

Allergy in Severe Asthma

Journal:	<i>Allergy</i>
Manuscript ID	ALL-2016-00434.R1
Wiley - Manuscript type:	Position Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	<p>Del Giacco, Stefano; University of Cagliari, Department of Medical Sciences and Public Health Bakirtas, Arzu; Gazi University Faculty of Medicine, Department of Pediatric Allergy and Asthma Bel, Elizabeth; Academic Medical Centre, University of Amsterdam, Department of Respiratory Medicine Custovic, Adnan; Imperial College Faculty of Medicine, Department of Paediatrics Diamant, Zuzana; University of Groningen, University Medical Centre Groningen, Department of General Practice and Department of Clinical Pharmacy & Pharmacology; Lund University, Department of Respiratory Medicine and Allergology Hamelmann, Eckard; Ruhr University Bochum, Allergy Center; Bethel Evangelisches Krankenhaus, Klinik für Kinder and Jugendmedizin Kinderzentrum Heffler, Enrico; University of Catania - AOU "Policlinico - Vittorio Emanuele", Dept. Clinical and Experimental Medicine - Respiratory Medicine and Allergy Kalayci, Ömer; Hacettepe University, School of Medicine Saglani, S.; Imperial College London, NHLI Sergejeva, Svetlana; University of Tartu, Institute of Technology Seys, Sven; Catholic University of Leuven, Clinical Immunology, Department of Immunology and Microbiology Simpson, Angela; University of Manchester, Centre Lead for Respiratory Medicine and Allergy, Education and Research Centre, University Hospital of South Manchester Bjermer, Leif; Lund University, Dept. of Clinical Sciences Lund, Respiratory medicine and Allergology</p>
Keywords :	asthma, atopy, allergy, severity, aetiology

1 Allergy in Severe Asthma

2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 Stefano R. Del Giacco¹, Arzu Bakirtas,² Elizabeth Bel³, Adnan Custovic⁴, Zuzana
2 Diamant^{5,13}, Eckard Hamelmann⁶, Enrico Heffler⁷, Ömer Kalayci⁸, Sejal Saglani⁹,
3 Svetlana Sergejeva¹⁰, Sven Seys¹¹, Angela Simpson¹², Leif Bjermer¹³

- 6 1. Department of Medical Sciences and Public Health, University of Cagliari, Italy
- 7 2. Department of Pediatric Allergy and Asthma, School of Medicine, Gazi University, Ankara, Turkey
- 8 3. Department of Respiratory Medicine, Academic Medical Centre, University of Amsterdam, The
9 Netherlands
- 10 4. Department of Paediatrics, Imperial College London, United Kingdom
- 11 5. University of Groningen, University Medical Centre Groningen, Department of General Practice and
12 Department of Clinical Pharmacy & Pharmacology, Groningen, The Netherlands
- 13 6. Allergy Center, Ruhr University Bochum, Klinik für Kinder and Jugendmedizin Kinderzentrum,
14 Bethel Evangelisches Krankenhaus, Bielefeld, Germany
- 15 7. Respiratory Medicine and Allergology - Department of Experimental and Clinical Medicine,
16 University of Catania, Italy
- 17 8. Hacettepe University, School of Medicine, Ankara, Turkey
- 18 9. National Heart & Lung Institute, Imperial College London, United Kingdom
- 19 10. Institute of Technology, University of Tartu, Estonia
- 20 11. Department of Microbiology and Immunology, Laboratory of Clinical Immunology, KU Leuven,
21 Belgium
- 22 12. Centre Lead for Respiratory Medicine and Allergy, University of Manchester, Education and
23 Research Centre, University Hospital of South Manchester, United Kingdom
- 24 13. Lund University, Department of Respiratory Medicine and Allergology, Lund, Sweden

28 **Corresponding author:**

29 **Stefano R. Del Giacco**

30 Cittadella Universitaria

31 Department of Medical Sciences and Public Health

32 University of Cagliari

33 09042 Monserrato (Cagliari), Italy

34 stedg@medicina.unica.it

35 Tel: +39-070-6754150

36 Fax: +39-070-6754086

37 **TOTAL WORD COUNT: 5727**

38
39 **Short title:** Allergy and Asthma Severity

40 **Keywords:** aetiology, asthma, allergy, atopy, severity

1
2
3 41 **ABSTRACT**
4
5 42
6
7 43 It is well recognized that atopic sensitisation is an important risk factor for asthma, both in adults
8
9 44 and in children. However, the role of allergy in severe asthma is still under debate. The term
10
11 45 “Severe Asthma” encompasses a highly heterogeneous group of patients who require treatment on
12
13 46 steps 4–5 of GINA guidelines to prevent their asthma from becoming "uncontrolled", or whose
14
15 47 disease remains "uncontrolled" despite this therapy. Epidemiological studies on emergency room
16
17 48 visits and hospital admissions for asthma suggest the important role of allergy in asthma
18
19 49 exacerbations. In addition, allergic asthma in childhood is often associated with severe asthma in
20
21 50 adulthood. A strong association exists between asthma exacerbations and respiratory viral
22
23 51 infections, and interaction between viruses and allergy further increases the risk of asthma
24
25 52 exacerbations. Furthermore, fungal allergy has been shown to play an important role in severe
26
27 53 asthma. Other contributing factors include smoking, pollution and work-related exposures. The
28
29 54 “Allergy and Asthma Severity” EAACI Task Force examined the current evidence and produced
30
31 55 this position document on the role of allergy in severe asthma.
32
33 56
34
35 57
36
37 58 **ABSTRACT WORD COUNT: 174**
38
39 59
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 60 **INTRODUCTION**
4
5 61
6

7 62 Numerous epidemiological studies have demonstrated that atopic sensitisation is a strong risk factor
8
9 63 for asthma in childhood(1, 2) and adulthood(3), both in the developed(3) and in the developing
10
11 64 countries(1, 2, 4), supporting the notion that asthma is in part an allergic disease. However, the role
12
13 65 of allergy in severe asthma remains the issue of considerable controversy. The term “severe
14
15 66 asthma” encompasses a highly heterogeneous group of patients, which is defined in various ways in
16
17 67 the literature(5). Recent international guidelines define “severe asthma” as asthma which requires
18
19 68 treatment at GINA steps 4–5 during the previous year or systemic corticosteroids (CS) for $\geq 50\%$ of
20
21 69 the previous year to prevent it from becoming “uncontrolled”, or asthma which remains
22
23 70 “uncontrolled” despite this therapy, or controlled asthma that worsens on tapering high doses of
24
25 71 inhaled corticosteroids (ICS), systemic CS or additional biologics(6).

26
27 72 Asthma exacerbations are one of the key features of severe asthma. Emergency room visits and
28
29 73 hospital admissions due to acute asthma attacks are increased in children who are sensitised and
30
31 74 exposed to high levels of inhalant allergens in their homes, emphasising the importance of “allergy”
32
33 75 in asthma exacerbations(7). The phenotypes of childhood onset allergic asthma and early
34
35 76 sensitisation are often associated with severe asthma in adulthood(8). However, some data indicated
36
37 77 that the proportion of severe asthma cases attributable to allergy may be overestimated, and that
38
39 78 aetiological mechanisms other than allergy may be important in the pathogenesis of severe asthma.
40
41 79 For example, numerous studies have reported a strong association between asthma exacerbations
42
43 80 and respiratory viral infections, suggestive of a viral-induced mechanism. Rather than being
44
45 81 mutually exclusive, viruses and allergens may interact in increasing the risk of asthma
46
47 82 development(9).

48
49 83 Furthermore, fungal sensitisation is strongly associated with severe asthma, hence, recently a new
50
51 84 subtype of Severe Asthma with Fungal Sensitization (SAFS) has been proposed(10).

52
53 85 Finally, the role of several co-factors, such as smoking, pollution and work-related exposures must
54
55 86 be considered when evaluating a patient with severe asthma.

56
57 87 The “Allergy and Asthma Severity” EAACI Task Force produced this position document on the
58
59 88 role of allergy in severe asthma, searching the literature of the last 10 years in the main databases
60
61 89 (MEDLINE, Scopus, ISI) and including milestone and important papers at the discretion of the
62
63 90 different co-authors.
64
65 91

92

DEFINITION AND ROLE OF INHALANT ALLERGENS IN ASTHMA

94

Atopy, allergy and asthma

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

108

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

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

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

1
2
3 124 both tests(17, 20). The level of sIgE is also associated with an increased risk of severe asthma
4 125 exacerbations requiring hospitalization among both children(17, 22) and adults(23). Finally, it has
5 126 been shown that there is a strong interaction between the levels of sIgE to inhalant allergens and
6 127 respiratory virus infections in increasing the risk of severe asthma exacerbations requiring hospital
7 128 admission(24), suggesting a synergism between quantitative sensitisation and respiratory virus
8 129 infections. This synergism has been indirectly confirmed in a study showing that pre-seasonal anti-
9 130 IgE- targeted therapy with omalizumab decreases seasonal exacerbations of asthma (“back-to-
10 131 school asthma”), which are almost certainly (rhino)virus-induced(25). In contrast, a recent study
11 132 showed that although impaired **IFN- β and IFN- λ** induction by rhinovirus was a feature of bronchial
12 133 epithelial cells from highly sensitised children with STRA(26), there was no relationship between
13 134 sensitisation and Th2-mediated inflammation with impaired interferon production, raising a
14 135 possibility of two independent mechanisms (atopy-related and virus-related).

15
16
17
18
19
20
21
22
23 136 All of the above data indicate that in the assessment of patients with asthma (including severe
24 137 asthma), the results of specific IgE measurement and SPT are not mutually exclusive but
25 138 complementary, and should not be reported as being “positive” or “negative”, but as the level of
26 139 sIgE and the size of SPT wheal diameter (*i.e.*, quantified). For SPTs, the size of the positive and
27 140 negative control should be taken into account. Recent data suggest that diagnostic accuracy of
28 141 specific IgE antibody measurement in the context of asthma and the distinction between “benign”
29 142 atopy (*i.e.*, sensitisation in the absence of allergic symptoms) and “pathologic” atopy (*i.e.*,
30 143 sensitisation related to allergic symptoms), may be improved by the measurement of allergen-
31 144 specific IgG antibody levels(27), although their measurement is not recommended routinely.

32
33
34
35
36
37
38
39
40
41

42 146 ***Heterogeneity of atopic sensitisation***

43 147 It has recently been proposed that “atopic sensitisation” maybe an umbrella term for a collection of
44 148 several different subgroups of sensitisation which differ in their association with asthma and other
45 149 allergic diseases(14). **Distinct subgroups (or classes) of sensitisation were described in one**
46 150 **population-based birth cohort (Manchester Asthma and Allergy Study) by applying a machine**
47 151 **learning approach with Bayesian inference to the SPTs and sIgE data collected longitudinally from**
48 152 **early life to school age(28), and similar latent structure was subsequently described using**
49 153 **comparable approach to longitudinal data on atopic sensitisation in another birth cohort (Isle of**
50 154 **Wight study)(14). Children who would be considered sensitised using conventional definitions were**
51 155 **clustered into four distinct subgroups characterised by a unique pattern of the responses to different**
52 156 **allergens and the timing of onset of allergen-specific sensitisation(28) (Figure 1). Importantly, the**

1
2
3 157 risk of asthma was increased more than 20-fold amongst children belonging to one of these
4 158 subgroups (those sensitised to multiple allergens in early life - comprising less than one third of the
5 159 sensitised children), but not amongst those in other classes(14, 28). Striking similarities were
6 160 observed in the association between different subgroups of atopic sensitisation in these two cohorts
7 161 in relation to asthma severity, with children in the subgroup of sensitisation characterised by IgE
8 162 responses to multiple allergens in early life having higher FeNO levels, more hyperreactive airways,
9 163 an increased risk of severe asthma exacerbations having significantly diminished lung function,
10 164 compared to all other classes(14, 28, 29). It is of note however, that such subtypes (clusters/classes)
11 165 of sensitisation can only be identified using statistical inference on longitudinal data(14, 28), and
12 166 that differentiation between different clusters at any single cross-sectional point is not yet
13 167 possible(30). Clinical translation of this important observation requires the development of specific
14 168 and sensitive biomarkers which can be measured at the time of presentation to clinic and which aid
15 169 differentiation between different sensitisation subgroups. Recent data indicate that IgE responses to
16 170 individual allergenic molecules rather than whole allergen extracts may prove useful in
17 171 differentiating the subtypes of sensitisation relevant to asthma onset and severity(31, 32).
18 172

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

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

1
2
3 191 putative mechanism where AD may increase the risk of subsequent sensitization, which in turn
4 192 increases the risk of asthma.

5
6 193 Filaggrin gene (FLG) mutations are associated both with atopic and nonatopic eczema starting in
7 the first year of life. FLG mutations combined with eczema in the first year of life are associated
8 194 with a later development of asthma and hay fever, and this may support the latter
9 195 mechanism(35). This more modern view of the atopic march is furthermore strongly supported by

10 196 recent data on the defective skin barrier function as the key factor for the pathogenesis of AD(36).
11 197 Skin barrier dysfunction facilitates transdermal dehydration and infiltration of allergens, bacteria
12 198 and bacterial toxins, thus inducing and enhancing allergen sensitization as a hallmark of the atopic
13 199 march(37). Skin sensitization is followed by airway sensitization to the same allergen and is one of
14 200 the most robust predictors for the development of childhood asthma(38). This is detailed further on
15 201 in this review. In conclusion, there is evidence for the hypothesis linking AD as an initial (but
16 202 probably not only) promoter of atopy/allergic sensitization with progression to asthma.
17 203
18 204

25 205 *Component-resolved diagnostics in asthma*

26 206 Recent advances in biochemistry and molecular biology have led to the isolation and
27 207 characterisation of numerous allergenic proteins (components), facilitating the profiling of IgE
28 208 reactivity to individual allergens at a molecular level. This new approach to allergy diagnosis has
29 209 been termed molecular diagnosis or component-resolved diagnostics (CRD), and its
30 210 commercialisation has facilitated the development of products in which sIgE to >100 allergen
31 211 components can be measured simultaneously. Component resolved diagnostics may help in
32 212 identifying patients at risk of developing more severe disease(31, 32). Sensitization to mite
33 213 allergens Der p 2 and Der f 2 has been reported to be more common in severe asthma (39). Latex
34 214 allergy and asthma is another example were sensitization to 3 out of 12 recombinant natural rubber
35 215 antigens (5, 6.01/6.02), was strongly linked to those with latex sensitization and asthma (40)

36 216 The role of these novel tools in clinical practice and how best to interpret the complex data they
37 217 generate is the subject of ongoing debate(41, 42). It has recently been reported that CRD may
38 218 improve the assessment of asthma(31, 43), and help better understanding the role of allergy in
39 219 severe asthma in childhood(44). However, it is likely that better interpretation algorithms are
40 220 needed to capitalise fully on the potential of this exciting new technology(43).
41 221
42 222
43 223

1
2
3 224

4
5 225 **SIMILARITIES AND DISTINCTIONS BETWEEN ADULT AND PAEDIATRIC SEVERE**
6
7 226 **ASTHMA**

8
9 227

10
11 228 A fundamental feature of severe asthma in both adults and children is its heterogeneity, with
12 229 multiple clinical phenotypes(6, 45-50). When unsupervised cluster analyses are performed, whether
13
14 230 in adults or children, several common clinical features provide phenotypic distinctions, including
15
16 231 the age of onset of disease, presence of co-morbidities, differences in lung function and the degree
17
18 232 of atopic sensitisation(50-52). Using this approach, it appeared that the role of atopic sensitisation
19
20 233 might be more important in the pathogenesis of severe asthma in early life. Severe atopy,
21
22 234 characterised by polysensitisation and high specific IgE levels, is integral to childhood severe
23
24 235 disease, such that >85% of children with severe asthma are severely atopic(53). In concurrence,
25
26 236 when phenotypic clusters are investigated in adults with severe asthma, the single most important
27
28 237 factor that repeatedly distinguishes the importance of allergy is age of disease onset(45). The
29
30 238 phenotype of childhood onset asthma is robust, is repeatedly identified in adult cluster analyses and
31
32 239 is undoubtedly associated with very severe allergic disease(8). In contrast, severe adult onset
33
34 240 asthma is a distinct phenotype that is usually not characterised by atopic sensitisation, but often
35
36 241 associated with nasal polyposis and sputum eosinophilia(54).

37
38 242

39
40 243 ***Atopy and paediatric severe asthma***

41
42 244 The importance of early atopic sensitisation contributing to childhood severe asthma is reflected in
43
44 245 the evidence of early sensitisation in preschool children being the main predictor of asthma
45
46 246 development by school age(19, 55). In addition, even though recurrent wheezing episodes caused
47
48 247 by rhinovirus infections in the first 3 years of life strongly predict asthma development(56), early
49
50 248 atopic sensitisation is the main risk factor determining progression to asthma(56). Moreover, the
51
52 249 pattern of atopic sensitisation to inhalant allergens, in particular to perennial ones, and the level of
53
54 250 specific IgE increase asthma risk(57).

55
56 251 The significant contribution of allergy to the pathogenesis of paediatric severe asthma is apparent
57
58 252 from the clinical features that distinguish patients with difficult asthma (who have underlying
59
60 253 modifiable factors) from those with genuine severe therapy resistant asthma (STRA)(58).
254 Significantly, more patients with STRA are polysensitised, and have food allergy. Perhaps the most
255 important distinctive feature of STRA becomes apparent when atopic sensitisation is quantified(18,

1
2
3 256 59). Patients with severe asthma have a much higher allergic burden(51, 60) suggesting that atopic
4 257 sensitisation plays a critical role in the development, progression and persistence of paediatric
5 258 severe disease.

6
7
8 259

9
10 260 ***Adult onset, severe asthma: an age-specific phenotype***

11 261 Adult onset asthma is a recognised phenotype of severe asthma, presenting with several sub-
12 262 phenotypes(61). Although it is considered predominantly non-allergic, a significant proportion of
13 263 patients with adult onset disease are atopic (34%)(61). In those with severe disease, a worse
14 264 prognosis is apparent in smokers and ex-smokers(62), and, as described later on, smoke exposure
15 265 has a detrimental effect on severe asthma, resulting in reduced corticosteroid responsiveness,
16 266 regardless of age(63). Distinguishing and specific features of adult onset asthma include association
17 267 with co-morbidities, such as obesity, and a predominance in middle-aged women(64). The adult-
18 268 onset obese, female predominant phenotype is characterised by the absence of inflammation and
19 269 atopic sensitisation. Although this specific set of features is seen in adults, mechanisms resulting in
20 270 obesity-associated asthma may not be dissimilar in children and adults. Children with severe asthma
21 271 who have a higher BMI are less likely to have detectable inflammatory Th2 cytokines and have
22 272 relatively higher lung function than those with lower BMI(53).

23
24
25 273 Another common adult-onset phenotype includes severe (non-allergic) eosinophilic phenotype,
26 274 which is the most prevalent phenotype of severe asthma in adults, associated with aspirin
27 275 sensitivity, nasal polyposis and eosinophilia, all persisting despite the treatment with high doses of
28 276 inhaled corticosteroids(54). Innate immune mechanisms underlying this phenotype have recently
29 277 been proposed since it has become apparent that patients respond to anti-IL-5 antibody
30 278 therapies(65).

31
32
33 279

34
35 280 ***Contribution of allergy to mechanisms underlying severe asthma***

36 281 The role of allergy in severe asthma needs to be understood to help identify underlying mechanisms
37 282 of disease progression which will impact both on the choice of add-on therapies and on the
38 283 discovery of novel therapeutics. Even though the majority of children and adults with early-onset
39 284 severe asthma are sensitised, it is interesting that not all respond to treatment with omalizumab(66,
40 285 67)suggesting several different mechanisms contributing to the development of different allergic
41 286 phenotypes.

1
2
3 287 Typically, the allergic asthma phenotype is associated with eosinophilia, elevated serum IgE and
4
5 288 Th2 cytokines. However, in adult-onset asthma, eosinophilia may be present without overt evidence
6
7 289 of allergy(65). The limited contribution of allergy to disease persistence is apparent in adults with
8
9 290 severe asthma who show a non-allergic, inhaled corticosteroid “resistant” eosinophilic phenotype,
10
11 291 which responds to systemic CS and targeted therapy with anti-IL-5 (mepolizumab)(68). Novel
12
13 292 mechanisms that may contribute to this adult-onset phenotype include epithelial innate cytokines
14
15 293 that directly induce the recruitment of innate lymphoid cells which secrete Th2/“allergic” cytokines
16
17 294 without the generation of IgE or an adaptive immune response(69). Interestingly, even though it is
18
19 295 thought that this is an innate, non-adaptive, non-allergic immune response, all murine experimental
20
21 296 models investigating the role of innate cytokines in asthma pathogenesis used allergen exposure as
22
23 297 the stimulus, suggesting allergy still plays a central mechanistic role in this phenotype(70). It is
24
25 298 possible that allergy is a risk factor in the development of adult-onset “non-allergic” eosinophilic
26
27 299 asthma, but the clinical manifestation of asthma changes with time and age, whereby it is less
28
29 300 overtly “allergic”, but remains eosinophilic.

301 In asthma, the effect of innate immunity eliciting Th2 responses seems to be strongly related to IL-
302 33(71), and is especially associated with severe disease. IL-33 expression is increased in bronchial
303 tissue from both adults(72, 73) and children(74)with severe asthma. Other important features of
304 innate cytokines that may contribute to the pathogenesis of severe disease in both adults and
305 children include their role in (relative) corticosteroid resistance(74) and their association with
306 angiogenesis and airway remodelling, in particular as regards IL-25 (74-76).

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

314

315

316 **CROSS-TALK BETWEEN ENVIRONMENTAL FACTORS, ATOPIC SENSITISATION** 317 **AND ASTHMA**

318

1
2
3 319 ***The airway epithelial barrier***

4
5 320 Environmental stimuli, such as viruses, bacteria and air pollutants, are known activators of innate
6
7 321 immunity and may thus enhance the airway inflammation in asthmatic patients. Allergens, apart
8
9 322 from being recognised by the adaptive immunity, may also play a crucial role in activating innate
10
11 323 immunity through proteases, biologically active glycolipids and enzymes(79). The airway epithelial
12
13 324 barrier, for long time perceived as only a mechanical barrier, is now also recognised as a gate to
14
15 325 initiate atopic sensitization and allergic inflammation(80). Epithelial cells recognise the allergens
16
17 326 with the help of pattern recognition receptors and produce an innate immune response. As apical
18
19 327 junctional complexes between the airway epithelium cells are being disrupted by viral infections
20
21 328 and inhaled airway irritants, they facilitate the entry of allergens from the lumen to be presented to
22
23 329 the dendritic cells.

24
25 330 In bronchial biopsies and brushings especially from more severe asthmatic patients, airway
26
27 331 epithelium cells showed structural and functional defects in apical junctional complexes compared
28
29 332 to healthy controls(81). However, this reduced barrier function was found to be reversible by
30
31 333 epidermal growth factor (EGF) treatment(81).

32
33 334

34
35 335 ***The role of microbiota***

36
37 336 Early life airway and gut microbiota and influencing factors such as the delivery method, feeding
38
39 337 practices, antibiotic use and living environment were shown to be related with allergic asthma
40
41 338 development(82). Both the microbial burden and diversity within the lower airways were shown to
42
43 339 be significantly higher in suboptimally controlled asthmatic patients compared to healthy
44
45 340 individuals(83). Protobacteria species significantly predominated in asthmatic patients using
46
47 341 inhaled corticosteroids and showed the strongest correlations with the degree of bronchial
48
49 342 hyperresponsiveness(82). In addition, corticosteroid resistance in asthmatic patients was found to be
50
51 343 related to airway microbiome diversity(84). In these patients, Haemophilus parainfluenza
52
53 344 dominated the microbiome, and was shown to inhibit the response to corticosteroid treatment
54
55 345 compared to corticosteroid responsive asthmatic patients. Microbial diversity was also shown to
56
57 346 increase the risk of rhinovirus-induced asthma exacerbations in children(85). If rhinovirus existed
58
59 347 concomitantly with Moraxella catarrhalis, Streptococcus pneumoniae, or Haemophilus influenzae
60
348 within the airways, the risk of asthma exacerbations was found to be significantly increased as
349 compared to children without these pathogens.

1
2
3 3504
5 351 ***Viruses***

6
7
8 352 The interaction between viral lower respiratory tract infections (LRTI) and atopic sensitization has
9 353 been recognized as a major factor contributing to asthma development and exacerbation(86, 87).
10 354 Birth cohort studies provide strong evidence for a synergistic effect of viral LRTI and atopic
11 355 sensitization on asthma inception particularly in predisposed children(56, 88). Other factors
12 356 reported to increase the risk of asthma development include the type of virus (more than 10-fold
13 357 increased risk for asthma development with rhinovirus compared to 5-fold with respiratory
14 358 syncytial virus), the severity of viral LRTI, the age during viral LRTI and the atopic
15 359 predisposition(89). Very recently, the number of respiratory episodes in the first years of life, but
16 360 not the particular viral trigger, was reported to be associated with later asthma development(90).

17
18 361 Respiratory viral infections in combination with atopic sensitisation and exposure to allergens
19 362 increase the risk of hospital admission due to asthma exacerbation both in children(91) and
20 363 adults(92). Rhinoviruses (RV), especially RV-C group, are the most frequent viruses detected
21 364 during an asthma exacerbation(22)including severe asthma exacerbations with near fatal and fatal
22 365 asthma(23). Also, allergic asthmatic individuals experience more severe and prolonged LRTI
23 366 symptoms with RV infection compared to non-atopic healthy controls(93). Biological mechanisms
24 367 including impaired innate or altered adaptive immune function, abnormal airway structure and
25 368 function following prior infections, genetic influences and extrinsic factors, such as maternal
26 369 smoking, air pollution and nutritional factors (vitamin D), may explain the altered immune response
27 370 to viral infections in asthmatic/allergic patients(87). Recently, antibody titers to species specific RV
28 371 infection in children during asthma exacerbation showed that antibody response to RV-C is low
29 372 even when the virus was detected, pointing to a divergent and possibly less efficacious immune
30 373 response to this subtype compared to RV-A and B(94). The association of susceptibility to RV
31 374 infection in asthma was also investigated in human bronchial epithelial cells showing impaired
32 375 interferon production to the virus in severe therapy resistant allergic asthmatic children(26) but
33 376 normal responses in well controlled asthmatic adults who were mostly atopic(95). In contrast to RV
34 377 data, interferon responses to influenza A virus and RSV in human bronchial epithelial cell cultures
35 378 were preserved in adults with mild to severe asthma(96).

36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57 380***Outdoor, indoor and food allergens***

1
2
3 381 Relationships between different types of allergens (outdoor, indoor, food) and the development and
4 382 severity of allergic disease, including asthma, have been studied(97). For instance, pollen allergy
5 383 has been found to be interrelated with various food allergies, digestive system Th2-inflammation
6 384 and asthma(98, 99). Cross-reactivity between pollen and several plant-derived foods, nuts, and
7 385 fruits has been well established(98). Food allergy without concomitant asthma has been found to be
8 386 associated with increased nonspecific bronchial hyperresponsiveness(100, 101), while several
9 387 studies report that children with asthma and concomitant food allergy have more severe disease,
10 388 poorer control, greater morbidity, and require more anti-asthma medications(102, 103).

11 389 The most common indoor allergens associated with asthma include house dust mites, domestic
12 390 animals (cats, dogs), and cockroaches(97, 104), while fungi can be found both indoor and outdoor.
13 391 In a cohort of 300 asthmatic children (aged 4-12 y), higher *Der p 1* and pet allergen levels were
14 392 found to be associated with greater asthma severity(105).

15 393 Fungal exposure is universal and fungi can be linked to asthma in a variety of ways. Fungal allergy
16 394 drives asthma severity and long-term or uncontrolled fungal infections are associated with a poor
17 395 control of asthma, complications such as bronchiectasies and chronic allergic bronchopulmonary
18 396 aspergillosis (ABPA)(106). In the general asthma population, sensitization to moulds ranges from 7
19 397 to 20%, in severe asthma patients from 35 to 75%, being 54-91% in life-threatening asthma
20 398 population(107-111). The first evidence of the link between the severity of asthma and fungal
21 399 sensitisation dates to 1978, when Schwartz et al. demonstrated a relationship between asthma
22 400 severity and *Aspergillus spp* sensitisation(112). *Alternaria* or *Cladosporium spp* sensitisation was
23 401 associated with asthma severity in the European Community Respiratory Health Survey.
24 402 Furthermore, a recent paper has shown that fungal sensitisation in children with persistent asthma is
25 403 associated with disease severity(113)and a 2014 review has shown increasing evidence that
26 404 sensitized asthmatic children may be susceptible to asthma exacerbations when exposed to outdoor
27 405 fungal spores and that the severity of exacerbation may vary with different fungi species(114).

28 406 The term “Severe Asthma with Fungal Sensitisation” (SAFS) was introduced by Denning et al. in
29 407 2006, to describe those patients who have persistent severe asthma (despite standard treatment) and
30 408 evidence of fungal sensitisation, as defined by positive SPT, or fungus or fungal antigen-specific
31 409 sIgE, and do not meet the criteria for ABPA(10). Proposed classification by an EAACI Task Force
32 410 sets the total IgE cut-off at <1000 IU/ml for SAFS and >1000 IU/ml for ABPA. ABPA was
33 411 accepted as an endotype(115), while SAFS remains a pragmatic definition(106). ABPA may
34 412 develop in asthmatics with a genetic predisposition and therefore SAFS may have the same

1
2
3 413 background. Carefully genotyping patients with different forms of asthma may allow a better
4 414 understanding of this disease.

5
6
7 415 “Trichophyton Asthma” is another clinical entity, where inhalation or the presence of cutaneous
8 416 infection (athlete’s foot, onychomycosis) in sensitised asthmatics is associated with disease
9 417 severity(106, 116).

10
11
12 418

13
14
15 419 ***Smoking***

16
17 420 Cigarette smoking itself may influence asthma, as it accelerates lung function decline(117), impairs
18 421 the response to CS (both inhaled and oral)(118), increases airway oxidative stress(119), perpetuates
19 422 symptoms despite of treatment(120) and induces the change of inflammatory phenotypes into more
20 423 aggressive ones(121), thereby resulting in a more severe disease(122).

21
22
23
24 424 Smoking also increases serum IgE levels, especially in men(123). This may result in an increased
25 425 risk of allergic sensitisation, at least for occupational allergens(124). However, the relationship
26 426 between cigarette smoking and allergy in severe asthma is still debated: some studies identify
27 427 smoking as a risk factor for allergic asthma(125), while others show a lower prevalence of atopic
28 428 sensitisation in smoking patients with severe asthma(121). According to a large epidemiological
29 429 survey (ECRHS II), smoking was more strongly associated with severe asthma in men than in
30 430 women, particularly if they were sensitised to moulds (*Cladosporium*), house dust mites or
31 431 cats(126). Even more conflicting data come from studies on the effect of passive smoking on the
32 432 risk of development of atopic sensitisation(127).

33
34
35
36 433 Cigarette smoking usually results in a more neutrophilic airway inflammation, which is less
37 434 responsive to ICS(121). Accordingly, alveolar macrophages from smokers have a reduced cellular
38 435 CS responsiveness, which is associated with reduced histone deacetylase activity, an essential
39 436 molecule for anti-inflammatory genes transcription(63, 128). In fact, they show an elevated
40 437 glucocorticoid receptors (GR) ratio in PBMC which is in favour of GR- β (not able to induce any
41 438 transcriptional activity) compared to GR- α (the active isoform with anti-inflammatory effects)(129).
42 439 These molecular events make smoking asthmatics less responsive to CS, currently the standard
43 440 controller therapy for asthma, leading them to a more probable evolution to severe asthma (figure
44 441 2).

45
46
47
48
49
50
51
52 442 Recently, a new distinct phenotype of severe asthma has been identified in frequent exacerbators,
53 443 and history of smoking seems to be a risk factor for this phenotype(130). A novel risk score for
54
55
56
57
58
59
60

1
2
3 444 asthma exacerbations developed and validated by Bateman *et al.* supports the evidence that
4 445 smoking status is a main predictor for uncontrolled asthma(131). Despite this well-known
5 446 relationship, active smoking is still surprisingly common among asthmatics(132). More efficient
6 447 smoking prevention programs and smoking cessation campaigns should be carried out to try to
7 448 reduce the risk of developing severe asthma. Moreover, most clinical trials with new drugs aimed
8 449 for severe asthma have been conducted in non-smoking patients, which results in incomplete
9 450 knowledge on the efficacy of such therapeutic approaches in smokers. Large “real life” studies in
10 451 severe asthma including smoking asthmatics should be encouraged. The complex relationship
11 452 between cigarette smoking and atopic sensitisation increasing the risk of severe asthma should be
12 453 better investigated as only few and conflicting data are presently available. However, this
13 454 relationship remains difficult to address, particularly in cross-sectional studies, because of the
14 455 potential selection bias (e.g. “healthy smoker effect”)(133). Prospective studies in lifetime smokers
15 456 with lifetime smoking are more appropriate to properly examine the relationships between smoking
16 457 and severe asthma.
17
18
19
20
21
22
23
24
25
26
27
28

29 459 ***Pollution***

30
31
32 460 The health effects caused by outdoor air pollution have been intensively studied during the last
33 461 decades. The term “outdoor air pollution” involves particulate matter (PM), gaseous pollutants
34 462 (nitrogen dioxide, sulphur dioxide and ozone) and traffic-related air pollution (elemental and carbon
35 463 black, road dust)(134).
36
37
38

39 464 Increased exposure to ultrafine particles and carbon monoxide within the previous 4-7 days was
40 465 associated with increased relative odds of a paediatric asthma visit(135). Other studies also indicate
41 466 that sudden increase or decrease of exposure to air pollution may affect asthmatic symptoms or
42 467 emergency department visits(136-138). Indeed, a decrease in the number of acute asthma events of
43 468 over 40% was found after reduction of air pollution during summer Olympic games(138). So far,
44 469 these studies were performed in children and included only a relatively low number of individuals.
45
46
47
48

49
50 470 Larger scale studies also demonstrated an adverse effect of outdoor air pollution on lung
51 471 function(139-141). A multicenter birth cohort study (ESCAPE) showed an association between
52 472 estimated levels of NO₂ and PM_{2.5} and decreases in FEV₁(139). In another birth cohort study
53 473 (MAAS), lifetime exposure to PM₁₀ and NO₂ was associated with significantly less growth in FEV₁
54 474 over time(140). In the same cohort, no association was found between long-term exposure to PM₁₀
55 475 and NO₂ and the prevalence of asthma or wheeze(142). In adult asthmatics, exposure to NO₂ and
56
57
58
59
60

1
2
3 476 PM₁₀ was associated with lower measures of FEV₁ and FVC(143)and exposure to ozone and PM₁₀
4 477 increased the risk of uncontrolled asthma(144). Overall, these studies thus provide evidence of an
5
6 478 inverse association between outdoor air pollution and lung function (Table 1). Whether asthma
7
8 479 severity is directly affected by outdoor air pollution is unclear.

9
10 480 Several studies showed a positive association between exposure to air pollution during infancy and
11 481 sensitisation to inhalant allergens(145-147). Although the mechanism underlying this association is
12 482 not fully understood, some evidence suggests that ultrafine carbon black particles can directly
13 483 induce maturation of dendritic cells *in vitro*(148), thereby facilitating sensitisation to inhalant
14 484 allergens. Alternatively, airborne pollutants can induce the influx of inflammatory cells to the lungs,
15 485 which might then lower the threshold for sensitisation. Indeed, it has recently been shown that
16 486 allergen-specific Th2/Th17 cells accumulate in the lungs of mice exposed to both diesel exhaust
17 487 particles and house dust mite extract(149). Diesel exhaust particles may also produce other
18 488 immunological effects(150, 151) (Table 2). Furthermore, exposure to moderate air pollution during
19 489 late pregnancy was found to cause increased cord blood IL-1 β (152). A recent meta-analysis,
20 490 however, showed no clear overall association between air pollution exposure and the development
21 491 of sensitisation in children up to 10 years of age(153).

22
23
24 492 In summary, in multi-sensitised asthmatics, daily exposure to allergens in combination with other
25 493 enhancing factors, including viral infections, environmental smoking, and/or pollution, will finally
26 494 determine the asthma course and severity.

27
28
29
30
31
32
33
34
35
36
37 495

38 39 496 ***Occupational/Work-Related***

40
41
42 497 Severe asthma may occur in patients affected by Work-Related Asthma (WRA).WRA encompasses
43 498 both Occupational Asthma (OA), defined as “asthma caused by the workplace” and “Work-
44 499 Exacerbated Asthma” (WEA), occurring in patients with pre-existing or concurrent asthma and
45 500 exacerbated by different work-related factors (i.e. aeroallergens, exercise, irritants)(154). OA can be
46 501 further divided into two subtypes: an allergic form (90% of all OA)(155), caused both by an IgE-
47 502 mediated mechanism towards high (HMW) and low (LMW) molecular weight agents(106), and a
48 503 non-IgE mediated form (Non-Allergic, Irritant-Induced [Occupational] Asthma (IIOA)), towards
49 504 specific LMW agents in which the mechanism has not been characterized yet. The non-allergic
50 505 IIOA can be further divided into the “Reactive Airway Dysfunction Syndrome” (RADS) and the
51 506 “IIOA after multiple exposures”. The first occurs after an acute, single exposure to very high
52
53
54
55
56
57
58
59
60

1
2
3 507 concentrations of irritating substances(156), while the second follows multiple exposure to irritants;
4 508 in this subtype, onset of asthma can follow the exposures after some time(157, 158).

5
6
7 509 WRA should be suspected in patients whose asthma worsens while working or begin at work. Here
8
9 510 a detailed occupational and medical history is fundamental(159, 160), while a clinical history only
10
11 511 shows a low specificity in the diagnosis of OA(161). The investigation of WRA follows a well-
12
13 512 defined protocol based on confirmation of bronchial asthma, work-related bronchoconstriction,
14
15 513 sensitisation to occupational agents and on the confirmation of the causal role of occupational
16
17 514 agents, being sensitisation *per se* not indicative of clinical symptoms(162) (Figure 3). Baseline
18
19 515 spirometry is mandatory and it is strongly recommended that this should be complemented with
20
21 516 non-specific bronchial hyperreactivity assessment with direct or indirect challenges. In individuals
22
23 517 with suspected WRA, presenting with a normal respiratory function and/or negative methacholine
24
25 518 challenge testing, serial lung function measurements and assessment of non-specific bronchial
26
27 519 hyperreactivity are strongly recommended(162, 163). Additionally, spirometry can be performed
28
29 520 during a work shift (Cross-shift spirometry). Furthermore, serial measurements of peak flow
30
31 521 expiratory rate (serial PEFr) have been used to objectively confirm the link between the workplace
32
33 522 and the asthmatic symptoms(164). Skin prick testing completes the diagnostic work-up, and the
34
35 523 selection of specific allergens related to the individual's job is fundamental. Specific IgE evaluation
36
37 524 is also of importance. The role of atopic mechanisms in severe occupational asthma has been
38
39 525 confirmed by a recent study where treatment with omalizumab was successful in 90% of severe
40
41 526 occupational asthma patients due to HMW and LMW agents, such as flour, animal dander, mites,
42
43 527 moulds, isocyanate or acrylates(165). It is worth noting that, at least in OA, allergen exposure levels
44
45 528 represent the major determinants both for the disease as such and for the severity of asthma(166,
46
47 529 167). Finally, specific inhalation challenges (SICs) or workplace inhalation challenges,
48
49 530 complemented by the assessment of airway inflammation by induced sputum and FeNO may be
50
51 531 considered.

52
53
54
55 532 Diagnosis of IIOA follows a well-defined protocol described in a recent EAACI Task Force
56
57 533 document(158).

58
59 534

60 535

536 **CONCLUSION**

1
2
3 537 There is increasing evidence for the important, but not exclusive, role of allergy in severe asthma.
4 538 Although some recent reports demonstrate that allergy may play only a limited role, this is likely
5
6 539 not true for childhood disease, where early atopic sensitisation is critical in determining the severity
7
8 540 of disease.

9
10 541 Mechanistic implications of co-factors interacting with allergy and asthma, such as virus infections,
11
12 542 pollution, smoking, and work-related exposures, still need to be completely uncovered to allow the
13
14 543 discovery of novel therapeutic targets.

15
16 544

17
18 545

19
20 546 **Author contributions:**

21
22
23 547 SRDG drafted the final version of this manuscript

24
25
26 548 All authors drafted different chapters and paragraphs of this work

27
28
29 549 All authors critically revised this work for important intellectual content

30
31
32 550 All authors approved the final version to be published

33
34
35 551 All authors agreed on accuracy and integrity of this work

36
37
38 552

39
40
41 553 **Conflict of interest disclosure:**

42
43
44 554 All authors declare that they have no conflict of interest regarding this work

45
46 555
47
48
49
50
51
52
53
54
55
56
57
58
59
60

556 REFERENCES

- 557 1. Addo-Yobo EO, Custovic A, Taggart SC, Craven M, Bonnie B, Woodcock A. Risk factors
558 for asthma in urban Ghana. *J Allergy Clin Immunol* 2001;**108**(3):363-368.
- 559 2. Al-Mousawi MS, Lovel H, Behbehani N, Arifhodzic N, Woodcock A, Custovic A. Asthma
560 and sensitization in a community with low indoor allergen levels and low pet-keeping frequency. *J*
561 *Allergy Clin Immunol* 2004;**114**(6):1389-1394.
- 562 3. Simpson BM, Custovic A, Simpson A, Hallam CL, Walsh D, Marolia H, et al. NAC
563 Manchester Asthma and Allergy Study (NACMAAS): risk factors for asthma and allergic disorders
564 in adults. *Clin Exp Allergy* 2001;**31**(3):391-399.
- 565 4. Stevens W, Addo-Yobo E, Roper J, Woodcock A, James H, Platts-Mills T, et al. Differences
566 in both prevalence and titre of specific immunoglobulin E among children with asthma in affluent
567 and poor communities within a large town in Ghana. *Clin Exp Allergy* 2011;**41**(11):1587-1594.
- 568 5. Custovic A, Johnston SL, Pavord I, Gaga M, Fabbri L, Bel EH, et al. EAACI position
569 statement on asthma exacerbations and severe asthma. *Allergy* 2013;**68**(12):1520-1531.
- 570 6. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International
571 ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J*
572 2014;**43**(2):343-373.
- 573 7. Sala KA, Carroll CL, Tang YS, Aglio T, Dressler AM, Schramm CM. Factors associated
574 with the development of severe asthma exacerbations in children. *J Asthma* 2011;**48**(6):558-564.
- 575 8. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma
576 phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care*
577 *Med* 2010;**181**(4):315-323.
- 578 9. Holt PG, Strickland DH, Sly PD. Virus infection and allergy in the development of asthma:
579 what is the connection? *Curr Opin Allergy Clin Immunol* 2012;**12**(2):151-157.
- 580 10. Denning DW, O'Driscoll BR, Hogaboam CM, Bowyer P, Niven RM. The link between
581 fungi and severe asthma: a summary of the evidence. *Eur Respir J* 2006;**27**(3):615-626.
- 582 11. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al.
583 GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization
584 patterns for inhalant allergens in Europe. *Allergy* 2009;**64**(10):1498-1506.
- 585 12. Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et
586 al. A revised nomenclature for allergy. An EAACI position statement from the EAACI
587 nomenclature task force. *Allergy* 2001;**56**(9):813-824.
- 588 13. Custovic A, Arifhodzic N, Robinson A, Woodcock A. Exercise testing revisited. The
589 response to exercise in normal and atopic children. *Chest* 1994;**105**(4):1127-1132.
- 590 14. Lazic N, Roberts G, Custovic A, Belgrave D, Bishop C, Winn J, et al. Multiple atopy
591 phenotypes and their associations with asthma: similar findings from two birth cohorts. *Allergy*
592 2013.
- 593 15. Lodrup Carlsen KC, Soderstrom L, Mowinckel P, Haland G, Pettersen M, Munthe Kaas
594 MC, et al. Asthma prediction in school children; the value of combined IgE-antibodies and
595 obstructive airways disease severity score. *Allergy* 2010;**65**(9):1134-1140.
- 596 16. Simpson A, Soderstrom L, Ahlstedt S, Murray CS, Woodcock A, Custovic A. IgE antibody
597 quantification and the probability of wheeze in preschool children. *J Allergy Clin Immunol*
598 2005;**116**(4):744-749.
- 599 17. Carroll WD, Lenney W, Child F, Strange RC, Jones PW, Whyte MK, et al. Asthma severity
600 and atopy: how clear is the relationship? *Arch Dis Child* 2006;**91**(5):405-409.
- 601 18. Marinho S, Simpson A, Marsden P, Smith JA, Custovic A. Quantification of atopy, lung
602 function and airway hypersensitivity in adults. *Clin Transl Allergy* 2011;**1**(1):16.
- 603 19. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, et al. Early
604 identification of atopy in the prediction of persistent asthma in children. *Lancet*
605 2008;**372**(9643):1100-1106.

- 1
2
3 606 20. Frith J, Fleming L, Bossley C, Ullmann N, Bush A. The complexities of defining atopy in
4 607 severe childhood asthma. *Clin Exp Allergy* 2011;**41**(7):948-953.
- 5 608 21. Just J, Gouvis-Echraghi R, Rouve S, Wanin S, Moreau D, Annesi-Maesano I. Two novel,
6 609 severe asthma phenotypes identified during childhood using a clustering approach. *Eur Respir J*
7 610 2012;**40**(1):55-60.
- 8 611 22. Murray CS, Poletti G, Keadze T, Morris J, Woodcock A, Johnston SL, et al. Study of
9 612 modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the
10 613 risk of asthma hospital admissions in children. *Thorax* 2006;**61**(5):376-382.
- 11 614 23. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism
12 615 between allergens and viruses and risk of hospital admission with asthma: case-control study. *BMJ*
13 616 2002;**324**(7340):763.
- 14 617 24. Murray CS PG, Ahlstedt S, Soderstrom L, Johnston SL, Custovic A. Probability of hospital
15 618 admission with acute asthma exacerbation increases with increasing specific IgE antibody levels.
16 619 *Allergy Clin Immunol Int: J World Allergy Org* 2007;**Suppl 2**:270-273.
- 17 620 25. Teach SJ, Gill MA, Togias A, Sorkness CA, Arbes SJ, Jr., Calatroni A, et al. Preseasonal
18 621 treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma
19 622 exacerbations. *J Allergy Clin Immunol* 2015;**136**(6):1476-1485.
- 20 623 26. Edwards MR, Regamey N, Vareille M, Kieninger E, Gupta A, Shoemark A, et al. Impaired
21 624 innate interferon induction in severe therapy resistant atopic asthmatic children. *Mucosal Immunol*
22 625 2013;**6**(4):797-806.
- 23 626 27. Holt PG, Strickland D, Bosco A, Belgrave D, Hales B, Simpson A, et al. Distinguishing
24 627 benign from pathologic TH2 immunity in atopic children. *J Allergy Clin Immunol* 2016;**137**(2):379-
25 628 387.
- 26 629 28. Simpson A, Tan VY, Winn J, Svensen M, Bishop CM, Heckerman DE, et al. Beyond atopy:
27 630 multiple patterns of sensitization in relation to asthma in a birth cohort study. *Am J Respir Crit Care*
28 631 *Med* 2010;**181**(11):1200-1206.
- 29 632 29. Belgrave DC, Buchan I, Bishop C, Lowe L, Simpson A, Custovic A. Trajectories of lung
30 633 function during childhood. *Am J Respir Crit Care Med* 2014;**189**(9):1101-1109.
- 31 634 30. Custovic A, Ainsworth J, Arshad H, Bishop C, Buchan I, Cullinan P, et al. The Study Team
32 635 for Early Life Asthma Research (STELAR) consortium 'Asthma e-lab': team science bringing data,
33 636 methods and investigators together. *Thorax* 2015;**70**(8):799-801.
- 34 637 31. Simpson A, Lazic N, Belgrave DC, Johnson P, Bishop C, Mills C, et al. Patterns of IgE
35 638 responses to multiple allergen components and clinical symptoms at age 11 years. *J Allergy Clin*
36 639 *Immunol* 2015.
- 37 640 32. Custovic A, Sonntag HJ, Buchan IE, Belgrave D, Simpson A, Prospero MC. Evolution
38 641 pathways of IgE responses to grass and mite allergens throughout childhood. *J Allergy Clin*
39 642 *Immunol* 2015;**136**(6):1645-1652 e1641-1648.
- 40 643 33. Belgrave DC, Granell R, Simpson A, Guiver J, Bishop C, Buchan I, et al. Developmental
41 644 profiles of eczema, wheeze, and rhinitis: two population-based birth cohort studies. *PLoS Med*
42 645 2014;**11**(10):e1001748.
- 43 646 34. van der Hulst AE, Klip H, Brand PL. Risk of developing asthma in young children with
44 647 atopic eczema: a systematic review. *J Allergy Clin Immunol* 2007;**120**(3):565-569.
- 45 648 35. Schuttelaar ML, Kerkhof M, Jonkman MF, Koppelman GH, Brunekreef B, de Jongste JC, et
46 649 al. Filaggrin mutations in the onset of eczema, sensitization, asthma, hay fever and the interaction
47 650 with cat exposure. *Allergy* 2009;**64**(12):1758-1765.
- 48 651 36. Peng W, Novak N. Pathogenesis of atopic dermatitis. *Clin Exp Allergy* 2015;**45**(3):566-574.
- 49 652 37. Hogan MB, Peele K, Wilson NW. Skin barrier function and its importance at the start of the
50 653 atopic march. *J Allergy (Cairo)* 2012;**2012**:901940.
- 51 654 38. Lodge CJ, Lowe AJ, Gurrin LC, Hill DJ, Hosking CS, Khalafzai RU, et al. House dust mite
52 655 sensitization in toddlers predicts current wheeze at age 12 years. *J Allergy Clin Immunol*
53 656 2011;**128**(4):782-788 e789.

- 1
2
3 657 39. Sylvestre L, Jegu J, Metz-Favre C, Barnig C, Qi S, de Blay F. Component-Based Allergen-
4 658 Microarray: Der p 2 and Der f 2 Dust Mite Sensitization Is More Common in Patients With Severe
5 659 Asthma. *J Investig Allergol Clin Immunol* 2016;**26**(2):141-143.
- 6 660 40. Vandenas O, Froidure A, Meurer U, Rihs HP, Riffart C, Soetaert S, et al. The role of
7 661 allergen components for the diagnosis of latex-induced occupational asthma. *Allergy*
8 662 2016;**71**(6):840-849.
- 9 663 41. Antonicelli L, Massaccesi C, Braschi MC, Cinti B, Bilo MB, Bonifazi F. Component
10 664 resolved diagnosis in real life: the risk assessment of food allergy using microarray-based
11 665 immunoassay. *Eur Ann Allergy Clin Immunol* 2014;**46**(1):30-34.
- 12 666 42. Nettis E, Bonifazi F, Bonini S, Di Leo E, Maggi E, Melioli G, et al. Molecular diagnosis and
13 667 the Italian Board for ISAC. *Eur Ann Allergy Clin Immunol* 2014;**46**(2):68-73.
- 14 668 43. Prosperi MC, Belgrave D, Buchan I, Simpson A, Custovic A. Challenges in interpreting
15 669 allergen microarrays in relation to clinical symptoms: A machine learning approach. *Pediatr*
16 670 *Allergy Immunol* 2013.
- 17 671 44. Konradsen JR, Nordlund B, Onell A, Borres MP, Gronlund H, Hedlin G. Severe childhood
18 672 asthma and allergy to furry animals: refined assessment using molecular-based allergy diagnostics.
19 673 *Pediatr Allergy Immunol* 2014;**25**(2):187-192.
- 20 674 45. Haldar A, Gupta UD, Majumdar KK, Laskar K, Ghosh S, Sen S. Community perception of
21 675 Dengue in slum areas of metropolitan city of West Bengal. *J Commun Dis* 2008;**40**(3):205-210.
- 22 676 46. Wu W, Bleecker E, Moore W, Busse WW, Castro M, Chung KF, et al. Unsupervised
23 677 phenotyping of Severe Asthma Research Program participants using expanded lung data. *J Allergy*
24 678 *Clin Immunol* 2014;**133**(5):1280-1288.
- 25 679 47. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat*
26 680 *Med* 2012;**18**(5):716-725.
- 27 681 48. Bell MC, Busse WW. Severe asthma: an expanding and mounting clinical challenge. *J*
28 682 *Allergy Clin Immunol Pract* 2013;**1**(2):110-121; quiz 122.
- 29 683 49. Moore WC, Fitzpatrick AM, Li X, Hastie AT, Li H, Meyers DA, et al. Clinical
30 684 heterogeneity in the severe asthma research program. *Ann Am Thorac Soc* 2013;**10** Suppl:S118-
31 685 124.
- 32 686 50. Fitzpatrick AM, Teague WG, Meyers DA, Peters SP, Li X, Li H, et al. Heterogeneity of
33 687 severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of
34 688 Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. *J Allergy Clin*
35 689 *Immunol* 2011;**127**(2):382-389 e381-313.
- 36 690 51. Just J, Deslandes-Boutmy E, Amat F, Desseaux K, Nemni A, Bourrat E, et al. Natural
37 691 history of allergic sensitization in infants with early-onset atopic dermatitis: results from ORCA
38 692 Study. *Pediatr Allergy Immunol* 2014;**25**(7):668-673.
- 39 693 52. Schatz M, Hsu JW, Zeiger RS, Chen W, Dorenbaum A, Chipps BE, et al. Phenotypes
40 694 determined by cluster analysis in severe or difficult-to-treat asthma. *J Allergy Clin Immunol*
41 695 2014;**133**(6):1549-1556.
- 42 696 53. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, et al. Pediatric severe
43 697 asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. *J Allergy Clin*
44 698 *Immunol* 2012;**129**(4):974-982 e913.
- 45 699 54. Amelink M, de Groot JC, de Nijs SB, Lutter R, Zwinderman AH, Sterk PJ, et al. Severe
46 700 adult-onset asthma: A distinct phenotype. *J Allergy Clin Immunol* 2013;**132**(2):336-341.
- 47 701 55. Illi S, von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U, et al. Perennial allergen
48 702 sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet*
49 703 2006;**368**(9537):763-770.
- 50 704 56. Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al.
51 705 Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J*
52 706 *Respir Crit Care Med* 2008;**178**(7):667-672.

- 1
2
3 707 57. Stoltz DJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Gern JE, et al. Specific patterns
4 708 of allergic sensitization in early childhood and asthma & rhinitis risk. *Clin Exp Allergy*
5 709 2013;**43**(2):233-241.
6 710 58. Sharples J, Gupta A, Fleming L, Bossley CJ, Bracken-King M, Hall P, et al. Long-term
7 711 effectiveness of a staged assessment for paediatric problematic severe asthma. *Eur Respir J*
8 712 2012;**40**(1):264-267.
9 713 59. Marinho S, Simpson A, Soderstrom L, Woodcock A, Ahlstedt S, Custovic A. Quantification
10 714 of atopy and the probability of rhinitis in preschool children: a population-based birth cohort study.
11 715 *Allergy* 2007;**62**(12):1379-1386.
12 716 60. Belgrave DC, Simpson A, Semic-Jusufagic A, Murray CS, Buchan I, Pickles A, et al. Joint
13 717 modeling of parentally reported and physician-confirmed wheeze identifies children with persistent
14 718 troublesome wheezing. *J Allergy Clin Immunol* 2013;**132**(3):575-583 e512.
15 719 61. Amelink M, de Nijs SB, de Groot JC, van Tilburg PM, van Spiegel PI, Krouwels FH, et al.
16 720 Three phenotypes of adult-onset asthma. *Allergy* 2013;**68**(5):674-680.
17 721 62. Westerhof GA, Vollema EM, Weersink EJ, Reinartz SM, de Nijs SB, Bel EH. Predictors for
18 722 the development of progressive severity in new-onset adult asthma. *J Allergy Clin Immunol*
19 723 2014;**134**(5):1051-1056 e1052.
20 724 63. Kobayashi Y, Bossley C, Gupta A, Akashi K, Tsartsali L, Mercado N, et al. Passive
21 725 smoking impairs histone deacetylase-2 in children with severe asthma. *Chest* 2014;**145**(2):305-312.
22 726 64. de Nijs SB, Venekamp LN, Bel EH. Adult-onset asthma: is it really different? *Eur Respir*
23 727 *Rev* 2013;**22**(127):44-52.
24 728 65. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: Eosinophilic airway
25 729 inflammation in nonallergic asthma. *Nat Med* 2013;**19**(8):977-979.
26 730 66. Grimaldi-Bensouda L, Zureik M, Aubier M, Humbert M, Levy J, Benichou J, et al. Does
27 731 omalizumab make a difference to the real-life treatment of asthma exacerbations?: Results from a
28 732 large cohort of patients with severe uncontrolled asthma. *Chest* 2013;**143**(2):398-405.
29 733 67. Deschildre A, Marguet C, Salleron J, Pin I, Rittie JL, Derelle J, et al. Add-on omalizumab in
30 734 children with severe allergic asthma: a 1-year real life survey. *Eur Respir J* 2013;**42**(5):1224-1233.
31 735 68. Bel EH, Ortega HG, Pavord ID. Glucocorticoids and mepolizumab in eosinophilic asthma.
32 736 *N Engl J Med* 2014;**371**(25):2434.
33 737 69. Vercelli D, Gozdz J, von Mutius E. Innate lymphoid cells in asthma: when innate immunity
34 738 comes in a Th2 flavor. *Curr Opin Allergy Clin Immunol* 2014;**14**(1):29-34.
35 739 70. Walker JA, McKenzie AN. Development and function of group 2 innate lymphoid cells.
36 740 *Curr Opin Immunol* 2013;**25**(2):148-155.
37 741 71. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, et al. IL-33 is more potent
38 742 than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway
39 743 contraction. *J Allergy Clin Immunol* 2013;**132**(4):933-941.
40 744 72. Prefontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J, et al. Increased IL-
41 745 33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010;**125**(3):752-754.
42 746 73. Traister RS, Uvalle CE, Hawkins GA, Meyers DA, Bleecker ER, Wenzel SE. Phenotypic
43 747 and genotypic association of epithelial IL1RL1 to human TH2-like asthma. *J Allergy Clin Immunol*
44 748 2015;**135**(1):92-99.
45 749 74. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes
46 750 airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol*
47 751 2013;**132**(3):676-685 e613.
48 752 75. Gregory LG, Jones CP, Walker SA, Sawant D, Gowers KH, Campbell GA, et al. IL-25
49 753 drives remodelling in allergic airways disease induced by house dust mite. *Thorax* 2013;**68**(1):82-
50 754 90.
51 755 76. Corrigan CJ, Wang W, Meng Q, Fang C, Wu H, Reay V, et al. T-helper cell type 2 (Th2)
52 756 memory T cell-potentiating cytokine IL-25 has the potential to promote angiogenesis in asthma.
53 757 *Proc Natl Acad Sci U S A* 2011;**108**(4):1579-1584.

- 1
2
3 758 77. Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene
4 759 expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin*
5 760 *Immunol* 2014;**133**(2):388-394.
6 761 78. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al.
7 762 Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;**365**(12):1088-1098.
8 763 79. Triggiani M, De Feo G, Cardamone C, Parente R. The Emerging Role of Innate Immunity in
9 764 Respiratory Allergy. *International Trends in Immunity* 2015;**3**(2):28-32.
10 765 80. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology
11 766 and allergic airway inflammation. *J Allergy Clin Immunol* 2014;**134**(3):509-520.
12 767 81. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, et al.
13 768 Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011;**128**(3):549-556 e541-
14 769 512.
15 770 82. Panzer AR, Lynch SV. Influence and effect of the human microbiome in allergy and asthma.
16 771 *Curr Opin Rheumatol* 2015;**27**(4):373-380.
17 772 83. Huang YJ, Nelson CE, Brodie EL, Desantis TZ, Baek MS, Liu J, et al. Airway microbiota
18 773 and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin*
19 774 *Immunol* 2011;**127**(2):372-381 e371-373.
20 775 84. Goleva E, Jackson LP, Harris JK, Robertson CE, Sutherland ER, Hall CF, et al. The effects
21 776 of airway microbiome on corticosteroid responsiveness in asthma. *Am J Respir Crit Care Med*
22 777 2013;**188**(10):1193-1201.
23 778 85. Kloepfer KM, Lee WM, Pappas TE, Kang TJ, Vrtis RF, Evans MD, et al. Detection of
24 779 pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms
25 780 and asthma exacerbations. *J Allergy Clin Immunol* 2014;**133**(5):1301-1307, 1307 e1301-1303.
26 781 86. Gavala ML, Bertics PJ, Gern JE. Rhinoviruses, allergic inflammation, and asthma. *Immunol*
27 782 *Rev* 2011;**242**(1):69-90.
28 783 87. James KM, Peebles RS, Jr., Hartert TV. Response to infections in patients with asthma and
29 784 atopic disease: an epiphenomenon or reflection of host susceptibility? *J Allergy Clin Immunol*
30 785 2012;**130**(2):343-351.
31 786 88. Kusel MM, de Klerk NH, Kebabdz T, Vohma V, Holt PG, Johnston SL, et al. Early-life
32 787 respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent
33 788 asthma. *J Allergy Clin Immunol* 2007;**119**(5):1105-1110.
34 789 89. Mackenzie KJ, Anderton SM, Schwarze J. Viral respiratory tract infections and asthma in
35 790 early life: cause and effect? *Clin Exp Allergy* 2014;**44**(1):9-19.
36 791 90. Bonnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between
37 792 respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin*
38 793 *Immunol* 2015;**136**(1):81-86 e84.
39 794 91. Papadopoulos NG, Christodoulou I, Rohde G, Agache I, Almqvist C, Bruno A, et al.
40 795 Viruses and bacteria in acute asthma exacerbations--a GA(2) LEN-DARE systematic review.
41 796 *Allergy* 2011;**66**(4):458-468.
42 797 92. Sandrock CE, Norris A. Infection in severe asthma exacerbations and critical asthma
43 798 syndrome. *Clin Rev Allergy Immunol* 2015;**48**(1):104-113.
44 799 93. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, et al. Frequency,
45 800 severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a
46 801 longitudinal cohort study. *Lancet* 2002;**359**(9309):831-834.
47 802 94. Iwasaki J, Smith WA, Khoo SK, Bizzantino J, Zhang G, Cox DW, et al. Comparison of
48 803 rhinovirus antibody titers in children with asthma exacerbations and species-specific rhinovirus
49 804 infection. *J Allergy Clin Immunol* 2014;**134**(1):25-32.
50 805 95. Sykes A, Macintyre J, Edwards MR, Del Rosario A, Haas J, Gielen V, et al. Rhinovirus-
51 806 induced interferon production is not deficient in well controlled asthma. *Thorax* 2014;**69**(3):240-
52 807 246.
53
54
55
56
57
58
59
60

- 1
2
3 808 96. Patel DA, You Y, Huang G, Byers DE, Kim HJ, Agapov E, et al. Interferon response and
4 809 respiratory virus control are preserved in bronchial epithelial cells in asthma. *J Allergy Clin*
5 810 *Immunol* 2014;**134**(6):1402-1412 e1407.
- 6 811 97. Baxi SN, Phipatanakul W. The role of allergen exposure and avoidance in asthma. *Adolesc*
7 812 *Med State Art Rev* 2010;**21**(1):57-71, viii-ix.
- 8 813 98. Bartra J, Sastre J, del Cuvillo A, Montoro J, Jauregui I, Davila I, et al. From pollinosis to
9 814 digestive allergy. *J Investig Allergol Clin Immunol* 2009;**19** Suppl 1:3-10.
- 10 815 99. Bousquet J, Schunemann HJ, Samolinski B, Demoly P, Baena-Cagnani CE, Bachert C, et al.
11 816 Allergic Rhinitis and its Impact on Asthma (ARIA): achievements in 10 years and future needs. *J*
12 817 *Allergy Clin Immunol* 2012;**130**(5):1049-1062.
- 13 818 100. Thaminy A, Lamblin C, Perez T, Bergoin C, Tonnel AB, Wallaert B. Increased frequency of
14 819 asymptomatic bronchial hyperresponsiveness in nonasthmatic patients with food allergy. *Eur Respir*
15 820 *J* 2000;**16**(6):1091-1094.
- 16 821 101. Krogulska A, Dynowski J, Jedrzejczyk M, Sardecka I, Malachowska B, Wasowska-
17 822 Krolikowska K. The impact of food allergens on airway responsiveness in schoolchildren with
18 823 asthma: A DBPCFC study. *Pediatr Pulmonol* 2016.
- 19 824 102. Krogulska A, Dynowski J, Funkowicz M, Malachowska B, Wasowska-Krolikowska K.
20 825 Prevalence and Clinical Impact of IgE-Mediated Food Allergy in School Children With Asthma: A
21 826 Double-Blind Placebo-Controlled Food Challenge Study. *Allergy Asthma Immunol Res*
22 827 2015;**7**(6):547-556.
- 23 828 103. Wang J, Liu AH. Food allergies and asthma. *Curr Opin Allergy Clin Immunol*
24 829 2011;**11**(3):249-254.
- 25 830 104. Custovic A, Simpson A, Woodcock A. Importance of indoor allergens in the induction of
26 831 allergy and elicitation of allergic disease. *Allergy* 1998;**53**(48 Suppl):115-120.
- 27 832 105. Gent JF, Belanger K, Triche EW, Bracken MB, Beckett WS, Leaderer BP. Association of
28 833 pediatric asthma severity with exposure to common household dust allergens. *Environ Res*
29 834 2009;**109**(6):768-774.
- 30 835 106. Denning DW, Pashley C, Hartl D, Wardlaw A, Godet C, Del Giacco S, et al. Fungal allergy
31 836 in asthma-state of the art and research needs. *Clin Transl Allergy* 2014;**4**:14.
- 32 837 107. Arbes SJ, Jr., Gergen PJ, Vaughn B, Zeldin DC. Asthma cases attributable to atopy: results
33 838 from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol*
34 839 2007;**120**(5):1139-1145.
- 35 840 108. Jaakkola MS, Ieromnimon A, Jaakkola JJ. Are atopy and specific IgE to mites and molds
36 841 important for adult asthma? *J Allergy Clin Immunol* 2006;**117**(3):642-648.
- 37 842 109. O'Driscoll BR, Hopkinson LC, Denning DW. Mold sensitization is common amongst
38 843 patients with severe asthma requiring multiple hospital admissions. *BMC Pulm Med* 2005;**5**:4.
- 39 844 110. Black PN, Udy AA, Brodie SM. Sensitivity to fungal allergens is a risk factor for life-
40 845 threatening asthma. *Allergy* 2000;**55**(5):501-504.
- 41 846 111. O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, et al.
42 847 Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients
43 848 with asthma. *N Engl J Med* 1991;**324**(6):359-363.
- 44 849 112. Schwartz HJ, Citron KM, Chester EH, Kaimal J, Barlow PB, Baum GL, et al. A comparison
45 850 of the prevalence of sensitization to Aspergillus antigens among asthmatics in Cleveland and
46 851 London. *J Allergy Clin Immunol* 1978;**62**(1):9-14.
- 47 852 113. Vicencio AG, Santiago MT, Tsirilakis K, Stone A, Worgall S, Foley EA, et al. Fungal
48 853 sensitization in childhood persistent asthma is associated with disease severity. *Pediatr Pulmonol*
49 854 2014;**49**(1):8-14.
- 50 855 114. Tham R, Dharmage SC, Taylor PE, Katelaris CH, Vicendese D, Abramson MJ, et al.
51 856 Outdoor fungi and child asthma health service attendances. *Pediatr Allergy Immunol*
52 857 2014;**25**(5):439-449.

- 1
2
3 858 115. Lotvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma
4 859 endotypes: a new approach to classification of disease entities within the asthma syndrome. *J*
5 860 *Allergy Clin Immunol* 2011;**127**(2):355-360.
- 6 861 116. Ward GW, Jr., Karlsson G, Rose G, Platts-Mills TA. Trichophyton asthma: sensitisation of
7 862 bronchi and upper airways to dermatophyte antigen. *Lancet* 1989;**1**(8643):859-862.
- 8 863 117. James AL, Palmer LJ, Kicic E, Maxwell PS, Lagan SE, Ryan GF, et al. Decline in lung
9 864 function in the Busselton Health Study: the effects of asthma and cigarette smoking. *Am J Respir*
10 865 *Crit Care Med* 2005;**171**(2):109-114.
- 11 866 118. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC.
12 867 Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J*
13 868 *Respir Crit Care Med* 2003;**168**(11):1308-1311.
- 14 869 119. Barnes PJ, Ito K, Adcock IM. Corticosteroid resistance in chronic obstructive pulmonary
15 870 disease: inactivation of histone deacetylase. *Lancet* 2004;**363**(9410):731-733.
- 16 871 120. Cerveri I, Cazzoletti L, Corsico AG, Marcon A, Niniano R, Grosso A, et al. The impact of
17 872 cigarette smoking on asthma: a population-based international cohort study. *Int Arch Allergy*
18 873 *Immunol* 2012;**158**(2):175-183.
- 19 874 121. Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, et al.
20 875 Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe
21 876 asthma. *J Allergy Clin Immunol* 2013;**131**(4):1008-1016.
- 22 877 122. Siroux V, Pin I, Oryszczyn MP, Le Moual N, Kauffmann F. Relationships of active smoking
23 878 to asthma and asthma severity in the EGEA study. Epidemiological study on the Genetics and
24 879 Environment of Asthma. *Eur Respir J* 2000;**15**(3):470-477.
- 25 880 123. Oryszczyn MP, Annesi-Maesano I, Charpin D, Paty E, Maccario J, Kauffmann F.
26 881 Relationships of active and passive smoking to total IgE in adults of the Epidemiological Study of
27 882 the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy (EGEA). *Am*
28 883 *J Respir Crit Care Med* 2000;**161**(4 Pt 1):1241-1246.
- 29 884 124. Nielsen GD, Olsen O, Larsen ST, Lovik M, Poulsen LK, Glue C, et al. IgE-mediated
30 885 sensitisation, rhinitis and asthma from occupational exposures. Smoking as a model for airborne
31 886 adjuvants? *Toxicology* 2005;**216**(2-3):87-105.
- 32 887 125. Polosa R, Knoke JD, Russo C, Piccillo G, Caponnetto P, Sarva M, et al. Cigarette smoking
33 888 is associated with a greater risk of incident asthma in allergic rhinitis. *J Allergy Clin Immunol*
34 889 2008;**121**(6):1428-1434.
- 35 890 126. Cazzoletti L, Marcon A, Corsico A, Janson C, Jarvis D, Pin I, et al. Asthma severity
36 891 according to Global Initiative for Asthma and its determinants: an international study. *Int Arch*
37 892 *Allergy Immunol* 2010;**151**(1):70-79.
- 38 893 127. Saulyte J, Regueira C, Montes-Martinez A, Khudyakov P, Takkouche B. Active or passive
39 894 exposure to tobacco smoking and allergic rhinitis, allergic dermatitis, and food allergy in adults and
40 895 children: a systematic review and meta-analysis. *PLoS Med* 2014;**11**(3):e1001611.
- 41 896 128. Adenuga D, Yao H, March TH, Seagrave J, Rahman I. Histone deacetylase 2 is
42 897 phosphorylated, ubiquitinated, and degraded by cigarette smoke. *Am J Respir Cell Mol Biol*
43 898 2009;**40**(4):464-473.
- 44 899 129. Livingston E, Darroch CE, Chaudhuri R, McPhee I, McMahon AD, Mackenzie SJ, et al.
45 900 Glucocorticoid receptor alpha:beta ratio in blood mononuclear cells is reduced in cigarette smokers.
46 901 *J Allergy Clin Immunol* 2004;**114**(6):1475-1478.
- 47 902 130. Kupczyk M, ten Brinke A, Sterk PJ, Bel EH, Papi A, Chanez P, et al. Frequent exacerbators-
48 903 -a distinct phenotype of severe asthma. *Clin Exp Allergy* 2014;**44**(2):212-221.
- 49 904 131. Bateman ED, Buhl R, O'Byrne PM, Humbert M, Reddel HK, Sears MR, et al. Development
50 905 and validation of a novel risk score for asthma exacerbations: The risk score for exacerbations. *J*
51 906 *Allergy Clin Immunol* 2015;**135**(6):1457-1464 e1454.
- 52 907 132. Accordini S, Janson C, Svanes C, Jarvis D. The role of smoking in allergy and asthma:
53 908 lessons from the ECRHS. *Curr Allergy Asthma Rep* 2012;**12**(3):185-191.

- 1
2
3 909 133. Becklake MR, Laloo U. The 'healthy smoker': a phenomenon of health selection?
4 910 *Respiration* 1990;**57**(3):137-144.
- 5 911 134. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. *Lancet* 2014;**383**(9928):1581-
6 912 1592.
- 7 913 135. Evans KA, Halterman JS, Hopke PK, Fagnano M, Rich DQ. Increased ultrafine particles
8 914 and carbon monoxide concentrations are associated with asthma exacerbation among urban
9 915 children. *Environ Res* 2014;**129**:11-19.
- 10 916 136. Weinmayr G, Romeo E, De Sario M, Weiland SK, Forastiere F. Short-term effects of PM10
11 917 and NO2 on respiratory health among children with asthma or asthma-like symptoms: a systematic
12 918 review and meta-analysis. *Environ Health Perspect* 2010;**118**(4):449-457.
- 13 919 137. Sarnat JA, Golan R, Greenwald R, Raysoni AU, Kewada P, Winquist A, et al. Exposure to
14 920 traffic pollution, acute inflammation and autonomic response in a panel of car commuters. *Environ*
15 921 *Res* 2014;**133**:66-76.
- 16 922 138. Friedman MS, Powell KE, Hutwagner L, Graham LM, Teague WG. Impact of changes in
17 923 transportation and commuting behaviors during the 1996 Summer Olympic Games in Atlanta on air
18 924 quality and childhood asthma. *JAMA* 2001;**285**(7):897-905.
- 19 925 139. Gehring U, Gruziova O, Agius RM, Beelen R, Custovic A, Cyrus J, et al. Air pollution
20 926 exposure and lung function in children: the ESCAPE project. *Environ Health Perspect*
21 927 2013;**121**(11-12):1357-1364.
- 22 928 140. Molter A, Agius RM, de Vocht F, Lindley S, Gerrard W, Lowe L, et al. Long-term exposure
23 929 to PM10 and NO2 in association with lung volume and airway resistance in the MAAS birth cohort.
24 930 *Environ Health Perspect* 2013;**121**(10):1232-1238.
- 25 931 141. Eeftens M, Hoek G, Gruziova O, Molter A, Agius R, Beelen R, et al. Elemental composition
26 932 of particulate matter and the association with lung function. *Epidemiology* 2014;**25**(5):648-657.
- 27 933 142. Molter A, Agius R, de Vocht F, Lindley S, Gerrard W, Custovic A, et al. Effects of long-
28 934 term exposure to PM10 and NO2 on asthma and wheeze in a prospective birth cohort. *J Epidemiol*
29 935 *Community Health* 2014;**68**(1):21-28.
- 30 936 143. Adam M, Schikowski T, Carsin AE, Cai Y, Jacquemin B, Sanchez M, et al. Adult lung
31 937 function and long-term air pollution exposure. ESCAPE: a multicentre cohort study and meta-
32 938 analysis. *Eur Respir J* 2015;**45**(1):38-50.
- 33 939 144. Jacquemin B, Kauffmann F, Pin I, Le Moual N, Bousquet J, Gormand F, et al. Air pollution
34 940 and asthma control in the Epidemiological study on the Genetics and Environment of Asthma. *J*
35 941 *Epidemiol Community Health* 2012;**66**(9):796-802.
- 36 942 145. Morgenstern V, Zutavern A, Cyrus J, Brockow I, Koletzko S, Kramer U, et al. Atopic
37 943 diseases, allergic sensitization, and exposure to traffic-related air pollution in children. *Am J Respir*
38 944 *Crit Care Med* 2008;**177**(12):1331-1337.
- 39 945 146. Gruziova O, Bellander T, Eneroth K, Kull I, Melen E, Nordling E, et al. Traffic-related air
40 946 pollution and development of allergic sensitization in children during the first 8 years of life. *J*
41 947 *Allergy Clin Immunol* 2012;**129**(1):240-246.
- 42 948 147. Nordling E, Berglind N, Melen E, Emenius G, Hallberg J, Nyberg F, et al. Traffic-related air
43 949 pollution and childhood respiratory symptoms, function and allergies. *Epidemiology*
44 950 2008;**19**(3):401-408.
- 45 951 148. de Haar C, Kool M, Hassing I, Bol M, Lambrecht BN, Pieters R. Lung dendritic cells are
46 952 stimulated by ultrafine particles and play a key role in particle adjuvant activity. *J Allergy Clin*
47 953 *Immunol* 2008;**121**(5):1246-1254.
- 48 954 149. Brandt EB, Biagini Myers JM, Acciani TH, Ryan PH, Sivaprasad U, Ruff B, et al. Exposure
49 955 to allergen and diesel exhaust particles potentiates secondary allergen-specific memory responses,
50 956 promoting asthma susceptibility. *J Allergy Clin Immunol* 2015;**136**(2):295-303 e297.
- 51 957 150. Acciani TH, Brandt EB, Khurana Hershey GK, Le Cras TD. Diesel exhaust particle
52 958 exposure increases severity of allergic asthma in young mice. *Clin Exp Allergy* 2013;**43**(12):1406-
53 959 1418.

- 1
2
3 960 151. De Grove KC, Provoost S, Hendriks RW, McKenzie AN, Seys LJ, Kumar S, et al.
4 961 Dysregulation of type 2 innate lymphoid cells and TH2 cells impairs pollutant-induced allergic
5 962 airway responses. *J Allergy Clin Immunol* 2016.
6 963 152. Latzin P, Frey U, Armann J, Kieninger E, Fuchs O, Roosli M, et al. Exposure to moderate
7 964 air pollution during late pregnancy and cord blood cytokine secretion in healthy neonates. *PLoS*
8 965 *One* 2011;**6**(8):e23130.
9 966 153. Gruzieva O, Gehring U, Aalberse R, Agius R, Beelen R, Behrendt H, et al. Meta-analysis of
10 967 air pollution exposure association with allergic sensitization in European birth cohorts. *J Allergy*
11 968 *Clin Immunol* 2014;**133**(3):767-776 e767.
12 969 154. Moscato G, Pala G, Barnig C, De Blay F, Del Giacco SR, Folletti I, et al. EAACI consensus
13 970 statement for investigation of work-related asthma in non-specialized centres. *Allergy*
14 971 2012;**67**(4):491-501.
15 972 155. Mapp CE, Boschetto P, Maestrelli P, Fabbri LM. Occupational asthma. *Am J Respir Crit*
16 973 *Care Med* 2005;**172**(3):280-305.
17 974 156. Brooks SM, Weiss MA, Bernstein IL. Reactive airways dysfunction syndrome (RADS).
18 975 Persistent asthma syndrome after high level irritant exposures. *Chest* 1985;**88**(3):376-384.
19 976 157. Tarlo SM, Broder I. Irritant-induced occupational asthma. *Chest* 1989;**96**(2):297-300.
20 977 158. Vandenplas O, Wiszniewska M, Raulf M, de Blay F, Gerth van Wijk R, Moscato G, et al.
21 978 EAACI position paper: irritant-induced asthma. *Allergy* 2014;**69**(9):1141-1153.
22 979 159. Cullinan P. Clinical aspects of occupational asthma. *Panminerva Med* 2004;**46**(2):111-120.
23 980 160. Vandenplas O, Ghezzi H, Munoz X, Moscato G, Perfetti L, Lemiere C, et al. What are the
24 981 questionnaire items most useful in identifying subjects with occupational asthma? *Eur Respir J*
25 982 2005;**26**(6):1056-1063.
26 983 161. Malo JL, Ghezzi H, L'Archeveque J, Lagier F, Perrin B, Cartier A. Is the clinical history a
27 984 satisfactory means of diagnosing occupational asthma? *Am Rev Respir Dis* 1991;**143**(3):528-532.
28 985 162. Vandenplas O, Toren K, Blanc PD. Health and socioeconomic impact of work-related
29 986 asthma. *Eur Respir J* 2003;**22**(4):689-697.
30 987 163. Tarlo SM, Balmes J, Balkissoon R, Beach J, Beckett W, Bernstein D, et al. Diagnosis and
31 988 management of work-related asthma: American College Of Chest Physicians Consensus Statement.
32 989 *Chest* 2008;**134**(3 Suppl):1S-41S.
33 990 164. Moscato G, Godnic-Cvar J, Maestrelli P, Malo JL, Sherwood Burge P, Coifman R.
34 991 Statement on self-monitoring of peak expiratory flow in the investigation of occupational asthma.
35 992 Subcommittee on Occupational Allergy of the European Academy of Allergology and Clinical
36 993 Immunology. *Allergy* 1995;**50**(9):711-717.
37 994 165. Lavaud F, Bonniaud P, Dalphin JC, Leroyer C, Muller D, Tannous R, et al. Usefulness of
38 995 omalizumab in ten patients with severe occupational asthma. *Allergy* 2013;**68**(6):813-815.
39 996 166. Baur X, Chen Z, Liebers V. Exposure-response relationships of occupational inhalative
40 997 allergens. *Clin Exp Allergy* 1998;**28**(5):537-544.
41 998 167. Jones MG. Exposure-response in occupational allergy. *Curr Opin Allergy Clin Immunol*
42 999 2008;**8**(2):110-114.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

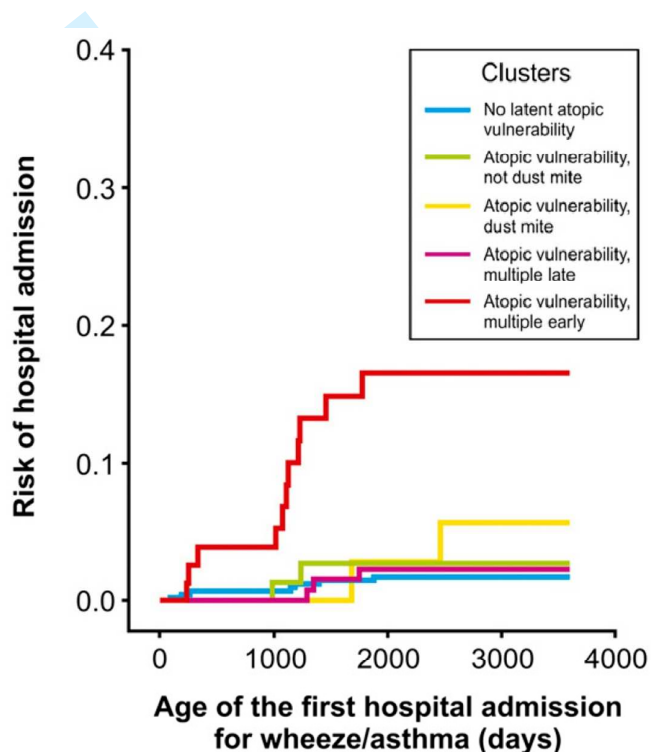


Table 1: Main pollutants and examples of their effects on respiratory function.

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)

Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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)

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

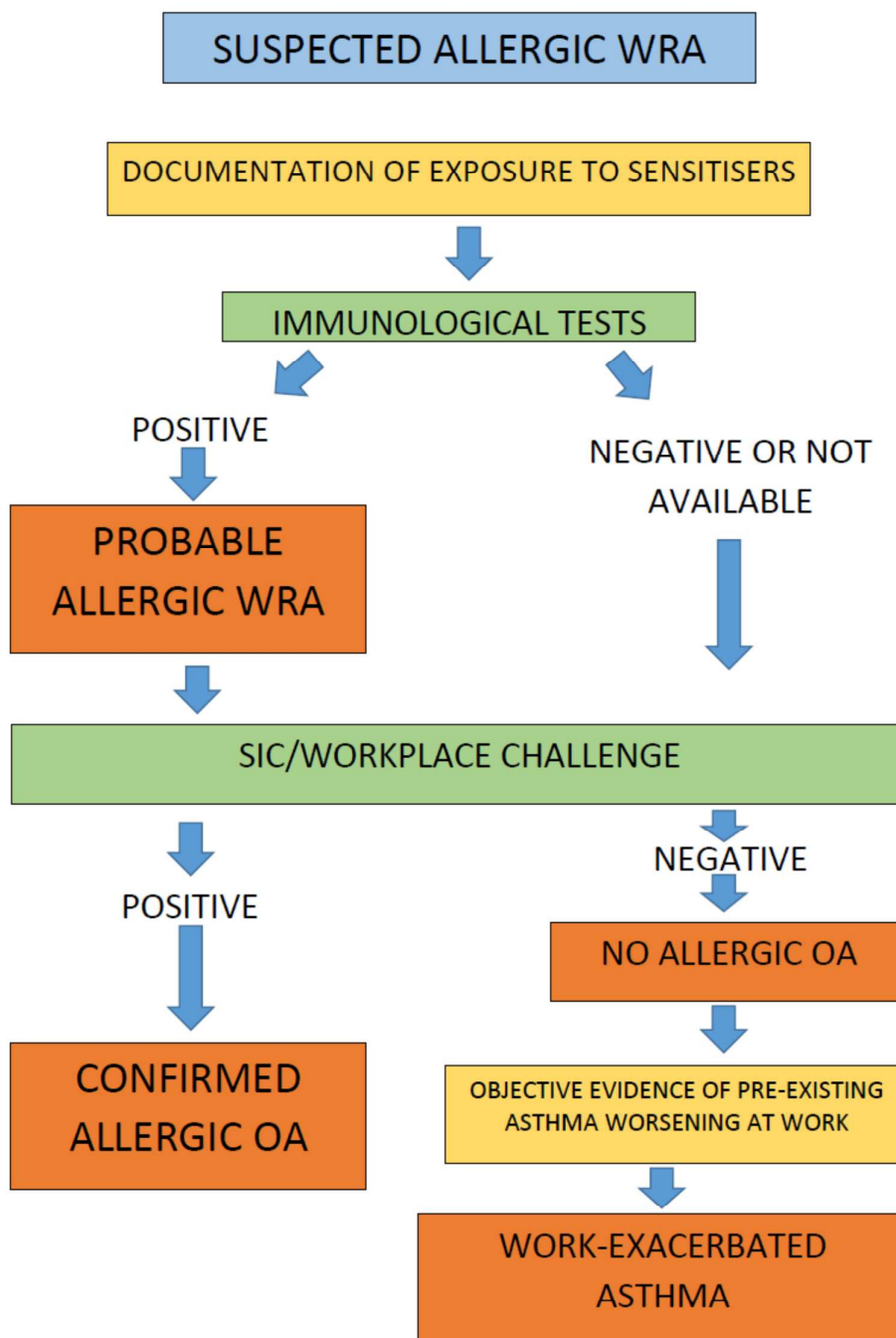
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

Stefano R. Del Giacco¹, Arzu Bakirtas,² Elizabeth Bel³, Adnan Custovic⁴, Zuzana Diamant^{5,13}, Eckard Hamelmann⁶, Enrico Heffler⁷, Ömer Kalayci⁸, Sejal Saglani⁹, Svetlana Sergejeva¹⁰, Sven Seys¹¹, Angela Simpson¹², Leif Bjermer¹³

1. Department of Medical Sciences and Public Health, University of Cagliari, Italy
2. Department of Pediatric Allergy and Asthma, School of Medicine, Gazi University, Ankara, Turkey
3. Department of Respiratory Medicine, Academic Medical Centre, University of Amsterdam, The Netherlands
4. Department of Paediatrics, Imperial College London, United Kingdom
5. University of Groningen, University Medical Centre Groningen, Department of General Practice and Department of Clinical Pharmacy & Pharmacology, Groningen, The Netherlands
6. Allergy Center, Ruhr University Bochum, Klinik für Kinder and Jugendmedizin Kinderzentrum, Bethel Evangelisches Krankenhaus, Bielefeld, Germany
7. Respiratory Medicine and Allergology - Department of Experimental and Clinical Medicine, University of Catania, Italy
8. Hacettepe University, School of Medicine, Ankara, Turkey
9. National Heart & Lung Institute, Imperial College London, United Kingdom
10. Institute of Technology, University of Tartu, Estonia
11. Department of Microbiology and Immunology, Laboratory of Clinical Immunology, KU Leuven, Belgium
12. Centre Lead for Respiratory Medicine and Allergy, University of Manchester, Education and Research Centre, University Hospital of South Manchester, United Kingdom
13. Lund University, Department of Respiratory Medicine and Allergology, Lund, Sweden

Corresponding author:

Stefano R. Del Giacco

Cittadella Universitaria

Department of Medical Sciences and Public Health

University of Cagliari

09042 Monserrato (Cagliari), Italy

stedg@medicina.unica.it

Tel: +39-070-6754150

Fax: +39-070-6754086

TOTAL WORD COUNT: 5727

Short title: Allergy and Asthma Severity

Keywords: aetiology, asthma, allergy, atopy, severity

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 $\geq 50\%$ 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 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 \geq 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

1
2
3 both tests(17, 20). The level of sIgE is also associated with an increased risk of severe asthma
4 exacerbations requiring hospitalization among both children(17, 22) and adults(23). Finally, it has
5 been shown that there is a strong interaction between the levels of sIgE to inhalant allergens and
6 respiratory virus infections in increasing the risk of severe asthma exacerbations requiring hospital
7 admission(24), suggesting a synergism between quantitative sensitisation and respiratory virus
8 infections. This synergism has been indirectly confirmed in a study showing that pre-seasonal anti-
9 IgE- targeted therapy with omalizumab decreases seasonal exacerbations of asthma (“back-to-
10 school asthma”), which are almost certainly (rhino)virus-induced(25). In contrast, a recent study
11 showed that although impaired IFN- β and IFN- λ induction by rhinovirus was a feature of bronchial
12 epithelial cells from highly sensitised children with STRA(26), there was no relationship between
13 sensitisation and Th2-mediated inflammation with impaired interferon production, raising a
14 possibility of two independent mechanisms (atopy-related and virus-related).
15
16
17
18
19
20
21
22

23 All of the above data indicate that in the assessment of patients with asthma (including severe
24 asthma), the results of specific IgE measurement and SPT are not mutually exclusive but
25 complementary, and should not be reported as being “positive” or “negative”, but as the level of
26 sIgE and the size of SPT wheal diameter (*i.e.*, quantified). For SPTs, the size of the positive and
27 negative control should be taken into account. Recent data suggest that diagnostic accuracy of
28 specific IgE antibody measurement in the context of asthma and the distinction between “benign”
29 atopy (*i.e.*, sensitisation in the absence of allergic symptoms) and “pathologic” atopy (*i.e.*,
30 sensitisation related to allergic symptoms), may be improved by the measurement of allergen-
31 specific IgG antibody levels(27), although their measurement is not recommended routinely.
32
33
34
35
36
37
38
39
40

41 ***Heterogeneity of atopic sensitisation***

42
43 It has recently been proposed that “atopic sensitisation” maybe an umbrella term for a collection of
44 several different subgroups of sensitisation which differ in their association with asthma and other
45 allergic diseases(14). Distinct subgroups (or classes) of sensitisation were described in one
46 population-based birth cohort (Manchester Asthma and Allergy Study) by applying a machine
47 learning approach with Bayesian inference to the SPTs and sIgE data collected longitudinally from
48 early life to school age(28), and similar latent structure was subsequently described using
49 comparable approach to longitudinal data on atopic sensitisation in another birth cohort (Isle of
50 Wight study)(14). Children who would be considered sensitised using conventional definitions were
51 clustered into four distinct subgroups characterised by a unique pattern of the responses to different
52 allergens and the timing of onset of allergen-specific sensitisation(28) (Figure 1). Importantly, the
53
54
55
56
57
58
59
60

1
2
3 risk of asthma was increased more than 20-fold amongst children belonging to one of these
4 subgroups (those sensitised to multiple allergens in early life - comprising less than one third of the
5 sensitised children), but not amongst those in other classes(14, 28). Striking similarities were
6 observed in the association between different subgroups of atopic sensitisation in these two cohorts
7 in relation to asthma severity, with children in the subgroup of sensitisation characterised by IgE
8 responses to multiple allergens in early life having higher FeNO levels, more hyperreactive airways,
9 an increased risk of severe asthma exacerbations having significantly diminished lung function,
10 compared to all other classes(14, 28, 29). It is of note however, that such subtypes (clusters/classes)
11 of sensitisation can only be identified using statistical inference on longitudinal data(14, 28), and
12 that differentiation between different clusters at any single cross-sectional point is not yet
13 possible(30). Clinical translation of this important observation requires the development of specific
14 and sensitive biomarkers which can be measured at the time of presentation to clinic and which aid
15 differentiation between different sensitisation subgroups. Recent data indicate that IgE responses to
16 individual allergenic molecules rather than whole allergen extracts may prove useful in
17 differentiating the subtypes of sensitisation relevant to asthma onset and severity(31, 32).

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

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

1
2
3 putative mechanism where AD may increase the risk of subsequent sensitization, which in turn
4 increases the risk of asthma.

5
6 Filaggrin gene (FLG) mutations are associated both with atopic and nonatopic eczema starting in
7 the first year of life. FLG mutations combined with eczema in the first year of life are associated
8 with a later development of asthma and hay fever, and this may support the latter
9 mechanism(35). This more modern view of the atopic march is furthermore strongly supported by
10 recent data on the defective skin barrier function as the key factor for the pathogenesis of AD(36).
11 Skin barrier dysfunction facilitates transdermal dehydration and infiltration of allergens, bacteria
12 and bacterial toxins, thus inducing and enhancing allergen sensitization as a hallmark of the atopic
13 march(37). Skin sensitization is followed by airway sensitization to the same allergen and is one of
14 the most robust predictors for the development of childhood asthma(38). This is detailed further on
15 in this review. In conclusion, there is evidence for the hypothesis linking AD as an initial (but
16 probably not only) promoter of atopy/allergic sensitization with progression to asthma.
17
18
19
20
21
22
23
24

25 26 ***Component-resolved diagnostics in asthma***

27
28 Recent advances in biochemistry and molecular biology have led to the isolation and
29 characterisation of numerous allergenic proteins (components), facilitating the profiling of IgE
30 reactivity to individual allergens at a molecular level. This new approach to allergy diagnosis has
31 been termed molecular diagnosis or component-resolved diagnostics (CRD), and its
32 commercialisation has facilitated the development of products in which sIgE to >100 allergen
33 components can be measured simultaneously. Component resolved diagnostics may help in
34 identifying patients at risk of developing more severe disease(31, 32). Sensitization to mite
35 allergens Der p 2 and Der f 2 has been reported to be more common in severe asthma (39). Latex
36 allergy and asthma is another example where sensitization to 3 out of 12 recombinant natural rubber
37 antigens (5, 6.01/6.02), was strongly linked to those with latex sensitization and asthma (40)
38
39
40
41
42
43
44

45
46 The role of these novel tools in clinical practice and how best to interpret the complex data they
47 generate is the subject of ongoing debate(41, 42). It has recently been reported that CRD may
48 improve the assessment of asthma(31, 43), and help better understanding the role of allergy in
49 severe asthma in childhood(44). However, it is likely that better interpretation algorithms are
50 needed to capitalise fully on the potential of this exciting new technology(43).
51
52
53
54
55
56
57
58
59
60

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,

1
2
3 59). Patients with severe asthma have a much higher allergic burden(51, 60) suggesting that atopic
4 sensitisation plays a critical role in the development, progression and persistence of paediatric
5 severe disease.
6
7

8 9 10 ***Adult onset, severe asthma: an age-specific phenotype***

11
12 Adult onset asthma is a recognised phenotype of severe asthma, presenting with several sub-
13 phenotypes(61). Although it is considered predominantly non-allergic, a significant proportion of
14 patients with adult onset disease are atopic (34%)(61). In those with severe disease, a worse
15 prognosis is apparent in smokers and ex-smokers(62), and, as described later on, smoke exposure
16 has a detrimental effect on severe asthma, resulting in reduced corticosteroid responsiveness,
17 regardless of age(63). Distinguishing and specific features of adult onset asthma include association
18 with co-morbidities, such as obesity, and a predominance in middle-aged women(64). The adult-
19 onset obese, female predominant phenotype is characterised by the absence of inflammation and
20 atopic sensitisation. Although this specific set of features is seen in adults, mechanisms resulting in
21 obesity-associated asthma may not be dissimilar in children and adults. Children with severe asthma
22 who have a higher BMI are less likely to have detectable inflammatory Th2 cytokines and have
23 relatively higher lung function than those with lower BMI(53).
24
25

26
27 Another common adult-onset phenotype includes severe (non-allergic) eosinophilic phenotype,
28 which is the most prevalent phenotype of severe asthma in adults, associated with aspirin
29 sensitivity, nasal polyposis and eosinophilia, all persisting despite the treatment with high doses of
30 inhaled corticosteroids(54). Innate immune mechanisms underlying this phenotype have recently
31 been proposed since it has become apparent that patients respond to anti-IL-5 antibody
32 therapies(65).
33
34
35
36
37
38
39
40
41
42
43
44
45

46 ***Contribution of allergy to mechanisms underlying severe asthma***

47
48 The role of allergy in severe asthma needs to be understood to help identify underlying mechanisms
49 of disease progression which will impact both on the choice of add-on therapies and on the
50 discovery of novel therapeutics. Even though the majority of children and adults with early-onset
51 severe asthma are sensitised, it is interesting that not all respond to treatment with omalizumab(66,
52 67)suggesting several different mechanisms contributing to the development of different allergic
53 phenotypes.
54
55
56
57
58
59
60

1
2
3 Typically, the allergic asthma phenotype is associated with eosinophilia, elevated serum IgE and
4 Th2 cytokines. However, in adult-onset asthma, eosinophilia may be present without overt evidence
5 of allergy(65). The limited contribution of allergy to disease persistence is apparent in adults with
6 severe asthma who show a non-allergic, inhaled corticosteroid “resistant” eosinophilic phenotype,
7 which responds to systemic CS and targeted therapy with anti-IL-5 (mepolizumab)(68). Novel
8 mechanisms that may contribute to this adult-onset phenotype include epithelial innate cytokines
9 that directly induce the recruitment of innate lymphoid cells which secrete Th2/“allergic” cytokines
10 without the generation of IgE or an adaptive immune response(69). Interestingly, even though it is
11 thought that this is an innate, non-adaptive, non-allergic immune response, all murine experimental
12 models investigating the role of innate cytokines in asthma pathogenesis used allergen exposure as
13 the stimulus, suggesting allergy still plays a central mechanistic role in this phenotype(70). It is
14 possible that allergy is a risk factor in the development of adult-onset “non-allergic” eosinophilic
15 asthma, but the clinical manifestation of asthma changes with time and age, whereby it is less
16 overtly “allergic”, but remains eosinophilic.
17

18
19 In asthma, the effect of innate immunity eliciting Th2 responses seems to be strongly related to IL-
20 33(71), and is especially associated with severe disease. IL-33 expression is increased in bronchial
21 tissue from both adults(72, 73) and children(74)with severe asthma. Other important features of
22 innate cytokines that may contribute to the pathogenesis of severe disease in both adults and
23 children include their role in (relative) corticosteroid resistance(74) and their association with
24 angiogenesis and airway remodelling, in particular as regards IL-25 (74-76).
25

26
27 An interesting distinction of adult asthma phenotypes based on gene expression of periostin by
28 airway epithelial cells includes the separation in Th2 high and Th2 low phenotypes(77), and the
29 utility of this biomarker to predict therapeutic response to antibodies that block Th2 cytokines(78).
30 Although biomarkers that allow such distinctions have not yet been identified in children, and while
31 in general children with severe asthma have low or undetectable Th2 cytokines in airway samples,
32 there is a sub-group in whom Th2 cytokines can be detected(53), emphasising similarities between
33 adult and childhood disease.
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

53 **CROSS-TALK BETWEEN ENVIRONMENTAL FACTORS, ATOPIC SENSITISATION** 54 **AND ASTHMA** 55 56 57 58 59 60

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

1
2
3 Relationships between different types of allergens (outdoor, indoor, food) and the development and
4 severity of allergic disease, including asthma, have been studied(97). For instance, pollen allergy
5 has been found to be interrelated with various food allergies, digestive system Th2-inflammation
6 and asthma(98, 99). Cross-reactivity between pollen and several plant-derived foods, nuts, and
7 fruits has been well established(98). Food allergy without concomitant asthma has been found to be
8 associated with increased nonspecific bronchial hyperresponsiveness(100, 101), while several
9 studies report that children with asthma and concomitant food allergy have more severe disease,
10 poorer control, greater morbidity, and require more anti-asthma medications(102, 103).

11
12 The most common indoor allergens associated with asthma include house dust mites, domestic
13 animals (cats, dogs), and cockroaches(97, 104), while fungi can be found both indoor and outdoor.
14 In a cohort of 300 asthmatic children (aged 4-12 y), higher *Der p 1* and pet allergen levels were
15 found to be associated with greater asthma severity(105).

16
17 Fungal exposure is universal and fungi can be linked to asthma in a variety of ways. Fungal allergy
18 drives asthma severity and long-term or uncontrolled fungal infections are associated with a poor
19 control of asthma, complications such as bronchiectasies and chronic allergic bronchopulmonary
20 aspergillosis (ABPA)(106). In the general asthma population, sensitization to moulds ranges from 7
21 to 20%, in severe asthma patients from 35 to 75%, being 54-91% in life-threatening asthma
22 population(107-111). The first evidence of the link between the severity of asthma and fungal
23 sensitisation dates to 1978, when Schwartz et al. demonstrated a relationship between asthma
24 severity and *Aspergillus spp* sensitisation(112). *Alternaria* or *Cladosporium spp* sensitisation was
25 associated with asthma severity in the European Community Respiratory Health Survey.
26 Furthermore, a recent paper has shown that fungal sensitisation in children with persistent asthma is
27 associated with disease severity(113)and a 2014 review has shown increasing evidence that
28 sensitized asthmatic children may be susceptible to asthma exacerbations when exposed to outdoor
29 fungal spores and that the severity of exacerbation may vary with different fungi species(114).

30
31 The term “Severe Asthma with Fungal Sensitisation” (SAFS) was introduced by Denning et al. in
32 2006, to describe those patients who have persistent severe asthma (despite standard treatment) and
33 evidence of fungal sensitisation, as defined by positive SPT, or fungus or fungal antigen-specific
34 sIgE, and do not meet the criteria for ABPA(10). Proposed classification by an EAACI Task Force
35 sets the total IgE cut-off at <1000 IU/ml for SAFS and >1000 IU/ml for ABPA. ABPA was
36 accepted as an endotype(115), while SAFS remains a pragmatic definition(106). ABPA may
37 develop in asthmatics with a genetic predisposition and therefore SAFS may have the same
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 background. Carefully genotyping patients with different forms of asthma may allow a better
4 understanding of this disease.
5

6
7 “Trichophyton Asthma” is another clinical entity, where inhalation or the presence of cutaneous
8 infection (athlete’s foot, onychomycosis) in sensitised asthmatics is associated with disease
9 severity(106, 116).
10
11

12 13 14 15 **Smoking**

16
17 Cigarette smoking itself may influence asthma, as it accelerates lung function decline(117), impairs
18 the response to CS (both inhaled and oral)(118), increases airway oxidative stress(119), perpetuates
19 symptoms despite of treatment(120) and induces the change of inflammatory phenotypes into more
20 aggressive ones(121), thereby resulting in a more severe disease(122).
21
22

23
24 Smoking also increases serum IgE levels, especially in men(123). This may result in an increased
25 risk of allergic sensitisation, at least for occupational allergens(124). However, the relationship
26 between cigarette smoking and allergy in severe asthma is still debated: some studies identify
27 smoking as a risk factor for allergic asthma(125), while others show a lower prevalence of atopic
28 sensitisation in smoking patients with severe asthma(121). According to a large epidemiological
29 survey (ECRHS II), smoking was more strongly associated with severe asthma in men than in
30 women, particularly if they were sensitised to moulds (*Cladosporium*), house dust mites or
31 cats(126). Even more conflicting data come from studies on the effect of passive smoking on the
32 risk of development of atopic sensitisation(127).
33
34

35
36 Cigarette smoking usually results in a more neutrophilic airway inflammation, which is less
37 responsive to ICS(121). Accordingly, alveolar macrophages from smokers have a reduced cellular
38 CS responsiveness, which is associated with reduced histone deacetylase activity, an essential
39 molecule for anti-inflammatory genes transcription(63, 128). In fact, they show an elevated
40 glucocorticoid receptors (GR) ratio in PBMC which is in favour of GR- β (not able to induce any
41 transcriptional activity) compared to GR- α (the active isoform with anti-inflammatory effects)(129).
42 These molecular events make smoking asthmatics less responsive to CS, currently the standard
43 controller therapy for asthma, leading them to a more probable evolution to severe asthma (figure
44 2).
45
46

47
48 Recently, a new distinct phenotype of severe asthma has been identified in frequent exacerbators,
49 and history of smoking seems to be a risk factor for this phenotype(130). A novel risk score for
50
51
52
53
54
55
56
57
58
59
60

1
2
3 asthma exacerbations developed and validated by Bateman *et al.* supports the evidence that
4 smoking status is a main predictor for uncontrolled asthma(131). Despite this well-known
5 relationship, active smoking is still surprisingly common among asthmatics(132). More efficient
6 smoking prevention programs and smoking cessation campaigns should be carried out to try to
7 reduce the risk of developing severe asthma. Moreover, most clinical trials with new drugs aimed
8 for severe asthma have been conducted in non-smoking patients, which results in incomplete
9 knowledge on the efficacy of such therapeutic approaches in smokers. Large “real life” studies in
10 severe asthma including smoking asthmatics should be encouraged. The complex relationship
11 between cigarette smoking and atopic sensitisation increasing the risk of severe asthma should be
12 better investigated as only few and conflicting data are presently available. However, this
13 relationship remains difficult to address, particularly in cross-sectional studies, because of the
14 potential selection bias (e.g. “healthy smoker effect”)(133). Prospective studies in lifetime smokers
15 with lifetime smoking are more appropriate to properly examine the relationships between smoking
16 and severe asthma.
17
18
19
20
21
22
23
24
25
26
27
28

29 **Pollution**

30
31
32 The health effects caused by outdoor air pollution have been intensively studied during the last
33 decades. The term “outdoor air pollution” involves particulate matter (PM), gaseous pollutions
34 (nitrogen dioxide, sulphur dioxide and ozone) and traffic-related air pollution (elemental and carbon
35 black, road dust)(134).
36
37
38

39
40 Increased exposure to ultrafine particles and carbon monoxide within the previous 4-7 days was
41 associated with increased relative odds of a paediatric asthma visit(135). Other studies also indicate
42 that sudden increase or decrease of exposure to air pollution may affect asthmatic symptoms or
43 emergency department visits(136-138). Indeed, a decrease in the number of acute asthma events of
44 over 40% was found after reduction of air pollution during summer Olympic games(138). So far,
45 these studies were performed in children and included only a relatively low number of individuals.
46
47
48
49

50
51 Larger scale studies also demonstrated an adverse effect of outdoor air pollution on lung
52 function(139-141). A multicenter birth cohort study (ESCAPE) showed an association between
53 estimated levels of NO₂ and PM_{2.5} and decreases in FEV₁(139). In another birth cohort study
54 (MAAS), lifetime exposure to PM₁₀ and NO₂ was associated with significantly less growth in FEV₁
55 over time(140). In the same cohort, no association was found between long-term exposure to PM₁₀
56 and NO₂ and the prevalence of asthma or wheeze(142). In adult asthmatics, exposure to NO₂ and
57
58
59
60

1
2
3 PM₁₀ was associated with lower measures of FEV₁ and FVC(143)and exposure to ozone and PM₁₀
4 increased the risk of uncontrolled asthma(144). Overall, these studies thus provide evidence of an
5 inverse association between outdoor air pollution and lung function (Table 1). Whether asthma
6 severity is directly affected by outdoor air pollution is unclear.
7
8

9
10 Several studies showed a positive association between exposure to air pollution during infancy and
11 sensitisation to inhalant allergens(145-147). Although the mechanism underlying this association is
12 not fully understood, some evidence suggests that ultrafine carbon black particles can directly
13 induce maturation of dendritic cells *in vitro*(148), thereby facilitating sensitisation to inhalant
14 allergens. Alternatively, airborne pollutants can induce the influx of inflammatory cells to the lungs,
15 which might then lower the threshold for sensitisation. Indeed, it has recently been shown that
16 allergen-specific Th2/Th17 cells accumulate in the lungs of mice exposed to both diesel exhaust
17 particles and house dust mite extract(149). Diesel exhaust particles may also produce other
18 immunological effects(150, 151) (Table 2). Furthermore, exposure to moderate air pollution during
19 late pregnancy was found to cause increased cord blood IL-1 β (152). A recent meta-analysis,
20 however, showed no clear overall association between air pollution exposure and the development
21 of sensitisation in children up to 10 years of age(153).
22
23
24
25
26
27
28
29
30

31 In summary, in multi-sensitised asthmatics, daily exposure to allergens in combination with other
32 enhancing factors, including viral infections, environmental smoking, and/or pollution, will finally
33 determine the asthma course and severity.
34
35
36
37
38
39

40 ***Occupational/Work-Related***

41
42 Severe asthma may occur in patients affected by Work-Related Asthma (WRA).WRA encompasses
43 both Occupational Asthma (OA), defined as “asthma caused by the workplace” and “Work-
44 Exacerbated Asthma” (WEA), occurring in patients with pre-existing or concurrent asthma and
45 exacerbated by different work-related factors (i.e. aeroallergens, exercise, irritants)(154). OA can be
46 further divided into two subtypes: an allergic form (90% of all OA)(155), caused both by an IgE-
47 mediated mechanism towards high (HMW) and low (LMW) molecular weight agents(106), and a
48 non-IgE mediated form (Non-Allergic, Irritant-Induced [Occupational] Asthma (IIOA)), towards
49 specific LMW agents in which the mechanism has not been characterized yet. The non-allergic
50 IIOA can be further divided into the “Reactive Airway Dysfunction Syndrome” (RADS) and the
51 “IIOA after multiple exposures”. The first occurs after an acute, single exposure to very high
52
53
54
55
56
57
58
59
60

1
2
3 concentrations of irritating substances(156), while the second follows multiple exposure to irritants;
4 in this subtype, onset of asthma can follow the exposures after some time(157, 158).
5
6

7 WRA should be suspected in patients whose asthma worsens while working or begin at work. Here
8 a detailed occupational and medical history is fundamental(159, 160), while a clinical history only
9 shows a low specificity in the diagnosis of OA(161). The investigation of WRA follows a well-
10 defined protocol based on confirmation of bronchial asthma, work-related bronchoconstriction,
11 sensitisation to occupational agents and on the confirmation of the causal role of occupational
12 agents, being sensitisation *per se* not indicative of clinical symptoms(162) (Figure 3). Baseline
13 spirometry is mandatory and it is strongly recommended that this should be complemented with
14 non-specific bronchial hyperreactivity assessment with direct or indirect challenges. In individuals
15 with suspected WRA, presenting with a normal respiratory function and/or negative methacholine
16 challenge testing, serial lung function measurements and assessment of non-specific bronchial
17 hyperreactivity are strongly recommended(162, 163). Additionally, spirometry can be performed
18 during a work shift (Cross-shift spirometry). Furthermore, serial measurements of peak flow
19 expiratory rate (serial PEFr) have been used to objectively confirm the link between the workplace
20 and the asthmatic symptoms(164). Skin prick testing completes the diagnostic work-up, and the
21 selection of specific allergens related to the individual's job is fundamental. Specific IgE evaluation
22 is also of importance. The role of atopic mechanisms in severe occupational asthma has been
23 confirmed by a recent study where treatment with omalizumab was successful in 90% of severe
24 occupational asthma patients due to HMW and LMW agents, such as flour, animal dander, mites,
25 moulds, isocyanate or acrylates(165). It is worth noting that, at least in OA, allergen exposure levels
26 represent the major determinants both for the disease as such and for the severity of asthma(166,
27 167). Finally, specific inhalation challenges (SICs) or workplace inhalation challenges,
28 complemented by the assessment of airway inflammation by induced sputum and FeNO may be
29 considered.
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

46 Diagnosis of IIOA follows a well-defined protocol described in a recent EAACI Task Force
47 document(158).
48
49
50
51
52
53
54

55 CONCLUSION

56
57
58
59
60

1
2
3 There is increasing evidence for the important, but not exclusive, role of allergy in severe asthma.
4 Although some recent reports demonstrate that allergy may play only a limited role, this is likely
5 not true for childhood disease, where early atopic sensitisation is critical in determining the severity
6 of disease.
7
8

9
10 Mechanistic implications of co-factors interacting with allergy and asthma, such as virus infections,
11 pollution, smoking, and work-related exposures, still need to be completely uncovered to allow the
12 discovery of novel therapeutic targets.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1
2
3 **Author contributions:**
4

5
6 SRDG drafted the final version of this manuscript
7

8
9 All authors drafted different chapters and paragraphs of this work
10

11
12 All authors critically revised this work for important intellectual content
13

14
15 All authors approved the final version to be published
16

17
18 All authors agreed on accuracy and integrity of this work
19

20
21
22
23
24 **Conflict of interest disclosure:**
25

26
27 All authors declare that they have no conflict of interest regarding this work
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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, Keadze 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, Prospero 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, Riffart 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.

- 1
2
3 57. Stoltz DJ, Jackson DJ, Evans MD, Gangnon RE, Tisler CJ, Gern JE, et al. Specific patterns
4 of allergic sensitization in early childhood and asthma & rhinitis risk. *Clin Exp Allergy*
5 2013;**43**(2):233-241.
- 6 58. Sharples J, Gupta A, Fleming L, Bossley CJ, Bracken-King M, Hall P, et al. Long-term
7 effectiveness of a staged assessment for paediatric problematic severe asthma. *Eur Respir J*
8 2012;**40**(1):264-267.
- 9 59. Marinho S, Simpson A, Soderstrom L, Woodcock A, Ahlstedt S, Custovic A. Quantification
10 of atopy and the probability of rhinitis in preschool children: a population-based birth cohort study.
11 *Allergy* 2007;**62**(12):1379-1386.
- 12 60. Belgrave DC, Simpson A, Semic-Jusufagic A, Murray CS, Buchan I, Pickles A, et al. Joint
13 modeling of parentally reported and physician-confirmed wheeze identifies children with persistent
14 troublesome wheezing. *J Allergy Clin Immunol* 2013;**132**(3):575-583 e512.
- 15 61. Amelink M, de Nijs SB, de Groot JC, van Tilburg PM, van Spiegel PI, Krouwels FH, et al.
16 Three phenotypes of adult-onset asthma. *Allergy* 2013;**68**(5):674-680.
- 17 62. Westerhof GA, Vollema EM, Weersink EJ, Reinartz SM, de Nijs SB, Bel EH. Predictors for
18 the development of progressive severity in new-onset adult asthma. *J Allergy Clin Immunol*
19 2014;**134**(5):1051-1056 e1052.
- 20 63. Kobayashi Y, Bossley C, Gupta A, Akashi K, Tsartsali L, Mercado N, et al. Passive
21 smoking impairs histone deacetylase-2 in children with severe asthma. *Chest* 2014;**145**(2):305-312.
- 22 64. de Nijs SB, Venekamp LN, Bel EH. Adult-onset asthma: is it really different? *Eur Respir*
23 *Rev* 2013;**22**(127):44-52.
- 24 65. Brusselle GG, Maes T, Bracke KR. Eosinophils in the spotlight: Eosinophilic airway
25 inflammation in nonallergic asthma. *Nat Med* 2013;**19**(8):977-979.
- 26 66. Grimaldi-Bensouda L, Zureik M, Aubier M, Humbert M, Levy J, Benichou J, et al. Does
27 omalizumab make a difference to the real-life treatment of asthma exacerbations?: Results from a
28 large cohort of patients with severe uncontrolled asthma. *Chest* 2013;**143**(2):398-405.
- 29 67. Deschildre A, Marguet C, Salleron J, Pin I, Rittie JL, Derelle J, et al. Add-on omalizumab in
30 children with severe allergic asthma: a 1-year real life survey. *Eur Respir J* 2013;**42**(5):1224-1233.
- 31 68. Bel EH, Ortega HG, Pavord ID. Glucocorticoids and mepolizumab in eosinophilic asthma.
32 *N Engl J Med* 2014;**371**(25):2434.
- 33 69. Vercelli D, Gozdz J, von Mutius E. Innate lymphoid cells in asthma: when innate immunity
34 comes in a Th2 flavor. *Curr Opin Allergy Clin Immunol* 2014;**14**(1):29-34.
- 35 70. Walker JA, McKenzie AN. Development and function of group 2 innate lymphoid cells.
36 *Curr Opin Immunol* 2013;**25**(2):148-155.
- 37 71. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, et al. IL-33 is more potent
38 than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway
39 contraction. *J Allergy Clin Immunol* 2013;**132**(4):933-941.
- 40 72. Prefontaine D, Nadigel J, Chouiali F, Audusseu S, Semlali A, Chakir J, et al. Increased IL-
41 33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010;**125**(3):752-754.
- 42 73. Traister RS, Uvalle CE, Hawkins GA, Meyers DA, Bleecker ER, Wenzel SE. Phenotypic
43 and genotypic association of epithelial IL1RL1 to human TH2-like asthma. *J Allergy Clin Immunol*
44 2015;**135**(1):92-99.
- 45 74. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes
46 airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol*
47 2013;**132**(3):676-685 e613.
- 48 75. Gregory LG, Jones CP, Walker SA, Sawant D, Gowers KH, Campbell GA, et al. IL-25
49 drives remodelling in allergic airways disease induced by house dust mite. *Thorax* 2013;**68**(1):82-
50 90.
- 51 76. Corrigan CJ, Wang W, Meng Q, Fang C, Wu H, Reay V, et al. T-helper cell type 2 (Th2)
52 memory T cell-potentiating cytokine IL-25 has the potential to promote angiogenesis in asthma.
53 *Proc Natl Acad Sci U S A* 2011;**108**(4):1579-1584.

- 1
2
3 77. Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene
4 expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin*
5 *Immunol* 2014;**133**(2):388-394.
- 6 78. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al.
7 Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;**365**(12):1088-1098.
- 8 79. Triggiani M, De Feo G, Cardamone C, Parente R. The Emerging Role of Innate Immunity in
9 Respiratory Allergy. *International Trends in Immunity* 2015;**3**(2):28-32.
- 10 80. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology
11 and allergic airway inflammation. *J Allergy Clin Immunol* 2014;**134**(3):509-520.
- 12 81. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, et al.
13 Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011;**128**(3):549-556 e541-
14 512.
- 15 82. Panzer AR, Lynch SV. Influence and effect of the human microbiome in allergy and asthma.
16 *Curr Opin Rheumatol* 2015;**27**(4):373-380.
- 17 83. Huang YJ, Nelson CE, Brodie EL, Desantis TZ, Baek MS, Liu J, et al. Airway microbiota
18 and bronchial hyperresponsiveness in patients with suboptimally controlled asthma. *J Allergy Clin*
19 *Immunol* 2011;**127**(2):372-381 e371-373.
- 20 84. Goleva E, Jackson LP, Harris JK, Robertson CE, Sutherland ER, Hall CF, et al. The effects
21 of airway microbiome on corticosteroid responsiveness in asthma. *Am J Respir Crit Care Med*
22 2013;**188**(10):1193-1201.
- 23 85. Kloepfer KM, Lee WM, Pappas TE, Kang TJ, Vrtis RF, Evans MD, et al. Detection of
24 pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms
25 and asthma exacerbations. *J Allergy Clin Immunol* 2014;**133**(5):1301-1307, 1307 e1301-1303.
- 26 86. Gavala ML, Bertics PJ, Gern JE. Rhinoviruses, allergic inflammation, and asthma. *Immunol*
27 *Rev* 2011;**242**(1):69-90.
- 28 87. James KM, Peebles RS, Jr., Hartert TV. Response to infections in patients with asthma and
29 atopic disease: an epiphenomenon or reflection of host susceptibility? *J Allergy Clin Immunol*
30 2012;**130**(2):343-351.
- 31 88. Kusel MM, de Klerk NH, Kebabdz T, Vohma V, Holt PG, Johnston SL, et al. Early-life
32 respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent
33 asthma. *J Allergy Clin Immunol* 2007;**119**(5):1105-1110.
- 34 89. Mackenzie KJ, Anderton SM, Schwarze J. Viral respiratory tract infections and asthma in
35 early life: cause and effect? *Clin Exp Allergy* 2014;**44**(1):9-19.
- 36 90. Bonnelykke K, Vissing NH, Sevelsted A, Johnston SL, Bisgaard H. Association between
37 respiratory infections in early life and later asthma is independent of virus type. *J Allergy Clin*
38 *Immunol* 2015;**136**(1):81-86 e84.
- 39 91. Papadopoulos NG, Christodoulou I, Rohde G, Agache I, Almqvist C, Bruno A, et al.
40 Viruses and bacteria in acute asthma exacerbations--a GA(2) LEN-DARE systematic review.
41 *Allergy* 2011;**66**(4):458-468.
- 42 92. Sandrock CE, Norris A. Infection in severe asthma exacerbations and critical asthma
43 syndrome. *Clin Rev Allergy Immunol* 2015;**48**(1):104-113.
- 44 93. Corne JM, Marshall C, Smith S, Schreiber J, Sanderson G, Holgate ST, et al. Frequency,
45 severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a
46 longitudinal cohort study. *Lancet* 2002;**359**(9309):831-834.
- 47 94. Iwasaki J, Smith WA, Khoo SK, Bizzantino J, Zhang G, Cox DW, et al. Comparison of
48 rhinovirus antibody titers in children with asthma exacerbations and species-specific rhinovirus
49 infection. *J Allergy Clin Immunol* 2014;**134**(1):25-32.
- 50 95. Sykes A, Macintyre J, Edwards MR, Del Rosario A, Haas J, Gielen V, et al. Rhinovirus-
51 induced interferon production is not deficient in well controlled asthma. *Thorax* 2014;**69**(3):240-
52 246.
- 53
54
55
56
57
58
59
60

- 1
2
3 96. Patel DA, You Y, Huang G, Byers DE, Kim HJ, Agapov E, et al. Interferon response and
4 respiratory virus control are preserved in bronchial epithelial cells in asthma. *J Allergy Clin*
5 *Immunol* 2014;**134**(6):1402-1412 e1407.
- 6 97. Baxi SN, Phipatanakul W. The role of allergen exposure and avoidance in asthma. *Adolesc*
7 *Med State Art Rev* 2010;**21**(1):57-71, viii-ix.
- 8 98. Bartra J, Sastre J, del Cuvillo A, Montoro J, Jauregui I, Davila I, et al. From pollinosis to
9 digestive allergy. *J Investig Allergol Clin Immunol* 2009;**19** Suppl 1:3-10.
- 10 99. Bousquet J, Schunemann HJ, Samolinski B, Demoly P, Baena-Cagnani CE, Bachert C, et al.
11 Allergic Rhinitis and its Impact on Asthma (ARIA): achievements in 10 years and future needs. *J*
12 *Allergy Clin Immunol* 2012;**130**(5):1049-1062.
- 13 100. Thaminy A, Lamblin C, Perez T, Bergoin C, Tonnel AB, Wallaert B. Increased frequency of
14 asymptomatic bronchial hyperresponsiveness in nonasthmatic patients with food allergy. *Eur Respir*
15 *J* 2000;**16**(6):1091-1094.
- 16 101. Krogulska A, Dynowski J, Jedrzejczyk M, Sardecka I, Malachowska B, Wasowska-
17 Krolikowska K. The impact of food allergens on airway responsiveness in schoolchildren with
18 asthma: A DBPCFC study. *Pediatr Pulmonol* 2016.
- 19 102. Krogulska A, Dynowski J, Funkowicz M, Malachowska B, Wasowska-Krolikowska K.
20 Prevalence and Clinical Impact of IgE-Mediated Food Allergy in School Children With Asthma: A
21 Double-Blind Placebo-Controlled Food Challenge Study. *Allergy Asthma Immunol Res*
22 2015;**7**(6):547-556.
- 23 103. Wang J, Liu AH. Food allergies and asthma. *Curr Opin Allergy Clin Immunol*
24 2011;**11**(3):249-254.
- 25 104. Custovic A, Simpson A, Woodcock A. Importance of indoor allergens in the induction of
26 allergy and elicitation of allergic disease. *Allergy* 1998;**53**(48 Suppl):115-120.
- 27 105. Gent JF, Belanger K, Triche EW, Bracken MB, Beckett WS, Leaderer BP. Association of
28 pediatric asthma severity with exposure to common household dust allergens. *Environ Res*
29 2009;**109**(6):768-774.
- 30 106. Denning DW, Pashley C, Hartl D, Wardlaw A, Godet C, Del Giacco S, et al. Fungal allergy
31 in asthma-state of the art and research needs. *Clin Transl Allergy* 2014;**4**:14.
- 32 107. Arbes SJ, Jr., Gergen PJ, Vaughn B, Zeldin DC. Asthma cases attributable to atopy: results
33 from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol*
34 2007;**120**(5):1139-1145.
- 35 108. Jaakkola MS, Ieromnimon A, Jaakkola JJ. Are atopy and specific IgE to mites and molds
36 important for adult asthma? *J Allergy Clin Immunol* 2006;**117**(3):642-648.
- 37 109. O'Driscoll BR, Hopkinson LC, Denning DW. Mold sensitization is common amongst
38 patients with severe asthma requiring multiple hospital admissions. *BMC Pulm Med* 2005;**5**:4.
- 39 110. Black PN, Udy AA, Brodie SM. Sensitivity to fungal allergens is a risk factor for life-
40 threatening asthma. *Allergy* 2000;**55**(5):501-504.
- 41 111. O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, et al.
42 Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients
43 with asthma. *N Engl J Med* 1991;**324**(6):359-363.
- 44 112. Schwartz HJ, Citron KM, Chester EH, Kaimal J, Barlow PB, Baum GL, et al. A comparison
45 of the prevalence of sensitization to Aspergillus antigens among asthmatics in Cleveland and
46 London. *J Allergy Clin Immunol* 1978;**62**(1):9-14.
- 47 113. Vicencio AG, Santiago MT, Tsirilakis K, Stone A, Worgall S, Foley EA, et al. Fungal
48 sensitization in childhood persistent asthma is associated with disease severity. *Pediatr Pulmonol*
49 2014;**49**(1):8-14.
- 50 114. Tham R, Dharmage SC, Taylor PE, Katelaris CH, Vicendese D, Abramson MJ, et al.
51 Outdoor fungi and child asthma health service attendances. *Pediatr Allergy Immunol*
52 2014;**25**(5):439-449.
- 53
54
55
56
57
58
59
60

- 1
2
3 115. Lotvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma
4 endotypes: a new approach to classification of disease entities within the asthma syndrome. *J*
5 *Allergy Clin Immunol* 2011;**127**(2):355-360.
- 6 116. Ward GW, Jr., Karlsson G, Rose G, Platts-Mills TA. Trichophyton asthma: sensitisation of
7 bronchi and upper airways to dermatophyte antigen. *Lancet* 1989;**1**(8643):859-862.
- 8 117. James AL, Palmer LJ, Kicic E, Maxwell PS, Lagan SE, Ryan GF, et al. Decline in lung
9 function in the Busselton Health Study: the effects of asthma and cigarette smoking. *Am J Respir*
10 *Crit Care Med* 2005;**171**(2):109-114.
- 11 118. Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC.
12 Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J*
13 *Respir Crit Care Med* 2003;**168**(11):1308-1311.
- 14 119. Barnes PJ, Ito K, Adcock IM. Corticosteroid resistance in chronic obstructive pulmonary
15 disease: inactivation of histone deacetylase. *Lancet* 2004;**363**(9410):731-733.
- 16 120. Cerveri I, Cazzoletti L, Corsico AG, Marcon A, Niniano R, Grosso A, et al. The impact of
17 cigarette smoking on asthma: a population-based international cohort study. *Int Arch Allergy*
18 *Immunol* 2012;**158**(2):175-183.
- 19 121. Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, et al.
20 Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe
21 asthma. *J Allergy Clin Immunol* 2013;**131**(4):1008-1016.
- 22 122. Siroux V, Pin I, Oryszczyn MP, Le Moual N, Kauffmann F. Relationships of active smoking
23 to asthma and asthma severity in the EGEA study. Epidemiological study on the Genetics and
24 Environment of Asthma. *Eur Respir J* 2000;**15**(3):470-477.
- 25 123. Oryszczyn MP, Annesi-Maesano I, Charpin D, Paty E, Maccario J, Kauffmann F.
26 Relationships of active and passive smoking to total IgE in adults of the Epidemiological Study of
27 the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy (EGEA). *Am*
28 *J Respir Crit Care Med* 2000;**161**(4 Pt 1):1241-1246.
- 29 124. Nielsen GD, Olsen O, Larsen ST, Lovik M, Poulsen LK, Glue C, et al. IgE-mediated
30 sensitisation, rhinitis and asthma from occupational exposures. Smoking as a model for airborne
31 adjuvants? *Toxicology* 2005;**216**(2-3):87-105.
- 32 125. Polosa R, Knoke JD, Russo C, Piccillo G, Caponnetto P, Sarva M, et al. Cigarette smoking
33 is associated with a greater risk of incident asthma in allergic rhinitis. *J Allergy Clin Immunol*
34 2008;**121**(6):1428-1434.
- 35 126. Cazzoletti L, Marcon A, Corsico A, Janson C, Jarvis D, Pin I, et al. Asthma severity
36 according to Global Initiative for Asthma and its determinants: an international study. *Int Arch*
37 *Allergy Immunol* 2010;**151**(1):70-79.
- 38 127. Saulyte J, Regueira C, Montes-Martinez A, Khudyakov P, Takkouche B. Active or passive
39 exposure to tobacco smoking and allergic rhinitis, allergic dermatitis, and food allergy in adults and
40 children: a systematic review and meta-analysis. *PLoS Med* 2014;**11**(3):e1001611.
- 41 128. Adenuga D, Yao H, March TH, Seagrave J, Rahman I. Histone deacetylase 2 is
42 phosphorylated, ubiquitinated, and degraded by cigarette smoke. *Am J Respir Cell Mol Biol*
43 2009;**40**(4):464-473.
- 44 129. Livingston E, Darroch CE, Chaudhuri R, McPhee I, McMahon AD, Mackenzie SJ, et al.
45 Glucocorticoid receptor alpha:beta ratio in blood mononuclear cells is reduced in cigarette smokers.
46 *J Allergy Clin Immunol* 2004;**114**(6):1475-1478.
- 47 130. Kupczyk M, ten Brinke A, Sterk PJ, Bel EH, Papi A, Chanez P, et al. Frequent exacerbators-
48 a distinct phenotype of severe asthma. *Clin Exp Allergy* 2014;**44**(2):212-221.
- 49 131. Bateman ED, Buhl R, O'Byrne PM, Humbert M, Reddel HK, Sears MR, et al. Development
50 and validation of a novel risk score for asthma exacerbations: The risk score for exacerbations. *J*
51 *Allergy Clin Immunol* 2015;**135**(6):1457-1464 e1454.
- 52 132. Accordini S, Janson C, Svanes C, Jarvis D. The role of smoking in allergy and asthma:
53 lessons from the ECRHS. *Curr Allergy Asthma Rep* 2012;**12**(3):185-191.
- 54
55
56
57
58
59
60

- 1
2
3 133. Becklake MR, Laloo U. The 'healthy smoker': a phenomenon of health selection?
4 *Respiration* 1990;**57**(3):137-144.
- 5 134. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. *Lancet* 2014;**383**(9928):1581-
6 1592.
- 7 135. Evans KA, Halterman JS, Hopke PK, Fagnano M, Rich DQ. Increased ultrafine particles
8 and carbon monoxide concentrations are associated with asthma exacerbation among urban
9 children. *Environ Res* 2014;**129**:11-19.
- 10 136. Weinmayr G, Romeo E, De Sario M, Weiland SK, Forastiere F. Short-term effects of PM10
11 and NO2 on respiratory health among children with asthma or asthma-like symptoms: a systematic
12 review and meta-analysis. *Environ Health Perspect* 2010;**118**(4):449-457.
- 13 137. Sarnat JA, Golan R, Greenwald R, Raysoni AU, Kewada P, Winquist A, et al. Exposure to
14 traffic pollution, acute inflammation and autonomic response in a panel of car commuters. *Environ*
15 *Res* 2014;**133**:66-76.
- 16 138. Friedman MS, Powell KE, Hutwagner L, Graham LM, Teague WG. Impact of changes in
17 transportation and commuting behaviors during the 1996 Summer Olympic Games in Atlanta on air
18 quality and childhood asthma. *JAMA* 2001;**285**(7):897-905.
- 19 139. Gehring U, Gruzieva O, Agius RM, Beelen R, Custovic A, Cyrus J, et al. Air pollution
20 exposure and lung function in children: the ESCAPE project. *Environ Health Perspect*
21 2013;**121**(11-12):1357-1364.
- 22 140. Molter A, Agius RM, de Vocht F, Lindley S, Gerrard W, Lowe L, et al. Long-term exposure
23 to PM10 and NO2 in association with lung volume and airway resistance in the MAAS birth cohort.
24 *Environ Health Perspect* 2013;**121**(10):1232-1238.
- 25 141. Eeftens M, Hoek G, Gruzieva O, Molter A, Agius R, Beelen R, et al. Elemental composition
26 of particulate matter and the association with lung function. *Epidemiology* 2014;**25**(5):648-657.
- 27 142. Molter A, Agius R, de Vocht F, Lindley S, Gerrard W, Custovic A, et al. Effects of long-
28 term exposure to PM10 and NO2 on asthma and wheeze in a prospective birth cohort. *J Epidemiol*
29 *Community Health* 2014;**68**(1):21-28.
- 30 143. Adam M, Schikowski T, Carsin AE, Cai Y, Jacquemin B, Sanchez M, et al. Adult lung
31 function and long-term air pollution exposure. ESCAPE: a multicentre cohort study and meta-
32 analysis. *Eur Respir J* 2015;**45**(1):38-50.
- 33 144. Jacquemin B, Kauffmann F, Pin I, Le Moual N, Bousquet J, Gormand F, et al. Air pollution
34 and asthma control in the Epidemiological study on the Genetics and Environment of Asthma. *J*
35 *Epidemiol Community Health* 2012;**66**(9):796-802.
- 36 145. Morgenstern V, Zutavern A, Cyrus J, Brockow I, Koletzko S, Kramer U, et al. Atopic
37 diseases, allergic sensitization, and exposure to traffic-related air pollution in children. *Am J Respir*
38 *Crit Care Med* 2008;**177**(12):1331-1337.
- 39 146. Gruzieva O, Bellander T, Eneroth K, Kull I, Melen E, Nordling E, et al. Traffic-related air
40 pollution and development of allergic sensitization in children during the first 8 years of life. *J*
41 *Allergy Clin Immunol* 2012;**129**(1):240-246.
- 42 147. Nordling E, Berglind N, Melen E, Emenius G, Hallberg J, Nyberg F, et al. Traffic-related air
43 pollution and childhood respiratory symptoms, function and allergies. *Epidemiology*
44 2008;**19**(3):401-408.
- 45 148. de Haar C, Kool M, Hassing I, Bol M, Lambrecht BN, Pieters R. Lung dendritic cells are
46 stimulated by ultrafine particles and play a key role in particle adjuvant activity. *J Allergy Clin*
47 *Immunol* 2008;**121**(5):1246-1254.
- 48 149. Brandt EB, Biagini Myers JM, Acciani TH, Ryan PH, Sivaprasad U, Ruff B, et al. Exposure
49 to allergen and diesel exhaust particles potentiates secondary allergen-specific memory responses,
50 promoting asthma susceptibility. *J Allergy Clin Immunol* 2015;**136**(2):295-303 e297.
- 51 150. Acciani TH, Brandt EB, Khurana Hershey GK, Le Cras TD. Diesel exhaust particle
52 exposure increases severity of allergic asthma in young mice. *Clin Exp Allergy* 2013;**43**(12):1406-
53 1418.

- 1
2
3 151. De Grove KC, Provoost S, Hendriks RW, McKenzie AN, Seys LJ, Kumar S, et al.
4 Dysregulation of type 2 innate lymphoid cells and TH2 cells impairs pollutant-induced allergic
5 airway responses. *J Allergy Clin Immunol* 2016.
6 152. Latzin P, Frey U, Armann J, Kieninger E, Fuchs O, Roosli M, et al. Exposure to moderate
7 air pollution during late pregnancy and cord blood cytokine secretion in healthy neonates. *PLoS*
8 *One* 2011;**6**(8):e23130.
9 153. Gruzieva O, Gehring U, Aalberse R, Agius R, Beelen R, Behrendt H, et al. Meta-analysis of
10 air pollution exposure association with allergic sensitization in European birth cohorts. *J Allergy*
11 *Clin Immunol* 2014;**133**(3):767-776 e767.
12 154. Moscato G, Pala G, Barnig C, De Blay F, Del Giacco SR, Folletti I, et al. EAACI consensus
13 statement for investigation of work-related asthma in non-specialized centres. *Allergy*
14 2012;**67**(4):491-501.
15 155. Mapp CE, Boschetto P, Maestrelli P, Fabbri LM. Occupational asthma. *Am J Respir Crit*
16 *Care Med* 2005;**172**(3):280-305.
17 156. Brooks SM, Weiss MA, Bernstein IL. Reactive airways dysfunction syndrome (RADS).
18 Persistent asthma syndrome after high level irritant exposures. *Chest* 1985;**88**(3):376-384.
19 157. Tarlo SM, Broder I. Irritant-induced occupational asthma. *Chest* 1989;**96**(2):297-300.
20 158. Vandenplas O, Wiszniewska M, Raulf M, de Blay F, Gerth van Wijk R, Moscato G, et al.
21 EAACI position paper: irritant-induced asthma. *Allergy* 2014;**69**(9):1141-1153.
22 159. Cullinan P. Clinical aspects of occupational asthma. *Panminerva Med* 2004;**46**(2):111-120.
23 160. Vandenplas O, Ghezzi H, Munoz X, Moscato G, Perfetti L, Lemiere C, et al. What are the
24 questionnaire items most useful in identifying subjects with occupational asthma? *Eur Respir J*
25 2005;**26**(6):1056-1063.
26 161. Malo JL, Ghezzi H, L'Archeveque J, Lagier F, Perrin B, Cartier A. Is the clinical history a
27 satisfactory means of diagnosing occupational asthma? *Am Rev Respir Dis* 1991;**143**(3):528-532.
28 162. Vandenplas O, Toren K, Blanc PD. Health and socioeconomic impact of work-related
29 asthma. *Eur Respir J* 2003;**22**(4):689-697.
30 163. Tarlo SM, Balmes J, Balkissoon R, Beach J, Beckett W, Bernstein D, et al. Diagnosis and
31 management of work-related asthma: American College Of Chest Physicians Consensus Statement.
32 *Chest* 2008;**134**(3 Suppl):1S-41S.
33 164. Moscato G, Godnic-Cvar J, Maestrelli P, Malo JL, Sherwood Burge P, Coifman R.
34 Statement on self-monitoring of peak expiratory flow in the investigation of occupational asthma.
35 Subcommittee on Occupational Allergy of the European Academy of Allergology and Clinical
36 Immunology. *Allergy* 1995;**50**(9):711-717.
37 165. Lavaud F, Bonniaud P, Dalphin JC, Leroyer C, Muller D, Tannous R, et al. Usefulness of
38 omalizumab in ten patients with severe occupational asthma. *Allergy* 2013;**68**(6):813-815.
39 166. Baur X, Chen Z, Liebers V. Exposure-response relationships of occupational inhalative
40 allergens. *Clin Exp Allergy* 1998;**28**(5):537-544.
41 167. Jones MG. Exposure-response in occupational allergy. *Curr Opin Allergy Clin Immunol*
42 2008;**8**(2):110-114.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

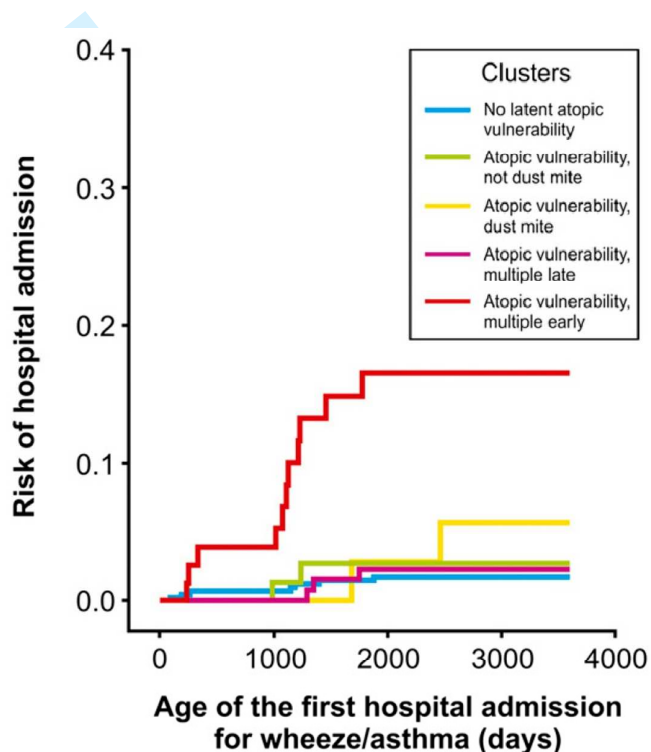


Table 1: Main pollutants and examples of their effects on respiratory function.

Pollutant	Outcome
Nitrogen dioxide (NO₂)	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)
PM₁₀	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 (O₃)	Increased risk of uncontrolled Asthma(144)

Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

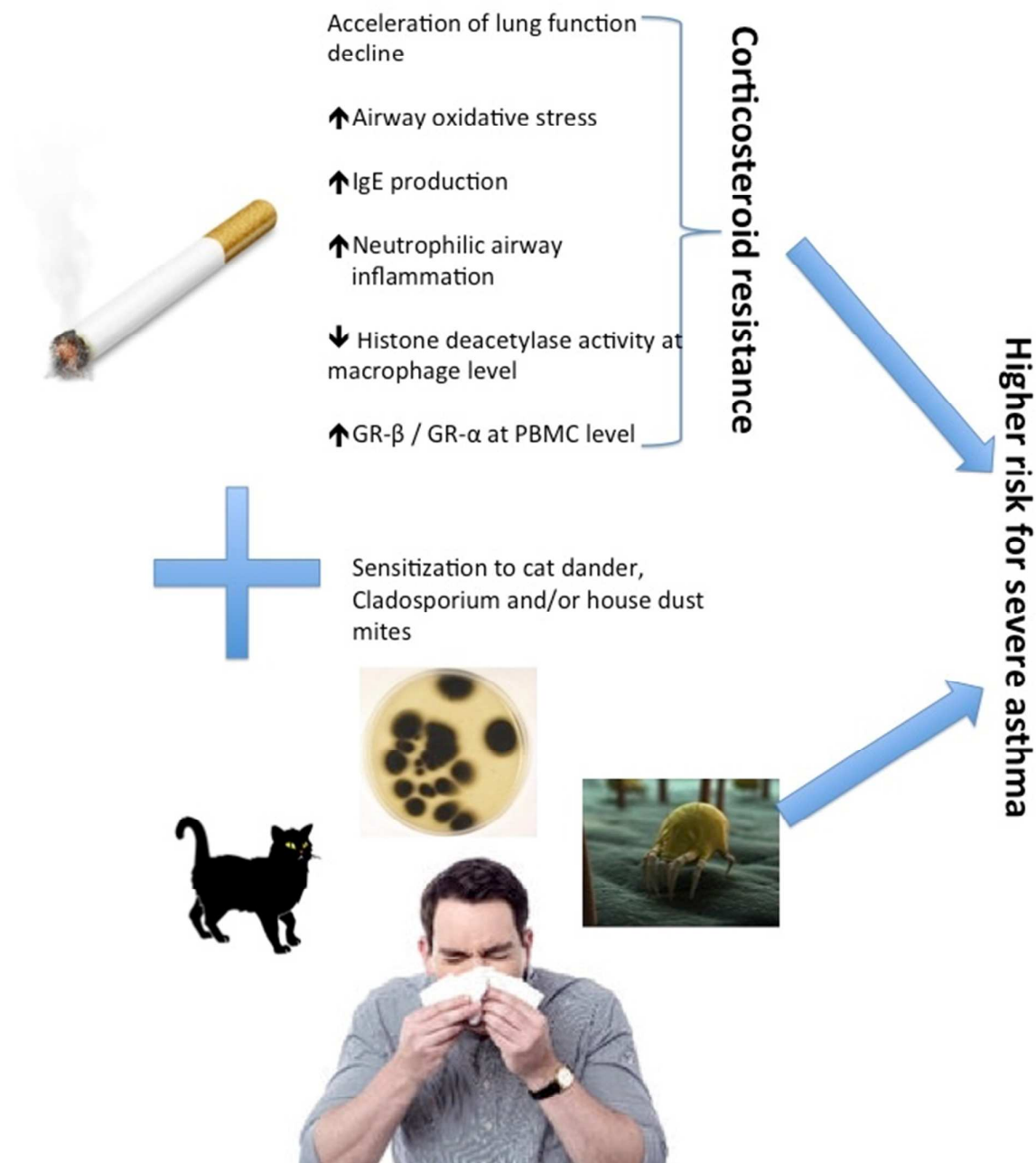
Table 2: Pollutants and examples of their effects on allergic inflammation

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)

For Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.

