



## Allergy in severe asthma

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### Abstract

It is well recognized that atopic sensitization 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.

Numerous epidemiological studies have demonstrated that atopic sensitization 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  $\geq 50\%$  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 sensitized and exposed to high levels of inhalant allergens in their homes, emphasizing the importance of ‘allergy’ in asthma exacerbations (7). The phenotypes of childhood onset allergic asthma and early sensitization 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 sensitization 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 cofactors, 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 sensitization is defined either when allergen-specific serum IgE (sIgE) is 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  $\geq 3$  mm. These standard allergy tests have high sensitivity, but in themselves do not signify disease. For example, a considerable proportion of such defined sensitized 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 'sensitized' 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 sensitization (14).

### Quantification of atopic sensitization 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 sensitization' has been confirmed in subsequent studies in adult asthma (18). It is now recognized that quantification of atopic sensitization in early life among 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 sensitization and respiratory virus infections. This synergism has been indirectly confirmed in a study showing that preseasonal 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- $\beta$  and IFN- $\lambda$  induction by rhinovirus was a feature of bronchial epithelial cells from highly sensitized children with STRA (26), there was no relationship between sensitization 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. sensitization in the absence of allergic symptoms) and 'pathologic' atopy (i.e. sensitization 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 sensitization

It has recently been proposed that 'atopic sensitization' may be an umbrella term for a collection of several different subgroups of sensitization which differ in their association with asthma and other allergic diseases (14). Distinct subgroups (or classes) of sensitization 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 sensitization in another birth cohort (Isle of Wight study) (14). Children who would be considered sensitized using conventional

definitions were clustered into four distinct subgroups characterized by a unique pattern of the responses to different allergens and the timing of onset of allergen-specific sensitization (28) (Fig. 1). Importantly, the risk of asthma was increased more than 20-fold among children belonging to one of these subgroups (those sensitized to multiple allergens in early life – comprising less than one-third of the sensitized children), but not among those in other classes (14, 28). Striking similarities were observed in the association between different subgroups of atopic sensitization in these two cohorts in relation to asthma severity, with children in the subgroup of sensitization characterized by IgE responses to multiple allergens in early life having higher FeNO levels, more hyper-reactive airways and 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 sensitization 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 sensitization subgroups. Recent data indicate that IgE responses to individual allergenic molecules rather than

whole-allergen extracts may prove useful in differentiating the subtypes of sensitization relevant to asthma onset and severity (31, 32).

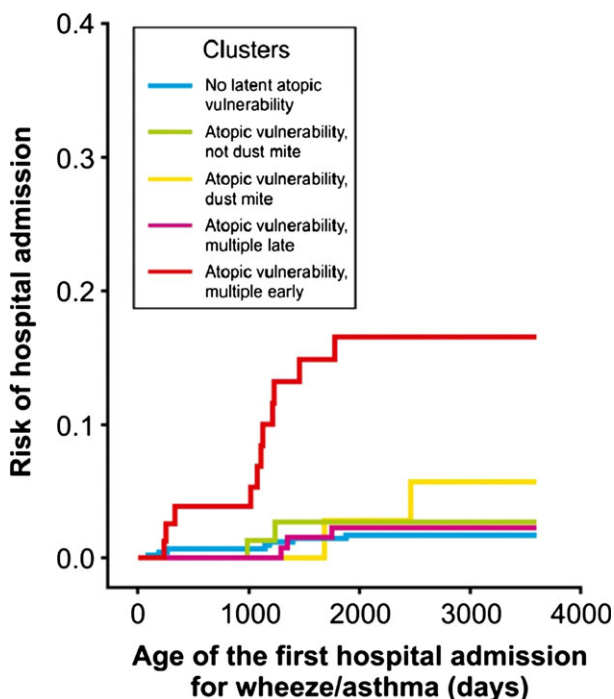
#### Progression from atopic dermatitis to allergic asthma – fact or myth?

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, while 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 (comanifestation), for example 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 with both 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 characterization of numerous



**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. Simpson et al. (28).

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 commercialization has facilitated the development of products in which sIgE to >100 allergen components can be measured simultaneously. CRD 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 where sensitization to 3 of 12 recombinant natural rubber antigens (5, 6.01/6.02) was strongly linked with 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 capitalize 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 comorbidities, differences in lung function and the degree of atopic sensitization (50–52). Using this approach, it appeared that the role of atopic sensitization might be more important in the pathogenesis of severe asthma in early life. Severe atopy, characterized by polysensitization 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 characterized by atopic sensitization, but often associated with nasal polyposis and sputum eosinophilia (54).

### Atopy and paediatric severe asthma

The importance of early atopic sensitization contributing to childhood severe asthma is reflected in the evidence of early sensitization 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 sensitization is the main risk factor determining progression to asthma (56).

Moreover, the pattern of atopic sensitization 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 STRA (58). Significantly, more patients with STRA are polysensitized and have food allergy. Perhaps the most important distinctive feature of STRA becomes apparent when atopic sensitization is quantified (18, 59). Patients with severe asthma have a much higher allergic burden (51, 60) suggesting that atopic sensitization 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 recognized phenotype of severe asthma, presenting with several subphenotypes (61). Although it is considered predominantly nonallergic, 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 comorbidities, such as obesity, and a predominance in middle-aged women (64). The adult-onset obese, female predominant phenotype is characterized by the absence of inflammation and atopic sensitization. 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 (nonallergic) 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 because 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 sensitized, 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 nonallergic, 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, nonadaptive, nonallergic 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 'nonallergic' 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 subgroup in whom Th2 cytokines can be detected (53), emphasizing similarities between adult and childhood disease.

### **Cross-talk between environmental factors, atopic sensitization 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 recognized 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 recognized as a gate to initiate atopic sensitization and allergic inflammation (80). Epithelial cells recognize 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). Proteobacteria species significantly predominated in asthmatic patients using inhaled corticosteroids and showed the strongest correlations with the degree of bronchial hyper-responsiveness (82). In addition, corticosteroid resistance in asthmatic patients was found to be related to airway microbiome diversity (84). In these patients, *Haemophilus parainfluenzae* 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 tenfold increased risk for asthma development with rhinovirus compared to fivefold 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 sensitization and exposure to allergens increase the risk of hospital admission due to asthma exacerbation both in children (91) and in 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 nonatopic 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 titres 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 with 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 hyper-responsiveness (100, 101), while several studies report that children with asthma and concomitant food allergy have more severe disease, poorer control and 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 years), 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 bronchiectases 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 sensitization dates to 1978, when Schwartz et al. (112) demonstrated a relationship between asthma severity and *Aspergillus* spp sensitization. *Alternaria* or *Cladosporium* spp sensitization was associated with asthma severity in the European Community Respiratory Health Survey. Furthermore, a recent paper has shown that fungal sensitization 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. (10) in 2006, to describe those patients who have persistent severe asthma (despite standard treatment) and evidence of fungal sensitization, as defined by positive SPT, or fungus or fungal antigen-specific sIgE, and do not meet the criteria for ABPA. 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 sensitized 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 sensitization, 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 sensitization 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 sensitized 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 sensitization (127).

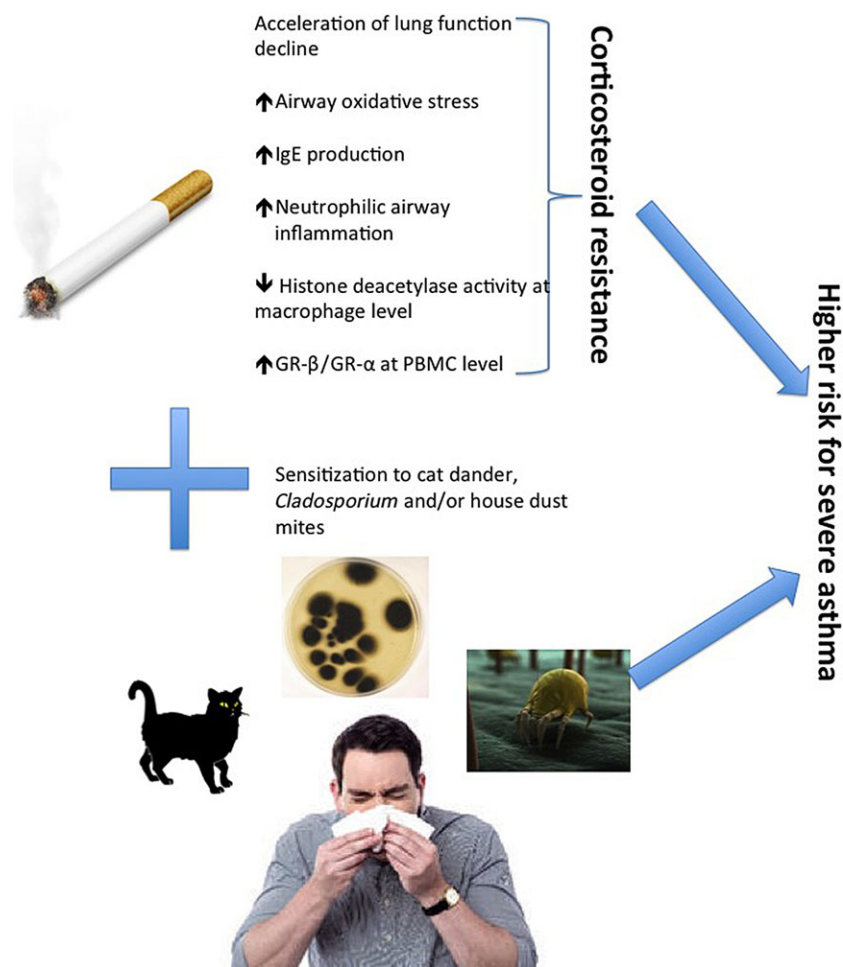
Cigarette smoking usually results in a more neutrophilic airway inflammation, which is less responsive to ICS (121). Accordingly, alveolar macrophages from smokers have a reduced cellular CS responsiveness, which is associated with reduced histone deacetylase activity, an essential molecule for anti-inflammatory 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 (Fig. 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. (131) supports the evidence that smoking status is a main predictor for uncontrolled asthma. Despite this well-known relationship, active smoking is still surprisingly common among asthmatics (132). More efficient smoking prevention programmes 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 nonsmoking 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 sensitization 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



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

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 in 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 multi-center birth cohort study (ESCAPE) showed an association between estimated levels of NO<sub>2</sub> and PM<sub>2.5</sub> and decreases in FEV<sub>1</sub> (139). In another birth cohort study (MAAS), lifetime exposure to PM<sub>10</sub> and NO<sub>2</sub> was associated with significantly less growth in FEV<sub>1</sub> over time (140). In the same cohort, no association was found between long-term exposure to PM<sub>10</sub> and NO<sub>2</sub> and the prevalence of asthma or wheeze (142). In adult asthmatics, exposure to NO<sub>2</sub> and PM<sub>10</sub> was associated with lower measures of FEV<sub>1</sub> and FVC (143) and exposure to ozone and PM<sub>10</sub> 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 sensitization 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 sensitization to inhalant allergens. Alternatively, airborne pollutants can induce the influx of inflammatory cells to the lungs, which might then lower the threshold for sensitization. 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-1 $\beta$  (152). A recent meta-analysis, however, showed no clear overall association between air pollution exposure and the development of sensitization in children up to 10 years of age (153).

In summary, in multisensitized 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 by both an IgE-mediated mechanism towards high (HMW) and low (LMW) molecular weight agents (106), and a non-IgE-mediated form (nonallergic, irritant-induced [occupational] asthma (HIOA)), towards specific LMW agents in which the mechanism has not been characterized yet. The nonallergic HIOA can be further divided into the ‘reactive airway dysfunction syndrome’ (RADS) and the ‘HIOA 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).

Work-related asthma 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, sensitization to occupational agents and on the confirmation of the causal role of occupational agents, being sensitization *per se* not indicative of clinical symptoms (162) (Fig. 3). Baseline

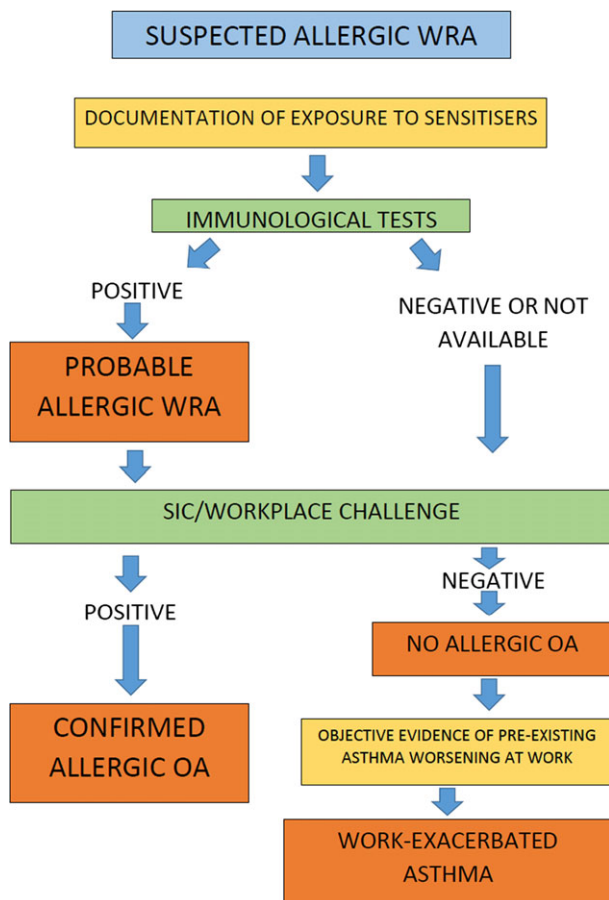
**Table 1** Main pollutants and examples of their effects on respiratory function

Pollutant	Outcome
Nitrogen dioxide (NO <sub>2</sub> )	Decreased FEV <sub>1</sub> (139)
	Less growth of FEV <sub>1</sub> over time (140)
	Lower measures of FEV <sub>1</sub> (143)
	Lower measures of FVC (143)
PM <sub>2.5</sub>	Decreased FEV <sub>1</sub> (139)
PM <sub>10</sub>	Less growth of FEV <sub>1</sub> over time (140)
	Lower measures of FEV <sub>1</sub> (143)
	Lower measures of FVC (143)
	Increased risk of uncontrolled asthma (144)
Ozone (O <sub>3</sub> )	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 <i>in vitro</i> (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 3** Allergic Occupational Asthma: diagnostic flow-chart. Moscato et al. (154).

spirometry is mandatory, and it is strongly recommended that this should be complemented with nonspecific bronchial hyper-reactivity 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 nonspecific bronchial hyper-reactivity 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 PEF) 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 sensitization is critical in determining the severity of disease.

Mechanistic implications of cofactors 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, and all authors drafted different sections and paragraphs of this work, critically revised this work for important intellectual content, approved the final version to be published and agreed on accuracy and integrity of this work.

### Conflict of interest disclosure

All authors declare that they have no conflict of interest regarding this work.

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