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Clinical phenotypes in asthma during childhood

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Introduction

In the 21st century, the heterogeneity of asthma and allergic diseases in childhood will be characterized by multiple phenotypes related to different pathophysiologic pathways or endotypes. From a medical point of view, improved understanding of disease etiology and mechanisms will lead to optimal management including personalized targeted treatment.

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The use of unsupervised, data-driven statistical methods, such as cluster analysis^{1,2,3} and latent class analysis,^{2, 3, 4} has emerged as a complementary approach to that based on the application of *a priori* definitions⁵, to help define objective, novel or previously unidentified phenotypes.

The recurrent question about asthma phenotypes concerns their stability throughout life. Phenotypes are the result not only of the patient's genetic background but also of environmental factors – such as pathogens (virus or bacteria), allergens, smoking, pollution and diet – that can vary over time thus possibly influencing asthma pathogenesis and consequently the phenotypes. Previously identified phenotypes therefore vary due to differences in the populations studied and the characteristics included in the models. However, data-driven approaches have yielded phenotypic classifications that are clinically meaningful and interpretable⁶ and that are relevant to prognosis^{7,8}.

Cluster analysis is an unsupervised statistical method to define groups of patients sharing several common parameters which are important to define the disease. The variability of the parameters used to construct the clusters or phenotypes partly explains the numerous phenotypes described in asthma. In this paper we will discuss the strengths of the parameters included in phenotype analysis in both pediatric and adult asthma patients: age of onset, gender, body mass index (BMI), atopy (according to definition, allergic disease, allergenic sensitizations (i.e., single or multiple) and type of allergen), bronchial obstruction and bronchial hyper-responsiveness, cortico-responsiveness, and biomarkers such as blood or sputum eosinophil (or other markers of eosinophil-driven inflammation) and neutrophil counts. The review takes into account the various findings from the Trousseau Asthma Program (TAP) – a Paris-based program exploring cohorts of children in a cross-sectional, prospective manner – which we discuss along with the results from similar cohort studies in both adults and children and recent publications.

Age of onset and asthma phenotypes

The hypothesis that the course of asthma differs according to whether onset occurs in early childhood (before 3 years) or later (between 3 and 6 years) was first described in the Tucson Children's Respiratory Study classification. Children with persistent and later onset wheezing are most likely to

experience asthma-like symptoms that persist into later childhood, adolescence, and adult life than those with early, transient wheezing. E. Bouzigon *et al.*⁹ confirmed a biological difference between age of onset of asthma and 17q21 variants which were more likely associated with early-onset asthma and exposure in early life to environmental tobacco smoke. This study showed that 17q21 risk genotypes increased the positive association between early respiratory infection and asthma, and that this was restricted to early-onset asthma and asthma that remits in young adulthood¹⁰. However, the function of the studied 17q21 genes is largely unknown, especially with respect to asthma. The 17q21 locus constitutes a relatively narrow region of interest on the chromosome and is composed of four genes including *ORMDL3* and *GSDML*. Hirota *et al.*¹¹ suggested a relationship between *ORMDL3*, viral infection, and asthma following a finding that *ORMDL3* expression was strongly induced in an experimental model of viral stimulation.

Classification of adult asthma into early- or late-onset (i.e., during childhood or adulthood, respectively) is widely used in the literature. A recent review¹² included 12 studies comparing early- and late-onset current asthma in adults. The most common age used to delineate the two age-of-onset phenotypes was 12 years. Adults with early-onset current asthma were more likely to be atopic and have more frequent symptoms compared with adults with late-onset disease, who were more likely to be female, smokers and with more marked fixed airflow obstruction. The prevalence of severe asthma was similar in both groups. Although early-onset adult asthma is more associated with atopy and potentially with genetic factors, late-onset adult asthma appears to be more related to environmental risk factors. The latter could therefore be more responsive to preventive strategies.

Single and multiple sensitization and asthma phenotypes

In a pediatric population from the Trousseau Asthma Program (TAP cohort)¹³, we defined the allergic asthma phenotype “*House dust mite (HDM) Sensitization and Mild Asthma*”. Ninety-eight percent of the children in this group were monosensitized and had mild asthma (74% of cases). In a previous study, we described a similar phenotype of asthma characterized by few allergic

sensitizations and mild asthma¹⁴. In the same manner, the Childhood Asthma Prevention Study (CAPS)¹⁵ and the Manchester Asthma and Allergy Study (MAAS)¹⁶ pediatric cohorts defined an HDM monosensitized population which confers a better prognosis of asthma than for children with multiple sensitizations. In the latter cohort¹⁶, children with a particular asthma phenotype (persistent wheeze, frequent asthma exacerbations, and multiple early atopy) had diminished lung function throughout childhood and were at higher risk of a progressive loss of lung function from age 3 to 11 years. In the same way, L Duijts et al.¹⁷ reported that early-onset wheezing phenotypes persisting after 18 months of age showed the strongest associations with asthma, poorer lung function (even worsening from mid-childhood), and higher FeNO levels in adolescence.

The prognosis for children with initial severe atopic phenotypes is worse than for other phenotypes. For example, none of the children with the “*Atopic multiple trigger wheeze*” phenotype in the TAP cohort became asymptomatic at 5 years. This poor prognosis of allergic asthma with early onset has also been described in numerous prospective birth cohorts^{18,19,20}. Furthermore, two population-based birth cohorts (MAAS and Isle of Wight - IoW)^{21,22} confirm that early onset of multiple sensitizations increases the risk of persistence of asthma with severe exacerbations during childhood.

More recently, numerous authors^{23,24,25} have shown that sensitization to multiple allergenic molecules was of greater value in predicting future allergic symptoms during childhood than sensitization to the crude extract. Furthermore, sensitization to both cat and dog allergenic molecules is associated with more prevalent cat- and dog-related symptoms and higher IgE levels to these molecules.

Finally, it appears that early multiple sensitization to multiple allergens, but also to allergenic molecules, conveys a higher risk of severe asthma phenotype especially in terms of asthma exacerbations^{26,27}.

Type of allergen sensitization and allergic asthma phenotypes

Pollen and food allergens and asthma exacerbations

We defined a “*Pollen Sensitization with Severe Exacerbations*” phenotype in the TAP cohort¹³ with 92% of the children in this group being sensitized to pollen and experiencing severe attacks. Erbas *et al.*²⁸ showed a linear increase in asthma emergency department presentations correlated with an increased concentration of ambient grass pollen ($p < 0.001$). Severe acute asthma in children requiring intensive care is also more frequently associated with food allergy²⁹ and food sensitization presents an increased risk of admission related to pollen exposure in girls³⁰. Furthermore, Garden *et al.* in the CAPS¹⁵ 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.

Mold sensitization and severe asthma

In the adult, mold sensitization in allergic asthma is associated with severe exacerbations requiring hospitalization and uncontrolled asthma despite high doses of ICS³¹. Near-fatal asthma and sensitization to the *Alternaria alternata* mold is a heterogeneous clinical entity and several profiles of patients have been described according to various clinical, pathophysiologic and histologic features. A recent cluster analysis applied to adults with near-fatal asthma³² described three clusters including one phenotype of younger patients characterized by insufficient anti-inflammatory treatment and frequent sensitization to *A alternata* and soybean, suggesting the existence of a specific pathogenic mechanism of these specific sensitizations. We have also recently described an association between *A alternata* sensitization and a severe asthma phenotype in children³³.

Staphylococcal enterotoxins and severe asthma phenotypes

Serum staphylococcal enterotoxin (SE)-specific IgE levels have been positively associated with the severity of asthma^{34,35}, particularly in late-onset asthma³⁶, linked to staphylococcal serine protease-like proteins^{37,38}. SE may be driving this phenotype of late-onset severe

asthma associated with chronic rhinosinusitis and nasal polyps, and sputum eosinophilia leading to raised SE-IgE and eosinophilic asthma. SEs are superantigens that can cause polyclonal activation of T cells in nasal and airway tissues, and can also activate other inflammatory cells such as B cells, eosinophils and epithelial cells³⁹. We recently described a particular phenotype in a TAP cohort of children with multiple allergic sensitization and greater sensitization to SE, associated with the steroid-refractory asthma phenotype.

Obesity, hormones and gender and asthma phenotypes

The male predominance of asthma during childhood switches to a female predominance during puberty⁴⁰. Accordingly, in the pediatric TAP cohort, the phenotype “*Atopic multiple trigger wheeze*” – associated with more allergic diseases such as eczema, allergic rhinitis and food allergy, more specific IgE positivity and a higher proportion of elevated values for total IgE – was more frequently encountered in boys. This phenotype is comparable to phenotypes identified by using other approaches. In the Avon Longitudinal Study of Parents and Children (ALSPAC) study, two phenotypes characterized by early- and intermediate-onset wheeze were associated with skin prick test positivity, bronchial hyperresponsiveness and reduced lung function⁴¹. The prevalence of boys in the allergic asthma group might be related to the association between male gender and allergic diseases^{42,43}. In the same manner, Belgrave *et al.*¹⁶ showed that children with frequent asthma exacerbations, and multiple early atopy are at risk of a progressive loss of lung function from 3 to 11 years and this effect is more marked in boys.

Conversely, female gender is more frequently associated with obesity which doubles the risk of developing asthma⁴⁴. Obese adults with asthma have poorer lung function and asthma control and more frequent exacerbations leading to hospitalization for their asthma^{45,46,47}. Furthermore, the female predominance in this asthma phenotype associated with obesity⁴⁸ drives the hypothesis that female sex hormones may play a role. Indeed, the incidence of asthma is higher in pubertal females than in males and this remains so throughout the reproductive years⁴⁹. In children, positive associations have been reported between BMI and an increased risk of new-onset asthma. Varraso *et al.*⁵⁰ demonstrated

an interaction between obesity, early puberty and more severe asthma symptoms and more hospitalizations for exacerbations. Sex hormones have an impact on the immune response, with progesterone antagonizing the Th-1 response and promoting the Th-2 response⁵¹. However, obesity is also associated with increased neutrophils both in the circulation and adipose tissue vasculature^{52,53}. Among the numerous proteins associated with obesity, the 146-amino acid protein, leptin, plays the central role. Leptin is a member of the interleukin-6 family of cytokines (pro-inflammatory cytokines). Secreted by adipocytes, the circulating level of leptin correlates with the amount of body fat and BMI and is a permissive factor for the initiation of pubertal events in both boys and girls⁵⁴. Nevertheless, the role of leptin in the association between BMI and asthma remains poorly understood. A cohort of obese reproductive-aged females⁵⁵ had a c-reactive protein concentration and sputum neutrophil count that were 8.4 and 1.7 times higher, respectively, than non-obese reproductive-aged females. In this study, higher neutrophilic airway inflammation was linked with higher CRP and IL-6, lower testosterone and no oral contraceptive pill use.

Asthma phenotypes and type of inflammatory cell

Eosinophilic asthma phenotype

Diagnosis of an eosinophilic phenotype is based on deep lung tissue exploration (bronchoalveolar lavage (BAL) or biopsy), induced sputum, blood assessment, or eosinophil cell-derived markers (such as FeNO, periostin, ...). In an earlier study of asthmatic children by our group, we showed a link between intra-alveolar eosinophilia and atopy⁵⁶. Moreover, it is known that blood eosinophilia is closely correlated to eosinophil inflammation in deep lung tissue⁵⁷. Most patients with asthma have predominantly eosinophilic inflammation, and respond well to a relatively small dose of inhaled corticosteroid (ICS)⁵⁸. Consequently, Hargreaves⁵⁹ showed that measurements of sputum eosinophil can be used to guide effective corticosteroid treatment in adult patients with asthma. Conversely, Flemming *et al.*⁶⁰ showed that incorporating sputum eosinophil measurements into the management algorithm of children with severe asthma did not significantly reduce overall exacerbations or

improve asthma control. However, some patients continue to exhibit eosinophilic airway inflammation despite high-dose ICS⁶¹ and have more marked impairment of lung function, evidence of airway remodeling, and a higher risk of severe or fatal asthma exacerbations in adult life^{62,63,64}. The mechanism underlying this persistent inflammation is unclear. Nevertheless, it is not totally steroid resistant as high-dose parenteral steroids have been shown to abolish airway eosinophilia in patients who have ongoing inflammation despite high-dose ICS.⁶⁵ In a recent study in children, we described an “*Eosinophilic steroid-refractory asthma phenotype*” defined as asthma uncontrolled despite high-dose ICS in the majority of cases (76%, $p<0.001$), with more multiple triggers (66%, $p<0.001$), more allergic comorbidities, more allergic rhinitis (AR) (82%, $p<0.001$), more active atopic dermatitis (40%, $p<0.001$), with both perennial and seasonal sensitization in 52% ($p<0.001$) and sensitization to special allergens (such as food allergies (31%, $p<0.001$), *A alternata* (29%, $p<0.001$) and SE (53%, $p<0.001$)) and finally a higher blood eosinophil count (mean $506/\text{mm}^3$, $p<0.001$) and higher percentage of eosinophils in BAL (mean 2.6%, $p<0.001$)³³. In the same manner, inflammatory phenotypes using previously established thresholds were found in a longitudinal observational study from two tertiary care centers⁶⁶ including 1 year of observation and at least three sputum samples. Adults with the persistent eosinophilic phenotype had a significantly shorter time to first exacerbation and a greater risk of exacerbation over the 1-year period than those with the non-eosinophilic phenotype based on the univariable and multivariable Cox proportional hazard model.

Neutrophilic asthma phenotype

Neutrophilic airway inflammation is associated with resistance to ICS medication in an adult asthma phenotype⁶⁷. Neutrophilic asthma represents up to 25% of symptomatic adult asthma patients and 59% of patients on high-dose ICS^{68,69}. However, to date, this phenotype has only been described in adult populations, mainly with late-onset asthma, rather than in children. Rackemann FM⁷⁰ first recognized the possible importance of respiratory infections on the etiology of *intrinsic* (non-allergic) asthma over 60 years ago. Other studies have also shown an association between the severity of asthma and neutrophilic inflammation detected by induced sputum particularly in the adult⁷¹.

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In the TAP cohort¹⁴, we described a “*Severe asthma with bronchial obstruction*” phenotype in children which is characterized by the lowest lung function and more blood neutrophils, IgG and IgA but less atopy. The children were also older and had a higher BMI. Nevertheless, the neutrophilic asthma phenotype is an uncommon childhood asthma phenotype and not stable over time⁷². Accordingly, Bossley *et al.*⁷³ showed that the pathology of pediatric severe therapy-resistant asthma was characterized by variable airway eosinophil counts but, unlike in adults, there was no neutrophilia.

Pathogen-host interactions have been demonstrated to lead to bronchial inflammation in asthma⁷⁴. Therefore, the term *intrinsic* asthma can be of clinical relevance and raises the possibility that these respiratory infections may play an important role in this asthma phenotype. Viral respiratory tract infections weaken local defenses against opportunistic bacterial pathogens, enhancing the risk of secondary pathogens breaching the mucosal barrier. The result is an amplification of immune-inflammatory responses associated with tissue damage⁷⁵. More specifically, a predominance of *M. catarrhalis* or members of the *Haemophilus* or *Streptococcus* genera have been found to be associated with a neutrophilic airway phenotype in treatment-resistant persistent asthma. In this particularly severe asthma phenotype, innate immune mechanisms may lead to a shift towards Th-1 or Th-17 mediated neutrophilic inflammation⁷⁶. More recent studies^{77,78} provide clinical support to our data, suggesting that the airway microbiota in non-eosinophilic (neutrophilic) asthma is different from eosinophilic phenotypes, and may be manipulated to improve clinical outcomes.

Gastroesophageal reflux disease (GERD) is a significant non-allergic comorbidity associated with steroid-refractory recurrent wheeze that has been well described in literature. The prevalence of GERD symptoms in patients with asthma is around 60%⁷⁹. In adult populations, a higher incidence of GERD⁸⁰ is associated with frequent hospitalizations, poor respiratory function and late-onset disease in non-allergic asthmatic patients. In the SARP cohort⁸¹, GERD with low pH was also independently associated with high BAL neutrophil counts. A chronic, undetected infection could also play a role in the neutrophilic

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inflammation and low pH⁸². In a recent study, we underline the role of GERD in younger children with severe recurrent wheeze, with neutrophil inflammation in the blood and BAL³³.

Knowledge is scant about the influence of occupational exposures on airway inflammation in patients with refractory asthma⁸³. Smoking is an obvious consideration as it is known to induce a neutrophilic inflammation that can persist despite cessation. In children, the influence of passive smoking on airway inflammation in asthma is less clear. However, several studies suggest that other factors such as environmental pollution or infection are important in driving the neutrophilic airway inflammation observed in late-onset asthma^{84,85,86}.

The prognosis of asthma is also poor in children with early-onset non-allergic severe asthma (as in the TAP cohort non-allergic uncontrolled wheeze phenotype). More than half (59%) of the children with this phenotype were still severe at 5 years. Spycher *et al.*⁸ found a third wheeze phenotype in two cohorts – identified as intermediate between the atopic persistent and the viral wheeze phenotype – associated with a poor prognosis in preadolescence similarly to our findings for the non-allergic uncontrolled wheeze group.

Evidence for the long-term effect of exposure in early life (especially to tobacco smoke) has also been confirmed from longitudinal birth cohorts. These show an early reduction in lung function by school age in children with asthma that subsequently continues into adulthood, without recovery. The relationship between childhood severe asthma and adult chronic obstructive pulmonary disease (COPD) appears even stronger⁸⁷. Tracking longitudinal measurements of growth and decline in lung function in patients with persistent childhood asthma, may reveal links between asthma and subsequent chronic airflow obstruction. Recently, the CAMP study described four characteristic patterns of lung-function growth⁸⁸ in children with asthma according to spirometric measurements performed from childhood into adulthood. In this study, 73 participants (11%) met the Global Initiative for Chronic Obstructive Lung Disease spirometric criteria for lung-function impairment that was consistent with COPD and these participants were more likely to have a reduced pattern of growth (18% vs. 3%, P<0.001). Childhood impairment of lung function and male sex were the most

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significant predictors of abnormal longitudinal patterns of lung-function growth and decline. In the same manner, in a cohort of patients recruited as children between the ages of 10 to 15 years, and followed up through adulthood until 60 to 65 years⁸⁹, childhood asthma and childhood wheezy bronchitis were found to increase the risk of COPD in the seventh decade along with reduced ventilatory function.

Paucicellular asthma phenotype

A paucicellular asthma phenotype has been defined as low sputum eosinophil and neutrophil counts (eosinophils < %3, neutrophils < 60%) in both children and adults⁷³. The low degree of airway inflammation in patients with this phenotype often translates into an absence of a decision to start controller medications such as ICS. The phenotype is poorly defined in preschool children but could correspond to the viral induced wheeze phenotype which we described in a cluster analysis performed in 551 early wheezers (average age of 18 months): the “*Mild Episodic Viral Wheeze*” (EVW) phenotype. EVW was prevalent in children with wheeze related to cold only, mild disease and with a high proportion of normal chest X-ray. Herr *et al.*⁹⁰ also described wheezer phenotypes in very young children in a French birth cohort including a mild episodic viral wheeze which was very similar to our EVW group. Similarly, Spycher *et al.*⁸ showed a wheeze phenotype with mild, virus-triggered symptoms in two independent cohorts. This is also consistent with findings from Tucson where children with early transient wheeze were less likely than persistent wheezers to have frequent episodes of wheeze or wheeze apart from colds in infancy¹⁸. Another study from the TAP cohort, revealed a good prognosis for children classified as having the EVW phenotype: at 5 years 69% were still in the EVW group or were asymptomatic. This finding is in accordance with many other studies which demonstrate that recurrent viral induced wheeze has a good prognosis with a low risk of asthma⁹¹ or mild asthma. In children of school age, besides two severe asthma phenotypes related to eosinophilic or neutrophilic inflammation, we also described an asthma phenotype characterized by a low degree of eosinophilic inflammation, mild disease, good lung function and without severe asthma

exacerbations¹⁴. In the same way, in adult subjects paucigranulocytic asthma may be seen as a low grade inflammatory disease although eosinophilic inflammation is still present^{92,93}.

In conclusion

Asthma is a heterogeneous disease with multiple courses during childhood. Even if the definition of severe asthma is somewhat heterogeneous and not often related to the global ERS/ATS consensus⁹⁴, poor disease prognosis is attached to particular severe phenotypes. These phenotypes can be summarized as early-onset asthma phenotypes (such as multiple allergic asthma but also poor lung function pattern frequently associated with neutrophilic asthma) or very rare phenotypes with late-onset asthma in the pubertal period (such as non-atopic hypereosinophilic asthma with nasal polyposis or neutrophilic asthma in obese girls).

Clinical implications

- Early-onset asthma during childhood is more associated with atopy.^{12,17}
- Early sensitization to multiple allergens, but also molecular allergens, conveys a higher risk of asthma exacerbations.^{21,22,26,27}
- Serum staphylococcal enterotoxin may drive a phenotype of late-onset severe asthma associated with chronic rhinosinusitis and nasal polyps.^{33,34,35,36,37}
- Patients with the persistent eosinophilic phenotype have a greater risk of asthma exacerbations.^{14,61,62,63,65}
- Neutrophilic airway inflammation is uncommon in children with asthma and not stable over time.^{71,72}
- There appears to be a strong relationship between severe childhood asthma and adult chronic obstructive pulmonary disease.^{86,87,88}.

- The mixed granulocytic phenotype is rarely observed in children. The instability of this phenotype may be due to environmental triggers.^{90,91}.
- Paucigranulocytic asthma may be seen as a low grade inflammatory disease.^{14,92, 93}

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