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Key points

- Severe asthma in children is a highly heterogeneous disorder, encompassing different clinical characteristics (phenotypes) and immunopathological pathways (endotypes).
- Research is focusing on the identification of noninvasive biomarkers able to predict treatment response and assist in designing personalised therapies for severe asthma.
- Blood and sputum eosinophils, serum IgE and exhaled nitric oxide fraction mostly reflect type 2 airway inflammation in children. However, knowledge regarding non-type 2 inflammation and related biomarkers is still lacking.

Educational aims

- To summarise the most recent evidence on biomarkers for severe asthma in children.
- To discuss their implementation in clinical practice through guiding patient identification and treatment decisions.

Review

Measuring inflammation in paediatric severe asthma: biomarkers in clinical practice

Severe asthma in children is a highly heterogeneous disorder, encompassing different clinical characteristics (phenotypes) and immunopathological pathways (endotypes). Research is focusing on the identification of noninvasive biomarkers able to predict treatment response and assist in designing personalised therapies for severe asthma. Blood and sputum eosinophils, serum IgE and exhaled nitric oxide fraction mostly reflect type 2 airway inflammation in children. However, in the absence of available point-of-care biomarkers, the diagnosis of non-type 2 asthma is still reached by exclusion. In this review, we present the most recent evidence on biomarkers for severe asthma and discuss their implementation in clinical practice. We address the methods for guiding treatment decisions and patient identification, focusing on the paediatric age group.

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Introduction

Asthma is one of the most common chronic diseases in childhood, with increasing incidence and prevalence [1]. Despite the recent advances in diagnostic and therapeutic strategies, asthma still poses a substantial health and socioeconomic burden, in particular in its uncontrolled and severe forms [2–5]. Severe asthma in children is characterised by sustained symptoms despite treatment with high doses of inhaled corticosteroids (ICS) or oral corticosteroids [6], and represents approximately 2–5% of childhood asthma cases [1]. Severe asthma is a heterogeneous disease encompassing different phenotypes, which can be defined in terms of both clinical and molecular characteristics [7]. Asthma phenotypes depend on age and may differ in triggers, clinical presentation, associated comorbidities, natural history and

response to treatment [1, 8–11]. The clinical diversity reflects different inflammatory pathways: type 2 and non-type 2 endotypes, which differ according to the presence or absence of eosinophilic airway inflammation, respectively (table 1) [7, 12]. The understanding of different endotypes of severe asthma has now led to the development of targeted immunomodulators and other biological therapies to more effectively treat the diverse patients encountered in clinical practice [13, 14].

In recent years, research has focused on the identification of biomarkers able to differentiate distinct endotypes and accurately predict response to treatment in patients with severe asthma. In this review, we present the most recent evidence on biomarkers for severe asthma and discuss their implementation in clinical practice in terms of guiding patient identification and treatment decisions, focusing on the paediatric age group.

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Noninvasive biomarkers should be integrated with clinical findings to assist in diagnosing and guiding personalised therapies for severe asthma in children <http://bit.ly/2JPvKFV>



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Table 1 Major endotypes of severe asthma in children

	Triggers	Major cellular drivers	Major molecular drivers	Related clinical features
Type 2 asthma				
Allergic eosinophilic	Allergens	Airway epithelial cells, Th2 lymphocytes, eosinophils	IL-25, IL-33, TSLP, IL-4, IL-5, IL-13, IgE	More common in children Good response to corticosteroids Reversible airway obstruction Associated with upper airway comorbidities
Nonallergic eosinophilic	Pollutants, microbes, glycolipids	Airway epithelial cells, ILC2s, eosinophils	IL-25, IL-33, TSLP, PGD ₂	Less common in children (late-onset) Significant AHR Relatively insensitive to corticosteroids No increased atopy
Non-type 2 asthma				
Paucigranulocytic	Environmental factors (cigarette smoke, allergens, contractile agonists)	ASM dysfunction; no cellular inflammation	High level of oxidative stress (mechanisms not known)	High AHR Insensitivity to corticosteroids
Neutrophilic	Infections	Th17 lymphocytes, neutrophils	IL-17, IL-21, IL-22	Bacterial airway colonisation Poor response to corticosteroids Severe airway obstruction

Th: T-helper cell; IL: interleukin; TSLP: thymic stromal lymphopoietin; IgE: immunoglobulin E; ILC2s: innate lymphoid cells type 2; PGD₂: prostaglandin D₂; AHR: airway hyperresponsiveness; ASM: airway smooth muscle cells.

Biomarkers in asthma: definition and sampling methods

Biomarkers are defined as quantitative measurements that allow clinicians to diagnose, assess the disease stage, predict clinical outcomes and monitor the treatment effects [15]. Biomarkers for asthma can be measured in different biological specimens, including exhaled breath, sputum, bronchoalveolar lavage (BAL), bronchial biopsy, peripheral blood and urine [7]. Each biomarker is specific to a particular sampling site, so different biomarkers do not provide comparable information on the pathways underlying inflammation in severe asthma. The strengths and limitations of each biomarker are discussed in table 2.

Sputum induction and BAL are used to detect airway inflammation at different sites of the bronchial tree. Conversely to biomarkers outside the respiratory tract, these tests provide a differential count of local inflammatory cells, including eosinophils, neutrophils, macrophages, lymphocytes and epithelial cells, with a reproducibility and validity that has been widely demonstrated [16]. While bronchoscopies with BAL are restricted to specialised centres due to the technical complexity, invasiveness and potential complications, sputum analysis offers a less invasive alternative for assessing

airway inflammation. However, it requires complex protocols, especially in patients with severe asthma and/or concomitant disorders. Measurement of biomarkers in exhaled breath (*i.e.* exhaled nitric oxide fraction (F_{ENO})) and exhaled breath condensate (EBC) (*i.e.* volatile organic compounds (VOCs)) are noninvasive and safe in children, given the necessity of repeatable measurements to monitor treatment efficacy and asthma progression [7]. Even these procedures are not bias-free, since several factors may hamper the interpretation of their results: factors relating to the collection procedure, as well as constitutive elements relating to the subject, may influence exhaled biomarker levels. Alternatively, peripheral blood may be easily obtained even in younger children, and blood eosinophils and neutrophils represent the most studied inflammatory cells linked to molecular pathways of asthma [7]. However, they have some limitations: blood eosinophilia is not as specific as sputum eosinophils, and confounding factors, including parasitic infections, allergen exposure and corticosteroid therapy, potentially affect blood eosinophil count; also, peripheral blood neutrophilia is considered not as accurate as sputum neutrophils to predict asthma severity [7]. Finally, novel sampling methods are exploring biomarkers obtained from saliva and nasal swabs, to increase the availability of noninvasive techniques in children [7].

Table 2 Summary of available biomarkers of childhood severe asthma

Biomarker	Sampling method	Strengths	Limitations
Type 2 asthma endotype			
Eosinophils	Bronchoscopy Bronchial biopsy BAL Induced sputum	Gold standard to measure airway inflammation Feasible in advanced clinical settings Sampling of central airway inflammation Cut-off of $\geq 3\%$ to indicate sputum eosinophilia May indicate steroid responsiveness	Invasive and not feasible in routine clinical practice Contraindicated in cases of severe airway obstruction No consensus on clear cut-off values Semi-invasive Time-consuming Requires specialised laboratory facilities and personnel Contraindicated in cases of severe airway obstruction
IgE	Peripheral blood Peripheral blood	Easily obtained, even in younger children Correlation with sputum eosinophil counts (except during systemic corticosteroid treatment) Cut-off of ~ 300 cells- μL^{-1} to indicate eosinophilic inflammation Easily obtained, even in younger children	Daily variations in number Affected by secondary causes of eosinophilia (<i>i.e.</i> parasitosis, common in children) No demonstrated correlation with airway eosinophilia Affected by secondary causes (<i>i.e.</i> parasitosis, common in children)
Periostin	Peripheral blood	Easily obtained, even in younger children	Baseline levels are higher in children, probably due to growth Conflicting results in children Not available in most laboratories May be affected by several factors
F_{ENO}	Exhaled breath	Noninvasive Fast measurement Repeatable and reproducible	No standardised methods for collection and analysis
VOCs	Exhaled breath	Noninvasive	Requires specialised laboratories Expensive To be validated in children with severe asthma
pH _i , markers of oxidative stress, leukotrienes, cytokines and chemokines	Exhaled breath condensate	Noninvasive	
Non-type 2 asthma endotype			
Neutrophils	Bronchoscopy Bronchial biopsy BAL Induced sputum	Gold standard to measure airway inflammation Correlation with better lung function in younger children with severe asthma Cut-off of $\geq 61\%$ to indicate sputum neutrophilia	Invasive and not feasible in routine clinical practice Contraindicated in cases of severe airway obstruction No consensus on clear cut-off values Semi-invasive Time-consuming Requires specialised laboratory facilities and personnel Contraindicated in cases of severe airway obstruction
	Peripheral blood	Easily obtained, even in younger children	No correlation with sputum or airway neutrophil counts

F_{ENO} : exhaled nitric oxide fraction; VOCs: volatile organic compounds.

Biomarkers of the type 2 asthma endotype

Most of the current established biomarkers available in clinical practice are related to type 2 asthma, since it is the best-characterised endotype in patients with asthma. The common biomarkers used in clinical practice are eosinophils, serum immunoglobulin (Ig)E, serum periostin and F_{ENO} [7].

Eosinophils

Eosinophils are central drivers of type 2 asthma, both in allergic and nonallergic forms, and represent the predominant inflammatory cell types in the airways of children with severe asthma [17]. Upon activation, eosinophils contribute to airway damage and chronic inflammation and promote remodelling, subepithelial membrane thickening and goblet cell metaplasia [17].

So far, sputum eosinophilia is the best-characterised and most useful biomarker. The normal range for sputum eosinophilia is defined as 1–2%, with female sex and atopic status associated with higher sputum eosinophil levels [18]. Some recent longitudinal studies have reported controversial results on temporal variability in sputum eosinophil counts in adult patients with severe asthma: while some authors have shown that 70% of patients with eosinophilic asthma (sputum eosinophils $\geq 2\%$) retained their sputum phenotype over 5 years [19], others revealed that 60% of the patients changed their phenotype from eosinophilic (sputum eosinophils $\geq 2\%$) to non-eosinophilic and *vice versa* [20]. A similar variability in sputum inflammatory cells over time was observed in 61% of children (including those with severe asthma) followed over 1 year in a longitudinal study by FLEMING *et al.* [21]. Specifically, they classified sputum samples into eosinophilic ($>2.5\%$ eosinophils), neutrophilic ($>54\%$ neutrophils), mixed granulocytic ($>2.5\%$ eosinophils, $>54\%$ neutrophils) or paucigranulocytic ($\leq 2.5\%$ eosinophils, $\leq 54\%$ neutrophils). Raised levels of inflammatory cells (up to 78%) were reported in at least one sputum sample of these children, regardless of asthma severity; also, the lack of temporal phenotype stability was not related to changes in asthma control or ICS therapy [21].

Sputum eosinophil count is increased in symptomatic patients with asthma, in patients with asthma during exacerbations, and in corticosteroid-naïve patients [22–24]. Increased eosinophil number in sputum has also been associated with airway hyperresponsiveness (AHR) and obstruction, and inversely correlated with forced expiratory volume in 1 s (FEV_1) [25].

In asthma, corticosteroids are effective at suppressing eosinophilic inflammation [26].

In general, sputum eosinophilia is associated with steroid responsiveness [26]; however, the persistence of elevated sputum eosinophilia may suggest either non-adherence or inadequate corticosteroid dosing [21]. In such cases, titrating ICS doses based on sputum eosinophilia may reduce the likelihood of asthma exacerbations [25]. Adjusting treatment based on sputum eosinophilia has been performed with various sputum eosinophil cut-offs, ranging from 2% to 8%; however, the most commonly used cut-off is sputum eosinophilia $\geq 3\%$ [18]. Unfortunately, there are insufficient data available to extend the clinical application of induced sputum eosinophilia in tailoring asthma medication in children [23].

The clinical utility of peripheral blood eosinophilia in the assessment of children with severe asthma has long been explored. Although there is no standardised cut-off, a blood eosinophil count of 300 cells μL^{-1} has commonly been used as a threshold to indicate eosinophilic inflammation [27].

Despite being influenced by some confounding factors (allergen exposure, parasitic infections and systemic corticosteroid treatment), blood eosinophils have shown the highest accuracy in the identification of sputum eosinophilia, compared with other biomarkers such as F_{ENO} and serum periostin [28]. Nevertheless, peripheral blood eosinophilia does not always reflect pulmonary (airway or mucosal) eosinophilia in children with severe asthma [29].

The correlation between blood eosinophil count, asthma severity and AHR has been demonstrated in several studies conducted in children [30, 31]. Analysing the 12-month longitudinal dataset of a cohort of children and adolescents with severe asthma exacerbations, SHAH *et al.* [32] recently found that elevated blood eosinophils were associated with acute asthma exacerbations and hospitalisations, but optimal cut-off points for blood eosinophils differed by age (≥ 150 versus ≥ 300 cells μL^{-1} for children versus adolescents, respectively). However, the addition of a second biomarker of type 2 inflammation, such as serum IgE and/or F_{ENO} , was useful in predicting asthma exacerbations only in adolescents [32].

In the Individualized Therapy for Asthma in Toddlers (INFANT) trial, the presence of blood eosinophilia (≥ 300 cells μL^{-1}), in combination with aeroallergen sensitisation, has been identified as a significant predictor of response to ICS in children with asthma [33]. Thus, blood eosinophil count could be useful to identify patients for ICS prescription and patients at high risk of exacerbations. Instead, higher eosinophil count has been assessed to be a sensitive and practical predictive biomarker for biological therapies targeting allergic and/or eosinophilic pathways in patients with severe asthma [7]. A high blood eosinophil count (>300 cells μL^{-1}) has been reported

as a potential biomarker to predict successful omalizumab treatment effects in children with severe allergic asthma [34]. In combination with F_{ENO} and periostin, blood eosinophils were shown to identify patient subgroups that may achieve more significant benefit from omalizumab therapy [34]. Blood eosinophil count has also evolved as a predictive biomarker for the efficacy of treatment with anti-interleukin (IL)-5 (mainly mepolizumab) in patients with severe eosinophilic asthma [35]; in particular, a baseline blood eosinophil threshold of ≥ 150 cells· μL^{-1} or a historical blood eosinophil threshold of ≥ 300 cells· μL^{-1} allow selection of patients with severe eosinophilic asthma who are most likely to achieve clinically significant reductions in the rate of exacerbations with mepolizumab treatment [36].

Sputum eosinophilia also adequately predicts response to biological drugs. Patients with refractory asthma are more likely to respond to anti-IL-5 or anti-IL-4/IL-13 treatment if they have sputum eosinophils of $>3\%$. A significant decrease in exacerbation rates, an improved quality of life and corticosteroid-sparing effects have been observed in adults treated with mepolizumab [35]. Hence, the newest Global Initiative for Asthma (GINA) document suggests assessment of sputum eosinophil count in adults with moderate or severe asthma who are managed in experienced centres [37]. No similar recommendations are available for children.

Serum IgE

IgE plays a critical pathophysiological role in allergy and asthma, influencing the functioning of several immune and structural cells of the bronchial wall [38–40]. By binding to its high- and low-affinity receptors, IgE exerts several biological functions: degranulation and mediator release by mast cells/basophils; promotion of mast cell maturation, survival and cytokine production; increase in allergen presentation by dendritic cells and B-cells; activation of eosinophils and T-helper type 2 (Th2) cells; activation, proliferation and cytokine release by smooth muscle cells; inhibition of type I interferon production by dendritic cells; and other indirect effects on innate immune cells. As a result, IgE is primarily responsible for both the acute and chronic phases of inflammation characteristic of allergic asthma [41].

Total IgE and allergen-specific IgE have been strongly associated with asthma in $>80\%$ of children [42]. In infants with viral-induced wheezing, a raised level of total IgE is considered a risk factor for asthma development [43], while high levels of allergen-specific IgE correlate well with asthma severity, mainly in children [42, 44]. Consequently, IgE measurements should be routinely performed in patients with asthma, and total IgE should be checked in every child diagnosed with severe

asthma, for the consideration of add-on therapies such as omalizumab [42].

Periostin

Periostin is an extracellular matrix protein secreted by airway epithelial cells, endothelial and smooth muscle cells, and lung fibroblasts, in response to IL-4 and IL-13. Following its release, periostin acts on keratinocytes, promoting the release of pro-inflammatory cytokines such as thymic stromal lymphopoietin (TSLP) and IL-24 and regulating epithelial–mesenchymal interactions. In this context, periostin actively participates in airway remodelling, eosinophil recruitment and mucus production from goblet cells [45, 46]. Thus, it has been associated with the type 2 asthma endotype.

Serum periostin levels ≥ 10 ng·mL $^{-1}$ identify an inflammatory status. However, age-dependent changes in periostin values commonly occur, probably due to bone turnover and growth, especially in children. Moreover, the presence of comorbidities, including rhinitis, rhinosinusitis with or without nasal polyposis, and atopic dermatitis, can affect its baseline values [47]. High levels of periostin have also been found in sputum or EBC, reflecting severe airway inflammation [47].

A meta-analysis reported high serum periostin levels in patients with asthma compared with healthy controls, despite a wide heterogeneity in periostin values among patients with asthma [47]. Particularly high serum periostin levels were found in those with corticosteroid-resistant asthma [48]. There appears to be a moderate or weak correlation between periostin and eosinophil count, F_{ENO} and serum total IgE [47]. Clinically, serum periostin levels have been inversely correlated with predicted FEV $_1$, probably reflecting the periostin-mediated fibrogenic effects [47]. In adults with severe asthma, periostin seems to be able to predict clinical response to monoclonal antibody therapies, including omalizumab and lebrikizumab [47].

In contrast to adult asthma, the usefulness of periostin in childhood asthma is still debated and controversial. Some studies on periostin have shown significantly higher values in children with asthma compared with healthy controls, a significant correlation between higher levels of serum periostin and induced AHR, and a moderate relationship between blood eosinophils and IgE in children with asthma [49, 50]. In contrast, KONRADSEN *et al.* [51] and INOUE *et al.* [52] did not detect correlation of serum periostin with blood eosinophils or F_{ENO} in childhood asthma patients. In a real-world setting, we recently demonstrated that serum periostin does not reflect asthma control grade in children with asthma [53]. It remains unclear if periostin has a predictive value for identifying severe asthma in children. Finally, serum periostin measurements cannot be readily assessed in most clinical laboratories.

Exhaled biomarkers

Exhaled nitric oxide fraction

As result of airway epithelium inflammation, inducible nitric oxide synthase increases the production of nitric oxide, which can be measured in exhaled breath and thus provide information about the inflammatory state of the airways [54, 55].

Measuring F_{ENO} has the advantages of being a noninvasive technique and being easy to perform with a minimal patient effort. However, several confounding factors, such as age, height, type of analyser, ethnicity, smoking, atopy, diet and corticosteroid treatment, may affect its reliability [54, 56]. F_{ENO} is a widely accepted biomarker for type-2-driven airway inflammation, displaying a specificity of 70% in asthma diagnosis [54]. In children, a F_{ENO} value <20 ppb is considered as normal, while levels >35 ppb are indicative of eosinophilic inflammation and associated with AHR, asthma symptoms, decline of lung function, asthma attacks and asthma-related emergency department visits [54]. In preschool children, it also correlates significantly with blood eosinophils and serum IgE levels. F_{ENO} values between 20 and 35 ppb should be interpreted cautiously and with reference to the clinical context [54].

Although F_{ENO} -guided management does not appear superior to clinically based management, elevated F_{ENO} levels predict response to ICS [57], mepolizumab [35] or benralizumab [58], while a F_{ENO} level ≥ 19.5 ppb is correlated with a response to omalizumab treatment [59]. A reduction in the F_{ENO} level during treatment with dupilumab is associated with an improvement in lung function [60].

Although the current guidelines do not recommend F_{ENO} in the routine management of children with severe asthma, it could be helpful in the stratification of patients in accordance with eosinophilic inflammation, and as a possible guide for a targeted therapeutic approach [6, 35].

Exhaled breath condensate

By saturating exhaled breath with water vapour and collecting the condensed material, EBC offers a noninvasive method of sample collection for volatile and nonvolatile compounds, such as nitric oxide products, hydrogen peroxide, leukotrienes and cytokines, as well as for investigating microRNA profiles and measuring pH and temperature [61].

Concentrations of exhaled hydrogen ions, nitric oxide products, hydrogen peroxide, leukotriene B_4 and 8-isoprostanes are increased in both adults and children with severe asthma; furthermore, they have been related to decreased lung function [62, 63]. Recent studies have shown that EBC pH ≤ 7.20 is indicative of poorly controlled asthma, although the performance of this biomarker is inferior to clinical evaluation of asthma control [62, 64].

The lack of a standardised collection technique or established cut-off values, the absence of a

correlation between EBC values and samples obtained by BAL, and the expensive costs of assays are the main disadvantages of EBC [65]. The EBC method is currently used only in research studies, and it requires further validation before being implemented in clinical practice [65].

Volatile organic compounds

The chronic inflammation processes and the oxidative stress generally associated with asthma result in reactive oxygen species synthesis. These reactive oxygen species promote the degradation of polyunsaturated fatty acids present in the bronchial epithelium cell membrane and induce the formation of VOCs. The latter enter the bloodstream and, subsequently, are excreted in the exhaled breath [66].

Gas chromatography and the electronic nose (e-Nose) are two different techniques able to measure exhaled VOCs [66]. The e-Nose is an artificial sensor system that provides a qualitative and/or quantitative analysis of VOCs. Based on chemical sensors, the e-Nose binds volatile substances, then generates and analyses specific pattern signals for detection of VOC mixtures [67].

VOCs may represent a safe, noninvasive, inexpensive and easy-to-sample tool for diagnosing and monitoring asthma; however, the lack of standardised methods for VOC collection and analysis still represent crucial issues. VOCs originate from 1) exogenous sources (the environment) and enter the human body *via* inhalation, ingestion or contact, 2) the metabolism of resident bacteria, and 3) endogenous metabolic processes of the patient. All these are excreted in exhaled breath, and it is not easy to discriminate each component according to the origin [66].

A systematic review and meta-analysis showed that VOCs have sensitivity and specificity values of 87% and 86%, respectively, for diagnosing asthma. Also, individuals with asthma have a risk six times higher than healthy controls of being diagnosed through exhaled VOC profiles [68]. Rufo *et al.* [68] reported that VOC levels are correlated to blood eosinophil and neutrophil counts and with eosinophils in BAL; therefore, VOCs can be used to differentiate asthma phenotypes based on sputum inflammatory profile. Lastly, VOCs may predict asthma control [69], disease course and oral corticosteroid treatment response [70]. With particular reference to the e-Nose, VOC analysis demonstrated greater accuracy in predicting corticosteroid responsiveness than sputum eosinophil counts or F_{ENO} in a small group of patients with mild/moderate asthma [70].

Urine metabolites

Urine metabolites, such as bromotyrosine and leukotriene E_4 , could potentially represent new clinical biomarkers to diagnose asthma.

Some studies have found increased urinary bromotyrosine and leukotriene E₄ levels in patients with airflow limitation and inadequately controlled asthma. Moreover, both these urine metabolites could predict risk of asthma exacerbations and response to treatments including ICS, leukotriene receptor antagonists and long-acting β -agonists [71–73].

Recently, the eosinophil granule-derived proteins, such as eosinophil protein X (EPX) (also known as eosinophil-derived neurotoxin), have been investigated in the urine samples of patients with asthma. Reflecting the eosinophil activation, these proteins have proved to be reliable for assessing airway inflammation. Over 70% of included studies in a recent meta-analysis reported higher EPX levels in children with asthma compared to controls [74]. Nevertheless, due to the variability of metabolic pathways associated with paediatric asthma and their weak correlation with other biomarkers, such as sputum cell count and F_{ENO} level, the utility of urine metabolites needs to be further investigated [75].

Biomarkers of the non-type 2 asthma endotype

Neutrophils

Despite neutrophils being the most commonly detected cell type in induced sputum samples, they do not yet represent established markers to define the non-type 2 asthma endotype [76]. The cut-off values used to define sputum neutrophilia are different between cohorts of patients with asthma, ranging from 40% to 76%. In healthy subjects, the detected median neutrophil percentage is about 37% (10th and 90th percentiles of 11% and 64%). Tobacco smoke, pollution exposure and acute/chronic infections can also contribute to increased sputum neutrophil counts. Generally, a sputum neutrophil count $\geq 61\%$ allows the diagnosis of neutrophilic asthma [77]. A cut-off of 5% in BAL neutrophils has been adopted to classify as “neutrophil-high” or “neutrophil-low” children with severe asthma who underwent bronchoscopy [78]. Currently, none of the proposed cut-offs have been approved [77].

Clinically, SHAW *et al.* [79] reported evidence that severity of asthma, as well as severe exacerbations, could be objectively assessed by the sputum neutrophil count. However, the prevalence of neutrophilia in severe asthma remains somewhat uncertain, especially in childhood asthma, in which neutrophilic airway inflammation has been recently associated with better lung function [80].

Conversely, TEAGUE *et al.* [81] reported that children with pauci-granulocytic asthma (with no evidence of increased numbers of eosinophils or neutrophils in sputum or blood and in whom anti-inflammatory therapies are ineffective at controlling

Self-evaluation questions

1. The proportion of patients with severe asthma is:
 - a) <2%
 - b) 2–5%
 - c) >5%
 - d) >10%
2. The clinical heterogeneity of severe asthma may reflect differences in:
 - a) Triggers
 - b) Associated comorbidities
 - c) Response to therapy
 - d) All of the above
3. Which of the following are significant drivers of bronchial inflammation in type 2 asthma?
 - a) Airway smooth muscle cells, oxidative stress
 - b) IL-4, IL-5, IL-13, IgE
 - c) IL-17, IL-21, IL-22
 - d) Neutrophils and Th17 lymphocytes
4. Which of the following biomarkers are considered invasive and not feasible in routine clinical practice in the paediatric age group?
 - a) Bronchoscopy with bronchial biopsy
 - b) Exhaled breath
 - c) Induced sputum
 - d) Peripheral blood sample

symptoms) experienced a lower post-bronchodilator airflow limitation, less blood eosinophilia, fewer doses of prednisone, and fewer infectious species than children with mixed granulocytic BAL [81]. Moreover, a strong relationship between sputum and blood neutrophils, as well as with asthma severity, was also reported, suggesting neutrophilic lung inflammation as a risk factor for poorly controlled asthma [82, 83].

Sputum neutrophil count can also reflect response to treatment. Cluster analyses from the Severe Asthma Research Program (SARP) found that the highest sputum neutrophil count was correlated with the worst lung function despite maximal bronchodilator and ICS treatment [84].

Given the limited accuracy and ability of available blood biomarkers to reflect sputum neutrophilia, as well as the unavailability of targeted therapy for neutrophilic asthma, sputum neutrophilia currently lacks a clinical application [85].

Other cells

Natural killer T (NKT) cells, a heterogeneous T-cell population, are involved in both the autoimmunity and adaptive immune responses [86]. Recently, their functional role has been investigated in asthma. By using flow cytometric analysis, HAMZAOUI *et al.* [87] reported a significant increase in the number of NKT cells in the sputum of patients with severe asthma compared with mild asthmatic and healthy

Suggested answers

1. b.
2. d.
3. b.
4. a.

control groups ($p < 0.05$). The invariant natural killer T (iNKT) cells, which feature the expression of T-cell receptors recognising lipid antigens, do not seem to differ significantly between children and adults with asthma compared to healthy subjects [88]. The mucosal-associated invariant T (MAIT) cells, which recognise microbial-derived vitamin B2 (riboflavin) metabolites, are significantly lower in the peripheral blood and sputum of patients with moderate to severe asthma, thus negatively correlate with clinical severity [89]. Mast cells can be detected in both eosinophilic and non-eosinophilic asthma. However, recently, BERGAMO DE ARAUJO-PAULINO *et al.* [90] provided evidence that mast cell subtypes and their related gene expression profiles are associated exclusively with airway eosinophilia and could predict clinical asthma outcomes as well as corticosteroid response.

Further investigations on the number and functional properties of lung iNKT, MAIT and mast cells will hopefully provide more information on asthma pathogenesis. To date, their clinical utility is still under debate.

Conclusion

The need for a more personalised and targeted therapy for severe asthma is pushing research to identify biomarkers for monitoring and/or prediction of treatment response in asthma through minimally invasive or noninvasive sampling methods. In daily clinical practice, few biomarkers are readily available and reliable to define severe asthma endotypes. Blood and sputum eosinophils, serum IgE and F_{ENO} are mostly reflective of type 2 airway inflammation in children. Conversely, knowledge regarding non-type 2 inflammation and related biomarkers is still lacking.

Even if a strong correlation between biomarkers and asthma endotype exists, uncertainty remains as asthma changes significantly over time among patients and with age. Given the pathophysiological complexity underlying severe asthma, it is desirable to adopt a combined strategy, in which biomarkers (alone or in combination) are used in conjunction with the clinical findings of each patient to identify the endotype and guide the clinician towards the most appropriate intervention.

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Conflict of interest

None declared.

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