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Noninvasive Biomarkers of Asthma

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Abstract

Asthma is a heterogeneous disease of the lower airways including various types of bronchial inflammation presenting with different phenotypes and endotypes. Therapeutic response of asthmatic phenotypes/endotypes can be predicted by the use of biomarkers of inflammation phenotyping, and in recent years, endotyping of asthmatics allows to predict who will best respond to anti-inflammatory treatment and optimize quality of life of asthmatics by reducing the risk of exacerbations. Based on noninvasive biomarkers of inflammations, several of them have been described that are useful in clinical practice. Some of the noninvasive biomarkers have a particularly important role in the diagnosis and treatment of asthmatics. Monitoring of noninvasive biomarkers, such as fraction of exhaled nitric oxide (FENO), cells in sputum, or biomarkers in exhaled breath condensate (EBC), two main inflammatory phenotypes have been described: eosinophilic phenotype and neutrophilic phenotype. In eosinophilic asthma, as the most prevalent inflammatory phenotype, asthmatics have more than 3% eosinophils in the sputum, elevated levels of FENO, and elevated leukotriene's cytokine levels in EBC. The most extensively studied biomarkers in asthma are TH2 or more generally T2-related asthmatic endotype. Their clinical benefit might be used to phenotype/endotype features of the underlying type of inflammation and selection of asthmatics, particularly with severe or difficult-to-treat asthma, which most likely will respond to additional biological therapy. In this chapter, we summarize the noninvasive biomarkers available for the management of asthmatics.

Keywords: asthma, biomarker, inflammation, phenotype, endotype

1. Introduction

Asthma is a heterogeneous disease which includes a spectrum of different subtypes with different inflammation patterns responding differently to different treatments. The most

recently updated GINA guidelines address these issues emphasizing the importance of standardized diagnosis and appropriate treatment strategies according to asthma phenotype and/or endotype [1]. Generally, biomarkers or physiological measures that precisely and conclusively define phenotypes and asthma endotypes are missing or are insufficient. Several biomarkers have been described in asthma, but most of them including noninvasive biomarkers are not commonly available or still need external validation [2]. Many of the potential noninvasive biomarkers will be described in this chapter. Normal ranges and validation need to be established for most of them, and stability over time must be examined in longitudinal studies. The new research is needed about the effects of asthma therapy on biomarker measurements, especially for biomarkers which are proposed to guide different treatments, like therapy with biologics [3]. Finally, and most importantly, the new biomarkers, especially noninvasive, need to be easily sampled and interpreted at the point of care in order to provide and improve the diagnosis and treatment of different asthma subtypes, especially those which are more severe and therapy resistant. The challenge is how to identify "high-quality" biomarkers which have high accuracy and robustness that could predict clinical outcomes and therapy response which is of essential in the application of the concept of precision medicine.

2. Fraction of exhaled nitric oxide (FENO)

Fraction of exhaled nitric oxide (FENO) (or fractional exhaled NO) is currently the most widely used biomarker in the exhaled breath, and it is often increased in asthma, even in mild and asymptomatic condition [4]. Fractional exhaled nitric oxide or the modeling of NO dynamics of the lung can give more information than a single FENO value. The synthesis of NO is mediated by constitutive (endothelial NOS or neuronal NOS) and inducible NO synthase (iNOS). Its production is due to oxidation of L-arginine to L-citrulline. iNOS is the only isoform correlated with exhaled nitric oxide, and FENO has been considered as a marker of eosinophilic inflammation involving small airways [5]. FENO was found to be strongly reduced by treatment with inhaled corticosteroids (ICS) [6]. In a general population of asthmatics, it was found that the FENO threshold that best identified a sputum eosinophil count ≥3% in patients receiving high dose of ICS was 27 ppb [7]. In a recent paper, it was shown that, in severe asthma, FENO, had a lower accuracy than blood eosinophils to identify eosinophilic asthma [8], but increased FENO levels have been associated with a good response to ICS [6], oral corticosteroids, anti-IgE [9], and anti-IL-4 and anti-IL-13 [10, 11]. FENO is established as a marker of inflammation in asthma, but more than 20 years of research have shown that it works in certain asthma endotypes (TH2) [12]. Therefore, there is an increasing need for useful biomarkers with predictive and prognostic value for the progression of the disease in asthmatic patients and their link with clinical treatments.

3. Exhaled breath condensate (EBC)

Exhaled breath condensate (EBC) is the product of cooling and condensation of the exhaled aerosol, collected by tidal breathing during 10–30 minutes into a specially designed cooling

device [13]. It represents a potentially very useful method for noninvasive diagnosis of asthma and other pulmonary diseases. EBC consists of three major components, first being distilled water that comes from condensed gas phase of the exhalate. The second component are non-volatile particles or droplets of different sizes that are aerosolized from the airway lining fluid (ALF), and final components are water-soluble volatiles that are exhaled and absorbed into the condensing breath [14]. Considering the origin of EBC, a large number of inflammation, oxidative stress, nitrosative stress biomarkers, or airway acidification indicators such as pH, adenosine, ammonia, free radicals, hydrogen peroxide, isoprostanes, leukotrienes, prostanoids, nitrogen oxides, peptides, and cytokines can be detected in EBC and have been studied last 20 years [15, 16]. These biomarkers can reflect the underlying state of lower airways as well as lung inflammation and can be altered in patients with asthma.

The collection procedure and requirements for the EBC collection devices as well as the storage and processing are still not standardized and validated due to many factors that influence the final outcome [15]. The final product of the collection is influenced by not only patient factors fluid and food intake timing, concurrent medication or drug intake, age, sex, weight, height, and disease but also external factors as room temperature, collection temperature, and device materials [15]. The volatiles and the nonvolatile particles of the EBC are highly diluted so the detection and analytics of the EBC are challenging tasks, furthermore due to the fact that there is no generally accepted dilution marker detected yet [16, 17].

4. Exhaled breath temperature (EBT)

Exhaled breath temperature (EBT) is a noninvasive method for detecting and monitoring pathological processes based on inflammation in bronchial lumen. According to the fact that heat is one of the cardinal signs of inflammation, the measurement of EBT was developed as a marker of airway inflammation and therefore used in the study of inflammatory respiratory diseases. The use of EBT devices is particularly attractive in patients with asthma who largely exhibit significantly higher EBT values compared with healthy subjects; these patients are therefore encouraged to use these exhaled thermometers in clinical practice for the maintenance of asthma control and in therapeutic management. In the currently available literature, there are almost 200 articles on the use of EBT in asthma and other respiratory diseases. Only few of these studies have assessed EBT measurements, and one recent study provides reliable reference values of EBT in healthy subjects. Despite the potential of EBT, it has not yet reached the clinical setting, partly because of a lack of standardization and validation of the method. However, a recent study deals with these obstacles. There are three groups of external temperatures influenced by EBT in different ways [18]. The first group considered the cases with external temperature ≤ 23°C In this case, the average EBT was 28.268 ± 2.872°C The second group considered cases measured with an external temperature of 23–28°C. In this case, EBT was 30.949 ± 2.511°C. The third group showed that if the test is performed with an external temperature > 28°C, the EBT was 32.558 ± 1.805°C. Authors did not report any influence by other variables, such as weight, height, blood oxygen saturation, lung function, area of residence, work, blood pressure, and axillary temperature, on EBT. Their findings are consistent with data from other authors [19]. There are several potential advantages of EBT

measurements: very easy for patients and requires only a few minutes; it is completely noninvasive and is therefore also suitable for children and patients with severe disease. The device is well accepted by patients and ethics committee, it is inexpensive, and it does not affect the underlying airway disease [20].

5. Electronic nose (e-Nose)

Exhaled breath contains thousands of volatile organic compounds (VOCs) in gaseous form that reflect the metabolic process occurring in the host, which may be used as markers of inflammation in the lung or systemically [21]. e-Nose is a portable device, which allows non-invasive, quick, and real-time pattern analysis of VOC spectra. Current e-Nose devices generally consist of an array of chemical sensors that specifically identify VOC mixture. Actually, e-Nose is a system of artificial sensor with chemical sensors that consists of an array for a qualitative and/or quantitative detection and description of VOC profiles or breath prints. Due to poor specificity to individual volatiles, chemical sensor arrays are not generally suitable for identifying single volatiles in complex mixtures of breath. Combining technologies, the high sensitivity of chemical sensor arrays with the high specificity of gas chromatography-mass spectrometry (GC-MS), which could mimic the performance of the natural olfactory system in e-Nose, can be used for identifying breath volatiles, as potential new markers of inflammations in different asthmatic sub-phenotypes [22, 23]. However, e-Nose technology has limitations. The optimal technique for breath collection, sampling, and analysis of single-breath volatiles indicating that future methodological studies are required is unknown. Miniaturized devices based on nanotechnology with micro- or nano-arrays are seen as a key in advancing a new e-Nose device.

Measurement of VOC by e-Nose can discriminate between patients with respiratory disease such as asthma and healthy controls. e-Nose breath prints are associated with the level of airway inflammation and might be useful in the assessment of asthma severity as well as can discriminate patients with fixed asthma from COPD patients with an 88% accuracy [24]. Longitudinal monitoring of exhaled metabolites measured by GC-MS and e-Nose can discriminate loss of asthma control [25]. The usefulness of measurement of VOC profiles by e-Nose in assessing asthma inflammatory phenotypes still needs to be confirmed.

6. Biomarkers in induced sputum

Induced sputum is a relatively noninvasive mode of airway sampling that provides an opportunity for analysis of cellular components and infective agents, including bacteria and viruses, together with fluid-phase constituents [26]. There are several standardized manuals that are available and help to educate health professionals how to perform the technique to the highest standard [27]. The application of induced sputum in the assessment of airway pathology has grown rapidly, especially after 2002, when European Respiratory Society (ERS) published the recommendations for standardization of sputum induction and processing [28, 29]. That is a

key component to provide valuable information for clinical decision-making. Sputum is collected after inhalations of hypertonic saline. Although relatively safe, induced sputum requires specialized training, equipment, and laboratory processing. Monitoring lung function during the induction procedure reduces the risk of excessive bronchoconstriction. Patient's active cooperation is needed for collection, making this technique unsuitable for some patients, especially for children below the age of 7 years. Induced sputum provides a rich source of soluble and cellular biomarkers. The sputum eosinophil percentage is a key biomarker which identifies patients who have eosinophilic and non-eosinophilic asthma phenotypes and correlates with severe exacerbations and AHR. Besides eosinophils, other sputum biomarkers are currently in research. Sputum neutrophils are often related to severe non-eosinophilic asthma with fixed airway obstruction. Soluble sputum biomarkers associated with asthma severity are IL-4, IL-5, IL-6, IL-12, IL-13, ECP, LT, TNF-α, CSF, TNF-α, and GM-CSF [30]. Biomarkers such as IL-8 and neurokinin A correlate with exacerbation, while procollagen synthesis peptides, tissue inhibitors of metalloproteinase, or THF-β have been associated with remodeling [31].

The widespread application of induced sputum in asthma proposed several disease phenotypes and defined which of these phenotypes respond to the current therapy. In neutrophilic asthma phenotype, the level of sputum mRNA expression of Toll-like receptors 2 and 4 as well as CD14 was high. Thus, this well-tolerated and safe method provides an additional tool to guide the clinical management of asthmatic patients [32]. To date induced sputum represents the only noninvasive measure of airway inflammation that has a clearly proven role in asthma management.

7. Biomarkers in urine of asthmatics

Urine is an easily accessible and noninvasive collectable biofluid containing many information about the current metabolic status of the body. Metabolic changes in the body of an asthmatic patient are reflected in the metabolite concentrations in urine, and the changes during asthma exacerbation can also be tracked well by urine metabolite analysis. Asthma, especially during exacerbation, causes a high level of oxidative stress due to the pulmonary reaction to exacerbation and resulting formation of reactive oxygen compounds that lead to cell damage. In children with asthma, the levels of LTE4 are increased in urine and are not altered under inhaled corticosteroid (ICS) therapy, but the 5-lipoxygenase inhibitors reduce the urinary LTE4 levels. Eosinophil protein X (EPX) is found in the urine of asthma patients, but the levels of the EPX fall within 3 months after anti-inflammatory therapy induction [33].

Prostaglandin D2 (PGD2) is released from mast cells, and it causes bronchoconstriction and vasodilatation in the airway. PGD2 is metabolized to $9\alpha,11\beta$-PGF2 and excreted in urine. It is also increased in patients with asthma, but its production, thus excretion, may be influenced by corticosteroid therapy [33]. Bromotyrosine (BrTyr) is another biomarker that originates from protein oxidation in eosinophils. Urinary levels of BrTyr are significantly higher in patients with asthma and even higher during exacerbation.

It has been suggested in a few studies with a small number of adult patients that during exacerbation the levels of threonine, alanine, carnitine, acetyl carnitine, and trimethylamine N-oxide

were slightly increased, which can be caused not only by the above-described oxidative stress but also by food and drug intake. Some metabolite urine concentrations were lower than usual: acetate, citrate, malonate, and others. Alkane and aldehyde levels were found to be increased in urine; also, the levels of carnitine and acetyl carnitine, which are essential in the process of fatty acid transport into mitochondria, were high in urine of patients with asthma [34]. Other potential biomarkers such as club cell protein 16 (CC16), as a biomarker of epithelial dysfunction, have been studied in urine of patients with asthma. One study in Chinese children showed lower levels of CC16 in asthmatic children [35].

8. Noninvasive biomarkers of asthmatic phenotypes

Over the past decade, the most important advance in the field of asthma has been the recognition of asthma as a syndrome or heterogeneous disease with several clinical presentations or phenotypes. Biomarkers help define the specific pathology of different asthma phenotypes and identify potential therapeutic targets. However, a number of biomarkers have been identified that help define asthma phenotypes most likely than reflect responsiveness to specific therapies. Noninvasive biomarkers such as FENO or sputum cells usually reflect the main inflammatory phenotypes of asthma. Eosinophilic phenotype having more than 3% eosinophils in the sputum is likely to reflect ongoing adaptive immunity in response to allergen. Several biomarkers of eosinophilic asthma, except the percentage of eosinophil, are easily available in clinical practice, such as blood eosinophils, serum-specific IgE, exhaled nitric oxide, or serum periostin level. A significant proportion of asthmatic patients, particularly those with severe disease, do not have a TH2-enhanced phenotype (TH-2 low) [9, 36]. Patients with a non-TH2 phenotype can be further split in two inflammatory phenotypes depending on the level of their airway neutrophilic inflammation: paucigranulocytic and neutrophilic [37]. Neutrophilic asthma as more than 76% neutrophils in the sputum is thought to reflect innate immune system activation in response to pollutants or infectious agents, mixed granulocytic asthma when both inflammatory cells are increased, and paucigranulocytic asthma is thought to be not inflammatory and characterized by smooth muscle dysfunction. Among severe asthmatics, a subgroup characterized by noneosinophilic inflammation was described [38]. We currently lack of user-friendly biomarkers of neutrophilic asthma and airway remodeling. This absence of biomarkers for these patterns of inflammation has made it difficult to recognized subjects who might respond to biologics that target this pathway [38].

9. Noninvasive biomarkers of asthmatic endotypes

Asthma is increasingly recognized as a heterogeneous group of diseases (syndrome) caused by multiple inflammatory pathogenic processes or endotypes. Recently, the definition of the term "endotype," describing a specific pathogenic mechanism leading to the clinical presentation of asthma. Two major asthmatic endotypes have been recognized: TH2-high, manifested by increased eosinophils in the sputum and airways, and TH2-low, with increased neutrophils or a paucigranulocytic cells. Using these classifications and specific biomarkers has led to promising

new therapeutics, often biologics, especially for TH2-high asthma. Many studies of asthmatic endotypes have assessed granulocyte populations in induced sputum. Increased percentage of sputum neutrophil usually represents an increase in IL-17-driven neutrophil recruitment or a relative reduction in other inflammatory cells such as eosinophils [39]. Thus, neutrophil activation state rather than number may be a more important indicator of their contribution to asthma severity, as an indicator of TH2-low endotype. From the other side, nitric oxide is produced by the action of iNOS encoded by the *NOS2* gene, and eosinophils are mobilized by chemokines such as eotaxin-3 encoded by the *CCL26* gene, highly correlated with the TH2 domination. Therefore, available noninvasive biomarkers such as sputum cell analysis or FENO can indirectly represent certain type 2-driven inflammation of asthmatic endotype [40]. The TH2-low endotype does not have any readily available point-of-care biomarkers, so TH2-low asthma is often diagnosed based on a lack of TH2-high biomarkers [41]. The TH2-low endotype characterized greater resistance to steroids and the development of therapies. Advances have been made with regard to sputum cytokine analysis and might also help to guide future treatment of severe asthma. Several other noninvasive biomarkers have been described in different asthma endotypes, but most of them are not commonly available or still need external validation [33].

10. Noninvasive biomarkers and asthma control

Asthma is a heterogeneous inflammatory disorder with several different phenotypes and a nonspecific clinical presentation. Even more, the usually used pulmonary function tests are insensitive and often normal or do not correspond to the disease evolvement [33]. Given the different etiology of asthma subtypes, the therapy is adjusted and needs to be evaluated throughout the duration of treatment. For that purpose a number of biomarkers have been studied for the last 30 years.

One of the longest in use is the measurement of the fraction of nitric oxide in exhaled breath (FENO). Nitric oxide (NO) is generated by three nitric oxide synthase isoenzymes, one of them being inducible (NOS2) that produces most of the exhaled NO. In patients with asthma, especially eosinophilic airway inflammation, the NOS2 overexpression can be reduced by inhaled corticosteroid therapy. This effect is used for predicting the efficacy and monitoring of the ICS therapy in patients with asthma [33]. However, the FENO measurement results can be influenced by flow rate, nasal contamination, ambient air, age, height, gender, race, spirometry or exercise before testing, diet, and smoke exposure [42]. In general, low FENO levels seem to be useful in predicting the asthma phenotypes that will respond poorly to ICS treatment [42]. When the asthma is responsive to ICS, the FENO levels correspond in a dose-dependent manner with ICS [42]. Nevertheless, the method still needs to be further evaluated in studies with standardized protocols.

Exhaled breath has recently been studied in a different setting; namely, after cooling the exhaled breath, a condensate (EBC) containing volatile and nonvolatile particles is produced and can be analyzed for existence of numerous biomarkers. The acidity of EBC is high in asthmatics, but after inducing anti-inflammatory therapy, it rapidly returns to normal values [33]. The total nitrite/nitrate levels have been found to be increased in pediatric asthma

patients compared to healthy controls; still, the results are conflicting regarding the association to asthma severity [42]. Levels of H_2O_2 increase with asthma severity so it plays a role in monitoring disease control and response to steroid treatment as the levels correspond with inducement of steroid therapy [42]. EBC in patients with asthma contains higher concentrations of 8-isoprostane both in children and in adults, when compared to healthy controls [42]. The level of 8-isoprostane is associated with asthma control and severity and, thus, can be used as a monitoring tool.

In combination with nitric oxide, interferon-gamma (IFN-γ), and interleukin-4 (IL-4) measurements in EBC, the level of 8-isoprostane can be used as a good marker for assessing asthma control [42].

However, there are many indices that the EBC contains biomarkers that could be used as a tool to control asthma progression and therapy adjustments; it is still a method primarily used in research setting. The metabolomic and proteomic methods are required in order to have EBC analysis in clinical use, which subsequently generates low-cost effectiveness of the methods at present [43].

Induced sputum is another source of biomarkers that can be used for asthma disease control. As a method it is safe, noninvasive, and thus usable in pediatrics. It contains cell phase (eosinophils and neutrophils) and supernatant with cytokines. Sputum eosinophil count is a key marker of asthma severity and responsiveness to steroid therapy. The number of eosinophils correlates well with asthma severity and is predictive of asthma exacerbation. Elevated levels of eosinophil cationic protein (ECP), IL-4, IL-5, IL-13, TNF-α, IL-6, IL-12, and granulocyte macrophage colony stimulator factor have been found in sputum supernatant of asthma patients [33]. Several studies have challenged the usage of induced sputum analysis for asthma control, but it seems that the results are contradictory so it needs further testing, and for the moment, the method has not been proven accurate enough to be used for asthma monitoring in childhood asthma [43].

Recently, urine has been studied as one of possible sources of biomarkers for asthma disease monitoring. The studies have, so far, found that only leukotriene E4 and bromotyrosine levels are high and associated with disease severity, exacerbations, and aspirin intake [33].

11. Noninvasive biomarkers and asthma therapy

The use of biomarkers in asthma is restrictive because knowledge of the asthma phenotypes is incomplete. The concept of better endotyping asthma can give precision medicine useful data necessary to develop new therapies. Recent trials evaluating biological therapies targeting IgE, IL-5, IL-4/IL-13, and IL-17 have utilized predictive markers to identify patients who might benefit from therapy. Multiple biomarkers including sputum eosinophil count, blood eosinophil count, FENO, and serum periostin have been used to identify patients with a good response to targeted medications (**Table 1**) [44–47]. Till now, relevant biomarkers that can be useful in the management of asthma are mainly related to TH2 response

	Biological sample	Exhaled air	Sputum		Blood						Urine
Target	Drug	FENO	eo	neu	eo	IgE[a]	Periostin	IL-13	IL-17	DPP-4	LT 4E
T2 biomarkers											
	Glucocorticoid	•	•								
IgE	Omalizumab	•	•		•		•				
IL-5	Mepolizumab	•			•						
IL-5	Reslizumab		•		•						
IL-5	Benralizumab	•			•						
IL4/IL-13	Dupilumab	•			•		•				
IL-13	Lebrikizumab	•					•				
IL-13	Tralokinumab						•	•			
DPA	Fevipiprant		•							•	
LT	LTA										•
Non-T2 biomarkers											
	Azithromycin	•		•[b]	•						
	Clarithromycin			•							
IL-17	Brodalumab			•[c]							
CXCR2	ACD5096			•[c]							

[a]Determines eligibility for omalizumab, but the level is not predictive for response

[b]Trial did not evaluate sputum neutrophil counts

[c]Trial did not demonstrate clinical improvement

Legend: eo, Eosinophils; neu, neutrophils; IL, interleukin; DPP-4, dipeptidyl peptidase-4; LT 4E, leukotriene 4E; CXCR2, CXC chemokine receptor 2 (Adapted according to Medrek et al. [45])

Table 1. Biomarkers predictive of response to different asthma therapies.

[45]. These biomarkers are considered more steroid-responsive [38]. The eosinophil counts has proven to be useful in the clinical arena in helping to predict short-term response to inhaled corticosteroids (ICS) and tailor the dose of ICS in the severe patients [32]. Sputum eosinophil percentage acts as a key marker and correlates with severe exacerbations and AHR. Also, it can be useful in a panel of biomarkers to select patients who may benefit from IL-5 targeted therapies, including reslizumab, mepolizumab, and benralizumab [48]. Blood eosinophils as surrogate markers for sputum eosinophilia are associated with relevant outcomes and are more readily measureable. New evidence supports fraction of exhaled nitric oxide (FENO)-based treatment algorithms for cost-effective maintenance of asthma control/ quality of life. Serum and sputum-derived periostin are biomarkers of lung function decline and associated with eosinophilic airway inflammation. Biomarker panels may improve predictive value as shown for the combination of FENO/urinary bromotyrosine in prediction

of steroid responsiveness. Novel biological therapies are proven effective in biomarker-selected populations. Biomarkers including blood eosinophils and FENO are proven to have utility for the effective administration of steroidal and novel biological therapies in asthma, allowing individualized treatment. According to experience of many investigators, the common cause of persistently elevated FENO despite therapy is poor compliance, but this marker is not validated [6]. The complexity and heterogeneity of the asthma request different approaches of phenotyping patients. Used and clustering omics data will provide a better chance of phenotyping asthma based on disease mechanism with composite set of markers obtained for each endotype. Probably, new biomarkers will replace currently available biomarkers and be more specific for both T2 and non-T2 pathways. According to some authors, novel approach is not based on developing new techniques than combining known biomarkers to increase their predictive values. Personalized medicine will allow more precision therapy and also provide novel targets and new treatment for each defined [46]. The future of personalized medicine will depend of availability of accurate and reliable predictive biomarkers.

12. Noninvasive biomarkers of childhood asthma

Asthma represents the most common chronic respiratory disease in children. Whereas preschool children present with multitrigger and viral wheeze, in school children, asthma is usually classified as allergic and non-allergic. For both, the underlying immunological mechanisms are not yet quite understood. Treatment is still prescribed unrelated of underlying mechanisms, and often asthma control in children has not been achieved. Nevertheless, the spectrum of asthma in clinical presentation is broad, and both symptoms and lung function may not always reflect the underlying airway inflammation or endotype [49]. Therefore, in recent years, following the example of adult asthmatics is trying to differentiate specific asthmatic phenotypes as well as endotypes in children. Several studies aiming to identify endotypes are underway, and their relevance for clinical monitoring and subsequent treatment options is still a subject of discussion [50]. For these reasons, the identification of objective biomarkers of childhood asthma phenotype/endotype, which may guide diagnosis, management, and treatment of asthmatic children and might have a role in the development of personalized approach [51]. That is why the availability of noninvasive and validated biomarkers to study and monitor disease is of relevance especially in childhood asthma [52]. Identification of clinically applicable noninvasive biomarkers such as biomarkers in EBC has been of particular interest in personalized diagnosis and treatment of asthma in children [53]. The utility of noninvasive biomarkers in routine clinical practice for monitoring inflammation in children with asthma is undefined, apart from FENO measurements. Sputum eosinophilia, EBC, and urinary leukotrienes are still not applied in routine clinical practice. Despite the development of new biomarkers or new immunological molecules, the complex puzzle of childhood asthma is still far from being completed (**Table 2**) [54].

Biological sample	Biomarker	Therapy response	Diagnostic	Prognostic
Exhaled air	FENO	x	x	
	EBC		x	x
	EBT		x	
	e-Nose		x	
Sputum	eo	x	x	x
	neu		x	
	IL-4		x	x
	IL-5			x
	IL-6			x
	IL-12			x
	IL-13			x
	ECP	x		x
	LT			x
	TNF-α			x
	CSF			x
	GM-CSF			x
	IL-8			x
	Neurokinin			x
	Metalloproteinase			x
	THF-β			x
	Procollagen synthesis peptides			x
Blood	eo	x	x	x
	IgE	x	x	
	Periostin	x	x	
	IL-13	x	x	
	IL-17	x	x	
	DPP-4	x	x	
Urine	LT E4	x	x	x
	EPX	x	x	
	11β-PGF2	x	x	
	BrTyr		x	x
	CC16		x	

Table 2. Biomarkers in asthma.

13. Conclusion

Over the past decades, a great emphasis has been put into developing and researching biomarkers, as well as noninvasive biomarkers in monitoring of inflammation and treatment of asthmatics. The three most promising biomarkers in clinical practice currently are analysis of cells in induced sputum, FENO, and biomarkers in EBC. In the past years, the progress has been made in the discovery, application, and implementation of new, especially noninvasive biomarkers in asthmatic patients. Although now-available noninvasive biomarkers have marked its benefits, their roles are still too limited and nonspecific for identifying at-risk patients, recognitions of specific asthma pattern (phenotype or endotype), and selection of specific and the most helpful treatment, particularly biologics. In the near future, the role of biomarkers in achieving personalized medicine will be critical.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

Abbreviations

ACQ   Asthma control questionnaire

AHR   Airway hyperreactivity

ALF   Airway lining fluid

BrTyr   Bromotyrosine

CC16   Club cell protein 16

CXCR2   CXC chemokine receptor 2

DPP-4   Dipeptidyl peptidase-4

EBC   Exhaled breath condensate

EBT   Exhaled breath temperature

ECP   Eosinophil cationic protein

e-Nose   Electronic nose

eo   Eosinophils

EPX   Eosinophil protein X

ERS   European Respiratory Society

FENO	Fraction of exhaled nitric oxide
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GC-MC	Gas chromatography-mass spectrometry
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
IL	Interleukin
iNOS	Inducible nitric oxide synthase
LT 4E	Leukotriene 4E
neu	Neutrophils
NO	Nitric oxide
PGD2	Prostaglandin D2
VOC	Volatile organic compound

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