of sIgE to inhalant allergens and respiratory virus infections in increasing the risk of severe asthma exacerbations requiring hospital admission(24), suggesting a synergism between quantitative sensitisation and respiratory virus infections. This synergism has been indirectly confirmed in a study showing that pre-seasonal anti-IgE- targeted therapy with omalizumab decreases seasonal exacerbations of asthma ("back-to-school asthma"), which are almost certainly (rhino)virus-induced(25). In contrast, a recent study showed that although impaired IFN-β and IFN-λ induction by rhinovirus was a feature of bronchial epithelial cells from highly sensitised children with STRA(26), there was no relationship between sensitisation and Th2-mediated inflammation with impaired interferon production, raising a possibility of two independent mechanisms (atopy-related and virus-related).

All of the above data indicate that in the assessment of patients with asthma (including severe asthma), the results of specific IgE measurement and SPT are not mutually exclusive but complementary, and should not be reported as being "positive" or "negative", but as the level of sIgE and the size of SPT wheal diameter (*i.e.,* quantified). For SPTs, the size of the positive and negative control should be taken into account. Recent data suggest that diagnostic accuracy of specific IgE antibody measurement in the context of asthma and the distinction between "benign" atopy (*i.e.,* sensitisation in the absence of allergic symptoms) and "pathologic" atopy (*i.e.,* sensitisation related to allergic symptoms), may be improved by the measurement of allergen-specific IgG antibody levels(27), although their measurement is not recommended routinely.

Heterogeneity of atopic sensitisation

It has recently been proposed that "atopic sensitisation" maybe an umbrella term for a collection of several different subgroups of sensitisation which differ in their association with asthma and other allergic diseases(14). Distinct subgroups (or classes) of sensitisation were described in one population-based birth cohort (Manchester Asthma and Allergy Study) by applying a machine learning approach with Bayesian inference to the SPTs and sIgE data collected longitudinally from early life to school age(28), and similar latent structure was subsequently described using comparable approach to longitudinal data on atopic sensitisation in another birth cohort (Isle of Wight study)(14). Children who would be considered sensitised using conventional definitions were clustered into four distinct subgroups characterised by a unique pattern of the responses to different allergens and the timing of onset of allergen-specific sensitisation(28) (Figure 1). Importantly, the

risk of asthma was increased more than 20-fold amongst children belonging to one of these subgroups (those sensitised to multiple allergens in early life - comprising less than one third of the sensitised children), but not amongst those in other classes(14, 28). Striking similarities were observed in the association between different subgroups of atopic sensitisation in these two cohorts in relation to asthma severity, with children in the subgroup of sensitisation characterised by IgE responses to multiple allergens in early life having higher FeNO levels, more hyperreactive airways, an increased risk of severe asthma exacerbations having significantly diminished lung function, compared to all other classes(14, 28, 29). It is of note however, that such subtypes (clusters/classes) of sensitisation can only be identified using statistical inference on longitudinal data(14, 28), and that differentiation between different clusters at any single cross-sectional point is not yet possible(30). Clinical translation of this important observation requires the development of specific and sensitive biomarkers which can be measured at the time of presentation to clinic and which aid differentiation between different sensitisation subgroups. Recent data indicate that IgE responses to individual allergenic molecules rather than whole allergen extracts may prove useful in differentiating the subtypes of sensitisation relevant to asthma onset and severity(31, 32).

Progression from Atopic Dermatitis to Allergic Asthma – fact or myth?

Although atopic dermatitis (AD) usually precedes allergic asthma or rhinitis, a clear causal relationship for the typical sequence in the development of these diseases – formerly termed as the 'atopic march' – remains to be confirmed. Recent analysis among 10,000 children followed from birth to school age, has demonstrated that, whilst point prevalence data for the whole population may show a profile consistent with the atopic march, modelling within individual data over the life course shows seven different patterns, with >94% of children with symptoms (AD, wheeze and rhinitis) during childhood not following the atopic march profile(33). Therefore, the atopic march may be just an epiphenomenon of different allergic subtypes occurring at similar time points of the individual development (co-manifestation), e.g. early-life wheeze and early-life sensitization. Evidence from longitudinal studies suggests that approximately one-third of patients with AD develop asthma and two-thirds develop allergic rhinitis support the hypothesis of an underlying common mechanism. A review of four population-based cohort studies with a minimum of 80% follow-up, confirmed that early-life AD (especially IgE-associated AD) is a significant risk factor for developing asthma later in life (pooled OR 2.14; 95% CI 1.76–2.75)(34). Interestingly, in two of these cohorts, the significant association of early-life eczema and asthma disappeared when adjusted for early-life wheeze and sensitization, but was still present when adjustment was confined to early-life wheeze, suggesting that sensitization is a major common factor. It also points to a

putative mechanism where AD may increase the risk of subsequent sensitization, which in turn increases the risk of asthma.

Filaggrin gene (FLG) mutations are associated both with atopic and nonatopic eczema starting in the first year of life. FLG mutations combined with eczema in the first year of life are associated with a later development of asthma and hay fever, and this may support the latter mechanism(35). This more modern view of the atopic march is furthermore strongly supported by recent data on the defective skin barrier function as the key factor for the pathogenesis of AD(36). Skin barrier dysfunction facilitates transdermal dehydration and infiltration of allergens, bacteria and bacterial toxins, thus inducing and enhancing allergen sensitization as a hallmark of the atopic march(37). Skin sensitization is followed by airway sensitization to the same allergen and is one of the most robust predictors for the development of childhood asthma(38). This is detailed further on in this review. In conclusion, there is evidence for the hypothesis linking AD as an initial (but probably not only) promoter of atopy/allergic sensitization with progression to asthma.

Component-resolved diagnostics in asthma

Recent advances in biochemistry and molecular biology have led to the isolation and characterisation of numerous allergenic proteins (components), facilitating the profiling of IgE reactivity to individual allergens at a molecular level. This new approach to allergy diagnosis has been termed molecular diagnosis or component-resolved diagnostics (CRD), and its commercialisation has facilitated the development of products in which sIgE to >100 allergen components can be measured simultaneously. Component resolved diagnostics may help in identifying patients at risk of developing more severe disease(31, 32). Sensitization to mite allergens Der p 2 and Der f 2 has been reported to be more common in severe asthma (39). Latex allergy and asthma is another example were sensitization to 3 out of 12 recombinant natural rubber antigens (5, 6.01/6.02), was strongly linked to those with latex sensitization and asthma (40)

The role of these novel tools in clinical practice and how best to interpret the complex data they generate is the subject of ongoing debate(41, 42). It has recently been reported that CRD may improve the assessment of asthma(31, 43), and help better understanding the role of allergy in severe asthma in childhood(44). However, it is likely that better interpretation algorithms are needed to capitalise fully on the potential of this exciting new technology(43).

SIMILARITIES AND DISTINCTIONS BETWEEN ADULT AND PAEDIATRIC SEVERE ASTHMA

A fundamental feature of severe asthma in both adults and children is its heterogeneity, with multiple clinical phenotypes(6, 45-50). When unsupervised cluster analyses are performed, whether in adults or children, several common clinical features provide phenotypic distinctions, including the age of onset of disease, presence of co-morbidities, differences in lung function and the degree of atopic sensitisation(50-52). Using this approach, it appeared that the role of atopic sensitisation might be more important in the pathogenesis of severe asthma in early life. Severe atopy, characterised by polysensitisation and high specific IgE levels, is integral to childhood severe disease, such that >85% of children with severe asthma are severely atopic(53). In concurrence, when phenotypic clusters are investigated in adults with severe asthma, the single most important factor that repeatedly distinguishes the importance of allergy is age of disease onset(45). The phenotype of childhood onset asthma is robust, is repeatedly identified in adult cluster analyses and is undoubtedly associated with very severe allergic disease(8). In contrast, severe adult onset asthma is a distinct phenotype that is usually not characterised by atopic sensitisation, but often associated with nasal polyposis and sputum eosinophilia(54).

Atopy and paediatric severe asthma

The importance of early atopic sensitisation contributing to childhood severe asthma is reflected in the evidence of early sensitisation in preschool children being the main predictor of asthma development by school age(19, 55). In addition, even though recurrent wheezing episodes caused by rhinovirus infections in the first 3 years of life strongly predict asthma development(56), early atopic sensitisation is the main risk factor determining progression to asthma(56). Moreover, the pattern of atopic sensitisation to inhalant allergens, in particular to perennial ones, and the level of specific IgE increase asthma risk(57).

The significant contribution of allergy to the pathogenesis of paediatric severe asthma is apparent from the clinical features that distinguish patients with difficult asthma (who have underlying modifiable factors) from those with genuine severe therapy resistant asthma (STRA)(58). Significantly, more patients with STRA are polysensitised, and have food allergy. Perhaps the most important distinctive feature of STRA becomes apparent when atopic sensitisation is quantified(18,

59). Patients with severe asthma have a much higher allergic burden(51, 60) suggesting that atopic sensitisation plays a critical role in the development, progression and persistence of paediatric severe disease.

Adult onset, severe asthma: an age-specific phenotype

Adult onset asthma is a recognised phenotype of severe asthma, presenting with several sub-phenotypes(61). Although it is considered predominantly non-allergic, a significant proportion of patients with adult onset disease are atopic (34%)(61). In those with severe disease, a worse prognosis is apparent in smokers and ex-smokers(62), and, as described later on, smoke exposure has a detrimental effect on severe asthma, resulting in reduced corticosteroid responsiveness, regardless of age(63). Distinguishing and specific features of adult onset asthma include association with co-morbidities, such as obesity, and a predominance in middle-aged women(64). The adult-onset obese, female predominant phenotype is characterised by the absence of inflammation and atopic sensitisation. Although this specific set of features is seen in adults, mechanisms resulting in obesity-associated asthma may not be dissimilar in children and adults. Children with severe asthma who have a higher BMI are less likely to have detectable inflammatory Th2 cytokines and have relatively higher lung function than those with lower BMI(53).

Another common adult-onset phenotype includes severe (non-allergic) eosinophilic phenotype, which is the most prevalent phenotype of severe asthma in adults, associated with aspirin sensitivity, nasal polyposis and eosinophilia, all persisting despite the treatment with high doses of inhaled corticosteroids(54). Innate immune mechanisms underlying this phenotype have recently been proposed since it has become apparent that patients respond to anti-IL-5 antibody therapies(65).

Contribution of allergy to mechanisms underlying severe asthma

The role of allergy in severe asthma needs to be understood to help identify underlying mechanisms of disease progression which will impact both on the choice of add-on therapies and on the discovery of novel therapeutics. Even though the majority of children and adults with early-onset severe asthma are sensitised, it is interesting that not all respond to treatment with omalizumab(66, 67)suggesting several different mechanisms contributing to the development of different allergic phenotypes.

Typically, the allergic asthma phenotype is associated with eosinophilia, elevated serum IgE and Th2 cytokines. However, in adult-onset asthma, eosinophilia may be present without overt evidence of allergy(65). The limited contribution of allergy to disease persistence is apparent in adults with severe asthma who show a non-allergic, inhaled corticosteroid "resistant" eosinophilic phenotype, which responds to systemic CS and targeted therapy with anti-IL-5 (mepolizumab)(68). Novel mechanisms that may contribute to this adult-onset phenotype include epithelial innate cytokines that directly induce the recruitment of innate lymphoid cells which secrete Th2/"allergic" cytokines without the generation of IgE or an adaptive immune response(69). Interestingly, even though it is thought that this is an innate, non-adaptive, non-allergic immune response, all murine experimental models investigating the role of innate cytokines in asthma pathogenesis used allergen exposure as the stimulus, suggesting allergy still plays a central mechanistic role in this phenotype(70). It is possible that allergy is a risk factor in the development of adult-onset "non-allergic" eosinophilic asthma, but the clinical manifestation of asthma changes with time and age, whereby it is less overtly "allergic", but remains eosinophilic.

In asthma, the effect of innate immunity eliciting Th2 responses seems to be strongly related to IL-33(71), and is especially associated with severe disease. IL-33 expression is increased in bronchial tissue from both adults(72, 73) and children(74)with severe asthma. Other important features of innate cytokines that may contribute to the pathogenesis of severe disease in both adults and children include their role in (relative) corticosteroid resistance(74) and their association with angiogenesis and airway remodelling, in particular as regards IL-25 (74-76).

An interesting distinction of adult asthma phenotypes based on gene expression of periostin by airway epithelial cells includes the separation in Th2 high and Th2 low phenotypes(77), and the utility of this biomarker to predict therapeutic response to antibodies that block Th2 cytokines(78). Although biomarkers that allow such distinctions have not yet been identified in children, and while in general children with severe asthma have low or undetectable Th2 cytokines in airway samples, there is a sub-group in whom Th2 cytokines can be detected(53), emphasising similarities between adult and childhood disease.

CROSS-TALK BETWEEN ENVIRONMENTAL FACTORS, ATOPIC SENSITISATION AND ASTHMA

The airway epithelial barrier

Environmental stimuli, such as viruses, bacteria and air pollutants, are known activators of innate immunity and may thus enhance the airway inflammation in asthmatic patients. Allergens, apart from being recognised by the adaptive immunity, may also play a crucial role in activating innate immunity through proteases, biologically active glycolipids and enzymes(79). The airway epithelial barrier, for long time perceived as only a mechanical barrier, is now also recognised as a gate to initiate atopic sensitization and allergic inflammation(80). Epithelial cells recognise the allergens with the help of pattern recognition receptors and produce an innate immune response. As apical junctional complexes between the airway epithelium cells are being disrupted by viral infections and inhaled airway irritants, they facilitate the entry of allergens from the lumen to be presented to the dendritic cells.

In bronchial biopsies and brushings especially from more severe asthmatic patients, airway epithelium cells showed structural and functional defects in apical junctional complexes compared to healthy controls(81). However, this reduced barrier function was found to be reversible by epidermal growth factor (EGF) treatment(81).

The role of microbiota

Early life airway and gut microbiota and influencing factors such as the delivery method, feeding practices, antibiotic use and living environment were shown to be related with allergic asthma development(82). Both the microbial burden and diversity within the lower airways were shown to be significantly higher in suboptimally controlled asthmatic patients compared to healthy individuals(83). Protobacteria species significantly predominated in asthmatic patients using inhaled corticosteroids and showed the strongest correlations with the degree of bronchial hyperresponsiveness(82). In addition, corticosteroid resistance in asthmatic patients was found to be related to airway microbiome diversity(84). In these patients, Haemophilus parainfluenza dominated the microbiome, and was shown to inhibit the response to corticosteroid treatment compared to corticosteroid responsive asthmatic patients. Microbial diversity was also shown to increase the risk of rhinovirus-induced asthma exacerbations in children(85). If rhinovirus existed concomitantly with Moraxella catarrhalis, Streptococcus pneumoniae, or Haemophilus influenzae within the airways, the risk of asthma exacerbations was found to be significantly increased as compared to children without these pathogens.

Viruses

The interaction between viral lower respiratory tract infections (LRTI) and atopic sensitization has been recognized as a major factor contributing to asthma development and exacerbation(86, 87). Birth cohort studies provide strong evidence for a synergistic effect of viral LRTI and atopic sensitization on asthma inception particularly in predisposed children(56, 88). Other factors reported to increase the risk of asthma development include the type of virus (more than 10-fold increased risk for asthma development with rhinovirus compared to 5-fold with respiratory syncytial virus), the severity of viral LRTI, the age during viral LRTI and the atopic predisposition(89). Very recently, the number of respiratory episodes in the first years of life, but not the particular viral trigger, was reported to be associated with later asthma development(90).

Respiratory viral infections in combination with atopic sensitisation and exposure to allergens increase the risk of hospital admission due to asthma exacerbation both in children(91) and adults(92). Rhinoviruses (RV), especially RV-C group, are the most frequent viruses detected during an asthma exacerbation(22)including severe asthma exacerbations with near fatal and fatal asthma(23). Also, allergic asthmatic individuals experience more severe and prolonged LRTI symptoms with RV infection compared to non-atopic healthy controls(93). Biological mechanisms including impaired innate or altered adaptive immune function, abnormal airway structure and function following prior infections, genetic influences and extrinsic factors, such as maternal smoking, air pollution and nutritional factors (vitamin D), may explain the altered immune response to viral infections in asthmatic/allergic patients(87). Recently, antibody titers to species specific RV infection in children during asthma exacerbation showed that antibody response to RV-C is low even when the virus was detected, pointing to a divergent and possibly less efficacious immune response to this subtype compared to RV-A and B(94). The association of susceptibility to RV infection in asthma was also investigated in human bronchial epithelial cells showing impaired interferon production to the virus in severe therapy resistant allergic asthmatic children(26) but normal responses in well controlled asthmatic adults who were mostly atopic(95). In contrast to RV data, interferon responses to influenza A virus and RSV in human bronchial epithelial cell cultures were preserved in adults with mild to severe asthma(96).

Outdoor, indoor and food allergens

Relationships between different types of allergens (outdoor, indoor, food) and the development and severity of allergic disease, including asthma, have been studied(97). For instance, pollen allergy has been found to be interrelated with various food allergies, digestive system Th2-inflammation and asthma(98, 99). Cross-reactivity between pollen and several plant-derived foods, nuts, and fruits has been well established(98). Food allergy without concomitant asthma has been found to be associated with increased nonspecific bronchial hyperresponsiveness(100, 101), while several studies report that children with asthma and concomitant food allergy have more severe disease, poorer control, greater morbidity, and require more anti-asthma medications(102, 103).

The most common indoor allergens associated with asthma include house dust mites, domestic animals (cats, dogs), and cockroaches(97, 104), while fungi can be found both indoor and outdoor. In a cohort of 300 asthmatic children (aged 4-12 y), higher *Der p* 1 and pet allergen levels were found to be associated with greater asthma severity(105).

Fungal exposure is universal and fungi can be linked to asthma in a variety of ways. Fungal allergy drives asthma severity and long-term or uncontrolled fungal infections are associated with a poor control of asthma, complications such as bronchiectasies and chronic allergic bronchopulmonary aspergillosis (ABPA)(106). In the general asthma population, sensitization to moulds ranges from 7 to 20%, in severe asthma patients from 35 to 75%, being 54-91% in life-threatening asthma population(107-111). The first evidence of the link between the severity of asthma and fungal sensitisation dates to 1978, when Schwartz et al. demonstrated a relationship between asthma severity and *Aspergillus spp* sensitisation(112). *Alternaria* or *Cladosporium spp* sensitisation was associated with asthma severity in the European Community Respiratory Health Survey. Furthermore, a recent paper has shown that fungal sensitisation in children with persistent asthma is associated with disease severity(113)and a 2014 review has shown increasing evidence that sensitized asthmatic children may be susceptible to asthma exacerbations when exposed to outdoor fungal spores and that the severity of exacerbation may vary with different fungi species(114).

The term “Severe Asthma with Fungal Sensitisation” (SAFS) was introduced by Denning et al. in 2006, to describe those patients who have persistent severe asthma (despite standard treatment) and evidence of fungal sensitisation, as defined by positive SPT, or fungus or fungal antigen-specific sIgE, and do not meet the criteria for ABPA(10). Proposed classification by an EAACI Task Force sets the total IgE cut-off at <1000 IU/ml for SAFS and >1000 IU/ml for ABPA. ABPA was accepted as an endotype(115), while SAFS remains a pragmatic definition(106). ABPA may develop in asthmatics with a genetic predisposition and therefore SAFS may have the same

background. Carefully genotyping patients with different forms of asthma may allow a better understanding of this disease.

"Trichophyton Asthma" is another clinical entity, where inhalation or the presence of cutaneous infection (athlete's foot, onychomycosis) in sensitised asthmatics is associated with disease severity(106, 116).

Smoking

Cigarette smoking itself may influence asthma, as it accelerates lung function decline(117), impairs the response to CS (both inhaled and oral)(118), increases airway oxidative stress(119), perpetuates symptoms despite of treatment(120) and induces the change of inflammatory phenotypes into more aggressive ones(121), thereby resulting in a more severe disease(122).

Smoking also increases serum IgE levels, especially in men(123). This may result in an increased risk of allergic sensitisation, at least for occupational allergens(124). However, the relationship between cigarette smoking and allergy in severe asthma is still debated: some studies identify smoking as a risk factor for allergic asthma(125), while others show a lower prevalence of atopic sensitisation in smoking patients with severe asthma(121). According to a large epidemiological survey (ECRHS II), smoking was more strongly associated with severe asthma in men than in women, particularly if they were sensitised to moulds (*Cladosporium*), house dust mites or cats(126). Even more conflicting data come from studies on the effect of passive smoking on the risk of development of atopic sensitisation(127).

Cigarette smoking usually results in a more neutrophilic airway inflammation, which is less responsive to ICS(121). Accordingly, alveolar macrophages from smokers have a reduced cellular CS responsiveness, which is associated with reduced histone deacetylase activity, an essential molecule for anti-inflammatory genes transcription(63, 128). In fact, they show an elevated glucocorticoid receptors (GR) ratio in PBMC which is in favour of GR-β (not able to induce any transcriptional activity) compared to GR-α (the active isoform with anti-inflammatory effects)(129). These molecular events make smoking asthmatics less responsive to CS, currently the standard controller therapy for asthma, leading them to a more probable evolution to severe asthma (figure 2).

Recently, a new distinct phenotype of severe asthma has been identified in frequent exacerbators, and history of smoking seems to be a risk factor for this phenotype(130). A novel risk score for

asthma exacerbations developed and validated by Bateman *et al.* supports the evidence that smoking status is a main predictor for uncontrolled asthma(131). Despite this well-known relationship, active smoking is still surprisingly common among asthmatics(132). More efficient smoking prevention programs and smoking cessation campaigns should be carried out to try to reduce the risk of developing severe asthma. Moreover, most clinical trials with new drugs aimed for severe asthma have been conducted in non-smoking patients, which results in incomplete knowledge on the efficacy of such therapeutic approaches in smokers. Large "real life" studies in severe asthma including smoking asthmatics should be encouraged. The complex relationship between cigarette smoking and atopic sensitisation increasing the risk of severe asthma should be better investigated as only few and conflicting data are presently available. However, this relationship remains difficult to address, particularly in cross-sectional studies, because of the potential selection bias (e.g. "healthy smoker effect")(133). Prospective studies in lifetime smokers with lifetime smoking are more appropriate to properly examine the relationships between smoking and severe asthma.

Pollution

The health effects caused by outdoor air pollution have been intensively studied during the last decades. The term "outdoor air pollution" involves particulate matter (PM), gaseous pollutions (nitrogen dioxide, sulphur dioxide and ozone) and traffic-related air pollution (elemental and carbon black, road dust)(134).

Increased exposure to ultrafine particles and carbon monoxide within the previous 4-7 days was associated with increased relative odds of a paediatric asthma visit(135). Other studies also indicate that sudden increase or decrease of exposure to air pollution may affect asthmatic symptoms or emergency department visits(136-138). Indeed, a decrease in the number of acute asthma events of over 40% was found after reduction of air pollution during summer Olympic games(138). So far, these studies were performed in children and included only a relatively low number of individuals.

Larger scale studies also demonstrated an adverse effect of outdoor air pollution on lung function(139-141). A multicenter birth cohort study (ESCAPE) showed an association between estimated levels of NO_2 and $PM_{2.5}$and decreases in FEV_1(139). In another birth cohort study (MAAS), lifetime exposure to PM_{10} and NO_2 was associated with significantly less growth in FEV_1 over time(140). In the same cohort, no association was found between long-term exposure to PM_{10} and NO_2 and the prevalence of asthma or wheeze(142). In adult asthmatics, exposure to NO_2 and

PM_{10} was associated with lower measures of FEV_1 and FVC(143)and exposure to ozone and PM_{10} increased the risk of uncontrolled asthma(144). Overall, these studies thus provide evidence of an inverse association between outdoor air pollution and lung function (Table 1). Whether asthma severity is directly affected by outdoor air pollution is unclear.

Several studies showed a positive association between exposure to air pollution during infancy and sensitisation to inhalant allergens(145-147). Although the mechanism underlying this association is not fully understood, some evidence suggests that ultrafine carbon black particles can directly induce maturation of dendritic cells *in vitro*(148), thereby facilitating sensitisation to inhalant allergens. Alternatively, airborne pollutants can induce the influx of inflammatory cells to the lungs, which might then lower the threshold for sensitisation. Indeed, it has recently been shown that allergen-specific Th2/Th17 cells accumulate in the lungs of mice exposed to both diesel exhaust particles and house dust mite extract(149). Diesel exhaust particles may also produce other immunological effects(150, 151) (Table 2). Furthermore, exposure to moderate air pollution during late pregnancy was found to cause increased cord blood IL-1β(152). A recent meta-analysis, however, showed no clear overall association between air pollution exposure and the development of sensitisation in children up to 10 years of age(153).

In summary, in multi-sensitised asthmatics, daily exposure to allergens in combination with other enhancing factors, including viral infections, environmental smoking, and/or pollution, will finally determine the asthma course and severity.

Occupational/Work-Related

Severe asthma may occur in patients affected by Work-Related Asthma (WRA).WRA encompasses both Occupational Asthma (OA), defined as "asthma caused by the workplace" and "Work-Exacerbated Asthma" (WEA), occurring in patients with pre-existing or concurrent asthma and exacerbated by different work-related factors (i.e. aeroallergens, exercise, irritants)(154). OA can be further divided into two subtypes: an allergic form (90% of all OA)(155), caused both by an IgE-mediated mechanism towards high (HMW) and low (LMW) molecular weight agents(106), and a non-IgE mediated form (Non-Allergic, Irritant-Induced [Occupational] Asthma (IIOA)), towards specific LMW agents in which the mechanism has not been characterized yet. The non-allergic IIOA can be further divided into the "Reactive Airway Dysfunction Syndrome" (RADS) and the "IIOA after multiple exposures". The first occurs after an acute, single exposure to very high

concentrations of irritating substances(156), while the second follows multiple exposure to irritants; in this subtype, onset of asthma can follow the exposures after some time(157, 158).

WRA should be suspected in patients whose asthma worsens while working or begin at work. Here a detailed occupational and medical history is fundamental(159, 160), while a clinical history only shows a low specificity in the diagnosis of OA(161). The investigation of WRA follows a well-defined protocol based on confirmation of bronchial asthma, work-related bronchoconstriction, sensitisation to occupational agents and on the confirmation of the causal role of occupational agents, being sensitisation *per se* not indicative of clinical symptoms(162) (Figure 3). Baseline spirometry is mandatory and it is strongly recommended that this should be complemented with non-specific bronchial hyperreactivity assessment with direct or indirect challenges. In individuals with suspected WRA, presenting with a normal respiratory function and/or negative methacholine challenge testing, serial lung function measurements and assessment of non-specific bronchial hyperreactivity are strongly recommended(162, 163). Additionally, spirometry can be performed during a work shift (Cross-shift spirometry). Furthermore, serial measurements of peak flow expiratory rate (serial PEFR) have been used to objectively confirm the link between the workplace and the asthmatic symptoms(164). Skin prick testing completes the diagnostic work-up, and the selection of specific allergens related to the individual's job is fundamental. Specific IgE evaluation is also of importance. The role of atopic mechanisms in severe occupational asthma has been confirmed by a recent study where treatment with omalizumab was successful in 90% of severe occupational asthma patients due to HMW and LMW agents, such as flour, animal dander, mites, moulds, isocyanate or acrylates(165). It is worth noting that, at least in OA, allergen exposure levels represent the major determinants both for the disease as such and for the severity of asthma(166, 167). Finally, specific inhalation challenges (SICs) or workplace inhalation challenges, complemented by the assessment of airway inflammation by induced sputum and FeNO may be considered.

Diagnosis of IIOA follows a well-defined protocol described in a recent EAACI Task Force document(158).

CONCLUSION

There is increasing evidence for the important, but not exclusive, role of allergy in severe asthma. Although some recent reports demonstrate that allergy may play only a limited role, this is likely not true for childhood disease, where early atopic sensitisation is critical in determining the severity of disease.

Mechanistic implications of co-factors interacting with allergy and asthma, such as virus infections, pollution, smoking, and work-related exposures, still need to be completely uncovered to allow the discovery of novel therapeutic targets.

Author contributions:

SRDG drafted the final version of this manuscript

All authors drafted different chapters and paragraphs of this work

All authors critically revised this work for important intellectual content

All authors approved the final version to be published

All authors agreed on accuracy and integrity of this work

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REFERENCES

1. Addo-Yobo EO, Custovic A, Taggart SC, Craven M, Bonnie B, Woodcock A. Risk factors for asthma in urban Ghana. *J Allergy Clin Immunol* 2001;**108**(3):363-368.
2. Al-Mousawi MS, Lovel H, Behbehani N, Arifhodzic N, Woodcock A, Custovic A. Asthma and sensitization in a community with low indoor allergen levels and low pet-keeping frequency. *J Allergy Clin Immunol* 2004;**114**(6):1389-1394.
3. Simpson BM, Custovic A, Simpson A, Hallam CL, Walsh D, Marolia H, et al. NAC Manchester Asthma and Allergy Study (NACMAAS): risk factors for asthma and allergic disorders in adults. *Clin Exp Allergy* 2001;**31**(3):391-399.
4. Stevens W, Addo-Yobo E, Roper J, Woodcock A, James H, Platts-Mills T, et al. Differences in both prevalence and titre of specific immunoglobulin E among children with asthma in affluent and poor communities within a large town in Ghana. *Clin Exp Allergy* 2011;**41**(11):1587-1594.
5. Custovic A, Johnston SL, Pavord I, Gaga M, Fabbri L, Bel EH, et al. EAACI position statement on asthma exacerbations and severe asthma. *Allergy* 2013;**68**(12):1520-1531.
6. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;**43**(2):343-373.
7. Sala KA, Carroll CL, Tang YS, Aglio T, Dressler AM, Schramm CM. Factors associated with the development of severe asthma exacerbations in children. *J Asthma* 2011;**48**(6):558-564.
8. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;**181**(4):315-323.
9. Holt PG, Strickland DH, Sly PD. Virus infection and allergy in the development of asthma: what is the connection? *Curr Opin Allergy Clin Immunol* 2012;**12**(2):151-157.
10. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006;**27**(3):615-626.
11. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. *Allergy* 2009;**64**(10):1498-1506.
12. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**(9):813-824.
13. Custovic A, Arifhodzic N, Robinson A, Woodcock A. Exercise testing revisited. The response to exercise in normal and atopic children. *Chest* 1994;**105**(4):1127-1132.
14. Lazic N, Roberts G, Custovic A, Belgrave D, Bishop C, Winn J, et al. Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts. *Allergy* 2013.
15. Lodrup Carlsen KC, Soderstrom L, Mowinckel P, Haland G, Pettersen M, Munthe Kaas MC, et al. Asthma prediction in school children; the value of combined IgE-antibodies and obstructive airways disease severity score. *Allergy* 2010;**65**(9):1134-1140.
16. Simpson A, Soderstrom L, Ahlstedt S, Murray CS, Woodcock A, Custovic A. IgE antibody quantification and the probability of wheeze in preschool children. *J Allergy Clin Immunol* 2005;**116**(4):744-749.
17. Carroll WD, Lenney W, Child F, Strange RC, Jones PW, Whyte MK, et al. Asthma severity and atopy: how clear is the relationship? *Arch Dis Child* 2006;**91**(5):405-409.
18. Marinho S, Simpson A, Marsden P, Smith JA, Custovic A. Quantification of atopy, lung function and airway hypersensitivity in adults. *Clin Transl Allergy* 2011;**1**(1):16.
19. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, et al. Early identification of atopy in the prediction of persistent asthma in children. *Lancet* 2008;**372**(9643):1100-1106.

20. Frith J, Fleming L, Bossley C, Ullmann N, Bush A. The complexities of defining atopy in severe childhood asthma. *Clin Exp Allergy* 2011;**41**(7):948-953.
21. Just J, Gouvis-Echraghi R, Rouve S, Wanin S, Moreau D, Annesi-Maesano I. Two novel, severe asthma phenotypes identified during childhood using a clustering approach. *Eur Respir J* 2012;**40**(1):55-60.
22. Murray CS, Poletti G, Kebadze T, Morris J, Woodcock A, Johnston SL, et al. Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* 2006;**61**(5):376-382.
23. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study. *BMJ* 2002;**324**(7340):763.
24. Murray CS PG, Ahlstedt S, Soderstrom L, Johnston SL, Custovic A. Probability of hospital admission with acute asthma exacerbation increases with increasing specific IgE antibody levels. *Allergy Clin Immunol Int: J World Allergy Org* 2007;**Suppl 2**:270-273.
25. Teach SJ, Gill MA, Togias A, Sorkness CA, Arbes SJ, Jr., Calatroni A, et al. Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. *J Allergy Clin Immunol* 2015;**136**(6):1476-1485.
26. Edwards MR, Regamey N, Vareille M, Kieninger E, Gupta A, Shoemark A, et al. Impaired innate interferon induction in severe therapy resistant atopic asthmatic children. *Mucosal Immunol* 2013;**6**(4):797-806.
27. Holt PG, Strickland D, Bosco A, Belgrave D, Hales B, Simpson A, et al. Distinguishing benign from pathologic TH2 immunity in atopic children. *J Allergy Clin Immunol* 2016;**137**(2):379-387.
28. Simpson A, Tan VY, Winn J, Svensen M, Bishop CM, Heckerman DE, et al. Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. *Am J Respir Crit Care Med* 2010;**181**(11):1200-1206.
29. Belgrave DC, Buchan I, Bishop C, Lowe L, Simpson A, Custovic A. Trajectories of lung function during childhood. *Am J Respir Crit Care Med* 2014;**189**(9):1101-1109.
30. Custovic A, Ainsworth J, Arshad H, Bishop C, Buchan I, Cullinan P, et al. The Study Team for Early Life Asthma Research (STELAR) consortium 'Asthma e-lab': team science bringing data, methods and investigators together. *Thorax* 2015;**70**(8):799-801.
31. Simpson A, Lazic N, Belgrave DC, Johnson P, Bishop C, Mills C, et al. Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years. *J Allergy Clin Immunol* 2015.
32. Custovic A, Sonntag HJ, Buchan IE, Belgrave D, Simpson A, Prosperi MC. Evolution pathways of IgE responses to grass and mite allergens throughout childhood. *J Allergy Clin Immunol* 2015;**136**(6):1645-1652 e1641-1648.
33. Belgrave DC, Granell R, Simpson A, Guiver J, Bishop C, Buchan I, et al. Developmental profiles of eczema, wheeze, and rhinitis: two population-based birth cohort studies. *PLoS Med* 2014;**11**(10):e1001748.
34. van der Hulst AE, Klip H, Brand PL. Risk of developing asthma in young children with atopic eczema: a systematic review. *J Allergy Clin Immunol* 2007;**120**(3):565-569.
35. Schuttelaar ML, Kerkhof M, Jonkman MF, Koppelman GH, Brunekreef B, de Jongste JC, et al. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction with cat exposure. *Allergy* 2009;**64**(12):1758-1765.
36. Peng W, Novak N. Pathogenesis of atopic dermatitis. *Clin Exp Allergy* 2015;**45**(3):566-574.
37. Hogan MB, Peele K, Wilson NW. Skin barrier function and its importance at the start of the atopic march. *J Allergy (Cairo)* 2012;**2012**:901940.
38. Lodge CJ, Lowe AJ, Gurrin LC, Hill DJ, Hosking CS, Khalafzai RU, et al. House dust mite sensitization in toddlers predicts current wheeze at age 12 years. *J Allergy Clin Immunol* 2011;**128**(4):782-788 e789.

39. Sylvestre L, Jegu J, Metz-Favre C, Barnig C, Qi S, de Blay F. Component-Based Allergen-Microarray: Der p 2 and Der f 2 Dust Mite Sensitization Is More Common in Patients With Severe Asthma. *J Investig Allergol Clin Immunol* 2016;**26**(2):141-143.
40. Vandenplas O, Froidure A, Meurer U, Rihs HP, Rifflart C, Soetaert S, et al. The role of allergen components for the diagnosis of latex-induced occupational asthma. *Allergy* 2016;**71**(6):840-849.
41. Antonicelli L, Massaccesi C, Braschi MC, Cinti B, Bilo MB, Bonifazi F. Component resolved diagnosis in real life: the risk assessment of food allergy using microarray-based immunoassay. *Eur Ann Allergy Clin Immunol* 2014;**46**(1):30-34.
42. Nettis E, Bonifazi F, Bonini S, Di Leo E, Maggi E, Melioli G, et al. Molecular diagnosis and the Italian Board for ISAC. *Eur Ann Allergy Clin Immunol* 2014;**46**(2):68-73.
43. Prosperi MC, Belgrave D, Buchan I, Simpson A, Custovic A. Challenges in interpreting allergen microarrays in relation to clinical symptoms: A machine learning approach. *Pediatr Allergy Immunol* 2013.
44. Konradsen JR, Nordlund B, Onell A, Borres MP, Gronlund H, Hedlin G. Severe childhood asthma and allergy to furry animals: refined assessment using molecular-based allergy diagnostics. *Pediatr Allergy Immunol* 2014;**25**(2):187-192.
45. Haldar A, Gupta UD, Majumdar KK, Laskar K, Ghosh S, Sen S. Community perception of Dengue in slum areas of metropolitan city of West Bengal. *J Commun Dis* 2008;**40**(3):205-210.
46. Wu W, Bleecker E, Moore W, Busse WW, Castro M, Chung KF, et al. Unsupervised phenotyping of Severe Asthma Research Program participants using expanded lung data. *J Allergy Clin Immunol* 2014;**133**(5):1280-1288.
47. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;**18**(5):716-725.
48. Bell MC, Busse WW. Severe asthma: an expanding and mounting clinical challenge. *J Allergy Clin Immunol Pract* 2013;**1**(2):110-121; quiz 122.
49. Moore WC, Fitzpatrick AM, Li X, Hastie AT, Li H, Meyers DA, et al. Clinical heterogeneity in the severe asthma research program. *Ann Am Thorac Soc* 2013;**10 Suppl**:S118-124.
50. Fitzpatrick AM, Teague WG, Meyers DA, Peters SP, Li X, Li H, et al. Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. *J Allergy Clin Immunol* 2011;**127**(2):382-389 e381-313.
51. Just J, Deslandes-Boutmy E, Amat F, Desseaux K, Nemni A, Bourrat E, et al. Natural history of allergic sensitization in infants with early-onset atopic dermatitis: results from ORCA Study. *Pediatr Allergy Immunol* 2014;**25**(7):668-673.
52. Schatz M, Hsu JW, Zeiger RS, Chen W, Dorenbaum A, Chipps BE, et al. Phenotypes determined by cluster analysis in severe or difficult-to-treat asthma. *J Allergy Clin Immunol* 2014;**133**(6):1549-1556.
53. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, et al. Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. *J Allergy Clin Immunol* 2012;**129**(4):974-982 e913.
54. Amelink M, de Groot JC, de Nijs SB, Lutter R, Zwinderman AH, Sterk PJ, et al. Severe adult-onset asthma: A distinct phenotype. *J Allergy Clin Immunol* 2013;**132**(2):336-341.
55. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U, et al. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006;**368**(9537):763-770.
56. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;**178**(7):667-672.

57. Stoltz DJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Gern JE, et al. Specific patterns of allergic sensitization in early childhood and asthma & rhinitis risk. *Clin Exp Allergy* 2013;**43**(2):233-241.
58. Sharples J, Gupta A, Fleming L, Bossley CJ, Bracken-King M, Hall P, et al. Long-term effectiveness of a staged assessment for paediatric problematic severe asthma. *Eur Respir J* 2012;**40**(1):264-267.
59. Marinho S, Simpson A, Soderstrom L, Woodcock A, Ahlstedt S, Custovic A. Quantification of atopy and the probability of rhinitis in preschool children: a population-based birth cohort study. *Allergy* 2007;**62**(12):1379-1386.
60. Belgrave DC, Simpson A, Semic-Jusufagic A, Murray CS, Buchan I, Pickles A, et al. Joint modeling of parentally reported and physician-confirmed wheeze identifies children with persistent troublesome wheezing. *J Allergy Clin Immunol* 2013;**132**(3):575-583 e512.
61. Amelink M, de Nijs SB, de Groot JC, van Tilburg PM, van Spiegel PI, Krouwels FH, et al. Three phenotypes of adult-onset asthma. *Allergy* 2013;**68**(5):674-680.
62. Westerhof GA, Vollema EM, Weersink EJ, Reinartz SM, de Nijs SB, Bel EH. Predictors for the development of progressive severity in new-onset adult asthma. *J Allergy Clin Immunol* 2014;**134**(5):1051-1056 e1052.
63. Kobayashi Y, Bossley C, Gupta A, Akashi K, Tsartsali L, Mercado N, et al. Passive smoking impairs histone deacetylase-2 in children with severe asthma. *Chest* 2014;**145**(2):305-312.
64. de Nijs SB, Venekamp LN, Bel EH. Adult-onset asthma: is it really different? *Eur Respir Rev* 2013;**22**(127):44-52.
65. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: Eosinophilic airway inflammation in nonallergic asthma. *Nat Med* 2013;**19**(8):977-979.
66. Grimaldi-Bensouda L, Zureik M, Aubier M, Humbert M, Levy J, Benichou J, et al. Does omalizumab make a difference to the real-life treatment of asthma exacerbations?: Results from a large cohort of patients with severe uncontrolled asthma. *Chest* 2013;**143**(2):398-405.
67. Deschildre A, Marguet C, Salleron J, Pin I, Rittie JL, Derelle J, et al. Add-on omalizumab in children with severe allergic asthma: a 1-year real life survey. *Eur Respir J* 2013;**42**(5):1224-1233.
68. Bel EH, Ortega HG, Pavord ID. Glucocorticoids and mepolizumab in eosinophilic asthma. *N Engl J Med* 2014;**371**(25):2434.
69. Vercelli D, Gozdz J, von Mutius E. Innate lymphoid cells in asthma: when innate immunity comes in a Th2 flavor. *Curr Opin Allergy Clin Immunol* 2014;**14**(1):29-34.
70. Walker JA, McKenzie AN. Development and function of group 2 innate lymphoid cells. *Curr Opin Immunol* 2013;**25**(2):148-155.
71. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol* 2013;**132**(4):933-941.
72. Prefontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010;**125**(3):752-754.
73. Traister RS, Uvalle CE, Hawkins GA, Meyers DA, Bleecker ER, Wenzel SE. Phenotypic and genotypic association of epithelial IL1RL1 to human TH2-like asthma. *J Allergy Clin Immunol* 2015;**135**(1):92-99.
74. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol* 2013;**132**(3):676-685 e613.
75. Gregory LG, Jones CP, Walker SA, Sawant D, Gowers KH, Campbell GA, et al. IL-25 drives remodelling in allergic airways disease induced by house dust mite. *Thorax* 2013;**68**(1):82-90.
76. Corrigan CJ, Wang W, Meng Q, Fang C, Wu H, Reay V, et al. T-helper cell type 2 (Th2) memory T cell-potentiating cytokine IL-25 has the potential to promote angiogenesis in asthma. *Proc Natl Acad Sci U S A* 2011;**108**(4):1579-1584.

77. Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol* 2014;**133**(2):388-394.
78. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;**365**(12):1088-1098.
79. Triggiani M, De Feo G, Cardamone C, Parente R. The Emerging Role of Innate Immunity in Respiratory Allergy. *International Trends in Immunity* 2015;**3**(2):28-32.
80. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. *J Allergy Clin Immunol* 2014;**134**(3):509-520.
81. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, et al. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011;**128**(3):549-556 e541-512.
82. Panzer AR, Lynch SV. Influence and effect of the human microbiome in allergy and asthma. *Curr Opin Rheumatol* 2015;**27**(4):373-380.
83. Huang YJ, Nelson CE, Brodie EL, Desantis TZ, Baek MS, Liu J, et al. Airway microbiota and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin Immunol* 2011;**127**(2):372-381 e371-373.
84. Goleva E, Jackson LP, Harris JK, Robertson CE, Sutherland ER, Hall CF, et al. The effects of airway microbiome on corticosteroid responsiveness in asthma. *Am J Respir Crit Care Med* 2013;**188**(10):1193-1201.
85. Kloepfer KM, Lee WM, Pappas TE, Kang TJ, Vrtis RF, Evans MD, et al. Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations. *J Allergy Clin Immunol* 2014;**133**(5):1301-1307, 1307 e1301-1303.
86. Gavala ML, Bertics PJ, Gern JE. Rhinoviruses, allergic inflammation, and asthma. *Immunol Rev* 2011;**242**(1):69-90.
87. James KM, Peebles RS, Jr., Hartert TV. Response to infections in patients with asthma and atopic disease: an epiphenomenon or reflection of host susceptibility? *J Allergy Clin Immunol* 2012;**130**(2):343-351.
88. Kusel MM, de Klerk NH, Kebadze T, Vohma V, Holt PG, Johnston SL, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007;**119**(5):1105-1110.
89. Mackenzie KJ, Anderton SM, Schwarze J. Viral respiratory tract infections and asthma in early life: cause and effect? *Clin Exp Allergy* 2014;**44**(1):9-19.
90. Bonnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin Immunol* 2015;**136**(1):81-86 e84.
91. Papadopoulos NG, Christodoulou I, Rohde G, Agache I, Almqvist C, Bruno A, et al. Viruses and bacteria in acute asthma exacerbations--a GA(2) LEN-DARE systematic review. *Allergy* 2011;**66**(4):458-468.
92. Sandrock CE, Norris A. Infection in severe asthma exacerbations and critical asthma syndrome. *Clin Rev Allergy Immunol* 2015;**48**(1):104-113.
93. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, et al. Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. *Lancet* 2002;**359**(9309):831-834.
94. Iwasaki J, Smith WA, Khoo SK, Bizzintino J, Zhang G, Cox DW, et al. Comparison of rhinovirus antibody titers in children with asthma exacerbations and species-specific rhinovirus infection. *J Allergy Clin Immunol* 2014;**134**(1):25-32.
95. Sykes A, Macintyre J, Edwards MR, Del Rosario A, Haas J, Gielen V, et al. Rhinovirus-induced interferon production is not deficient in well controlled asthma. *Thorax* 2014;**69**(3):240-246.

96. Patel DA, You Y, Huang G, Byers DE, Kim HJ, Agapov E, et al. Interferon response and respiratory virus control are preserved in bronchial epithelial cells in asthma. *J Allergy Clin Immunol* 2014;**134**(6):1402-1412 e1407.
97. Baxi SN, Phipatanakul W. The role of allergen exposure and avoidance in asthma. *Adolesc Med State Art Rev* 2010;**21**(1):57-71, viii-ix.
98. Bartra J, Sastre J, del Cuvillo A, Montoro J, Jauregui I, Davila I, et al. From pollinosis to digestive allergy. *J Investig Allergol Clin Immunol* 2009;**19 Suppl 1**:3-10.
99. Bousquet J, Schunemann HJ, Samolinski B, Demoly P, Baena-Cagnani CE, Bachert C, et al. Allergic Rhinitis and its Impact on Asthma (ARIA): achievements in 10 years and future needs. *J Allergy Clin Immunol* 2012;**130**(5):1049-1062.
100. Thaminy A, Lamblin C, Perez T, Bergoin C, Tonnel AB, Wallaert B. Increased frequency of asymptomatic bronchial hyperresponsiveness in nonasthmatic patients with food allergy. *Eur Respir J* 2000;**16**(6):1091-1094.
101. Krogulska A, Dynowski J, Jedrzejczyk M, Sardecka I, Malachowska B, Wasowska-Krolikowska K. The impact of food allergens on airway responsiveness in schoolchildren with asthma: A DBPCFC study. *Pediatr Pulmonol* 2016.
102. Krogulska A, Dynowski J, Funkowicz M, Malachowska B, Wasowska-Krolikowska K. Prevalence and Clinical Impact of IgE-Mediated Food Allergy in School Children With Asthma: A Double-Blind Placebo-Controlled Food Challenge Study. *Allergy Asthma Immunol Res* 2015;**7**(6):547-556.
103. Wang J, Liu AH. Food allergies and asthma. *Curr Opin Allergy Clin Immunol* 2011;**11**(3):249-254.
104. Custovic A, Simpson A, Woodcock A. Importance of indoor allergens in the induction of allergy and elicitation of allergic disease. *Allergy* 1998;**53**(48 Suppl):115-120.
105. Gent JF, Belanger K, Triche EW, Bracken MB, Beckett WS, Leaderer BP. Association of pediatric asthma severity with exposure to common household dust allergens. *Environ Res* 2009;**109**(6):768-774.
106. Denning DW, Pashley C, Hartl D, Wardlaw A, Godet C, Del Giacco S, et al. Fungal allergy in asthma-state of the art and research needs. *Clin Transl Allergy* 2014;**4**:14.
107. Arbes SJ, Jr., Gergen PJ, Vaughn B, Zeldin DC. Asthma cases attributable to atopy: results from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2007;**120**(5):1139-1145.
108. Jaakkola MS, Ieromnimon A, Jaakkola JJ. Are atopy and specific IgE to mites and molds important for adult asthma? *J Allergy Clin Immunol* 2006;**117**(3):642-648.
109. O'Driscoll BR, Hopkinson LC, Denning DW. Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. *BMC Pulm Med* 2005;**5**:4.
110. Black PN, Udy AA, Brodie SM. Sensitivity to fungal allergens is a risk factor for life-threatening asthma. *Allergy* 2000;**55**(5):501-504.
111. O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, et al. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* 1991;**324**(6):359-363.
112. Schwartz HJ, Citron KM, Chester EH, Kaimal J, Barlow PB, Baum GL, et al. A comparison of the prevalence of sensitization to Aspergillus antigens among asthmatics in Cleveland and London. *J Allergy Clin Immunol* 1978;**62**(1):9-14.
113. Vicencio AG, Santiago MT, Tsirilakis K, Stone A, Worgall S, Foley EA, et al. Fungal sensitization in childhood persistent asthma is associated with disease severity. *Pediatr Pulmonol* 2014;**49**(1):8-14.
114. Tham R, Dharmage SC, Taylor PE, Katelaris CH, Vicendese D, Abramson MJ, et al. Outdoor fungi and child asthma health service attendances. *Pediatr Allergy Immunol* 2014;**25**(5):439-449.

115. Lotvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 2011;**127**(2):355-360.
116. Ward GW, Jr., Karlsson G, Rose G, Platts-Mills TA. Trichophyton asthma: sensitisation of bronchi and upper airways to dermatophyte antigen. *Lancet* 1989;**1**(8643):859-862.
117. James AL, Palmer LJ, Kicic E, Maxwell PS, Lagan SE, Ryan GF, et al. Decline in lung function in the Busselton Health Study: the effects of asthma and cigarette smoking. *Am J Respir Crit Care Med* 2005;**171**(2):109-114.
118. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC. Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J Respir Crit Care Med* 2003;**168**(11):1308-1311.
119. Barnes PJ, Ito K, Adcock IM. Corticosteroid resistance in chronic obstructive pulmonary disease: inactivation of histone deacetylase. *Lancet* 2004;**363**(9410):731-733.
120. Cerveri I, Cazzoletti L, Corsico AG, Marcon A, Niniano R, Grosso A, et al. The impact of cigarette smoking on asthma: a population-based international cohort study. *Int Arch Allergy Immunol* 2012;**158**(2):175-183.
121. Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, et al. Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe asthma. *J Allergy Clin Immunol* 2013;**131**(4):1008-1016.
122. Siroux V, Pin I, Oryszczyn MP, Le Moual N, Kauffmann F. Relationships of active smoking to asthma and asthma severity in the EGEA study. Epidemiological study on the Genetics and Environment of Asthma. *Eur Respir J* 2000;**15**(3):470-477.
123. Oryszczyn MP, Annesi-Maesano I, Charpin D, Paty E, Maccario J, Kauffmann F. Relationships of active and passive smoking to total IgE in adults of the Epidemiological Study of the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy (EGEA). *Am J Respir Crit Care Med* 2000;**161**(4 Pt 1):1241-1246.
124. Nielsen GD, Olsen O, Larsen ST, Lovik M, Poulsen LK, Glue C, et al. IgE-mediated sensitisation, rhinitis and asthma from occupational exposures. Smoking as a model for airborne adjuvants? *Toxicology* 2005;**216**(2-3):87-105.
125. Polosa R, Knoke JD, Russo C, Piccillo G, Caponnetto P, Sarva M, et al. Cigarette smoking is associated with a greater risk of incident asthma in allergic rhinitis. *J Allergy Clin Immunol* 2008;**121**(6):1428-1434.
126. Cazzoletti L, Marcon A, Corsico A, Janson C, Jarvis D, Pin I, et al. Asthma severity according to Global Initiative for Asthma and its determinants: an international study. *Int Arch Allergy Immunol* 2010;**151**(1):70-79.
127. Saulyte J, Regueira C, Montes-Martinez A, Khudyakov P, Takkouche B. Active or passive exposure to tobacco smoking and allergic rhinitis, allergic dermatitis, and food allergy in adults and children: a systematic review and meta-analysis. *PLoS Med* 2014;**11**(3):e1001611.
128. Adenuga D, Yao H, March TH, Seagrave J, Rahman I. Histone deacetylase 2 is phosphorylated, ubiquitinated, and degraded by cigarette smoke. *Am J Respir Cell Mol Biol* 2009;**40**(4):464-473.
129. Livingston E, Darroch CE, Chaudhuri R, McPhee I, McMahon AD, Mackenzie SJ, et al. Glucocorticoid receptor alpha:beta ratio in blood mononuclear cells is reduced in cigarette smokers. *J Allergy Clin Immunol* 2004;**114**(6):1475-1478.
130. Kupczyk M, ten Brinke A, Sterk PJ, Bel EH, Papi A, Chanez P, et al. Frequent exacerbators--a distinct phenotype of severe asthma. *Clin Exp Allergy* 2014;**44**(2):212-221.
131. Bateman ED, Buhl R, O'Byrne PM, Humbert M, Reddel HK, Sears MR, et al. Development and validation of a novel risk score for asthma exacerbations: The risk score for exacerbations. *J Allergy Clin Immunol* 2015;**135**(6):1457-1464 e1454.
132. Accordini S, Janson C, Svanes C, Jarvis D. The role of smoking in allergy and asthma: lessons from the ECRHS. *Curr Allergy Asthma Rep* 2012;**12**(3):185-191.

133. Becklake MR, Lalloo U. The 'healthy smoker': a phenomenon of health selection? *Respiration* 1990;**57**(3):137-144.
134. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. *Lancet* 2014;**383**(9928):1581-1592.
135. Evans KA, Halterman JS, Hopke PK, Fagnano M, Rich DQ. Increased ultrafine particles and carbon monoxide concentrations are associated with asthma exacerbation among urban children. *Environ Res* 2014;**129**:11-19.
136. Weinmayr G, Romeo E, De Sario M, Weiland SK, Forastiere F. Short-term effects of PM10 and NO2 on respiratory health among children with asthma or asthma-like symptoms: a systematic review and meta-analysis. *Environ Health Perspect* 2010;**118**(4):449-457.
137. Sarnat JA, Golan R, Greenwald R, Raysoni AU, Kewada P, Winquist A, et al. Exposure to traffic pollution, acute inflammation and autonomic response in a panel of car commuters. *Environ Res* 2014;**133**:66-76.
138. Friedman MS, Powell KE, Hutwagner L, Graham LM, Teague WG. Impact of changes in transportation and commuting behaviors during the 1996 Summer Olympic Games in Atlanta on air quality and childhood asthma. *JAMA* 2001;**285**(7):897-905.
139. Gehring U, Gruzieva O, Agius RM, Beelen R, Custovic A, Cyrys J, et al. Air pollution exposure and lung function in children: the ESCAPE project. *Environ Health Perspect* 2013;**121**(11-12):1357-1364.
140. Molter A, Agius RM, de Vocht F, Lindley S, Gerrard W, Lowe L, et al. Long-term exposure to PM10 and NO2 in association with lung volume and airway resistance in the MAAS birth cohort. *Environ Health Perspect* 2013;**121**(10):1232-1238.
141. Eeftens M, Hoek G, Gruzieva O, Molter A, Agius R, Beelen R, et al. Elemental composition of particulate matter and the association with lung function. *Epidemiology* 2014;**25**(5):648-657.
142. Molter A, Agius R, de Vocht F, Lindley S, Gerrard W, Custovic A, et al. Effects of long-term exposure to PM10 and NO2 on asthma and wheeze in a prospective birth cohort. *J Epidemiol Community Health* 2014;**68**(1):21-28.
143. Adam M, Schikowski T, Carsin AE, Cai Y, Jacquemin B, Sanchez M, et al. Adult lung function and long-term air pollution exposure. ESCAPE: a multicentre cohort study and meta-analysis. *Eur Respir J* 2015;**45**(1):38-50.
144. Jacquemin B, Kauffmann F, Pin I, Le Moual N, Bousquet J, Gormand F, et al. Air pollution and asthma control in the Epidemiological study on the Genetics and Environment of Asthma. *J Epidemiol Community Health* 2012;**66**(9):796-802.
145. Morgenstern V, Zutavern A, Cyrys J, Brockow I, Koletzko S, Kramer U, et al. Atopic diseases, allergic sensitization, and exposure to traffic-related air pollution in children. *Am J Respir Crit Care Med* 2008;**177**(12):1331-1337.
146. Gruzieva O, Bellander T, Eneroth K, Kull I, Melen E, Nordling E, et al. Traffic-related air pollution and development of allergic sensitization in children during the first 8 years of life. *J Allergy Clin Immunol* 2012;**129**(1):240-246.
147. Nordling E, Berglind N, Melen E, Emenius G, Hallberg J, Nyberg F, et al. Traffic-related air pollution and childhood respiratory symptoms, function and allergies. *Epidemiology* 2008;**19**(3):401-408.
148. de Haar C, Kool M, Hassing I, Bol M, Lambrecht BN, Pieters R. Lung dendritic cells are stimulated by ultrafine particles and play a key role in particle adjuvant activity. *J Allergy Clin Immunol* 2008;**121**(5):1246-1254.
149. Brandt EB, Biagini Myers JM, Acciani TH, Ryan PH, Sivaprasad U, Ruff B, et al. Exposure to allergen and diesel exhaust particles potentiates secondary allergen-specific memory responses, promoting asthma susceptibility. *J Allergy Clin Immunol* 2015;**136**(2):295-303 e297.
150. Acciani TH, Brandt EB, Khurana Hershey GK, Le Cras TD. Diesel exhaust particle exposure increases severity of allergic asthma in young mice. *Clin Exp Allergy* 2013;**43**(12):1406-1418.

151. De Grove KC, Provoost S, Hendriks RW, McKenzie AN, Seys LJ, Kumar S, et al. Dysregulation of type 2 innate lymphoid cells and TH2 cells impairs pollutant-induced allergic airway responses. *J Allergy Clin Immunol* 2016.
152. Latzin P, Frey U, Armann J, Kieninger E, Fuchs O, Roosli M, et al. Exposure to moderate air pollution during late pregnancy and cord blood cytokine secretion in healthy neonates. *PLoS One* 2011;**6**(8):e23130.
153. Gruzieva O, Gehring U, Aalberse R, Agius R, Beelen R, Behrendt H, et al. Meta-analysis of air pollution exposure association with allergic sensitization in European birth cohorts. *J Allergy Clin Immunol* 2014;**133**(3):767-776 e767.
154. Moscato G, Pala G, Barnig C, De Blay F, Del Giacco SR, Folletti I, et al. EAACI consensus statement for investigation of work-related asthma in non-specialized centres. *Allergy* 2012;**67**(4):491-501.
155. Mapp CE, Boschetto P, Maestrelli P, Fabbri LM. Occupational asthma. *Am J Respir Crit Care Med* 2005;**172**(3):280-305.
156. Brooks SM, Weiss MA, Bernstein IL. Reactive airways dysfunction syndrome (RADS). Persistent asthma syndrome after high level irritant exposures. *Chest* 1985;**88**(3):376-384.
157. Tarlo SM, Broder I. Irritant-induced occupational asthma. *Chest* 1989;**96**(2):297-300.
158. Vandenplas O, Wiszniewska M, Raulf M, de Blay F, Gerth van Wijk R, Moscato G, et al. EAACI position paper: irritant-induced asthma. *Allergy* 2014;**69**(9):1141-1153.
159. Cullinan P. Clinical aspects of occupational asthma. *Panminerva Med* 2004;**46**(2):111-120.
160. Vandenplas O, Ghezzo H, Munoz X, Moscato G, Perfetti L, Lemiere C, et al. What are the questionnaire items most useful in identifying subjects with occupational asthma? *Eur Respir J* 2005;**26**(6):1056-1063.
161. Malo JL, Ghezzo H, L'Archeveque J, Lagier F, Perrin B, Cartier A. Is the clinical history a satisfactory means of diagnosing occupational asthma? *Am Rev Respir Dis* 1991;**143**(3):528-532.
162. Vandenplas O, Toren K, Blanc PD. Health and socioeconomic impact of work-related asthma. *Eur Respir J* 2003;**22**(4):689-697.
163. Tarlo SM, Balmes J, Balkissoon R, Beach J, Beckett W, Bernstein D, et al. Diagnosis and management of work-related asthma: American College Of Chest Physicians Consensus Statement. *Chest* 2008;**134**(3 Suppl):1S-41S.
164. Moscato G, Godnic-Cvar J, Maestrelli P, Malo JL, Sherwood Burge P, Coifman R. Statement on self-monitoring of peak expiratory flow in the investigation of occupational asthma. Subcommittee on Occupational Allergy of the European Academy of Allergology and Clinical Immunology. *Allergy* 1995;**50**(9):711-717.
165. Lavaud F, Bonniaud P, Dalphin JC, Leroyer C, Muller D, Tannous R, et al. Usefulness of omalizumab in ten patients with severe occupational asthma. *Allergy* 2013;**68**(6):813-815.
166. Baur X, Chen Z, Liebers V. Exposure-response relationships of occupational inhalative allergens. *Clin Exp Allergy* 1998;**28**(5):537-544.
167. Jones MG. Exposure-response in occupational allergy. *Curr Opin Allergy Clin Immunol* 2008;**8**(2):110-114.

Figure 1. For those children who suffered a hospital admission with wheeze or asthma after age 3 years, a highly significant increase in the risk was seen only among children in the multiple early sensitisation subgroup (HR 9.2; 95% CI, 3.5–24; P < 0.001), but not other atopy classes.

From (28): Simpson A, Tan VY, Winn J, et al. Beyond atopy: multiple patterns of sensitization in relation to asthma in a birth cohort study. Am J Respir Crit Care Med 2010;181(11):1200-6, with permission

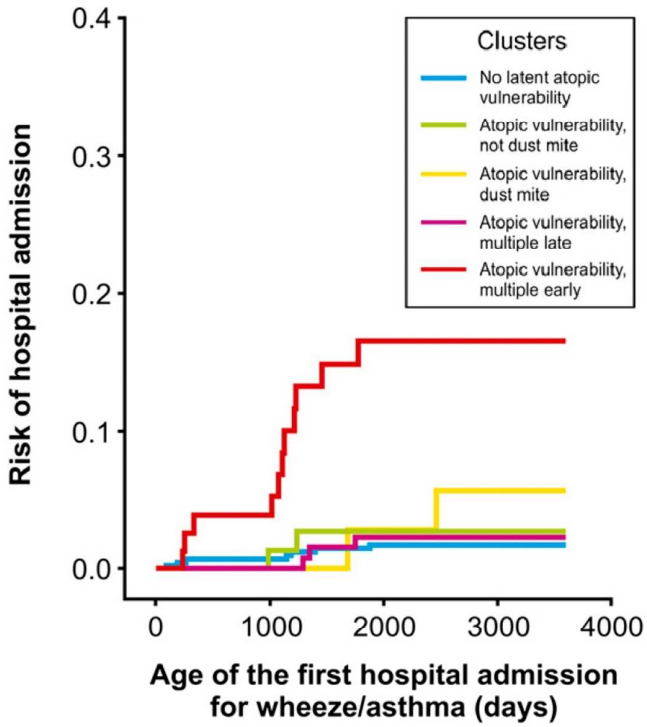

Table 1: Main pollutants and examples of their effects on respiratory function.

Pollutant	Outcome
Nitrogen dioxide (NO_2)	Decreased FEV_1(139)
	Less growth of FEV_1 over time(140)
	Lower measures of FEV_1(143)
	Lower measures of FVC(143)
$PM_{2.5}$	Decreased FEV_1(139)
PM_{10}	Less growth of FEV_1 over time(140)
	Lower measures of FEV_1(143)
	Lower measures of FVC(143)
	Increased risk of uncontrolled Asthma(144)
Ozone (O_3)	Increased risk of uncontrolled Asthma(144)

Table 2: Pollutants and examples of their effects on allergic inflammation

Pollutant	Outcome
Ultrafine Carbon Black Particles	Induced maturation of Dendritic Cells in vitro (148)
Diesel Exhaust Particles and House dust Mite Extract	Increased allergen-specific IgE and other cardinal features of asthma (150)
	Accumulation of allergen-specific Th2/Th17 cells in lungs (149)
	Both Th2 and ILC2 contribute to DEP-enhanced airway inflammation (151)

Figure 2: Influence of smoking and atopy in determining more severe asthma.

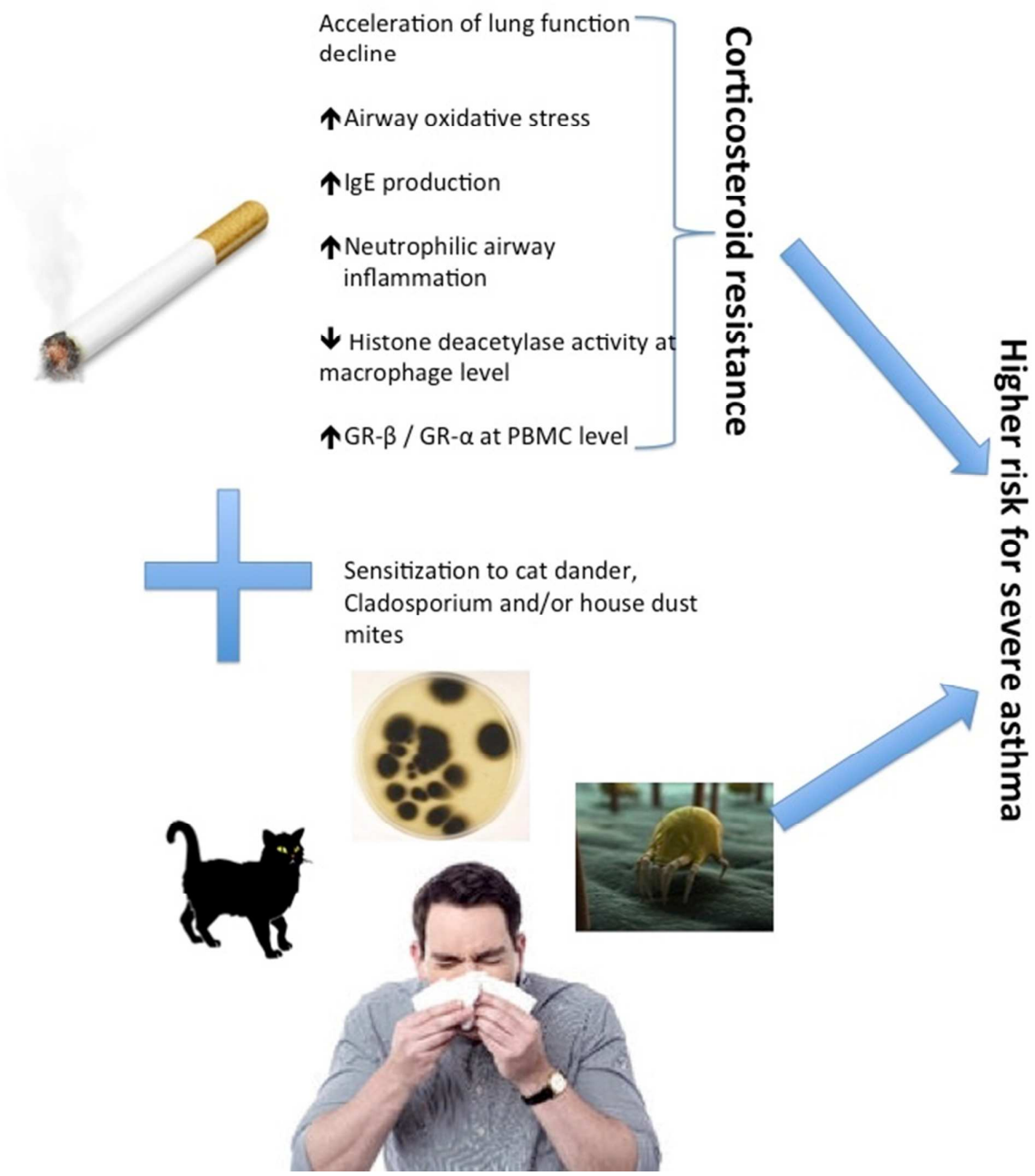

Figure 3: Allergic Occupational Asthma: diagnostic flow-chart.

From: Moscato G, Pala G, Barnig C, De Blay F, Del Giacco SR, Folletti I, et al. EAACI consensus statement for investigation of work-related asthma in non-specialized centres. Modified from: Allergy. 2012;67(4):491-501, with permission.