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**What lessons can be learned about asthma phenotypes in children
from cohort studies?**

**J Just^{1,2}, F Amat^{1,2}, P Saint Pierre³, R Gouvis-Echraghi¹, N Guillemot^{1,2}, T
Guiddir^{1,2}, R Couderc⁴, I Annesi Maesano²**

¹Allergology department, Centre de l'Asthme et des Allergies. Hôpital d'Enfants
Armand-Trousseau (APHP) –

²Sorbonne Universités, UPMC Univ Paris 06, UMR_S 1136, Institut Pierre Louis
d'Epidémiologie et de Santé Publique, Equipe EPAR, F-75013, PARIS 75571 Cedex

³Laboratoire de statistiques théoriques et appliquées, Université Pierre et Marie Curie -
Paris 06, 4 place Jussieu, 75252 PARIS cedex 05, France.

⁴Service de biochimie et biologie moléculaire, Hôpital d'Enfants Armand-Trousseau -
26, Avenue du Dr. Arnold Netter, 75571 PARIS Cedex 12 – UPMC Univ Paris 06.
France.

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Address for correspondence:

Pr. Jocelyne JUST

Centre de l'Asthme et des Allergies. Hôpital d'Enfants Armand-Trousseau (APHP) -
26, Avenue du Dr. Arnold Netter, 75571 PARIS Cedex 12. France.

Tel. +33 1 44 73 63 17

24 Fax: +33 1 44 73 66 35

25 E-mail: jocelyne.just@trs.aphp.fr

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28 **Abstract**

29 “Phenotyping” asthma by multivariate analyses and more recently by unsupervised
30 analysis has been performed in children cohorts. We describe the key findings that
31 have emerged from these cohorts. In very young children 3 wheeze phenotypes seem
32 to exist: the mild episodic viral wheeze phenotype, the severe atopic wheeze and more
33 inconstantly encountered the severe nonatopic wheeze. Early onset of allergy in
34 asthma (more frequently encountered in boys) is associated with poor prognosis unlike
35 the severe non-atopic wheeze phenotype with a female predominance. The prognosis
36 of the severe nonatopic wheeze depends on time of onset (early or late) of allergic
37 expression. At school age, the risk of severe asthmatic exacerbations is associated with
38 eosinophil predominant inflammation frequently related to allergic asthma, whereas
39 neutrophil inflammation is associated with moderate-to-severe asthma with poorer
40 lung function. Nevertheless, Allergic asthma is also a heterogeneous disease with
41 severe allergic phenotype strongly associated with atopic dermatitis and very
42 high eosinophil-driven inflammatory markers. Further studies are required to find non-
43 invasive biological markers to better define wheezing phenotypes in very young
44 children associated with an elevated risk of developing severe asthma with a view to
45 personalizing treatment.

46

47 **Introduction**

48 Asthma is the most common disease during childhood. It affects 200–300 million
49 people worldwide, and its prevalence has increased over the past few decades¹. It is a
50 heterogeneous disease in terms of triggers, severity, inflammation and age of onset².
51 To date, asthma classification has mainly been related to asthma severity: it is
52 generally accepted that phenotypes related to asthma severity follow a track during
53 childhood and even during the whole of life³. This observation supports the notion that
54 phenotypes defined by asthma severity remain stable.

55 Asthma severity is also an important parameter because the major healthcare burden of
56 this disease is related to severe asthma. Nevertheless, severe asthma includes multiple
57 features and it is important to distinguish between the different severe phenotypes to
58 determine targeted and effective treatment⁴.

59 Anti-inflammatory treatment is the pedestal of asthma treatment but there is ongoing
60 debate about whether children, especially preschool children, should be treated with
61 continuous or intermittent inhaled corticosteroid treatment. On the other hand,
62 uncontrolled asthma despite high levels of controlled medication should be treated
63 with an additional treatment such as an anti-IgE⁵ or various anti-cytokine treatments.

64 There is currently growing evidence that personalized medicine based on phenotypes
65 and endophenotypes (characterized by physiopathological pathways) is important in
66 the management of all chronic diseases⁶. For all these reasons, and especially to
67 contribute to decreasing the burden of the disease, phenotypes deserve to be described
68 in asthma.

69 Against this background of “phenotyping” asthma, multivariate analyses and, more
70 recently, unsupervised analyses have been performed in birth cohorts⁷⁸⁹¹⁰¹¹¹²¹³¹⁴¹⁵. In
71 the same manner, phenotyping analyses have also been performed in cohorts of

72 children comprising moderate to severe asthma phenotypes¹⁶¹⁷¹⁸¹⁹²⁰²¹²²²³. In relation to
73 these data, it appears that age of onset, hyperresponsiveness, asthma control under
74 treatment and multiple sensitizations are important parameters to define phenotypes of
75 asthma during childhood.

76 In this article we highlight the main results, which have emerged from these cohorts in
77 preschool then in school age wheezers

78

79 ***In very young children, severe asthma phenotypes exist and are related to gender***
80 ***(Table n°1)*** The Pollution and Asthma Risk an Infant Study (PARIS), a birth cohort
81 composed of 3500 full-term newborns recruited in five public Parisian maternity
82 hospitals in Paris and its close suburbs, described wheezer phenotypes²⁴: severe atopic
83 wheezers and non-atopic severe wheezers as well as a mild nonatopic wheezer
84 phenotype. Trousseau Asthma Program (TAP) cohorts are independent cohorts of
85 several hundred consecutive children explored in a cross-sectional and prospective
86 manner by unsupervised analysis. In a TAP cohort study of 551 early wheezers with
87 an average age of 18 months described similar asthma phenotypes by cluster
88 analysis: “*Mild Episodic Viral Wheeze*” (*EVW*) and “*Atopic Multiple-Trigger*” (*AMT*)
89 and “*Non-atopic uncontrolled-wheeze*” (*NAUW*)¹⁴ (Fig 1). These phenotypes were
90 validated by another independent TAP cohort¹⁵ which also applied a new approach
91 including exhaled nitric oxide (FeNO) determinants together with severity and atopy
92 by cluster analysis. Furthermore

93 *EVW* consisted of children with wheeze related to cold only, mild disease. These
94 findings are consistent with The Tucson Children's Respiratory Study. This birth
95 cohort is a long-term, longitudinal, prospective study of the risk factors for acute lower
96 respiratory tract illnesses in early childhood and for chronic obstructive airways
97 disease in later life. It includes 1,246 newborns enrolled between May 1980 and
98 January 1984²⁵. For the first time these authors described early transient wheeze with
99 rare episodes of wheeze apart from colds in infancy compared to persistent
100 wheezers²⁶. In the same manner, Spycher *et al* ²⁷. in two independent cohorts (nested
101 samples of total population of 1,650 white children recruited in 1990 at the age of 0–5
102 years in Leicestershire, UK) showed a phenotype with mild, virus-triggered symptoms
103 wheeze. The *AMT* phenotype, includes more children with allergic diseases such as

104 eczema, allergic rhinitis (AR) food allergy and more specific IgE positivity and a
105 higher proportion of elevated values for total IgE. This phenotype is associated with
106 severe wheezing disease, defined by a greater percentage of multiple trigger wheezes
107 (*MTW*). The *AMT* phenotype is comparable to phenotypes identified by using other
108 approaches. For example, in the Avon Longitudinal Study of Parents and Children
109 (ALSPAC)⁶, a birth cohort in which the children of 14 541 pregnancies were recruited
110 antenatally between 1990–92, two phenotypes characterized by early and
111 intermediate-onset of wheeze persisting during childhood were characterized by
112 allergy features (with skin prick test positivity) and severity of the disease (with
113 bronchial hyperresponsiveness and reduced lung function)²⁸. This description was also
114 confirmed across two independent cohorts of children previously described by
115 Spycher *et al*²¹. The TAP cohort found that more boys fell into the *AMT* group. This
116 might be related to the well-known association between male gender and allergic
117 diseases²⁹³⁰. In a British birth cohort, The National Asthma Campaign Manchester
118 Asthma and Allergy Study (MAAS cohort) parents were screened at ‘booking’
119 antenatal visits between October 1995 and July 1997. A total of 3,618 mothers and
120 2,172 fathers underwent skin-prick testing and newborns were allocated to allergic risk
121 groups according to parental atopic status. The main result of this cohort was the
122 impact of early onset allergy on asthma prognosis. Effectively, Belgrave *et al.*³¹
123 showed that children with frequent asthma exacerbations and multiple early atopy are
124 at risk of a progressive loss of lung function from 3 to 11 years and this effect is also
125 more marked in boys.

126 *NAUW* was characterized mainly by moderate to severe disease and uncontrolled
127 wheeze despite high doses of ICS, a higher proportion of parental asthma and higher
128 ferritine values. This phenotype has been less described by other teams. However,

129 Spycher *et al.*²¹, showed an intermediate phenotype between the atopic persistent and
 130 the viral wheeze phenotype in terms of prognosis in preadolescence.

131 Moreover, in the TAP cohort the NAUW phenotype was only encountered in girls.

132 Isozaki *et al.*³² demonstrated a gender difference in phenotypes with atopic wheezing
 133 more frequently encountered in boys and non-allergic wheezing resistant to treatment
 134 more prevalent in girls. This phenotype has been known for a long time in adults and
 135 is described as 'Intrinsic asthma' with a female predominance though the aetiology is
 136 still not understood³³.

137 In the TAP cohort, with only two parameters related to atopy and asthma severity (i.e.
 138 total IgE and asthma severity), 90% of boys and 83% of girls were assigned to the
 139 appropriate cluster (Fig 2). This result underlines the impact of atopy but also of the
 140 “intrinsic” aetiology in disease severity, with a higher percentage of boys in severe
 141 atopic phenotypes and girls in severe non-atopic phenotypes.

142

143 ***Stability of asthma phenotypes in very young children during preschool age depends***
 144 ***on allergic expression (Table n°1)***

145 Several rules have been developed to predict whether preschool children will have
 146 asthma at school age such as the stringent and loose forms of the Asthma Predictive
 147 Index (API) in the Tucson Cohort³⁴. The Prevention and Incidence of Asthma and
 148 Mite Allergy (PIAMA) is a birth cohort study initiated in 1996 which enrolled
 149 children born to allergic mothers in a double-blind placebo-controlled trial to evaluate
 150 the use of mite-impermeable mattress and pillow covers³⁵. In this cohort, the API
 151 scores were externally validated with predictions comparable to the original Tucson
 152 study. However, these prediction rules are difficult to apply in clinical practice
 153 because of an overall low positive predictive value³⁶.

154 Another approach of the TAP cohort was to investigate if prospectively defined
155 phenotypes could have a different course during childhood and thus improve
156 prediction of the course of asthma (Fig 3)¹⁷. This study revealed a good prognosis for
157 children classified as having the *EVW* phenotype: at 5 years old 69% were still in the
158 *EVW* group or were asymptomatic. This finding is in accordance with many other
159 studies which demonstrate that recurrent viral induced wheeze has a good prognosis
160 with a low risk of asthma³⁷ or mild asthma. Spycher *et al*²¹ in particular noted that
161 despite mild symptoms in early life, children with transient wheeze were more likely
162 than controls to continue to have current wheeze and use bronchodilators in
163 preadolescence. This finding underlines the fact that remission
164 can occur in this phenotype, a fact which is important for the clinician in the
165 management of early wheezing.

166 The prognosis of the initial severe phenotypes (*AMT* and *NAUW*) is worse than for
167 *EVW*. This finding is in accordance with the notion of tracking asthma severity during
168 childhood³⁸. None of the children in the *AMT* phenotype became asymptomatic at 5
169 years and more than half of *NAUW* children were still severe at 5 years (Fig 3). This
170 poor prognosis of allergic asthma with early onset has already been described in
171 numerous prospective birth cohorts²⁰³⁹⁴⁰. The MAAS⁴¹⁴² demonstrated that atopy, and
172 especially early onset of multiple sensitizations, increases the risk of persistence of
173 asthma with severe exacerbations during childhood. In contrast, only Spycher *et al*.²¹
174 found a third wheeze phenotype in both their cohorts – identified as intermediate
175 between the atopic persistent and the viral wheeze phenotype – associated with a poor
176 prognosis in preadolescence similarly to the TAP findings for the *NAUW* group.
177 Possibly, this phenotype is associated with persistent wheezing during preschool and
178 school age in relation with late-onset atopy.

179 More globally, the lesson to retain about prognosis of the early wheezer is that
180 changes observed in each initial phenotype in preschool aged children depend not only
181 on the expression of allergy but also on the time of onset (early or late). Finally,
182 although none of the phenotypes identified in the TAP corresponded to the GINA
183 definition of asthma severity, it is worth noting that their most recent guidelines⁴³ have
184 approved a “phenotyping approach” in asthma management by referencing the TAP
185 article¹⁷.

186

187 ***Asthma severity at school age depends on inflammatory cellular type (Table n°1 et***
188 ***n°2)***

189 Asthma in children is highly heterogeneous and is related not only to lung function
190 and atopy but also to systemic inflammation. Another TAP cohort recruited 309
191 children of school age and identified three independent clusters of asthma in a cluster
192 analysis applied to 15 variables⁴⁴.

193 The “*Asthma with multiple allergic sensitizations*” cluster was more atopic, with more
194 frequent severe exacerbations but relatively normal lung function. This type of severe
195 asthma is associated with an inflammation predominantly of “allergic type” (with
196 eosinophil and basophil cells) in combination with multiple allergic sensitizations and
197 elevated total IgE. Many studies, mainly in children, have confirmed that asthma at
198 risk of severe exacerbations or difficult to control is associated with allergic asthma⁴⁵.

199 The “*Severe asthma with bronchial obstruction*” cluster was characterized by the
200 lowest lung function and more blood neutrophils, IgG and IgA but less atopy. The
201 children were also older and had a higher BMI. Other studies have shown an
202 association between the severity of asthma and neutrophilic inflammation detected by
203 induced sputum in particular in adult^{46,47} The TAP results underline that neutrophilic

204 asthma exists also in children with unknown aetiology³⁸. This finding supports those
 205 of the Epidemiological study on the Genetics and Environment of Asthma, bronchial
 206 hyperresponsiveness and atopy (EGEA). The EGEA family sample consisted of 388
 207 French nuclear families that included 253 families ascertained through offspring with
 208 asthma (one offspring proband in 90% of families and two in the remainder) and 135
 209 families ascertained through a parent with asthma. EGEA asthma phenotypes showed
 210 that the phenotype of “active treated allergic childhood-onset asthma” is associated
 211 with blood eosinophilia, while "active treated adult-onset asthma" is associated to
 212 blood neutrophils⁴⁸. Another group linked inflammation to asthma severity. The
 213 Severe Asthma Research Program (SARP) funded by the The National Heart, Lung,
 214 and Blood Institute (NHLBI) recruited subjects with asthma of all ages who met the
 215 ATS workshop definition of severe asthma. An additional group of subjects with
 216 asthma that did not meet the criteria for severe asthma (not severe) was also studied. In
 217 the same manner as in previous studies, a multivariate approach identified a severity
 218 spectrum related to sputum cellular inflammation from mild-to moderate allergic
 219 asthma with minimal or eosinophil predominant inflammation to moderate-to-severe
 220 asthma with neutrophil-predominant or mixed granulocytic inflammation⁴⁹.

221

222 ***Allergic asthma is a heterogeneous disease (Table n°2)***

223 Allergy expression defines the prognosis of asthma during childhood but the problem
 224 is how should atopy be defined? Atopy depends on allergic sensitization (single or
 225 multiple but also the type of allergen and the date of onset of sensitization) and the
 226 association with allergic comorbidities (AR, eczema, food allergy).
 227 In a recent article¹⁶, a TAP cohort study performed in 125 children of school age with
 228 allergic asthma described four phenotypes.

229 The “*House dust mite (HDM) Sensitization and Mild Asthma*” phenotype, 98% of
230 whom were monosensitized and had mild asthma (74% of cases). In a previous study,
231 we had described a similar phenotype of asthma with few allergic sensitizations and
232 mild asthma³⁸. Moore *et al.*⁵⁰ also defined a large cluster (82% of the study
233 population) of adults with early-onset mild atopic asthma. The Childhood Asthma
234 Prevention Study (CAPS)⁷ included pregnant women whose unborn children were at
235 high risk of developing asthma because of a parent or a sibling with a current
236 diagnosis of asthma or frequent wheeze recruited from the antenatal clinics of six
237 hospitals in Sydney, Australia. Six hundred and sixteen women were enrolled in the
238 main trial between September 1997 and December 1999⁵¹ Both the CAPS and MAAS
239 cohorts³⁰ as well as the TAP cohort defined an HDM monosensitized population which
240 confers a better prognosis of asthma than children with multiple sensitizations.

241 In the TAP cohort, the “*Pollen Sensitization with Severe Exacerbations*” phenotype
242 comprised 92% of children with severe exacerbations and pollen sensitization. Erbas
243 *et al.*⁵² showed a linear increase in asthma emergency department presentations
244 correlated with an increased concentration of ambient grain of grass pollen ($p < 0.001$).
245 More specifically, pollen-allergic children seem to be admitted due to food-induced
246 anaphylaxis more often during the pollen season ($p = 0.015$)^{53,54}.

247 In the TAP cohort “*Multiple Allergies and Severe Asthma*” phenotype includes
248 children with a multiple allergic phenotype (100% of the children had eczema and
249 multiple sensitizations) and higher values of IgE (1123 kU/L) and FeNO (67 ppb).
250 Moreover, the severity of asthma is attested by the highest proportion of moderate to
251 severe asthma (95%) and a significant decrease in FEF₂₅₋₇₅. The clinical relevance of
252 FeNO in asthma severity was also identified in the PIAMA cohort⁵⁵ and by Sonnappa
253 S *et al*⁵⁶: FeNO measured at 8 years was associated with persistent, intermediate, and

late-onset wheeze only in children with allergic sensitization at 8 years. The German Multicentre Allergy Study (MAS) cohort consists of 1,314 children born in 1990 and followed at the ages of 1, 3, 6, 12, 18, and 24 months and then at yearly intervals thereafter until age 13 years⁵⁷. This cohort found that eczema associated with filaggrin loss-of-function mutations conveyed a greater risk of severe asthma phenotype with a significant decrease in pulmonary function at puberty. Moreover, in accordance with the TAP results, children (especially boys) with persistent wheeze, frequent asthma exacerbations, and multiple early atopy in the MAAS cohort²⁵ had diminished lung function throughout childhood and were at higher risk of a progressive loss of lung function from age 3 to 11 years. Furthermore, Garden *et al.* (the CAPS cohort)⁴⁷ showed that asthma risk is related to the type of allergen sensitization, with a strong association between the mixed food and inhalant sensitization class and poor asthma control at age 8 years.

Finally the last phenotype discovered in the TAP cohort was the “*Multiple Allergic Sensitizations and Mild Asthma*” phenotype, in which 97% of the children had multiple sensitizations and 100% mild asthma. Several reasons could explain this novel result linking multiple sensitizations and mild asthma severity. Firstly, this phenotype is associated with a low percentage of eczema and it is known that eczema plus allergic asthma confers an intrinsically severe asthma. Secondly, multiple allergic sensitizations could be related to biological sensitizations rather than to real allergy explaining why total IgE and FeNO values were lower in this group.

To summarize, severe allergic asthma in children could consist of two phenotypes depending on the type of sensitization and the association with other allergic comorbidities: severe phenotype with acute exacerbations and related to pollens and/or

278 food sensitizations and a severe phenotype with very high inflammatory markers
279 constantly associated with atopic dermatitis.

280

281 ***Asthma phenotypes and personalized medicine***

282 The Childhood Asthma Management Program (CAMP) is a multicenter, randomized,
283 double-masked clinical trial designed to explore the clinical relevance of the clustering
284 approach in assessing the long-term effects of three inhaled treatments for mild to
285 moderate childhood asthma: budesonide (a glucocorticoid used daily) and albuterol (a
286 short-acting β -agonist bronchodilator used as needed); nedocromil (a nonsteroid anti-
287 inflammatory agent used daily) and albuterol; and placebo and albuterol¹¹. Overall, in
288 this cohort Howrylak *et al.*⁵⁸ found five reproducible patient clusters that could be
289 differentiated on the basis of atopic burden, degree of airway obstruction, and history
290 of exacerbation. Moreover, the clustering approach predicted long-term asthma control
291 as well as longitudinal differences in pulmonary function and response to long term
292 treatment.

293

294 ***Conclusions***

295 As already suggested⁵⁹, our analysis confirms that existing cohorts studies have
296 provided data useful for the ascertainment of early life asthma phenotypes. Further
297 studies should be designed to find noninvasive biological markers to better define
298 wheezing phenotypes in very young children associated with an elevated risk of
299 developing asthma to define personalized medicine based on targeted treatment.

300

301 **FIG 1: Representation of the three clusters according to the percentage of**
 302 **Multiple Trigger Wheeze, Severity and Atopy for the entire population (n=551)**

303 MTW (Multiple Trigger Wheeze) is defined as wheezing during colds and with other
 304 triggers such as house dust, grass, pets, tobacco smoke, exercise or cold air; Severity is
 305 defined as percentage of moderate to severe asthma according to GINA classification;
 306 Circled area represents the percentage of atopy defined as percentage of positive
 307 Phadiatop Infant® ≥ 0.35 U/ml. (Ref 14)

308

309 **FIG 2: Classification tree for entire population using two variables i.e. asthma**
 310 **severity according to GINA classification and Phadiatop Infant®**

311 Subjects are assigned to the three clusters that range from milder recurrent wheeze
 312 (Cluster 1) to more severe disease (Clusters 2 and 3) with 76% of the subject assigned
 313 to the appropriate cluster. Tree performances are given. (Ref 14)

314

315 **FIG 3: Change of wheeze phenotypes from under 3 years until 5 years of age**

316 Y axis represents change (expressed in percentage) of separate 3 clusters towards the 4
 317 clusters at 5 years of age. At 3 years of age : *Mild EVW*: Episodic Viral Wheeze
 318 (wheezing only during colds and asymptomatic between episodes), *Atopic MTW*:
 319 Multiple Trigger Wheeze (wheezing during colds and with other triggers such as
 320 house dust, grass, pets, tobacco smoke, exercise or cold air), *NAUW*: Non-atopic
 321 Uncontrolled Wheeze. The Fisher exact test rejects the independence assumption
 322 ($p < 0.001$). (Ref 17)

323

324

325 **Table n° 1: Asthma phenotypes in very young children**

326

	Gender	High risk of	Prognosis in term	Predominant
	Predominance	exacerbation	of asthma	inflammation
			persistence during	/Response to
			childhood	corticosteroid
<i>Mild Episodic Viral</i>	Male ¹⁸	No ^{14,15,18}	Good ^{17,20,21,31}	None ¹⁴
<i>Wheeze</i>				/High ¹⁴
<i>Atopic Multiple-</i>	Male ^{14,21,25}	Yes ^{25,14,18}	Poor ^{14,21,17,,22,19,25,28,29}	Eosinophilic ^{14,18,15,19}
<i>Trigger</i>			^{33,34,35,36}	/Intermediate ¹⁴
<i>Nonatopic wheeze</i>	Female ^{14,26}	No ^{14,18,15}	Intermediate	Neutrophilic ?
<i>phenotype</i>			(depending of late	/Low ¹⁴
			onset of allergic	
			expression) ^{17,21}	

327

328

329 **Table n° 2: Asthma phenotypes in children at school age**

330

	Gender	Age of asthma onset	High Risk of exacerbation	Prognosis in tem of lung function decline	Predominant inflammation /Response to corticosteroid
<i>House dust mite Sensitization and Mild Asthma</i>	M	Early ^{16,46,30}	–	–	Eosinophil /High ¹⁶
<i>Pollen Sensitization with Severe Exacerbations”</i>	M	Late ¹⁶	Yes ^{16,39}	–	Eosinophil ^{16,,46,47,48} /High ¹⁶
<i>Multiple Allergic Sensitizations and eczema associated with Severe Asthma</i>	M	Early ^{16,25,38,51}	–	Poor ^{16,51,45}	High Eosinophil ^{16,38,49} /Low ¹⁶
<i>Multiple Allergic Sensitizations and Mild Asthma</i>	M	Early ¹⁶	–	–	Eosinophil ¹⁶ /High ¹⁶
<i>Severe asthma with bronchial obstruction</i>	F	Late ^{38,42,43,44}	–	Poor ^{43,44}	Neutrophil ³⁸ /Very low ^{38,42,44}

331

332

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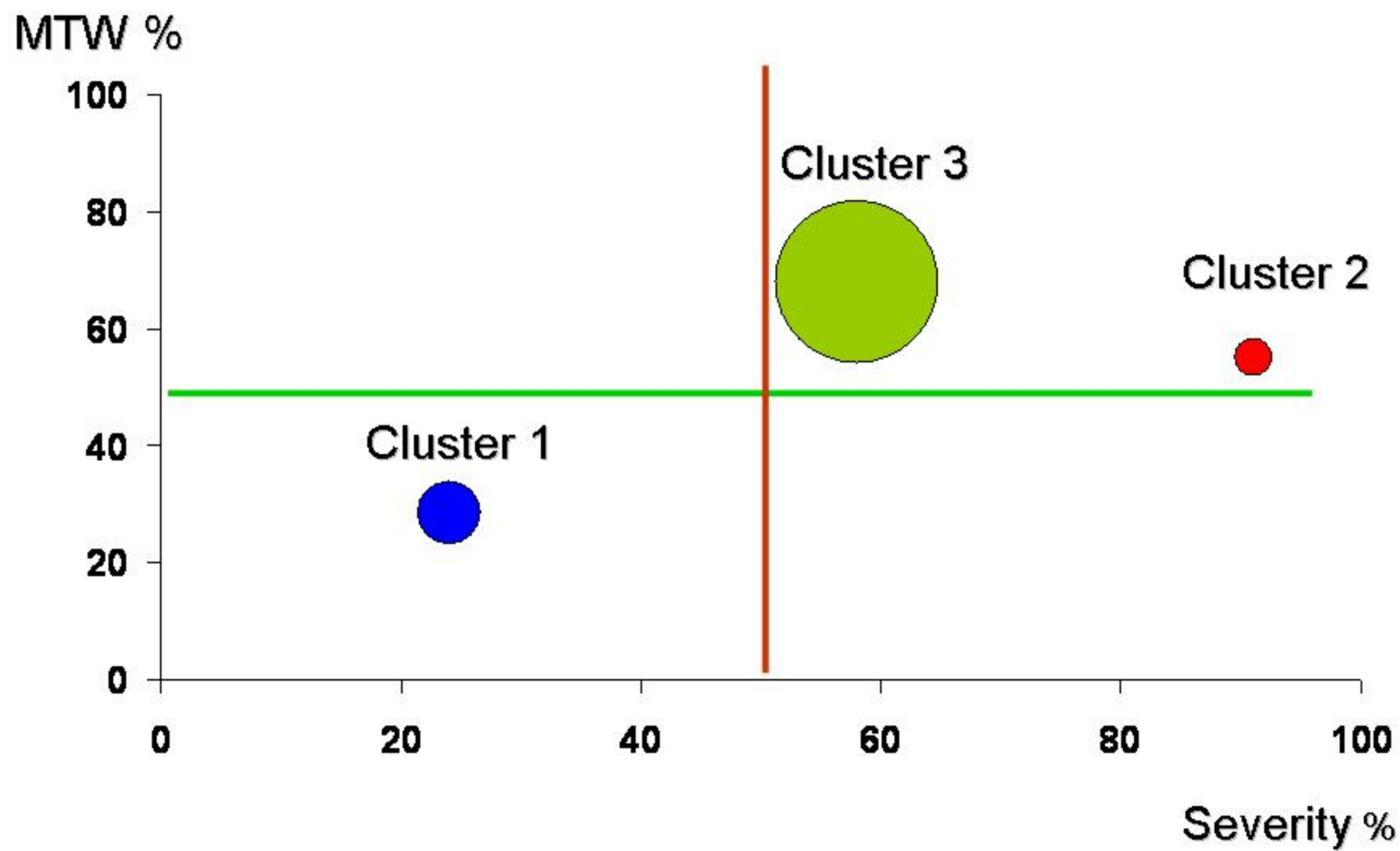
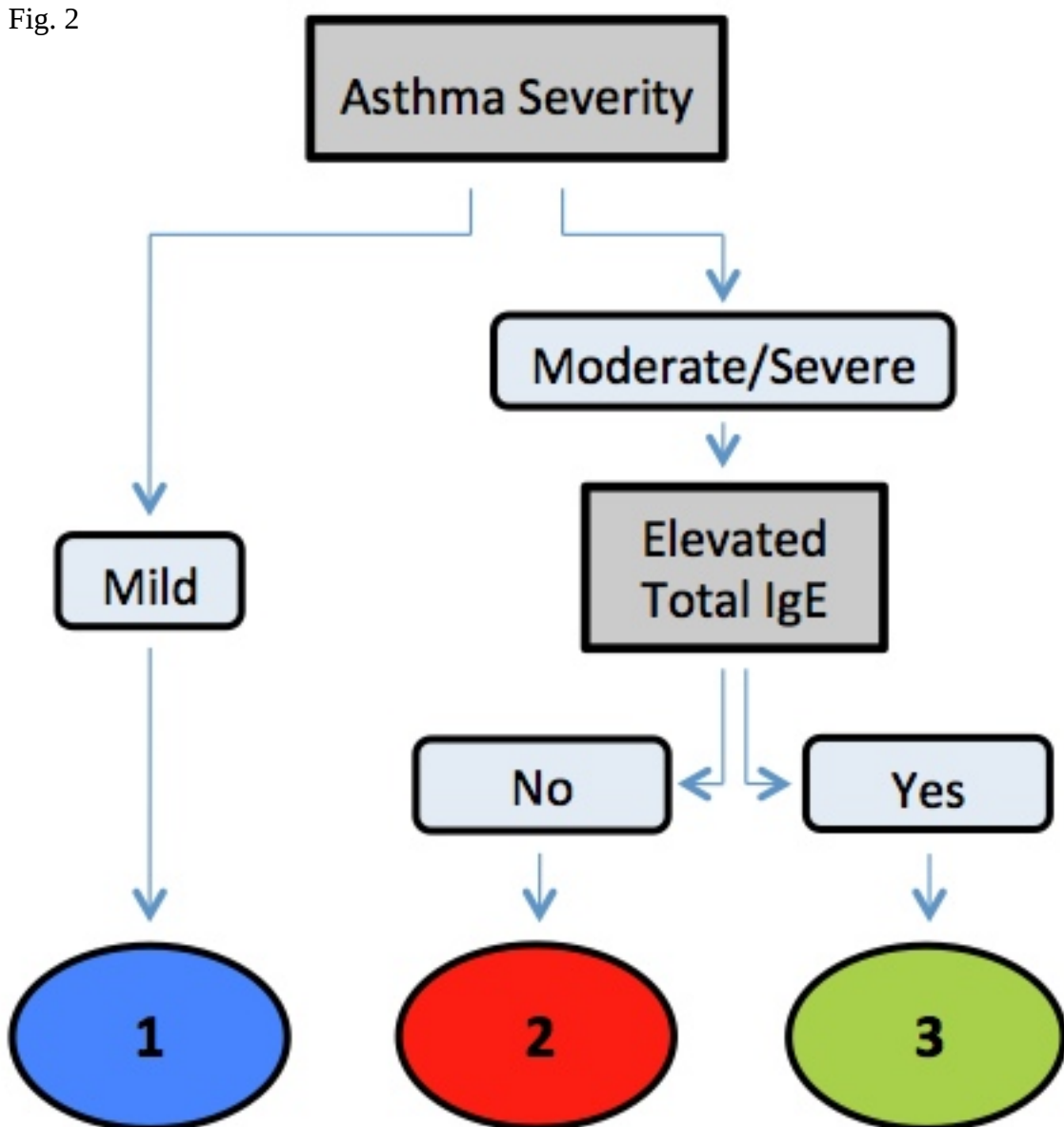


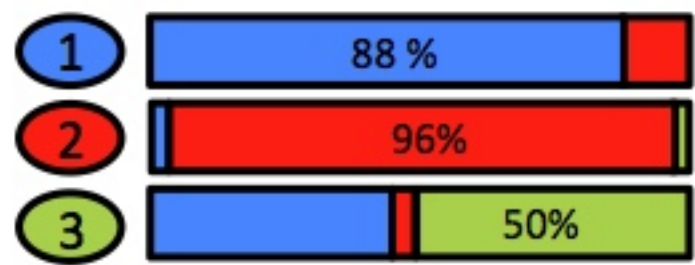
Fig. 1

Fig. 2



Population		Prediction		
		1	2	3
Cluster label	1	70	10	0
	2	1	53	1
	3	14	2	16

Percentage		Prediction		
		1	2	3
Cluster label	1	0.88	0.12	0
	2	0.02	0.96	0.02
	3	0.44	0.06	0.5



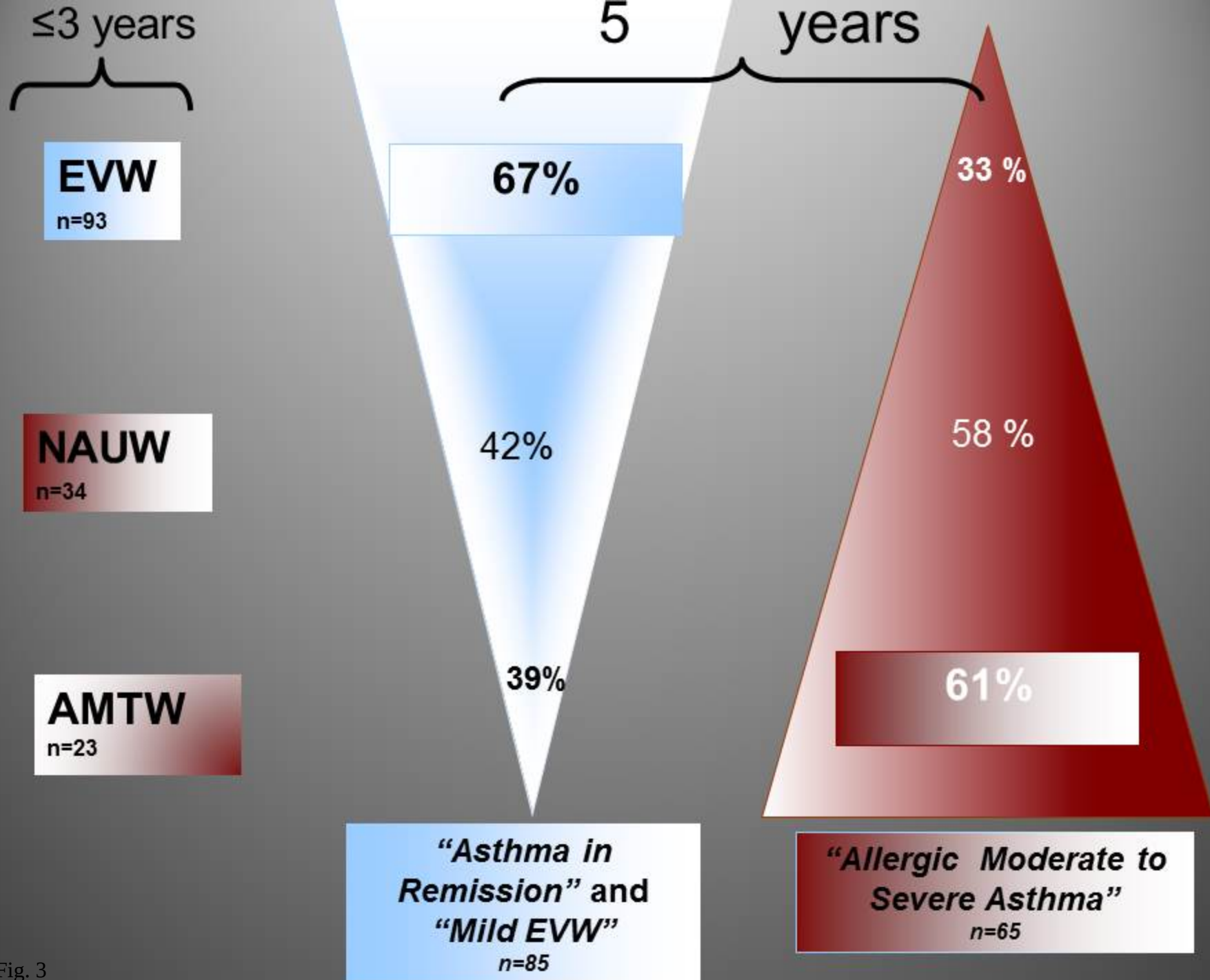


Fig. 3