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REVIEW

Asthma Endotyping and Biomarkers in Childhood Asthma

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Childhood asthma represents a heterogeneous challenging disease, in particular in its severe forms. The identification of different asthma phenotypes has stimulated research in underlying molecular mechanisms, such as the endotypes, and paved the way to the search for related specific biomarkers, which may guide diagnosis, management, and predict response to treatment. A limited number of biomarkers are currently available in clinical practice in the pediatric population, mostly reflecting type 2-high airway inflammation. The identification of biomarkers of childhood asthma is an active area of research that holds a potential great clinical utility and may represent a step forward toward tailored management and therapy: the so-called Precision Medicine. The aim of the present review is to provide an updated overview of asthma endotyping, mostly focusing on novel noninvasive biomarkers in childhood asthma.

Keywords: asthma, children, biomarkers, airway inflammation, eosinophils, exhaled nitric oxide, IgE

Introduction

Childhood asthma is a common chronic airway disease characterized by airway inflammation, airway hyperresponsiveness (AHR), and reversible airway obstruction, affecting around 15% of school-aged children in Europe, with increasing incidence and prevalence.[1,2] Symptoms include wheezing, shortness of breath, chest tightness, and cough, ranging in severity from mild symptoms to life-threatening exacerbations. 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 exacerbations and medication-related side effects, and preventing the progression of obstructive lung damage during growth and then later in life.[1] While the majority of asthmatic children have mild or moderate disease and can be adequately controlled with standard medications, a minority (around 5%) of children with asthma suffer from a severe uncontrolled disease, carrying a significant health and socioeconomic burden, and requiring additional but still limited therapeutic options.[3]

The recognition of different disease variants (phenotypes), even in childhood, has recently allowed to overcome the so far rooted and simplified view of asthma as a single disease.[4,5] Childhood asthma phenotypes may differ in clinical presentation, natural history, inflammatory mechanisms, response to treatment, and depend on age.[6] The increasing awareness on heterogeneity of childhood asthma has also led to the recognition of underlying pathophysiological and/or molecular mechanisms (endotypes) and paved the way to the search for related specific indicators (biomarkers), which may guide diagnosis, phenotyping, management, and predict response to treatment.[7–9] The identification of biomarkers of childhood asthma is an active area of research that holds a potential great clinical utility and may represent a step forward toward tailored management and therapy: the so-called Precision Medicine, which is an emerging approach for disease treatment targeted to the needs of individual patients on the basis of genetic, biomarker, phenotypic, or psychosocial characteristics that distinguish a given patient from other patients with similar clinical presentations.[10,11]

Phenotypes in Childhood Asthma

Starting from the results of historical longitudinal cohort studies, epidemiologic and symptom-based criteria have been conventionally used to describe childhood asthma phenotypes.[12–17] Atopy, reduced lung function, and viral and bacterial respiratory infections in wheezing infants have been identified as major risk factors for the persistence of asthma. Furthermore, a greater magnitude of atopy and lower lung function have been recognized as the main features of children with severe asthma in comparison with those with mild-to-moderate disease.[4–6] However, tracking the course of the disease within longitudinal studies also revealed a limited practical utility of these disease variants, because of their complex heterogeneity, the possibility of overlapping features, and their instability over time.[18]

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Advanced statistical methods, such as cluster analysis and latent class analysis methodology, have recently renewed the interest in investigating and identifying new childhood asthma phenotypes.[19–24] Phenotyping in children has been set on the basis of multiple variables: the presence or absence of atopy, the temporal patterns and the triggers of symptoms, the severity of the disease, the patterns of airway inflammation, and the response to medications, in particular for severe asthma.[18,23–31] Results from cluster analyses studies on severe asthma highlighted that childhood-onset severe asthma is characterized by eosinophilic airway inflammation, male predominance, severe atopy with multiple sensitizations, airflow limitation, and early signs of airway remodeling, presenting itself differently from adult-onset phenotype and rapidly changing over time or in response to treatment.[32–35] However, the large amount of data derived from these statistical categorizations is still more useful for researchers than clinicians, and has to be interpreted with caution, due to the variability of statistical methods used and to the lack of validation in several populations.[36] Furthermore, the current identification of asthma phenotypes does not provide insight into the underlying pathogenic mechanisms and has limited clinical value in predicting outcomes and directing therapy.

Although it is already known that asthma has a strong genetic component, recent research studies indicate that variability in genotype contributes significantly to the heterogeneity of asthma phenotype and morbidity.[37] Research on asthma susceptibility genes highlighted the role of *ADAM33*, a disintegrin and metalloproteinase domain 33 expressed in fibroblasts and smooth muscle cells, in regulating susceptibility of lung epithelium/fibroblasts to remodeling in response to allergic inflammation.[38] Gene polymorphisms of *ADAM 33* have been associated with an increased risk for childhood asthma, in particular in the Asian population, and with an excess decline in lung function in asthmatic subjects, representing potential markers of susceptibility and severity of disease.[39] Genetic variants may also influence the response to therapy: one of the most investigated pharmacogenetic effects has been the effect of polymorphisms at the gene encoding the β2-adrenergic receptor, *ADRB2*, on the bronchodilator response to inhaled short- and long-acting β agonists. Of particular interest is the Arg16Gly polymorphism of the *ADRB2* gene, which is associated with enhanced downregulation and uncoupling of β2-receptors and with differences in pulmonary function responsiveness to short-acting β agonists in children.[40] Furthermore, the use of long-acting β agonists as add-on controller in asthmatic children carrying the Arg16Gly polymorphism has been associated with increased risk of asthma exacerbation.[41,42] Thus, Arg16Gly polymorphism in the β2-receptor might be considered a potential marker for optimizing therapy in pediatric asthma.

Clinical application of these genetic findings, as well as a better understanding of the modifying effects of environment on these genetic susceptibilities, may potentially lead to identification of new biologic pathways involved in the pathogenesis of the disease, the development of new therapeutic approaches, and the identification of at-risk individuals.

Endotypes in Childhood Asthma

In relation to asthma heterogeneity, the term ''endotype'' has recently been introduced to describe ''a subtype of a condition defined by a unique or distinctive functional or pathophysiologic mechanism.''[43,44] Therefore, the definition of inflammatory patterns of the disease is essential for endotyping asthma and better directing therapy.[8]

There are two major different endotypes defined for asthma: type 2 (T2)-high asthma and T2-low asthma, based on the type of underlying airway immune-mediated inflammation.[44,45] T2-high asthma is typically characterized by eosinophilic inflammation, initiated by ''alarmins,'' such as interleukin-25 (IL-25), IL-33, and thymic stromal lymphopoietin, all of which are secreted after a trigger in bronchial epithelium (ie, allergens, microbes, pollutants), and subsequently sustained by the release of signature cytokines IL-4, IL-5, and IL-13 from cells of both the innate and adaptive immune systems, including T helper 2 (Th2) cells, invariant T cells, natural killer (NK) cells, eosinophil/basophil progenitor cells, and type 2 innate lymphoid cells (ILC2s).[45,46] Type 2 cytokines actively recruit eosinophils, mast cells, and basophils in the airways, and directly mediate immunoglobulin E (IgE) synthesis, then contributing to the hallmarks of asthma pathophysiology, such as mucus production, subepithelial fibrosis, bronchial remodeling, and AHR.[47] T2-high asthma endotype typically displays a good response to corticosteroid therapy and, because probably there are more readily available biomarkers for its identification, has become the target of biological therapies (ie, anti-IgE, anti-IL-5).[35] Although less known and studied, T2-low asthma is driven by either a neutrophilic or, less commonly, paucigranulocytic inflammatory pattern, sustained by IL-8, IL-17A, IL-2,2, and other T cell-related cytokines, as well as epithelial cell-derived cytokines.[48,49] T2-low asthma endotype is considered rare but mainly seen in patients with severe disease and shows a typical corticosteroid insensitivity, such as corticosteroid resistance.[50]

In contrast with adults, in whom the cellular pattern of airway inflammation has been extensively studied with invasive and semi-invasive techniques [bronchial biopsies, bronchoalveolar lavage (BAL), and induced sputum], in children the majority of analyses were performed in the field of severe asthma.[32,51–53] Three major endotypes of airway inflammation have been described in children with severe asthma: eosinophilic, neutrophilic, and paucigranulocytic inflammation.[51–56]

The eosinophilic endotype is the most common in childhood; it clinically matches with the early-onset severe asthma, characterized by uncontrolled symptoms, more atopy, impaired lung function, increased AHR, increased number of exacerbations, and steroid responsiveness compared with the other phenotypes.[57] Unlike in adults, the levels of inflammatory cells in induced sputum have been reported to significantly vary over time in children with severe asthma and these variations were not related to changes in asthma therapy or asthma control.[58] However, a persistent airway eosinophilia has been also described in a small subset of children with severe asthma, even after high-dose systemic corticosteroids.[59] Bossley et al. recently characterized the pathology and mediators of inflammation and remodeling in a large cohort of 69 pediatric patients with severe therapy-resistant asthma (STRA), who underwent fiberoptic bronchoscopy, BAL, and endobronchial biopsy before a therapeutic trial with corticosteroids. Airway eosinophilia to varying degrees, without neutrophilia or increased mast cell counts, and initial features of remodeling were demonstrated in children with STRA; importantly, signature T2 cytokines were absent in

the majority of children, with the exception of a small subgroup, confirming the disease heterogeneity even in the pediatric population.[60] Furthermore, this eosinophilic airway inflammation was likely to persist despite systemic steroids in the majority of patients.[61,62]

In another study, Andersson et al. reported two subgroups within the pediatric STRA phenotype, which differ in the number of intraepithelial neutrophils detected in the bronchial biopsy; in the subgroup with increased intraepithelial airway neutrophilia, an exaggerated epithelial response to IL-17A was demonstrated, together with increased submucosal and epithelial expression of IL-17 receptor (IL-17R).[63] In the same group, an interesting novel finding was that the number of intraepithelial airway neutrophils correlated with better lung function, better symptom control, and lower dose maintenance inhaled steroids.[63]

Recently, ILC2s have been identified in BAL, induced sputum, and peripheral blood from children with STRA[64]; these preliminary findings suggest a potential pivotal role of ILC2s in the molecular mechanisms of pediatric allergic severe asthma.

As in adults, the functional role of airway neutrophils in mediating pediatric asthma pathophysiology is still unclear. Neutrophilic infiltration may be a feature of airway inflammation at all ages and may be mostly triggered by exposure to viruses and bacterial endotoxins in the pediatric age, subsequently leading to asthma symptoms.[65] Airway neutrophils have been assessed in a minority of children with severe asthma through induced sputum cytology.[53] More recently, an increased number of intraepithelial neutrophils, together with an increased submucosal and epithelial expression of IL-17R, have been determined in the lung biopsies of a subgroup of children with STRA; this finding was associated with better lung function, better symptom control, and lower dose maintenance inhaled corticosteroids (ICS[63]); even if these results may question for the first time the association of neutrophils and poor response with corticosteroids, they require further validation. Finally, other studies reported the coexistence of neutrophils with eosinophils in the airway tissue, contributing to highlight the complexity of defining inflammatory endotypes in children with asthma.[32,60]

Biomarkers in Childhood Asthma

A biomarker is a quantifiable biological indicator that provides an objective measure of health status or disease. The ideal biomarker should be ''measured in an analytical test system with established performance characteristics and should have a scientific body of evidence that elucidates the physiologic, pharmacologic, or clinical significance of the test results.''[66] Furthermore, a valid biomarker should have practical availability and reliability.[9]

Biomarkers for asthma can be measured in different biological specimens, including sputum, BAL, exhaled breath condensate (EBC), bronchial biopsy, urine, and blood.[67] Currently, BAL with bronchoscopy and bronchial biopsy are 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.[68,69] Sputum induction still has limited use outside the research setting, as it is considered semi-invasive as well as technically complex, especially in children younger than 8 years.[70] Thus, the availability of noninvasive methods to study and monitor disease inflammation is of main relevance especially in childhood asthma.

Biomarkers for asthma have potential utility for distinguishing the inflammatory endotype (T2-high versus T2-low asthma), predict responsiveness to specific treatments (in particular, T2 cytokine-targeted therapy), monitor success of a selected treatment option, and assess the risk of disease progression.[47,71] Single and combination biomarkers are now being recommended for use in the assessment of patients with asthma, and in particular with severe asthma (Table 1).

T2-high asthma biomarkers

Most of the current established biomarkers available in clinical practice are related to T2-high inflammation.[72,73]

Table 1. Current Biomarkers in Childhood Asthma

Biomarker	*Sample type*	*Associated asthma endotype*	*Proposed use*
Eosinophil	Serum, sputum	T2-high	Disease phenotyping Severity of clinical symptoms Monitoring of asthma control Prediction of treatment response
Neutrophil	Sputum	T2-high/T2-low	Disease phenotyping Under investigation
IgE	Serum	T2-high	Disease phenotyping Severity of clinical symptoms
Periostin	Serum	T2-high	Disease phenotyping Severity of clinical symptoms Diagnosis Prediction of treatment response
FeNO	Exhaled air	T2-high	Disease phenotyping Severity of clinical symptoms Monitoring of asthma control
IL-17	Serum	T2-low	Disease phenotyping
EBC	Exhaled air	Not yet determined	Under investigation
VOCs	Exhaled air	Not yet determined	Under investigation

EBC, exhaled breath condensate; FeNO, fractional exhaled nitric oxide; IgE, immunoglobulin E; IL, interleukin; T2, type 2; VOCs, volatile organic compounds.

These include blood or sputum eosinophils, serum IgE, serum periostin, and fractional exhaled nitric oxide (FeNO).[46]

Eosinophils. Eosinophil is the central driver of T2 inflammation and represents the predominant inflammatory cell type in the airways of children with severe asthma, playing a major role in maintaining chronic inflammation.[60,61,74–76] Although there is no standardized cutoff for eosinophilic inflammation, a blood eosinophil count of around 300 cells/μL or a sputum eosinophil cell count above 2%–3% of the total cell count has been used as thresholds.[77–79]

Several studies demonstrated that blood eosinophil count well correlates with asthma severity and AHR in children.[80–82] The presence of blood eosinophilia and high FeNO can be indicative of a good response to ICS, although less so for oral corticosteroid therapy.[83] In the Individualized Therapy for Asthma in Toddlers (INFANT) trial, blood eosinophil counts of 300 cells/μL or greater, together with aeroallergen sensitization, have been recently identified as predictors of best response to daily ICS in preschool asthmatic children requiring step 2 asthma treatment[84]; these results encourage further studies to better tailor treatments on preschool children with asthma, which present with numerous and variable phenotypic presentations that correspond to different outcomes and still suffer from significant therapeutic gaps.[85] Elevated eosinophil numbers in peripheral blood (>400 cells/μL) have been linked to a higher rate of severe asthma exacerbations.[86] Recently, the use of blood eosinophil count has been assessed as a sensitive and practical predictive biomarker for biologic treatment in patients with severe asthma. A decrease in blood eosinophil count has been associated with a consistent pattern of improved clinical outcomes in patients with severe asthma receiving omalizumab (anti-IgE)[87]; with particular reference to the pediatric population, Busse et al. reported a high eosinophil count (>300 cells/mL) to be a potential biomarker to predict successful omalizumab treatment effects.[88] Furthermore, blood eosinophils, in combination with FeNO, and periostin were shown to identify patient subgroups that may achieve greater benefit from omalizumab therapy.[89] Baseline blood eosinophil count threshold of 150 cells/μL or greater and/or a historical blood eosinophil count threshold of 300 cells/μL have been established as a biomarker to allow selection of adult patients with severe asthma who are most likely to benefit from mepolizumab (anti IL-5) therapy.[90,91] However, this result cannot be translated in the pediatric population and clinical studies to assess the efficacy, pharmacokinetics, and pharmacodynamics of mepolizumab in children with severe asthma are currently ongoing (NCT03292588 and NCT02377427).[92]

Peripheral blood eosinophilia is considered not as specific as sputum eosinophil, being potentially influenced by several confounding factors, such as allergen exposure, parasitic infections, and current corticosteroid therapy. However, the results of an external validation in two independent cohorts of patients with mild to moderate asthma showed that blood eosinophil had the highest accuracy in the identification of sputum eosinophilia, compared with FeNO and serum periostin.[93] Nevertheless, peripheral blood eosinophilia does not always reflect pulmonary (airway or mucosal) eosinophilia in children with severe asthma.[94]

When feasible, sputum analysis can provide further information about the airway cellular composition and cytokines. Increased eosinophil number in sputum is a hallmark feature of atopy and asthma,[95,96] is associated with AHR and airway obstruction,[97,98] inversely correlates with forced expiratory volume in 1 second (FEV_1),[99] and acts as a predictor of severe asthma exacerbations,[100–102] both in adults and children.

Sputum eosinophilia may also predict clinical response to corticosteroid therapy (both ICS and systemic treatment) and to biologic therapy.[78,103,104] In a recent Cochrane revision, sputum analysis for the evaluation of percentage of sputum eosinophilia is considered beneficial in objectively monitoring asthma and guiding tailored therapeutic interventions to maintain control and reduce exacerbations in adults with asthma, while insufficient data are currently available for children.[58,105,106]

Total and allergen-specific IgE. Total IgE and especially allergen-specific IgE are the signatures of atopic status, associated with asthma. More than 80% of children with asthma show an allergic component; high levels of total IgE increase the risk of later asthma development in infants with viral-induced wheezing,[107] while high levels of allergen-specific IgE (in particular for aeroallergens, such as house dust mites or furry animals) well correlate with asthma severity, mainly in children.[108] Consequently, allergy screening should be routinely performed in patients with asthma, and total IgE should be checked in every child diagnosed with severe asthma to eventually address an add-on therapy with omalizumab.[109,110]

Periostin. Periostin is a secreted extracellular matrix protein that was originally identified in cells of the periosteum and involved in bone growth and repair.[111] In addition, periostin is also an IL-4- and IL-13-inducible protein that is secreted by airway epithelial cells and lung fibroblasts and can be detected in peripheral blood, as well as in sputum and EBC.[112] In this context, periostin plays a role as a mediator of several pathogenic processes in asthma, such as airway remodeling, subepithelial fibrosis, eosinophil recruitment, and regulation of mucus production from goblet cells.[113–115] Several reports have suggested that serum periostin could be a useful biomarker of T2-high inflammation in adult asthmatic patients, since it has been demonstrated in overexpression in epithelial cells, upregulation by classic T2 cytokines such as IL-4 and IL-13, and ability to predict a clinical response to lebrikizumab (anti IL-13) and omalizumab treatment in severe asthmatics.[88,116] However, periostin levels are known to be higher in children than in adults, most likely due to bone growth, and may overlap with local production within the airways, thus impairing the clinical utility in children; periostin levels may be also elevated in other concomitant diseases, such as rhinosinusitis with or without polyposis and atopic dermatitis.[117] With these limitations, pediatric studies on periostin showed significantly higher values in children with asthma compared with healthy controls, a correlation between levels of serum periostin and induced-AHR, and a moderate relationship with blood eosinophilia and IgE in asthmatic children.[118,119] It still remains unclear if periostin has a predictive value for identifying severe asthma in children.

Fractional exhaled nitric oxide. Nitric oxide (NO) is a signaling molecule produced by respiratory epithelial cells, is found in exhaled breath, functions as a vasodilator and bronchodilator in the lungs, and is synthesized from L-arginine by inducible NO synthase enzymes in response to inflammatory cytokines.[120] In particular, in asthmatic patients, allergen exposure results in IL-4 and IL-13 expression, which, acting through signal transducer and activator of transcription 6, induces iNOS, resulting in significant

increases in NO levels.[121] FeNO measured in exhaled breath is one of most studied noninvasive biomarkers in recent years. Its measurement is simple, safe, and well tolerated, and it has been standardized in school-aged children.[120,122] FeNO levels may be influenced by several factors, including smoking, diet, obesity, somatic variables, spirometry or exercise before testing, flow rate, nasal contamination, and ambient air.[123]

FeNO level may be useful as a predictive factor for new-onset asthma in preschool children[124] and has been shown to correlate with AHR, blood eosinophils, and serum IgE levels in children.[125,126] 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 who are likely to respond to ICS treatment.[122] Multiple studies have demonstrated that an increased FeNO value at baseline or increasing FeNO values during ICS reduction accurately predict an asthma exacerbation.[121] According to the cutoff values published in the American Thoracic Society (ATS) guidelines, an FeNO of >35 ppb suggests a likely response to ICS, while an FeNO of <20 ppb in children indicates a less likely responsiveness to ICS treatment.[122] Although it has been suggested as a useful tool to guide treatment in childhood asthma, many studies show contradictory results in terms of its utility, mainly due to the differences in the design of the trials and in the selection of patients.[127] The efficacy of tailoring asthma interventions based on FeNO has been evaluated in comparison with primary guideline management in asthmatic children in a recent Cochrane systematic review: the analysis of nine pediatric studies shows that the use of FeNO to guide asthma therapy significantly decreased the number of children who had one or more exacerbations over the study period but did not impact on the day-to-day clinical symptoms or ICS doses, so that its use cannot be recommended in routine clinical practice to tailor the dose of ICS for all children with asthma.[128]

Combination of biomarkers. Combination of these biomarkers has been evaluated to improve the identification of T2-high inflammation in asthma and to predict response to therapy. Prior studies in older children have shown the association of markers of allergic inflammation, such as IgE levels (>200 kU/L), exhaled NO (values >25 ppb), and eosinophilic cationic protein with response to ICS in older asthmatic children.[129] In a cross-sectional study by Konradsen et al., FeNO, in combination with blood eosinophils, had a high predictive value for the identification of children with the highest asthma morbidity, while there was no association between asthma morbidity and serum levels of periostin.[130] In another recent Swedish study, simultaneous increase of both FeNO and blood eosinophil count was correlated with a higher likelihood of AHR and uncontrolled asthma in a large cohort of young asthmatic patients.[131] The combination of blood eosinophils and FeNO has been also investigated in the setting of pediatric severe asthma of Severe Asthma Research Program (SARP) study, showing increased values only in 30%–40% of subjects.[24]

Exhaled breath condensate. The EBC collection is a novel noninvasive technique that can be used to investigate several asthma biomarkers, including markers of oxidative stress (8-isoprostane, hydrogen peroxide, aldehydes, and nitrite/nitrate), markers of inflammation (eicosanoids), pH, temperature, microRNA profiles, and other cytokines.[69] EBC analyzes microdroplets collected after cooling exhaled air and can be easily performed even in younger children with severe disease.[69] Elevated levels of hydrogen peroxide, nitrites and nitrates, and leukotrienes B4 have been demonstrated in the EBC of children with asthma[132,133] and higher levels of 8-isoprostane and leukotrienes were both detected in children with severe asthma.[134,135] EBC technology is actually dedicated to research studies in specialized centers and requires further standardization of the methodologies used for sample collection and analysis, before moving it to clinical practice.

Volatile organic compounds. Assessing volatile organic compounds (VOCs) in exhaled breath is another promising metabolomics approach for investigating airway inflammation in asthma.[69] VOC analysis captures gaseous molecules (ie, hydrocarbons such as ethanol, acetone, isoprene, benzene, and many others) from exhaled air, originating from three main sources: the external environment, and endogenous metabolic processes both human and nonhuman (the microbiome).[136] Considered a ''molecular fingerprint'' of breath, a combination of VOCs may represent a safe, noninvasive, and easy-to-sample tool for diagnosing and monitoring pediatric pulmonary diseases such as asthma. Previous studies performed in adults with asthma suggested good predictive accuracy of exhaled VOCs for asthma diagnosis and that several compounds, mainly alkanes, may be significantly associated with asthma inflammation.[137,138]

Although still limited to research settings, preliminary studies have proved the reliability of VOC assessment in distinguishing atopic and asthmatic from healthy children and in predicting exacerbations in asthmatic children.[139,140] A recent revision of pediatric literature on VOC analysis in exhaled breath performed on wheezing or asthmatic children confirmed a moderate to good prediction accuracy (80%–100%, with a combination of VOCs) in pediatric asthma diagnosis.[136] However, there are still various constraints associated with standardization of the different breath analysis techniques and further prospective cohort studies are needed to validate and introduce exhaled VOC profiling in a clinical scenario.

Other biomarkers. Novel biomarkers that may be associated with inflammation, but especially with remodeling processes, are emerging, since recent studies from pediatric severe asthma cohort studies showed that the pathophysiological abnormalities of asthma, inflammation, AHR, and remodeling may develop in parallel.[141] However, a current limitation to the path of recognizing mechanisms of remodeling and related phenotypes is the relative difficulty in obtaining repeated invasive biopsies to assess longitudinal structural changes over time, especially in the pediatric population. Recently, the high mobility group box type 1 (HMGB1) protein has been proposed as a blood biomarker potentially able to elucidate one of the mechanisms of chronic airway dysfunction in asthma.[142] HMGB1 is an inflammation marker of the alarmins family promoting immediate immune response to tissue damage,[142] and is one of the most important damage-associated molecular pattern molecules, initiating and perpetuating immune responses in infectious and noninfectious inflammatory diseases.[143] Its role is to act as a ''danger signal'' orchestrating homeostatic defensive responses in damaged tissues.[142] Major structural features of HMGB1, a 30 kDa nuclear and cytosolic ubiquitous protein, are its two DNA-binding domains, termed A and B box, and a negatively charged C-terminal acidic

region. HMGB1 contains two nuclear localization sequences, resides in the nucleus, and functions as a nonhistone chromatin-binding protein.[144] Early work demonstrated that HMGB1 stabilizes chromatin structure and modulates gene transcription by bending the DNA helical structure.[145] HMGB1 can also be localized to the cytosolic compartment, implicating that it might also have important functions outside the nucleus.[144] As a consequence of infection or apoptosis, HMGB1 is released in the extracellular compartment either by passive release from necrotic cells or active production by macrophages, dendritic cells, and NK cells.[146] By binding to toll-like receptors (TLR) 2 and 4, and the receptor for advanced glycation end-products,[147] HMGB1 upregulates the synthesis of inflammatory cytokines, elicits chemotaxis of inflammatory cells, and supports proliferation, chemotaxis, and synthesis of metalloproteinases by stromal fibroblasts,[148] thereby contributing to the pathogenesis of both acute and chronic disorders.[149] As for the potential pathogenic role of HMGB1 in the respiratory tract, a recent study has shown increased levels of the protein in children with stable, off-therapy, allergic asthma.[150] Particularly, authors investigated the relationship between HMGB1 levels and lung function parameters, showing that sputum HMGB1 levels were higher in children with asthma than in healthy controls and, moreover, sputum HMGB1 levels also positively related to the serum total IgE levels in children with asthma. Finally, an inverse and strict correlation between sputum HMGB1 levels and pulmonary function indices was also observed in children with mild, moderate, and severe asthma.[150]

Existing data on further possible biomarkers of Th2-mediated asthma need to be validated and their usefulness for clinical practice remains to be elucidated, in particular in the pediatric population.

T2-low asthma biomarkers

To date, biomarkers of T2-low asthma have not yet been established, at least in clinical practice.[35] Unlike the eosinophilic counterpart, sputum neutrophils do not represent an established marker to define the T2-low asthma endotype, widely varying the different cutoff values used in the literature from as low as 40% to as high as ≥76%.[50] Moreover, blood neutrophils have limited accuracy and ability to predict sputum neutrophils across the spectrum of asthma severity.[151] Serum progranulin, an epithelial-derived protein known to inhibit neutrophil degranulation, has been recently proposed as novel biomarker of neutrophilic inflammation in severe asthma patients with airflow limitation,[152] but the exact mechanism of its anti-inflammatory action still remains unclear.

IL-17 has been found to play an important role in the pathogenesis of T2-low asthma, both in adults and children.[50,62] It has been reported that IL-17 and its related cytokines are highly upregulated in bronchial and nasal mucosa of adult subjects with neutrophilic asthma prone to exacerbations.[153] High levels of serum IL-17 have been also detected in children with asthma and, together with serum IgE and blood eosinophils, they could have a predictive value in diagnosing childhood asthma.[154] Furthermore, both serum IL-17 and IL-17$^+$ T cells have been associated with asthma severity in children.[155,156]

Among the many biomarkers investigated in the airways and blood of T2-low asthmatics, some of them are giving promising results for their future use in clinical practice. Human tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a protein expressed in various cell types, including inflammatory cells, such as monocytes, macrophages, dendritic cells, T cells, and NK cells; acting through its highly inducible receptor, named fibroblast growth factor-inducible 14 (Fn14), TWEAK may contribute to the development of airway inflammation and, in particular, potentially stimulate human bronchial epithelial cells to produce proinflammatory IL-8 and granulocyte-macrophage colony-stimulating factor.[157] Kim et al. evaluated the airway TWEAK levels in a large population of 230 children with noneosinophilic asthma: sputum TWEAK levels were significantly elevated in children with asthma and higher in

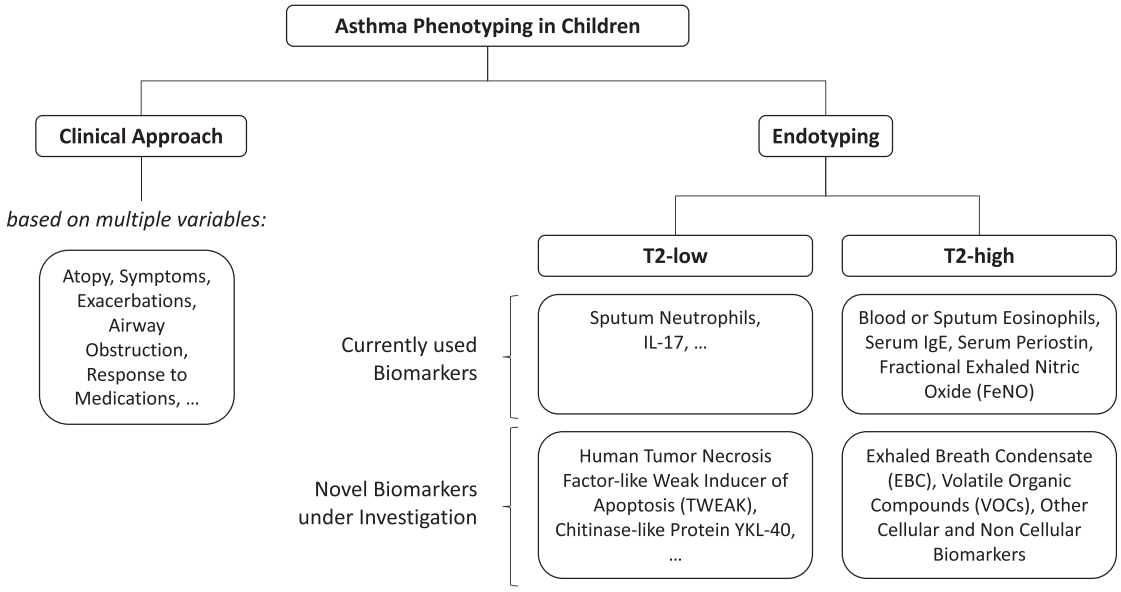

FIG. 1. Novel approach to phenotype and endotype childhood asthma.

children with greater asthma severity and poorer control status; moreover, the authors found a negative correlation between sputum TWEAK levels and the spirometric parameters of bronchial obstruction, supporting the possible association of TWEAK with airway obstruction and remodeling.[158] Thus, the TWEAK/Fn14 axis may also represent a future therapeutic target for limiting airway remodeling in asthma. YKL-40 is a chitinase-like protein that has a role in the inflammation and tissue remodeling in several human diseases. In adults, YKL-40 levels are increased in the blood and lungs of patients with asthma and correlate with lung function deficits, disease severity, and persistence.[159] Besides, circulating levels of YKL-40 are also elevated in children with persistent and severe asthma.[160–162] Although YKL-40 represents a feasible biomarker for T2-low asthma, the exact mechanisms linking YKL-40 with asthma remain to be determined.

Conclusions

Childhood asthma represents a heterogeneous challenging disease, in particular in its severe forms. Clinical and morphologic characteristics of asthma phenotypes, as well as unique responses to different treatments, do not necessarily provide insights into the underlying pathophysiologic processes of airway inflammation. A careful assessment of inflammatory endotypes should be considered a central component of the workup and management of severe asthma in children. A limited number of biomarkers are currently available in clinical practice in the pediatric population, including blood and sputum eosinophils, serum IgE, periostin, and FeNO, mostly reflecting different molecular components of type 2-high airway inflammation (Fig. 1). Individually or in combination, they may help to improve diagnosis and predict severity of the disease and response to both conventional and novel biological targeted therapies. The type-2 low inflammatory endotype is still poorly characterized in particular in the pediatric population.

Current research efforts are aimed to integrate clinical characteristics and available combination of biomarkers to characterize asthma endotypes. Further research in childhood asthma biomarkers is needed to improve endotyping asthma, predicting major clinical outcomes and ultimately leading to personalized therapies.

Author Disclosure Statement

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