DIFFICULT AND SEVERE ASTHMA IN CHILDREN

EDITED BY: Andrew Bush, Giorgio Piacentini, Francesca Santamaria, Nicola Ullmann and Renato Cutrera

PUBLISHED IN: Frontiers in Pediatrics

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88945-986-5
DOI 10.3389/978-2-88945-986-5

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DIFFICULT AND SEVERE ASTHMA IN CHILDREN

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Globally, severe asthma is defined by the WHO [1] as either (A) untreated severe asthma; (B) difficult-to-treat severe asthma; and (C) treatment-resistant severe asthma. Untreated severe asthma is a political problem: the children do not have access to the basic tools for asthma management, and when this is corrected, asthma outcomes are transformed [2]. The problem in difficult-to-treat severe asthma is not the airway disease, but co-morbidities and behavioral factors. This is the group in which there are most asthma deaths [3], underscoring that severe asthma cannot be solely defined by levels of prescribed therapy. Treatment-resistant severe asthma is rare and challenging, and the problem is the airway pathology. These children require new and innovative therapies.

Many current approaches to paediatric asthma have been challenged by the recent *Lancet* commission [4]. Asthma is a clinical umbrella term, comprising the

symptoms of wheeze, breathlessness, chest tightness and sometimes cough. The next step is to ask the question 'what sort of asthma is this?' In most developed world schoolchildren, the answer is that they have the treatable traits of ß-2 agonist responsive bronchoconstriction and airway eosinophilia, which latter should be responsive to inhaled corticosteroids (ICS). So managing asthma in most children is straightforward. If a correct diagnosis has been made (by no means always the case [5, 6]), then most children will respond to low dose ICS, perhaps with the addition of a long-acting ß-2 agonist [7, 8]. Many children do not respond, however, and then, rather than uncritically increasing the ICS dose as per 'evidence-based' stepwise guidelines, the paediatrician needs to consider why not, in a logical manner. The diagnosis should be reviewed, and then, since the airway pathology should not be a problem, reasons for non-responsiveness should be sought in the domains of extrapulmonary comorbidities (asthma plus, for example obesity, exercise induced laryngeal obstruction (EILO) and social and environmental (difficult asthma, the usual example is poor adherence [9, 10]). When all these factors have been assessed [11-13], many children are found to be treatment responsive. In some, basic management steps cannot be achieved, and these children are placed into categories of refractory difficult asthma, and refractory asthma plus [14]. There remains a group of children with true severe, therapy resistant asthma, about 5-10% of all asthmatics, also pose a considerable problem, and who, with some refractory asthmatics, usually go on to bronchoscopic airway phenotyping and consideration of biological therapies.

This Research Topic addresses all aspects of refractory severe asthma and severe therapy resistant asthma in children. The distinguished authors review the burden of the disease [https://www.frontiersin.org/articles/10.3389/fped.2018.00186/full] and the difficulties surrounding definitions and classification [https://www.frontiersin.org/articles/10.3389/fped.2018.00170/full], important interventions to improve basic management, including the significant topics of getting the diagnosis right [https://www.frontiersin.org/articles/10.3389/fped.2018.00276/full], implementing basic guideline recommendations [https://www.frontiersin.org/articles/10.3389/fped.2018.00234/full], improving adherence [https://www.frontiersin.org/articles/10.3389/fped.2018.00232/full], assessment of comorbidities and phenotyping the disease [[https://www.frontiersin.org/articles/10.3389/fped.2019.00068/full and https://www.frontiersin.org/articles/10.3389/fped.2018.00258/full] using big data and modern mathematical, data driven approaches. The *Lancet* commission [4] stressed the need for a measurement culture, and there are reviews on measuring airway obstruction and inflammation [https://www.frontiersin.org/articles/10.3389/fped.2018.00189/full and https://www.frontiersin.org/articles/10.3389/fped.2018.00196/full]. Pathophysiological mechanisms of severe disease are discussed, including the important role of allergy [https://www.frontiersin.org/articles/10.3389/fped.2019.00028/full] and macrophage biology [https://www.frontiersin.org/articles/10.3389/fped.2018.00206/full]. Innovative approaches to treatments are reviewed, including all aspects of pharmacotherapy colleagues [https://www.frontiersin.org/articles/10.3389/fped.2018.00432/full]. The need to assess innovative approaches is stressed by data on the use of Astragalus on Tregs [https://www.frontiersin.org/articles/10.3389/fped.2018.00255/full], and the role of immunotherapy [https://www.frontiersin.org/articles/10.3389/fped.2018.00231/full], the only potentially disease modifying approach we have.

We believe that our distinguished authors have produced a wide-ranging and stimulating volume, which summarizes the present situation and points to areas of work which will move the field forward. We thank them all for their dedication, and the Publishers for their help and forbearance. Our chief hope is that this Topic will

be an important step along the road to making severe asthma a thing of the past, of historical importance only.

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Citation: Bush, A., Piacentini, G., Santamaria, F., Ullmann, N., Cutrera, R., eds. (2019). Difficult and Severe Asthma in Children. Lausanne: Frontiers Media.
doi: 10.3389/978-2-88945-986-5

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frontiers in Pediatrics

EDITORIAL
published: 17 May 2019
doi: 10.3389/fped.2019.00205

Editorial: Difficult and Severe Asthma in Children

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Keywords: allergy, atopy, endotype, phenotype, eosinphil, airway inflammation, airway obstruction

Editorial on the Research Topic

Difficult and Severe Asthma in Children

The global burden of asthme and its treatment is huge (1), and is reviewed by Ferrante and La Grutta. Part of the problem requires a political solution; in large parts of the world, children do not have access to basic asthma treatments (2) [and shamefully, this is not just a low and middle income setting problem; children in the so-called developed world die of asthma because their parents cannot afford to pay for treatment (3), and there is no uniform health coverage].

Of course, asthma is an umbrella term covering a multiplicity of airways diseases in children (4, 5), many of which differ from adult disease (6, 7). Oksel et al. cover the topic of phenotype discovery and its clinical relevance. They explore the complimentary approaches of unsupervised discovery and investigator-imposed approaches. They stress the need for cross-disciplinary approaches if we are to maximize the opportunities offered by big data. Licari et al. also underline the heterogeneity of the disease and highlight the difficulties of measuring control and risk, especially in severe asthma. Importantly, their focus is on factors which can be modified (8).

There can be few greater challenges than the child with asthma who does not respond to treatment as anticipated, and this is the main theme of this Topic. The human misery of chronic symptoms and the ever-present risks of acute attacks including death[1], impaired lung growth with the likely consequence of chronic obstructive pumonary disease (9), and side-effects of medications, especially systemic corticosteroids, are a huge burden to a thankfully small, but certainly significant, group of children.

GETTING BASIC MANAGEMENT RIGHT

This is clearly essential, because if this is done, many children with apparently severe asthma turn out to respond to the usual basic strategies (10–12).

Is It Asthma, and Is It Only Asthma?

Ullmann et al. tackle this topic, and make the important point that pediatricians consider themselves able to diagnose asthma clinically, and all too often commit the child to long term treatment without making a single objective measurement! It is impossible to think of another chronic condition in which simple tests can be made to support the diagnosis, but are omitted (13). The result, that a diagnosis of asthma is frequently wrong, is all too predictable. They also highlight "asthma plus," co-morbidities which may mimic or worsen asthma, and may lead to inappropriate

[1] https://www.rcplondon.ac.uk/projects/national-review-asthma-deaths

OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *16 April 2019*
Accepted: *02 May 2019*
Published: *17 May 2019*

Citation:
Bush A, Cutrera R, Piacentini G, Santamaria F and Ullmann N (2019) Editorial: Difficult and Severe Asthma in Children. Front. Pediatr. 7:205. doi: 10.3389/fped.2019.00205

escalation of treatment. As discussed, the differential diagnosis of asthma in children is wide; and inhaled foreign body is rarely helped by even the highest dose of inhaled corticosteroids (ICS)!

Getting a Measurement Culture Established

Twenty-first century asthma management *must* be based on a measurement culture. Two important reviews are measurement of airway obstruction by Calogero et al. and assessment of airway inflammation by Tenero et al. Calogero et al. review the role of spirometry and forced oscillation in the assessment of children with asthma. The bedrock of physiology is spirometry, including the acute response to a short-acting β-2 agonist. Of course there is no "gold standard" physiological test, but the more tests that are done which fail to document variable airflow obstruction (including home lung function monitoring and exercise challenge testing) the more carefully another diagnosis should be sought. Persistent airflow limitation will almost certainly track throughout life, and the child with severe asthma is the father of the man with chronic obstructive pulmonary disease. We also need to look beyond spirometry to more sophisticated physiological testing to understand really severe asthma. Treatment of eosinophilic airway inflammation is with ICS, but so often we fail to measure what we are treating; would a cardiologist treat hypertension without measuring blood pressure? Furthermore, there is increasing evidence that untreated Type 2 inflammation even in apparently well controlled asthmatics is a marker of risk for an acute asthma attack (14). There is most evidence on exhaled nitric oxide and induced sputum and peripheral blood eosinophil count, but Tenero et al. have rightly gone beyond these measures to discuss many other potential inflammometry tools. If we are to move from phenotypes to endotypes, much more specific markers using the techniques they discuss will be very important.

Getting the Basics Right

It is clear that guidelines on their own, without consideration of implementation strategies, are insufficient to deliver improved outcomes, but paying heed to them is really important. Tesse et al. review both pharmacological and, equally importantly, non-pharmacological guideline recommendations; following these is an essential pre-requisite before diagnosing severe, therapy resistant asthma. However, the best medications in the world are useless unless they are taken, and poor adherence to treatment is difficult to detect and even more difficult to correct. Boutopoulou et al. carried out a systematic review of the various strategies to improve adherence. There was a great diversity of methods, testifying to how frustrating a problem this is to deal with. Encouragingly, in general all of the interventions improved adherence to some extent, but none was perfect, and the authors clearly identified a need for more research. Certainly the experience of most pediatric pulmonologists is that, if adherence could be got right, a lot of apparently therapy resistant asthma would disappear. The commonest cause of resistance to asthma therapy is that the therapy is gathering dust on the shelf.

Getting the Basic Treatment Right

There is of course more to treatment than just reaching for more medications; treatment of severe asthma includes attention to social and environmental factors, and co-morbidities (15). Early multiple aeroallergen sensitization is associated with progression to preschool wheeze (16), but in established allergic asthma, the combination of aeroallergen sensitization and exposure, and viral infection are strongly associated with risk of a severe attack (17). The relationship between allergy and severe asthma is reviewed by Arasi et al., who also discuss how other risk factors such as smoking play into the pathophysiology of severe asthma, and rightly stress the need for more research.

WHAT ARE THE PATHOPHYSIOLOGICAL MECHANISMS?

Phenotypes and Endotypes

This subject was reviewed by Bush. Currently, all too many patients are treated without any measurements being made. The current approach should be to phenotype the airway, looking for treatable traits such as eosinophilic airway inflammation. However, we cannot assume that airway eosinophilia is synonymous with Type 2 inflammation (18, 19), and we need to move to determining endotypes, specific pathobiological pathways, especially as a multiplicity of new biologicals will become available.

New Kid on the Block: Macrophage Biology?

Alveolar macrophages are a key player in host defense, but have been little studied in asthma. Kulkarni et al. used induced sputum at sea level and then in a low allergen environment at altitude. They show that allergen or possibly lipopolysaccharide may drive enhanced phagocytosis by asthmatic alveolar macrophages. More work is needed to see how this fits into the Type 2 inflammation paradigm, which was unchanged.

BEYOND GUIDELINES: INNOVATIVE THERAPIES IN SEVERE ASTHMA

The Dawning of the Age of Biological

After years of just having "the blue and brown inhaler" or variants thereof, we are now in a new age of in particular novel biologicals, reviewed by Maglione et al. However, they rightly highlight the vital importance of getting safety and efficacy data in children; we cannot assume that severe asthma is the same across the whole life course. If the biologicals deliver on their promise, then oral corticosteroids as long term therapy in children may become consigned to the history books.

Immunotherapy?

Corticosteroids are potent improvers of symptoms, whether given orally or by inhalation, but multiple studies have shown they are palliative, not curative of asthma (20–22). The only truly disease-modifying approach is allergen immunotherapy (23). Tosca et al. review the roles of immunotherapy in preventing

the development of asthma (surely our ultimate aim) and also in established disease, which may be a more risky approach.

From Left Field: Herbal Medications

Probably few if any readers know much about the herbal medication *Astrologus*. In an intriguing study, Wang et al. treated children for 6 months with this medication and showed an increase in spirometry and quality of life; they also showed impressive effects on regulatory T-cells, proposing very plausible mechanistic biological pathways. This herbal approach is not yet ready for prime time in the West, but is an important reminder of how much we still have to learn from Eastern practice.

SUMMARY AND CONCLUSIONS

This Topic offers diverse perspectives on really severe asthma in children, especially focusing on the treatable. The 60 distinguished authors have brought a unique collective wisdom for which we are grateful. The Editors have certainly learned a lot as the Topic has evolved, and we commend it to future readers; the numbers who have already gone to these articles testify to the great job the authors have done.

AUTHOR CONTRIBUTIONS

AB wrote the initial draft. All authors reviewed and approved the manuscript.

ACKNOWLEDGMENTS

AB is an Emeritus NIHR Senior Investigator, and acknowledges funding from the Asthma UK Centres for Applied Research and Allergic Mechanisms in Asthma.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

MINI REVIEW
published: 22 June 2018
doi: 10.3389/fped.2018.00186

The Burden of Pediatric Asthma

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Asthma is the most common chronic disease in children, imposing a consistent burden on health system. In recent years, prevalence of asthma symptoms became globally increased in children and adolescents, particularly in Low-Middle Income Countries (LMICs). Host (genetics, atopy) and environmental factors (microbial exposure, exposure to passive smoking and air pollution), seemed to contribute to this trend. The increased prevalence observed in metropolitan areas with respect to rural ones and, overall, in industrialized countries, highlighted the role of air pollution in asthma inception. Asthma accounts for 1.1% of the overall global estimate of "Disability-adjusted life years" (DALYs)/100,000 for all causes. Mortality in children is low and it decreased across Europe over recent years. Children from LMICs particularly suffer a disproportionately higher burden in terms of morbidity and mortality. Global asthma-related costs are high and are usually are classified into direct, indirect and intangible costs. Direct costs account for 50–80% of the total costs. Asthma is one of the main causes of hospitalization which are particularly common in children aged < 5 years with a prevalence that has been increased during the last two decades, mostly in LMICs. Indirect costs are usually higher than in older patients, including both school and work-related losses. Intangible costs are unquantifiable, since they are related to impairment of quality of life, limitation of physical activities and study performance. The implementation of strategies aimed at early detect asthma thus providing access to the proper treatment has been shown to effectively reduce the burden of the disease.

Keywords: asthma, burden, children, cost, epidemiology, prevalence, morbidity, mortality

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *08 May 2018*
Accepted: *06 June 2018*
Published: *22 June 2018*

Citation:
Ferrante G and La Grutta S (2018) The Burden of Pediatric Asthma. Front. Pediatr. 6:186. doi: 10.3389/fped.2018.00186

INTRODUCTION

Asthma is the most prevalent chronic respiratory disease worldwide, affecting more than 300 million people of all ethnic groups throughout all ages (1). It is the most common chronic disease in children, imposing an increasingly consistent burden on health system (2). Despite the various asthma phenotypes described in children, this condition is overall recognized as a chronic inflammatory disease of the airways characterized by variable symptoms of wheeze, breathlessness, chest tightness and/or cough associated with expiratory airflow limitation that may resolve spontaneously or in response to medication (3).

The understanding of the global burden of pediatric asthma has increased over the last two decades thanks to national and international studies on general populations. In defining asthma prevalence, epidemiological surveys have focused on self-reported (or parent-reported) symptoms by using standardized questionnaires, rather than on doctor diagnosis.

In particular, wheezing has been considered the most important symptom in the identification of asthma (4). The International Study of Asthma and Allergies in Childhood (ISAAC) consisted of 3 phases. Phase I was conducted during the period 1991–1995 in children and adolescents aged 6–7 and 13–14 years, respectively, in 56 countries throughout the world (5); Phase III, including 237 centers in 98 countries, was performed 5 years later, in order to examine changes in prevalence of symptoms after Phase I (6). Data on respiratory health in the younger age group were obtained from questionnaires fulfilled by parents, whereas the older age group was able to self-complete the questionnaires, which were constructed in order to be accessible to people from different countries and income levels. This accurate methodology explains the high response rate with 721.601 children involved in Phase One and 1.187.496 in Phase Three. The Italian part of ISAAC, the SIDRIA (Italian acronym for Studi Italiani sui Disturbi Respiratori nell'Infanzia e l'Ambiente) Study, was performed in two different phases between 1994 and 2002 and provided information on the epidemiology of childhood asthma in Italy, basing on questionnaires including the ISAAC core module (7, 8). Data collected from these surveys contributed to improve knowledge in the epidemiology of childhood asthma through a standardized approach. A survey to examine variations in prevalence rates of childhood asthma, wheeze and wheeze with asthma in Europe has been recently carried out (9), but further studies are needed for a more comprehensive and ongoing assessment of disease prevalence, morbidity and hospitalization all over the world (10).

GLOBAL AND TIME TRENDS OF ASTHMA IN CHILDREN

Overall, "asthma ever" was more common in High-Income Countries (HICs), even though the highest prevalence of severe symptoms among children with current (past 12 months) wheeze were found in Low- and Middle-Income Countries (LMICs) (**Figure 1**) (11). Conspicuous variations in the lifetime prevalence of asthma have been reported between countries all over the world (6). Between ISAAC Phase One and Phase Three prevalence of asthma symptoms became globally increased in children and adolescents ranging from 11.1 to 11.6% and from 13.2 to 13.7%, respectively (12). Generally, prevalence of "asthma ever" significantly increased in children and adolescents, probably due to a greater awareness of this condition and changes in diagnostic practice. However also host (genetics, atopy) and environmental factors (microbial exposure, exposure to passive smoking, and air pollution), seemed to contribute to the observed increasing trends. In particular, lifetime prevalence of asthma remained the same or even decreased in HICs, whereas it increased in many LMICs, especially in Eastern European as well as in Latin American and Northern African countries (6, 13). In Europe, increased prevalence has been reported in children (12).

Between SIDRIA Phase One and Phase Two, prevalence of "asthma ever" tended toward a stabilization in children, ranging from 9.1 to 9.5%, whereas in adolescents it increased from 9.1 to 10.4%. Prevalence of "current wheeze" resulted to be little increased in children, ranging from 7.8 to 8.6%, whereas it slightly decreased in adolescents, ranging from 10.5 to 9.7%. Noteworthy, asthma prevalence in adolescents increased only in those living in large metropolitan areas, with a percent prevalence change of 3.3% (14). Prevalence of asthma symptoms in migrant children was found to increase with the number of years of living in Italy, with risk of lifetime asthma and current wheeze very similar to Italian children (15). This finding adds further evidence to the critical role that environmental exposures may play in the development of asthma in childhood. Lastly, prevalence of asthma was found to be higher in males as in children as in adolescents, whereas asthma was more common in girls after puberty. These gender differences might be attributed to a narrower airways caliber in males than females in early life due to the effects of different hormonal factors (7).

RISK FACTORS FOR ASTHMA IN CHILDHOOD

Genetics and Epigenetics

Differences in asthma prevalence observed in vary ethnic groups all over the world may be explained by differences in the genetic susceptibility. Although the specific contribution of genetics to asthma has not thoroughly clarified, a large number of genetic markers reliably associated with asthma and airway inflammation have been identified to date. Particularly, different polymorphisms in the 17q21 locus have been found to be associated with asthma. This is considered one of the strongest loci for asthma, even though its function is still not known. Risk alleles of some single nucleotide polymorphisms (SNPs) in this locus were associated with an increased number of CD4+ cells as well as with the number of eosinophils in the airway wall biopsies of asthmatics, suggesting the involvement of these genes in the Th2 pathway in asthma (16). A new locus associated with time to asthma onset was identified at position 16q12 (17). A recent study found novel genetic factors to potentially explain sex-specific asthma effects during childhood (18). Moreover, a SNP on chromosome 8 was associated with early lung function decline (19). No specific models of genetic transmission have been identified so far.

A multi-factorial model characterized by complex relationships between genes and environment has been instead proposed. In this context, greater and greater interest on epigenetics has been focused in recent years, finding that environmental exposures are able to modulate genes expression in a complex interaction that may be even transferred from mother to child (20). More recently, traffic related air pollution's influence on asthma was showed to be associated with DNA demethylation of a CpG site in the promoter region of the Ten-eleven translocation 1 gene, a possible biomarker for childhood asthma (21).

Atopy

Data from epidemiological studies showed the strong link between asthma and atopy. Indeed, the family history of atopy is considered one of the most relevant risk factors for developing asthma. The relationship between allergic sensitization and

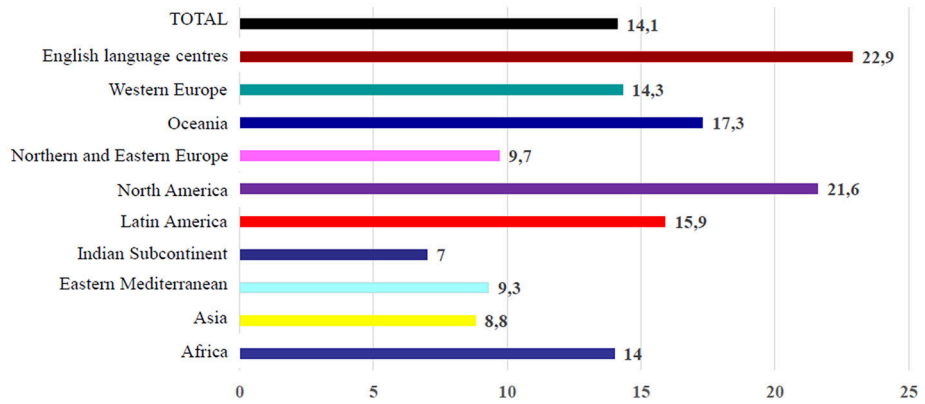

FIGURE 1 | Prevalence of current wheezing in adolescents 13–14 years: data from ISAAC Phase III.

asthma onset has been well-documented (22, 23). However, the causal relationship between individual allergen exposure and the development of symptoms has been not clearly delineated, probably due to the complexity of interactions between timing and dose of exposure and genetic predisposition. Almost 60% of schoolchildren with asthma are allergic, mainly to perennial allergens such as house dust mites, animal dander and molds. These indoor allergens play an important role in eliciting asthma exacerbations, by increasing airway inflammation and bronchial hyper-responsiveness (24). Lastly, it was recently showed that aeroallergen sensitization before the age of 5 years significantly increased the risk of asthma with persistence into adolescence (25).

Microbial Exposure

A reduced microbial exposure since early life through improved sanitation and increased rates of immunization have been linked to the increased prevalence of asthma observed in childhood. Changes in environment and/or lifestyle have been suggested to alter the development of the immune system increasing the risk of asthma in genetically susceptible subjects, basing on the so called "hygiene hypothesis." Accordingly, children raised in modern environment with a scanty natural microbial burden may be prone to develop allergic diseases in view of an under-stimulation of the immune system. Indeed, recent evidences showed that exposure to some microbes can protect from atopy, whereas others seem to promote allergic diseases. The timing of exposure to as well as the properties of the infectious agent, in addition to the genetic susceptibility of the host, may influence the future development of asthma (26). There is evidence for a critical role of respiratory virus infections in early life for asthma development. In particular, Respiratory Syncytial Virus (RSV) and Human Rhinovirus (HRV) are most frequently associated with wheezing episodes in preschool children and with asthma development in the next years. Approximately 50% of the infants with lower respiratory tract infections caused by RSV during the first 12 months of life developed persistent asthma at school age (27). In children at high risk for development of asthma, HRV-induced wheezing in infancy resulted to be the strongest predictor of doctor diagnosed asthma at age 6 years (28, 29). In addition to viral causes, some studies suggested that infections by atypical bacterial, such as *Mycoplasma pneumoniae* and *Chlamydia pneumonia*, may play a role in inducing and exacerbating asthma (30, 31).

Environmental Exposure

The increased asthma prevalence in metropolitan areas with respect to rural ones and, overall, in industrialized countries, highlighted the role of air pollution in asthma inception (14). Exposure to both outdoor and indoor pollutants has been associated with increased asthma exacerbations, rates of hospitalization and reduced lung function (32–34). Even though a cross-sectional study on 5 European birth cohorts recently showed no associations between air pollutants exposure and asthma prevalence (35), a European population-based birth cohort study on more than 14,000 children found that increasing exposure to nitric dioxide (NO_2) and particulate matter with a diameter of less than 2.5 μm ($PM_{2.5}$) at the birth address was associated with increased asthma incidence at age 14–16 years (36). Further evidence comes from a meta-analyses of birth cohort studies showing that increased childhood exposure to $PM_{2.5}$ and black carbon was associated with increased risk of asthma at age 12 years (37). The most dangerous environmental exposure in children derives from Environmental Tobacco Smoke (ETS), which is universally recognized as a major risk factor for asthma. Exposure to prenatal and early postnatal passive smoke may have adverse effect on both the immune system and the structural and functional development of the lung; this may explain the subsequent increased risk of incident asthma (38). Exposure to ETS at school age is associated with an increased risk of severity and exacerbations and may be considered a risk factor for asthma persistence in later life (39). In recent years increasing interest raised the novel concept of Third-Hand Smoke (THS), the combination of tobacco smoke pollutants which remain in an indoor environment after tobacco has been smoked. Since infants and children are prone to the risks related to THS exposure, investigations are warranted to study the health effects of THS relevant to different exposure pathways

and profiles occurring also in pre-natal life (40). Lastly, a new threat to the respiratory health of children and adolescents has been raised from the spread of electronic cigarettes, the most commonly used tobacco product among adolescents (41). Recent studies have shown an association with asthma symptoms in adolescents (42, 43). Further research on the health effects of electronic cigarettes is advised.

THE GLOBAL BURDEN OF DISEASE

Lifelong Outcomes

Asthma can appear at any stage throughout life, but it generally develops in childhood (44). Data from the Melbourne Asthma Study reported that 47% of individuals with persistent asthma, and even 75% of subjects classified with severe asthma, at age 6 still had asthma symptoms at age 50 years (45). Noteworthy, children with severe asthma were those at increased risk of developing Chronic Obstructive Pulmonary Disease (COPD) (45–47). Therefore, asthma can be considered a lifelong disease with a major burden especially in subjects suffering from severe asthma.

Morbidity

In 1990, the Global Burden of Disease Study (GBD) proposed the "Disability-adjusted life years" (DALYs) as a measure of disease burden. DALYs quantify how many years of life are lost due to death and/or non-fatal illness or impairment. This health gap measure can be considered as the sum of two components: years of life lost plus years lived with disability (YLDs) (48). The latter measure is calculated as the prevalence of each disease sequela multiplied by the disability weight for that sequela. Asthma was the 14th highest ranked cause of global YLDs at all ages, but specific data for children were not available (49). In the GBD 2015, it accounted for 1.1% of the overall global estimate of DALYs/100,000 for all causes. Overall, asthma represents the second most important respiratory disease after COPD when considering the burden of disease as measured by both YLDs and DALYs.

Mortality

Mortality for asthma is relatively low at all ages. In Europe, asthma is responsible for 0.4% of all deaths (43,000 persons), with wide differences among countries (50). In the GDB 2015, a decrease of 26.7% was observed in comparison with 1990. The decrease in age-standardized death rates was 58.8% between 1990 and 2015. The greatest decrease was observed in HICs, reflecting a better access to health services as well as better treatment options following international guidance (1). Asthma mortality in children is low and is significantly associated with symptoms prevalence and hospital admissions (51). Hence, when comparing childhood asthma mortality between countries, any reduction in prevalence has to be taken into account. Over recent years, asthma mortality in children decreased across Europe, with little difference between countries. This would be attributable to a better control of symptoms due to improvements in treatment of asthma attacks together with the more widespread use of inhaled corticosteroids which have been shown to reduce mortality at all ages (52). Noteworthy, data from the National Review of Asthma Deaths Confidential Enquiry Report showed that in United Kingdome 80% of asthma deaths occurred in people with poor adherence to treatment and in those who had taken more bronchodilators (53).

SOCIO-ECONOMIC COST OF CHILDHOOD ASTHMA

Asthma is a chronic condition that can assume different severity degrees throughout patient's life, with significant social impact and economic burden. In fact, this disease can be associated with limitations on physical and social aspects of daily life of children and their caregivers, especially when symptoms are not controlled (3). Overall, global asthma-related costs are high and significantly vary across countries, depending on several factors, such as the type of health system, financial resources on Public Health and methods of data collection (54).

Usually, asthma-related costs are classified into direct, indirect and intangible costs (**Figure 2**). Direct costs generally account for 50–80% of the total costs and include: disease management (e.g., outpatient visits, visits to emergency services, hospital admission, medications), complementary investigations or treatment and other costs (e.g., assistance in home care, transportation to medical visits) (54, 55). In children and adolescents with asthma the number of outpatient visits as well as the number of visits to emergency services is higher than in non-asthmatics, increasing according to the disease severity degree (56). Asthma is one of the main causes of hospitalization in children which are usually at least twice than in adults. Hospitalizations are particularly common in children aged < 5 years with a prevalence that has been increased during the last two decades, mostly in LMICs (57). Medications account for variable costs, which differ across countries depending on health system and public or private insurance coverage (58). Greater use of asthma drugs, particularly

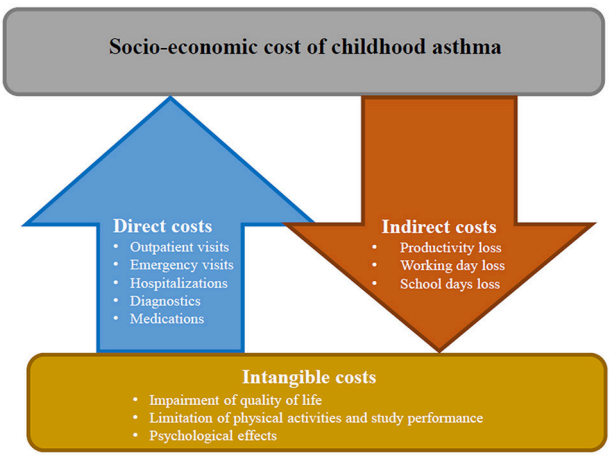

FIGURE 2 | Socio-economic cost of childhood asthma: direct, indirect, and intangible costs.

inhaled steroids, occurred in recent years globally increased costs related to asthma medications (54).

Indirect costs include work-related losses and early mortality. Although loss of working days is not directly applicable in children, absenteeism from school is a comparable consequence. In childhood asthma indirect costs are usually higher than in older patients: a child with an exacerbation of asthma loses on average 3–5 days school days and at least one of her/his caregivers loses the same working time (59).

Intangible costs are unquantifiable, since they are related to impairment of quality of life, limitation of physical activities, and study performance, with consequent psychological effects such as depression and anxiety. Nonetheless, the social burden of asthma is considerable, not only for the child but also for parents. Therefore, when assessing quality of life in asthmatic children, it is important also to assess the quality of life of the caregivers (52).

CONCLUSIONS

During last decades, asthma prevalence has been increasing worldwide. As a chronic condition that usually starts in early childhood, it imposes a high lifetime burden on individuals, their caregivers and the community. Despite significant progress in health care reached in last decades, there are still consistent disparities between countries. Children from LMICs particularly suffer a disproportionately higher burden in terms of morbidity and mortality (54). The implementation of strategies aimed at early detect asthma thus providing access to the proper treatment has been shown to effectively reduce the burden of the disease (50). The Global Asthma Network (GAN) was established in 2002 as a collaboration between individuals from ISAAC and the International Union Against Tubercolosis and Lung Disease in order to globally improve asthma care through enhanced surveillance, research collaboration, funding and capacity building, access to medical care and quality-assured essential medicines, and education of health professionals and public (60). A survey undertaken by GAN in 120 countries during 2013–2014 showed that only about one in four countries had a national asthma strategy, with a lower proportion in LCMIs than in HICs. In countries with a high prevalence of current wheeze, adopting an asthma strategy was significantly more common than what occurred in low prevalence countries. This may be due to lack of interest or to difficulties in engaging in world-wide epidemiological studies. However, extension of asthma strategies in all countries is strongly recommended, since such an approach could have a big impact on the burden of the disease, by decreasing severity and improving symptoms control (61). In this perspective, asthma, as other non-communicable chronic respiratory diseases, must be included in the agenda of each national authority. It is therefore important that monitoring of prevalence and severity continue globally. Further studies focusing on estimates of asthma costs are warranted, especially in LMICs. Moreover, standard methods of data collection are desirable in order to obtain comparable information from different countries (2). Finally, future asthma research should integrate both pediatric and adult populations in longitudinal studies, with the aim of better understand the role of risk and protective factors on disease onset and severity throughout life (1, 62, 63).

AUTHOR CONTRIBUTIONS

GF and SL provided substantial contributions to the conception or design of the work, revised the manuscript for important intellectual content, approved the final version, and agreed to be accountable for all aspects of the work.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 19 March 2019
doi: 10.3389/fped.2019.00068

Pathophysiological Mechanisms of Asthma

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The recent *Lancet* commission has highlighted that "asthma" should be used to describe a clinical syndrome of wheeze, breathlessness, chest tightness, and sometimes cough. The next step is to deconstruct the airway into components of fixed and variable airflow obstruction, inflammation, infection and altered cough reflex, setting the airway disease in the context of extra-pulmonary co-morbidities and social and environmental factors. The emphasis is always on delineating treatable traits, including variable airflow obstruction caused by airway smooth muscle constriction (treated with short- and long-acting β-2 agonists), eosinophilic airway inflammation (treated with inhaled corticosteroids) and chronic bacterial infection (treated with antibiotics with benefit if it is driving the disease). It is also important not to over-treat the untreatable, such as fixed airflow obstruction. These can all be determined using simple, non-invasive tests such as spirometry before and after acute administration of a bronchodilator (reversible airflow obstruction); peripheral blood eosinophil count, induced sputum, exhaled nitric oxide (airway eosinophilia); and sputum or cough swab culture (bacterial infection). Additionally, the pathophysiology of risk domains must be considered: these are risk of an asthma attack, risk of poor airway growth, and in pre-school children, risk of progression to eosinophilic school age asthma. Phenotyping the airway will allow more precise diagnosis and targeted treatment, but it is important to move to endotypes, especially in the era of increasing numbers of biologicals. Advances in -omics technology allow delineation of pathways, which will be particularly important in TH2 low eosinophilic asthma, and also pauci-inflammatory disease. It is very important to appreciate the difficulties of cluster analysis; a patient may have eosinophilic airway disease because of a steroid resistant endotype, because of non-adherence to basic treatment, and a surge in environmental allergen burden. Sophisticated –omics approaches will be reviewed in this manuscript, but currently they are not being used in clinical practice. However, even while they are being evaluated, management of the asthmas can and should be improved by considering the pathophysiologies of the different airway diseases lumped under that umbrella term, using simple, non-invasive tests which are readily available, and treating accordingly.

Keywords: biomarker, transcriptomics, bronchial biopsy, bronchial brushings, induced sputum, airway inflammation, asthma phenotype, endotype

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *04 January 2019*
Accepted: *19 February 2019*
Published: *19 March 2019*

Citation:
Bush A (2019) Pathophysiological Mechanisms of Asthma. Front. Pediatr. 7:68. doi: 10.3389/fped.2019.00068

INTRODUCTION: APPROACHING AIRWAYS DISEASE

The recent Lancet *Asthma* Commission (1) was predicated on the assumption that the term "asthma" was no more a diagnosis than is "arthritis" or "anemia." It is an umbrella term that should be used to describe a constellation of clinical symptoms, namely wheeze, breathlessness, chest tightness and cough, and should be followed by the question "what sort of asthma is this?" Dissecting out the individual asthmas is increasingly important as novel biologicals with different modes of action are increasingly being deployed. The ultimate aim is to discover endotypes of asthma, but currently we have not yet got to this point. The importance of endotyping is illustrated by the extraordinary achievements when the endotypes and the gene-class specific sub-endotypes of cystic fibrosis (CF) were first separated from the generality of conditions with chronic airflow infection and inflammation. The result has been the specific, molecular therapies (2–4), none of which would have come to the bedside if they had been tested on every child with a chronic wet cough. It is also important, but largely outside the scope of this chapter, to set airway disease in the context of extra-pulmonary co-morbidities such as obesity, and environmental and lifestyle factors, such as adverse environmental exposures and adherence (5).

The conventional view of at least school age and adult asthma is that the root cause is airway inflammation, which leads to airway hyper-responsiveness, and, secondary to repeated episodes of inflammation, airway remodeling. However, a critical review of the evidence shows that this view is untenable. There is only a weak correlation at baseline between eosinophilic inflammation and bronchial hyper-responsiveness (6, 7). The anti-IgE monoclonal omalizumab reduces airway eosinophilia, but has no effect on bronchial hyper-responsiveness (8), whereas the anti-TNF monoclonal etanercept reduces hyper-responsiveness but has no effect on airway inflammation (9). Furthermore, there is no relationship between the extent of airway remodeling, specifically reticular basement membrane thickness, and the degree or duration of any inflammatory parameter (10). Indeed, there is evidence that remodeling may be protective under some circumstances, discussed in more detail below. Thus, the relationships between the three classic components of asthma are more complex than previously thought, and this is highly relevant to considerations of pathophysiology.

FIRST PRINCIPLE: DECONSTRUCTING AIRWAY DISEASE

Adverse stimuli can affect any biological tube in relatively limited and stereotypical ways. These are:

- Narrowing to cause fixed obstruction
- Narrowing to cause variable obstruction which changes spontaneously over time, and with treatment
- Inflammation with various cell types predominant; inflammation may be harmful or beneficial
- The tube may become infected with combinations of bacterial, viral and fungal pathogens
- There may be increased "twitchiness" of the tube—this is different from variable obstruction. An increased reflex expulsive effort (cough) may not be accompanied by transient airflow obstruction
- The tube contents may be abnormal: including being too wet, too many solids, or too dry.

Furthermore, there are domains of risk, which also need to be considered in any discussion of pathophysiology:

- Risk of acute asthma attacks, which may be fatal
- Risk of impaired trajectories of lung growth, which may sometimes but not inevitably be associated with asthma attacks
- (in pre-school children) risk of progressing from episodic wheeze to eosinophilic atopic school age asthma
- A fourth risk, about which little is known and will not be discussed here, is the risk of failing to remit

Clearly not all are relevant to all pediatric airways diseases: the hallmark of CF is the effects of the airway being too dry ["low volume hypothesis" (11)] and infection and neutrophilic inflammation, whereas some at least of the asthmas are dominated by eosinophilic airway inflammation. What is also clear is that we need modern–omics or genetic tools to try to dissect out these components—and these are sadly lacking. Indeed, currently we are not even trying routinely to identify treatable traits in airway disease, instead haphazardly making diagnoses and embarking on therapeutic trials without making simple measurements in order objectively to phenotype the airway disease. The three important treatable traits, which will be considered in turn, are:

- Does the child have the treatable trait of eosinophilic airway inflammation which is likely to respond to inhaled corticosteroids (ICS)?
- Does the child have the treatable trait of (usually short-acting, β-2 agonist sensitive) reversible airflow obstruction? And conversely, does the child have the untreatable trait, meaning treatment should be discontinued, of fixed airflow obstruction?
- Does the child have the treatable trait of bacterial infection which is driving the disease and can be treated with antibiotics?

The contention of this chapter is that the isolated questions "does my child have asthma?" and (for example) "do survivors of preterm birth have a higher risk of asthma?" are meaningless in isolation. The correct questions are "does this child have an airway disease at all, or are the symptoms in fact due to deconditioning or some other cause (12)?" and, if the child has an airway disease, "what is the nature of this particular airway disease?"

CURRENT BEST PRACTICE: PHENOTYPING THE AIRWAY

A phenotype is defined as the set of observable characteristics of an individual resulting from the interaction of its genotype with the environment. It is important to make the distinction between phenotyping which is of clinical value (changes treatment,

prognostic value) from those determining mechanistic pathways. However, if phenotyping does not help in either domain, it cannot be said to be useful. An endotype is defined as a subtype of a condition, which is defined by a distinct functional or pathobiological mechanism. In general, phenotyping leads to rather non-specific treatment, whereas endotyping opens up an exciting vista of pathway specific therapies, as I will show.

Airway Inflammation, and the Potentially Treatable Trait of Airway Eosinophilia

Airway Eosinophilia

ICS are amongst the most effective agents in the whole of therapeutics for the vast majority of children with eosinophilic asthma. Low dose treatment, if taken efficiently and regularly, will result in complete control of asthma in most children (13), while accepting there are steroid resistant phenotypes. The most direct evidence of airway eosinophilia is of course obtained at fibreoptic bronchoscopy (FOB) with broncho-alveolar lavage (BAL) and endobronchial biopsy. This is of course not ethical or practical in most children, and non-invasive methods must be used. Induced sputum was initially the most popular technique, and although this is time-consuming, it is perfectly feasible as a diagnostic test for infection even in resource poor areas (14). Although in older children sputum and BAL eosinophilia are tightly correlated (15), this is not the case in pre-schoolers (16). Furthermore, there is a failure rate of up to 20% (17), and induced sputum does not reflect mucosal inflammation.

Of the other non-invasive methods, peripheral blood eosinophil count has become most popular. It reflects BAL eosinophilia (15, 18), and, even more importantly, is an excellent biomarker predicting response to anti-TH2 monoclonal antibody strategies (19–21). Peripheral blood eosinophil count can be measured on a finger prick sample with point of care equipment (22). Significantly, in the first attempt at personalized medicine in pre-school children, the combination of a peripheral blood eosinophil count $>300/\mu\lambda$ and aeroallergen sensitization was the strongest predictor of response to ICS (23). Exhaled nitric oxide (FeNO) has also been used as a surrogate for airway eosinophilia. The utility of this method in reducing asthma attacks has been demonstrated (24), but although there is a relationship with induced sputum eosinophil count, it varies between individuals and is inconsistent in the same individual over time (25). Clearly FeNO and induced sputum are complimentary and useful, but exactly how they should be used in combination is unclear.

However, the presence of airway eosinophils should prompt critical thought. Firstly, airway eosinophilia, although often related to Type 2 inflammation, is not synonymous with that endotype, and this needs to be borne in mind when contemplating anti-TH2 monoclonal therapies. In our cohort of severe, therapy resistant asthmatics, those with steroid resistant airway eosinophilia had very little evidence of ongoing secretion of the signature TH2 cytokines interleukin (IL)-4, IL-5 and IL-13, in either induced sputum supernatant, BAL or immunohistochemistry of endobronchial biopsy (26). Also, the U-BIOPRED group, using sputum transcriptomics in adult asthmatics documented a group which included patients with moderate sputum eosinophilia, who instead of having the expected TH2 handprint, were characterized by genes of metabolic pathways, ubiquitination and mitochondrial function, as well as, in another study, an IL-6 modulated pathway (below).

Furthermore, if eosinophils are present in the airway, their role or otherwise in disease causation should be carefully considered. In adults at least, eosinophilic bronchitis is a cause of chronic cough, but with no evidence of reversible airflow obstruction; treatment is with ICS, but not β-2 agonists (27). In a challenging study, airway biopsies were compared in patients with active asthma, normal controls, and patients who by any criteria had outgrown their previously diagnosed asthma (28). The airway wall histology, in terms of eosinophilia and reticular basement membrane thickness was the same irrespective of whether the patient had active asthma or had "outgrown" the disease. This leads to the challenging question as to what is the "X-factor" that is needed to convert airway eosinophilia into airway disease? At the moment this remains completely unknown.

Having said all this, clearly if there is no airway eosinophilia, it seems to make little sense to prescribe an anti-eosinophil strategy such as ICS. Although it is true that corticosteroids have numerous genomic and non-genomic effects (29) which hypothetically could be beneficial in airway disease, this has never been shown, and there may be potentially adverse effects in at least some airway diseases, for example reducing neutrophil apoptosis and prolonging the survival of this cell in the airway (30). Surely in the twenty-first century we should not prescribe anti-eosinophilic medications if there is no airway eosinophilia to treat, any more than anti-hypertensives should be prescribed to people who have a normal blood pressure?

Neutrophilic Asthma?

This is another area which illustrates the danger of extrapolating adult studies to children. Asthma characterized by mucosal and sputum neutrophilia is well described in adults (31), who tend also to have severe asthma with less evidence of atopy. Unsurprisingly, neutrophilic asthma is steroid non-responsive. By contrast, in our cohort of children with severe asthma, multiple atopic sensitization was common, but there was no evidence of mucosal, sputum or BAL neutrophilia (26). However, in a subgroup of patients neutrophils were found within the epithelium (32), and, quite unlike what might be expected from adult data, these patient had better symptom control (Asthma control test, ACT) and better first second forced expired volume (FEV_1) while being prescribed a lower dose of ICS. Although it is always dangerous to move from cross-sectional associations to hypotheses, nonetheless it would seem that, whatever the role of neutrophils in adult asthma, in pediatric asthma neutrophils are having a beneficial effect. This raises the intriguing possibility that bacterial infection may have a role in some pediatric asthmas. Again highly speculatively, is it possible that excessively high doses of ICS might actually worsen "bacterial asthma" (if it exists!) by causing topical mucosal immunosuppression (33), leading to a positive feedback loop of worsening symptoms leading to higher ICS doses leading to worsening symptoms? Further data are needed to explore

this. However, a practical clinical message is that the finding of BAL or mucosal neutrophilia should prompt a search for another diagnosis.

Fixed and Variable Airflow Obstruction

Variable Airflow Obstruction

Wheeze is a frequently sought, and often misinterpreted sound. Even when a polyphonic, musical predominantly expiratory noise is heard by the physician, all it betokens is narrowing of the airway lumen. It is not synonymous with airway smooth muscle constriction. Causes include intraluminal airway secretions; airway malacia which may be localized or generalized; and extraluminal compression, for example by a mass of lymph nodes. Most of the asthmas as defined above have the hallmark of either or both of obstructive physiology which improves with acute administration of a short acting β-2 agonist, or normal physiology but with an exaggerated response to stimuli such as cold air or allergen challenge.

In terms of variable airflow obstruction, as a profession we have been remiss in failing to document this objectively. Even pre-school children can perform spirometry, and criteria for adequate curves (34) and definitions of bronchodilator responsiveness (35) for this age group have been published. Simple field tests, like acute response to a short acting β-2 agonist, a short period of home peak flow or increasingly electronic spirometry monitoring, and an exercise challenge may be informative. Although there is no one definitive diagnostic test for asthma, and all the above have a high specificity but low sensitivity for asthma (36), the more tests that fail to demonstrated variable airflow obstruction, the less likely is the treatable trait of bronchodilator responsive airflow obstruction to be present.

AHR correlates poorly with inflammation (above), but of course there is a relationship which is best described by a two-component model, "inflammatory" and "anatomical." Three major longitudinal studies (37–39) have measured AHR very early in life, before any significant exposure to infection or allergen, and certainly before there is any evidence of airway inflammation or remodeling (40). Each has shown a strong association between early AHR and adverse long term respiratory outcomes. The likely pathological basis is increased airway length and reduced radius, leading to a baseline increase in airway resistance (41), which with further narrowing by a constrictor stimulus leads to an exaggerated reduction in airflow. There is also evidence in later life that airway inflammation causes a component of AHR, and that anti-inflammatory therapies can improve AHR (42).

In terms of the practical value of measuring AHR in the clinic, population studies have shown that, although in group data, AHR relates to asthma severity, many normal people can be shown to have AHR but have no symptoms (43). Thus, "abnormal" AHR of itself does not lead to disease; by analogy with the airway eosinophil story, what is the "X-factor" that converts asymptomatic AHR to an airway disease? Again, this is completely unknown. However, what is certain is that failure to demonstrate AHR in a patient said to be symptomatic with asthma should lead to reconsideration of the diagnosis; certainly, if a child is thought to have eosinophilic airway inflammation but AHR cannot be demonstrated, then the child's symptoms are not due to eosinophilic asthma.

Fixed Airflow Obstruction

The importance of this *untreatable* trait is that ICS and other therapies should not be escalated when there is no hope of benefit. There is no generally accepted pediatric definition of fixed airflow obstruction. In general, it should be defined as an abnormal FEV_1 (more than 1.96 Z-scores below normal) after a systemic corticosteroid trial and the acute administration of short-acting β-2 agonist, but neither the dose, duration or route of administration of systemic steroids, nor the dose of short-acting β-2 agonist is agreed. We use a single injection of intramuscular triamcinolone (40 mg in a child weighing <40 kgm, 80 mgm in the rest) to ensure adherence, and 1 mgm of salbutamol via a spacer (44). We found very little evidence of benefit from adding extra doses of triamcinolone (45). It should be noted that the measurement of steroid responsiveness in children with asthma encompasses more than just measuring spirometry; we use a multi-domain approach (46, 47).

One component of airway obstruction is determined antenatally. Maternal nicotine exposure (active and passive smoking, vaping) has been shown in animal models and humans to lead to structural changes with a readout of airflow obstruction shortly after birth, before the first viral infection (41, 48–52). Other adverse factors include maternal exposure to environmental pollution (53), maternal hypertension (54), and any factor leading to a low birth weight or prematurity or both (55, 56). The second component is the structural airway wall changes in established asthma, including increased airway smooth muscle, reticular basement membrane thickening, increased numbers of goblet cells and increased airway vascularity. Conventionally, these are considered to result from cycles of airway inflammation and contribute to airflow obstruction (above). However, at least in pediatrics, most studies linking inflammation and remodeling are cross-sectional and observational, and demonstrating association is a long step from proving causation. It is currently not possible to synthesis a coherent account of the pathophysiology of remodeling, and one can only present a few statements which require integration and explanation.

1. At least some aspects of remodeling, for example increased reticular basement membrane thickening, plateau in childhood, and are non-progressive into adult life (10).
2. No pediatric studies have shown eosinophilic airway inflammation with no evidence of remodeling. Remodeling has been described in the absence of current airway eosinophilia, but these children have been prescribed usually high dose ICS, and these data are equally consistent with the hypothesis of ICS having successfully treated airway eosinophilia as the alternative, that remodeling precedes airway eosinophilia (57).
3. Although airway remodeling can be partially attenuated by prolonged use of high-dose ICS (58, 59), the changes are much more steroid resistant than is airway eosinophilia.

4. Reticular basement thickening is inversely correlated with AHR, (60) and thus may be a protective response of the airway, protecting against life-threatening bronchoconstriction.
5. It is at least conceivable that reticular basement membrane thickening is a protective measure designed to limit penetration of cytokines and chemokines into the systemic circulation, and possibly also protect the airway mucosa from tissue damaging enzymes within the lumen (61).
6. On the other hand, the increase in airway smooth muscle in asthma (62), and the beneficial responses seen in adults with bronchial thermoplasty together with reduction in smooth muscle in animals submitted to thermoplasty (63, 64), suggests this aspect of remodeling is adverse.

Airway Infection and the Asthmas

This is another area that is currently difficult to understand. Some issues are clear; acute attacks of wheeze are usually precipitated by respiratory viral infections at all ages (65). Recent studies have shown that bacteria are isolated equally frequently during a wheeze attack (66), but it is unclear whether bacteria cause the attack, or are the result of a transient mucosal immunoparesis secondary to viral infection or iatrogenic as a result of ICS treatment (33). The general failure of antibiotics to impact acute wheeze attacks to any great degree is a strong pointer against a causal role for bacteria. We also know that the airway microbiome differs between normal and asthmatic children (67), and that airway neutrophilia in children appears to be beneficial rather than the reverse (32), implying a more important role of infection than previously thought. It is also clear that very early nasopharyngeal bacterial colonization with bacteria is associated with a mixed TH1/TH2/TH17 mucosal response, and subsequent adverse respiratory outcomes (68–72), although whether bacterial colonization is causal, or a marker of a subtle immune deficiency, is unclear. In a study using bronchoscopy and BAL to measure airway inflammation and infection, and the airway microbiota, in severe pre-school wheezers, we demonstrated two separate microbiota-based clusters; a Moraxella positive, airway neutrophilic group, and a mixed microbiota, macrophage and lymphocyte predominant group (73). Interestingly, these did not relate to clinical phenotype or markers of atopic status. Long term follow up will be needed to determine the significance of these clusters. There are some *in vitro* data lending plausibility to a link between bacterial infection and airway eosinophilia; in a nasal polyp model, *Staph aureus* binds via TLR2 leading to epithelial release of the alarmins TSLP and IL33, and the TH2 signature cytokines IL5 and IL13 (74). However, we need more data about the role of bacteria and the interactions with airway eosinophilia to try to understand asthma pathophysiology.

Of more immediate practical clinical significance is the need to determine whether the child with respiratory symptoms has an underlying chronic bacterial infection which will respond to oral antibiotics ["persistent bacterial bronchitis (PBB)"] (75, 76). Typically the symptoms are of wet cough not wheeze, but secretions narrowing the lumen may cause wheeze, and non-wheeze noises may be misinterpreted by the family. For most of these children, invasive sampling is not appropriate. We have shown that the yield of organisms is much greater with induced sputum, even in young children, compared to cough swabs, and indeed induced sputum results are comparable to FOB (16). It should be noted that PBB, like bronchiectasis, is a description not a diagnosis, and should prompt a focused diagnostic work-up (77).

The Time Domain: Often Unappreciated

The two important pathophysiological areas are firstly, the stability of measurements over time, and secondly, the extent to which sophisticated analysis of simple measurements over a long time period can help us understand asthma pathophysiology and understand risk.

Temporal Stability of Measurements

Adult studies in which sputum cell counts are used to guide treatment have given promising results, in particular in the reduction of asthma attack frequency (78). When we attempted to replicate this study in pediatric asthma (79), we actually found that sputum cellular phenotypes were very variable in the same individual over time, in both severe and mild-moderate asthma (80). These discrepancies are unexplained, but underscore the importance of not critically extrapolating from adults to children. Likely sputum cell counts reflect not merely the underlying disease, but environmental factors and treatment adherence. The U-BIOPRED group used breath-omics (the eNose) to define three clusters, but also noted that many patients changed cluster as peripheral blood eosinophil count changed over time (81). Cluster stability is discussed in more detail below.

Fluctuation Analyses

The first major paper used time series analyses of fluctuations in peak flow to develop a quantitative basis for objective risk prediction of acute asthma attacks and for evaluating treatment effectiveness (82). Subsequent manuscripts from this group confirmed that by considering measurements of peak flow and spirometry in isolation, rather than as part of a series, resulted in important information being lost. These and other mathematical techniques could be used to predict the response to β-2 agonists (83) and whether ICS withdrawal was likely to be successful (84). Furthermore, the method distinguished between asthma and healthy controls, partly independent of atopy, and inflammation but related to the *17q21* locus (85). These mathematical techniques have not found their way into the routine asthma clinic, but clearly this sort of mathematical analysis is giving potentially important information about asthma pathophysiology which may aid management.

Summary: What Is Current Best Practice?

The days of diagnosing and treating asthma without making objective measurements are past. Is there any other chronic condition in which simple tests are available, but are routinely not performed before committing a child to long term treatment? We should use our knowledge of the pathophysiology of asthma to phenotype the airway, even in young children, focusing on the treatable traits. It is out with the scope of this article, but

we should also consider any extra-pulmonary co-morbidities and social and environmental factors when planning management.

SPECIAL "ASTHMAS": HOW DOES THE LANCET COMMISSION HELP US?

Most of the phenotyping issues arise in children who solely have an airway disease. However, there are some situations in which airway phenotyping is helpful in understanding pathophysiology and planning treatment.

Pre-school Wheeze: Is It Asthma, Dr?

As discussed, this question is without meaning, as is the statement that asthma cannot be diagnosed until a given and entirely arbitrary age. The proper approach is to determine which (if any) treatable traits the infant exhibits. The INFANT study (23) was discussed above; so at the very least, rather than asking pointless questions or making fatuous statements, a blood eosinophil count should be performed and aeroallergen sensitization determined. Ideally spirometry should be measured, but at the very least, if the child becomes acutely wheezy, this should be documented by a physician, and the response of wheeze intensity and oxygen saturation to inhaled β-2 agonist determined. If airway infection and PBB is suspected, documentation with cough swab or induced sputum that there is actually infection present should be mandatory, especially if multiple or prolonged antibiotic courses are being used.

Wheeze in the First Year of Life: What Is It, Dr?

Very little is known about this asthma. We know it is not related to airway eosinophilia (40), so ICS should not be prescribed. We know it is common, and the prevalence across the world is highly variable (86). This is an area where a lot more work is needed on pathophysiology.

Does the Child With Another Pulmonary or a Systemic Disease Also Have Asthma?

Very frequently, ICS are prescribed to children with reasons other than asthma for being symptomatic, often either preceding the realization that a non-asthma disease is present, or on the "just in case" principle. This is to be deplored; ICS have potential serious adverse events, not least the increased risk of pneumonia (87), tuberculous (88) and non-tuberculous *Mycobacterial* infection (89), at least from adult studies.

"Asthma" in Other Pulmonary Diseases

The classical quandary is whether survivors of premature birth have "asthma" (90). Again, phenotyping resolves this. It is clear from a number of studies that these children have fixed and variable airflow obstruction (91, 92). However, FeNO is normal not raised, even in the absence of ICS (93), nor is there an elevation of exhaled breath temperature (94), a non-specific sign of inflammation ("calor" in the parlance of yesteryear). Thus, unless there is evidence in a given individual of a second diagnosis of atopic, allergic asthma (raised FeNO and peripheral blood eosinophil count, aeroallergen sensitization), ICS should not be prescribed. The same principles of investigation apply to other airway diseases, such as obliterative bronchiolitis and the airway disease after neuroendocrine cell hyperplasia of infancy; these are discussed in detail elsewhere (95).

"Asthma" in Children With a Systemic Disease

Exemplar diseases are CF, primary ciliary dyskinesia (PCD) and sickle cell anemia (SCD). We have shown that the prescription of ICS in PCD is haphazard and bears no relationship to any marker of atopic sensitization or airway eosinophilia (96). In both CF and PCD, where there is evidence that airway neutrophilia leads to tissue damage from the release of proteases and other enzymes, inhibiting neutrophil apoptosis with ICS (above) may have particularly adverse consequences. In these situations also, determining the presence or otherwise of the treatable traits airway eosinophilia and β-2 responsive bronchoconstriction should be used to determine treatment. SCD is a particularly interesting airway disease. Compared with controls, SCD children had fixed but not variable airflow obstruction, and no evidence of AHR or airway eosinophilia (97) we speculate that the airway disease may be on the basis of airway ischaemia due to microinfarcts secondary to sickling, analogous to what is seen more dramatically when the bronchial arteries are stripped off the airway during the unifocalisation procedure (98). So in all systemic conditions, if a treatable trait is present it should be treated, but treatment for a non-existent problem should be withheld.

Obesity Asthma—Not Lean Asthma in a Fat Body

The impact of the obesity epidemic across the world is well known, and the question as to whether obesity "causes" asthma is hotly debated (99). Again, phenotyping and looking at pathophysiology sheds light on the subject. The first question in a child who is breathless and obese is, does the child have an airway disease at all? Even in lean children, exertional breathlessness is more often due to deconditioning than asthma or exercise-induced laryngeal obstruction (EILO), and many non-asthmatics were treated with inhaled medication (12). A cardiopulmonary exercise test with measurements of any post-exercise bronchoconstriction may be informative.

If the obese child truly has an airway disease, then its nature should be characterized. Of course, obesity does not prevent the development of atopy, and the child may have standard atopic, eosinophilic pediatric asthma. However, obese asthma may be relatively ICS resistant, suggestive of another phenotype in some cases. Dysanaptic airway growth is defined as a normal FEV_1 with a greater than normal FVC, and thus a reduced FEV_1/FVC ratio (100). Essentially airways are of normal caliber but increased length, the latter thought to be determined by lung size. In a study of six adult cohorts, four with longitudinal data, dysanapsis was found to be commoner in the obese, and associated with worse outcomes including severe asthma attacks and use of oral prednisolone. Studies on whether obese asthma is associated with Type 2 inflammation are conflicting (101, 102). It is well known that obesity is a pro-inflammatory condition. There are intriguing data suggesting that the airway may be the target of systemic inflammation, instead of the source of inflammatory

cytokines spilling into the systemic circulation. In a study of two adult cohorts, plasma IL-6 was measured as a marker of systemic inflammation and related to BMI and asthma outcomes (103) Patients who were IL-6 high were more likely (but not inevitably) obese, and had worse FEV_1 and more likely a history of asthma attacks. There was no relationship between IL-6 and serum IgE or sputum eosinophils, demonstrating that the effects of systemic inflammation were not mediated via Type 2 inflammation. Again these studies indicate the need to go back to pathophysiology, and the utility of airway phenotyping when considering airway disease, especially if it is non-responsive to conventional therapy.

THE PATHOPHYSIOLOGY OF ASTHMA RISK

Increasingly the importance of future risk as a domain to be considered in asthma management[1]. If risk is to be managed, it must be measured, and the underlying pathophysiology of the risk be understood.

Asthma Attacks

Asthma attacks are all too common, may cause death, impair quality of life, incur a huge burden of health care cost and are associated with worsening respiratory and lung growth trajectories. Asthma attacks are not "exacerbations," a futile word implying a reversible inconvenience (104, 105); they are lung attacks. A recent meta-analysis of the risk factors for an asthma lung attack (106), as did the UK National Review of Asthma Deaths[2], highlighted that having had one bad attack, the patient was at high risk of having another. Many asthma fatalities related to social factors, such as poor adherence and failure to engage with regular follow up reviews. However, the underlying pathophysiology of asthma attacks is also important. Specifically, the concept that asthma control may be good, but risk of a future attack high, is pivotal.

Asthma attacks may be driven purely by respiratory viral infection, with no background Type 2 inflammation, usually in pre-school children with episodic viral wheeze (107). A huge surge in environmental allergen burden in the absence of viral infection may also rarely cause acute asthma attacks, as in the Barcelona soya bean epidemics (108), and thunderstorm asthma (109). The vast majority of attacks are respiratory viral driven in patients who have background ongoing type 2 inflammation; thus the combination of respiratory viral infection, allergic sensitization and allergen exposure was very strongly predictive of an asthma attack (110). It has been shown that using FeNO and (in adults) induced sputum eosinophil count to titrate ICS treatment leads to a reduction in asthma attacks (24). Inadequate ICS treatment (usually related to non-adherence) was another strong predictor of an asthma attack (106). Omalizumab therapy in the summer given to children on step 5 therapy who had had an asthma attack in the previous year ameliorated the autumnal rise in asthma attacks driven by returning to school and winter viral infections (111). Finally, in a proof of concept, double blind, randomized controlled study, mite impermeable bedding led to a reduction in oral corticosteroid use in the year after an asthma attack in children sensitized to house dust mite (112). This allows a risk prediction index for asthma attacks (112). The Seasonal Asthma Exacerbation Prediction Index (SAEPI) has been validated as a means of predicting children at risk for an asthma attack (113, 114). For those aeroallergen sensitized, an attack in the prior season and reduced spirometry predict a further asthma attack irrespective of season. Measures such as increased numbers of positive allergen skin prick tests, high prescribed ICS doses, increased FeNO, blood eosinophil counts and total and specific IgE levels may predict a seasonal asthma exacerbation. In summary, uncontrolled Type 2 inflammation, even in the face of good asthma symptom control, is a major risk factor for future asthma attacks.

There are other factors of importance which have been reviewed elsewhere (115). There are some asthma patients who never have an attack, implying either genetic protection or susceptibility factors, which are poorly understood. One example is the gene for the epithelial protein CDHR3, which is the receptor for RV-C (116), and gene mutations may convey increased susceptibility to attacks (117, 118). Indoor and outdoor air pollution, including tobacco, and vitamin D deficiency potentially through multiple immunological and other pathways (119), are all associated with increased risk of asthma attacks.

Interestingly, many severe asthma patients never have an attack, for reasons which are unclear. Analysis of the SARP-3 cohort showed that nearly half never had an asthma attack, but a quarter had at least three attacks per year (120). Peripheral blood eosinophil count, body mass index, and bronchodilator responsiveness were positively associated with frequency of attacks, but not asthma duration, age, sex, race, and socioeconomic status. The findings were replicated in previous SARP patient cohorts.

Adverse Trajectories of Lung Function

There is an extensive literature on tracking of lung function, from a series of overlapping birth cohorts (121). Although there are discrepancies, some due to methodological issues such as lung function measurements in infancy, the balance of the evidence is that spirometry tracks from the pre-school years to late middle age at least, with possible deviations from tracking if puberty is late with a subsequent fast growth trajectory (122). In summary, spirometry rises to a plateau at about 20–25 years of age and thereafter declines (123). Adult studies have shown that failure to reach a normal spirometric plateau carries a 26% risk of COPD, compared with a 6% risk in those who attain their full growth potential (and who develop COPD because of an abnormally rapid rate of decline of spirometry) (124). A number of cohort studies have shown that some children have persistently low spirometry during childhood, putting them in the high risk category. The pathophysiology of this phenotype is poorly understood (125–127). The Tucson group used latent class analysis to determine that there were two trajectories, normal, and low lung function. Risk factors for the low lung function group included a history of maternal asthma (20.0 vs. 9.9%; $P = 0.02$); early life RSV lower respiratory tract infection

[1]https://ginasthma.org/

[2]https://www.rcplondon.ac.uk/projects/national-review-asthma-deaths/

(41.2 vs. 21.4%; $P = 0.001$); and physician-diagnosed active asthma (whatever the value of that label) at age 32 years (43.9 vs. 16.2%; $P < 0.001$) (125). In the Tasmanian cohort (126), there were three low trajectories (early below average, accelerated decline; persistently low; and below average); predictors included childhood asthma, bronchitis, pneumonia, allergic rhinitis, eczema, parental asthma, and maternal smoking. This group were followed up into the sixth decade, and COPD risk could be calculated. Odds ratios were 35·0, 95% CI 19·5-64·0 (early below average, accelerated decline): 9·5, 4·5–20·6 (persistently low); and 3·7, 1·9–6·9 (below average). In a combined analysis of the MAAS and ALSPAC cohorts (127), the persistently low trajectory was associated with severe wheeze attacks, early allergic sensitization, and tobacco smoke exposure. However, although it is clear that there is a group of asthmatics with low trajectory lung function who are at risk of COPD, it is not clear that anything can be done to reverse this. The Tasmanian group showed that risks were exacerbated in those children who went on to smoke, and certainly general advice should be given about risk avoidance; but this is clearly an area of asthma pathophysiology which merits further work. Also of note, and meriting further work is the association of high early all-cause mortality in populations with impaired spirometry (128, 129); it may well be that low spirometry should be used as an important signal of systemic disease (130).

Risk of Progressive Disease

Many if not all children who develop atopic eosinophilic asthma by school age start with acute discrete episodes of pre-school wheeze before progressing to a multiple trigger pattern of symptoms. A proportion of children with viral wheeze progress to school age eosinophilic airway disease. The pathways to progression are very poorly understood (40, 107, 131, 132). Important factors associated with progression include multiple early atopic sensitization and severe attacks of wheeze (133). We know that those with no personal or family atopic history are unlikely to progress, but although predictive indices (134–136) have a good negative predictive value, unfortunately the positive predictive value not much better than 50%. Currently we know that in the pre-school years those who develop school age asthma lose lung function, which is never regained throughout life (137, 138); and they develop airway remodeling and eosinophilic inflammation. Although there is active research in the field (139) we do not have good predictive biomarkers nor do we understand the endotypes of progression or regression of the disease, nor do we have any therapeutic interventions. We know that ICS used early are not disease-modifying (140–142), but we do not know what might be useful. There are tantalizing hints that risk reduction is possible, from a randomized controlled trial of fish-oil supplementation (143) and the differences in atopic risk between the Amish and Hutterite communities, related to environmental exposures (144). This is another aspect of pathophysiology that requires more work.

Risk of Side-Effects

Clearly ICS doses should be the minimum required to control Type 2 inflammation. There is some evidence that the risk of side-effects relates more to an excessive dose of ICS relative to the degree of airway inflammation rather than the absolute dose prescribed. In a group of adult asthmatics, there was no difference in the pharmacokinetics of an intravenous dose of fluticasone, with similar area under the curves (145). However, when the same dose was given by inhalation, there was far greater absorption into the systemic circulations in the non-asthmatics. There was unfortunately no objective measurement of inflammation, but it is not unreasonable to suppose that overdosing relative to inflammation leads to side-effects.

CONCLUSION: AIRWAY PHENOTYPING

Clearly we can learn a lot about the pathophysiology of the asthmas, and use this knowledge to improve diagnosis and treatment. We are still not achieving this in routine clinical practice, and this is shameful. However, even if we were phenotyping all patients and understanding pathophysiology with currently available tools, we need to progress to the next step, namely endotyping. The current position is described in the next section of this article.

OUR TARGET: ENDOTYPING THE AIRWAY

The general approach to airway endotyping has been to collect and characterize as far as possible large groups of patients, for example the U-BIOPRED (146, 147) and SARP (148) cohorts, and use sophisticated—omics technologies to perform cluster analyses to try to determine the endotypes driving disease. However, caution is needed; whether a child is in a particular cluster will be driven by the underlying endotype, but also the effects of adverse environmental, infective or other factors which may vary over time, the contrasting effects of prescribed treatment, and whether the treatment is actually used by the patient. Environmental factors are unstable over time, and changes may be dramatic (108, 109, 149, 150). Treatment adherence is highly variable, difficult to measure and will affect the airway. Hence an eosinophilic airway may be the final common pathway of combinations of a steroid resistant endotype; poor adherence to ICS treatment; and increased environmental allergen exposure. Clearly the management of these three factors is very different. Very few groups have studied longitudinal stability of phenotypes or endotypes, and a second, validation cohort is rarely used; when done, the evidence for stability is weak (151). Thus, our challenge is to differentiate what truly reflects a real endotype and what represents non-disease attributes (above). This is rarely addressed or even often identified as a problem.

Furthermore, most of these analyses are in patients with severe asthma, because mild asthma hardly merits study. However, most patients referred with "severe" asthma would have mild asthma if they took their treatment (152–154). In this regard, in a recent GWAS there was substantial overlap between mild and moderate to severe asthma (155). Although the authors speculated that this related to epigenetic silencing of genes, and was therefore not reflective of gene expression, an alternative explanation is that many diagnosed as moderate to severe asthma in fact were non-adherent to treatment.

Endoyping Asthma With Omics Technology

The U-BIOPRED (146, 147) and SARP (148) investigators, who have largely focused on severe asthma, albeit with additional controls, are the major groups exploiting -omics technology. Systems biology is increasingly used to allow clusters and phenotypes to emerge from the data (103, 156) using an unbiased analysis, not confounded by pre-set ideas. This is impossible; there is inevitable investigator bias in selecting the data to collect and analyse. For example, until relatively recently, bacterial infection was not thought relevant to asthma, but this has been challenged (above); prior to this work, bacterial samples would not have been collected and analyzed.

Cluster Analyses: What Do They Tell Us?

The SARP investigators (157) identified four clusters: mild to moderate early onset asthma, normal body mass index and no or eosinophilic airway inflammation; the second had the same inflammatory characteristics, but the patients older, more likely to be obese, with impaired airway obstruction on spirometry and African Americans were over-represented. The last two exhibited had predominantly neutrophilic sputum; sputum eosinophilia was also sometimes seen. One of these clusters also contained older patients who were more likely to be obese and have severe asthma, obstructive spirometry and to be treated with oral corticosteroids. The final cluster was the oldest, with males over-represented, and more likely to be obese and prescribed complex medication regimes. As well as a lack of replication and assessment of cluster stability over time, the investigators could not dissociate the effects of disease from those of treatment. Furthermore, association does not prove causation, which could not be determined. Critically, this sort of cluster analysis did not appear to help us make progress in understanding or treating disease. This last point is underscored by another SARP analysis revealing this time five clusters (120) with no differences in outcomes, again questioning the usefulness of this approach.

The U-BIOPRED investigators identified four clusters in adults using sputum cell transcriptomics (158, 159). They used clinical clustering and training and validation cohorts to define phenotypes (159) which were then used to assess differences in sputum proteomics and transcriptomics data. The first were well-controlled patients with moderate-to-severe asthma. The second was in smokers with late onset severe asthma, further characterized by chronic airflow obstruction. The only difference between this and the third cluster was that the latter contained non-smokers. The fourth cluster contained obese women who had uncontrolled severe asthma, normal lung function but multiple asthma attacks. There were differences in gene expression in these clusters, which adds validity to their findings.

Assessment of the temporal stability of clusters was performed by the ADEPT group (160). They performed a baseline cluster analysis in adults which were then re-assessed over time and also validated in a U-BIOPRED subset (161). They used sophisticated mathematical techniques which included fuzzy-partition-around-medoid clustering. They included including clinical and biomarker profiles. They also classified the patients into $TH2_{hi}$ and $TH2_{lo}$ using gene expression profiles on bronchial biopsies. They identified four phenotypic clusters. The first was characterized by mild, early onset disease, good spirometry, and little in the way of inflammation; that present was predominantly Type 2. The second contained moderately well controlled asthmatics who had mild airflow limitation and moderate airway responsiveness; they also had Type 2 inflammation. The third group were only moderately well controlled, had minor Type 2 inflammation or a non-eosinophilic, neutrophilic phenotype airway phenotype with predominantly fixed airflow obstruction. The fourth cohort had severe asthma with uncontrolled reversible airflow obstruction, a mixed and type 2 inflammatory picture. However, there was huge overlap between the clusters for almost every marker; this does not invalidate the study, but suggests that cluster analysis may be excellent for determining groups linked by common mechanisms, but has yet to been shown in any cluster analysis. Hence the role of cluster analyses is yet to be defined. There are commonalities and differences between studies, and they have not delivered endotypes.

Asthma Diagnosis Using Omics Technology

It is well known that asthma is poorly diagnosed (162–164), often objective diagnostic tests are not used, and those that are available are so crude when compared with the gene signature approaches used, for example, in tuberculosis diagnostics (165, 166). Blood transcriptomics would be the ideal. Red cedarwood triggered asthma in adults has a gold standard diagnostic test, unlike most of the asthmas outside the workplace, namely bronchial challenge. In a small study split into two cohorts, discovery and validation, adults with red cedarwood asthma could be reliably diagnosed using a gene signature in peripheral blood (167). Confirmation in other settings is needed, but a gene signature approach would be a major step change on current diagnostic approaches.

The U-BIOPRED group (168) also used a training and validation set. They identified 1693 genes differentially expressed in adult asthmatics as against controls, with a bigger effect size in severe asthmatics. Unfortunately, and reducing the value of this approach, around 90% of the differences could be related solely to differences in peripheral blood white cell count. Pathway analysis showed that genes related to chemotaxis, migration and myeloid cell trafficking, and decreased development of B-cells, haematopoietic progenitor cells and lymphoid organs were involved in the differences, in both training and validation cohorts. The results were similar but less pronounced in mild-moderate asthmatics. Gene signatures of corticosteroid responsiveness also differed. However, the transcriptomics did not map to any clinical cluster (169). Again this calls into question the utility of this approach, certainly as a clinical tool, and highlights our lack of understanding of the complexity of asthma. It is possible that the results might have been different if airway cells had been used, as being closer to the pathological process.

Asthma Pathophysiology: Hypothesis Generating Studies

Gene expression is regulated in part by non-coding RNA, and this has been a subject of asthma research. In adults with severe asthma, activation status of CD4 and CD8 lymphocytes was related to non-coding RNA expression. There were significant changes in CD8 but not CD4 cells, Multiple pathways involved in T-cell activation were enhanced and there were many changes in miRNA expression (170). This is observational study, and very preliminary, but an important starting point. The rapidly expanding field of the role of micro-RNAs has been reviewed in detail elsewhere (171).

Asthma Pathophysiology: Exploring Endotypes of Inflammation

Although the ideal is one endotype susceptible to a single biological, the reality is likely to be much more complex. Cytokines and chemokines were measured in sputum from subjects in the SARP group with varying severities of asthma, and unbiased factor analysis was used to try to define specific inflammatory pathways (172). There were complex inflammatory protein interactions identified by factor analysis. Severe asthma patients had nine increased and four decreased proteins compared to mild asthma subjects. Twenty-six mediators were significantly associated with an increasing single induced sputum leucocyte type: sixteen with neutrophils; 5 with lymphocytes; IL-15 and CCL15/MIP1δ with macrophages; interestingly, only IL-5 with eosinophils; and IL-4 and TNFSF10/TRAIL with airway epithelial cells. Forty three cytokines, chemokines, and growth factors which had no missing data were mapped onto the first 10 factors, containing mixes of Th1, Th2, Th9, and Th17 inflammatory and anti-inflammatory proteins, rather than pure pathways. Hence focus on a single specific mediator or pathway is likely an oversimplification of the complex reality of the asthmatic airway.

In a further study, the U-BIOPRED investigators started by defining phenotypes from sputum cytology, either eosinophil- and neutrophil-predominant. Next, they used sputum plugs to generate Affymetrix arrays and analyzed the data were analyzed using hierarchical, unsupervised clustering. They identified three transcript associated clusters (TACs). The first was contained oral corticosteroid dependent patients who had frequent asthma attacks, severe airflow obstruction, and the highest sputum eosinophil counts and FeNO levels. Immunologically, the receptors Il33R, CCR3 and TSLPR were upregulated and there was the strongest IL-13/TH2 and ILC2 gene signatures. The second cluster was clinically characterized by sputum neutrophilia, a raised serum CRP and eczema, and immunologically by IFN-, TNF-α- and inflammasome related genes being upregulated. The final cluster had moderate sputum eosinophilia and better spirometry, but despite sputum eosinophilia was immunologically characterized by upregulation of genes of metabolic pathways, ubiquitination and mitochondrial, with surprisingly, no TH2 signature. This important paper again highlights that eosinophilia is not synonymous with TH2 activation, confirming our own findings in severe asthma (26).

A pioneering study used transcriptomics of bronchial brushings and biopsies to determine $TH2_{hi}$ and $TH2_{lo}$ subgroups of mild to moderate asthmatics based on TH2 gene signatures (173). The $TH2_{hi}$ subgroup had elevated peripheral blood levels of periostin (which is also derived from growing bone, so cannot be used in pediatrics), CLCA1 and Serpin B2, and eosinophilic airway inflammation which was ICS responsive. A subsequent study (174) using airway epithelial cell gene expression in adults confirmed this finding, but found that non-invasive biomarkers such as periostin were not sufficiently sensitive. The U-BIOPRED subsequently identified two steroid-resistant, eosinophilic subgroups in severe asthmatics (175); one with high mucosal eosinophilia, raised FeNO, asthma attacks and oral corticosteroid use; by contrast, the second eosinophilic group was more obese. We previously noted that sputum and BAL eosinophils correlate with each other, but not with mucosal biopsy eosinophils (15), but which is most important under what circumstances has not been determined. The U-BIOPRED investigators also described two non-eosinophilic groups, and developed model to predict the likelihood of the patient being steroid responsive. It is very clear that there are non-inflammatory phenotypes of severe asthma, and also that mucosal and BAL eosinophilia is not synonymous with Type 2 inflammation (176).

A further U-BIOPRED study highlighted the IL6 pathway as a potential cause of eosinophilic inflammation independent of TH2 cytokines (177). Activation of IL-6 trans signaling in air-liquid interface cultures of bronchial epithelial cells reduced the integrity of the epithelium. Associated with this was a specific signature enriched in airway remodeling genes. This signature identified a subgroup of adult asthmatics with increased epithelial expression of these inducible genes in the absence of systemic inflammation. There was an overrepresentation of patients with frequent attacks, peripheral blood eosinophilia, and submucosal of T cells and macrophage infiltration. TLR receptor pathway genes were upregulated, but cell junction genes expression was reduced. Sputum sIL6R and IL6 levels correlated with sputum markers of innate immune activation and airway remodeling. This study further evidence that there is a subset of asthmatic patients with no evidence of Type 2 inflammation; it may be that IL6 is driving airway inflammation and epithelial dysfunction in this group of patients.

The IL1 pathway may also be important (178). Sputum transcriptomics were compared in severe and mild-moderate adult asthmatics with eosinophilic and neutrophilic asthma. The investigators reported that IL1RL1 gene expression was associated with severe eosinophilic asthma, whereas NLRP3 inflammasome expression was highest in those with severe, neutrophilic asthma. These changes were only seen in induced sputum, not in bronchial brushings or biopsy specimens, underscoring the need to study multiple tissues if pathophysiology is to be understood.

Finally, FeNO is a well-known as an asthma biomarker, but whether more than one pathway results in increases has been little studied. The SARP team used a microarray

platform to relate FeNO to bronchial airway epithelial cell gene expression (179). They identified 549 genes whose expression correlated with FeNO. They used k-means to cluster the patient samples and found that a total of 1,384 genes were identified in nine gene groups. Although type 2 inflammation genes were present, novel pathways, including those related to neuronal function, WNT pathways, and actin cytoskeleton, were also discovered, suggesting novel and as yet poorly characterized inflammatory pathways were at play in asthma.

Taken together, these studies suggest that in particular in severe asthma, there are multiple endotypes, possibly co-existing in some patients. There is far more to asthma pathophysiology than Type 2 inflammation. We have much more to learn from harnessing omics technology to the study of the asthmas.

Asthma Pathophysiology: Persistent Airflow Limitation

As discussed above, there are multiple contributory factors to persistent airflow limitation, including congenital and acquired remodeling, so it is likely that multiple genes are involved. The UBIOPRED group (180) used Gene Set Variation Analysis (GSVA), as a means of detecting underlying endotypes in such heterogeneous samples. Severe adult asthma patients from the U-BIOPRED cohort with persistent airflow limitation defined as post-bronchodilator FEV_1/FVC below the lower limit of normal) were compared with asthmatics with normal spirometry. Gene expression was assessed on the total RNA of sputum cells, nasal brushings, and endobronchial brushings and biopsies. Fourteen differentially enriched gene signatures were identified that were associated with ICS, eosinophils, IL13, IFN-α, specific $CD4^+$ T-cells and airway remodeling. There was a differentially expressed gene network associated with remodeling solely in the airway wall.

Asthma Attacks: Can We Do Better?

Chitinase-like protein YKL-40 modulates airway inflammation and serum levels are associated with asthma severity (181). In another SARP study (182), adult asthmatics were analyzed to determine if there were clusters based on YKL-40 levels, and the findings were validated in SARP. Sputum transcriptome analysis were used to demonstrate molecular pathways associated with YKL-40 clusters, of which four could be identified. Those with high serum YKL-40 were associated with earlier onset and longer duration of disease, severe airflow obstruction, and near-fatal asthma attacks. The cluster with the highest serum YKL-40 levels had adult onset disease and less airflow obstruction, but frequent attacks. Interestingly, and despite the fact that attack frequency was an important correlate of these clusters, an airway transcriptome analysis showed activation of non-type 2 inflammatory pathways. This study provides further evidence for the importance of non-TH2 pathways, and, although this needs validation, possibly suggests that serum YKL-40 levels may help risk-stratify patients.

Future Risks: Progression From Pre-school Episodic Wheeze to School Age Eosinophilic Airway Disease

This is an extremely complicated subject which is largely beyond the scope of this review. We know that antenatal and postnatal tobacco and pollution exposure are important factors impacting future lung health, but we know little or nothing of the molecular pathways to disease [see review Bush (121)]. Furthermore, although we can predict who are low risk children, we are poor at predicting high risk, what the pathways to eosinophilic asthma actually are, and how we can reduce risk, either on a population or individual level. We have some largely descriptive –omics data which hint at pathways, but our knowledge gaps are huge.

Gene expression profiles were studied in transient and persistent wheezers using peripheral CD4+ve cells, and compared to normal, non-wheezing controls (183). The study was observational and descriptive, but did describe differences in gene expression between the two wheezing groups, with some commonalities in the paths involving proliferation and apoptosis of T-cells. Another group prospectively followed 202 preschool wheezers to school age, and testing the hypothesis that the use of volatile organic compounds (VOCs) and exhaled breath condensate would enhance the prognostic value of conventional predictive indices (184). They showed that VOCs and possibly inflammation related genes (TLR-4, catalase, TNF-α) improved predictive of persistent wheeze, but this study is also realm, and hypothesis generating, requiring validation in another cohort.

HOW WILL WE MAKE OMICS WORK IN PRACTICE?

When clinically indicated, invasive techniques can be used to discover novel mechanisms and pathways, but these will be only applicable in really severe cases, not in more mildly affected infants and children. For most cases, non-invasive approaches must be found, especially in children. Blood, urine and induced sputum can and should be routine clinical tests, there are other accessible biosamples which should be evaluated. Exhaled breath analysis is non-invasive, requires only passive co-operation, and with modern analytical techniques can give point of care answers. Investigators can distinguish different airway diseases in adults (COPD, asthma) from a breathprint of VOCs (185, 186). In children, 8 of 945 compounds studied could differentiate asthmatics from controls with a sensitivity of 89% and a specificity of 95% (187). There has been considerable interest in sophisticated mass spectrometry techniques, which can be applied for example to skin secretions, in order to detect airway infection in CF (188). The true test of the utility of these techniques will be whether they can differentiate children with non-specific respiratory symptoms from true asthmatics, and predict steroid responsiveness in the asthmatics. Perhaps in the future we will have a pediatric "Breathalyzer" which will give a readout of the important biomarkers to tell us the diagnosis, what endotypes are at play, and how best to treat the child.

SUMMARY AND CONCLUSIONS

There is no doubt we have incredible opportunities within reach to transform the diagnosis and treatment of the asthmas. We have powerful tools and –omics technologies available to us, as well as pathway specific monoclonals. These need to be targeted rationally. We need to reflect that the specific designer molecules which are transforming CF (2–4) would have been discarded as ineffective if they had been applied indiscriminately to all CF patients, and not to gene class specific sub-endotypes. The CF community are progressing to *ex vivo* testing of novel compounds (189, 190) and we must do the same for the asthmas, to produce truly personalized airway medicine.

There are questions specifically pertaining to the asthmas that we need to address. We need to understand steroid resistant, particularly non-TH2 driven eosinophilia, and apparently non-inflammatory asthma pathways. We have argued elsewhere that children with refractory difficult asthma (for example, those persistently not taking basic medications) should not be denied biologicals to prevent them from dying (191); even in this group, identification of the endotype will be needed to ensure the right child gets the right biological. But we also need to appreciate the diversity of the asthmas—Type 2 inflammation, although obviously important, is only one part of the picture, and we need to better are specific appreciate the whole.

But finally we must appreciate that the more sophisticated and expensive approaches to monitoring and treatment are available, the more clinical skills become relevant (192). We will need better get the basics right, rather than immediately deploy the latest gene probe test and expensive therapeutic molecule. At bottom, most pediatric asthma is a simple disease to diagnose and treat if basic measurements are made and the child is given low dose therapy appropriately and regularly. We should never lose sight of this reality, and never stop using our clinical skills, and honing those skills, to get the basics right, working in a multidisciplinary team alongside the child and family.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 20 September 2018
doi: 10.3389/fped.2018.00258

Classification of Pediatric Asthma: From Phenotype Discovery to Clinical Practice

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Advances in big data analytics have created an opportunity for a step change in unraveling mechanisms underlying the development of complex diseases such as asthma, providing valuable insights that drive better diagnostic decision-making in clinical practice, and opening up paths to individualized treatment plans. However, translating findings from data-driven analyses into meaningful insights and actionable solutions requires approaches and tools which move beyond mining and patterning longitudinal data. The purpose of this review is to summarize recent advances in phenotyping of asthma, to discuss key hurdles currently hampering the translation of phenotypic variation into mechanistic insights and clinical setting, and to suggest potential solutions that may address these limitations and accelerate moving discoveries into practice. In order to advance the field of phenotypic discovery, greater focus should be placed on investigating the extent of within-phenotype variation. We advocate a more cautious modeling approach by "supervising" the findings to delineate more precisely the characteristics of the individual trajectories assigned to each phenotype. Furthermore, it is important to employ different methods within a study to compare the stability of derived phenotypes, and to assess the immutability of individual assignments to phenotypes. If we are to make a step change toward precision (stratified or personalized) medicine and capitalize on the available big data assets, we have to develop genuine cross-disciplinary collaborations, wherein data scientists who turn data into information using algorithms and machine learning, team up with medical professionals who provide deep insights on specific subjects from a clinical perspective.

Keywords: asthma, phenotypes, disease progression, machine learning, longitudinal data, big data

OPEN ACCESS

Edited by:
Renato Cutrera, Bambino Gesù Ospedale Pediatrico (IRCCS), Italy

Reviewed by:
Francesca Santamaria, Università degli Studi di Napoli Federico II, Italy
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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *08 June 2018*
Accepted: *29 August 2018*
Published: *20 September 2018*

Citation:
Oksel C, Haider S, Fontanella S, Frainay C and Custovic A (2018) Classification of Pediatric Asthma: From Phenotype Discovery to Clinical Practice. Front. Pediatr. 6:258. doi: 10.3389/fped.2018.00258

INTRODUCTION

Asthma is a term describing a heterogeneous medical condition characterized by variable symptom expression, airway inflammation and therapeutic responses, making the clinical diagnosis challenging and long-term prognosis uncertain (1). Identifying genetic risk factors, environmental associates and pathophysiological mechanisms of asthma is further complicated by the fact that there is no uniform definition of this condition (2–4). In research settings, different studies use different definitions, which may lead to the under- or over-estimation of cases, and any signal in

genetic of environmental association studies may be diluted as a consequence of the heterogeneity of the primary outcome measure (5). For example, Van Wonderen et al. reviewed 122 published articles and reported a staggering 60 different definitions of childhood asthma used in cohort studies (4). After selecting four common definitions used in the literature and applying them to a single cohort, the authors found that prevalence estimates varied from 15.1 to 51.1% (4). For the clinical setting, the UK National Institute of Health and Care Excellence (NICE) guidance recommends algorithm for diagnosing childhood asthma which is based on sequential assessment of four objective tests of lung function/airway inflammation (spirometry, bronchodilator reversibility, fractional exhaled nitric oxide, and peak flow variability; https://www.nice.org.uk/guidance). However, a recent study has found a poor agreement between the proposed algorithm and a strict epidemiological definition of asthma (physician diagnosis, current symptoms, and regular use of inhaled corticosteroids) in a birth cohort study (6). The authors suggested that the proposed NICE guidance on asthma diagnosis in children should not be implemented, emphasizing the uncertainties of how to accurately diagnose asthma, and which objective tests are useful (6).

There is increasing recognition that asthma is not a single disease, but a collective noun used to describe a set of clinical symptoms and features which may arise through different pathophysiological mechanisms (7, 8). While the subtypes of asthma sharing similar observable characteristics are often labeled as "phenotypes," "asthma endotypes" are defined on the basis of pathophysiological mechanisms associated with discrete subtypes. There is a general consensus in the medical community that different endotypes of asthma do exist, however, there is no consensus as to what these endotypes are, or how to define them (9). One approach to endotype discovery capitalizes on the advances in computer sciences and software engineering and uses unbiased, data-driven approaches in an attempt to uncover different "phenotypes" of asthma, with the assumption that patterns of clinical symptoms are a reflection of specific underlying pathophysiological mechanisms (9). It is important to emphasize that disease subtypes discovered using data-driven approaches are not observed, but latent (i.e., hidden) by nature, and ideally should not be referred to as "phenotypes" (i.e., observable characteristics). However, as the term "phenotype" has been used in this context for more than a decade (10), we will maintain this nomenclature in this review. A thorough review of the implementation of data-driven methods for phenotype discovery in pediatric asthma has been conducted recently, with a particular focus on childhood wheezing illness and different "wheezing phenotypes" at a population level (11, 12). We will expand the discussion beyond the existing approaches to understanding phenotypic complexity in asthma, and highlight the role of clinical context and clinical experience in linking latent "phenotypes" to underlying biological mechanisms and tailored treatment approaches. We start by highlighting the heterogeneity of asthma and its phenotypic expression, and then discuss potential solutions to maximize the gain from different sources of data, and their clinical utility in asthma research.

DISENTANGLING ASTHMA HETEROGENEITY: FROM SUBJECTIVE TO DATA-DRIVEN APPROACHES

The idea of characterizing asthma subtypes based on the temporal pattern of symptoms through the life-course is not new (13), but has gained momentum in recent years with emerging of data-driven analytic approaches. Over the past two decades, subtyping approaches have progressed from subjective sub-typing to statistical classification techniques. **Table 1** summarizes different approaches for discovering pediatric asthma phenotypes. In subjective sub-typing, phenotypes are identified using predefined or hypothesized criteria based on investigators' insights about clinical features, symptoms, age of onset, and progression rate (14, 23). The main limitation of this approach is that less obvious or rare patterns may be missed. A risk of artificially limiting the set of inputs or imposing a structure on the data is that it may limit the predictive ability of a model by missing associations which do, in fact, exist (9). In contrast, data-driven classification relies on techniques and algorithms that mine the large data sets to uncover the underlying structures and patterns "hidden" in the data. Statistical methods such as cluster analysis and latent class analysis (LCA) (11, 24–26), principal component analysis (20, 27), and exploratory factor analysis (21), have been widely applied to discover homogeneous subtypes of asthma. These procedures ranged from univariate approaches (a single symptom measured over time) to more sophisticated, multivariate approaches that simultaneously model several variables, including symptoms and other clinical and environmental characteristics. By incorporating the longitudinal structure of data, the latter has enabled investigators to capture the multidimensionality of the disease and to characterize phenotypic heterogeneity across the life-course (28).

Nowadays, big data set containing many thousands of variables (such as clinical variables, objective tests, various biomarkers, genome-wide genotyping, proteomics etc.), are extensively used in medical research. In particular, the concept of "big" is difficult to pin down and relative to each field. Big data in healthcare refers to the large volumes of data accumulated from numerous sources, patients and populations that can no longer be easily handled by traditional statistical analysis methods due to its complexity. One of the advantages of big data in medicine is its capacity to examine heterogeneity between diverse populations, build better predictive models around individual patients and deliver more personalized and effective care. As an example, big data could be used to develop analytical tools that can help identify at-risk asthma patients before an attack occurs[1], to identify patients with exacerbations and inadequately controlled asthma (29) and to understand how variations in environmental factors influence childhood asthma hospitalization (30).

In the context of "big data analytics," it is not possible to define *a priori* all possible causal and associational mechanisms (9). By allowing algorithms to model a large number of potential associations in an unsupervised way, patterns can be identified

[1]http://www.propellerhealth.com

TABLE 1 | Different approaches for phenotypic discovery with the associated advantages and disadvantages.

References	Age (years)	Sample size	Methodology	Strengths	Limitations
(13)	1–6	54	Subjective sub-typing	- Phenotypes are observable expressions - Choice of cutoff guided by investigator expertise - Simple	- Predefined or hypothesized criteria needed - Rare patterns may be missed - Risk of over- or under- fitting as there are no objective statistical criteria for judging fit - Subjective cut-offs need to be recalibrated when new data becomes available - Un-validated cut-offs pose challenge for comparing findings across studies
(14)	1–6	826			
(15)	1–6	6265	Latent class analysis	- Probabilistic class allocation. - No prior knowledge is needed. - Hidden patterns may be uncovered that could not be a priori. - Hypothesis generating - Objective statistical criteria for judging whether phenotypes represent true variation	- Discovered sub-types are latent and retrospective by nature - Within-class heterogeneity arising from individuals whose patterns do not exemplify any phenotype - Meaningful clinical interpretation required to explain the patterns - Number of derived phenotypes may be related to the frequency and timing of data collection - Unclear to what extent established phenotype labels convey temporal patterns
(10)	1–7	689			
(16)	1–9	953			
(17)	1–8	5760			
	1–8	2810			
(18)	1–8	1184			
(19)	8–12	3890			
(20)	3–5	946	Principal component analysis	- Accounts for - coexisting symptoms - Reduces the variable dimensions in complex diseases	- Difficult clinical interpretation - Not useful for categorical and longitudinal data unless properly specified
(21)	7–35	925	Exploratory factor analysis		
(22)	6–18	613	Hierarchical clustering	- No a priori info about the number of classes required	- Risk of misclassifying distinct phenotypes that are present at low frequency

that could not have been predicted in advance, even by experts in the field. As such, data is allowed to speak for itself, often without relying on any prior knowledge. However, a danger of this approach is that it may become divorced from rigorous scientific scrutiny and meaningful clinical interpretation (9), since big data can only explain part of the picture (31). In the absence of guidance about the clinical plausibility of findings, there is a risk of identifying false positive associations as the number of relationships being tested increases (32). To be genuinely successful, the "data-driven" approach should encompass making decisions based on both data analysis and interpretation **(Figure 1)**, which can only be achieved through a true synergy between the expertise in data science and clinical domain (22).

LATENT VARIABLE MODELING PARADIGMS FOR "PHENOTYPE" IDENTIFICATION

One way to address the complexity of asthma is to derive asthma phenotypes that differentiate groups of patients presenting with similar combinations of symptoms, and to understand how biological factors shape each of these disease "phenotypes" (5). One such approach is latent class trajectory modeling, a class of probabilistic models in which repeated measurements of observable symptoms are modeled to identify homogeneous sub-populations within the larger heterogeneous population. Over the last few decades, latent modeling approaches [reviewed in (9, 11, 12)] have been extensively used to identify longitudinal trajectories of childhood wheeze (10, 17, 33, 34), atopy (34–37), and asthma (11, 12, 19), and to evaluate their associations with early life risk factors. For example, recent studies which used data from several population-based birth cohort studies have described four discrete trajectories of lung function from early childhood to young adulthood (38, 39), providing evidence that early life influences might be crucial not only for childhood asthma, but also for the pathogenesis of COPD in adulthood (28).

However, despite the increasing utilization of (and reliance on) latent class methods to stratify asthma and allergic diseases, there is a striking lack of enquiry into the extent of between-individual variation within the supposedly homogeneous "phenotypes." Latent class methods use posterior probabilities which provide researchers with an objective basis for assigning individuals to classes (phenotypes) that best typify their pattern of symptom development. As these probabilities collectively measure specific individual's likelihood of belonging to each of the classes discovered by a model, a class (or "phenotype") membership is not fixed, and all individuals are assigned a non-zero probability of belonging to each class. It is a common practice to then assign individuals to one of the latent classes according to the maximum posterior probability for an individual belonging to a particular class. Once classified in such way, the individuals are often considered as members of a single class, despite occasionally a considerable variations in posterior probabilities and marginal class assignments.

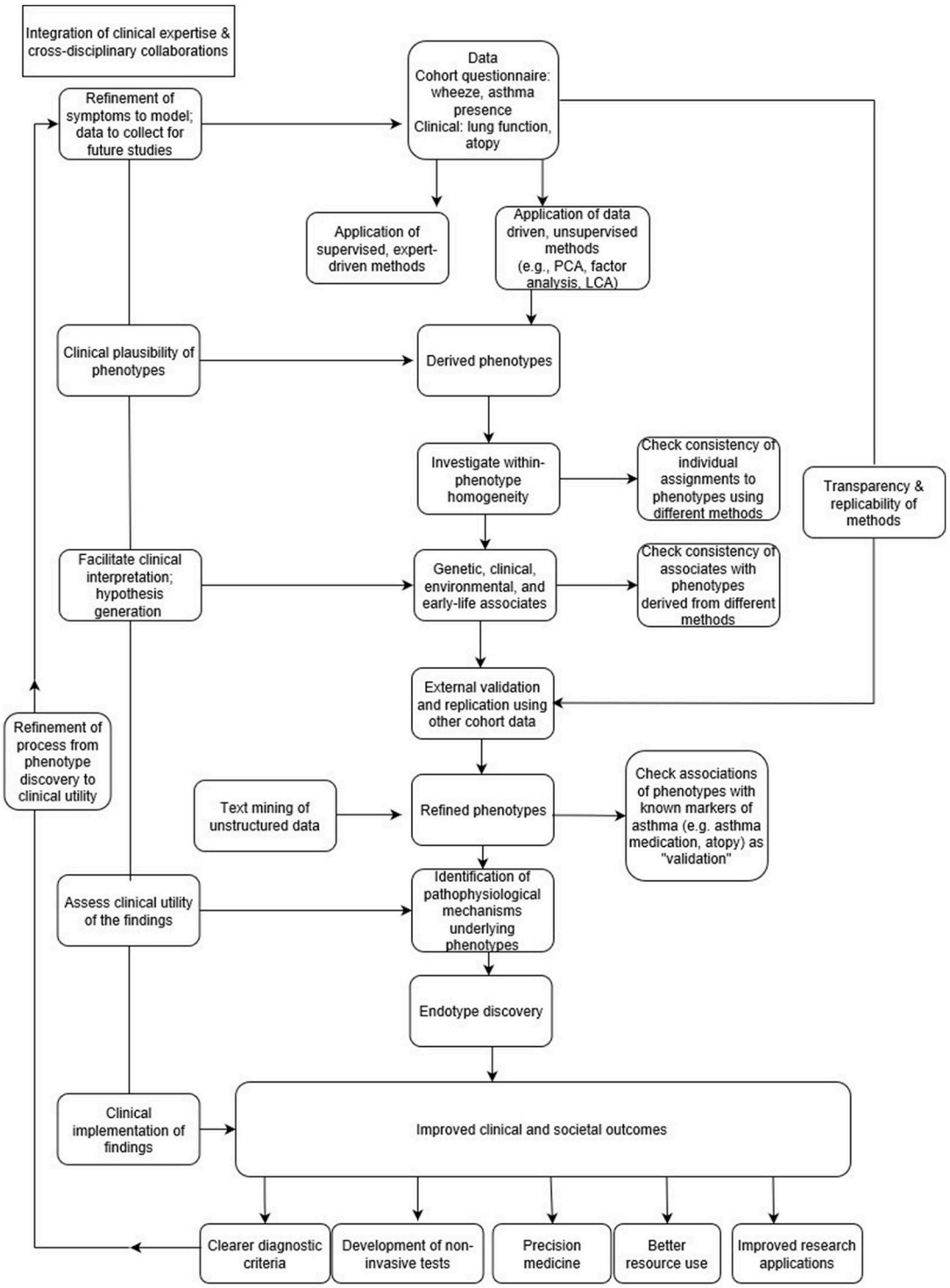

FIGURE 1 | From phenotype discovery to clinical utility.

However, in some cases, there may be subjects who have low posterior probabilities for all classes, and/or whose patterns do not exemplify any phenotype. As an example, an individual may have a 0.5 probability of belonging to "phenotype 1," 0.30 probability of belonging to "phenotype 2," and 0.20 probability of being in "phenotype 3," yet such classification would assign a person into "phenotype 1," ignoring the underlying uncertainty in class assignment. An implication of this is that the latent

classes may not, in fact, reflect homogeneous patterns. If we are to understand pathophysiological processes underpinning different phenotypes, then each phenotype should include only individuals whose patterns fit well within the assigned class with a high probability (close to 1), and with a very low probability of belonging to other classes.

Furthermore, it is unclear to what extent the phenotypic nomenclature adequately conveys the temporal characteristics of individuals assigned to the classes, for example, whether "persistent" wheeze means long and/or uninterrupted spells of wheeze, and/or whether "early transient" means absolutely no recurrence of wheeze later in life. Also, one has to be careful not to assume that a persistence of symptoms (such as wheeze) necessarily reflects a persistence of the same pathophysiological process. For example, children with "persistent wheeze" may develop symptoms in early life due to impaired anti-virus responses (40), while the cause of the wheezing later in the school age may be related to other mechanisms such as IgE-mediated sensitisation (41). We would also like to highlight that phenotypes derived from different birth cohort studies often share the same nomenclature (such as "transient early," "late-onset" and "persistent"), but phenotypes with the same assignment often differ substantially in terms of their age of onset, temporal trajectory and distributions within a population. Although common labels are frequently ascribed to latent classes (phenotypes) across studies, it has not been established whether individuals with similar longitudinal profiles are classified to the "same" phenotype in different cohorts sharing similar time points (or even within the same cohort). Moreover, classifications derived from latent class methods appear to be based on a combination of the timing of onset of symptoms and their frequency, but there has been a lack of research into whether there are different levels of disease "severity" within each phenotype, and how would the addition of information on severity impact classification.

A recent review of childhood wheeze phenotypes discovered using data-driven methods found a lack of consistent associations with risk factors and associates across different studies (18). We propose that within-class heterogeneity may be a in part responsible for these discrepancies, and may mask potentially important and consistent associations. Given that the optimal solution for the number of phenotypes may be an artifact of the underlying assumptions of the methods employed, idiosyncrasies particular to a cohort, and within-class heterogeneity, we encourage researchers to investigate the characteristics of the individual trajectories assigned to the phenotypes, and in doing so, question whether the model assumptions are appropriate for the data at hand. In order to reduce misclassification and derive more holistic phenotypes which reflect a" real life" and clinical practice, we would also suggest that rather than focusing on a single symptom (e.g., wheeze), we should employ methods that can incorporate a more comprehensive set of symptoms and/or comorbidities (for example, rhinitis, atopic dermatitis) (42). Thus, in order to achieve more consistency in phenotype discovery (in particular with respect to the role of different risk factors), it may be necessary to move beyond LCA and employ other methods for phenotype discovery.

ADVANCING PHENOTYPE DISCOVERY: THE CASE FOR A MORE REFINED APPROACH

As outlined above, the identification of asthma phenotypes and their underlying distinct pathophysiological mechanisms is crucial for the development of targeted therapeutic strategies (1, 5, 8, 9). In order to achieve this goal, it is imperative that researchers derive asthma phenotypes that are truly homogenous. Whilst data-driven approaches have provided a framework for unearthing a structure within large datasets, there is a risk of assuming that the results represent the "truth," in particular when this assumption is based on a reliance on objective statistical criteria, such as the Bayesian information criterion (BIC), Akaike information criterion (AIC), etc. For the clinical community, the proliferation of machine learning techniques and their associated language inventory of "new" terms [hidden Markov models (34), random forest (42), Bayesian networks (42), latent variable modeling (42), clustering (22), etc.,] are complex to comprehend, even by the statistically literate. Rigorous scientific assessment, reproducibility and transparency of models are increasingly challenging with the availability of diverse programming languages (R, Python, Stata, MATLAB, Infer.Net, MPlus, etc.,). The density of code underlying some algorithms makes it difficult to replicate and validate models (43). Although performance measures to compare the predictive adequacy of various machine learning techniques (area under the curve [AUC], sensitivity, specificity, positive and negative predictive values [PPV and NPV respectively], etc.,) are routinely published, studies rarely demonstrate how numeric improvements in prediction translate into better outcomes for patients. Hence, there is a pressing need for big data research to include data's relationship to improved outcomes at its core. In addition, steps need to be taken to improve the statistical literacy of healthcare professionals through greater education to bridge the divide with the big data "industry." It is essential that clinicians embrace new findings and engage in debates surrounding big data and healthcare.

Birth cohorts have been instrumental in shedding light on asthma heterogeneity, but they alone cannot address all important questions, particularly in relation to severe disease, and the pathophysiological mechanisms underlying different phenotypes. Patient cohorts contain data which complement the information from birth cohorts, and bringing together these data assets may be essential to disaggregate asthma. Such a multi-cohort approach would enhance the credibility, reproducibility and generalizability of phenotyping results, while maximizing the benefits of accumulated and readily available evidence, but methodological challenge of how best to co-analyse the data from different contexts remains unanswered.

THE CLINICAL UTILITY OF DATA-DRIVEN PHENOTYPES

To date, numerous asthma classifications have been proposed based on observable clinical characteristics, disease severity,

triggers, age of onset and inflammatory markers. For example, various atopic phenotypes (pollen sensitization with severe exacerbations, multiple allergies with severe asthma, house dust mite, multiple early/late, and late mixed inhalant) were defined based on asthma severity and allergic sensitization in pediatric populations from the TAP (44), MAAS (45), and CAPS (37) cohorts. Similarly, several inflammatory phenotypes (46) such as eosinophilic asthma, neutrophilic asthma, pauci-granulocytic asthma (24, 47–50), and Th2-high asthma (51), and trigger-induced asthma phenotypes such as cigarette smoke-induced asthma (52), air pollution-induced asthma (53), and exercise-induced asthma (54) have been identified in different populations. Although the long-term goal of the phenotype-driven approach is to broaden the personalized management of asthma, translation into clinically actionable endotypes is not readily apparent. This may, in part, be due to the limited ability to identify causative pathophysiological mechanisms of distinct subgroups of childhood asthma. The clinical utility of phenotype classification and their use in everyday clinical practice requires an improved understanding of pathophysiological mechanisms that underlie each asthma subgroup.

One way of bridging the findings from data-driven analytics into day-to-day clinical practice is by linking identified phenotypes to a specific underlying pathology, and tailoring treatment choices based on pathophysiologic mechanisms. Recent advances in molecular techniques offer promising opportunities to link phenotypes with underlying pathological mechanisms. For example, by employing machine learning, a recent study has described an architecture of multiple cytokine responses by human blood mononuclear cells to rhinovirus stimulation comprising six response profiles, and observed major differences in trajectories of asthma, allergic sensitization and lower respiratory tract infections during childhood between these profiles, suggesting that impaired anti-virus immunity may contribute to the development of a specific phenotype of troublesome childhood asthma (41). In another study, Bønnelykke et al (45). identified a novel gene (*CDHR3*) that was associated with a specific phenotype of early onset asthma with severe exacerbations. In subsequent studies, the risk variant in *CDHR3* has been reported to facilitate rhinovirus-C binding and replication (55), suggesting that the *CDHR3* may pose a risk to early-onset asthma with severe exacerbations and hospitalisations through an interaction with RV-C infection (56). Collectively, these findings highlight how the use of umbrella term "asthma" masks the complexity of disease heterogenity, and that the derivation of more precise and internally-homogenous phenotypes may be useful for providing more accurate assessment of underlying pathophysiology. Several recent studies which used machine learning-based methodologies applied to a large amount of data generated by multiplex arrays measuring IgE to more than 100 individual allergenic proteins suggest that it may be possible to develop better diagnostic algorithms to help practicing physicians differentiate between benign and clinically important allergic sensitisation to help asthma diagnosis (57–59).

In short term, the continued validation and replication of asthma phenotypes in different populations, and the integration of novel approaches such as whole genome sequencing and omics profiling to tease out pathological mechanisms underlying different phenotypes are needed to help deliver personalized medicine in clinical practice. In the longer-term, findings from large-scale data have the potential for the development of non-invasive and quick diagnostic assessments for use in clinics (57, 60).

FUTURE POTENTIAL FOR REFINING PHENOTYPES: INTEGRATING TEXT MINING APPROACHES INTO ASTHMA RESEARCH

The exponential growth in the amount of data which is being generated in healthcare setting often makes it difficult to extract knowledge and value from a vast amount of unstructured data, or to understand whether these insights are relevant to the clinical setting (9). To date, over 119,200 scientific articles are indexed in the PubMed database under the "asthma" label, with a publication rate of more than 3,000 asthma-related papers each year (https://www.ncbi.nlm.nih.gov/pubmed/). Methodologies such as text mining are usually seen as a specialization of the broader data-mining field, with the ultimate aim of extracting useful information from unstructured data and unlocking full insight contained in huge volumes of data. They commonly rely on Natural Language Processing (NLP) methods, a key component of many Artificial Intelligence systems, dedicated to the automatic treatment of written, typed or spoken resources. The biomedical field has extended NLP solutions to biological and medical domain (also known as bioNLP) (61, 62), and demonstrated its potential use for performing extraction of asthma candidate genes (63, 64), biological and clinical concepts (65), protein-protein interactions (66), and gene-disease associations (67). Earlier applications of bioNLP in asthma research were limited to text-searching from clinical notes to characterize patients with asthma exacerbation (68), and asthma as a principal diagnosis (69). More recent studies have extended their use to include classification components of NLP which help to classify asthma status at a patient level (70).

The application of NLP to clinical problems holds out great promise of extracting biomedical relations from scientific literature and clinical narratives, and unlocking clinical information from various medical documents such as consultation notes, patient narratives or medical admission and discharge records. However, such clinical information is commonly omitted in phenotyping studies, mostly due to the unstructured nature of the data. While the integration of bioNLP methodologies with machine learning tools may help tackle the inconsistency in asthma ascertainment over many studies, one of the key limitation of bio-text mining approaches is that they still require manual curation and shared annotated datasets which are currently very limited in asthma research (71). The collaborative efforts of biomedical community toward shared objectives and tasks (72), may help overcome the current limits in BioNLP, unlock its full potential for deciphering complex disease, and provide solutions to medical problems that are too complex for a single discipline or method to resolve.

CONCLUSION

Despite a significant contribution of recent phenotyping studies to our understanding of asthma heterogeneity, the translation of findings to clinical practice is hampered by a number of methodological challenges. The promise of data-driven "revolution" to support clinical decision making will not be fulfilled by technological and methodological advances alone, but by a fundamental change in medical culture, and the advancement of a team science approach (5). If we are to make a step change toward personalized medicine and capitalize on the available big data assets, we have to develop genuine cross-disciplinary collaborations, wherein data scientists who turn data into information using algorithms and machine learning, team up with medical professionals who provide deep insights on specific subjects from a clinical perspective, and prioritize which problems to solve. This may facilitate more meaningful and robust disease classification through, for example, a more informed choice of prognostic indicators, and inform the clinical decision-making process. Bringing together diverse disciplines and skill sets is a challenge for medical science in general, and complex heterogeneous long-term conditions such as asthma may offer an example ofs how targeting a particular health problem by looking at it from multiple perspectives can achieve insights that translate to patient benefit through the delivery of personalized medicine.

AUTHOR CONTRIBUTIONS

AC, CO, and SH conceived the idea; SF and CF provided input on the methodology; All authors wrote the report.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 11 February 2019
doi: 10.3389/fped.2019.00028

Severe Asthma and Allergy: A Pediatric Perspective

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Severe asthma in children is associated with significant morbidity and lung function decline. It represents a highly heterogeneous disorder with multiple clinical phenotypes. As its management is demanding, the social and economic burden are impressive. Several co-morbidities may contribute to worsen asthma control and complicate diagnostic and therapeutic management of severe asthmatic patients. Allergen sensitization and/or allergy symptoms may predict asthma onset and severity. A better framing of "allergen sensitization" and understanding of mechanisms underlying progression of atopic march could improve the management and the long-term outcomes of pediatric severe asthma. This review focuses on the current knowledge about interactions between severe asthma and allergies.

Keywords: allergy, allergic rhinitis, atopic dermatitis, children, difficult-to-treat asthma, food allergy, severe asthma

OPEN ACCESS

Edited by:
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Comenius University, Slovakia

Reviewed by:
Vladimir Pohanka,
Slovak Medical University, Slovakia
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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *29 November 2018*
Accepted: *23 January 2019*
Published: *11 February 2019*

Citation:
Arasi S, Porcaro F, Cutrera R and Fiocchi AG (2019) Severe Asthma and Allergy: A Pediatric Perspective. Front. Pediatr. 7:28. doi: 10.3389/fped.2019.00028

INTRODUCTION

Atopic sensitization is a well-established, but not exclusive, risk factor for severe asthma both in children (1, 2) and adults (3), all over the world (1–4). Although its role in determining asthma severity has been considered limited in the past years, some reports confirm that allergy may play a significant role especially in childhood, when early atopic sensitization is crucial to determine the severity of disease.

Though most asthmatic children achieve symptoms' control through occasional bronchodilator (BD) use or low to medium dose of inhaled corticosteroids (ICSs), a small but significant subset of patients remains with uncontrolled asthma despite treatment with high-dose inhaled glucocorticoids (**Table 1**) or requiring such a treatment to remain well-controlled (5). This group of children with chronic symptoms and episodic exacerbation requiring short-acting $beta_2$ agonists (SABA) is defined as affected by "difficult-to-treat asthma." This definition includes poorly-controlled asthma due to at least one of the following: an incorrect diagnosis; comorbidities; poor adherence to therapy because of adverse psychological or environmental factors (6). "Severe asthma" is considered a specific subset of "difficult-to-treat asthma." It is characterized by the need of higher intensity therapy in order to maintain symptom control or uncontrolled symptoms despite such therapy (6), proper diagnosis (7), and management of comorbidities and correction of unsuitable behavior for control disease.

In 2014, a task force of the European Respiratory Society (ERS) and the American Thoracic Society (ATS) updated the definition of severe asthma in pediatric patients. According to the latter, children affected by severe asthma require treatment with high-dose ICSs and either a long-acting beta-agonist (LABA) or a leukotriene antagonist for the previous year or systemic corticosteroids for at least 50% of the previous year to prevent uncontrolled asthma or asthma that remains uncontrolled despite this therapy (5).

TABLE 1 | High-dose ICD dosages for children (mcg/d) according to Global Initiative for Asthma (GINA) guidelines.

Drug name	GINA (6–11 y)	GINA (>12 y)
Beclomethasone dipropionate (HFA)	>200	>400
Budesonide (DPI)	>400	>800
Budesonide (nebules)	>1,000	
Ciclesonide (HFA)	>160	>320
Fluticasone propionate (DPI)	>400	>500
Fluticasone propionate (HFA)	>500	>500
Mometasone furoate (DPI)	>440	>440

DPI, dry powder inhalers; HFA, hydrofluoroalkane.

TABLE 2 | Characteristics of severe bronchial asthma in children and adults.

Characteristics	Pediatric asthma	Adult-onset asthma
IgE-sensitization	+++	+
Poly-sensitization	+++	+
High specific IgE levels	+++	+
Clinical heterogeneity (i.e., multiple phenotypes)	+++	+++
Severe non-allergic obese female prevalent phenotype	–	+++
Severe non-allergic eosinophilic phenotype (nasal polyposis, sputum eosinophilia, and aspirin sensitivity)	–	+++

In a birth cohort study, the prevalence of severe asthma has been estimated about 0.5 and 4.5% in all 10-year-olds and current asthmatic children when assessed in 10 year olds, respectively (8). Notwithstanding, it is associated with a significant economic burden related to more and severe symptoms needing of adjunctive medical resource use and higher health costs (9). Furthermore, the increased number of parent's working days lost during child asthma exacerbations is accompanied by less global economic productivity (10).

Since only a small percentage of asthmatic patients is affected by severe asthma, this clinical entity is still poorly known even if associated with notable morbidity. Both in children and adults, severe asthma is a heterogeneous disorder with multiple clinical phenotypes (11). However, elegant cluster analyses have shown that the role of atopic sensitization might be more important in the pathogenesis of severe asthma specifically in childhood onset asthma: more than 85% of children with severe asthma are severely atopic (12). In contrast, severe adult-onset asthma is a distinct phenotype that is usually not characterized by atopic sensitization, but often associated with nasal polyposis and sputum eosinophilia (13, 14). A brief overview of characteristics and differences between pediatric and adult-onset severe asthma is provided in **Table 2**.

Though it is well-recognized that atopic sensitization is an important risk factor mainly for pediatric asthma, the role of allergy in children affected by severe asthma is still under debate. This review aims to focus the role of allergy in pediatric severe asthma.

THE ATOPIC MARCH

Though atopic manifestations may persist for several years and then resolve over time (15), in atopic children, adolescents, and adults allergy manifestations may evolve according to a predetermined sequence, characterized by the progression from atopic dermatitis (AD) to allergic rhinitis (AR) and asthma (16–18).

Therefore, it seems that atopic predisposition represents a major risk factor for developing all atopic diseases in patients for which the progression from AD to asthma defines the well-known "atopic march" (19). However, the temporal presentation of allergic diseases may differ from the usual progression of the atopic march due to genetic influences and environmental factors (20).

Foremost, allergens may penetrate easier a defective skin barrier, therefore leading to transcutaneous sensitization and subsequentially initiating the atopic march (21). Indeed, IgE sensitization to food or airborne allergens is a significant cofactor to induce the progression of the atopic march in patients with AD (22–24). Moreover, it is widely described that the risk of developing asthma in patients with AD is strictly related to both the clinical expression of IgE sensitization and the severity of eczema (25–27).

It seems that transcutaneous IgE-sensitization may precede airway sensitization (28) and that IgE-associated AD might represent the first step of the atopic march and, therefore, it may predict the upcoming development of allergic diseases, including food allergy, AR, and asthma (please see **Figure 1**) (28). Since AR is a further major risk factor for bronchial hyper-reactivity and asthma, it can precede asthma onset in the natural history of the atopic march (29–31).

ALLERGIC COMORBIDITIES

Several factors and co-morbidities may contribute to worsen asthma control and complicate diagnostic and therapeutic management of severe asthmatic patients (**Figure 2**) (32).

Atopic Dermatitis

Several studies reported that family history of atopy, early onset AD, higher initial severity of atopic eczema, hens' egg sensitization and male sex are associated with an increased risk of asthma in childhood (33). Furthermore, the percentage of patients with severe asthma and concomitant eczema is greater than expected and close relationship between asthma and atopic dermatitis severity has been reported (34).

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; AERD, aspirin exacerbated respiratory disease; AD, atopic dermatitis; AIT, allergen specific immunotherapy; AR, allergic rhinitis; ATS, American Thoracic Society; BD, bronchodilator; ERS, European Respiratory Society; FA, food allergy; HDM, house dust mites; ICS, inhaled corticosteroid; IgE, immunoglobulin E; LABA, long-acting beta2-agonist; MAAS, Manchester Asthma and Allergy Study; MAS, Multicentre Allergy Study; NSAIDs, non-steroidal anti-inflammatory drugs; SABA, short-acting beta2-agonist; SAFS, Severe asthma with fungal sensitization.

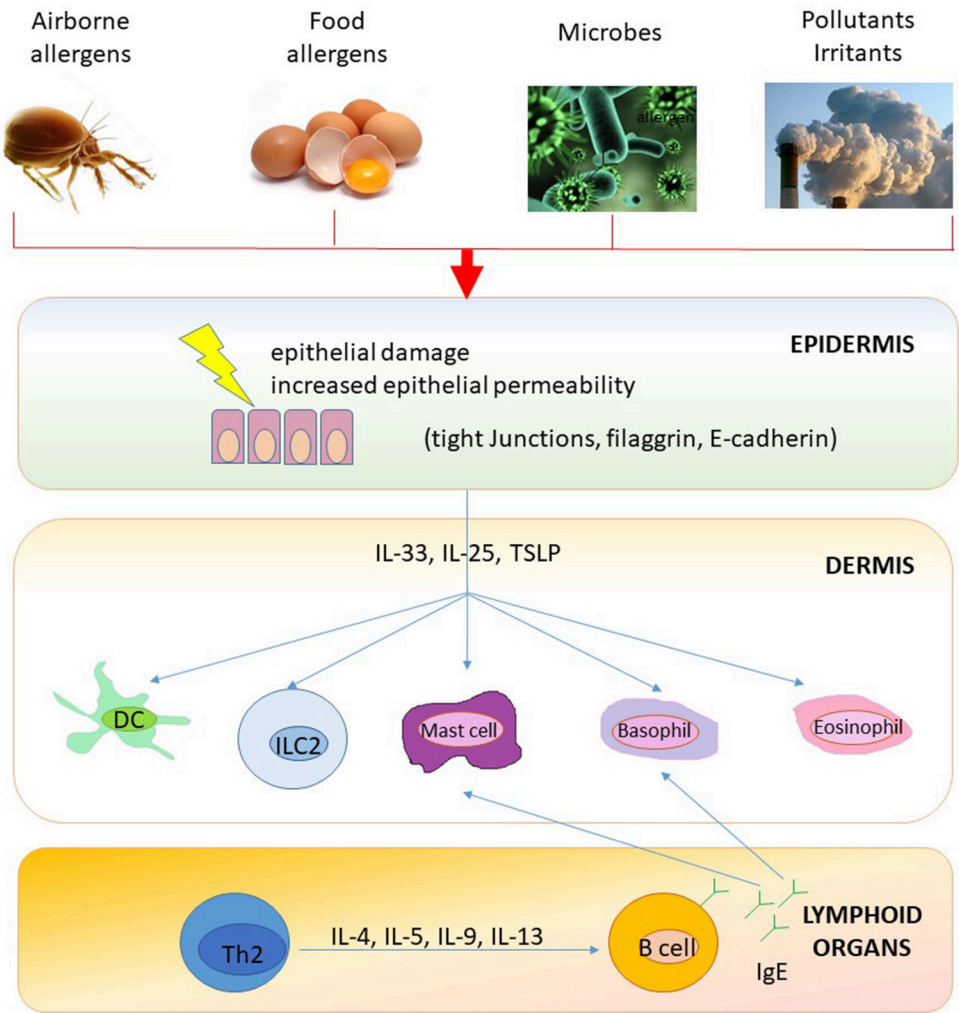

FIGURE 1 | A model of epithelial barrier damage and skin IgE-sensitization. Impaired skin barrier (e.g., eczema) promotes foreign antigen (e.g., airborne and food allergens) penetration and activation of innate and specific immune responses. Epithelial cell-derived cytokines (such as TSLP, IL-33, and IL-25) license antigen presenting cells (i.e., dendritic cells) to drive type 2 immune responses and stimulate several cell types (including basophils, eosinophils, mast cells, and ILCs) to start and maintain allergic inflammation also in regional draining lymph nodes (e.g., B-cell IgE skewing). Furthermore, T cells circulate back to infiltrate the skin or are distributed peripherally to other end organs to initiate diverse atopic disorders. DC, dendritic cells; Ig, Immunoglobulin; IL, interleukin; ILC, Innate Lymphoid cells; Th, T helper cells; TSLP, thymic stromal lymphopoietin.

Therefore, as AD and the subsequent atopic march mostly present in early infancy, primary, and secondary prevention should be attempted as early as possible to prevent asthma symptoms onset (35).

Allergic Rhinitis

Allergic rhinitis is almost ubiquitous in children with asthma living in urban areas. The presence of allergic sensitization to inhalant allergens and rhinitis symptoms is typically associated with early onset of severe asthma (36). Patients with AR report poorer asthma control, more exacerbations and emergency visits (37) and have more difficulty in achieving symptom control (38).

Perennial allergic rhinitis with seasonal exacerbations is considered the most severe phenotype and most likely to be associated with difficult-to-control asthma (39).

This means that treating coexisting allergic rhinitis could improve asthma control and reduce healthcare resource utilization (40).

Food Allergy

Respiratory symptoms as clinical manifestation of IgE-mediated food allergy (FA), usually, occur immediately after exposure to the offensive food and are accompanied by skin and/or gastrointestinal manifestations. Food allergen exposure occurs usually by ingestion, but the inhalation of food proteins (through dust or aerosolized particles) may also trigger respiratory symptoms (41).

Among patients with FA, asthmatic symptoms are more frequent in children, and especially in those with concomitant atopic dermatitis. In addition to respiratory symptoms occurring

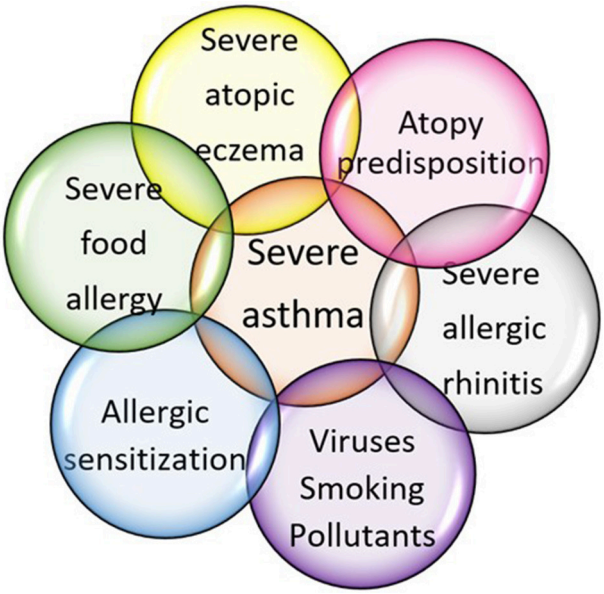

FIGURE 2 | Factors contributing to severe asthma development in childhood.

as a presentation of FA, patients with FA are at increased risk for developing asthma and often severe asthma as expression of progression of the atopic march. Symptomatic FA and food allergen sensitization are associated with asthma development both in younger and older children: it was reported that this association is stronger among children with multiple or severe FAs (42–44). Moreover, children with FA develop asthma earlier and at a higher prevalence than children without FA. The opposite is also true, as asthma is a risk factor for the persistence of food allergy (45–47).

Food allergy and food sensitization can be also considered as important markers to predict asthma severity. Indeed, it is reported that children with FA and sensitization to at least one food (e.g., egg, milk, soy, peanut, wheat, and fish) had worse lung function (48), higher rates of hospitalization, emergency department visits, use of systemic glucocorticoids (49), or need of mechanical ventilation for severe asthma exacerbation (50, 51).

Aspirin Sensitization

Aspirin-exacerbated respiratory disease (AERD) is a chronic medical condition, usually in adults and adolescents, consisting of three clinical features: sinus disease with recurrent nasal polyps, asthma, and sensitivity to aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Even though asthma is not always present in AERD, asthma symptoms develop 1–3 years after the development of rhinitis or later. When present, asthma is severe and difficult to treat and often characterized by increased residual volume and diminished diffusing capacity due to increased airway remodeling (52).

ROLE OF ATOPIC SENSITIZATION IN SEVERE ASTHMA

Previous studies showed that atopic sensitization is a major risk factor for severe asthma in children (53–55). Overall, the expression "atopic sensitization" refers to the positivity either of serum allergen-specific IgE (sIgE) or a positive skin prick test (SPT) to allergen extracts. Arbitrary cut-off points have been set: levels of sIgE >0.35 KU/l (56) and a mean wheal diameter ≥3 mm (SPT) (57). Though these tests are highly sensitive, their mere positivity do not mean itself clinical disease. Quantification of atopic sensitization increases the specificity in relation to childhood asthma presence and severity (4), and asthma persistence in adulthood (58–60). The severity of asthma correlates with both sIgE levels and the number of sensitizations, also on the molecular level. The number of component-specific sensitizations correlate with disease severity in **grass** allergic children (61) as well as in **house dust mites** allergic (HDM) pediatric patients. In the Multicentre Allergy Study (MAS) cohort, a birth cohort started in 1990 in Germany, the number of HDM-component specific sensitizations increased with disease severity and with age. Sensitization to Der p 1 and Der p 23 before the age of 5 years was predictive of asthma at school age (62). Similarly, in the Manchester Asthma and Allergy Study (MAAS) birth cohort, asthmatic children were characterized by more complex molecular patterns of IgE sensitization to grass and mite molecules (Der p 1 and Der p 2) (63). In a French study, atopic sensitization to Der p 2 and Der f 2 was more common in severe asthma. In a cohort of 300 asthmatic children (age range, 4–12 years), higher levels of Der p 1 and pet allergen [cat (Fel d 1), dog (Can f 1)] were found to be associated with greater asthma severity (64). Similarly, in **latex** allergy sensitization to 3 (5, 6.01/6.02) of the 12 recombinant natural rubber antigens so far known was strongly linked with asthma (65).

Fungal Allergy

Fungal allergy drives asthma severity, too (66). Sensitization to molds has been estimated: 7–20% in the general asthma population; 35–75% in severe asthma patients; 54–91% in life-threatening asthma population (67–69). The severity of exacerbation relates to the different fungi species: in particular, Aspergillus or Alternaria or *Cladosporium* spp. sensitization has been linked to severe asthma (70, 71).

Long-term or uncontrolled fungal infections are associated with a poor controlled asthma, bronchiectasis, and chronic allergic bronchopulmonary aspergillosis (ABPA) (71). The term "Severe asthma with fungal sensitization" (SAFS), introduced by Denning et al. (71), describes a specific phenotype in patients with persistent severe asthma (despite standard treatment) and evidence of fungal sensitization, and do not meet the criteria for ABPA. An EAACI Task Force sets the total IgE cut-off at <1,000 IU/ml for SAFS and >1,000 IU/ml for ABPA, a specific endotypes of asthma, with a genetic predisposition.

ROLE OF VIRUSES, SMOKING, AND POLLUTANTS

In poli-sensitized asthmatics, daily exposure to allergens combined with other enhancing factors, such as viral infections, smoking (even tertiary one), and/or environmental pollution, influences the asthma course and severity (**Figure 2**). There is robust evidence concerning the synergistic effect of **viral**

lower respiratory tract infections (LRTI) and IgE sensitization on asthma development, particularly in children predisposed to atopy (72) and asthma exacerbation (73). Increased risks of asthma inception in atopic predisposed children 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; and the age during viral LRTI (74). The risk of hospital admission due to asthma exacerbation is increased by the interaction among respiratory viral infections in combination with atopic sensitization and exposure to allergens (75).

Cigarette Smoking

Cigarette smoking itself may influence asthma severity, through different patho-mechanisms (76). There is evidence that smoking increases itself serum IgE levels, especially in male adults (77), and rises the risk of IgE sensitization, mainly to occupational allergens (78). Nevertheless, in severe asthmatic patients, the complex association between cigarette smoking and allergy remains currently controversial.

Data reports that children exposed to **air pollution** are at major to develop IgE sensitization to inhalant allergens (79, 80). However, the immunological mechanisms underlying this link remain to be better clarified. Notwithstanding, some data suggest that ultrafine carbon black particles may induce maturation of dendritic cells *in vitro* (81), which might then facilitate sensitization to airborne allergens. On the other side, airborne pollutants may modulate the inflammatory cellular response in the lungs, thereby lowering the threshold for sensitization.

PREVENTION STRATEGIES

As widely described above and resumed in **Table 2**, children with early onset atopy, high specific IgE-sensitization and multiple IgE-sensitizations are at increased risk for developing severe asthma in childhood. In addition, it is well-established that severe allergic diseases more frequently coexist (**Figure 2**).

Based on these premises, it is reasonable that prevention strategies and proper treatments of atopic diseases could prevent occurrence of severe asthma and viceversa. However, asthma development depends on complex and still not fully known interception of genes and environment. Therefore, effective primary prevention strategies for asthma, and especially for severe asthma in children, -though highly desirable- might be difficult to be identified both at population and individual level.

Primary Prevention

Some studies have suggested that maternal consumption of allergenic food (such us cow's milk, peanut, or fish) and vitamin D and E intake during pregnancy could be associated to decreased risk of allergy and wheezing in their offspring, respectively (82–84).

Conflicting results have been reported about the beneficial effect of maternal breastfeeding on pediatric asthma development (85). The role of prebiotics, probiotics, and synbiotic interventions (86) and other dietary supplements (such as nucleosides and nucleotides) (87) is under investigation. Overall, the level of evidence remains currently low or even very low because of the risk of bias, heterogeneity among studies, imprecision, and inconsistency of results, as well as indirectness of available research.

Instead, there is stronger evidence concerning maternal smoking and tobacco post-natal exposition. They are both associated to increased risk of asthma in offspring (88).

Atopic Dermatitis

Since the epithelium plays an important role in protecting against the development of allergic diseases and the occurrence of transcutaneous IgE-sensitization may precede airway sensitization (**Figure 1**), proactive emollient therapy able to make stronger the epithelial barrier may prevent or delay the development of IgE-sensitization in children affected by AD (89).

Allergic Rhinitis

It is well-known that AR and asthma often coexist. As they share genetic background, chronic airway inflammation pathway and similar triggers (allergen exposure, viral infections, cold air, and air pollution) (90), treatment of rhinitis can be beneficial for preventing severe asthma exacerbations.

The role of exposure to airborne allergens on AR and asthma is well-established and strict avoidance of the culprit allergen(s) is desirable though it is often hard or even impossible. Symptomatic drugs for AR, such as H1-antihistamines and intranasal corticosteroids are not recommended for asthma management (40). However, data suggest that the use of H1-antihistamines in AR children is associated with delayed asthma development (91) and improvement of asthma outcomes (92). Similarly, a significant reduction of asthma symptom scores and rescue medication use has been reported for patients with AR and coexisting asthma by using intranasal corticosteroid therapy (93). Moreover, anti-leukotrienes target both upper and lower airways and could be beneficial in patients with asthma and concomitant AR (94).

Biological drugs—such as anti-IgE therapy (i.e., omalizumab) and antibody against the α-subunit of receptor for IL-4 and IL-13 (i.e., dupilumab)- have been approved for a few specific phenotypes of severe asthmatic patients at different ages (**Table 3**). They could be beneficial on both diseases: severe asthma and severe AR (95, 96). Notwithstanding, allergen immunotherapy (AIT) is considered the only etiological treatment able to prevent asthma development (97), to improve asthma symptoms in AR affected children (98), and to prevent new sensitizations in already sensitized patients (99). Furthermore, since adverse events are more common during the escalation or build-up phases of AIT, omalizumab has been suggested as "add-on therapy" to AIT (100). Nevertheless, larger studies are needed to identify patients who would benefit the addition of omalizumab to AIT, as well as optimal dosing strategies and duration treatment (101).

Food Allergy

Strict avoidance of the culprit food(s) represents currently the standard therapeutic option for FA (102). However, accidental exposure is possible and related to severe adverse events. Allergen

TABLE 3 | Main biological targeted treatments for severe asthma in children.

Drug (trade name), dosage	Mechanism of action	Suggested population	Adverse effects
APPROVED			
Omalizumab (Xolair), s.c. injections every 2–4 wks, depending on body weight and IgE levels	Anti- IgE; binds Fc receptor of free circulating IgE and downloads IgE production	Age >6 yrs; 30 UI < IgE < 700 UI* ; positive skin test or elevated specific IgE level toward a perennial	Anaphylaxis (∼0.2% pts); monitor for helmintic infection
Mepolizumab (Nucala), 100 mg in pts aged 12 yrs or older (40 mg in pts aged 6–11 yrs) by s.c. injections every 4 wks	Anti- IL-5; binds circulating IL-5	Age >12 yrs; eosinophlic asthma	Zoster (rare); avoid if active helminithic infection
UNDER INVESTIGATION			
Reslizumab (Cinqair), approved for adults (3 mg/kg by i.v. injections every 4 wks)	Anti- IL-5; binds circulating IL-5	Eosinophilic asthma	Anaphylaxis (rare); avoid if active helmintic infection
Dupilumab (Dupixent), approved for adults with atopic dermatitis	Anti- IL-4 and anti-IL-13; binds common α-subunit of receptor for IL-4 and IL-13	Eosinophlic asthma	Eosinophilia (rare); avoid live vaccines; avoid if active helmintic infection

*IL, interleukin; i.v., intravenous; pt, patient; s.c., subcutaneous; wk, week; yr, year. *Upper limit varies according to body weight and regulatory authorities.*

specific immunotherapy (AIT) alone or combined with adjuvants (including probiotics and anti-IgE monoclonal antibodies) is nowadays the only active treatment for FA (103). Nevertheless, it is highly demanding, especially in patients with a severe phenotype, who usually have allergies to multiple foods and concomitant severe asthma. For these severe patients, biologicals might be a better potential therapy alone or in combination with AIT (104, 105). However, more data are needed.

AERD

Careful avoidance of aspirin and other NSAIDs is mandatory in patients affected by AERD in order to prevent asthmatic exacerbations. The use of acetaminophen or selective COX-2 inhibitors should be encouraged as alternative drugs. Furthermore, the addition of a leukotriene receptor antagonist (e.g., montelukast) and 5-lipoxygenase inhibitors (e.g., zileuton) to standard asthma treatment have been shown to be effective in improving asthma outcomes (106). Aspirin desensitization is currently the only causative treatment in AERD affected patients improving both upper and lower airway symptoms; however, only a very small percentage of patients benefit from this therapeutic option (107). Biologic therapies (such as anti-IgE, anti-IL-5 monoclonal antibodies, IL-4α receptor antagonist, and anti-thymic stromal lymphopoietin) could be promising therapeutic options for AERD patients given their effectiveness in nasal polyposis and asthma (108).

Unfortunately, there is currently lack of data about any specific role of the treatment of allergic comorbidities in preventing the development of severe asthma in atopic children.

OPTIMIZING TREATMENT OF SEVERE ASTHMA

The current guideline-based drug therapy of pediatric severe asthma is based primarily on data extrapolated from adult studies. High dose of inhalant **corticosteroids** (or oral corticosteroids) combined with a second controller (such as a LABA or leukotriene modifier/theophylline) are the mainstay of treatment (5).

However, the current challenge facing physicians and researchers is to provide a "**personalized medicine**," which is tailored to the diverse patho-mechanisms underlying clinical presentations (109).

Allergen Specific Immunotherapy

AIT has been demonstrated to have beneficial effects in the management of childhood allergic asthma, including effects on symptom control, medication use, and airway hyperresponsiveness (110). However, trials involve children with mild-moderate allergic asthma, and studies specifically examining the efficacy of AIT in children with severe asthma are missing. Notwithstanding, the majority of studies involved monosensitized patients, whereas most children with severe asthma are polysensitized, mainly in Southern Europe. In addition, allergen immunotherapy should be commenced in patients with well-controlled asthma, a situation which is less common among children with severe asthma. Since 2003, several **targeted therapies** have been approved for severe asthma and others are still under investigation (**Table 3**). Furthermore, they could be beneficial in other allergic comorbidities. However, their cost is often expensive. Therefore, a precise identification and selection of good responders is pivotal.

A **regular longitudinal assessment** of outcomes of children with severe asthma is pivotal. Follow-up appointments should be devoted not only to reduce maintenance therapy to the minimal amount required to achieve control of asthma symptoms, but also to assess the atopic status of the patient and any concomitant atopic co-morbidities and, therefore, address any modifiable factors (including allergen exposure, basics of inhaler technique, and adherence).

CONCLUSIONS

Asthma is a common disease in childhood with a minority of affected children having severe asthma. Several data suggest

that allergies may play a key role in children with severe asthma. Many children with severe asthma have coexisting allergic disease(s). Allergies to foods, molds, pollens, and pets have been associated with both asthma inception and severe asthma exacerbations. A better understanding of interactions between asthma and allergy and mechanistic implications of cofactors, such as virus infections, pollution, and smoking will allow the development of novel therapeutic targets and, therefore, additional strategies for improving disease control.

AUTHOR CONTRIBUTIONS

SA and FP wrote the first draft of the manuscript. AF and RC critically reviewed the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 19 June 2018
doi: 10.3389/fped.2018.00170

Difficult vs. Severe Asthma: Definition and Limits of Asthma Control in the Pediatric Population

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Evaluating the degree of disease control is pivotal when assessing a patient with asthma. Asthma control is defined as the degree to which manifestations of the disease are reduced or removed by therapy. Two domains of asthma control are identified in the guidelines: symptom control and future risk of poor asthma outcomes, including asthma attacks, accelerated decline in lung function, or treatment-related side effects. Over the past decade, the definition and the tools of asthma control have been substantially implemented so that the majority of children with asthma have their disease well controlled with standard therapies. However, a small subset of asthmatic children still requires maximal therapy to achieve or maintain symptom control and experience considerable morbidity. Childhood uncontrolled asthma is a heterogeneous group and represents a clinical and therapeutic challenge requiring a multidisciplinary systematic assessment. The identification of the factors that may contribute to the gain or loss of control in asthma is essential in differentiating children with difficult-to-treat asthma from those with severe asthma that is resistant to traditional therapies. The aim of this review is to focus on current concept of asthma control, describing monitoring tools currently used to assess asthma control in clinical practice and research, and evaluating comorbidities and modifiable and non-modifiable factors associated with uncontrolled asthma in children, with particular reference to severe asthma.

Keywords: asthma control and severity, severe asthma, difficult to treat asthma, asthma, treatment adherence, severe asthma risk factors

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *21 April 2018*
Accepted: *24 May 2018*
Published: *19 June 2018*

Citation:
Licari A, Brambilla I, Marseglia A, De Filippo M, Paganelli V and Marseglia GL (2018) Difficult vs. Severe Asthma: Definition and Limits of Asthma Control in the Pediatric Population. Front. Pediatr. 6:170. doi: 10.3389/fped.2018.00170

INTRODUCTION

Asthma is one of the most common chronic diseases affecting all age groups, with up to 20% of children aged 6–7 years experiencing severe wheezing episodes within a year (1). Childhood asthma is currently defined as a clinical umbrella syndrome encompassing symptoms such as wheezing, shortness of breath, chest tightness, and cough, ranging in severity from mild symptoms to life-threatening attacks (2). In this context, several pathophysiologic components (traits) have been identified in children such as airflow limitation, eosinophilic airway inflammation, airway infection and impaired airway defenses, some of which being treatable (2). The primary goal of asthma management and treatment is to achieve the control of symptoms and underlying airway inflammation, aiming at minimizing the risk of future asthma attacks and medication-related side effects, and preventing the progression of obstructive lung damage during growth and then later in life (3).

International guidelines emphasize the importance of evaluating asthma control, rather than asthma severity, in order to guide asthma management decisions (3–5). Asthma control is defined as the degree to which manifestations of the disease are reduced or removed by therapy (6, 7). Two components of asthma control are identified in the guidelines: symptom control and risk. Symptom control domain refers to daytime symptoms and nighttime symptoms (i.e., cough, wheeze, exercise limitation), reliever use (short-acting β2 agonists, SABA) for the treatment of symptoms, and deviation from normal levels of activity (i.e., playing, sleeping, attending work or school), preserving normal or near-normal lung function, and meeting patient and parent expectations. Future risk of poor asthma outcomes domain includes preventing severe asthma attacks (requiring systemic corticosteroids, emergency medical treatment or hospitalization), loss of lung function or impairment of normal lung growth in childhood, and adverse effects caused by medication use (3–7). Asthma severity reflects the intrinsic "activity" of the disease and should be typically assessed both prior to beginning treatment with controller medications, and then at regular intervals to determine degree of responsiveness to treatment. Thus, asthma severity is usually defined from the level of treatment needed to achieve and maintain adequate control (7).

Over the past decade, the definition and the tools of asthma control have been substantially implemented so that the majority of children with asthma have their disease well controlled with standard therapies. However, a small subset of asthmatic children (<5% of all pediatric asthma) still requires maximal therapy to achieve or maintain symptom control and experience considerable morbidity (8–11). The identification of the factors that may contribute to the gain or loss of control in asthma is essential in differentiating children with difficult-to-treat asthma from those with severe asthma that is resistant to traditional therapies. The aim of this review is to focus on current concept of asthma control, describing monitoring tools currently used to assess asthma control in clinical practice and research, and evaluating comorbidities and modifiable and non modifiable factors associated with uncontrolled asthma in children, with particular reference to severe asthma.

ASSESSMENT OF ASTHMA CONTROL

Evaluating asthma control starts with taking a detailed history of frequency of asthma symptoms, sleep disturbances, limitation of activity, and frequency of reliever use in the past 4 weeks. Treatment issues (adherence to therapy and inhaler technique) as well as comorbidities (i.e., nasal disease, eczema, food allergy, obesity, etc.) and factors that may complicate care should be also periodically reviewed (3).

Asthma attacks are characterized by an acute worsening of asthma symptoms in response to a trigger such as allergen exposure, viral infection, environmental irritants (including pollution and cigarette smoke) or a combination of these. Given that asthma attacks can occur also on the background of seemingly good control and normal lung function (12), the concepts of acute asthma attacks and asthma baseline control, although overlapping, are not the same.

Symptom Control

Common tools for monitoring asthma available in a clinical practice setting can be distinguished in subjective measures, such as asthma diaries and questionnaires-based composites asthma scores, and objective measures, including spirometry, bronchial hyperreactivity tests and biomarkers of airways inflammation (7).

Subjective Measures

Subjective measures of asthma control include asthma diaries and asthma symptom questionnaires; moreover, visual analog scale (VAS) may be introduced in assessing patient's airways obstruction perception. These tools were initially introduced in clinical research; nevertheless, they are spreading also in clinical practice, since they enable the physicians to strictly monitor their patients' symptoms trends. Each of these tools presents pros and cons, that are briefly reported also in **Table 1**.

Asthma diaries are created to obtain data regarding asthma-related events on a daily basis. So far asthma diaries validated for clinical practice or for clinical trials are the Pediatric Asthma Diary (PAD) (13), the Pediatric Asthma Caregiver Diary (PACD) (14), the daytime and nocturnal asthma symptom diary scales (15) and the Asthma Control Diary (ACD) (16). Asthma diaries collect data day-to-day, so that they do not depend on patient recall. This feature enables the physician to monitor asthma-related events in real time and to promptly adjust therapy. The main concern regarding paper diaries is represented by the risk of missing large amounts of data, since this kind of diaries can be retrospectively filled-in by the patient, either with invented data or with false data. In order to solve this problem, the diaries should be administered day-by-day through telematics-primed device, for instance telephone-administered diaries or online diaries (17–19). This solution has been already adopted in allergic rhinitis (20): as a matter of fact, the MASK-rhinitis study collected symptoms referred by patients, by means of a user-friendly smartphone application; so similar "apps" might be developed in order to be administered to asthmatic patients. Each asthma diary collects a large amount of data for each patient, so another concern about this tool regards the way to evaluate and analyse data derived from asthma diaries in clinical trials. Mixed model analyses should be adopted in order to report the large amount of longitudinal data collected by asthma diaries (21). Furthermore, there is not a standardized method to account for outcome data from asthma diaries: some studies used either "symptom-free days" (22) or "symptom days" (23) so as to obtain information about cost-benefit analysis. Other studies reported the proportion of patients with at least one event or the rate of annual events (24). Both these methods reveal the efficacy of medication in the population level, although a small number of patients may be responsible for a large number of events. Another method to report diaries-derived data is "time to first event" (25), which can estimate the impact of treatment on disease progression.

Asthma symptom questionnaires are intended to monitor asthma-related events over a period between 1 and 4 weeks.

TABLE 1 | Subjective measures of asthma control.

	Pros	Cons
Asthma Diaries	Do not depend on patient recall Monitor asthma-related events in real time	They can be retrospectively filled in Each diary collects large amount of data Lack of a standardized method to account for outcome data
Asthma Symptom Questionnaires		
ACQ, ACT, cACT, TRACK	Changes in composites asthma scores correlate to clinical deterioration and to the need of step-up therapy Provide data easy to analyze	Depend on patient recall Focus on a small-time window
CASI	Identify differences between patients who could seem similar only on the basis of their clinical symptoms Useful in clinical research to test impact of additional treatment in the context of standard care	Need for additional studies to validate the application of this tool Its role in clinical practice is unclear It does not include some relevant aspects of disease control
APGAR	Provides a wider spectrum of information than other questionnaires, about features that can be crucial in therapeutic planning It is linked to a care algorithm, which can help the physician to undertake a therapy	Need for additional studies to validate the application of this tool in clinical practice or in clinical research
VAS	Quick and feasible tool for "real-life" monitoring VAS <6 is a reliable marker of uncontrolled asthma Provides information about symptom perception	Need for additional studies to validate the application of this tool in clinical practice or in clinical research

ACQ, Asthma Control Questionnaire; ACT, Asthma Control Test; APGAR, Activities, Persistent, triGGers, Asthma medications, Response to therapy; cACT, Childhood Asthma Control Test; CASI, Composite Asthma Severity Index; TRACK, Test for Respiratory and Asthma Control in Kids; VAS, Visual Analog Scale.

They can be administered only to patients (i.e. Asthma Control Questionnaire–ACQ; Asthma Control Test–ACT), or both to patients and their caregivers (i.e., Childhood Asthma Control Test–ACT), or only to caregivers (i.e., Asthma Therapy Assessment Questionnaire–ATAQ; Test for Respiratory and Asthma Control in Kids–TRACK). Their items usually include the frequency and impact of daytime symptoms, nocturnal symptoms, limitation of normal activities; some questionnaires include also questions about exercise induced-dyspnea (cACT and TRACK) and just one questionnaire evaluate also a measure of lung function (ACQ) (7). From all these data, each questionnaire elaborates a composite score, which express the level of asthma control. Four questionnaires (ACQ, ACT, cACT, TRACK) provide a cut-off value that enables the physician to distinguish between uncontrolled vs. controlled asthma, while ACT and ATAQ have a cut-off value to identify poorly controlled asthma (7). In clinical practice it may be useful to monitor for each patient how the answer to a specific question changes, so as to understand whether the disease is under control or not. Some changes in composites asthma scores correlate to clinical deterioration in asthma control and, consequently, to the need of a step-up of therapy (26). In clinical trials asthma questionnaires are more useful than asthma diaries because their provided data are more easily analyzable than those derived from asthma diaries. As a matter of fact, they have been used to assess the efficacy of different treatments (27, 28).

The answers given to asthma questionnaires depend on patient recall. Moreover, the questionnaires focus on a small-time window, which can not entirely reflect the level of disease control, because patients could improve their adherence to therapy just before clinical visit, recent events or recent period of bad control may bias reporting of the whole recall period and the trend of disease attacks can be inconstant through different seasons. In c-ACT, that is administered both to patients and to their caregivers, usually children give lower scores to asthma control than those given by their parents; in addition to that, some studies demonstrated that the correlation between c-ACT score and forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) is stronger in newly diagnosed asthma, while it becomes weaker in patients who undergo treatment for asthma (29).

VAS is a measurement instrument designed as 10 cm–long ruler, on which the patient is asked to put a mark corresponding to his symptom perception, considering that "0" represents "severe airflow obstruction perception," while "10" represents "no respiratory symptoms." VAS has been already introduced in some allergic conditions (such as rhinitis) as a quick, achievable instrument to monitor in "real-life" setting whether the disease is under control or not (30). Recently VAS has been suggested as a useful tool also for evaluating asthma symptoms perception: there are some evidence that a VAS <6 is a reliable marker of uncontrolled asthma in adults (31) and that, in children with bronchial obstruction, poor VAS scores well correlate with respiratory function decrease; moreover, after bronchodilation test, children with initial bronchial obstruction usually report a significant improvement in VAS scores (32). However, the perception of dyspnea has been little studied in severe pediatric asthma (33) and additional studies are needed to validate the application of this tool in clinical research and then in clinical practice.

Composite Asthma Severity Index (CASI) is a subjective measure based on multiple aspects of asthma severity, such as

impairment, risk and treatment. It accounts for five domains of disease, which include symptoms (day symptoms and night symptoms), controller medication use, lung function tests and asthma attacks. The final score can go from 0 to 20: the higher it is, the more severe the disease is quantified (34). CASI estimates asthma severity by means of the level of medication needed to obtain the present clinical situation, in addition to impairment features and future risk measures. This peculiarity enables the examiner to identify differences between patients who, otherwise, could seem similar only on the basis of their clinical symptoms. Therefore, it is useful for clinical research, since it is sensitive in discriminating among treatment groups even in a well-controlled population, and it can detect the effect of a specific intervention in addition to guidelines-based treatment (35). However, this score needs to be validated on a larger population and its feasibility in clinical practice is unclear. Finally, it does not include some relevant aspects of disease control (i.e. quality of life and economic costs of asthma) (35).

The Asthma APGAR is a newly introduced tool for primary assessment of asthma. The acronym "APGAR" stands for "Activities, Persistent, triGGers, Asthma medications, Response to therapy," it consists in a sequence of questions regarding not only the number of asthma attacks, the presence of diurnal or nocturnal symptoms or the limitation of normal activities due to asthma symptoms, but also the triggers of attacks, the way and the frequency with which the patients take their medicine and whether they think their medicines work or not. Its main distinctive feature is that it gathers a wider spectrum of information than other questionnaires: it mainly focuses on some peculiar aspects of the disease, which are not investigated by means of other questionnaires, but which can be pivotal for tailoring therapy (36). APGAR's questionnaire is also linked to a care algorithm, which is thought to help the physician to undertake a therapy, while all of the other questionnaires so far adopted only give the physician a simple score. Further evidences are needed to validate this tool as a reliable instrument in clinical research and/or in clinical practice.

Beyond electro-monitoring devices, mobile phone-based apps, gadgets and wearable devices, another tool assessing the degree of asthma disease control may be represented by Telemedicine. According to the American Telemedicine Association definition, it consists in "the remote delivery of health care services and clinical information using telecommunications technology" (37). It makes use of internet monitoring, text messages, email reminders. Its efficacy in controlling asthma is still unclear, and it may be cost-effective in rural communities or in underserved areas.

Objective Measures

No objective gold-standard measure is currently available to diagnose asthma. Among objective measures commonly used to diagnose and monitor asthma, we can identify lung function tests, airway hyperresponsiveness (AHR) tests (**Table 2**) and biomarkers of airways inflammation.

Lung function tests, particularly spirometry, are objective, noninvasive, and extremely helpful in the diagnosis and follow-up of patients with asthma. Examination of the FVC, FEV_1, and forced expiratory flow rate over 25%−75% of the FVC (FEF_{25-75}) is a reliable way to detect baseline airway obstruction. An obstructive airflow pattern is defined by a reduced FEV_1 (<0.80), a reduced FEV_1/FVC ratio (normally >0.75 to 0.80, and usually >0.90 in children) and a concavity of the expiratory flow volume loop during a spirometry test (38). FEV_1/FVC ratio is the most sensitive tool to assess the airflow obstruction and it is related to asthma severity; as a matter of fact, it can predict asthma-related morbidity and mortality even when FEV_1 is still normal (39). However, younger children may have briefer exhalation times and/or lower lung volumes than adults, so that their FEV_1 may be comparable to their FVC and, consequently, their FEV_1/FVC ratio may result normal even in case of airways obstruction (40). FEF_{25-75} has been proposed as a sensitive indicator of small airway obstruction and a better indicator of a response to bronchodilators and AHR than either FEV_1 or FVC; it can be considered normal when it is ≥70% of predicted FEF_{25-75} (41). Unfortunately, its great variability makes it unreliable as exclusive tool to assess airflow obstruction. In patients affected by asthma, it decreases earlier than other spirometry parameters and is able to predict long-term asthma persistence (42).

TABLE 2 | Obejctive measures of lung function.

	Pros	Cons
Spirometry	Objective, noninvasive, helpful for diagnosis and follow-up FEV_1/FVC ratio can predict asthma-related morbidity and mortality even when FEV_1 is still normal FEF_{25-75} is a sensitive indicator of small airway obstruction and a better indicator of a response to bronchodilators	It relies on patients' ability to carry out the test (unlikely applicable in children younger than 6 years old) Children may have briefer exhalation times and/or lower lung volumes than adults, consequently, their FEV_1/FVC ratio may result normal even in case of airways obstruction Its great variability makes it unreliable as exclusive tool to assess airflow obstruction
PEF	Useful information about obstruction in the large central airways	The test is extremely effort-dependent
AHR	Objective, replicable, useful for diagnosis Exercise-induced bronchoconstriction is more specific than other provocation tests in detecting asthma among pediatric population	Unlikely applicable in young children It relies on patients' ability to carry out the test

AHR, airway hyperresponsiveness; FEF_{25-75}, forced expiratory flow rate over 25–75% of the FVC; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; PEF, peak of expiratory flow.

Documentation of reversibility of air flow obstruction following inhalation of a bronchodilator is central to the definition of asthma. After bronchodilation, an improvement of at least 12% and 200 mL in the FEV_1 is considered a positive response and is indicative of reversible air flow obstruction; however, in children, an improvement of 10% may be adequate to indicate significant improvement. It has been proved that the persistence of a significant degree of bronchodilator responsiveness despite regular treatment according to guidelines may represent a marker of worse asthma control (43, 44). Higher bronchodilator reversibility has been added as an additional independent risk factor for asthma attacks in both adults and children in 2018 update of the Global Strategy for Asthma Management and Prevention (3). Besides, reversible or partially reversible airflow obstruction seems to be a distinctive spirometry feature of pediatric severe asthma clinical phenotypes, as demonstrated by the results from severe asthma cohort studies (45, 46).

Although spirometry is still considered the gold standard for use in diagnosing and monitoring change in airway function in patients with asthma, other modalities may have particular application in both younger and older children. The Forced Oscillation Technique (FOT) is a lung function modality based on the application of an external oscillatory signal in order to determine the mechanical response of the respiratory system. The method is noninvasive and requires minimal patient cooperation, which makes it suitable for use in young pediatric patients. In the context of asthma, FOT may be more sensitive than spirometry in identifying disturbances of peripheral airways and assessing the level of asthma control or the effectiveness of therapy at the long term (47). Further research is required to determine the exact role of FOT as an objective monitoring tool in pediatric asthma.

Measuring peak of expiratory flow (PEF) may provide some useful information about obstruction in the large central airways. An exaggerated variability in PEF (morning-to-evening more than 20%) can confirm the diagnosis of asthma; however, the test is extremely effort-dependent and should not be used alone to diagnose asthma. Variability in PEF was suggested as an indicator of poor asthma control (48); however, studies evaluating the efficacy of PEF monitoring for improving various outcome measures in childhood asthma have yielded conflicting results (49–51).

AHR can be demonstrated either by direct inhaled agents (i.e., methacholine) or by indirect stimulants (i.e., exercise). Provocation tests with direct inhaled agents result positive if they cause a 20% fall in baseline FEV_1, but they are not routinely used in pediatric population (52). Exercise challenge testing consists in assessing the FEV_1 variations after 6–8 min of treadmill exercise; a decrease in FEV_1 of more than 10% is diagnostic of exercise induced bronchoconstriction (53). There is some evidence that exercise-induced bronchoconstriction is more specific than other provocation tests in detecting asthma among pediatric population (54).

Airway inflammation is the hallmark of disease pathophysiology in asthma. Currently bronchoscopy, bronchial biopsy, and bronchoalveolar lavage (BAL) are considered the gold standard to assess airway inflammation and remodeling in asthma; however, the invasiveness of these diagnostic methods limits their use in pediatric age in daily clinical practice. Over the last 10 years, there has been an explosion of interest in the research of non-invasive biomarkers to assess of airway inflammation. Biomarkers for asthma have potential utility for distinguishing the inflammatory and/or molecular pattern or "endotype," predict and monitor responsiveness to specific treatments, and assess of the risk of disease progression (55). Thus, the identification of non-invasive methods to study and monitor disease inflammation represents a relevant challenging field of research in childhood asthma. Most of the current established biomarkers available in clinical practice are related to allergic eosinophilic (Th2-high) inflammation. These include blood or sputum eosinophils, serum IgE, and fractional exhaled nitric oxide (FeNO) (55). Single and combination biomarkers are now being recommended for use in the assessment of children with asthma, in particular with severe asthma, and have been extensively reviewed elsewhere (55). Among these, fractional exhaled NO (FeNO) monitoring has a validated role in current clinical practice as a biomarker useful in asthma diagnosis, monitoring control, and predicting asthma attacks (56, 57). Its measurement is simple, safe, and well tolerated, and it has been standardized in school-aged children (58, 59). In pediatric asthma, FeNO is now recognized as a surrogate marker of eosinophilic airway inflammation and it is used to identify children with allergic asthma that are likely to respond to ICS treatment (60). Multiple studies have demonstrated that an increased FeNO value at baseline or increasing FeNO values during ICS reduction accurately predict an asthma attack (60). According to the cut-off values published in the American Thoracic Society (ATS) guidelines, a FeNO of > 35 ppb suggests a likely response to ICS, while a FeNO of <20 ppb in children indicates a less likely responsiveness to ICS treatment (61). Although FeNO-guided treatment has been associated with significantly fewer asthma attacks and lower attack rate than treatment based on current guidelines in childhood asthma, it not routinely recommended for the general asthma population at present (62). Identifying the populations most likely to benefit from FeNO-guided treatment, as well as establishing the optimal frequency of monitoring FeNO, still require further studies.

Risk Factors for Poor Asthma Outcomes

The evaluation of symptom control should be always combined with the assessment of risk factors of adverse outcome in children with asthma, both at diagnosis and periodically thereafter.

First of all, having uncontrolled asthma symptoms, experiencing more than 1 episode of asthma attack in last year and/or admission to intensive care unit for asthma are the main independent risk factors for future attacks (63–67); other risk factors that are potentially modified have been recently updated and include: (i) high SABA use (more than 3 canisters/year) (68) and inadequate inhaled corticosteroids (ICS) therapy (not prescribed, poor adherence, incorrect inhaler technique) (69, 70); (ii) low FEV_1 (even if normal spirometry does not exclude severe asthma in children) (71, 72) and higher bronchodilator reversibility (44); (iii) viral infections (rhinovirus and other respiratory viruses), allergen exposure in atopic children, tobacco smoke exposure and outdoor air pollution

(65). Eosinophilic airway inflammation has been associated with risk of attacks that can be prevented with corticosteroid treatment (2). Blood eosinophils and FeNO have been identified as indirect measures of eosinophilic airway inflammation both in adults and children. These reliable biomarkers may provide a better perspective on risk of attacks and the likely response to treatment with corticosteroids than traditional physiological measures as lung function and asthma symptoms (2). However, blood eosinophilia and elevated FeNO have been recognized as risk factors for acute attacks mainly in adults with allergic asthma taking ICS (3). Overall, it has also been recently established that the presence of any of these mentioned conditions increases the risk of asthma attacks, even if the patient has few asthma symptoms (3).

The severity of asthma attack is a risk marker of both subsequent attacks and mortality from asthma (73). A recent revision of risk factors for severe asthma attacks identified nonwhite race, psycho-social stress and obesity as additional clinical predictors in children (67). Furthermore, cadherin-related family member 3 (CDHR3) has been identified as a novel susceptibility gene for recurrent severe asthma attacks in children ages 2–6 years: in particular, variants of CDH3 seem to alter the integrity of airway epithelium and subsequently promote entry and replication of respiratory viruses (74). However, with the exception of history of one recent severe attack, no current clinical or biological markers has been shown to be highly predictive of severe asthma attacks in children (67). Finally, the recent UK National Review of Asthma Deaths (NRAD) identified the conditions at high risk for death from asthma: (i) a single severe attack; (ii) recent discharge from hospital after an acute asthma attack; (iii) use of hospital urgent care facilities in the previous year; (iv) utilization of more than 6 canisters of SABA/year; and (v) failure to attend follow-up appointments[1]. Thus, an asthma attack (even one) should be carefully considered a significant immediate red flag signaling a high risk of future attacks and asthma deaths, in particular in primary care. Moreover, considering that NRAD reported that around 60% of those who died from asthma were classified as "mild to moderate," the definition of severity by level of treatment should need to be questioned. All these mentioned factors should be added to the conventional definitions of risk as "high risk factors" with the aim to improve care and hopefully reduce the number of deaths.

Early growth characteristics such as pre-term birth, low birth weight and greater infant weight gain have been recently added to the list of risk factors of poor asthma outcomes, all being determinants of airflow limitation and associated with increased risk of childhood asthma (3, 75).

Children with uncontrolled asthma, in particular if treated with high-dose ICS and oral corticosteroids (OCS), may experience medication side-effects (i.e. local and systemic side-effects) (76, 77). The risk factors for medication side-effects include frequent use of oral steroids, long-term, high-dose and/or potent ICS use, or concomitant use of P450 inhibitors (such as ritonavir, ketoconazole, itraconazole) that can markedly increase both bioavailability and decrease clearance of most of the ICS (3). Ongoing monitoring of medication side-effects should be an essential component of a comprehensive childhood asthma management program.

UNCONTROLLED ASTHMA: DIFFICULT VS. SEVERE ASTHMA IN CHILDREN

The actual asthma management is control-based, including evaluation of symptom control and risk domains; therapeutic strategies are based on a stepwise approach and adjusted in a continuous cycle involving assessment, treatment and review (3). Asthma severity should be determined before the patient is treated, while the assessment of asthma control should be performed after treatment has been instituted; then, both of them should be re-determined at every visit. Step up and step down of treatment should be adapted to every patient in order to maintain asthma control with the minimum dose of medication. The frequency of the assessment of asthma control is variable and depends on disease activity but typically is every 1–6 months. Patients with asthma may be classified into three broad categories as well controlled, partly controlled, or uncontrolled, according to established criteria (**Table 3**) (3).

Identifying children and adolescents with uncontrolled severe asthma is important because they potentially need close monitoring and additional treatment with advanced biological therapies (78–82). Although accounting for <5% of all pediatric asthma (83), uncontrolled severe asthma carries the majority of morbidity and accounts for nearly 50% of all asthma healthcare costs, and even mortality (8). Children with persistent uncontrolled asthma, despite maximal therapy, are defined as having problematic severe asthma, an umbrella term comprising asthma-mimicking conditions, asthma that is difficult-to-treat because of comorbidities, improper inhaler technique or poor therapeutic adherence, and other environmental factors, and true severe therapy-resistant asthma, as defined by the latest European Respiratory Society/American Thoracic Society (ERS/ATS) definition (8, 84, 85): after confirming a diagnosis of asthma

TABLE 3 | Levels of asthma symptom control (to assess retrospectively in the past 4 weeks). adapted from Global Initiative for Asthma (3).

	Well-controlled	Partly controlled	Uncontrolled
Daytime asthma symptoms more than twice a week	None of these	1 or 2 of these	3 or 4 of these
Night waking due to asthma			
Need for reliever* for symptoms more than twice a week			
Limitation of activity due to asthma			

Excluding before exercise.

[1]Data from National Review of Asthma Deaths' (2014) Available online at: https://www.rcplondon.ac.uk/projects/outputs/why-asthma-still-kills.

and addressing comorbidities, true severe therapy-resistant asthma is that which requires treatment with high dose inhaled glucocorticoids plus a second controller and/or systemic glucocorticoids to prevent asthma from becoming 'uncontrolled' or which remains 'uncontrolled' despite this therapy (8). The identification of the factors that may contribute to the gain or loss of control in asthma is essential in differentiating children with difficult-to-treat asthma from those with severe asthma that is resistant to traditional therapies.

Monitoring and Managing Comorbidities

Once alternative diagnoses have been excluded and the diagnosis of asthma confirmed, the contribution of comorbid conditions to disease severity should be evaluated. Childhood asthma is associated with several comorbidities (**Table 4**), variably presenting and depending on the age of the subject (86–89). Observations from severe asthma international registries and cohort studies highlighted a distinct picture for pediatric age. In the National Heart, Lung and Blood Institute's Severe Asthma Research Program (SARP) the highest prevalence of comorbid conditions, namely sinus disease, gastroesophageal reflux and obesity, has been found in only 20% of children studied (clustered as "Group 3") (90). In the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED), around 65% of school-aged children with severe asthma had a diagnosis of concomitant allergic rhinitis, while 40% of children reported food allergy (91). Unlike upper airway pathology, the relationship between gastroesophageal reflux and asthma is still a matter of debate and there is not enough evidence to support causality; besides, it has been demonstrated that treating reflux does not actually improve asthma outcomes (92). Likewise, the relationship between food allergy and respiratory symptoms is still unclear.

Dysfunctional breathing has been reported as common in some asthma cohort; however, the available data suggest that 5.3% or more of children with asthma have dysfunctional breathing and that, unlike in adults, it is associated with poorer asthma control (93). Besides, the results of an observational cohort study conducted on 71 children with problematic asthma reported that 15% of them has dysfunctional breathing, including hyperventilation and vocal cord dysfunction (94). Although it is not possible to draw conclusive estimates on the prevalence of comorbidities in childhood severe asthma, it is well established that the presence of one and/or more comorbid conditions contributes to the loss of asthma control and may complicate the assessment of asthma and potentially impact on its management, outcome and healthcare expenditure (8, 86, 88). Thus, addressing comorbidities is an essential step in the approach to the child with uncontrolled asthma.

Adherence to Treatment

Determining if the child is receiving the prescribed controller medication with the adequate inhaler technique is another essential cornerstone of the management of childhood asthma. Poor adherence to treatment is common in asthmatic children, as well as in adults, with reported rates varying from 30 to 70% (85, 94, 95) and it should be considered in all children with uncontrolled asthma. Medication issues do not only concern about therapeutic adherence, but also affect management of prescriptions, parental supervision, and use of inhaler devices (70, 94, 96). These are all potentially modifiable factors that have been associated with loss of asthma control (97). Interventions to improve patient adherence have been tested in several clinical trials and demonstrated a variable efficacy (94). Identifying non-adherent patients and establishing an educational intervention can be complex and time-consuming and require an integrated strategy in everyday clinical practice including regular follow-up assessment at the doctor office, effective patient-centered behavioral interventions, improvement of doctor-patient communication, and new instruments of control such as electronic monitoring (85).

Review of Environmental Factors

Ongoing exposure to environmental factors is an important cause of poor asthma control in some patients (98). For patients with severe asthma who have an allergic component to their disease, allergen control measures will have an important effect. Potential triggers should be identified during evaluation and need renewed strategies for control. Common inhalant allergens that can contribute to poor asthma control and cause attacks include animal allergens (both pets and pests: cats, dogs, rodents), house dust mites, cockroaches, indoor and outdoor fungi and outdoor plant allergens (tree, grass, ragweed pollen) (99–101). In particular, home and school exposures to pets, house dust mites, mold are associated with severe asthma attacks in children, supporting the importance of atopy in this population (98).

Fungal exposure and sensitization have been recently associated with a sub-phenotype of childhood asthma characterized by increased disease severity, AHR, airway eosinophilic inflammation, more exacerbations and relative

TABLE 4 | Asthma comorbidities in childhood.

Comorbid Conditions	Potential mechanism contributing to worsen asthma
Obesity	Mechanical effects on lung functions; pro-inflammatory state contributing to airway inflammation; corticosteroid resistance
Gastroesophageal reflux	Direct contamination of the lower airway; esophago-bronchial reflex; reduced efficiency of the lower esophageal sphincter due to altered configuration of the diaphragm during respiratory disease
Food allergy	Unclear (consider anaphylaxis at rest and on exercise in the differential diagnosis)
Rhinosinusitis	Shared complex inflammatory mechanisms between upper and lower airways, according to the "United Airways Disease" concept
Upper Airway Obstruction/Sleep Disordered Breathing	Obesity-associated (common); increased neutrophilic inflammation of the airways
Dysfunctional Breathing	Unclear (also consider vocal cord dysfunction and other hyperventilation syndromes in the differential diagnosis)

steroid resistance (102). Severe asthma with fungal sensitization (SAFS) is a recognized subphenotype of severe asthma in both children and adults. SAFS is defined as severe, therapy-resistant asthma with fungal sensitization demonstrated by a positive skin prick test response or specific IgE to at least one of 7 fungi (i.e., Aspergillus fumigatus, Alternaria alternata, Cladosporium herbarum, Penicillium chrysogenum, Candida albicans, Trichophyton mentagrophytes, or Botrytis cinerea), with IgE <1,000 and with negative IgG Aspergillus serology (102). Little is known about the mechanisms mediating this subphenotype. Recent evidence suggests a role for innate epithelial cytokine IL-33 in the pathogenesis of SAFS, as higher levels of IL-33 have been found on BAL and airway samples from children with SAFS compared to children without SAFS (102). Till date there are no guidelines for the diagnosis and treatment of SAFS in pediatric population: reduction of environmental fungal exposure in the home and antifungal therapy have been anecdotally successful in some children.

There are also non-allergic factors that potentially contribute to asthma such as exposure to tobacco smoke (including active and passive smoking, as well as vaping), environmental pollution and other irritants (i.e., incense, joss sticks, air fresheners and other aerosol sprays) (103). Tobacco smoke exposure is common in children with asthma and it has been reported in 25% of cases in a cohort of children having problematic severe asthma (94); it is also associated with loss of asthma control, higher incidence of respiratory infections, increased likehood of asthma attack-related hospitalizations (104–107); furthermore, it was found that parental (passive) smoking impairs histone deacetylase-2 function, which could contribute to increased corticosteroid-resistant inflammation in children with severe asthma (108). In addition, exposure to tobacco smoke exacerbates inflammatory airway responses to allergens (109). Exposures at home, daycare, school and/or work should be reviewed.

Evaluation of Psychosocial Factors

Asthma attacks may be triggered by both acute and chronic stress, through a supposed effect of enhancement of allergic eosinophilic airway inflammatory response (110). Among psychosocial issues, anxiety and depression have been more frequently reported in children with persistent and severe asthma rather than mild or intermittent asthma, and in their families (94, 111, 112). Given the complexity of relationship between these two entities, it is yet to be determined whether anxiety and depression are the cause or result of severe asthma; however, both should be treated on their individual merits.

CONCLUSIONS

Over the past 10 years, there has been an increasing interest on the concept of asthma control, with development and validation of new and promising "asthma control tools."

Uncontrolled persistent asthma in children represents a clinical challenge and requires a multidisciplinary systematic assessment, including the assessment of comorbid conditions, treatment- related issues, environmental exposures and psychosocial factors. The presence or absence of these factors may contribute to the gain or loss of control and differentiate children with difficult-to-treat asthma from those with true severe therapy-resistant asthma. Finally, the early identification of modifiable factors contributing to childhood uncontrolled asthma is essential to avoid a further and useless escalation of treatment; likewise, addressing a correct diagnosis of true severe therapy-resistant asthma avoids deferring of invasive testing and advanced biological therapies.

AUTHOR CONTRIBUTIONS

All authors made substantial contribution to the conception of the work, reviewed the literature on the subject, and drafted the final version of the manuscript; AL, VP, and GM revised it critically for important intellectual content. All authors finally approved the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 03 October 2018
doi: 10.3389/fped.2018.00276

Asthma: Differential Diagnosis and Comorbidities

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Childhood asthma remains a multifactorial disease with heterogeneous clinical phenotype and complex genetic inheritance. The primary aim of asthma management is to achieve control of symptoms, in order to reduce the risk of future exacerbations and progressive loss of lung function, which results especially challenging in patients with difficult asthma. When asthma does not respond to maintenance treatment, firstly, the correct diagnosis needs to be confirmed and other diagnosis, such as cystic fibrosis, primary ciliary dyskinesia, immunodeficiency conditions or airway and vascular malformations need to be excluded. If control remains poor after diagnostic confirmation, detailed assessments of the reasons for asthma being difficult-to-control are needed. Moreover, all possible risk factors or comorbidities (gastroesophageal reflux, rhinosinusitis, dysfunctional breathing and/or vocal cord dysfunction, obstructive sleep apnea and obesity) should be investigated. At the same time, the possible reasons for poor symptom control need to be find in all modifiable factors which need to be carefully assessed. Non-adherence to medication or inadequate inhalation technique, persistent environmental exposures and psychosocial factors are, currently, recognized as the more common modifiable factors. Based on these premises, investigation and management of asthma require specialist multidisciplinary expertise and a systematic approach to characterizing patients' asthma phenotypes and delivering individualized care. Moreover, since early wheezers are at higher risk of developing asthma, we speculate that precocious interventions aimed at early diagnosis and prevention of modifiable factors might affect the age at onset of wheezing, reduce the prevalence of persistent later asthma and determine long term benefits for lung health.

Keywords: differential diagnosis, asthma, diagnosis, comorbidities, wheeze, asthma mimics

OPEN ACCESS

Edited by:
Michael David Shields, Queen's University Belfast, United Kingdom

Reviewed by:
Surendran Thavagnanam, The Royal London Hospital, United Kingdom
Louise Jane Fleming, Imperial College London, United Kingdom

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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *05 May 2018*
Accepted: *12 September 2018*
Published: *03 October 2018*

Citation:
Ullmann N, Mirra V, Di Marco A, Pavone M, Porcaro F, Negro V, Onofri A and Cutrera R (2018) Asthma: Differential Diagnosis and Comorbidities. Front. Pediatr. 6:276. doi: 10.3389/fped.2018.00276

INTRODUCTION

Childhood asthma is a multifactorial disease with heterogeneous clinical phenotype and complex genetic inheritance. The primary aim of asthma management is to make an early diagnosis and to achieve a prompt control of symptoms, in order to reduce the risk of future exacerbations and progressive loss of lung function (1). When asthma is correctly diagnosed, low-dose inhaled corticosteroids can easily control symptoms in most of patients. When asthma does not respond to usual treatment and higher maintenance doses are needed or not sufficient, we enter in the "problematic severe asthma" field (2, 3). However, in some cases, we would probably better define it as "problematic respiratory symptoms unresponsive to prescribed asthma therapy" (4). Nowadays,

all pediatricians feel probably confident in clinically diagnose a child with asthma and functional respiratory tests, as a simple spirometry, are often not even performed before starting the basic treatment. Pediatric asthma increased in numbers due to a greater awareness, however, lately we moved from the problem of under diagnosing to a possibly worse condition of over diagnosing asthma (4). The known expression: "Not all that wheezes is asthma" continues to be relevant. In front of a child with ongoing symptoms despite correct treatment, the first consideration is to ensure if the diagnosis of asthma is in fact correct. Depending on the clinical signs and symptoms, alternative diagnosis needs to be taken into consideration, a new correct diagnosis might be made and a different management followed. Differently, when the detailed re-evaluation confirms the diagnosis of asthma, all possible risk factors or comorbidities need to be considered and, sometimes treated, to ensure the maximal effort to obtain symptoms control. At the same time, doctors should verify that basic asthma management strategies are in place. Often the poor symptom control is a consequence of modifiable factors which need to be carefully assessed such as: (1) non-adherence to medication, (2) inadequate inhalation technique, (3) persistent environmental exposures and (4) psychosocial factors. Anytime our asthma management is not satisfying and we categorize our patient as "difficult to treat asthmatic," we need to remember that the first step is always to go right back to square one, instead of keep increasing the corticosteroid treatment: the differential diagnosis, with the most positive attitude of looking for our possible own "mistakes."

The main aim of this paper is to offer a clinical overview of all possible asthma mimickers which need to be taken into consideration and of the comorbidities that might contribute at the poor symptoms control.

PROBLEMATIC SEVERE ASTHMA OR PROBLEMATIC RESPIRATORY SYMPTOMS

As respiratory pediatricians we all experienced that low-dose inhaled corticosteroids can easily control asthma symptoms in most of patients. Moreover, exacerbations in children are often concentrated in some periods of the year due to viral trigger or aeroallergens, and some little patients can even stop their treatment in summer time without clinical deterioration. Nevertheless, children and their parents sometimes keep complaining of recurrent respiratory symptoms despite the correct and continuous treatment, and various courses of antibiotics, beta-2 agonists and oral corticosteroids are frequently prescribed by pediatrician. As a consequence of the poor symptoms control, asthma treatment is often increased and the patient categorized as "problematic severe asthmatic." However, an earlier step could be identified and this condition better defined as "problematic respiratory symptoms unresponsive to prescribed asthma therapy" (4). In fact, the known expression: "Not all that wheezes is asthma" continues to be relevant and the asthma diagnosis should be clinically reconsidered. In the last few decades, we possibly moved from under diagnosing to over diagnosing asthma. Nowadays, a thriving child with history of recurrent cough and a need of frequent beta-2 and inhaled corticosteroid (ICS) treatment is often diagnosed with "probable asthma" and ICS in ever increasing doses immediately prescribed. The initial suspected diagnosis, made with insufficient features and no spirometry testing, gradually, and with no evidence, becomes a definitive diagnosis rarely questioned in the future. Over diagnosis of asthma is a significant problem both in primary and secondary care (5, 6). With this new concept, before an unjustified referral for difficult asthma is made, all the components of the airway disease need to be reconsidered and atypical signs or symptoms which point to an alternate diagnosis recognized. "False asthmas" are often diagnosed years after the onset of clinical symptoms, leading to excessive use of anti-asthmatic medications other than dangerous progression of the misdiagnosed chronic primary disease (7). As often suggested, the first step is to go back to the beginning and take a really detailed history and perform a complete physical examination.

THE HISTORY AND PHYSICAL EXAMINATION

Asthma diagnosis is often suspected when a constellation of typical features is present, however, as most guidelines highlight, diagnosis has to be confirmed either by objective tests or an objective response to treatment. One of the initial major error that occurs is that insufficient features of the asthma pattern are sought before the diagnosis is made. Patient's history should always give enough answers to the following question: "Does the child have any or all of cough, wheeze and breathlessness?." A persistent cough (especially if non-productive) alone in an otherwise well-child is highly unlikely be due to asthma (8). The medical term wheezing is often used by parents to describe different sounds and care should be taken to be sure what they actually mean (9, 10). The habit to ask the family to try to demonstrate the sound of wheezing yourself, could help to avoid misunderstanding. It is also important to understand if the child is noisy and breathless at the same time and to assess the severity of symptoms. After an intense exercise, breathlessness is common but a formal diagnosis of asthma or exercise-induced laryngeal obstruction (EILO) is correct only in about half of those complaining of symptoms. The rest of adolescents are just deconditioned or exaggerating their symptoms (11). The possibility of fabricated and induced illness should be also excluded and possible personal or family psychological factors should be investigated. If symptoms are not clear and an uncontrolled symptomatic asthma need to be ruled out, instead of an extra increase of ICS or of a useless beta-2 prescription, an exercise test may be useful. One of the most "typical" asthma symptoms, such as cough is often present but, at the same time, is definitively a non-specific symptom. In fact, a cough may be the first symptom of many diseases or conditions affecting the respiratory tract. Almost all diseases of the respiratory tract, and in some cases of the extra-respiratory tract, can cause cough (12).

If symptoms are referred just during daytime (no cough or wheezing at night or first hours of morning) and with a sudden

onset of symptoms in the absence of obvious triggers, asthma cannot be definitively excluded but other possible diagnosis should be seriously considered. Finally, pattern of symptoms need to be explored with important implications for therapy. For example, of particular importance is distinguishing children only with asthma induced by viral symptoms from those with elapsing symptoms between colds, the latter being more compatible with a chronic disease. At this stage, the atopic status of the family and of the patient should already being stated or it would be necessary to do so, in fact, non-atopic pediatric severe asthma exists but it is rare.

Moreover, history should always include specific questions on parents' smoking habits, pets and home environment. Information on pregnancy, delivery and neonatal period could lead our attention to rule out different diseases: persistent symptoms from birth or first weeks of life are rarely due to an asthmatic condition (13).

A careful physical examination is as important as a careful history. Examination is usually normal, however atypical signs and symptoms need to be identified to think of alternative possible diseases. The diagnosis of asthma should be reconsidered in case of the following: (1) presence of clubbing, cyanosis, significant anemia or nasal polyps; (2) failure to thrive (13).

The presence of symptoms "typical" of asthma plus the finding on physical examination of wheezing strongly point to a diagnosis of asthma. Confirmation of the diagnosis of asthma is based on three key additional elements:

- The demonstration of variable expiratory airflow limitation, preferably by spirometry, when possible.
- Documentation of reversible obstruction.
- Exclusion of alternative diagnoses (see "Alternative Diagnosis" below).

Evidence of airway obstruction on spirometry, especially if reversible with a bronchodilator, can confirm the diagnosis of asthma or assess the severity (14, 15). However, normal spirometry does not exclude the diagnosis. Moreover, young or non-compliant patients are often unable to perform. Improvement on a trial of medications is often used to confirm the diagnosis in these patients (16).

Measurements of peak expiratory flow using a peak flow meter are more variable and effort dependent. Thus, peak flow measurements alone should not be used to diagnose asthma but is more often used in the follow-up of patients to monitor patients' symptoms and response to therapy.

Since, especially in young patients, there is no gold standard test for asthma diagnosis, other possible tests could be performed such as: allergy testing, done either by skin or *in vitro*. Outdoor aeroallergens are unusual triggers in infants and very young children but may be triggers in older children. In addition, when indicated testing reveals the presence of IgE antibody to any allergen, an atopic diathesis is demonstrated, increasing the likelihood that chest symptoms are due to asthma.

In older children bronchoprovocation testing (with methacholine, cold air, or exercise) could be performed when the clinical features are suggestive of asthma but spirometry is normal and there is no response to asthma medications. An exercise challenge of sufficient magnitude may provoke symptoms in children with asthma (17). A negative bronchoprovocation study may also be useful in reducing the likelihood that a child has asthma, although it cannot be used to exclude the diagnosis. Chest radiograph should be performed only in children whit persistent symptoms who do not respond to treatment. In those children, the chest radiograph may display findings suggestive of causes for wheezing other than asthma. Exhaled nitric oxide testing is not recommended as a diagnostic tool but is mostly used in the follow up of asthmatic patients.

TABLE 1 | Clinical clues to alternative diagnosis in children with wheezing.

Clinical clue	Possible diagnosis
PERINATAL AND FAMILY HISTORY	
Symptoms present from birth	Chronic lung disease of prematurity, PCD, CF
Family history of unusual chest disease	CF, Neuromuscular disorders, PCD
Severe upper respiratory tract disease	PCD
SYMPTOMS AND SIGNS	
Persistent moist cough	PBB, Bronchiectasis, Recurrent aspiration, PCD, CF
Excessive vomiting	GERD (w/without aspiration)
Dysphagia	Swallowing problems (w/without aspiration)
Breathlessness with light headedness and peripheral tingling	Dysfunctional breathing, Panic attacks
Inspiratory stridor	Tracheal or laryngeal disorder
Abnormal voice or cry	Laryngeal problems
Focal signs in chest	Developmental anomaly, FB, Post-infective syndrome
Persistent wheeze	Extrinsic intra thoracic airway compression, Airway-malacia, Luminal obstruction, CF, FB
Finger clubbing	CF, Bronchiectasis
Failure to thrive	CF, GERD

CF, cystic fibrosis; FB, foreign body; GERD, gastro-esophageal reflux disease; PBB, protracted bacterial bronchitis; PCD, primary ciliary dyskinesia.

ALTERNATIVE DIAGNOSIS

When to Look for Other Diagnosis

As specified above, the persistent condition of "problematic respiratory symptoms unresponsive to prescribed asthma therapy" need to motivate a clinical full reassessment to exclude that the patient was wrongly labeled with the diagnosis of asthma. To be able to do so, all possible conditions which might mimic asthma need to be considered. Some diseases, better defined as comorbidities, might also coexist with a diagnosis of asthma and will be discussed in a specific section.

Table 1 modified from British Guideline on the Management of Asthma, enlists the clinical clues to alternative diagnosis in asthmatic young children (18). **Table 2** lists alternative diagnosis for "persistent respiratory symptoms not responding to asthma treatment" and the main clinical characteristics for each.

TABLE 2 | When to suspect specific alternative diagnosis and initial useful diagnostic examinations.

Alternative diagnosis	When to suspect	Useful diagnostic examinations
Cystic fibrosis and bronchiectasis	Daily cough productive of sputum, clubbing, malabsorption and failure to thrive, recurrent chest infections, airways bacterial colonization	Sweat chloride test, Genetic tests, Swab culture, Lung Function tests, Chest CT
Immunodeficiency	Recurrent airway infections, Systemic infections (from a few months of age)	Immunoglobulins and specific tests
Primary ciliary dyskinesia	Neonatal upper airway symptoms, Chronic rhinosinusitis, Recurrent otitis media, Daily wet cough, Laterality defects	Nasal NO, HSVM, EM, Genetic tests, Immunofluorescence, Chest CT
Protracted Bacterial Bronchitis	Cronich wet cough, Poor response to Beta-2 agonists, Good response to prolonged course of antibiotics	Often no need of examinations, Swab culture, Bronchoscopy with BAL
Airway malacia	Monophonic wheeze when the child is active, High risk setting (i.e., pt operated for tracheo-esophageal fistula or vascular ring), Presence of associated stridor	Lung function test (truncated expiratory flow in spirometry), Flexible bronchoscopy, Dynamic CT
Airway foreign body	Abrupt onset of symptoms, History of choking, Unilateral monophonic wheeze, Focal hyperinflation of lung	Bronchoscopy, chest x-ray
Habit cough	Prolonged dry, honking cough; Absence of cough during sleep; Absence of any physical findings	Medical investigations should be avoided
Vocal cord dysfunction	Absence of structural abnormalities, Sudden worsening of "asthma" symptoms, No response to asthma medications	Video of an attack, Laryngoscopy during attack
Bronchiolitis obliterans	History of severe viral respiratory infection in the first 3 years of life	CT scan (characteristic mosaic pattern and air trapping)

CT, computed tomography; EM, eletcron microscopy; HSVM, high speed video microscopy; NO, nitric oxide.

Symptoms and clinical signs present from birth, such as: daily productive cough, recurrent upper and lower airway infections could suggest Cystic Fibrosis, Primary Ciliary Dyskinesia or an Immunodeficiency condition. When a chronic wet cough responds to prolonged antibiotic treatment a diagnosis of Protracted Bacterial Bronchitis should be made. Inspiratory sounds or difficult breathing suggest extra-thoracic pathological conditions (tracheal or laryngeal disorders). Respiratory problems combined with significant gastresophageal symptoms should prompt investigations to rule out: GER, recurrent aspiration, swallowing problems. Moreover, the sudden onset of respiratory symptoms with mono lateral thoracic sounds could be indicative for airway foreign body.

Finally, doctors should also remember that a child who presented early on his life one or few episodes of preschool wheeze or dry cough related to colds or upper airway infections should not be labeled with a diagnosis of asthma. Many of these patients in fact are just "healthy" children that will stop experiencing wheezing and respiratory symptoms at school age. This correct medical attitude will possibly avoid overtreatment, medicalization, and anxiety in parents.

Cystic Fibrosis, Non-CF Bronchiectasis and Immunodeficiency

One of the typical symptoms of a patient affected by Cystic Fibrosis (CF) or diffuse non-CF bronchiectasis is chronic cough with the production of abundant sputum which is definitively atypical for a severe asthmatic child. Moreover, CF patients suffer from recurrent chest infections from a very young age and might present episodes of bronchial asthma, which could lead to some diagnostic confusion (19–22).

In non-CF bronchiectasis, the most frequently reported symptoms are productive cough; sputum expectoration; respiratory distress; growth retardation; night sweats (23, 24).

Similarly, respiratory symptoms and complications present a significant cause of morbidity and also mortality among patients suffering from different forms of Primary immunodeficiencies. Frequent and severe infections of either upper (e.g., sinusitis and otitis media) or lower respiratory tract (e.g., pneumonia, bronchitis, bronchiectasis, and interstitial lung diseases) are typical of an immunodeficiency condition. (25–27). Failure to thrive signs and sign of malabsorption could be observed both in CF and immunodeficiency patients.

Primary Ciliary Dyskinesia

Lower airways are commonly involved in Primary Ciliary Dyskinesia (PCD). One of the most common manifestation is chronic asthma, that is generally unresponsive to maintenance treatment, especially at school-age and during adolescence (28). A mild to moderate obstructive pattern is frequently found at spirometry. Possible pathological changes accounting for this include airway smooth muscle hypertrophy and fibrosis, intraluminal secretions and altered lung mechanics secondary to repeated infection (29). Frequently, heterogeneous clinical presentation asthma symptoms let to a challenging diagnosis. More worrying, many patients remain not diagnosed until adulthood when they might already have developed lung bronchiectasis. Lower and upper recurrent respiratory symptoms with no obvious other cause, such as prematurity should suggest to rule out this diagnosis (30). Later in childhood, these children suffer of chronic moist cough often triggered by viral infections, which is one of the reason for years of misdiagnosis with bronchial asthma.

A history of unexplained respiratory distress at birth, recurrent otitis, lower and upper respiratory infections require further examinations. Nasal nitric oxide (nNO) measurement represents a helpful screening tool for PCD diagnosis. nNO levels are extremely low in PCD compared to healthy and disease controls (31). Otherwise, there is no single gold standard diagnostic test for PCD. A complete diagnostic work-up, including high-speed video microscopy analysis, transmission electron microscopy, immunofluorescence, sinus and chest TC is mandatory when PCD is highly suspected (32).

The management of PCD patients involves mainly airway clearance, infection control, and elimination of exposure to inflammatory triggers, also including passive smoke, without which asthma could not improve.

Protracted Bacterial Bronchitis

In children with chronic cough (lasting ≥4 weeks) not responding to asthma treatment, protracted bacterial bronchitis (PBB) should be clinically suspected and a prolonged (minimum of 2 weeks) course of antibiotic should be prescribed. PBB is often misdiagnosed as asthma, resulting in inappropriate and often high doses of inhaled Corticosteroids. Children with PBB were usually young and scarce systemic symptoms, without evidence of sinusitis or ear disease. They typically appeared well, with normal growth and development (33, 34).

Untreated, this condition over time can lead to suppurative lung disease and bronchiectasis. Lung function tests are usually normal (35).

Airway Malacia

Medical awareness for this condition is unfortunately still low and a good number of children are still misdiagnosed as asthmatic after several years of fruitless asthma treatment. (36). Malacic airways (laryngomalacia, tracheomalacia, bronchomalacia) are the most common congenital abnormalities of the pediatric airway and are characterized by excessive softness of tissues leading to increased airway compliance and excessive dynamic collapse during the respiratory cycle (37). Laryngomalacia can be suspected when positional stridor occurs in infants. Even though it is usually a benign condition resolving spontaneously, symptom persistence associated to feeding disturbance, poor weight gain, chest retraction and progressive deformation of rib cage require other investigation to exclude neurologic, genetic and cardiac disorders. When malacia involves intra thoracic airways barking cough, diffuse (tracheomalacia) or unilateral (bronchomalacia) monophonic wheeze, and prolonged expiratory phase expiratory symptoms may occur and must be distinguished from asthma. Sometimes, excessive dynamic collapse can also cause cyanotic spells, apnea and difficulty weaning ventilator support (38).

Congenital tracheomalacia may occur in isolation but has also been associated with other airway abnormalities (tracheoesophageal fistulas, laryngeal clefts, laryngomalacia, and bronchomalacia). Differently, acquired tracheomalacia occurs in the normally developed trachea that undergoes damage from external compression (tumors, cysts, goiter, vascular structures), trauma (tracheostomy), positive pressure ventilation, infection, or inflammation (38). The specific characteristic cough sound often triggered after viral respiratory infection and the flow-volume spirometry pattern (reduction in peak expiratory flow) can help to suspect this condition (39). Other more invasive tests are needed for final diagnosis, such as flexible bronchoscopy and dynamic CT scan with contrast enhancement (40). Observation and conservative management are typically all that are required. However, surgical intervention can be necessary in the most severe cases, and can result in significant improvement in symptoms (37).

Airway Foreign Body

Usually, the sudden onset of respiratory symptoms is more suggestive for a diagnosis of an accidental aspiration of foreign body than of a sudden onset of asthma. Definitively, it is more common in young children (<4 years old). History could be positive for choking and immediate distress but the absence of such history does not certainly rule out the diagnosis. Clinically patient might present persistent cough, unilateral and monophonic wheeze and respiratory distress. However, symptoms depend on the grade of airway obstruction (complete or partial).

Habit Cough

Diagnosis can usually be suspected when the patient coughs repeatedly during the visit, especially when the cough is noising and referred exclusively during daily hours. Usually these children would have received many therapies, including asthma drugs, with no benefit on the honking, brassy cough of many weeks duration, often occurring after a respiratory tract infection. It is more common in children older than 8 years and can cause undue distress to children and both parents. The cough disappears as soon as the child is distracted and always absent during sleep. The origins of the habit cough are often obscure, in some patients can also occur in the background of mild asthma. The diagnosis is made after exclusion of other causes. Some patients might respond to control of breathing exercises from a physiotherapist or speech therapist. Psychological stresses need to be sought in the child's school or home life. The help of a psychologist may be needed to identify and resolve the problem.

Psychological Issues

There is a growing evidence in literature that stress could be a mediator of atopy and that worsens asthma in children. On one hand, asthma control shall be reduced in stressed patients and families due to a poor adherence to treatment; on the other hand, neuroimmunological mechanisms may influence asthma when a psychological distress occurs (41). Lind et al. found that stress, exhaustion, anxiety, depression, were higher than normal in allergic asthma and atopic dermatitis, but not in nonallergic asthma. The vicious circle between psychological issues and symptoms is well-established: inflammatory and non-inflammatory mechanisms contribute to enhance atopy and asthma symptoms (42, 43). Finally, has been demonstrated the strong relationship between panic disorder and asthma. The typical presentation of panic disorder is the hyperventilation:

rapid expiration causes dehydration of the airway contributing to induce asthma by airway narrowing (44).

Vocal Cord Dysfunction

This condition is characterized by inappropriate movement of the vocal cords (adduction during inspiration) induced by: (a) exercise (b) psychological stress (c) local irritation–i.e., reflux. Often vocal cord dysfunction coexists with asthma but it is unresponsive to short-acting beta-2 agonists and it presents with intermittent inspiratory symptoms. The diagnosis of vocal cord dysfunction involves an accurate medical history and specific exams. Patients report air hunger, intermittent aphonia or dysphonia, sensation of choking, chest tightness, chest pain, difficulty swallowing, fatigue and throat clearing. Endoscopic examination with direct visualization of the vocal cords via flexible, transnasal fiber-optic laryngoscopy, possibly after bronchial challenge or during an acute attack, is the gold standard for the diagnosis. Spirometry may show an abnormal shape of inspiratory loop consistent with a variable extrathoracic obstruction (45). Treatment depends on the underlying cause, however, often children are taught breathing control exercises, encouraging nose breathing, and appropriate use of the diaphragm (46). This intervention is often leaded by a respiratory physiotherapist or speech therapist and may help resolving the problem (47–49).

Bronchiolitis Obliterans

Bronchiolitis obliterans (BO) is frequently secondary to influenza, parainfluenza, measles, respiratory syncytial virus, varicella, and Mycoplasma pneumoniae. However, adenovirus is by far the most common agent linked to the development of BO, and can present with persistent wheezing, rather than paroxysmal symptoms. CT scan will show a characteristic mosaic pattern and air trapping (50).

ASTHMA PLUS: CO-MORBIDITIES

In cases where symptoms remain uncontrolled despite maximal guideline-recommended treatment, and all alternate diagnosis have been excluded, possible comorbidities need to be investigated as they may be a coincidental finding or they may contribute directly for the severity of asthma (51).

Establishing the real impact and the causative effect of comorbidities on asthma control it is complicated, and a medical treatment is sometimes necessary to assess their role (52). In most cases, although the ability to improve pediatric severe asthma by treating comorbidities remains unconfirmed, a therapeutic trial should be prescribed.

Obesity

Obesity is a risk factor for poor asthma control, but the relationship between the two conditions is still under debate. It is evident that breathlessness due to simple deconditioning may lead to an erroneous diagnosis of asthma, however, there might be some interesting recent findings. Both, overweight and deconditioning merit treatment in their own right, however weight reduction is known to be very difficult to achieve. Recently, it was found an increased prevalence of asthma in obese children and this group of patients showed more severe exacerbations (greater risk of admission to pediatric intensive care unit) suggesting that asthma in obesity could be a specific disease (53). Moreover, obesity seems to be possible associated with steroid resistance (54). However, we need to be cautious to consider a real association between severe asthma and obesity, as results might be confounded by the known association of obesity with low socio-economic conditions and all what this brings (55). Finally, obesity is known to be associated with variable increased inflammatory phenotypes and airway may be the target of systemic inflammation (56). Obese asthmatic patients probably will also need to be investigated for obstructive sleep apneas and treated appropriately (57). In conclusion, behavioral and weight reduction programs should be offered to asthmatic obese patients to obtain weight reduction. At the same time, it is important to assess airway disease and, before steroid therapy is escalated, look for evidence of uncontrolled airway inflammation (4).

Rhinosinusitis

Pediatric chronic rhinosinusitis (CRS) represent different stages of one chronic inflammatory disease of the mucosa of the nasal cavity and paranasal sinuses, resulting from repeated acute bacterial rhinosinusitis, and leading to a "maladaptive-eosinophilic" stage disease. CRS is a major disease condition with high morbidity and can influence lower airway disease status in adults. In a cross-sectional Korean study, including 17,506 participants, CRS was significantly related to asthma, in particular CRS without nasal polyps was related to childhood-onset asthma (onset before 18 years) or early-onset asthma (onset before 40 years) in adults (58). Given this potential evolution toward more irreversible disease, a more aggressive early intervention it is hoped, to prevent these long-term consequences. Coexistence of chronic rhinosinusitis with nasal polyps and asthma and rather similar characteristics of inflammation support assumption that chronic rhinosinusitis and nasal polyps and asthma may be, at least in part, the same disease process. Chronic rhinosinusitis with nasal polyps is estimated to occur in 7% of all asthmatics, whereas asthma is reported to occur in 26–48% of patients with Chronic rhinosinusitis with nasal polyps (59). Patients with allergic rhinitis and/or chronic rhinosinusitis report poorer asthma control, more exacerbations and emergency visits and have more difficulty achieving symptom control (60), and increased asthma severity has also been shown to be associated with sinonasal inflammation (61). Upper airway symptoms can significantly affect patients' quality of life and should be treated irrespectively of any benefit on asthma control, which could also be obtained (62). A study by Penn et al. examined the efficacy of anti-IgE therapy for the treatment of Chronic rhinosinusitis with nasal polyps. Despite the small number of patients, the nasal polyp scores significantly improved in the anti-IgE group (63, 64). In a recent randomized, double-blind, placebo-controlled trial, Gevaert et al. reported a reduction of nasal polyp size and an improvement of symptoms, compared with placebo in patients with Chronic rhinosinusitis with nasal polyps independent of atopic status (65). Otherwise, despite these promising results,

further studies are needed in order to confirm them, as well as of other biologics.

Gastro-Esophageal Reflux

Symptoms of GER often coexist in children with severe asthma. Micro-aspiration, acid stimulation of the esophagus and vagus nerve stimulation are the mechanism proposed to explain how the GER can trigger asthma (66). Furthermore, GER can trigger VCD with consequent laryngeal dysfunction mimicking asthma symptoms. Anyway, it has been proven that GER treatment does not improve asthma control (67). This may suggest that symptoms triggered by GER are mimics of asthma symptoms, rather than exacerbating airway inflammation or airway hyperresponsiveness. Specific examinations assessing for GER, such as impedance-pHmetry and/or gastroesophageal endoscopy must be request in order to exclude this comorbidity (68). A trial of treatment with PPI is recommended as the initial diagnostic step in symptomatic children (69).

Food Allergy

It is still debated whether there is a real relationship between difficult asthma and food allergy, however, there is a higher incidence of food allergy in asthmatic patients admitted to intensive care units and thus it could be considered as a comorbid condition (70). In the literature evidences that support the close relationship between food allergy and asthma as well as the early food sensitization or allergy preceding the development of asthma and other atopic disease can be found (71).

Sometimes patients with food allergy report bronchospasm caused by ingestion or inhalation of the offending food. Even though it is a rare condition (72) linked to an underlying bronchial reactivity, it may develop either in children suffering from asthma or in non-asthmatic patients as part of an allergic reaction to food. When the ingestion of the offending food occurs closely to physical exertion, asthma and anaphylactic symptoms may develop configuring the clinical picture of food-dependent exercise-induced anaphylaxis (73).

It is already showed that symptomatic sensitization to foods is associated with asthma (74) and that the risk of asthma morbidity (daytime symptoms, hospital admissions, lower percent predicted forced expiratory volume in 1-s, asthma persistence, severe asthma exacerbation) is higher in children with food allergy. This risk is especially increased in children with high levels of food specific IgEs and multiple or severe food allergy (75, 76).

When food allergy is diagnosed (suggestive clinical history, positive skin prick tests and specific IgE, positive oral food challenge), the avoidance of the offending food should be suggested, even though specific immunotherapy can be also considered in selected cases to alter the course of atopic march (77).

PROBLEMATIC ASTHMA OR TRUE SEVERE UNCONTROLLED ASTHMA

During the previous process of considering alternative diagnosis and the possible presence of comorbidities that may impact asthma clinical expression and control in childhood, doctors should always verify that basic asthma management strategies are in place (78). Often, the poor symptom control is a consequence of modifiable factors which need to be carefully assessed before proceeding to more invasive investigations. The most frequent conditions that might justify a poor control of symptoms are: non-adherence to medication, inadequate inhalation technique, persistent environmental exposures and psychosocial factors (79). Finally, all patients and carers must be given an individualized written asthma action plan that details current treatment, how to recognize and treat an exacerbation and when to seek appropriate help (47).

SUMMARY AND CONCLUSIONS

In conclusion, since it is known that the majority of asthmatic children can be well-controlled with the basic treatment, if a patient keeps complaining of severe or recurrent respiratory symptoms despite all the basic asthma management strategies are in place, alternative diagnosis need to be considered. Any deviation from common typical patterns should alert one to keep a high index of suspicion for diseases that mimic asthma. Moreover, all possible co-morbidities that might contribute to the uncontrolled clinical condition, need to be excluded and eventually treated. To do so, we need to remember that there is no substitute for a good clinical evaluation that can be performed by all doctors before an unnecessary referral is made.

AUTHOR CONTRIBUTIONS

NU, VM, and FP drafted the initial manuscript, searched for bibliography and revised the final manuscript. VN, AD, and MP were involved in drafting the manuscript, critically revised the manuscript, and approved the final manuscript. RC and NU made substantial contributions to conception and design of the study. RC reviewed and approved the final manuscript. All authors read and approved the final manuscript as submitted.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

MINI REVIEW
published: 26 June 2018
doi: 10.3389/fped.2018.00189

Measuring Airway Obstruction in Severe Asthma in Children

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Lung function is an important tool in the diagnosis and monitoring of patients with asthma at all ages. Airway obstruction is a typical feature of asthma and it can be assessed with several lung function techniques. Spirometry, respiratory resistance and reactance, and lung volumes are available to measure it at different ages and in children. The assessment of a bronchodilator response is always recommended to show the reversibility of the obstruction. Poor lung function is a predictor of poor asthma outcome and a low Forced Expiratory Volume in the first second of expiration percent predicted measured with spirometry, has been shown to be associated with a higher risk of having an exacerbation during the following year independently of the presence of asthma symptoms. In severe asthma lung function assessment is used to distinguish different phenotypes, children with severe asthma have worse airflow limitation prior to administration of a bronchodilator than children with non severe asthma. Airway resistance and reactance are indirect measurements of airway obstruction and they can be measured with the forced oscillation technique, which is feasible also in non-collaborative children. This technique can be more informative in discriminating patients with asthma from healthy controls and is able to indicate a more peripheral involvement of the airways. The role of this technique in severe asthma is still debated. In conclusion lung function is useful in the clinical management of children with severe asthma.

Keywords: severe asthma, lung function, children, respiratory resistance, reactance

OPEN ACCESS

Edited by:
Renato Cutrera,
Bambino Gesù Ospedale Pediatrico (IRCCS), Italy

Reviewed by:
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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *29 April 2018*
Accepted: *07 June 2018*
Published: *26 June 2018*

Citation:
Calogero C, Fenu G and Lombardi E (2018) Measuring Airway Obstruction in Severe Asthma in Children. Front. Pediatr. 6:189. doi: 10.3389/fped.2018.00189

INTRODUCTION

Lung function measurements are very important in the diagnosis and monitoring of patients with asthma, especially those with severe asthma. Several techniques are available for children and adults to measure airway obstruction, which is a typical feature of asthma. Furthermore, tracking of lung function, from childhood to adulthood, has been shown in several studies, highlighting that reduced lung function at young age is associated with the development of chronic obstructive pulmonary disease (COPD) in adulthood (1–3). This article will cover the main pulmonary function techniques used in children to detect airway obstruction and their clinical utility in the diagnosis and management of severe asthma in children.

SPIROMETRY

Spirometry indices are obtained with a forced expiratory maneuver according to the technical European Respiratory Society (ERS)/American Respiratory Society (ATS) recommendations (4). Modified acceptability criteria for spirometry in preschool children (2–5 years) have also been published (5). The Forced Expiratory Volume in the first second of expiration (FEV_1) and its

ratio to Forced Vital Capacity (FEV_1/FVC) are considered the gold standard to assess airway obstruction in adults and children (6). These indices are sufficient to grade the severity of obstruction, which is very important in the evaluation of subjects with severe asthma. Obstruction is shown by an abnormal FEV_1/FVC (<80%) and is defined as mild when FEV_1 is >70% predicted, moderate 60–69%, moderately severe 50–59%, severe 35–49%, very severe <35% (7).

Once a baseline measure is obtained, the evaluation of the reversibility of the obstruction with the assessment of the bronchodilator response (BDR) is recommended, as this is a typical feature of asthma. A positive BDR is defined by the ERS/ATS recommendations as an increase in FEV_1 ≥12% and ≥200 mL with respect to baseline after administration of 400 mcg of salbutamol given by metered dose inhaler via a spacer (7). However, it has been described by Tse et al. that in children this cut-off can be not enough sensitive to detect airway obstruction and can lead to a misdiagnosis of asthma (8), suggesting that a lower cut-off (8%) should be used in children.

Regarding the clinical utility of spirometry, the American Academy of Asthma, Allergy and Immunology states in the list of the Choosing Wisely Initiative "do not diagnose or manage asthma without spirometry"[1]. Also, according the most recent update of the GINA Guidelines (9) the diagnosis of asthma should be made not only based on the clinical features of the patient, such as clinical history and symptoms, but also using pulmonary function testing to detect the presence of airflow limitation. Furthermore, the NICE guidelines (10) recommend to perform spirometry in children and adults with a diagnosis of asthma to show an obstructive pattern on the test. The assessment of a bronchodilator response is also recommended to show the reversibility of the obstruction. Several guidelines on asthma also recommend to perform spirometry for monitoring asthma at each visit (10) or at least after 3 or 6 months from the beginning of treatment and on a regular basis afterwards (1–2 years) (9).

Measuring lung function is also useful as a predictor of asthma exacerbations. In the GINA guidelines (9) a low value of FEV_1 is mentioned as a predictor of poor asthma outcome. In a retrospective study in children with asthma, a low FEV_1 percent predicted (particularly when <60%) has been shown to be associated with a higher risk of having an exacerbation during the following year independently of the presence of symptoms (11). Also, Schifano et al. have described in a population of 894 asthmatic children a low concordance between symptoms and lung function results and a higher grade of asthma severity was given when spirometry was taken into account (12). This is very important in children especially when a poor awareness of symptoms is detected.

Spirometry is able to measure flows at low lung volumes that are considered to be more representative of the small airways such as the forced expiratory flow between 25 and 75% of FVC (FEF_{25-75}), at 50% of FVC ($FEF_{50,}$), and at 75% of FVC (FEF_{75}). The role of these indices in detecting airway obstruction in clinical practice is however controversial. Some authors have shown that these indices are more sensitive than FEV_1 in detecting peripheral obstruction [(13)], while others have shown that they do not affect the clinical decision more than FEV_1/FVC (14). For this reason, a recent ATS Technical Statement recommends that FEF_{25-75} should not be shown in the pulmonary function report (15).

The definition of severe asthma, according to an ERS/ATS Task Force (16), is the presence of uncontrolled symptoms despite high dose of corticosteroids and includes difficult to treat asthma and asthma resistant to therapy. Lung function assessment is necessary for confirming the diagnosis of severe asthma and to establish the phenotype. From a practical point of view, spirometry is useful in confirming the diagnosis of severe asthma in children and it should be always performed with the administration of a bronchodilator to detect airway obstruction and its reversibility. The aspect of the Flow-Volume inspiratory and expiratory loops can be also useful in the differential diagnosis of other causes of obstruction, intrathoracic or extrathoracic airway obstruction (17).

Different phenotypes of severe asthma have been described (18, 19). These phenotypes change according to age and are different in children and adults (20). Assessing airway obstruction is useful to identify the phenotypes at all age (21). Airway obstruction however, can be not present or only mild in children with severe asthma especially in between exacerbations (22–24). In respect to adults with severe asthma, children tend to have better lung function, but a greater decline overtime (25). It has been recently reported that children with severe asthma have worse airflow limitation prior to administration of a bronchodilator than children with non-severe asthma, but have similar values after BD showing an increased BDR (20).

In the TENOR Study, that included also children and adolescents, the authors have shown that acute asthmatic exacerbations can have a role in the decline of lung function, as the inflammation cascade can lead to airway remodeling and a fixed non reversible bronchoconstriction (26). In children this mechanism can be different and especially when there is eosinophilic inflammation remodeling of airways is not always present (27). Contrarily, a recent study published by Ortega et al. on lung function data retrospectively collected from the DREAM and MENSA studies on mepolizumab in severe asthmatics with eosinophilic inflammation (mainly adults with a very small proportion of adolescents), shows that a higher number of exacerbations is associated with a worsening of lung function measurements over time (28). These features can identify a more severe phenotype of asthma and each exacerbation can contribute with a loss in FEV_1 of about 50 mL/year (28). The mechanism involved is hypothesized to be a reduced elasticity of the lung as a result of airway narrowing and remodeling associated to deposition of collagen.

Lung function measurements can be used to monitor response to therapy but few data are available on severe asthma in children. Deschildre et al. published data on 1 year of real life experience on 104 children with severe asthma treated with omalizumab showing an increase of 4.9% predicted of FEV_1 at the end of the 12 months (29). A recent longitudinal study on lung function in severe asthma conducted in Brazil on 65 severe asthmatic

[1]http://choosingwisely.org/wp-content/uploads/2015/02/AAAAI-Choosing-Wisely-List.pdf

patients (between 6 and 18 years of age) receiving high dose of corticosteroids showed only a small improvement of airway reversibility (FEV_1 after bronchodilator) (30).

BODY PLETHYSMOGRAPHY

Lung function obtained with the body plethysmography allows to measure airway resistance and lung volumes, with particular interest to Total Lung Capacity (TLC) and Residual Volume (RV). RV is a good index of air trapping and is a marker of hyperinflation when the value is greater than 120% predicted. This measure can add information to the conventional spirometry especially in severe asthmatic patients. It has been shown that 49% of children having airflow obstruction with an FEV_1/FVC <80% have a significant hyperinflation (31). Sorkeness et al. in the SARP cohort, showed that children with severe asthma were more hyperinflated and air-trapped in respect to children with non severe asthma that had normal RV and TLC (32).

RESPIRATORY RESISTANCE AND REACTANCE

Airway resistance, which is an indirect measurement of airway obstruction, can be evaluated with the body plethysmograph, but its measurement requires the panting maneuver which is substantially infeasible in preschool children. Several techniques have been developed, such as the interrupter technique (Rint), the forced oscillation technique (FOT), and the measurement of specific airway resistance (sRaw) using the body plethysmograph, which are performed during tidal breathing. These techniques require only minimal collaboration and are feasible also in preschool children when spirometry cannot be performed or other techniques cannot be used. In addition to respiratory resistance, FOT offers the advantage of also measuring respiratory reactance, which can be thought of as the distensibility of the respiratory system. Technical recommendations for each technique are available (5, 33) and several reference equations have been published (34–36).

The evaluation of the bronchodilator response is also possible and different cut-off values have been reported in the literature (35, 37, 38). Those techniques, with particular regard to FOT has been suggested to be more sensitive in detecting airway obstruction because of no "deep inspiration" effect on bronchial tone that is required with a forced maneuver (39, 40).

In a study conducted from Heijkenskjold Rentzhog et al. (41), the authors showed that FOT measurements were more informative in discriminating patients with asthma from healthy controls. FOT measurements were able to indicate a more peripheral involvement of the airways. Similar results are also available form previous studies on BDR in FOT resistance measurements and spirometry in children (39, 42) and adults (43). IOS results compared to spirometry have been shown to be able to predict loss of control in a group of 54 children with asthma (44). Data from 252 children with mild to moderate persistent asthma, demonstrated that the area under the reactance curve measured with IOS, was able to detect changes in airway mechanics after weeks of treatment in respect to other oscillations indices or spirometry results (45). There are some data that show that sRaw measured at preschool age correlates with mild obstruction measured with low volumes (6), also in a recent study, although on a small number of asthmatic children, sRaw is a better indices of bronchoconstriction in respect to FEV1 after a challenge test (46).

The role of these techniques in severe asthma in children remains to be assessed and further studies are needed.

MULTIPLE BREATH WASHOUT (MBW)

The Multiple Breath Washout technique measures the Lung Clearance Index (LCI), which is the cumulative expired volume required to clear an inert gas from the lungs, divided by FRC or the number of times the lung volume has to be "turned over" to wash out an inert gas such as nitrogen (N_2) or sulfur hexafluoride (SF_6). LCI is an index of inhomogeneity of ventilation and it has been demonstrated to be able to detect early lung damage in particular in children chronic lung disease such as Cystic Fibrosis (47), however it has some limits in measuring airway obstruction. In severe asthma with severe obstruction some areas of the lung can be excluded from ventilation giving a false value of LCI that can even appear within the normal range. S_{cond} is another index measured with MBW that refers to the inhomogeneity of the conducting airways and this might be more useful in asthmatic patients. The limits of this technique are also related to the lack of extended reference equations with particular respect to the pediatric population.

BRONCHIAL HYPERRESPONSIVENESS

Bronchial hyperresponsiveness (BHR) can be considered as the variation of the bronchial tone that can vary after exposition to different stimuli. The stimuli that can be used as provocation test can be direct (chemicals such as histamine or methacholine, or inhaled allergens) or indirect (physical exercise, mannitol, inhaled cold air or hyperventilation with dry cold air). After a brochoprovocation test a fall in FEV_1 of 20 or 15%, depending on the test used, is considered as a cut off value. A maximal fall of FEV_1 between 10 or more in respect to the baseline value on two consecutive measurements after a maximal effort is considered diagnostic for exercised induced bronchoconstriction (48). In children exercise testing can be very important for the assessment of asthma in the diagnostic process but also in monitoring the response to therapy, especially when exercise symptoms are reported or there is a poor perception of symptoms (9). Methacholine bronchoprovocation test is more sensitive but less specific than exercise testing in asthmatics (49).

In a prospective study Part et al, showed in 1,041 children, the CAMP Cohort, that the percent recovery index (the percent increase in FEV_1 after bronchodilator) in response to a bronchial challenge with methacholine was able to predict an asthmatic exacerbation in the following year (50). In severe asthma, both methacholine and exercise tests can be used to asses BHR when

the diagnosis is not clear in difficult cases (16). The role of BHR in the clinical evaluation of the child with severe asthma is debatable and it is not safe to evaluate when baseline FEV_1 is low (51).

CONCLUSION

In conclusion, pulmonary function testing assessing airway obstruction is important in the clinical management of children with asthma and severe asthma during diagnosis, monitoring and evaluating response to treatment.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This article was funded by the Meyer Foundation, Florence, Italy.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

MINI REVIEW
published: 06 July 2018
doi: 10.3389/fped.2018.00196

Measuring Airway Inflammation in Asthmatic Children

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Asthma is the most common chronic respiratory disease in children characterized by airways inflammation, bronchial hyperresponsiveness, recurrent reversible airways obstruction, and respiratory symptoms. The diagnosis of the disease is based on clinical history, airways obstruction at spirometry, and bronchial reversibility. Asthma treatment is aimed to disease control, through the use of controller treatment and monitoring lung function. However, lung function and symptoms not always reflect the underlying airways inflammation and response to the therapy. Objective parameters of asthma inflammation could be important for the clinician in the management of patients with asthma. In the last years, some studies were focused on biomarkers to identify phenotype, inflammation, and pathobiological pathways to help the clinician in the diagnosis and in personalizing the management. Accordingly, clinically feasible tests are represented by the collection of exhaled breath condensate (EBC) and measurement of exhaled nitric oxide (FeNO). Other—methods such as the evaluation of volatile organic compound (VOCs), that reflect airways inflammation and treatment efficacy, are currently used for research purposes For some of these methods, The lack of standardization in pre-collection, collection, post-collection of samples, and interpretation of the results may a problem in clinical practice. Improved these limitations, several biomarkers will be useful to distinguish patients with a different disease condition to personalize the treatment.

Keywords: biomarkers, inflammation, FeNO, exhaled breath condensate, exhaled breath volatile compounds

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *29 April 2018*
Accepted: *18 June 2018*
Published: *06 July 2018*

Citation:
Tenero L, Zaffanello M, Piazza M and Piacentini G (2018) Measuring Airway Inflammation in Asthmatic Children. Front. Pediatr. 6:196. doi: 10.3389/fped.2018.00196

INTRODUCTION

Asthma is a chronic respiratory disease characterized by inflammation of the airways, bronchial hyperresponsiveness, recurrent reversible airway obstruction, and respiratory symptoms (1). Asthma is the most common chronic respiratory disease in childhood with an incidence between 1 and 18% in people from different countries.

The diagnosis of asthma is based on anamnestic data, clinical evaluation, limitation of the airflow, and bronchial reactivity (1). The goal of asthma treatment is the control of respiratory symptoms with controller daily drugs, reduction of rescue medications needed to maintain a regular daily activity and reaching a normal lung function (1). Though airways inflammation is a pivotal characteristic of the disease, it is not directly related to lung function test or symptoms (2) and with the real response to the therapy (3).

Therefore, objective parameters of airway inflammation should be considered relevant for the treatment choice in asthmatic patients.

The assessment of biomarkers in the exhaled breath of patients with asthma and other lung diseases is a very attractive approach to monitor airways inflammation. Biomarkers are objectively measurable indicators of the biological and pathological processes as well as of the pharmacological responses to the therapeutic intervention (4).

An ideal biomarker should be easy to collect and measure, inexpensive, noninvasive, and feasible in children capable to contribute to the phenotyping of the disease and to the assessment of treatment response (4, 5).

In order to have a clinically useful tool, there are important issues regarding sensibility, reproducibility, and variability of the methods, that need to be evaluated before moving from bench to bedside. In the next future, a number of recently proposed methods are expected to be clinically useful to predict the progression of the disease, to the phenotyping and endotyping of the disease in order to move toward a personalized treatment in asthma (**Figure 1**).

Bronchoscopy with bronchoalveolar lavage (BAL) and endobronchial biopsy are considered the gold standards for assessing airway inflammation and remodeling in asthma but, being invasive methods, they have a limited use in the clinical setting, in particular in pediatric care (2).

In recent years, therefore, researchers have focused their studies to define surrogate biomarkers to be proposed in the assessment of airway inflammation in asthmatic children and are shortly revised in the following sections (4–6) (**Figure 1**).

INDUCED SPUTUM

The method of induced sputum is a relatively noninvasive diagnostic procedure able to harvest cells and mediators from the lower airway (2) which, however, requires a level of expertise in the collection in order to obtain adequate samples and reliable results. Sputum can be induced in children older than 6 years after inhalation of nebulized hypertonic saline solution at increasing concentration (7, 8).

The procedure to collect sputum is standardized by European Respiratory Society Task Force and is applicable to children older than 8 years (9, 10).

However, the practical use of this method is limited due to technical issues which can make the feasibility at clinical setting difficult to perform for regular assessment of airways inflammation in children (11, 12). In particular, hypertonic saline inhalation may cause bronchoconstriction, especially in asthmatic children, for whom pre-medication with β2-agonist may be necessary. Furthermore, the post-collection analysis needs skilled laboratory personnel for mediator assay and cell specimens to be transferred onto slides and properly stained. Trained physicians are also needed in order to have a correct reading of the results from the specimens.

Several studies demonstrated a strong correlation between cellular elements of BAL and cells collected from in the airways through the induced sputum method.

The dominant cell in sputum from normal children is the macrophages, and the normal ratio for eosinophils in sputum from children is 2.5% (7).

Different inflammatory patterns are being identified from sputum of asthmatic children and adults: eosinophilic, neutrophilic, mixed, and paucigranulocytic types (13).

Sputum eosinophilia is a marker of severity of allergic inflammation in asthma and is characterized by elevated numbers of eosinophils, and eosinophil cationic protein concentration, as well as increased nitric oxide and hydrogen peroxide levels in exhaled breath. The patients with an eosinophilic pattern are expected to have a better response to corticosteroids therapy (14).

Also, children with stable asthma show increased number of eosinophils and bronchial epithelial cells in their sputum (15, 16). During asthma exacerbation, eosinophils and mast cells are more represented in the samples obtained from the airways through the method of induced sputum and eosinophil cationic protein concentrations are higher in the fluid phase of the samples. Some patients have sputum neutrophilia with improved levels of interleukin 8 (17). On the other hand, children with asymptomatic airway hyperresponsiveness are expected to have normal cell counts, whereas patients with cystic fibrosis have sputum neutrophilia (15, 18).

EXHALED BREATH CONDENSATE

Exhaled breath condensate (EBC) is a noninvasive method aimed to evaluate volatile markers and inflammatory mediators that may contribute to evaluate asthma pathophysiology.

EBC collects particles from airway lining fluid by the condensation of warm humid breath onto a cold surface in a condensing device.

EBC collection, as described in ATS/ERS guidelines, requires a refrigerated device (19) and patient are requested to breath at tidal volume for 10–15 min. During this time the airways lining fluid undergoes an aerosolization process and the exhaled fraction is condensed in a cooling device (0 to −20°C) (20).

The most frequently evaluated parameters in EBC are pH, exhaled markers of oxidative stress and inflammation.

EBC is composed of water vapor, unstable volatiles such as CO_2 and H_2O_2, inorganic (O_2, N_2), and organic (CO_2) particles, exogenous, and endogenous organic compounds, protein, and cytokines (21). In the respiratory tract, H_2O_2 may be released from inflamed cells—including neutrophils, macrophages, eosinophils, and epithelial cells. Nitrogen redox forms such as nitrite (NO_2 –) and nitrate (NO_3 –) are present in the epithelial lining fluid of the human respiratory tract.

High concentrations of NO_2 and NO_2+NO_3 were showed in patients with asthma, CF and bronchiectasis compared with healthy controls (21).

The pH of EBC is a non-specific marker of airway disease being a median normal pH value of 8.0 in children from 0 to 20 years (22). Some studies showed a lower pH in children with stable asthma than healthy controls and a lower pH value in children with severe than mild asthma (23, 24). In addition, asthmatic patients not adequately treated with ICS have been demonstrated to have a lower pH than those properly treated. Patients with acute exacerbation had a higher pH value after the treatment with budesonide (23, 24).

At present, no association has been reported between asthma symptoms, lung function, FeNO, and airway hyperresponsiveness (25, 26).

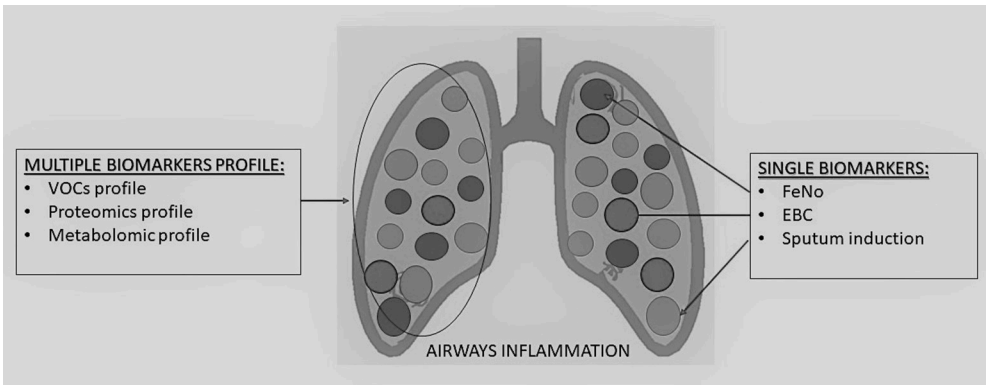

FIGURE 1 | Summary of single and multiple biomarkers detection methods to assess airways inflammation in children.

Biomarkers related to oxidative stress like H_2O_2, 8-isoprostane, asymmetric dimethylarginine (ADMA), aldehydes, and nitrite/nitrate are important to be evaluated in EBC in a number of airway diseases, including asthma.

Asymmetric dimethylarginine (ADMA) is an EBC marker of oxidative stress which can be assessed by the UPLC-MS/MS technique. It is an analog of L-arginine that reduces the synthesis of NO and increases superoxide from inhibition of NOS. A previous study showed that asthmatic children had higher values of ADMA than healthy ones with no difference with ICS treatment (27).

The oxidation of the phospholipid membrane and polyunsaturated fatty acid produces aldehydes and lipid hydroperoxides. One study showed high levels of glutathione in the EBC of asthmatic children with exacerbation and after 5 days of prednisolone therapy, the malondialdehyde level dropped, while glutathione rose (28). Malondialdehyde levels also correlate with air pollution, lung function and inflammatory markers. These results suggest that during exacerbations there is an imbalance between oxidative and antioxidant agents in the airways.

H_2O_2 is released from cells in the airways as superoxide anions, an unstable and reactive particle. In the respiratory system, H_2O_2 can be released from both inflammatory cells—including neutrophils, macrophages, eosinophils—and epithelial cells. In non-asthmatic, non-smoking children, the normal value of this molecule is 0.09 μmol (19). H_2O_2 was higher in asthmatic children during exacerbations and decreased after ICS treatment, supporting the hypothesis that H_2O_2 is a marker of airways inflammation (29, 30). However, other studies failed to demonstrate its ability to predict exacerbations (31, 32).

8-isoprostane, a suitable marker of oxidative stress, is a product of arachidonic acid (33). Children and adults with severe asthma or asthma exacerbation have high levels of this 8-isoprostane (34). The concentrations of 8-isoprostane failed to show any correlation with lung function, FeNO, ICS or leukotriene receptor antagonist therapy (35, 36).

Eicosanoids are another group of markers derived from arachidonic acid that play a role in asthmatic inflammation. The presence of these markers in EBC is confirmed by specific enzyme immunoassay and radioimmunoassays (37).

Asthmatic children have high levels of leukotriene B4 (LTB4), cysteinyl leukotrienes (LTC4, LTD4, and LTE4) in EBC (36, 37). The role of cysteinyl leukotrienes (CysLT) in response to ICS therapy is under debate (38–40). Some authors showed a significant reduction of CysLT after a course of oral corticosteroids and after 6 months of ICS therapy, whereas others did not confirm this result. A significant reduction of CysLTs has been reported after montelukast therapy (41).

Several other markers of inflammation and oxidative stress, such as cytokines and adenosine, have been investigated. Asthmatic children showed high Th2 cytokines and low Th1 cytokines in EBC (42, 43). Moreover, children with asthma had a high IL-4/INFγ ratio related to Th2 inflammation (43). IL-4 was high in asthmatic and atopic children as a predictor of asthma condition, whereas IL-5 could predict exacerbations (32).

FRACTIONAL EXHALED NITRIC OXIDE (FENO)

Nitric oxide in airways is mainly produced by two enzymes: constitutive nitric oxide synthase (cNOS), that produces low quantities of NO, and epithelial inducible NOS (iNOS) that is induced by various inflammatory cytokines (44). FeNO is a marker of eosinophilic airway inflammation, able to evaluate the level of inflammation and the response of anti-inflammatory therapy (45).

FeNO is a noninvasive, repeatable and reproducible method (46) applicable in the pediatric practice. The gold standard technique for cooperative children is the single breath on-line method (47) but also other techniques have been proposed for uncooperative children or in sedated infants (47). Nevertheless, at present, no clear evidence is available regarding the potential clinical application of FeNO measurements in uncooperative children. In this age group, the method deserves additional efforts to standardization because it its potential application to predict asthma (33).

FeNO levels can be influenced by different factors such as patient's age, height, gender, and race, nasal contamination, exhalation flow ambient and air pollution (48). The execution of spirometry or exercise before the measurement, diet or exposure to smoke also need to be considered (48).

The standardization of techniques permits to collect comparable data from different centers in healthy children and subjects with diseases. For this purpose, the first document on FeNO evaluation in children was published in 2002 (47), subsequently revised by ATS/ERS in 2005 (48).

Several studies demonstrated that FeNO correlates with airway hyperresponsiveness, IgE serum levels, bronchodilator response, skin prick tests, asthma symptoms, and lung function (49, 50).

Airway inflammation in allergic asthma results from the activation of Th2-mediated pro-inflammatory cytokine mechanism involving IL-4, IL-5, and IL-13. This mechanism causes the expression of epithelial inducible NO synthase up-regulated via STAT-6, a process which is corticosteroid sensitive (51, 52).

Furthermore, other studies showed that FeNO levels are correlated with serum eosinophilic cation protein, eosinophils in induced sputum, blood eosinophilia, eosinophil infiltration of the airways, and IgE levels in atopic patients (52). High FeNO values characterize Th2-mediated airway inflammation, eosinophilia, and responsiveness to ICS (52).

FeNO is helpful during evaluation of the patients characterizing asthma of the eosinophilic phenotype and predicting asthma exacerbation. In children less than 5-year-old with recurrent coughing and wheezing, increased FeNO levels can predict physician diagnosed asthma at school age (53). Furthermore, increased FeNO levels at the age of 4 years, predict higher risk for wheezing, asthma and need of ICS by school age (54).

The ATS guidelines recommend the use of FeNO for monitoring airway inflammation and for addressing the choice of anti-inflammatory treatment (52, 55, 56). It has been extensively proved a rapid decrease of FeNO values when ICS treatment is started, with a dose-dependent mechanism and a sudden rise when ICS therapy is withdrawn (57). This trend may be helpful in monitoring patient adherence to the therapy (46).

High FeNO values are not always linked to eosinophilic asthma but also to allergic rhinitis, eosinophilic bronchitis and allergen or viral exposure and a correct interpretation is stressed in the ATS/ERS document (55).

In patient treated with omalizumab, FeNO values, blood eosinophils and BMI can predict the response to the therapy (58).

Authors, for a long time, have been very cautious to support the use of FeNO as coadjutant to standard symptom-based management (59, 60).

However, in recent Cochrane review it has been showed that FeNO guided treatment in asthmatic children was associated with a significant reduction in exacerbation as compared with guideline-based treatment (61). On these bases, the most recent GINA document in 2018 included FeNO guided treatment as a proposed for tailoring treatment to be considered in children (1).

Therefore, FeNO can be regarded a useful method to categorize patients with eosinophilic and Th2-mediated asthma evaluating the response to ICS therapy, predicting exacerbations and the compliance to the therapy.

EXHALED BREATH VOLATILE ORGANIC COMPOUNDS (VOCS)

Exhaled breath volatile organic compounds (VOCs) are analyzed by breathomics science and they represent a noninvasive tool to evaluate the lung inflammation. VOCs have a metabolic origin from larger molecules. Airway VOCs originate not only from the upper and lower airways but also from a capillary bed near the alveoli (62). Their concentration in exhaled breath is influenced by blood gas coefficient, cardiac output and alveolar minute volume.

The methodological approach to collect VOCs from exhaled breath requires attention to exclude organic compounds from the ambient air, type of sampling (total vs. alveolar breath), type of collecting materials and other confounding factors (62).

In particular, the collection of airway VOCs needs an inhalation filter to exclude ambient VOCs. The patient is asked to breath into a system which can collect online samples directly via inert tubes into an analyzer or off-line by collecting exhaled air into bags, tubes or syringes. These devices are made of inert materials such as Tedlar bags (63).

Gas chromatography-mass spectrometry (GC-MS) and flame ionization detection (GC-FID) are the most widely used techniques to analyze the samples after collection. These methods can differentiate and quantify VOCs at low concentrations, but they require both qualified technicians and expensive technology (33).

A new non-selective approach to analyze VOCs in exhaled breath is metabolomic profiling that identify and quantify all metabolites in a biological sample without *a priori* hypothesis.

Metabolomic profiles describe the interaction among environmental exposure, medication, nutrition, and toxic substances, genetic expression and microorganisms (33, 62). This method is an interesting approach to patient characterization and personalized medication (33). This approach simultaneously evaluates many metabolites in a sample and generates a profile capable of discriminating between different groups of individuals characterizing the biochemical processes underway in each biological system.

More recently sensor-based device such as the electronic nose, colorimetric sensor array, and gold nanoparticle sensors have been proposed. They adopt specific sensors with optical, chemical or electronic properties that analyze VOCs in the EB (62). Recently, some studies have demonstrated the clinical application of these instruments in respiratory disease (64, 65). VOCs in the EB discriminate between asthma and healthy and between atopic and non-atopic children (64, 65). VOCs profile in exhaled breath was able to discriminate healthy, transient wheezing and asthmatic children starting from the age of 2-3-year-old (66). In

the pediatric field, VOCs can also predict asthma exacerbations (67, 68).

Nevertheless, further studies are necessary to evaluate the clinical utility of VOCs in evaluating asthma severity and monitoring asthma symptoms and response to ICS therapy.

CONCLUSIONS

Noninvasive techniques to collect and analyze airways inflammatory biomarkers are helpful in evaluating the airway pathophysiology of asthmatic children.

In clinical practice, FeNO evaluation has been suggested as the only valid and non-invasive technique to test for underlined eosinophilic inflammation, but it would need to be adopted in combination with other useful markers.

The standardization of the new techniques to collect biomarkers in EB and EBC remains problematic. Low reproducibility of exhaled biomarkers and the lack of standardization of the methods of pre-collection, collection, post-collection of the samples, along with the correct interpretation of the results, represent critical issues in clinical practice.

The identification and utilization of ideal and defined biomarkers in asthmatic children remains debated. The reasons for this are the biological aspects of each prospective biomarker, the disease pathobiology, and methods and invasiveness of sample collection. Therefore, the development of novel biomarker with more sensitivity and specificity may lead to prompt diagnosis of severe asthma in future.

AUTHOR CONTRIBUTIONS

MZ, MP, and LT: drafting of the manuscript; GP, MZ, and LT: critical revision.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 02 August 2018
doi: 10.3389/fped.2018.00206

Macrophage Phagocytosis and Allergen Avoidance in Children With Asthma

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Background and Objective: Airway macrophages perform the crucial functions of presenting antigens, clearing pathogens, and apoptotic cells. Macrophage phagocytosis is increased in adults with mild asthma and allergen exposure is known to activate macrophages. However, it is not clear whether the mechanism behind this is due to a primary defect or environmental factors such as allergen or lipopolysaccaride (LPS) exposure. Our aim was to assess the phagocytic function of airway macrophages in children with mild to moderate asthma after residence in a low allergen\LPS environment at high altitude.

Methods: Sputum induction was performed in children with asthma at baseline and after residence for a 3 weeks' period at a high-altitude asthma center that has very low ambient allergen levels. The markers of eosinophilic inflammation (including percentage of macrophage cytoplasm with red hue) and phagocytosis of fluorescein isothiocyanate-labeled, heat-killed *Staphylococcus aureus* by airway macrophages was analyzed. Internalized bacteria were quantified using confocal microscopy.

Results: The median bacterial count [mean (standard deviation)] per macrophage was significantly lower [39.55 (4.51) vs. 73.26 (39.42) ($p = 0.006$)] after residence at high altitude. No association was observed between markers of eosinophilic inflammation and bacterial phagocytosis.

Conclusions: The results suggest that the mechanism behind the enhanced phagocytosis of bacteria in childhood asthma may be secondary to allergen or possibly LPS exposure.

Keywords: macrophages, phagocytosis, asthma, children, allergen avoidance

OPEN ACCESS

Edited by:
Milos Jesenak, Jessenius Faculty of Medicine, Comenius University, Slovakia

Reviewed by:
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Giuseppe Pingitore, Allergy Unit "G.B.Grassi" Hospital, ASL Roma D, Italy

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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *21 May 2018*
Accepted: *02 July 2018*
Published: *02 August 2018*

Citation:
Kulkarni N, Kantar A, Costella S, Ragazzo V, Piacentini G, Boner A and O'Callaghan C (2018) Macrophage Phagocytosis and Allergen Avoidance in Children With Asthma. Front. Pediatr. 6:206. doi: 10.3389/fped.2018.00206

INTRODUCTION

In recent years, knowledge of macrophage biology has vastly improved. The normally protective alveolar macrophages can turn into pathogenic cells in adverse lung environment demonstrating their plasticity (1). The transcriptome and epigenetic landscape of tissue macrophages have been demonstrated as being determined by the tissue-specific microenvironment. Macrophages are

constantly sensing the milieu of the adjacent stroma to regulate the tissue homeostasis both during steady and inflammatory states. (2). Lavin at all have shown that local tissue environment can alter the macrophages to acquire the identity and function of tissue resident macrophages e.g., transfer of bone marrow derived alveolar macrophages or mature peritoneal macrophages into alveolar space converted to alveolar macrophage-like cells (3).

Irrespective of the origin of macrophages (fetal, adult or differentiated tissue) once in lung environment they can fully function as alveolar macrophages and are similar at genomic and epigenomic levels (4, 5). Various studies have revealed that the development and resolution of lung injury is accompanied by remarkable changes in the numbers and types of macrophages (6, 7).

The lung is exposed to the environmental toxins like, microorganisms, chemical agents (including environmental LPS), allergens, and antigens. The alveolar and airway macrophages perform a dual role of activation and suppression of inflammation (8).

Asthma research has long been focused on cells that appear to be the major contributors of airway inflammation, including eosinophils, neutrophils, and lymphocytes. However, few studies have endorsed the mechanisms of function and regulation of macrophages that may orchestrate the inflammatory process. Macrophages are front line defenders of innate immunity and also play a crucial role in organ development, tissue turnover, and regeneration (9). The macrophages are capable of sensing viral, microbial, parasitic antigens, immune complexes, and apoptotic or necrotic cells which helps in immune surveillance (9–11).

Airway macrophage phagocytosis in adults with mild asthma is increased compared to healthy individuals and is possibly related to higher levels of activation of macrophages (12). The enhanced phagocytic function could be related to increased levels of cysteinyl leukotrienes in allergic lung (13) or other inflammatory mechanisms. However, Alexis et al. (12) observed adults with mild asthma, and those with higher eosinophil counts had lower phagocytosis (12). This suggests that macrophage phagocytic function may be related to an interaction with allergen and eosinophilic inflammation. Thus far, there have been no studies on allergen exposure reduction to test this as a possible cause of enhanced phagocytosis. In addition, LPS is well known to activate macrophages resulting in increased chemotactic and phagocytic activity (14) and therefore reduction of LPS would reduce phagocytic activity.

Because of the low relative humidity at high altitudes, the environment is free of house dust mites and molds (15). A number of studies in children (16–20) and adults with asthma have revealed a beneficial effect of high altitude residence on symptoms, lung function, and eosinophilic inflammation (21). However, no study has explored the effect of high altitude on macrophage phagocytosis, which results in a marked reduction in allergen and also possibly LPS exposure.

In adults with high levels of airway eosinophilic inflammation (defined as a sputum eosinophil count greater than 5%), macrophage phagocytosis has been shown to be lower, indicating that eosinophilic inflammation may affect phagocytosis (12). The toxic effect of eosinophil proteins on the epithelium and other cells (22, 23) is well known. These proteins may impair macrophage function.

We have previously shown, however, that sputum eosinophil count alone may be insufficient to identify ongoing eosinophilia (24). In children with asthma glucocorticoid treatment results in reduction of sputum eosinophil count, this could also be due to drop in recruitment of eosinophils because of natural resolution of disease process. Corticosteroid therapy increases eosinophil apoptosis and uptake by macrophages, thereby increasing the eosinophil protein content within the macrophages. To differentiate between these processes and to identify ongoing eosinophilia, we recently developed a novel marker of eosinophilic inflammation (eosinophil protein content in airway macrophages) (24).

In this study we hypothesized that airway macrophage phagocytosis would be lower after reduction of allergen exposure in children with mild to moderate asthma. To test our hypothesis, we compared airway macrophage phagocytosis before and after allergen avoidance during residence at the High Altitude Paediatric Asthma Centre in Misurina (1,756 m), which offers residential treatment for children with asthma. In addition, we assessed the relationship between airway macrophage phagocytosis and markers of eosinophilic inflammation (including eosinophilic proteins in airway macrophages) before their high-altitude residence. According to our understanding, this is the first study that explores the effect of a high altitude, with allergen and possibly LPS avoidance, on the phagocytic function of sputum macrophages in pediatric or adult asthma.

MATERIALS AND METHODS

Study Patients

The study enrolled children aged 7–17 years ($n = 62$) admitted to Istituto Pio XII, Misurina, Belluno (High Altitude Paediatric Asthma Centre) from various cities in Italy between June and September 2010. Children with a diagnosis of asthma and residing in the center for 3 weeks or more were included. The diagnosis of asthma was supported by clinical symptoms and reversibility testing ($>$ 12% increase in FEV_1 after short acting bronchodilator treatment) (25). The exclusion criteria were respiratory infection in the preceding 6 weeks, congenital heart disease and chronic suppurative lung disease, or associated respiratory conditions such as cystic fibrosis and primary ciliary dyskinesia.

Study Design

On the day of arrival (T0) the demographic data, exposure to tobacco smoke, and medications including the doses of inhaled steroids were recorded. All children underwent clinical examination; spirometry, FeNO, and skin prick tests were performed. Spirometry was carried out as per the American Thoracic Society Guidelines (26). Sputum induction and blood eosinophil blood counts were performed within 2 days of arrival. At the end of the stay (T1) the symptoms during the stay and the treatment received were recorded and sputum induction was performed. The inhaled corticosteroid dose was converted to an estimated equipotent daily dose, in accordance with the

guidelines of the Global Initiative for Asthma (GINA) (25) to compare the groups. Ethical approval was obtained by the Ethics Committee for Clinical Research of the Local Heath Authority in Belluno, and the parents provided written informed consent for the study. Our investigation was restricted to children with asthma because no healthy children resided at a high altitude for the time required by the study.

METHODS

Reagents and Chemicals

All reagents, culture media, and latex beads were purchased from Sigma–Aldrich (Milan, Italy) unless otherwise specified.

EXHALED NITRIC OXIDE MEASUREMENT

FeNO was measured using a standard technique complying with the recommendations of the European Respiratory Society/American Thoracic Society (27), using a chemiluminescence analyzer (Logan LR 2149; Logan Research Ltd., Rochester, Kent, UK), and expressed as parts per billion (ppb).

SPUTUM INDUCTION AND PROCESSING

Sputum was induced and processed as described previously (28, 29). Air-dried cytospins were stained with Diff-Quik. The total cell count, cell viability, and level of squamous cell contamination were assessed. The eosinophil differential count was obtained by counting 400 non-squamous cells and expressed as a percentage. The children were divided into 2 groups depending on the differential count on arrival (T0): eosinophilic ($\geq$ 3%) and non-eosinophilic ($<$ 3%).

Macrophage Eosinophil Protein Content

The image analysis method used was as previously described (24). Please refer to the online supporting information for details.

Macrophage Culture and Phagocytosis Assays

The macrophage culture and phagocytosis are described in the online supporting information.

CONFOCAL MICROSCOPY AND IMAGE ANALYSIS OF *STAPHYLOCOCCUS AUREUS* PHAGOCYTOSIS

After adherence, macrophages were incubated for 2 h with fluorescein isothiocyanate conjugated, heat-killed *Staphylococcus aureus* (Invitrogen Milan Italy) resuspended in RPMI 1640 supplemented with 5% FBS (10:1 ratio of staph aureus/Airway Macrophage). Please refer to online supporting information for details of further processing of sample, confocal microscopy, and image analysis to quantify internalized bacteria. The median bacterial count/airway macrophage and median maximum intensity\airway macrophage was calculated for each subject.

Latex Bead Phagocytosis: Phagocytic Index

After adherence, macrophages were incubated with 2-μm latex beads (airway macrophage: bead = 1:10) resuspended in RPMI 1640 (supplemented with 5% FBS) for 2 h. Please refer to the online supporting information for details on the counting of internalized bacteria. The phagocytic index (beads/100 airway macrophages) and number of phagocytic macrophages were calculated.

Statistics

Statistical analysis was performed using GraphPad Prism 6 (GraphPad, San Diego, CA, USA). Patient characteristics are presented as mean (standard error), median (range or interquartile range) or percentages. The between-group comparisons for non-parametric data were made using the Mann–Whitney test for unpaired data and the Wilcoxon matched-pair test for paired data as appropriate and proportions were assessed using Fisher's exact/chi-square test. Differences were considered significant when $p < 0.05$. Linear regression and Spearman's co-relations were used to explore the association between the macrophage eosinophil protein content and phagocytosis.

RESULTS

Patient Characteristics

The clinical characteristics of 54 children who produced adequate sputum for cell counts on arrival (T0) are as shown in **Table 1**. All children were in GINA Groups 1–3 (with 77.7% of children in GINA Groups 1 and 2) depending on the treatment in the previous 3 months prior to the start of residency. Thirty children had sputum eosinophil counts over 3% and were therefore assigned to the eosinophilic group, with 24 in the non-eosinophilic group. The median (range) sputum eosinophil content in the eosinophilic group was 12% (3–52%). Boys were predominant in the eosinophilic group. No difference was observed in FEV_1 between the groups. However, the predicted FEF_{25-75}% was lower and blood eosinophil and FeNo content were higher in the eosinophilic phenotype (**Table 1**).

Fifty children produced adequate sputum samples on both occasions (T0 and T1) for differential counts. No statistical difference was observed in lung function measurements before or after their high-altitude stay in a very low ambient allergen environment. However, after exposure to a high altitude (T1), they had a significantly lower FeNO ($p < 0.001$) and eosinophil counts ($p = 0.001$) (**Table 2**, **Figure 1d**). No difference was observed in percentage cell viability (**Table 1**) (all cell types) in the eosinophilic and non-eosinophilic groups or in the T0 or T1 samples. The patients who produced an adequate sample for phagocytosis assay did not differ from those who did in age, lung function parameters, blood eosinophil counts, or sputum cell counts. The equipotent inhaled corticosteroid dose was reduced ($p = 0.01$) (**Table 2**) during their stay at the center because the children were exhibiting fewer symptoms.

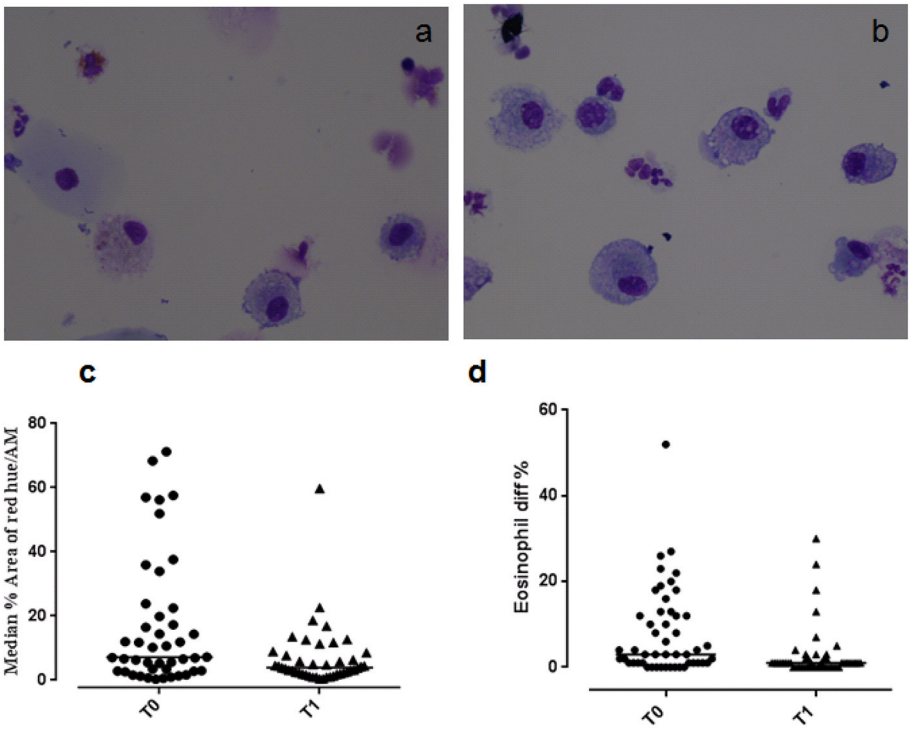

FIGURE 1 | Eosinophilic staining of macrophages at **(a)** T0 and **(b)** T1. Graphs showing **(c)** the median % red hue/airway macrophage ratio, and **(d)** sputum eosinophil differential counts at T0 and T1.

Macrophage Eosinophilic Proteins

Example images of red hue in macrophages before and after residence in Misurina, as an index of eosinophil uptake, are shown in **Figures 1a,b**. No significant difference was observed ($p = 0.39$) between median airway macrophage red hue percentage in eosinophilic ($n = 30$) [median (range)] [5.9 (0.6–57.6)] and non-eosinophilic asthma ($n = 24$) [8.7 (0.34–71.2)]. After time spent at a high altitude (T1), the children ($n = 43$ pairs) had a significantly lower airway macrophage red hue percentage [$p = 0.005$] than before T0 (**Table 2**, **Figure 1c**).

Macrophage Phagocytosis

Examples of confocal images showing internalized bacteria and light microscopy images showing latex beads are presented in **Figures 2**, **3**, respectively. No significant difference ($p = 0.3$) was observed between the median bacterial counts in eosinophilic ($n = 16$) and non-eosinophilic asthma ($n = 8$) at T0. After exposure to a high altitude (T1), the children ($n = 19$ pairs) exhibited significantly lower median bacterial counts [Mean (standard deviation)] [$p = 0.006$, 39.55 (4.51) vs. 73.26 (39.42)] than at T0 (**Table 3**).

Eosinophilic Inflammation and Phagocytosis

No significant correlation or association was observed between age, FeNo, pre- and post-bronchodilator lung function (FEV_1% predicted, FVC% predicted, FEF_{25-75}% predicted), blood counts (total white count, eosinophil count), or sputum parameters (viability, eosinophil and neutrophil counts) and bacterial uptake by macrophages (median bacterial count) and median maximum intensity (bacterial fluorescence). Latex bead phagocytosis (number of phagocytic macrophages (%) and phagocytic index) were significantly positively associated with FeNo ($p = 0.028$) and blood eosinophils ($p = 0.028$), but were not associated with any other parameter. There was a positive correlation between number of phagocytic macrophages (%) and blood eosinophils ($r = 0.508$, $p = 0.046$). The median airway macrophage red hue area percentage was not associated with bacterial or latex phagocytosis parameters. Please refer to online Supplement Figures 1–4.

DISCUSSION

Alveolar macrophages line the luminal surface and are primary defense mechanism to protect the lungs from invading pathogens and pollutants. They initiate and evolve the immune response in the lung. Overall, the number of macrophages in patients with asthma is similar to that in healthy individuals (30).

In asthma, two major functionally distinct subsets of macrophages have been intensively investigated: the M1 and M2 macrophages. Macrophages have been grouped into M1 and M2 types depending on their function in subjects with asthma. M1 macrophages are differentiated by bacteria-derived mediators such as interferon-c and lipopolysaccaride (LPS)

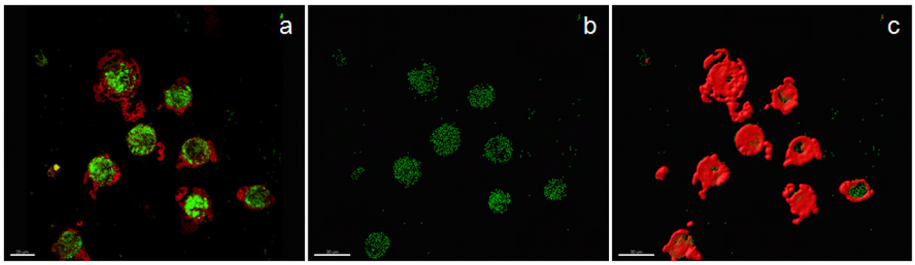

FIGURE 2 | Confocal image of sputum macrophages cultured with heat-killed fluorescein isothiocyanate-labeled *Staphylococci aureus* (airway macrophage:bacteria = 1:10) showing internalized bacteria. **(a)** Confocal image of bacteria (green) and cytoplasm (red), **(b)** bacteria alone, and **(c)** the surface view of macrophages showing that a majority of the bacteria are internalized.

TABLE 1 | Baseline characteristics of patients.

	Eosinophilic group ($n = 30$)	Non-eosinophilic group ($n = 24$)	*p*-value
Mean age (yr) (SD)	13.51 (± 3.1)	13.02 (± 2.9)	0.55
Sex (male), No. (%)	25 (83.3)	13 (54.1)	**0.034**
BMI	18.99 (14.4–31.9)	19.99 (12.8–41.1)	0.7
Smoking in house (yes) No. (%)	12 (40)	7 (29.2)	0.56
Food allergy (yes) No. (%)	14 (46.6)	9 (37.5)	0.58
GINA Classification No. (%)			0.30
1	6 (20)	9 (37.5)	
2	17 (56.7)	10 (41.7)	
3	7 (23.3)	4 (16.7)	
5	0	1 (4.1)	
FEV_1 % predicted mean (SE)	106.8 (2.97)	113.7 (3.7)	0.13
FVC% predicted mean (SE)	104.5 (2.22)	107.2 (2.8)	0.45
FEF_{25-75}% predicted Mean (SE)	98.27 (5.03)	115.9 (6.5)	**0.034**
Blood eosinophils (%)	7.8 (3.9–12.1)	5.05 (1.9–13.1)	**0.014**
Median FeNo (ppb) (range)	23.75 (6.3–79.3)	12.8 (12.7–41.1)	**0.001**
MEDIAN INDUCED SPUTUM (RANGE)			
Total cell count × 10^6 \g sputum	2.3 (0.39–9.5)	1.7 (0.05–7.2)	0.48
Cell viability (%)	86.5 (59.7–96.4)	88.9 (57.9–100)	0.27
Macrophages (%)	62.5 (1–88)	72.5 (14–97)	0.17
Neutrophils (%)	22 (8–72)	26.5 (3–86)	0.46

(Eosinophilic group ≥ 3% sputum eosinophil differential count). Bold P-values indicates $p < 0.05$.

TABLE 2 | Lung function, FeNo, sputum differential counts, inhaled corticosteroids, and macrophage red hue percentage results on arrival (T0), and after the allergen reduction intervention (3-week residence at a high-altitude asthma center) (T1).

	T0 ($n = 50$)	T1 ($n = 50$)	*p*-value
FEV_1% predicted mean (SE)	109.5 (2.4)	116.0 (2.04)	0.67
FVC% predicted mean (SE)	105.8 (1.81)	105.6 (1.89)	0.91
FEV/FVC ratio % predicted mean (SE)	103.1 (1.29)	101.8 (1.3)	0.56
FEF_{25-75}% predicted mean (SE)	105.3 (4.38)	102.9 (4.01)	0.72
Median FeNo (ppb) (range)	18.85 (2.6–79.3)	11.5 (2.1–52.2)	**0.0004**
ICS treatment*			**0.01**
No ICS treatment No. (%)	13 (26)	20 (40)	
Low daily dose No. (%)	21 (42)	26 (52)	
Medium daily dose No. (%)	16 (32)	4 (8)	
MEDIAN INDUCED SPUTUM (RANGE)			
Total cell count × 10^6\g sputum	2.2 (05–9.5)	2.25 (0.26–11.2)	0.24
Macrophages (%)	65 (1–97)	63 (6–87)	0.15
Neutrophils (%)	24.5 (3–86)	32.5 (11–90)	**0.006**
Eosinophils (%)	3 (0-52)	1 (0–30)	**0.001**
Lymphocytes (%)	0 (0–2)	0 (0–8)	0.7
Median % area red hue/airway macrophage (range) ($n = 43$ pairs)	7.04 (0.72–71.23)	3.82 (0.41–59.82)	**0.005**

**GINA classification of equipotent daily dose of inhaled glucocorticosteroids for children older than 5 years (25). Bold P-values indicates $p < 0.05$.*

and release various inflammatory cytokines and chemokines, whereas M2 macrophages have increased phagocytic activity but are poor at clearing intracellular pathogens. Moreover, experimental data suggests both M1 and M2 subsets are involved in asthma (31). In addition there are regulatory macrophages performing different physiological functions and develop in response to different stimuli (32). These are vital in regulating immune responses and reduce inflammation (33). Macrophages are critical for both innate and acquired immunity and play a pivotal role in lung defense. Lung macrophages recognize a wide variety of pathogenic antigens, immune complexes, and apoptotic or necrotic cells (11). Increased infection in asthma has led to increased investigation of the clearance of bacterial, cellular, and inhaled particles by macrophages in patients with asthma.

Alexis et al. have demonstrated that phagocytosis of opsonised particles in subjects with mild asthma with airway eosinophilia was lower as compared to those without eosinophilia. Overall phagocytosis was comparable between controls and those with mild intermittent asthma (12). In a similar subjects, phagocytic

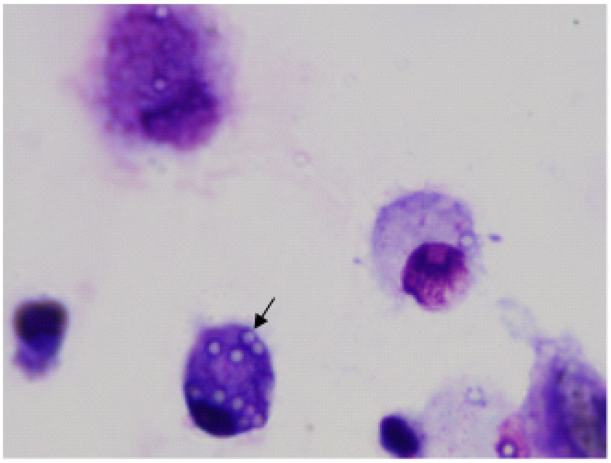

FIGURE 3 | Image of airway macrophages cultured with latex beads (airway macrophage: bead = 1:10) for 2 h. External beads were digested with xylene. Internalized beads are preserved (arrow).

TABLE 3 | Phagocytosis assay on arrival (T0) and after the allergen reduction intervention (T1) (3-week residence at a high-altitude asthma center).

Bacterial phagocytosis (n = 19 pairs)	**T0**	**T1**	***p***
Mean bacterial count mea (SD)	73.26 (39.42)	39.55 (4.51)	**0.006**
Mean max intensity (SD)	0.044 (0.010)	0.038 (0.01)	0.08
LATEX BEAD PHAGOCYTOSIS (n = 10 pairs)			
Median beads/100 cells (range)	201.0 (71.56–1268)	44.52 (9.8–246.8)	**0.003**
Mean % phagocytic AM (SD)	57.70 (16.24)	27.86 (16.36)	**0.001**

Bold P-values indicates $p < 0.05$.

activity in airway macrophages decreased by about 50% 6 h after endotoxin (LPS) inhalation (34).

Fitzpatrick et al. sampled bronchoalveolar lavage macrophages from children with severe asthma and demonstrated impaired phagocytosis of *S. Aureus*. Interestingly this remained unchanged after LPS stimulation. Moreover, they have shown that children with severe asthma also had increased alveolar macrophage apoptosis (35). These later findings were attributed to imbalance in glutathione homeostasis (36). Similar findings of reduced phagocytosis of *H influenzae* and *S aureus* were seen in bronchoalveolar lavage and monocyte-derived macrophages from adults with severe asthma (37). Not only bacterial uptake but apoptotic cell clearance by lavage macrophages is reduced in severe asthma as compared to healthy controls or mild-moderate asthma patients (38).

Studies have demonstrated that severe asthma is associated with inflammatory mediators such as prostaglandin E2 and D2, which can suppress the phagocytic activity of alveolar macrophages (39–41). Recently, Brugha et al. investigated the phagocytosis of inhalable carbonaceous particle matter by airway macrophages obtained from the induced sputum of asthmatic children. Their results demonstrated a 51% lower carbon content in the macrophages of children with moderate to severe asthma compared with mild asthma and healthy children (42). Moreover, the study demonstrated an inverse association between alveolar macrophage carbon and urinary metabolites of prostaglandin E2 and prostaglandin D2.

The effect of a high altitude on improvement of asthma symptoms has been attributed to the very low levels of ambient allergens. In this study, we described for the first time the effect of residence at a high altitude with markedly reduced allergens and possibly pollutants (e.g., LPS) on the phagocytic function of sputum macrophages and markers of eosinophilic inflammation in children with mild to moderate asthma. According to our understanding, this is the original study exploring the effect of reduced allergen\LPS exposure on the phagocytic function of sputum macrophages in pediatric or adult asthma. The phagocytosis of heat-killed bacteria in this group of children decreased after residence at a high altitude and was similar in eosinophilic and non-eosinophilic phenotypes. We also found that bacterial phagocytosis was not associated with parameters of eosinophilic inflammation, including the median airway macrophage red hue percentage.

Our study strongly suggests that allergen and possibly LPS exposure is a major contributor to enhanced macrophage phagocytosis in asthma. The induced sputum macrophage capacity to phagocytose particles has been demonstrated to be higher in subjects with mild asthma (12, 43) than in healthy controls. One possible explanation is that activation of macrophages is enhanced in asthma. Silva et al showed that cysteinyl leukotrienes enhanced Fcγ-mediated phagocytosis in sensitized rats (13). **(author?)** (28) showed that children with asthma, after residence in a very low ambient allergen environment at a high altitude had lower exhaled breath cysteinyl leukotrienes and leukotriene B4 (28). This might partially explain our finding of reduced phagocytosis in children with mild asthma staying in similar conditions. Along with reduction in allergens there is also possibility of lower level of ambient pollution including LPS. LPS is known to stimulate macrophages and therefore reduction would lower phagocytic capacity.

Fitzpatrick et al. sampled bronchoalveolar lavage macrophages from children with severe asthma and demonstrated impaired phagocytosis (35) but did not include children with mild asthma. We were unable to compare our results of phagocytosis assays with other studies assessing sputum macrophage phagocytosis because this is the first such study on children with asthma. In addition, no studies have included reduction of allergens as an intervention to assess phagocytosis of sputum macrophages. No difference was observed in the viability of sputum cells on arrival or after residence at a high altitude that could have affected the macrophage phagocytic function.

We recently described a novel marker (macrophage eosinophil protein content measured as red hue percentage) that identifies ongoing sputum eosinophilia in patients with asthma and a normal sputum eosinophil count (24). On admission, children without sputum eosinophilia had similar airway macrophage red hue percentages as those with sputum eosinophilia. This suggests the presence of ongoing eosinophilia in the non-eosinophilic group. Because airway macrophages

acquire eosinophilic proteins by phagocytizing apoptotic eosinophils, a lower airway macrophage red hue suggests that macrophages have ingested fewer eosinophils. Therefore, the combination of low airway macrophage red hue and low sputum eosinophil counts suggests a reduction of eosinophil recruitment to the airways. The reduction of sputum eosinophilia after residence at a high altitude has previously been demonstrated (20, 28). In children with asthma glucocorticoid treatment results in reduction of sputum eosinophil count, this could also be due to drop in recruitment of eosinophils because of natural resolution of disease process. Corticosteroid therapy increases eosinophil apoptosis and uptake by macrophages, thus increasing the eosinophil protein content within macrophages. It could be argued that when admitted to the center that supervises administration of treatment, the improved adherence to therapy could have contributed to the reduction in eosinophilic inflammation. However, this is unlikely because the airway macrophage red hue percentage dropped after residence at a high altitude, suggesting reduction of eosinophil ingestion by sputum macrophages and reduced recruitment. Therefore, using the macrophage red hue measurement has the added value of helping to differentiate between a reduced sputum eosinophil count related to treatment and that related to reduced recruitment of eosinophils.

Although evidence (12) indicates that increased eosinophilic inflammation is inversely associated with phagocytosis, we did not find an association between bacterial phagocytosis and markers of eosinophilic inflammation (including the eosinophilic proteins in airway macrophages). However, this relationship must be explored further in children with severe asthma who have persistent inflammation.

Our study had some limitations. We were unable to recruit healthy children because they did not reside for 4 weeks at the high-altitude asthma center. Because of the small amount of sputum samples obtained from the children, insufficient numbers of macrophages were obtained to analyze the macrophages for cell surface markers of activation. It is possible that *in vivo* phagocytosis may be affected by other factors that we did not measure. However, our study results indicating higher phagocytosis in patients with mild asthma are like those of inhaled particles in adults in *in vivo* experiments (43).

In conclusion, induced sputum was successfully used to study macrophage phagocytosis in children with asthma. The sputum macrophages in children with mild to moderate asthma after allergen and possibly lipopolysaccaride avoidance at a high altitude are less phagocytic. Because activation of macrophages by allergens in mild asthma enhances phagocytosis, removal of this stimulus appears to reduce phagocytosis. No association was observed between phagocytosis and eosinophilic inflammation markers. Further research is essential to dissect the mechanism of various types of alveolar macrophage phagocytosis in asthma.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

The authors thank all staff at High Altitude Children's Asthma Center in Misurina, Claire Smith, Louise Donnelly (Imperial College London for advice on methodology), Will Monteiro (Institute for Lung Health in Glenfield Hospital). This research is supported by the NIHR GOSH BRC. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health. NK is the recipient of a European Respiratory Society Fellowship (Number 774).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2018.00206/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 23 August 2018
doi: 10.3389/fped.2018.00234

Treating Pediatric Asthma According Guidelines

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Asthma is a common chronic inflammatory disorder of the lower respiratory airways in childhood. The management of asthma exacerbations and the disease control are major concerns for clinical practice. The Global Strategy for Asthma Management and Prevention, published by GINA, updated in 2017, the British Thoracic Society/Scottish Intercollegiate Guideline Network, revised in 2016, the National Institute for Health and Care Excellence asthma guideline consultation, available in 2017, are widely accepted documents, frequently implemented, with conflicting advices, and different conclusion on asthma definition and treatment. An International Consensus on Pediatric Asthma was carried out in 2012 by a Committee with expertise in the field, to critically review differences on current guidelines. In addition, the specific issue of treating severe and difficult asthma has been recently highlighted throughout the International European Respiratory Society/American Thoracic Society guidelines on severe asthma. The aim of this paper is to describe conventional treatments and some new therapeutic approaches to pediatric asthma according to guidelines, highlighting key aspects, and differences on proposed clinical recommendations for asthma management. Age specific therapy are proposed in steps, according to clinical severity and the level of disease control. If control is not achieved within 3 months, stepping-up should be considered; otherwise, if control is achieved after 3 months, stepping down may be considered. The most used drug classes of asthma medications are beta-2 adrenergic agonists, corticosteroids, and leukotriene modifiers. Intramuscolar triamcinolone has been used for severe asthma treatment. Chromones and xanthines have been extensively used in the past, but they have shown limits related to their efficacy and safety profile. Omalizumab, a monoclonal antibody against IgE, is an immunomodulatory biological agent, used as new drug in patients with confirmed IgE-mediated allergic asthma, only for patient's specific range of total IgE level. There are low evidences in the efficacy of metotrexate, as well as macrolide antibiotics in children with asthma. Antifungal agents are also not recommended in asthmatic patients. Non-pharmacological measures that may improve patient's quality of life should also be attempted. We conclude that treatment decisions on childhood asthma management should be critically made, pondering the differences suggested by agreed international consensus documents.

Keywords: asthma, pharmacology, guidelines, asthma management, children

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *30 May 2018*
Accepted: *01 August 2018*
Published: *23 August 2018*

Citation:
Tesse R, Borrelli G, Mongelli G, Mastrorilli V and Cardinale F (2018) Treating Pediatric Asthma According Guidelines. Front. Pediatr. 6:234. doi: 10.3389/fped.2018.00234

INTRODUCTION

Asthma is a heterogeneous chronic airway disease very common in childhood, usually characterized by respiratory symptoms including wheeze, breathlessness, chest tightness and cough, together with variable expiratory airflow obstruction (1, 2). There is widespread concern about symptom control in asthmatic patients, as well as risk of adverse outcomes into clinical practice. It is therefore not surprising that several guidelines are available to support health care professionals on asthma management of children and adults.

The Global Strategy for Asthma Management and Prevention (GINA) and the British Thoracic Society (BTS)/Scottish Intercollegiate Guideline Network (SIGN) are widely accepted documents, released in the USA and in Europe, respectively. Since 2002 the GINA Science Committee was established to review published research on asthma. Expert leaders in adult and pediatric asthma research, regularly meet with the American Thoracic Society (ATS) and European Respiratory Society (ERS) international conferences, to critically review asthma-related scientific literature. The major revision of GINA has been updated in 2017, suggesting recent conclusions on asthma definition and treatment (3).

The first BTS guideline dated back to 1990 and the SIGN guidelines to 1996, but in 1999 the two Society jointly produced new asthma guideline, updated annually, until 2016, and made available on websites (4).

Both guidelines are frequently implemented, providing recent updates recommendations for asthma management and prevention, with conflicting advices and different conclusion on asthma definition and treatment (5, 6). In 2015 the United Kingdom (UK) National Institute for Health and Care Excellence (NICE) also released a consultation document for diagnosis and monitoring of asthma, and later, on December 2016, for the management of chronic asthma, including cost benefit analysis that were not considered in the BTS/SIGN guidance (7).

An International Consensus on Pediatric Asthma was carried out in 2012 by a working Committee with expertise in the field (8), to critically review differences on the current abovementioned International guidelines, but also including other reports like the Australian Asthma Management document and the Japanese Guideline for Childhood Asthma (9, 10).

Non pharmacological measures that may improve quality of life and reduce symptoms in asthmatic people should be attempted, particularly in children. They include avoidance of exposure to environmental tobacco smoke or cessation of smoking among adolescents, avoidance of food or drug triggers, in people sensitive to them, avoidance of indoor and/or outdoor pollution and irritants (11, 12). Weight loss in overweight patients should be advised; breathing exercise programmes, following physiotherapist methods should also be encouraged.

However, pharmacotherapy represents the fundament of asthma treatment in adults and children. The international reports define the principles of pharmacological asthma management and indicate age specific treatments in steps, according to clinical severity and the level of disease control, which is determined by the interaction between the patient's genetic background, the ongoing treatment, environment, and psychosocial factors. Asthma severity is assessed retrospectively from the level of treatment required to control symptoms and exacerbations, that may change over months.

Asthma phenotypes have been defined based on specific pathologic features, demographic and clinical patterns or therapy responses and personalized phenotype-guided asthma treatments are under investigation, with the acknowledgment of the patient's adherence to therapy issues (13).

This review gives a practical perspective on the most used drugs and the basic steps of asthma management in children and adolescents, based on current international guidelines recommendations, highlighting the key aspects and differences of these documents.

CATEGORIES OF ASTHMA MEDICATIONS AND STEP-WISE STRATEGIES OF TREATMENT

The pharmacological options for treatment of asthma include, according to their use, reliever medications, which are drugs that allow relief of symptoms within few minutes, during worsening asthma or exacerbations, also used for prevention of exercise-induced bronchoconstriction; controller medications, that are used for maintenance treatment: they control symptoms and reduce airway inflammation and future risks of exacerbations; add-on therapies, proposed for patients with severe persistent asthma symptoms and exacerbations, despite treatment with high dose controller medications.

The most used drug classes of asthma medications are beta-2 adrenergic agonists, corticosteroids, and leukotriene modifiers, usually montelukast.

A stepwise approach for pharmacotherapy management in asthmatic patients has been proposed (**Table 1**). Treatment starts at the step most appropriate to the initial severity of asthma. If control is not achieved within 3 months, stepping-up should be tried, after reconsidering adherence to therapy, environment factors, and associated co-morbidities; otherwise, treatment step down may be attempted once good asthma control and the patient's lowest effective level of treatment has been found and maintained for about 3 months. Each recommendation has been assessed for adults adolescents (over 12 years) and children (5–12 years, and under 5 years) in all guidelines (3, 4).

Medications Used for Rapid Relief of Symptoms

At present, **Step 1** treatment is with as-needed inhaled short-acting beta2-agonists (SABAs) alone, commonly salbutamol. SABAs are used for acute relief of asthma symptoms, mainly in patients with occasional daytime symptoms and with normal lung function. Inhaled anticholinergic agents, usually ipratropium, are second-line relievers (14); they are less effective than SABA, but may have synergistic effects when added to SABA during severe exacerbations in reducing patients hospitalization (3, 4).

TABLE 1 | Stepwise pharmacotherapy management in asthmatic children.

	STEP 1	STEP 2	STEP 3	STEP 4	STEP 5
Reliever therapy	As-needed SABA		As-needed SABA or low dose ICS/ LABA		
Controller therapy		Low dose ICS	Low /medium ICS/LABA	Medium/high ICS/LABA	Add-on treatment (omalizumab)
Other common controller options	Low dose ICS	LTRA	Medium/high dose ICS Low ICS + LTRA	High dose ICS + LTRA	Low dose OCS

SABA, short-acting beta$_2$-agonist; ICS, inhaled corticosteroids; LABA, long-acting beta$_2$-agonist; LTRA, leukotriene receptor antagonists; OCS, oral corticosteroids.

In adults, oral SABA or short-acting theophylline are potential alternatives to SABA, however, they have a slower onset of action than inhaled SABA and a higher risk of side-effects, and they are not recommended in children (15).

Medications Used for Long-Term Asthma Symptoms Control

Inhaled Corticosteroids (ICS)

More frequent symptoms or the presence of any exacerbation risk factors indicate that regular controller treatment is needed. For long term asthma control in children, a maintenance treatment with therapeutic doses of ICS in addition to as-needed SABA, should be considered. Regular low dose ICS improve asthma symptoms and lung function, decrease need for additional medication and hospital admission. In the international reports this combination of drugs is the first option of treatment included into **Step 2** (3, 4). ICS differ in potency and bioavailability; beclomethasone dipropionate (BDP) and budesonide have approximately equivalent effects in clinical practice, although there may be some variations using different delivery devices. Fluticasone propionate and mometasone appear to provide the same clinical activity, compared to BDP and budesonide, at half the dosage. The initial dose of ICS should be appropriate to the severity of disease (**Table 2**). In children and adolescent, the starting ICS dose will usually be less or equal 200 micrograms BDP or equivalent per day, given initially twice daily (except ciclesonide proposed once daily). More than 200–400 μg BDP or equivalent would be considered a pediatric moderate dose, and more than 400 μg a pediatric high dose. The dose of ICS should then be titrate the to the lowest effective dose at which control of asthma is maintained. There is an increasing evidence showing that, at recommended doses, ICS are also safe and effective in young children with asthma (16).

However, long term follow-up studies have demonstrated some effect of the chronic ICS use at intermediate-high doses with growth retardation in pre-pubertal children in the first years of treatment, and the reduction in adult final height (17–19). Poorly-controlled asthma itself may affect patient's growth (20). Therefore, after symptoms control has been achieved, ICS therapy should be gradually tapered to the lowest effective dose.

Clinical adrenal suppression has also been described in a small number of children who had been treated with ICS (21). The duration of ICS treatment that may expose a child at risk of clinical adrenal insufficiency is unknown but is likely to occur at high ICS dose per day (at ≥800 μg BDP per day or equivalent). Tests of adrenal function such as the low-dose adrenocorticotrophic hormone (ACTH) test, may be useful in predicting clinically relevant adrenal insufficiency in a child using high-dose ICS, but it is unknown how frequently would need to be repeated in children.

TABLE 2 | Low, medium, and high inhaled corticosteroids doses in asthmatic children and adolescents.

Inhaled corticosteroid	Total daily dose (mcg)		
	Low	Medium	High
SCHOOL AGED CHILDREN			
Beclometasone dipropionate	100–200	200–400	>400
Budesonide	100–200	200–400	>400
Ciclesonide	80	80–160	>160
Fluticasone propionate	100–200	200–400	>400
Mometasone furoate	110	220–440	>440
Triamcinolone acetonide	400–800	800–1,200	>1,200
ADOLESCENTS			
Beclometasone dipropionate	200–500	500–1,000	>1,000
Budesonide	400–200	400–800	>800
Ciclesonide	80–160	160–320	>320
Fluticasone propionate	100–250	250–500	>500
Mometasone furoate	110–220	220–440	>440
Triamcinolone acetonide	400–1,000	1,000–2,000	>2,000

Moreover, the use of extra-fine (diameter <2 μm) vs. fine particle BDP administered as mono-therapy or in combination with LABA, have shown reduction in exacerbation rates in severe asthmatic patients, in a meta-analysis of observational studies (22). Corticosteroid insensitivity is also another specific issue under investigation: molecular mechanisms including glucocorticoid receptor alteration, have been proposed (23).

For patients with persistent symptoms or recurrent exacerbations despite the use of low dose ICS, may be considered a step up, after checking for medication adherence, inhaler technique, continuous allergen exposure and comorbidities such as rhinosinusitis, gastroesophageal reflux, obesity, and obstructive sleep apnea.

Long-Acting Beta-2 Adrenergic Agonists (LABA)

In adolescents likewise in adults, the combination of ICS and LABA, including salmeterol and formeterol, improves asthma outcomes more than higher doses of ICS, and it should be considered before increasing the dose of 400 μg BDP or equivalent per day and certainly before administering 800 μg of

BDP. A LABA should not be used as monotherapy for asthma but only in fixed-dose combination devices also containing an ICS. Recent studies have shown that the risk of adverse events related to the use of LABA when combined with steroids is similar to the risk of using corticosteroids alone in asthmatic patients (24, 25). However, it is more expensive and does not reduce the risk of further exacerbations compared with ICS alone. In addition to studies reassuring about the use of LABA in adolescent and adults (25), an international trial that followed for 26 weeks 6,208 children, aged 4–11 years, with asthma and an exacerbation in the previous year, has also shown that addition of salmeterol to fixed fluticasone doses is effective and does not lead to increased risk of serious asthma-related adverse outcomes than using the ICS alone (26).

Leukotriene Receptor Antagonists (LTRAs)

LTRAs work by blocking some inflammatory responses, such as tightening of airway muscles and the production of mucus secretion, mediated by leukotriens, which are released during asthmatic reaction by cells involved in the pathogenesis of asthma. In international guidelines LTRA are recommended in monotherapy, as second choice after low dose ICS, for the initial step of chronic asthma treatment. Into the next steps, they are also considered as add-on medications, usually in addition to ICS for improving symptoms and pulmonary function, by an increase in antinflammatory activity (27). They may be considered especially for some patients who experienced side-effects using ICS or children under 5 years old (28), and they are effective especially in exercise-induced asthma (29, 30). A review by Bisgaard et al. on more than 2,700 children and adolescents enrolled in several studies, suggested that the safety profile for montelukast was similar to that of placebo or other usual care therapy, and did not change with long-term use (31).

The options of treatment at **Step 3** differ depending on age group. In young children, a medium dose of ICS plus as-needed SABA is the preferred solution, whereas in adolescents, as well as in adults, the combination of low dose ICS/LABA (BDP/formoterol or budesonide/formoterol) as maintenance treatment with as-needed SABA as reliever, or low dose ICS/LABA (BDP/formoterol) as both maintenance and reliever treatment may be considered. Adding LABA to the same low dose of ICS seem to improve, in this age group, symptoms and lung function and reduce risk of exacerbations, compared with a fixed dose of ICS/LABA as maintenance treatment or a higher dose of ICS, both with as-needed SABA. Another option for adults and adolescents if asthma control remains suboptimal, is to increase ICS to medium dose, but this is less effective than adding a LABA. Other less efficacious options are low dose ICS plus either LTRA or low dose, sustained-release theophylline (3).

For adolescent patients as well as adults, low dose maintenance and reliever ICS/LABA with as-needed SABA, is suggested for **Step 4;** if necessary, in patients with not complete symptoms control, may be considered the use of medium dose ICS. For children <12 years, if asthma control is not achieved using moderate dose ICS, the recommendation is to refer the child for expert assessment. A high dose is recommended only when good asthma control cannot be achieved with medium dose ICS plus LABA and/or a third controller, such as LTRA. However, the increase in ICS dose generally provides little additional benefit, and there is an increased risk of side-effects.

The NICE publication include some conflicting advices on key issues of asthma management in primary care. One controversial difference is about the use of LTRA as first choice add-on therapy for patients whose asthma is not well controlled with low dose ICS. LABA are considered marginally more effective than LTRA, in the analysis by NICE, to justify the additional cost (7). The Primary Care Respiratory Society UK has suggested an initial trial of LTRA as add-on treatment to low dose ICS, but underlie that it should not be changed the treatment of patients already favorably established on a LABA regimen (5).

When patients experience persistent symptoms or exacerbations despite high-dose ICS or ICS/LABA, or other options of Step 4 treatment, there are different pharmacological options that may be considered at **Step 5**, as following listed.

Oral Corticosteroids

Add-on low dose oral corticosteroids (≤7.5 mg/day prednisone equivalent) may be considered, but may be associated with potential side effects especially in long-term treatments. Prednisolone is the most used steroid for maintenance therapy in patients with chronic asthma (32). Blood pressure, urine or blood sugar, cholesterol, bone mineral density, growth (height and weight centile), should be regularly monitored, and cataracts should be screened in patients using steroid tablets.

Omalizumab

Anti-immunoglobulin E (anti-IgE) treatment for patients >12 years of age, with severe allergic asthma, impaired lung function, and proven IgE-mediated sensitivity to inhaled allergens, is also a treatment option. Omalizumab is a humanized monoclonal antibody that interferes with the inflammatory cascade by reducing serum IgE levels and inhibiting IgE binding to receptors. It is given as a subcutaneous injection every 2 or 4 week depending on patient weight and total serum IgE levels. It exhibit a suitable clinical outcome reducing exacerbations, and improving asthma control (33). School-age children and adolescent with moderate-to-severe asthma treated with omalizumab in long-term trials had a significantly reduced number of exacerbation attacks, improved the quality of their life and reduced the need for other standard medications to control asthma (34, 35). Omalizumab is safe and well tolerated but it has the inconvenience of the subcutaneous administration and elevated costs.

Immunotherapy

Studies using both subcutaneous and sublingual allergen immunotherapy (SCIT and SLIT) have demonstrated benefit in reducing asthma symptoms and bronchial hyperreactivity in children who do not completely respond to other preventative strategies including ICS (36). The safety profile of SLIT seems to be better than SCIT. There are, however, few studies of adding immunotherapy to ICS so there is difficulty precisely defining where it should sit in step-wise asthma management.

Mepolizumab

The use of specific anti-IL5 monoclonal antibodies treatment (mepolizumab), based on eosinophilia (>3%) in induced sputum as biomarkers to identify responsive patients is a debated topic. Some trials indicate that anti-eosinophil biologic drug can be effective for treatment of the severe systemic corticosteroid-dependent asthma, with a "eosinophilic" endotype, whose disease is largely dependent on eosinophil pathophisiology, by a beneficial effect on airway remodeling (37).

OTHER PHARMACOLOGICAL OPTIONS

Theophylline and Chromones

Other drug solutions not recommended for routine use are theophylline, the most used methylxanthine, which has low efficacy in asthma bronchoconstriction and common side-effects, especially when administered at higher doses (38), and chromones (nedocromil sodium and sodium cromoglycate) used as mast cell stabilizer, having a suitable safety profile, but low efficacy (39). They are included in guidelines as second-line medications in initial treatment steps and prevention of exercise-induced asthma.

Intramuscolar Triamcinolone

Intramuscolar treatment with triamcinolone has been used for severe asthma treatment. Pediatric studies suggest that it may affect eosinophilic inflammation, improve airway obstruction and prevent exacerbation attacks in children with severe asthma (40, 41), but side effects using it are worse than after treatment with oral corticosteroids (42, 43).

Anticholinergic Agents

The addition of tiotropium bromide, a long-acting inhaled anticholinergic agent, to ICS plus LABA has been shown to improve lung function and asthma symptom control in patients with severe persistent asthma in adults (44). There have been no studies of the use of tiotropium in asthmatic children.

Metotrexate, Antifungal Agents, Macrolides

There are low evidences in the efficacy of metotrexate in children with asthma. A meta-analysis investigating the steroid-sparing effect of oral metotrexate in asthmatics showed a small benefit despite a high recurrence of adverse effect (45).

Antifungal agents are not recommended in asthmatic patients without allergic broncopulmonary aspergillosis (46). Vicencio and coworkers recently documented that a high percentage of children with refractory asthma had fungal sensitization correlated to the severity of their disease. Based to a case report of a positive response to itraconazole treatment of a child with severe asthma, the Authors suggest that antifungal therapy may represent a potential successful treatment in some patients with evidence of fungal sensitization, after eliminating molds in the environment (47, 48).

Macrolides have been also proposed in combination to the treatment regimen of asthmatic children, with the aim of decreasing the need of the patient's daily dosage of corticosteroids, due to its antinflammatory and antimicrobial activity (49). A recent study including 40 school-aged asthmatic children showed that a 3-week course of clarythromicyn given as add-on therapy to regular treatment, was associated with an increased control of symptoms and a decrease in the duration of the asthma exacerbations (50). Since the use of this antibiotic is safe compared to other medications, it may be indicated in severe asthmatic patients who have mainly neutrophilic airway inflammation and show resistance to other therapy. However, in long term treatment the development of macrolide resistance among respiratory pathogens should be considered.

ASTHMA SEVERITY ASSESSMENT

Asthma severity can be assessed when the patient is in a regular control of symptoms with continuous treatment:

- Mild asthma is asthma that is well controlled with Step 1 or Step 2 treatment, i.e., with as-needed reliever medication alone, or with low-dosage controller treatment such as low dose ICS, LTRA, or chromones.
- Moderate asthma is asthma that is well controlled with Step 3 treatment e.g., low dose ICS/LABA.
- Severe asthma is asthma that requires Step 4 or 5 treatment, e.g., high-dose ICS/LABA, to prevent it from becoming "uncontrolled," or asthma that remains "uncontrolled" despite this treatment. The ERS/ATS Task Force on Severe Asthma has published International guidelines providing definition and guidance about the management of patients with uncontrolled asthma (51).

CONCLUSIONS

We conclude that due to heterogeneity of asthma characteristics, treatment decisions should be critically made, pondering the differences highlighted by agreed international consensus documents.

Symptoms control of asthmatic patients should be closely monitored, as well as risk factors and frequency of exacerbations, and the response to any treatment adjustment should be documented and regularly reviewed by specialists. A step up in treatment may be considered if patients do not respond suitably to initial treatment, after checking for comorbidities, or alternative therapy options may be attempted. A follow-up visit within 1 week after an exacerbation attack should be scheduled, and a written asthma action plan should be completed by the patient as part of a personal asthma management education. An occasional short-term step up for weeks in maintenance pharmacological doses may be necessary, for example, during viral infections or seasonal allergen exposure. Sometimes a daily dose adjustment of the maintenance therapy dosage may be needed according to symptoms. Ongoing monitoring adherence to asthma therapy and asthma control by spirometry in children who can perform it, and self-monitoring at home by peak expiratory flow evaluation, together with avoidance of triggers, is also encouraged.

AUTHOR CONTRIBUTIONS

RT designed the review and drafted the manuscript. GB, GM, and VM contributed to data collection and interpretation of the literature data. FC revised the work and provided critical input to the manuscript. All Authors approved the final version of the manuscript as submitted.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

MINI REVIEW
published: 21 August 2018
doi: 10.3389/fped.2018.00231

Immunotherapy and Asthma in Children

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Allergen immunotherapy (AIT) is still the only disease-modifying treatment strategy for IgE-mediated allergic diseases, with consolidated evidence both in adults and children. AIT is effective in determining clinical improvement of allergic rhinitis and asthma, such as reduced symptoms, medication use, and improvement of quality of life, with a long-lasting effect after cessation of treatment. Results from recent clinical studies have implemented the evidence of effectiveness and safety of allergen immunotherapy for the treatment of allergic asthma, so that the current asthma guidelines now recommend sublingual immunotherapy as an add-on therapy for asthma in adults and adolescents with house dust mite allergy, allergic rhinitis, and exacerbations despite low-to-moderate dose ICS, with forced expiratory volume in 1 second more than 70% predicted. AIT may also reduce the risk of progression from allergic rhinitis to asthma in children and prevent the onset of new sensitizations, thus representing a potentially preventive method of treatment. The aim of this review is to present an updated overview of the clinical indications of AIT, with particular reference to pediatric asthma, of the mechanisms of clinical and immunological tolerance to allergens, and of the potential biomarkers predicting clinical response.

Keywords: allergen immunotherapy, subcutaneous immunotherapy, sublingual immunotherapy, allergic rhinitis, asthma

OPEN ACCESS

Edited by:
Renato Cutrera, Bambino Gesù Ospedale Pediatrico (IRCCS), Italy

Reviewed by:
Yusei Ohshima, University of Fukui, Japan
Elisabetta Calamelli, Università degli Studi di Bologna, Italy

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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *24 May 2018*
Accepted: *30 July 2018*
Published: *21 August 2018*

Citation:
Tosca MA, Licari A, Olcese R, Marseglia G, Sacco O and Ciprandi G (2018) Immunotherapy and Asthma in Children. Front. Pediatr. 6:231. doi: 10.3389/fped.2018.00231

INTRODUCTION

Allergen immunotherapy (AIT) is the administration of the causal allergen to control allergic inflammation and symptoms. AIT has been used for over a century and it is actually considered the only disease-modifying treatment strategy for IgE-mediated allergic diseases, as causing a persistent immunological and clinical tolerance toward the causal allergen (1).

Both subcutaneous AIT (SCIT) and sublingual (SLIT) are used and accepted as effective treatments for adults and children with allergic rhinitis (AR) with or without asthma (2, 3). Historically, SCIT was early proposed as the first route of AIT administration, since first report (4). However, despite its proven effectiveness, the use of SCIT is still limited by the need for frequent injections by a doctor over a minimum of 3 years, and, mostly important, the potential occurrence of systemic severe reactions. Consequently, SCIT should be administered in a medical setting by clinicians able to manage anaphylaxis (1). Furthermore, the risk of systemic reactions to SCIT is greater in subjects with uncontrolled asthma and with accelerated dosing schedules (5). Given these disadvantages, SLIT may represent a viable alternative to SCIT, mainly in children, allowing safe self-administration at home (6–8). So, route selection vaccine is based on availability or approval, cost, and the patient's age or the physician's or patient's preference (9).

SCIT or SLIT products cannot be actually compared due to their heterogeneous composition and allergen concentration (10). Existing studies suggest that both may induce similar immunologic changes (11). Allergens are used for SCIT as aqueous or physically-adsorbed (depot) extracts, as well as chemically modified allergens (allergoids) as depot formulations. Allergens for SLIT are used as drops or tablets. Waiting for a harmonized and international AIT products regulation, they are still available most commonly either by being distributed as "named patient products" (NPP), prepared simply in compliance with Good Manufacturing Practice, and by obtaining a formal marketing authorization (12). SLIT tablets for grass pollen and house dust mite (HDM) have been recently registered for use in children, adolescents, and adults (13, 14).

Through complex molecular and cellular mechanisms inducing immune tolerance, effective AIT may modify the natural course of allergic disease, both preventing the onset of new sensitizations and the clinical disease progression (from rhinitis to asthma). AIT may also control allergic symptoms that are unresponsive to avoidance strategies and medications, reduce medication use, and improve quality of life, with a long-lasting effect after cessation (15, 16). Results from recent trials have implemented the evidence of effectiveness and safety of AIT for the treatment of allergic asthma, so that updated asthma guidelines now recommend SLIT as an add-on therapy for asthma in adults and adolescents with HDM allergy, under certain conditions (3).

The aim of this review is to present an updated overview of the clinical indications of AIT, with particular reference to pediatric asthma, of the mechanisms of clinical and immunological tolerance to allergens, and of the potential biomarkers predicting clinical response. A literature search was performed through Medline via Pubmed to identify all relevant articles published in English, on the basis of the following three search terms: "allergen immunotherapy", "children," and "asthma." From the articles retrieved in the first round of search, additional references were identified by a manual search among the cited references.

OVERVIEW OF THE MECHANISMS OF ALLERGEN IMMUNOTHERAPY

AIT targets the upper and lower respiratory allergic symptoms by modulating the IgE-mediated response consequent to allergen exposure. Through multiple mechanisms involving both innate and adaptive immunity, AIT regulates T- and B-cells, changing antibody isotypes, decreases mediator release, and migration of inflammatory cells to tissues (17–19).

A key mechanism in inducing immunologic tolerance is the upregulation of allergen-specific T-regulatory (Treg) cells and B-regulatory (Breg) cells, which primarily down-regulate the Th_2 response (19) (**Table 1**). Regulatory cells inhibit the activation of allergen-specific Th_2 lymphocytes, suppress allergic inflammation, and ultimately shift toward a Type 1-mediated immune response, releasing cytokines, interleukin (IL)-10 and transforming growth factor-β (TGF-β) (20).

TABLE 1 | Mechanisms of immunologic tolerance mediated by T and B regulatory cells during allergen immunotherapy.

Treg-mediated mechanisms
Release regulatory cytokines (IL-10, TGF-β, and IL-35)
Induce tolerogenic DCs subsets
Reduced number of ILC2
Suppress activation of allergen-specific Th_2 lymphocytes
Downregulate the expression of FCεRI receptors on mast cells,
Decrease allergen-specific IgE synthesis
Promote B-cell production of IgG_4 antibody
Breg-mediated mechanisms
Release regulatory cytokines (IL-10, TGF-β)
Induce the synthesis of IgG4 blocking antibodies
Inhibit activation and proliferation of effector T lymphocytes
Suppress Th_2-dependent inflammation
Promote T-cell expression of Foxp3 and generation of functional Treg cells

Breg, B regulatory; DCs, dendritic cells; FCεRI, high-affinity receptor for the Fc region of IgE; Foxp3, forkhead box P3; IgE, immunoglobulin E; IgG_4, immunoglobulin G subtype 4; IL, interleukin; ILC2, innate lymphoid cells type 2; TGF, transforming growth factor; Th_2, T helper type 2; Treg, T regulatory.

After high-dose allergen administration by AIT, dendritic cells (DCs) produce IL-12, IL-27, and IL-10, generating and activating distinct phenotypes of Tregs: natural (nTreg) and inducible (iTreg) cells (17, 19). Both nTreg and iTreg cells suppress allergic response through direct and indirect mechanisms: release regulatory cytokines (IL-10, TGF-β, and IL-35), directly induce tolerogenic DCs subsets, suppress activation of allergen-specific Th_2 lymphocytes, downregulate the expression of FCεRI receptors on mast cells, decrease allergen-specific IgE synthesis, and promote B-cell production of IgG4 in an allergen-independent manner (17, 19). IL-10 directly inhibits T-cell-associated cytokines, including IL-4 and IL-5, reduces proinflammatory cytokine from mast cells and eosinophils, decreases allergen-specific IgE production, and increases IgA and IgG_4 levels (19). Competitively binding to the same site epitopes recognized by IgE, IgG_4 exert a sort of "immunologic blockade" inhibiting mast cell and basophil degranulation. In addition, IgG_4 has been proposed to co-stimulate the inhibitory IgG receptor FcγRIIb, which can negatively regulate FcεRI signaling and in turn inhibit effector cell activation. IgG_4 also inhibit IgE-mediated facilitation of allergen presentation to T lymphocytes (20). TGF-β suppresses both activity and proliferation of Th_2 cells and innate lymphoid cells type 2 (ILC2), thus inhibiting Th_2-cytokines (IL-4, IL-5, IL-9, and IL-13), and consequently decreasing the activation of eosinophils, basophils, mast cells and IgE-secreting B lymphocytes (17, 19).

Bregs play a key role in inducing immune tolerance to allergens, directly promoting the synthesis of allergen-specific IgG_4, inhibiting activation and proliferation of effector T lymphocytes, suppressing Th_2-dependent inflammation, increasing Treg cells (21). Overall, AIT is able to restore the impaired allergen tolerance.

CLINICAL INDICATIONS OF AIT IN CHILDREN WITH RESPIRATORY ALLERGY

AIT should be considered in patients who have AR with or without conjunctivitis, and/or asthma with documented sensitization consisting with symptoms after sensitizing-allergen exposure (22). Candidates for AIT are patients with uncontrolled symptoms by medications and/or environmental prevention or experiencing drug adverse effects or wishing reduction of long-term treatment (22).

The evidence of efficacy and safety of AIT in AR and/or asthma have been documented both in adults and children (6, 23, 24).

Allergic Rhinitis

AIT is the established treatment of choice for AR patients experiencing failed allergen avoidance and/or medical therapy (2, 25). European Academy of Allergy and Clinical Immunology (EAACI) 2018 guidelines recommend AIT in AR patients, with or without conjunctivitis, with evident sensitization to one or more clinically relevant allergens and moderate-to-severe symptoms despite regular and/or avoidance strategies (6, 26). This statement is based on a body of international trials evidence providing a comprehensive assessment of AIT in AR: both SCIT and SLIT are effective for seasonal and perennial AR in achieving short-term improvements in symptom, medication use, and combined symptom and medication scores (27, 28). The evidence of longer-term effectiveness is documented for grass AIT, especially for tablets (26). Thus, standardized and validated AIT products should be used when available, and a product-specific evaluation of evidence is actually recommended before initiating treatment with a specific product (6, 26).

With particular reference to children, AIT should be considered similarly to adults (26). Although major gaps still exist for the pediatric age (29), it can be actually recommended: (i) continuous, pre- and pre/co-seasonal SCIT for children with seasonal AR; (ii) continuous SCIT for perennial AR (weak recommendation due to the lack of exclusive pediatric data); (iii) pre-co-seasonal and continuous SLIT for seasonal AR (both tablet and drop); (iv) poor evidence for perennial AR, although the effectiveness of SLIT tablet approach has been demonstrated in the short term in mixed adult/adolescent studies (26). It is also recommended a minimum of 3-year course to achieve long-term efficacy (6).

Asthma

Asthma is a common chronic disease affecting all age groups, with up to 20% of children aged 6–7 years experiencing severe wheezing episodes within a year (30). The actual asthma management is control-based: therapeutic strategies are based on a stepwise approach and adjusted in a continuous cycle involving assessment, treatment and review (3, 31–33). However, standard pharmacotherapy does not affect the underlying pathogenetic immune response, as it is withdrawn symptoms and inflammation occur again.

SLIT is actually recommended as add-on treatment option in adult HDM-sensitized asthmatics with concomitant AR who have exacerbations despite inhaled corticosteroids (ICS] treatment, with forced expiratory volume in 1 s (FEV_1) more than 70% predicted, as stated in the latest Global Initiative for Asthma Report (GINA) update (3). Asthma must be mild-to-moderate and allergic, and well or "partially" controlled by standard pharmacotherapy; asthma control has to be maintained throughout AIT course (34). Conversely, AIT is still restricted in patients with uncontrolled asthma, as it represents a significant risk factor for serious and even fatal adverse reactions (5). Coupling anti-IgE biological therapy (omalizumab) with AIT has been proposed as a suitable option to increase SCIT effectiveness and safety (35–38).

This significant change in the GINA strategy draws upon recent Phase III clinical trial evaluating the treatment of asthma with the standardized quality (SQ) HDM SLIT-tablet in adults: the addition of HDM SLIT to maintenance therapies reduced ICS or the time to first exacerbation upon ICS reduction, suggesting that SQ HDM SLIT-tablet may improve overall asthma control (39). While these data require further studies to confirm long-term efficacy and safety of this product in adults, less information is available for adolescents (40, 41) and studies are in progress in children (42). HDM sensitization in early childhood represents an important risk factor for the development of asthma (43) and is linked to the impairment of lung function in school-age asthmatic children (43, 44) and asthma persistence into adulthood (45). So, other single HDM SLIT preparations have been tested in children and adolescents with allergic asthma. Among others, SLIT with the 300 index of reactivity (IR)-standardized HDM extract, in children aged 6–18 years with AR with or without allergic asthma, improved rhinitis and/or asthma symptoms scores, together with a reduction of rescue medications (46).

Meta-analyses confirmed the effectiveness and the safety profile of AIT in allergic asthma for adults and children (27, 47–51), mainly concerning SLIT in children (22, 52–54). Consistent reduction in combined AR and asthma symptom and medication scores have been demonstrated in pediatric patients with asthma and comorbid AR treated with SLIT (48, 53, 55). These clinical effects have been also demonstrated to be persistent after AIT discontinuation up to 5 years (56). SCIT has demonstrated effectiveness in controlling asthma and reducing medication use (49, 50, 57). Meta-analyses of randomized clinical trials using SCIT in asthma have demonstrated a significant reduction in symptoms, medication use, and AHR both in children and adults, while the effect on lung function showed conflicting results (49, 50). Studies conducted in children showed similar results (58–61). SCIT may have a long-term impact on childhood asthma, as demonstrated in a prospective study using HDM SCIT: after 3 years of SCIT discontinuation, a global remission of asthma (in particular reduced doses of ICS, lower asthma symptom scores, higher quality of life scores, less AHR, and higher FEV_1) was reported in treated patients compared to controls (62). In another retrospective study, asthmatics allergic to either HDM or grass pollen, treated with SCIT in childhood, were re-evaluated 9 years after the discontinuation, showing a three-time lower risk of frequent asthma symptoms than controls,

but without any difference in lung function or medication use (63).

Overall, both SCIT and SLIT appear to be effective for the treatment of AR and asthma in children (64, 65). Of particular interest for the pediatric population, it is the AIT ability (both SCIT and SLIT) to reduce ICS doses together with the impact on asthma control. These promising results highlight the immunomodulating pivotal role of AIT in controlling and inducing remission of disease activity. Furthermore, the persistence of these clinical effects after discontinuation separates AIT uniquely from other anti-allergic therapies.

Prevention of Allergy Progression and Asthma Onset

Over the last decade, the disease-modifying properties of AIT have been largely investigated, mainly focusing on the prevention of allergic sensitization and asthma onset.

Developing new sensitizations is characteristic of the natural history of allergy. A preventive effect of AIT on the onset of new sensitizations has been reported (1, 66) and demonstrated in asthmatic children mono-sensitized to HDM (67, 68). However, a recent systematic review and meta-analysis on this topic reported a low level of evidence related to the heterogeneity and the high risk of bias of the included studies (69, 70). A recent EAACI-funded meta-analysis highlighted a reduced risk of developing new sensitizations at least over the short period, but none on the long-term (71).

Children with AR have an increased risk of developing asthma later on in life when compared to those without AR, especially those with AHR (72). Few but significant studies investigated disease modification in children, mainly concerning AIT (73–77). The prevention of allergy (PAT) study was a large prospective randomized controlled study to evaluate the preventive effect of SCIT in children (aged 6–14 years) with grass and/or birch AR without asthma (73–75). Actively-treated children had a significantly reduced risk of developing asthma and fewer asthma symptoms after 3-year treatment compared to controls (73). This preventive effect persisted at 5 years (74) and 7 years (75) after SCIT discontinuation. The preventive effect of SLIT was evaluated in two open trials conducted on children with AR with or without asthma: a 3-year course of SLIT improved AR symptoms, reduced onset of asthma, and decreased AHR (78, 79). Although promising, these findings had a major limitation as derived from open studies with a limited number of subjects. The results of the grass tablet asthma prevention (GAP) study, the first randomized, double-blind, placebo-controlled trial, have been recently published (77). The GAP study involved 812 children (aged 5–12 years), with grass-pollen AR and without asthma, who received 3-year SQ grass-SLIT-tablet or placebo and were followed for 2 years after discontinuation (77). Treated children significantly reduced the risk of experiencing asthma symptoms or using asthma medication, as well as AR-related symptoms and medications, at the end of the trial, during the 2-year post-treatment follow-up, and during the entire 5-year trial period (77). Taken together, these studies suggest that AIT might reduce the risk of developing asthma symptoms in children, especially in those with AR. EAACI guidelines on the PAT recommend a 3-year course of AIT in children with moderate-to-severe AR and grass/birch pollen allergy, uncontrolled with pharmacotherapy for short-term and possibly long-term prevention of asthma symptoms in addition to improving the control of AR (6, 16).

Finally, the role of AIT in the primary PAT is currently under investigation. In a recent proof of concept study, 111 young children (aged 5–9 months), not sensitized, but at high risk of atopy, were treated with prophylactic HDM SLIT (80). A significant sensitization prevention was demonstrated in the active group; however, no significant preventive effect was observed on HDM sensitization or allergy-related symptoms (80). Further studies are expected to clarify the role of AIT as early-intervention in high-risk children.

PATIENT SELECTION AND BIOMARKERS OF RESPONSE

Since AIT is allergen-specific, a detailed clinical history and appropriate allergy diagnostic tests are essential to properly identify the triggering clinical relevant allergen(s) (2). In case of polysensitized patients, the identification of major allergens should be supported by the use of component-resolved diagnostics (81). Uncontrolled AR symptoms despite antihistamines and/or topical corticosteroids and allergen avoidance measures and/or side-effects of medication, the duration of AR symptoms, as well as the assessment of asthma control, are essential to consider AIT as a therapeutic option (2). Each patient should be evaluated individually by considering the benefits and the risks, and the ability to comply/cooperate with AIT (26). Although some clinical studies have demonstrated efficacy and safety of AIT in preschool children (82, 83), there is no consensus on a specific lower age limit for initiating AIT for respiratory allergy (8). However, this issue still deserves further extensive studies in the perspective of preventive strategies.

Although highly effective, some patients could not respond to AIT treatment. Thus, the identification and validation of potential predictive biomarkers of AIT effectiveness is an active filed of research and could enhance the selection and the clinical management of patients receiving AIT. Biomarkers are quantitative measurement predicting clinical and immunological effects of AIT (84). In particular, ideal biomarkers for AIT should assist in patient selection and identification of responders and predict clinical and immunological response during treatment and after discontinuation of treatment (84). The EAACI Taskforce recently reviewed all candidate biomarkers used in clinical trials of AR patients with or without asthma (18, 84) (**Table 2**). Markers can be cellular (Tregs), humoral (allergen-specific IgG_4 ($sIgG_4$), IgE/IgG_4), molecular (interleukins), or functional (IgE FAB and blocking factor). Although several studies have included biomarkers as secondary outcomes, specifically AIT biomarker studies are still lacking. To date, raised serum allergen-specific IgE (sIgE) are considered the only useful biomarker to select candidates for AIT, in the

TABLE 2 | Potential biomarkers for allergen immunotherapy (AIT).

Categories	Candidate biomarkers	Domain	Advantages	Disadvantages, unmet need	Possible applications
Biomarkers for diagnosis	IgE (sIgE, tIgE, sIgE/tIgE)	Antibodies	Elevated serum IgE levels in the context of a clear history of allergic symptoms is a biomarker for selection of patients for AIT	No clear correlation with clinical outcome Lack of validation in RDBPCT Lack of standardization of assay platform and reference ranges/cut-off values	Prediction of disease severity and/or progression
	CD63, CD203c, DAO, basophil histamine release				
Biomarkers predictive of AIT safety	CD63, CD203c, DAO, basophil histamine release	Basophil activation	Small amount of blood (<2 ml) is required to perform the test	Mechanism of allergen induced basophil hyperresponsiveness during AIT not completely known Limited number of studies Need for standardized assays	Reduced risk of side effects and improvement of patients' compliance to treatment
Biomarkers of AIT efficacy	IgG subclasses ($sIgG_1$, $sIgG_4$, $sIgE/IgG_4$)	Antibodies	$sIgG_4$ is a biomarker of immunologic response of AIT	No clear correlation with clinical outcome Limited data on local antibody levels and activities	Prediction of patients' compliance
	sIgE/total IgE	Antibodies	Potential positive predictive biomarker of response for AIT	Lack of validation in RDBPC	Prediction of clinical response
	IgE FAB, IgE-BF	Serum Inhibitory activity for IgE	Highly reproducible serum-based assay Association with clinical outcome has been reported in some studies	Availability limited to specialized centers or laboratories	
Not yet determined	CCR3, ECP, eotaxin, IFN-γ, IL-2, IL-2R, IL-4/5/6, IL-8/9/10, IL-13/18, MCP-1, TARC, transthyretin	Cytokines and chemokines	May be useful to further explore mechanisms of AIT	No correlation with clinical outcome	Not known
	DCs, Breg, Treg	Cellular biomarkers	Early biomarkers of immunologic response	Not routinely performed, limited applications in clinical practice No clear correlation with clinical outcome	Prediction of immunological response
	SPT, Id, In, chamber studies	*In vivo* biomarkers	Provocation tests used as surrogate biomarkers of clinical response to AIT Provocation methods are recommended as primary endpoints in proof-of-concept and dose-finding trials of AIT	Standardization and validation differ from the various challenge protocols Comparison between provocation test results and symptoms after natural exposure are currently lacking	Prediction of clinical response

Adapted from Shamji et al. (84). BF, binding factor; Breg, B regulatory cell; CCR, chemokine receptor; CD, cluster of differentiation; DAO, diamine oxidase; DC, dendritic cell; ECP, eosinophil cationic protein; FAB, facilitated antigen binding; Id, intradermal test; IFN, interferon; Ig, immunoglobulin; IL, interleukin; In, intranasal test; MCP, monocyte chemoattractive protein; RDBPCT, Randomized double-blinded placebo-controlled trial; sIg, allergen-specific Ig; SPT, skin prick test; TARC, thymus and activation-regulated chemokine; tIg, total Ig; Treg, T regulatory cell.

context of a clear history of symptoms on exposure to the relevant allergen (84). In particular, preliminary data suggest that higher levels of sIgE in children could be helpful to predict AIT efficacy (85). Furthermore, sIgG4, sIgE/total IgE ratio, and IgE-FAB are in the pipeline as candidate biomarkers for compliance and response to AIT, respectively (84). More research is needed to confirm and interpret the possible association of biomarkers with both clinical response and persistence of clinical benefit after discontinuation of AIT.

CONCLUSIONS

AIT represents a valuable therapeutic option, especially in childhood, to modify the progression of allergic disease. AIT may be particularly useful in children with AR and new-onset asthma because it may modify the long-term prognosis of their airway disease. Both SCIT and SLIT seem to be effective in pediatric allergic asthma, showing a promising steroid-sparing effect of which patients treated with high-dose pharmacotherapy for a long term could benefit most. To date, uncontrolled asthma

remains a clear contraindication for AIT treatment; however, coupling novel biological therapies with AIT could represent a novel approach to treat these patients with high risk of adverse reactions. Over the last decade considerable advances in the AIT approach have been also made to move forward this therapeutic field in the context of personalized medicine. The advanced knowledge of the mechanisms of sustaining clinical and immunological tolerance toward allergens, the implementation of vaccination strategies (using recombinant allergen extracts, or modified extracts at increased safety/efficacy, or adjuvants to further stimulate the immune system), the implementation and diffusion of international guidelines, the definition of regulatory aspects such as standardization and registration of AIT products, the standardization of clinical trial outcomes, as well as the planning of future dedicated pediatric studies, will all implement the evidence of efficacy and safety of AIT for allergic children.

AUTHOR CONTRIBUTIONS

All authors made substantial contribution to the conception of the work, reviewed the literature on the subject, and drafted the final version of the manuscript. AL and MT revised it critically for important intellectual content. All authors finally approved the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

SYSTEMATIC REVIEW
published: 21 August 2018
doi: 10.3389/fped.2018.00232

Interventions on Adherence to Treatment in Children With Severe Asthma: A Systematic Review

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OPEN ACCESS

Edited by:
Renato Cutrera, Bambino Gesù Ospedale Pediatrico (IRCCS), Italy

Reviewed by:
Gian Luigi Marseglia, Policlinico San Matteo Fondazione (IRCCS), Italy
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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *22 May 2018*
Accepted: *31 July 2018*
Published: *21 August 2018*

Citation:
Boutopoulou B, Koumpagioti D, Matziou V, Priftis KN and Douros K (2018) Interventions on Adherence to Treatment in Children With Severe Asthma: A Systematic Review. Front. Pediatr. 6:232. doi: 10.3389/fped.2018.00232

Introduction: Poor adherence to inhaled medication is a commonly encountered problem among children with asthma. However, there is a relatively paucity of data regarding the adherence of children with severe asthma, as well as the merit of any interventions to improve this adherence.

Objectives: The aim of this systematic review was to identify the available literature on the rate of adherence and the influence of interventions in improving adherence to controller inhaled medication, in children with severe asthma.

Methods: A systematic literature search was performed in MEDLINE/PubMed, Cochrane Library, and Scopus databases. Studies were included in the present review if their target population were children and/or adolescents with severe asthma and presented data on medication adherence before and after a given intervention.

Results: A total of seven studies, conducted in USA, Canada, and UK, and published between 2012 and 2018, met the inclusion criteria. Adherence to controller medication was assessed via either objective or subjective measures (questionnaires), or a combination of them. Interventions included communication during pediatric visits and audio-taped medical visits, individualized care programs, electronic monitoring devices, interactive website and peak–flow prediction with feedback. Adherence rates for the baseline (before intervention) or for the control groups ranged from 28 to 67%. In general, there was a significant improvement of adherence after intervention with rates increasing to 49–81%.

Conclusion: Adherence rate in children with severe asthma is not satisfactory but it can be improved after proper interventions. Nevertheless, the heterogeneity among adherence assessment tools, and the variety of interventions, in combination with the lack of studies focusing on severe asthma, highlight the need for further research in this field.

Keywords: children, severe asthma, difficult asthma, inhaled treatment, adherence

INTRODUCTION

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough that vary over time and in intensity, together with variable expiratory airflow limitation (1). Asthma affects 1–18% of the population in different countries. It is one of the main causes of disability, health care services utilization, and quality of life impairment (2–5). It is estimated that about 14% of the children worldwide experience asthma symptoms. Asthma management aims to achieve good symptom control; maintain normal activities; minimize asthma attacks; reduce the side effects of treatment and as a result prevent the progression of obstructive lung damage during growth and then later in life (2).

Although the majority of asthma patients can be effectively controlled with the available medications, a substantial subset remains uncontrolled despite being offered the optimal therapy (6).

Severe asthma, according to European Respiratory Society (ERS)/American Thoracic Society (ATS) is asthma which requires Step 4 or 5 treatment [according to Global Initiative for Asthma (GINA) guidelines], e.g., high dose inhaled corticosteroids (ICS) and long-acting beta agonists (LABA) or leukotriene modifier/theophylline for the previous year or systemic ICS for $\geq$50% of the previous year to prevent it from becoming "uncontrolled" or which remains "uncontrolled" despite this therapy (6). Severe asthma includes patients with refractory or treatment-resistant asthma, or patients with incomplete response to treatment due to comorbidities (5). The primary approach of a child with problematic, severe respiratory symptoms, who is unresponsive to prescribed asthma therapy should be the confirmation of asthma diagnosis; secondly, one should explore if the child belongs to the category of "difficult-to-treat asthma" (2). The latter term is reserved for patients with ongoing factors that interfere with achieving good asthma control (allergen exposure, poor adherence), severe therapy-resistant asthma, asthma plus comorbidities (gastroesophageal reflux, obesity, obstructive apnea), or any combination of the above (7). Clinicians should also be aware of a prevalent cluster of chronic upper airway comorbidities, such as chronic rhinosinusitis and allergic rhinitis, which is recognized to patients with severe asthma but also seems that contribute to worsen asthma control and complicate asthma diagnosis and management (8). Distinguishing between severe asthma and uncontrolled asthma is crucial since the latter can be due to causes that can be more or less easily improved, such as the correction of a faulty inhaler technique and poor adherence (2, 9, 10).

Adherence is defined as the extent to which the patient's behavior matches the agreed recommendations from the prescriber. Patients can follow or not their doctors' recommendations, but failure to do so should not be a reason to blame the patient (11, 12). Adherence of asthmatics to long-term inhaled treatment has contributed substantial to asthma control and to morbidity reduction, yet, in general, it still remains suboptimal (13–16). The suboptimal adherence leads to poorer clinical outcomes and increased health care costs (17, 18).

Poor adherence (<60%) (19) to inhaled medication should be considered in all "difficult to control" patients. It has been reported that only 55% of children with moderate/severe persistent asthma use their controller medication daily (20).

Low adherence rates suggest an urgent need to increase adherence in order to reduce the burden of the disease. The improvement of adherence will result in better asthma control, and therefore, in a reduction of asthma severity (16, 21, 22). Shared decision making for medication/dose choice (23), inhaler reminders (24), home visits (25), prescribing ICS once daily versus twice (26), are all some of the interventions that are conducive to adherence improvement (2).

Although there are quite a few published reviews on the adherence of asthmatic children to controller medication and the effects of various interventions thereupon, there is still a lack of focus on severe asthma. Our aim in this systematic review was to identify the available literature on the rate of adherence and the influence of various interventions in improving adherence to controller inhaled medication, in children with severe asthma.

METHODS

This systematic review was based on the Preferred Reporting Items for Systematic Reviews and Meta—Analyses (PRISMA) statement, which aims to be the most accurate elaboration of a systematic review (27).

Two independent reviewers searched for publications in three of the most commonly used databases in medicine: Pubmed/Medline, Cochrane Library and Scopus. Key word combinations of "children," "severe asthma," "difficult asthma," "inhaled treatment," and "adherence," were used to retrieve articles with these key words in title and abstract.

The criteria included were as follows:

1) Articles which were published from January of 2012 to March of 2018;
2) Articles written in English;
3) Studies that targeted children and/or adolescents;
4) Studies which have focused on severe asthma (as their main aim or as a subcategory of the study population); and
5) Studies on the effect of an intervention on adherence rate (as their main aim or as a subcategory of the study population).

Data Extraction

The data extraction was conducted by two reviewers. The characteristics collected for each study were references, sample characteristics, study design, duration, adherence assessment, intervention tools, and outcomes. **Table 1** illustrates the main studies' characteristics.

RESULTS

Studies Selection

The search of the 3 databases retrieved 644 articles. Of these, 284 were duplicates and were excluded. The remaining 360 articles were screened for relevance. The full texts of 23 articles were

TABLE 1 | Study characteristics.

References	Sample characteristics	Study design	Duration	Adherence assessment	Intervention tool	Outcomes
Jochmann et al. (19)	93 children (STRA $n = 21$, Difficult asthma $n = 51$), 5–17 years, outpatient	Prospective observational cohort	6 months	Electronic monitoring device (smartinhaler) MARS-5 rating scale for self-reported adherence	Electronic monitoring	Median adherence for whole population was 74%. Good adherence (≥80%) in 42% of patients, Suboptimal adherence (<80%) in 58% ($p < 0.0065$).
Feldman et al. (14)	192 children (severe persistent $n = 52$), 7–15 years (feedback group), 7–12 years (no feedback group), outpatient	Prospective longitudinal	6 weeks	Doser CT (MediTrack)	PEF prediction with feedback	Adherence to PEF feedback group 48.8 ± 4.5(%) and to no PEF feedback group 27.5 ± 4.9 (%) ($p = 0.002$).
Duncan et al. (29)	48 youth (severe persistent $n = 8$), 9–15 years, outpatient	Randomized controlled trial	5 months	Electronic monitoring device (MDILog-II)	Teamwork intervention (TI) Asthma education (AE) Standard care (SC)	Mean daily adherence for TI group (20-weeks) was 81%, while for the AE group 33.6% and for the SC group 37%.
Sleath et al. (28)	259 children (moderate/severe persistent $n = 185$), 8–16 years, outpatient	Prospective interventional	1 month	Questionnaire	Audio-taped medical visit and home visit interview	Children reported average control medication adherence was 72.4% (SD = 32.9; range, 0–100), while caregivers reported average control medication adherence 84.7% (SD = 26.1; range, 0–100).
Christakis et al. (31)	603 children (severe persistent $n = 14$) , 2–10 years, outpatient	Randomized controlled trial	6 months	Questionnaire	Tailored interactive website	Controller medicine users with persistent asthma (intervention group) at both time points had significantly better adherence than the control group ($p = 0.01$).
Guénette et al. (32)	61 adolescents, 12–17 years ($N = 349$, aged 12–45 years)	Pragmatic controlled clinical trial	12 months	Morisky Medication Adherence Scale (MMAS −4) Medication Possession Rate (MPR)	Integrated care program	The integrated program had statistically significant effectiveness on ICS adherence, $p = 0.0197$ (mean MMAS–4 at baseline/12 month follow-up = 1.98/1.70, $p = 0.051$, mean MRP at baseline/12 month follow-up 19.41/22.86, $p = 0.0629$).
Ellis et al. (30)	167 adolescents, 12–16 years, outpatient	Randomized controlled trial	12 months	Medication Adherence subscale	Multisystemic Therapy-Health Care (MST-HC)	MST-HC was associated with better controller medication adherence at 6 months postintervention ($p < 0.01$).

STRA, Severe therapy-resistant asthma; ICS, Inhaled Corticosteroids; PEF, Peak expiratory flow.

assessed for eligibility; and finally 7 articles were chosen for the systematic review.

The reasons for the exclusions are listed on the flow chart (**Figure 1**) which also provides the publication retrieval process.

Studies Description

Seven articles were included in the systematic review. Five of them were conducted in USA (14, 28–31); 1 in Canada (32); and 1 in UK (19).

Three studies were randomized controlled clinical trials (29–31), 1 was a controlled pragmatic clinical trial (32), 1 was a prospective observational cohort (19), 1 was a prospective longitudinal study (14), and 1 was a prospective interventional study (28).

Patients' recruitment was held mainly during outpatient visits. Guénette et al. (32) recruited patients with mediation of community pharmacists. Patients were children and their age ranged from 2 to 16 years old.

The total sample for severe asthma from the 7 studies was $n = 508$ children/adolescents, with the lowest sample size being $n = 8$ (29) and the highest $n = 185$ (28).

Severe asthma was a subcategory of the selected sample in all seven studies (14, 19, 28–32).

Adherence Assessment

Adherence in ICS treatment was evaluated through objective (14, 29), or subjective measures (28, 30, 31) or as a combination of both (19, 32). Objective measures of adherence assessment included electronic monitoring devices (Smartinhaler, Doser CT, MDILog-II) used in 4 of the studies (14, 19, 29, 32) and Medication Possession Ratio (MPR) used by Guénette et al. (32).

All three pre-mentioned devices developed as a canister attachment that fits on top of the majority of inhalers. They can provide accurate information on medication usage, including timing and number of doses taken, and all these recorded data can be used to guide asthma management (19).

Medication Possession Ratio (MPR) is a validated objective measure based on pharmacy records expressing the percentage of days supply received divided by a period of time and has been found to be more accurate than self-report (33).

Questionnaires are subjective measures for assessing adherence that are convenient and relatively unobtrusive and rely on self-report (34). The questionnaires used in the studies reported in this review were:

(1) Brief Medication Questionnaire, a tool for screening patients' adherence as well as their barriers to adherence. The tool includes a 5-item Regimen Screen that asks patients how they took each medication in the past week, a 2-item Belief Screen that asks about drug effects and bothersome features, and a 2-item Recall Screen about potential difficulties remembering (35);

(2) Morisky medication adherence scale (MMAS-4) which is a generic self-reported, medication-taking behavior scale using four questions about past medication use patterns (36);

(3) Medication Adherence Rating Scale (MARS-5) is a self-reported measure which evaluates both attitudes about medications and actual medication-taking behavior and consists of 10 items (37); and

(4) Medication Adherence subscale which measures adherence to controller medication (38).

Intervention Tools

Among the 7 studies, 3 had as a main research objective the increase of adherence rate after an intervention (28, 29, 31) whereas in the other 4 studies (14, 19, 30, 32) the adherence improvement rate was a subcategory of the findings.

Interventions design addressed solely the children (14, 19, 32), or there was also an element of parental involvement (28–31).

Briefly, Sleath et al. (28) audio-taped and coded communication during pediatric visits whether the provider included child or caregiver input into the asthma management treatment plan. Individualized care programs where health teams assess patients' and their caregivers' individual needs, share information and improve knowledge on asthma, were implemented in 3 of the studies (29, 30, 32). Christakis et al. (31) created a web-based tailored intervention aiming to increase children's positive beliefs about asthma management and Feldman et al. (14) used a piece of equipment where peak—flow prediction with feedback encouraged children to receive daily their inhaled treatment. Electronic monitoring devices were used in 1 study as an intervention (19) with the perception that adherence could improve following a period of monitoring.

The mean duration of interventions was 22 weeks, whereas the maximum (30, 32) and minimum duration (28) was 48 and 4 weeks, respectively.

Outcomes

Across the 7 studies, adherence rates for the baseline (before intervention) or for the control groups ranged from 28 to 67% (14, 29, 31); there was a remarkable improvement after intervention with adherence rates increasing to 49–81% (14, 28, 29, 31).

Feldman et al. (14) found a significant positive difference between intervention and the control group ($p = 0.02$) using PEF prediction with feedback. Christakis et al. (31) using an interactive website, also showed a significant correlation between intervention and adherence improvement. Concerning the implementation of individualized care programs, Guénette et al. (32) showed statistically significant effectiveness measuring the adherence with MMAS-4 Scale ($p = 0.0151$). Statistically significant correlation was also demonstrated in Ellis et al. (30) measurements ($p < 0.01$). Team Intervention in Duncan et al. (29) study had a positive impact in adherence rate in comparison with Standard Care (81 vs. 37% respectively). Suboptimal adherence rates after intervention were presented in Jochman et al. (19) (median adherence was 74%) similarly with Sleath et al. (28) (average control medication adherence as reported by children was 72.4%).

DISCUSSION

This systematic review isolated seven articles from the recent literature, which focused on the improvement of adherence to inhaled medication after specific intervention, in children with severe asthma. Overall, the results of this systematic review

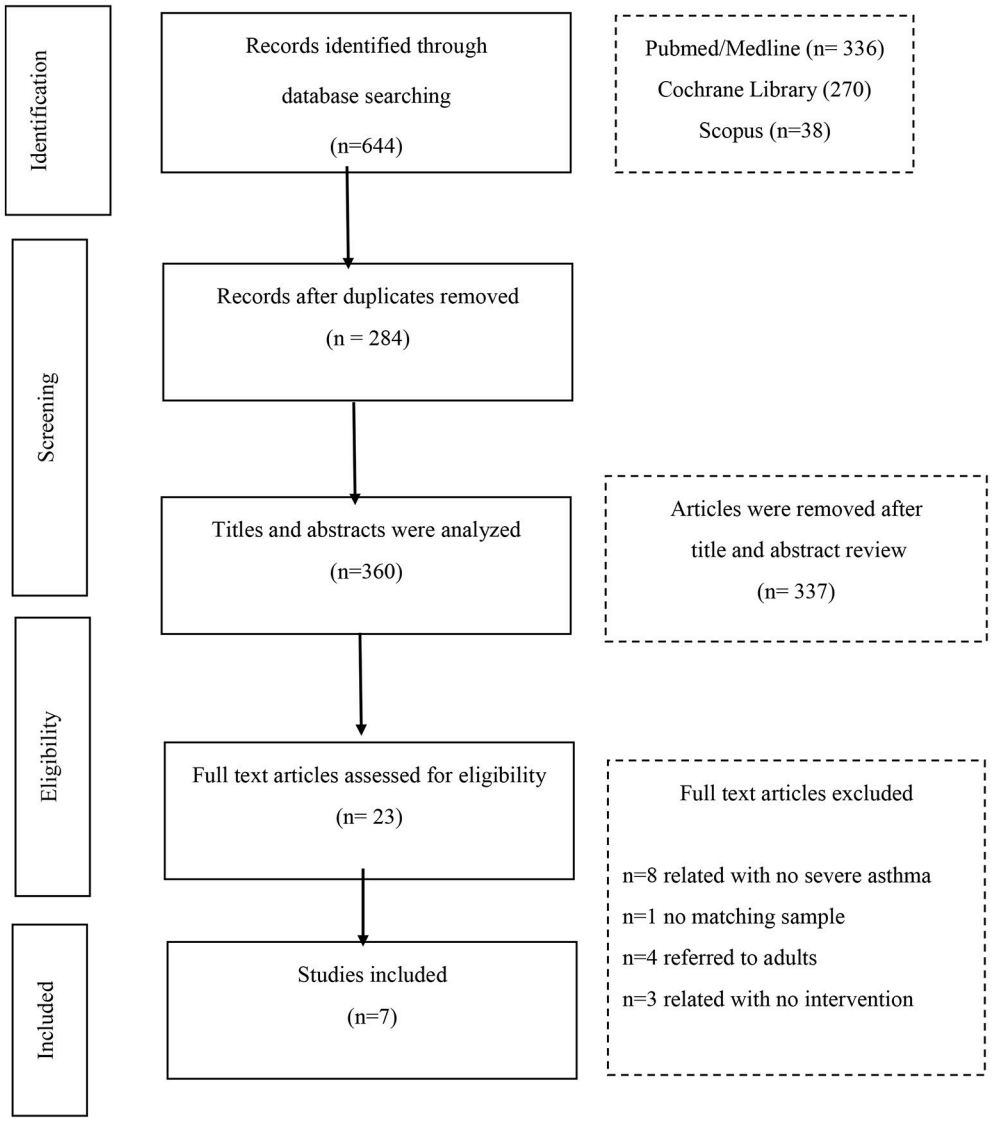

FIGURE 1 | Flow diagram of studies included in systematic review.

highlight the importance of interventions in respect of adherence improvement, in children suffering from severe asthma.

Adherence can be assessed with objective or subjective measures (34). Objective measures used in some of the studies included in the current review were different kinds of electronic monitoring devices and the Medication Possession Ratio. There have also been included studies that used questionnaires for the subjective assessment of adherence. Some of the main objective adherence measuring tools for asthma medication adherence are the electronic monitoring devices, the canister weight, and the pharmacy refill data (39). Electronic monitoring has been labeled as the "gold standard" for assessing adherence due to the objective and detailed data it provides, but the cost and technology requirements (e.g., equipment, staff training) prohibit its widespread routine clinical use (40, 41). At the same time, some of the subjective measuring tools are interviews, questionnaires and diary/self-reporting (34). Subjective measures are inexpensive, convenient, and relatively unobtrusive and have the potential to provide information on related issues. Although, they mostly rely on self-report, their accuracy depends on psychometric properties and may mask variability of adherence across regimen components if assessed globally (40–42). **Table 2** shows advantages and disavantages of methods for adherence assessment (34). As a great heterogeneity has appeared in asthma population regarding individualized capabilities, needs and preferences (43), researchers have concluded that more targeted and personalized methods of assessment are required (44).

Severe asthma in children is known to cause great morbidity, and raise asthma costs. The exact prevalence is unknown

TABLE 2 | Summary of objective and subjective methods for adherence assessment (34).

Objective methods
ELECTRONIC MONITORING
Advantages
• Potentially measuring a variety of adherence behaviors (e.g., timing of dose, technique)
Disadvantages
• Usually not measuring actual consumption of medication
• Difficult to use
• Costly
• Associated with technological issues (e.g., battery failure and malfunction)
• Doubtful acceptability to patients and families
PHARMACY REFILL DATA
Advantages
• Inexpensive
• Fairly accurate (correlating with electronic monitoring data)
Disadvantages
• Not measuring consumption
• May be patients use other pharmacies, stockpiling medications, or family members' medications
• Difficulty in logistics (e.g., staff time, privacy regulations) to obtain records
PILL COUNT/CANISTER WEIGHT
A**dvantages**
• Inexpensive
• Fairly accurate (correlating with electronic monitoring data)
Disadvantages
• Patients may forget to bring their medication or miss their appointment
• May be manipulated by patient
• Not confirming that medication was taken
• Can be cumbersome for staff collecting and calculating
Subjective methods
INTERVIEWS
Advantages
• Can obtain extra information (e.g., regimen components, family issues), not only for medication use
• Can be administered over the telephone
Disadvantages
• Relies on self-report; subject to recall bias
• Psychometric properties and structure of the interview determine accuracy
DIARY/SELF-MONITORING
Advantages
• Reduces demands on memory
• Inexpensive
• Flexible-monitoring a range of variables in relation to a variety of adherence components
Disadvantages
• Relies on self-report; can be fabricated by patient
• Requires "adherence" to recording information, when adherence is a general concern
QUESTIONNAIRES
Advantages
• Inexpensive
• Convenient to be administered
Disadvantages
• Relies on self-report; subject to bias and social desirability
• May mask variability of adherence across regimen if assessed globally

although it is estimated that 2–5% of asthmatic children have severe disease (45, 46). There is an interrelated relationship between severe asthma and adherence and clinicians can overestimate the severity of asthma if they do not assess adherence. Furthermore, poor adherence can lead to severe asthma if it is not corrected (47). According to our findings, adherence rate at the baseline for children with severe asthma ranged between 28 and 67%, which is in agreement with Celano et al. (48) who studied children with persistent asthma. The adherence of children with any kind of asthma is approximately 30–70% (49).

In our systematic review, we retrieved studies with interventions aimed at improving adherence. Some of the intervention tools that had been used were: home interviews and audio-taped medical visits (28); individualized care programs (29, 30, 32); electronic monitoring device use (19); interactive website (31) and peak–flow prediction with feedback. In another systematic review and meta-analysis various behavioral interventions, e.g., providing families with specific strategies to manage the regimen; educational interventions providing basic information to families about the patient's illness and the importance of adherence; organizational interventions such as introducing calendars for self-monitoring and facilitating discussion with caregivers about their child's illness or supporting caregiver-health care provider interactions were meta-analyzed and discussed (50). Furthermore, two other studies investigated the efficacy and safety of text messages for dose reminding (51, 52).

Asthma control is associated with adherence level. Jochmann et al. (19) found that children with poor adherence maintained poor control, while Ellis et al. (30) showed that children with asthma knowledge and controller device use skills had better medication adherence. Similarly, in other studies, adolescents report that it is more likely to adhere to treatment when they feel hopeful (53), view management tasks as important and feel competent (54), or when they intend to follow treatment recommendations (55, 56). Increased levels of self-efficacy are associated with better adherence, as well (57, 58). Adherence to ICS was an independent strong predictor of long term asthma control, with highest levels of asthma control found when the adherence raised above 80% of prescribed doses (16).

Our review showed that adherence rate in children with severe asthma can be increased after a proper intervention. In the studies included in our review, there was a significant increase of adherence rate, from 28–67% to 49–81%. There is a relatively lack of studies focusing on severe asthma, but the above findings are in agreement with the results of studies dealing with all kinds of asthma in children (24, 59). Although, the majority of studies have shown a positive effect, there are some instances where intervention with electronic asthma medication reminders did not improve the adherence rate (32, 51). Also, a scheduled follow up visit in combination with a comprehensive asthma management care program implemented in preschool children with asthma in the Netherlands did not correct adherence rates (60). A systematic review and meta-analysis found the studies that applied adherence education as intervention achieved a benefit of 20% points over control, while electronic trackers

or reminders led to better adherence rates of 10% points. Researchers concluded that interventions' results depend on the group target, method and duration of intervention (61).

The main limitations of this review is the lack of studies focusing on severe asthma in children as most of the studies investigate adherence in children in the community and adult severe asthmatics rather than severely asthmatic children. Additionally, there is heterogeneity in the definitions of severe asthma among studies, a problem that has already been noticed by other researchers (62). Studies used different adherence assessment tools, therefore results were presented in various ways.

A weak point of all 7 studies is that it is not clear whether the adherence improvement was clinically meaningful. Literature suggests that in order to maintain asthma control, adherence rates have to be in excess of 80% (63).

CONCLUSION

Adherence interventions have a positive impact on adherence rate in children with severe asthma. The remarkable heterogeneity between adherence assessment tools, and interventions, combined with the lack of studies focused on severe asthma, highlight the research gap in this field. There is a great need for further research focused exclusively on severe asthma and adherence treatment, in children.

AUTHOR CONTRIBUTIONS

BB and DK contributed equally to this review. They did the literature research and the main writing of the article. VM, KP, and KD did all the academic support and the corrections during the whole process. KD did the finally fixing in English also.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

ORIGINAL RESEARCH
published: 20 September 2018
doi: 10.3389/fped.2018.00255

Astragalus Oral Solution Ameliorates Allergic Asthma in Children by Regulating Relative Contents of $CD4^+CD25^{high}CD127^{low}$ Treg Cells

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Objective: To explore the effects of *Astragalus* oral solution (AOS) on allergic asthma in children by investigating relative contents of $CD4^+CD25^{high}CD127^{low}$ Treg cells.

Methods: The contents of Astragaloside A in AOS were detected by using HPLC. Eighty children with allergic asthma were recruited from February 2016 to June 2017, and randomly assigned into the control group (received placebo, 0.1% quinine chloride in deionized water, daily) and the AOS group (received 10 mL AOS daily). After 6-month treatment, therapeutic results were compared between the two groups. Serum levels of IL-10 and TGF-beta, Th1 cytokines (IL-2 and IFN-γ), and Th2 cytokines (IL-4 and IL-6) were measured by using ELISA kits. Relative contents of $CD4^+CD25^{high}CD127^{low}$ Treg cells were determined by using flow cytometry.

Results: Astragaloside A was the main ingredient of AOS with 0.216 ± 0.027 mg/mL from six-batch samples. After 6-month therapy, the AOS group showed improved forced expiratory volume in 1 s (FEV1) and the Pediatric Asthma Quality of Life Questionnaire (PAQLQ) scores compared with the control group ($P < 0.05$). Serum level of IL-10 was higher and the levels of TGF-beta, Th1 cytokines (IL-2 and IFN-γ), and Th2 cytokines (IL-4 and IL-6) were lower in the AOS group than in the control group ($P < 0.05$). AOS treatment increased the percentage of gated $CD4^+$ T cells, $CD4^+CD25^+$ T cells, $CD4^+CD25^{high}$ Treg cells, $CD4^+CD25^+FoxP3^+$ Treg cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells when compared with the control group ($P < 0.05$).

Conclusions: Astragaloside A was the main component of AOS, and AOS ameliorated allergic asthma in children by regulating relative contents of $CD4^+CD25^{high}CD127^{low}$ Treg cells.

Keywords: ***Astragalus*** **oral solution, Astragaloside A, allergic asthma, $CD4^+CD25^{high}CD127^{low}$ Treg cells, children**

OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *26 February 2018*
Accepted: *28 August 2018*
Published: *20 September 2018*

Citation:
Wang W, Jing W and Liu Q (2018) Astragalus Oral Solution Ameliorates Allergic Asthma in Children by Regulating Relative Contents of $CD4^+CD25^{high}CD127^{low}$ Treg Cells. Front. Pediatr. 6:255. doi: 10.3389/fped.2018.00255

BACKGROUND

Asthma is a common inflammatory disorder of the lungs and is often characterized by reversible airflow obstruction and bronchospasm. Allergic asthma is a main threat to public health as it perturbs children's respiratory system. More than 300 million people worldwide have allergic asthma (1, 2). According to the data from the World Health Organization (WHO), asthma affects most children, and the number is expected to increase to 400 million in 2025 (3). With the

development of society and industrialization, the incidence and mortality of allergic asthma in children have been increasing. Approximately 30–90% of children with allergic asthma have allergic rhinitis (AR) (4). AR is one of the related factors in the pathogenesis of allergic asthma, which leads to a direct result of asthma onset (5). With their similarities in etiology, location, pathogenesis, and pathophysiology, both AR and allergy asthma should be treated together (6). Seasonal perennial allergens, such as grass, trees, pollen, house dust (HD), mold, and animal fur, can often cause allergic asthma in children (7, 8). In allergic patients, respiratory allergies often lead to systemic allergies. In nasal allergic inflammation, Th1, Th2, and Th17 cells migrate into the bone marrow, stimulating the production of inflammatory cells, mast cells, IL-5, IL-13, IL-17, IL-22, and IL-33 (9). Inflammatory cells and cytokines enter the nasal mucosa and lungs, triggering respiratory airway inflammation and causing allergic symptoms (10). At the same time, they can also increase the expression of adhesion molecules in the nasal and bronchial mucosa, thereby aggravating respiratory allergies (11).

Studies have shown that eosinophils and mast cells play important roles in the pathogenesis of allergic asthma (12). Th2-like cytokines are mainly secreted by T cells and regulate asthma airway inflammation. T cells and interleukins have been found to play an important role in the progression of allergic asthma (13).

The regulatory T cell (Treg) is a subpopulation of T cells that regulate the immune system, maintain tolerance to self-antigens, and prevent autoimmune disorders. An earlier report found decreased Treg cells in patients with asthma, and there was a significant correlation between change in airway Tregs cells and asthma. Improving Treg cells may be a novel strategy in the prevention of asthma and other allergic disorders (14). $CD4^+CD25^+$ Treg cells are a unique population of Treg cells that can independently modulate adaptive and innate autoimmune responses (15). Foxp3 is a cell marker of $CD4^+CD25^+$ Treg cells and is closely related to the differentiation and function of regulatory Treg cells (16, 17), which can regulate the immune responses. In the pathogenesis of asthma, Treg cells secrete a variety of cytokines that can suppress the proliferation of T cells and the synthesis of IgE through the transmission of inflammatory cells, such as IL-10 and TGF-β. In different stages of allergic asthma, the analysis of the effects of cytokines and chemokines becomes very important for asthma prevention and treatment.

Astragalus, a large genus of herbs, belongs to the legume family Fabaceae and has potent immune boosting and health-promoting properties (18, 19). *Astragalus membranaceus* (AM) has been widely used for thousands of years in China to treat asthma. Animal model tests showed that the extracts of AM can increase the levels of $CD4^+$ $CD25^{high}$ Treg cells and Foxp3 mRNA expression in an asthmatic animal model (20). *Astragalus* was further found to prevent the recurrence of asthma by modulating Th1/Th2 cytokines in asthmatic children (21). *Astragalus* oral solution (AOS) is made by the Affiliated Nanjing Hospital of Nanjing Medical University, and one of active ingredients is Astragaloside A, which regulates immune responses. Despite the high efficacy of AOS, little is known about the changes of IL-10, TGF-β, Th1, and Th2 cytokines, and $CD4^+CD25^{high}CD127^{low}$ Treg cells in the children with allergic asthma. Children with allergic asthma were recruited at our hospital from 2016 to 2017, and treated by using AOS. Related indicators and immunological changes were examined to evaluate the value of AOS treatment for the children with allergic asthma.

MATERIALS AND METHODS

Materials

Astragaloside A was purchased from China Pharmaceutical and Biological Laboratory (lot number 11078-200703, Beijing, China). AOS was purchased from Nanjing Hospital of Traditional Chinese Medicine (patches numbers: 100234,100406, 100609, 100815, 100987, and 101009; Nanjing, China). AOS was prepared as follows: AM slices were dried to a constant weight, 450 g. After soaking in 5 liters of distilled water overnight, the main ingredients of AM were extracted by using the ultrasonic constant temperature ultrasonic extractor (Cat. No., Scientz-5TQL4, Ningbo Scientz Biotech., Ningbo, China) twice. The first extract was obtained at 30 min (1000 W), and the second tract at 45 min and 50°C, and the extracts were concentrated to 450 mL. The extracts were further centrifuged at 12,000 × *g* for 30 min, and supernatants were filtered through a 10 kDa membrane (Millipore, Bedford, MA, USA).

Chromatographic Conditions

The HPLC system consisted of a Kromasil C18 column (4.6 × 200 mm, id 5 μm) with a guard column (10 × 4.6 mm, id 5 μm) (Waters Chromatography Division of Millipore Corp., Milford, MA, USA), a Waters 515 HPLC Pump, a full-wavelength UV detector, a Waters 717 plus autosampler injector and an Empower workstation. The mobile phase was composed of acetonitrile-water (35:65, v/v), the determination wavelength was 203 nm, and the column temperature was 30°C. All reagents were of chromatographic grade. To achieve the baseline separation and accurate quantitative analysis, the theoretical plate number of Astragaloside A was not less than 3,000.

Measurement of Astragaloside A in AOS

One-milliliter of AOS was added to a 10 mL volumetric flask and extracted twice with petroleum. The petroleum ether extract was discarded, and the lower solution was extracted four times with saturated n-butanol (20, 15, 15, and 10 mL). The combined n-butanol solution was washed with 5% sodium bicarbonate solution twice, each for 30 mL. The washings were discarded and n-butanol solution was collected, and evaporated to dry. The residue was dissolved in 10-mL ethanol. Approximately 0.05 g Astragaloside A standard was added to a 25 mL volumetric flask, and ethanol was added to achieve 1.967 mg/mL Astragaloside A as a reference. A series of the stock solution (0.2, 0.4, 0.6, 0.8, and 1.0 mL) was added to 10 mL volumetric flask, and ethanol was added to achieve a series of Astragaloside A standards with different concentrations (0.0393, 0.0786, 0.118, 0.157, 0.196 mg/mL). Ten microliter of solution was injected into HPLC and the peak area of Astragaloside A was determined. The measurements were repeated twice. The peak area was used as the ordinate, Astragaloside A concentration was used as abscissa, and

the standard curve was plotted. The regression equation was y = 1.245x + 7.526C, $r = 0.9996$. The standard was repeated for five times with RSD 0.7% to ensure the method was accurate. AOS was measured by using the same situation and the results showed perfect repeatability. The average content of Astragaloside A was 0.216% with RSD 1.5%. The tests were stable at 0, 2, 4, 6, and 8 h.

Patients

Before the experiment, all procedures were approved by the human research ethical committee of the Affiliated Hospital of Changchun University of Traditional Chinese Medicine (Changchun, China, approval no. 20170128x). A written consent form approved by our committee and signed by all subjects. Eighty children with allergic asthma, were recruited at the Department of Pediatrics, Affiliated Hospital of Changchun University of Traditional Chinese Medicine. The clinical diagnostic criteria of the "Children's Asthma Diagnosis and Prevention Guide" was used to examine allergic asthma in children (22), and the children had the following symptoms: wheezing, shortness of breath, chest pain or cough, and allergic asthma caused by cold air, physical and chemical stimulation, viral upper respiratory tract infection and exercise. Wheezing episodes in the lungs could be heard and the expiratory phase was prolonged. The typical clinical manifestations, such as no clear wheezing or signs should have at least the following positive test: bronchial provocation test or motor positive test, and or bronchial diastolic positive test (forced expiratory volume in 1 s FEV1 increased).

Allergy Skin Test

The hypersensitivity response of each patient was assessed by using conventional skin prick tests against 16 common aeroallergens according to an earlier report (23). Skin prick tests were performed according to the methods introduced by Gislason (24). The test would be regarded as clinically significant if the allergen reactions were more than 10.

Measurement of Total IgE

Serum IgE levels were measured by using the Human IgE ELISA Kit from Abcam (Shanghai, China). Serum IgE levels were positively associated with the color intensity of the test and calibrated against the WHO standard for IgE (25).

The Test for Allergen-Specific IgE

Allergen-specific IgE was detected in the patients' serum against HD, *D. pteronyssinus* (DP), *D. farinae* (DF), and *B. tropicalis* (BT) by using the AllergyScreen system and ImmunoCAP 100 system (Amersham Pharmacia Biotech, Uppsala, Sweden). The test was calibrated against the WHO Standard for IgE ranging from 0.35 to 100 KU/L for specific IgE.

Inclusion Criteria

The following inclusion criteria were used:

(1) Male or female patients between the ages of 3–12 years; (2) Allergens reactions more than 10. (3)Total serum IgE levels more than 350 IU/mL; (4) competency to perform pulmonary function tests and pulmonary function FEV1 <80% predictive value; (5) children not taking the medicine such as aspirin and other drugs that may trigger asthma symptoms within 4 weeks before enrollment; (6) children able to receive treatment under the guardianship of a parent who signed an informed consent form.

Exclusion Criteria

Children who had the following conditions were excluded: (1) various psychiatric disorders; (2) severe congenital heart disease; (3) pneumothorax, pleural effusion, active pulmonary tuberculosis, acute exacerbation within the past month and received emergency treatment; (5) infectious rhinitis and sinusitis; (6) anaphylactic shock or eczema of unknown etiology; (7) received immunotherapy and (8) adverse reactions after enrollment.

Patients Grouping

The extracts of AM were evaporated to dry, and the residue was dissolved in 10 mL ethanol. AOS was prepared by the 10-fold dilution of AM ethanol solution. Residual petroleum and n-butanol compounds were measured by gas chromatography (Agilent 6890, Agilent Tech., USA) with a flame ionization detecting system. None of the residual compounds were found in the AOS solution.

Based on inclusion and exclusion criteria, 80 children with allergic asthma caused by dust mites were recruited at our hospital from February 2016 to June 2017. The sample size was analyzed by using PASS version 13 (NCSS Statistical Software, Kaysville, UT, USA), and the power of sample size was 85%. The children were evenly and randomly assigned into two groups: the AOS group, where the patients received 10 mL AOS daily and the control group, where the patients received placebo (0.1% quinine chloride in deionized water) daily. The duration of the experiment was 6 months.

Evaluation of Allergic Asthma Symptoms

Lung function was measured by using FEV1, which was compared with predicted values. Most symptoms of allergic asthma were investigated by using Pediatric Asthma Quality of Life Questionnaire (PAQLQ) scores as shown in **Table 1** (26).

The severity of asthma was classified into four levels according to previous report (27): mild intermittent, mild persistent, moderate persistent, and severe persistent based on symptom frequency and either spirometric (FEV_1) or peak expiratory flow (PEF). The primary outcome was also measured according to asthma-related clinical events, including cough, wheeze, and need of intervention. Allergic sensitization to common dietary and respiratory allergens were measured according to an earlier report (28). Serum IgE of 0.2 or 0.35 IU/mL were regarded as positive and predictive for allergic asthma. Eosinophils (29) and serum levels of ECP (30) are increased in allergic asthma.

Measurement of Serum Levels of IL-10 and TGF-β

Five millimeters of venous blood was obtained from each patient. Two millimeters of blood was placed at room temperature for 1 h. The serum was isolated via centrifugation at 5000 × *g* for 10 min. The serum levels of IL-10 and TGF-β were measured by using the

TABLE 1 | The Pediatric Asthma Quality of life questionnaire (PAQLQ score).

Scores	Aspects
LIMITED ACTIVITY	
1	Strong physical activity
2	Medium physical activity
3	Social activity
4	Work-related activities
5	Sleepiness
SYMPTOMS	
8	Shortness of breath
14	Dull aching chest
18	Expiratory or inspiratory difficulties, early morning symptoms of asthma
24	Night arousal
ENVIRONMENT	
9	Smoke,
17	Dust
23	Air pollution environment
26	Strong smell symptoms,
25	Air pollution of the environment
28	Strong smell had to be avoided
EMOTION	
6	Chest tightness caused by the degree of discomfort
7	Worried about suffering from asthma
12	Cough caused by discomfort
13	Asthma and worry
15	Medication
16	Clear throat
21	Worry about no asthma medication
22	Heavy breathing
27	Fear of breathlessness
29	Remuneration,
30	Desperately breathing,
10	Limitation of conscious activities

ELISA kits from R & D Systems, Inc. (Minneapolis, MN, USA). The serum Th1 cytokines (IL-2 and IFN-γ), and Th2 cytokines (IL-4 and IL-6) (31) were measured by using the kits from Abcam (Shanghai office, China).

Measurement of the Percentage of Gated $CD4^+$ T Cells, $CD4^+CD25^+$ T Cells, $CD4^+CD25^{high}$ Treg Cells, $CD4^+CD25^+$ $FoxP3^+$ Treg Cells and $CD4^+CD25^{high}$ $CD127^{low}$ Treg Cells

Three millimeters of blood was added to an anticoagulant-containing (EDTA-K2) tube. Peripheral blood mononuclear cells (PBMCs) were isolated from human peripheral blood via Ficoll-Hypaque (Sigma, St. Louis, MO, USA) density gradient centrifugation. Mouse anti-human CD4 monoclonal antibody was purchased from Zhongshan Golden Bridge Biotechnology (Beijing, China) CD127 (BD Pharmingen #558598, San Jose, CA) was conjugated to ALEXA FLUOR 488. Goat-anti-mouse FITC IgG was purchased from Kangwei (Beijing, China). CD25 PE-Cy7 (BD, clone M-A251), CD25 APC (clone 2A3), and FoxP3 PE (clone PCH101) were purchased from BD Biosciences (Franklin, NJ, USA). CD4+ T cells were gated on side scatter height vs. CD4. The percentage of $CD4^+CD25^+$ T cells, $CD4^+CD25^{high}$ Treg cells, $CD4^+CD25^+$ $FoxP3^+$ Treg cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells in PBMCs was measured by using FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA).

Statistical Analysis

SPSS20.0 statistical software was used to perform statistical analyses. All data were expressed as mean values ± standard deviation (S.D.). Normal distribution and variance homogeneity were analyzed by a paired *t*-test. Normal distribution was analyzed by ANOVA between groups. Non-normal distribution was analyzed by Wilcoxon rank test. Count data were compared by using contingency χ^2 test. The statistical differences were significant if $P < 0.05$.

RESULTS

Recovery Rate of Astragaloside A

AOS was taken from six different batches. With two copies per group, each group was added to a concentration of 0.197 mg/mL Astragaloside A control solution 0.8, 1.0, and 1.2 mL. The average recovery rate of Astragaloside A was 96.2% with RSD 2.65% (**Table 2**).

Average Contents of Astragaloside A in AOS

Table 3 showed that the contents of Astragaloside A were 0.216 ± 0.027 mg/mL from six batches of AOS.

Clinical Demographic Characteristics

The AOS and control groups were composed of 80 cases with 43 males and 37 females, and aged 3–12 years. Age is associated with the risk of allergic asthma (32, 33). In our study, age distribution (5-7, 8-10, and 11-12) was similar between the two groups ($P > 0.05$). The experimental group comprised 23 males and 17 females, with an average age of 8.7 ± 3.3 years. The control group comprised 22 males and 18 females, with an average age of 8.9 ± 2.7 years. The statistical difference was not statistically significant in all parameters (**Table 4**, $P > 0.05$). The most allergic responses to common inhalants included *Cocos nucifera, Brassica nigra*, cat dander, HD, DP, DF, and BT. The statistical difference for these inhalants was insignificant between two groups (**Table 5**, $P > 0.05$). The total serum IgE levels were 428.49 ± 58.61 and 431.27± 60.45 IU/mL between two groups. The statistical difference for allergen-specific IgE reactivity against HD, DP, DF, and BT was insignificant between the two groups (**Table 6**, $P > 0.05$).

No significant differences were found for symptoms of allergic asthma and biochemical characterization of allergic asthma between the two groups (**Tables 7**, **8**). Significant differences of asthma-related events were found after 6-month follow-up (**Table 7**). The severity of allergic asthma was significantly

TABLE 2 | Recovery rate of Astragaloside A.

Times	Sampling volume/ml	Sample content/mg	Addition/mg	Measured values/mg	Recovery rate	Average value	RSD/%
1	10	0.215	0.1574	0.35420	0.9511	0.962	2.65
2	10	0.215	0.1574	0.36540	0.9812		
3	10	0.215	0.1967	0.39870	0.9684		
4	10	0.215	0.1967	0.37540	0.9118		
5	10	0.215	0.2360	0.44670	0.9905		
6	10	0.215	0.2360	0.43650	0.9678		

TABLE 3 | The average contents of Astragaloside A from 6 batches of AOS.

Batches	1	2	3	4	5	6
Contents of Astragaloside A, mg/ml	0.203	0.242	0.213	0.189	0.243	0.204

AOS, astragalus oral solution.

TABLE 4 | Clinical demographic characteristics.

	AOS group	Control group	χ^2 values or *t* values	*P*-values
Age, years	8.7 ± 3.3	8.9 ± 2.7	0.124	0.658
5-7 year, *n* (%)	6	4	0.630	0.730
8-10 year, *n* (%)	26	29		
11-12 year, *n* (%)	8	7		
Male/Female	23/17	22/18	0.051	0.822
BMI	22.67 ± 3.18	23.98 ± 2.75	0.578	0.209
Disease duration, months	36.90 ± 4.28	38.91 ± 5.27	2.397	0.009
Respiratory rate, times/min	19.74 ± 1.54	20.33 ± 1.49	−1.908	0.153
Heart rate, time/min	76.82 ± 3.77	77.02 ± 3.03	−0.292	0.768
Systolic pressure, mmHg	121.68 ± 9.35	123.83 ± 11.55	−1.039	0.326
Diastolic pressure, mmHg	77.04 ± 7.11	75.46 ± 6.51	1.127	0.235

BMI, body mass index = weight (kg)/height (m^2).

TABLE 5 | Allergic response to common inhalants.

	AOS group	Control group	*P*-values
Pollens			0.936
Cocos nucifera	31 (77.5)	33 (82.5)	
Brassica nigra	20 (50)	24 (60)	
Delonix sp.	19 (47.5)	17 (42.5)	
Azadirachta indica	17 (42.5)	15 (37.5)	
Caesalpinia sp	15 (37.5)	18 (45)	
Molds			0.915
Aspergillus fumigatus	11 (27.5)	12 (30)	
Aspergillus niger	8 (20)	9 (22.5)	
Candida albicans	5 (12.5)	4 (10)	
Cladosporium sp.	6 (15)	7 (17.5)	
Alternaria Iternate	4 (10)	2 (5)	
Others			0.623
Dog dander	6 (15)	9 (22.5)	
Cat dander	20 (50)	21 (52.5)	
House dust	40 (100)	32 (80)	
Dermatophagoides pteronyssinus	32 (80)	39 (97.5)	
Dermatophagoides farinae	21 (52.5)	19 (47.5)	
Blomia tropicalis	24 (60)	22 (55)	

Chi-square test was performed and there will be significant difference if $P < 0.05$ between two groups.

TABLE 6 | Allergen-specific IgE reactivity against HD, DP, DF, and BT Allergens.

	AOS group	Control group	*P*-values
HD	28 (70)	25 (62.5)	0.478
DP	24 (60)	20 (50)	0.369
DF	30 (75)	33 (82.5)	0.412
BT	35 (87.5)	28 (70)	0.056

HD, House dust; DP, D. pteronyssinus; DF, D. farinae; BT, B. tropicalis. Chi-square test was performed and there will be significant difference if $P < 0.05$ between two groups.

lower in the experimental group than in the control group ($P < 0.05$).Similarly, biochemical indices of allergic asthma were significantly lower in the experimental group than in the control group (**Table 8**, $P < 0.05$).

AOS Treatment Improved the Allergic Asthma Symptoms in Children

Before treatment, the FEV1% in the AOS group was comparable with that in the control group ($P > 0.05$, **Figure 1A**). After treatment, the FEV1% were significantly increased in the AOS group than in the control group ($P < 0.05$, **Figure 1A**). Similarly, PAQLQ scores were comparable between two groups before treatment ($P > 0.05$, **Figure 1B**). After treatment, PAQLQ scores were increased significantly in the AOS group when compared with those in the control group ($P < 0.05$, **Figure 1B**). The results suggest that AOS reduces the symptoms of allergic asthma by improving FEV1% and PAQLQ.

AOS Increased the Serum Level of IL-10 and Reduced the Level of TGF-β

The serum levels of IL-10 were comparable between the AOS (24.12 ± 3.68 pg/mL) and control groups (26.25 ± 3.79 pg/mL) (**Figure 2A**, $P > 0.05$). Comparatively, the serum levels of TGF-β were same between the AOS (932.67 ± 148.43 pg/mL) and

TABLE 7 | Comparison of symptoms of allergic asthma between two groups.

	AOS group, $n = 40$	Control group, $n = 40$	*P*-values
BEFORE THERAPY			
No. of wheezing episodes	1.10 (0.7–1.6)	1.08 (0.6–1.5)	0.85
Days with wheeze	7.58 (1.36–13.9)	6.95 (0.90–12.3)	0.25
Days on inhalative betamimetics	13.8 (3.37–24.6)	13.2 (8.18–17.2)	0.78
Days on inhalative steroids	10.0 (7.23–24.9)	9.80 (6.99–17.5)	0.89
No. of rescue-free days	128.7 (108.4–158.6)	121.5 (112.0–163.0)	0.93
No. of symptom-free days	123.0 (126.4–162.6)	128.5 (115.7–160.3)	0.71
Allergic Asthma Classification, Cases (%)			
Mild intermittent	13 (32.5)	15 (37.5)	0.968
Mild persistent	12 (30)	11 (27.5)	
Moderate persistent	8 (20)	7 (17.5)	
Severe persistent	7 (17.5)	7 (17.5)	
AFTER THERAPY			
No. of wheezing episodes	0.51 (0.3–1.9)	1.10 (0.6–1.9)	0.03
Days with wheeze	5.26 (3.03–13.7)	11.3 (7.15–14.8)	0.01
Days on inhalative betamimetics	10.7 (6.3–30.2)	13.9 (2.68–18.9)	0.02
Days on inhalative steroids	10.5 (6.3–22.7)	14.8 (4.6–25.6)	0.01
No. of rescue-free days	148.5 (108.9–168.1)	126.0 (99.6–172.4)	0.02
No. of symptom-free days	142.5 (102.6–152.5)	104.0 (86.2–141.8)	
Allergic Asthma Classification, Cases (%)			
Mild intermittent	21 (52.5)	12 (30)	0.02
Mild persistent	10 (2.5)	6 (1.5)	
Moderate persistent	4 (10)	14 (3.5)	
Severe persistent	5 (12.5)	8 (20)	

The statistical difference was significant if $P < 0.05$.

TABLE 8 | Comparison of biochemical characterization of allergic asthma between two groups.

	AOS group, $n = 40$	Control group, $n = 40$	*P*-values
BEFORE THERAPY			
Total IgE (IU/mL)	416.7 (312.8–556.4)	412.2 (310.0–560.6)	0.86
Eosinophils (%)	2.6 (2.3–3.9)	2.8 (2.3–3.5)	0.32
ECP (μg/mL)	22.3 (14.9–29.9)	21.9 (12.6–28.2)	0.69
AFTER THERAPY			
Total IgE (IU/mL)	214.7 (162.3–290.5)	420.2 (316.2–481.3)	0.01
Eosinophils (%)	2.2 (1.5–4.0)	3.0 (2.5–3.6)	0.01
ECP (μg/mL)	16.7 (9.89–23.8)	24.8 (16.6–32.4)	0.01

The statistical difference was significant if $P < 0.05$.

control groups (968.27 ± 150.64 pg/mL) (**Figure 2B**, $P > 0.05$). After treatment, the serum level of IL-10 in the experimental group was significantly increased and the level of TGF-β was significantly decreased compared with those in the control group (**Figure 2**, $P < 0.05$). The results suggest that AOS treatment increases the serum levels of IL-10 and reduces the levels of TGF-β.

AOS Treatment Reduced Serum Level of Th1 (IL-2 and IFN-γ) and Th2 Cytokines (IL-4 and IL-6)

Before the treatment, the statistical difference for the serum level of Th1 (IL-2, **Figure 3A**, and IFN-γ, **Figure 3D**) and Th2 cytokines (IL-4, **Figure 3B** and IL-6, **Figure 3C**) was insignificant between AOS and control groups ($P > 0.05$). After the treatment, the serum levels of Th1 (IL-2, **Figure 3A**, and IFN-γ, **Figure 3D**) and Th2 cytokines (IL-4, **Figure 3B** and IL-6, **Figure 3C**) were lower in the AOS group than the control group ($P < 0.05$).

Percentage of Gated $CD4^+$ T Cells, $CD4^+CD25^+$ T cells, $CD4^+CD25^{high}$, $CD4^+CD25^+FoxP3^+$ Treg Cells and $CD4^+CD25^{high}CD127^{low}$ Treg Cells

Before treatment, the percentage of gated $CD4^+$ T cells (14.26 ± 3.51%, **Figure 4A**), $CD4^+CD25^+$ T cells (9.15 ± 2.28%, **Figure 4B**), $CD4^+CD25^{high}$ (5.67 ± 0.75%, **Figure 4C**), $CD4^+CD25^+FoxP3^+$ Treg cells (1.53 ± 0.21%, **Figure 4D**), and $CD4^+CD25^{high}CD127^{low}$ (0.72 ± 0.28%, **Figure 4E**) Treg cells in the AOS group were comparable with those in the control group, gated $CD4^+$ T cells (13.89 ± 4.01%, **Figure 4A**), $CD4^+CD25^+$ T cells (9.29 ± 2.37%, **Figure 4B**), $CD4^+CD25^{high}$ (5.38 ± 0.64%, **Figure 4C**), $CD4^+CD25^+FoxP3^+$ Treg cells (1.62 ± 0.30%, **Figure 4D**), and $CD4^+CD25^{high}CD127^{low}$ (0.79 ± 0.25%, **Figure 4E**) ($P > 0.05$). After treatment, the percentage of gated $CD4^+$ T cells (18.63 ± 6.27%, **Figure 4A**), $CD4^+CD25^+$ T cells (12.34 ± 4.61%, **Figure 4B**), $CD4^+CD25^{high}$ (9.65 ± 0.83%, **Figure 4C**), $CD4^+CD25^+FoxP3^+$ Treg cells (2.46 ± 0.37%, **Figure 4D**) and $CD4^+CD25^{high}CD127^{low}$ (1.34 ± 0.31%, **Figure 4E**) Treg cells in the AOS group were significantly higher than those in the control group, gated $CD4^+$ T cells (13.99 ± 4.22%, **Figure 4A**), $CD4^+CD25^+$ T cells (9.12 ± 2.05%, **Figure 4B**), $CD4^+CD25^{high}$ (5.63 ± 0.47%, **Figure 4C**), $CD4^+CD25^+FoxP3^+$ Treg cells (1.58 ± 0.24%, **Figure 4D**), and $CD4^+CD25^{high}CD127^{low}$ (0.81 ± 0.28%, **Figure 4E**) ($P < 0.05$) ($P < 0.05$). The results suggest that AOS consumption increases the percentage of gated $CD4^+$ T cells, $CD4^+CD25^+$ T cells, $CD4^+CD25^{high}$, $CD4^+CD25^+FoxP3^+$ Treg cells, and $CD4^+CD25^{high}CD127^{low}$ Treg cells in children with allergic asthma.

DISCUSSION

The results showed that the content of Astragaloside A in AOS varied from 0.19 to 0.24 mg/mL. The quality of the different batches of the mixture was influenced by the quality of the decoction pieces, processing and other factors. HPLC was an effective method to detect the quality of AOS and provided a basis for high-quality standards.

Allergic asthma is caused by a variety of cells, including inflammatory cells (neutrophils, T lymphocytes, eosinophils, and mast cells) (34), airway structural cells (airway smooth muscle cells and epithelial cells) (35), and cell components. AR is the most common symptoms of allergic asthma after individuals come in contact with allergens. Many inflammatory cells and

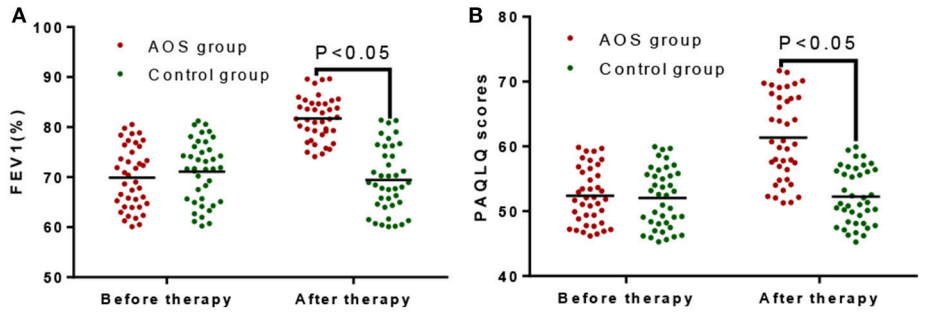

FIGURE 1 | The effects of AOS on FEV1% and PAQLQ scores in the children with allergy asthma. **(A)** The effects of AOS on FEV1% in the children with allergy asthma. **(B)** The effects of AOS on PAQLQ scores in the children with allergy asthma. FEV1, forced expiratory volume in 1 s; PAQLQ, Pediatric Asthma Quality of Life Questionnaire. $n = 40$ for each group. The statistical difference was significant for $P < 0.05$.

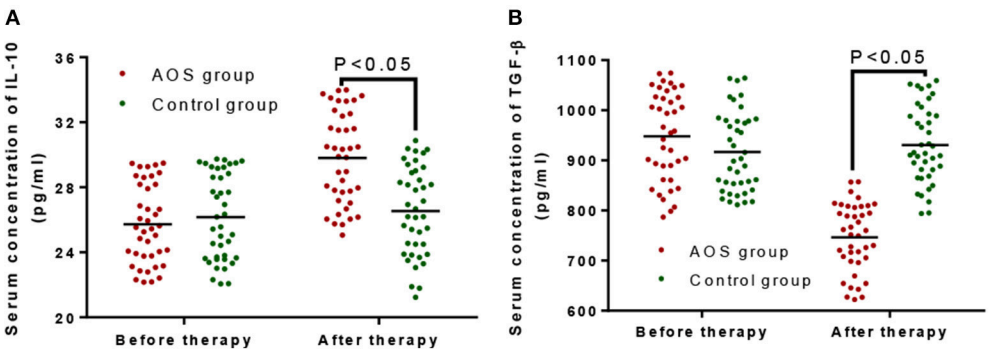

FIGURE 2 | The effects of AOS on the serum levels of IL-10 and TGF-β in the children with allergy asthma. **(A)** The effects of AOS on serum level of IL-10 in the children with allergy asthma. **(B)** The effects of AOS on TGF-β in the children with allergy asthma. $n = 40$ for each group. The statistical difference was significant for $P < 0.05$.

cytokinesare involved in the inflammatory responses, including nasal itching, sneezing, running nose, and stuffy nose. The incidence of childhood allergic asthma has been increasing, but its pathogenesis is not yet clear. The traditional Th1/Th2 imbalance theory cannot explain all the pathogenesis. Presently, a number of studies have focused on Treg cells to investigate the pathognesis (36, 37). Our findings demonstrated that AOS treatment reduced the serum levels of Th1 and Th2 cytokines (**Figure 3**). Analyses of childhood allergic asthma by using $CD4^{+}CD25^{high}CD127^{low}$ Treg cells will have important clinical values.

IL-10 regulates allergic asthma and is directly involved in the regulation of inflammatory cells. IL-10 factor can inhibit the proliferation, secretion of Th cells, and the proliferation and differentiation of antigen-presenting cells (38). IL-10 cells inhibit T cells, antigen presentation (39), and the expression of IL-8 (40). IL-10 was reported to inhibit eosinophil-induced inflammatory effects of asthma, suggesting that IL-10 plays a key negative regulatory role in the development of allergic asthma (41). The level of IL-10 was found to be reduced significantly in T cells of peripheral blood during the onset of allergic asthma (42). In our study, the IL-10 level in the AOS group was comparable with that in control group before treatment ($P > 0.05$, **Figure 2**). After treatment, the level of IL-10 in the AOS group was significantly increased ($P < 0.05$). After AOS treatment, IL-10 may inhibit the antigen-presenting process and T cells response and play a critical role in the prevention of asthma.

TGF-β is a kind of stimulating factor with many biological effects, and it plays a critical role in regulating inflammation in various organs and tissues. Thus, TGF-β is widely studied in the transforming growth factor family and involved in the fibrosis formation of tissues and organs. Its overexpression in cardiac tissue can cause cardiac hypertrophy or myocardial fibrosis, which is involved in the process of ventricular remodeling (43, 44).TGF-β is a multifunctional cytokine that can participate in the process of cell proliferation, apoptosis and differentiation (45). In the immune system, TGF-β is a regulator of immunity with both pro- and anti-inflammatory effects and is involved in a variety of airway inflammation and immune responses. Many studies on TGF-β in the pathogenesis of asthma have been performed. However, t many controversies at different stages of development are still present. Most of researchers think that TGF-β can inhibit or promote inflammation (46, 47).

Elevated levels of TGF-β were reported in patients with atopic asthma (48, 49), which may be caused by the continued stimulation of allergens and airway remodeling. TGF-β also has a chemotactic function and can promote the proliferation

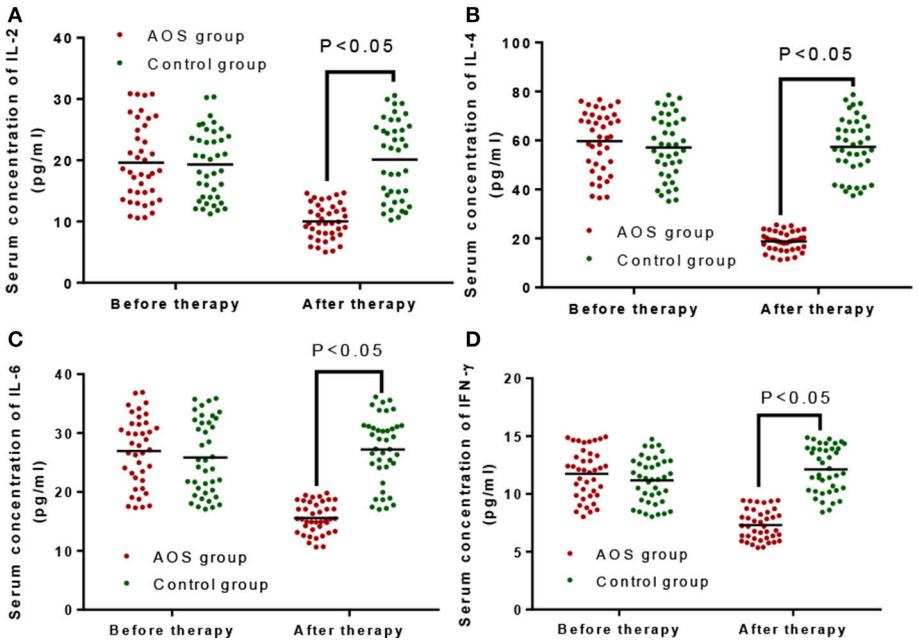

FIGURE 3 | The effects of AOS on the serum levels of Serum Th1 cytokines (IL-2 and IFN-γ), and Th2 cytokines (IL-4 and IL-6). **(A)** The effects of AOS on serum level of IL-2. **(B)** The effects of AOS on serum level of IL-4. **(C)** The effects of AOS on serum level of IL-2. **(B)** The effects of AOS on serum level of IL-6. **(D)** The effects of AOS on serum level of IFN-γ. $n = 40$ for each group. The statistical difference was significant for $P < 0.05$.

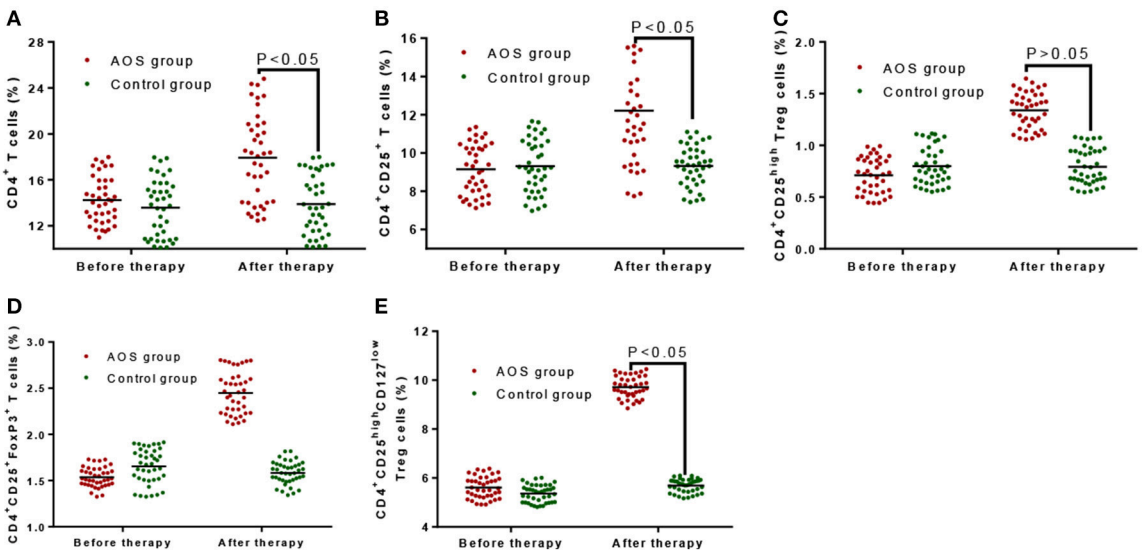

FIGURE 4 | The effects of AOS on the contents of T cells in the children with allergy asthma. **(A)** The effects of AOS on the contents of gated $CD4^+$ T cells in the children with allergy asthma. **(B)** The effects of AOS on the contents of $CD4^+CD25^+$ T cells in the children with allergy asthma. **(C)** The effects of AOS on the contents of $CD4^+CD25^{high}$ Treg cells in the children with allergy asthma. **(D)** The effects of AOS on the contents of $CD4^+CD25^+FoxP3$ Treg cells in the children with allergy asthma. **(E)** The effects of AOS on the contents of $CD4^+CD25^{high}CD127^{low}$ Treg cells in the children with allergy asthma. $n = 40$ for each group. The statistical difference was significant for $P < 0.05$.

and differentiation of inflammatory cytokines (50). In previous studies, the severity of asthma was found to be related to the concentration of TGF-β in the peripheral blood (51). In the present study, the serum level of TGF-β in the experimental group was comparable with that in the control group ($P > 0.05$). After AOS consumption, the level of TGF-β was significantly decreased ($P < 0.05$), suggesting that AOS ameliorates allergic asthma by reducing the serum level of TGF-β.

Treg cells are a subset of T-cells that control the autoimmune reactivity *in vivo* and play a key role in the inhibition of autoimmunity (52, 53). They exert immunosuppressive effects through the specific binding of their surface molecules (CTLA-4, CD25) to the corresponding ligands on the cells (54). IL-10 can inhibit the proliferation of T cells, the synthesis of cytokines such as IL-2 by Th1 and Th2 cells, and the expression of MHCII in macrophages (55). Immune regulatory T cells may maintain the immune tolerance via several mechanisms. $CD4^+CD25^+$ Treg cells are able to bind to target cells and participate in immune regulation. In previous studies, $CD4^+CD25^+$ Treg cells and $CD4^+CD25^+$ T cells were increased simultaneously with $CD4^+CD25^+$ Treg proliferation (56). The percentage of $CD4^+CD25^+$ T cells and $CD4^+CD25^{high}CD127^{low}$ was decreased during acute episode of childhood allergic asthma (57). In the present study, the percentage was comparable for $CD4^+CD25^+$ T cells, $CD4^+CD25^{high}$, $CD4^+CD25^+FoxP3^+$ Treg cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells between two groups before the treatment (**Figure 4**, $P > 0.05$). After the treatment, AOS consumption increased the percentage of $CD4^+CD25^+$ T cells, $CD4^+CD25^{high}$, $CD4^+CD25^+FoxP3^+$ Treg cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells (**Figure 4**, $P < 0.05$). The results suggest AOS consumption reduces the risk of allergic asthma by increasing the percentage of $CD4^+CD25^+$ T cells, $CD4^+CD25^{high}$, $CD4^+CD25^+FoxP3^+$ Treg cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells in PBMCs.

Th2 cytokines are associated with allergic airway inflammation (2) while Th1 cells inhibit Th2 immune activities (3). The imbalance of Th1/Th2 cytokines indicates the risk of asthma (58). Peripheral blood eosinophils increase the levels of Th1 and Th2 cytokines (4). By contrast, AOS therapy will reduce the levels of peripheral blood eosinophils and may result in the down-regulation of Th1 and Th2 cytokines. AOS may also increase the level of Treg cells, which will suppress the level of blood eosinophils (5).

The present study has some limitations to. For the side effects of AOS were still unclear. According to previous reports, *Astragalus* induces some side effects, including anemia, neutropenia, thrombocytopenia, fatigue, poor appetite, nausea, and vomiting (1). However, these side effects were not found in the present study. Although Astragaloside A is the main bioactive component in AOS, other components were not analyzed in AOS. The functions of certain components should be confirmed in the future work. In this experiment, we only explored the effects of AOS on the serum levels of IL-10, TGF-β and the percentage of $CD4^+CD25^+$ T cells, $CD4^+CD25^{high}$, $CD4^+CD25^+FoxP3^+$ Treg cells and $CD4^+CD25^{high}CD127^{low}$ Treg cells in PBMCs. However, modern studies show that the expression level of Treg cells is associated with a variety of autoimmune diseases. The study of Treg alone cannot fully explain the exact mechanism for its action. In recent years, the research on the immune balance between Treg/Th17 has been increased. In the future, further work is highly demanded to reveal the relationship between the impact of the balance and allergic asthma in children.

CONCLUSIONS

The average contents of Astragaloside A were 0.216 ± 0.027 mg/mL from six batches of AOS. After 6-month therapy, AOS treatment was more effective in the experimental group than in the control group ($P < 0.05$). AOS reduced the symptoms of allergy asthma in children group by improving FEV1% and PAQLQ scores. The children with allergic asthma have a lower level of serum IL-10 and higher level of TGF-β. AOS consumption increased the level of serum IL-10 and reduced the level of TGF-β. AOS can be effective in the treatment of allergic asthma in children by increasing the percentage of $CD4^+CD25^+$ T cells, $CD4^+CD25^{high}$, $CD4^+CD25^+FoxP3^+$ Treg cells, and $CD4^+CD25^{high}CD127^{low}$ Treg cells.

AUTHOR CONTRIBUTIONS

WW conceived, designed the study, and wrote the manuscript. QL and WJ performed the experiments, analyzed the data, and contributed reagents, materials, analysis tools.

ACKNOWLEDGMENTS

We are very grateful to two reviewers for their critical and strategic comments, which have significantly improved the quality of our paper.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 16 January 2019
doi: 10.3389/fped.2018.00432

New Drugs for Pediatric Asthma

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Asthma is the most common chronic disease in children. As suggested by international guidelines, the main goals of asthma treatment are symptoms control and lung function preservation, through a stepwise and control-based approach. The first line therapy based on inhaled corticosteroids may fail to reach control in more than one third of patients, especially adolescents, and in these lung function and quality of life may progressively worsen. Treatment with omalizumab, the first anti-immunoglobulin E recombinant humanized monoclonal antibody, has been definitely approved in pediatric uncontrolled asthma. In this review, we discuss the mechanisms and potential roles of emerging therapies for pediatric severe asthma. Novel biologic drugs (i.e., dupilumab, mepolizumab, reslizumab, and benralizumab) seem to be promising in reducing annual exacerbation rates and steroid-use in glucocorticoid-dependent cases, but available data are few and limited to adolescents and adults. Evidences on the use of the muscarinic antagonist tiotropium as controller medication in pediatric settings are progressively growing, sustaining an application as asthma maintenance treatment in children aged >6 years and in preschool children with persistent asthmatic symptoms, but well powered trials are needed to confirm its safety and efficacy. New inhaled corticosteroids (i.e., ciclesonide and mometasone) are effective as once-daily controller therapy, but long-term studies in the different pediatric ages are needed to compare effectiveness and safety to usual treatments. At present, the role of macrolides in pediatric severe asthma is controversial and their administration is not recommended routinely, but may be considered in children with neutrophilic asthma for reducing daily oral steroids administration and improving lung function. Despite the availability of several novel therapeutic strategies for uncontrolled asthma, future trials targeted at specific pediatric age subgroups are needed to support evidences of safety and efficacy also in children.

Keywords: severe asthma, therapy, biologics, inhaled corticosteroids, muscarinic antagonists, macrolides, children, adolescents

OPEN ACCESS

Edited by:
Michael David Shields, Queen's University Belfast, United Kingdom

Reviewed by:
Basil Elnazir, Tallaght Hospital, Ireland
Ignacio Tapia, Children's Hospital of Philadelphia, United States

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Specialty section:
This article was submitted to Pediatric Pulmonology, a section of the journal Frontiers in Pediatrics

Received: *18 October 2018*
Accepted: *27 December 2018*
Published: *16 January 2019*

Citation:
Maglione M, Poeta M and Santamaria F (2019) New Drugs for Pediatric Asthma. Front. Pediatr. 6:432. doi: 10.3389/fped.2018.00432

INTRODUCTION

Asthma is the most common chronic condition of childhood, and its management represents part of the daily activity of most professionals who deal with pediatric care. At any age, the main purposes of asthma treatment are to reduce exacerbations and to limit the progressive loss of lung function, thus decreasing the use of health resources and improving quality of life. In few words, asthma therapy aims to achieve good symptom control (1).

National and international guidelines on asthma management of adults and children agree in indicating inhaled corticosteroids (ICS) as the most effective and safe medications, which may be used alone or associated with other controller therapies, in a stepwise, control-based approach (2–4).

Nevertheless, despite this approach, in more than one third of all patients with asthma the persistence of clinical symptoms, often associated with overt lung function abnormalities, indicate poor control, with the proportion increasing to >50% in adolescents (5, 6). This variability in treatment response is partly genetically determined. Pharmacogenomic studies, through the characterization of genes relevant in asthma treatment response, are paving the way toward the personalization of therapy. This would entail a dramatic change in asthma treatment from the actual "one size fits all" approach to the so called "precision medicine," that is the tailoring of healthcare to the individual through the identification of clinical, biological, or genetic markers (7). Therefore, the interest in the development of new drugs and in the application of therapies currently used in other conditions for pursuing asthma control is high. This is particularly true in the pediatric setting, due to the lack of adequately designed trials including children. Indeed, even though results from adult studies may be sometimes translated to adolescents, this is not the case for younger asthmatic patients for whom evidence on the safety and efficacy of new treatments is still sparse.

The present review will go through the new therapeutic approaches to pediatric asthma, highlighting available evidence on their efficacy, the related risks, and the areas of uncertainty that in many cases still limit their regular application in the clinical practice.

BIOLOGICS

Over the last decades, the development of monoclonal antibodies specifically designed to bind determined targets has deeply changed the approach to a number of conditions, also affecting children (8). In pediatric respiratory medicine, this novelty has been embodied by omalizumab, a recombinant DNA-derived humanized anti-IgE monoclonal antibody, which is the only biologic drug recommended in children with moderate-to-severe asthma (3, 9). Omalizumab, which was approved by the United States (US) and the European Union in 2003 and 2005, respectively, is able to decrease the quantity of cell-bound IgE, to downregulate the IgE receptors on mast cells, basophils, and dendritic cells, thus preventing mediator release from effector cells (10, 11). At present, its use is licensed as an add-on treatment for patients aged > 6 years with severe persistent allergic asthma and positive skin test or specific IgE to perennial aeroallergens, FEV_1 < 80% predicted, frequent daytime symptoms, or nighttime awakenings, and multiple severe asthma exacerbations despite traditional maintenance therapy (12, 13). In comparison to the robust body of literature supporting the efficacy and safety of omalizumab in severe, inadequately controlled adult asthma (14, 15), evidence in children is far more limited. Nevertheless, even though no studies have been published to date in preschoolers, only few well-designed clinical trials have addressed the role of omalizumab in uncontrolled asthma affecting children older than 6 years. A large, multicenter randomized controlled trial (RCT) assessed the efficacy of omalizumab in 419 subjects (mean age 10.9 years) with persistent allergic asthma, and showed that anti-IgE treatment increases the number of symptoms-free days and reduces the number of exacerbations and the need for controller therapies (16). Similar results were achieved by other pediatric studies which have strengthened the role of omalizumab in limiting asthma exacerbations, and to a lesser extent, in improving patients' lung function (17–21). Despite reported cases of anaphylaxis in children (22), which warrant hospital administration, omalizumab is basically safe, whereas high cost represents a more relevant issue.

The scenario of biologics applied to asthma treatment has recently added to the more "consolidated" omalizumab, a number of novel drugs for which evidence of safety and efficacy is still very sparse and generally limited to adult studies. Dupilumab, an anti–interleukin-4 receptor α monoclonal antibody blocking both interleukin-4 and interleukin-13 signaling, is probably the most promising for a future application in pediatric asthma, as shown by two recent trials (23, 24). These large, multicenter RCT have enrolled patients aged >12 years with moderate-to-severe uncontrolled asthma (23) or glucocorticoid-dependent severe asthma (24). In such subjects dupilumab has proven effective in decreasing exacerbations and improving asthma control, also resulting in better lung function. Similar results have been provided for mepolizumab, which has been recently approved for severe eosinophilic asthma in adults and adolescents. This anti-interleukin-5 antibody has been shown to reduce severe asthma exacerbations (25) and need for oral steroids (26), even though evidence for its use in children < 12 years is virtually absent. Mepolizumab is not the only anti-interleukin-5 antibody under investigation for improving asthma control. Reslizumab and benralizumab are anti-interleukin-5 antibodies whose efficacy has been evaluated by few recent studies including patients with asthma aged >12 years (27–31). Both treatments have proven safe and effective in improving asthma control and lung function in selected patients with severe uncontrolled asthma and high blood eosinophil count, but, again, available data are few and limited to adolescents and adults.

MUSCARINIC ANTAGONISTS

The increased cholinergic tone typical of asthma makes muscarinic receptors an obvious target for therapeutic strategies aimed at reducing airway hyperresponsiveness. In children with severe asthma exacerbations, inhaled short-acting antimuscarinic agents, namely ipratropium, are a widely accepted therapeutic option whose efficacy on lung function is well documented (32). Used in addition to nebulized albuterol, ipratropium has proven effective in reducing the risk for hospital admission as well as in improving spirometry in children with asthma exacerbations (33). Less frequent is the use of muscarinic antagonists as controller therapy, even though tiotropium,

the most widely used long-acting muscarinic antagonist is mentioned as a possible add-on therapeutic option in step 4 of GINA guidelines for children older than 12 years taking the combined treatment of ICS and long-acting beta^2-agonists (LABA), but still reporting inadequate asthma control (3). Inhaled tiotropium (Spiriva Respimat®), first indicated in adult COPD treatment, was shown to improve pulmonary function and respiratory symptoms in adult moderate-to-severe asthma (34, 35). Nevertheless, when added to ICS, tiotropium appears to be slightly less effective in improving quality of life in comparison to the traditional combination ICS/LABA (36). Tiotropium was recently approved by the US Federal Drug Administration (FDA) as an asthma maintenance treatment in children aged >6 years (37), whereas in Europe its use is still limited to adults. However, available evidence in children and adolescents is progressively growing, thus making a future wider application in pediatric asthma likely. In particular, school-aged children with severe symptomatic asthma have shown improvement of lung function and good tolerability and safety when tiotropium was added to medium or high-doses of ICS (38, 39). Furthermore, positive trends in asthma control and FEV_1 were also observed in adolescents with moderately severe asthma treated with tiotropium as an add-on drug to ICS and other controllers for 3 months (40, 41). Finally, a recent small RCT showed the potential to reduce asthma exacerbation risk in children aged 1–5 years with persistent asthmatic symptoms, with tolerability similar to that of placebo, although mean daytime asthma symptom scores were not significantly different between groups (42). Despite its debated role within the group of controller drugs in childhood asthma, tiotropium remains an attractive option, both for the possibility of once-daily administration and for its peculiar way of delivery. Indeed, the drug is administered by a device named Respimat® in form of a mist of extremely fine particles (diameter $< 6\,\mu m$), whose main advantages are the lower speed of delivery, the limited pharyngeal deposition and the enhanced pulmonary deposition in comparison to the common metered dose inhalers. Additional well powered trials are needed to further assess the safety and efficacy of tiotropium especially in young children with uncontrolled asthma symptoms.

NEW INHALED CORTICOSTEROIDS

Inhaled corticosteroids represent the cornerstone of asthma therapy at all ages (3). Despite the long and large experience with traditional molecules such as beclometasone, flunisolide, fluticasone, and budesonide, all with high safety and efficacy profiles, some new ICS have been recently approved, but only few of these are allowed for the pediatric use.

Ciclesonide, licensed from age 4 years in the US and from age 12 years in Europe, is a pro-drug activated by esterases within the lung to form the active compound (des-ciclesonide). The possibility of a single daily administration, and evidence supporting its efficacy in improving asthma control and in reducing airway inflammation make this steroid a valid option as asthma controller therapy (43). Nonetheless, given the lack of relevant differences in the efficacy of ciclesonide vs. fluticasone or budesonide, long-term superiority trials are needed to identify the usefulness and safety of ciclesonide compared to other ICS (44). Similarly, mometasone, which has the same FDA approval as ciclesonide and may be used down to the age of 4 years also in Europe, has proven effective as once-daily controller therapy in several pediatric studies (45), with evidence supporting a significant functional improvement in school-aged children with persistent asthma (46).

MACROLIDE ANTIBIOTICS

Macrolides are widely used antibiotics with both antimicrobial and anti-inflammatory activities (47). Indeed, in addition to their well-known antibiotic effect, there is evidence that macrolides modulate the expression of cellular adhesion molecules, may attenuate inflammatory cell migration and inhibit the respiratory burst in polymorphonuclear cells. Furthermore, macrolides clearly affect neutrophil function, even though the exact mechanisms have not been elucidated (48). For these reasons, macrolides have been initially recommended in diffuse panbronchiolitis (49), cystic fibrosis (50), and non-cystic fibrosis bronchiectasis (51). As airways infection is a possible cause for asthma, macrolides have been supposed to be used as long-term treatment for improving the disease control and reducing the need for steroids (52). Actually, the early troleandomycin—no longer recommended because of adverse effects on liver function tests—was reported to work as "steroid-sparing" drug by reducing the catabolism of steroids, but indeed no steroid reduction was demonstrated (53). Most recently, clarithromycin was found to widely suppress severe, steroid-insensitive allergic airways disease in a mouse model through its anti-inflammatory effects on tumor necrosis factor-α/interleukin-17 immune responses that are largely independent of its antimicrobial effects (54). Indeed, macrolides may reduce airway inflammation either by acting on pro-inflammatory cytokines or by controlling intracellular infection which may trigger and maintain inflammation (55).

At present, the role of macrolides in severe asthma is controversial. In a large multicentre RCT azithromycin did not reduce the rate of severe exacerbations and lower respiratory tract infections in a population of severe asthma adults not including children or adolescents (56). Finally, a systematic review and meta-analysis did not show a benefit of macrolides over placebo on rates of exacerbations, quality of life or participants' need for rescue medications (57).

Looking specifically at the pediatric literature, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* have been suggested to play a role in the pathogenesis of severe asthma (58). Preschool-aged children with frequent, severe exacerbations may benefit from sporadic use of azithromycin (59). Regrettably, very few RCTs investigated the role of macrolides in school-age children or adolescents with asthma, and failed to demonstrate a beneficial effect probably because of the low power of the study

TABLE 1 | Summary of novel drugs for treatment of severe pediatric asthma.

Drug	Mechanism of action	Novel evidence in children
BIOLOGICS		
Dupilumab	Anti–IL-4 receptor α monoclonal antibody, blocks both IL-4 and IL-13 signaling.	Two trials also involving children >12 years. Decreases exacerbations and improves lung function in moderate-to-severe uncontrolled asthma (22). In glucocorticoid-dependent asthma reduces steroid use (23).
Mepolizumab	Anti-IL-5 monoclonal antibody, selectively inhibits eosinophilic inflammation.	Two trials also involving children >12 years. Effective and well tolerated. Reduces the risk of asthma exacerbations in patients with severe eosinophilic asthma (24, 25).
Reslizumab	Anti-IL-5 monoclonal antibody, inhibits activity within the IL-5 signaling pathway and reduces blood and tissue eosinophils.	Three trials also involving children >12 years (26, 27, 29). Improves lung function, asthma control and symptoms, quality of life in uncontrolled asthma.
Benralizumab	Anti-IL-5 receptor α monoclonal antibody, induces direct, rapid, and nearly complete depletion of eosinophils.	Two trials also involving children >12 years (28, 30). Significantly reduces annual exacerbation rates in patients with severe, uncontrolled asthma with blood eosinophils >300 cells per μL.
MUSCARINIC ANTAGONISTS		
Tiotropium	Long-acting muscarinic antagonist, decreases airway tone binding the muscarinic receptors.	Evidence of improved lung function in children >12 years receiving tiotropium in addition to standard therapy (39, 40), as well as in children aged 6–11 years (37, 38). Weaker evidence of reduction in exacerbations rate in children aged 1–5 years (41).
NEW INHALED CORTICOSTEROIDS		
Ciclesonide	Reduces airway inflammation through a single day administration	Improves airway inflammation and asthma control in atopic children (42). Longer-term trials needed to identify the usefulness and safety compared to other ICS (43).
Mometasone	Reduces airway inflammation through a single day administration	Functional improvement in school-aged children with persistent asthma (45).
MACROLIDES		
	Reduce airway inflammation by acting on pro-inflammatory cytokines or by controlling intracellular infection.	May reduce daily oral steroid administration and improve FEV_1 (63). Routine use not recommended in children or adolescents with severe asthma (8, 62).

(60–62). Actually, the possibility that macrolides work well in pediatric severe asthma is not definitely excluded, yet at present the evidence is quite poor. It should be also kept in mind that an inappropriate use of macrolides unavoidably results in antibiotic resistance that is a major worldwide concern, especially in the pediatric population where the respiratory infection rate is as high as in the elderly (63).

Although an official document and a recent pragmatic review do not recommend the routine use of macrolides for children with severe asthma (9, 64), they may be useful for reducing daily oral steroid administration and improving FEV_1 (65), and, for this reason, can be proposed as *ex-juvantibus* trial in children with neutrophilic asthma (66). In conclusion, further well-designed and large RCTs are warranted before routine use of macrolides is recommended or definitely condemned in pediatric severe asthma.

A list of new medications for treating children with severe, uncontrolled asthma, also including the novel evidence for their use, is provided in **Table 1**.

OTHER INTERVENTIONS

Immunosuppressive drugs including cyclosporine, azathioprine and methotrexate, commonly used in several immune-mediated disorders, have been proposed for severe asthma management, but no recommendation may be formulated at present due to the insufficiency of data, particularly in children (3).

Allergen immunotherapy has proven effective in improving symptom control in mild to moderate asthmatic children, especially in the presence of a clear association between symptoms and exposure to a specific allergen, but its applicability in the clinical practice is strongly limited by the requirement for patients to have stable (and not uncontrolled) asthma symptoms due to the risk of severe reaction (67).

A surgical procedure named bronchial thermoplasty, consisting in the ablation of the airway smooth muscle layer, has shown to provide some benefits in adults unresponsive to conventional therapies (68). Nevertheless, evidence in children

or adolescents is completely lacking at present, and such intervention is therefore not recommended in these age groups.

Finally, the association between worse symptom control and fungal sensitization in severe asthma has led to studies assessing the efficacy of antifungal drugs on asthma outcomes. However, data are still conflicting and not conclusive and insufficient to support formal recommendations (69).

CONCLUSIONS

Severe asthma identifies children or adolescents who need high-dose ICS therapy and a second controller therapy in the previous year, or systemic corticosteroids for 50% of the year, to prevent asthma from being uncontrolled or that remains uncontrolled notwithstanding these medications (9). Unfortunately, once excluded any comorbidity or optimized patients' adherence to treatment and inhalation technique, about 5–10% of the asthmatic pediatric population continue to have severe symptoms or signs and the loss of lung function may be progressive and irreversible (70). The latter point is of paramount importance in view of the fact that a stringent relationship between the childhood insults to the lung and the accelerated aging that can occur in adult chronic obstructive lung disease has been postulated (71). Finally, asthma not responding to treatment may result in significant morbidity and frequent healthcare utilization (1, 72). For all the above, there is considerable need for robust studies of children with uncontrolled asthma confirming the clinical efficacy and safety of medications increasingly used in the adult population, but not allowed in patients <12 years of age because of the paucity of literature data.

AUTHOR CONTRIBUTIONS

MM has made substantial contributions to conception and design, has been involved in drafting the manuscript, and has given final approval of the version to be published. MP conceived the idea, has been involved in drafting the manuscript and has given final approval of the version to be published. FS has made substantial contributions to conception and design, has been involved in drafting the manuscript and revising it critically for important intellectual content, and has given final approval of the version to be published.

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