



Adult asthma biomarkers

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Purpose of review

A variety of novel asthma treatments have been developed based on phenotypes, and the clinical trial results show promising responses. This review summarizes the current knowledge of biomarkers for the determination of asthma phenotypes.

Recent findings

Eosinophilic inflammation is the most focused phenotype because most novel asthma treatments have targeted T-helper type 2 (Th2) pathway. Fractional-exhaled nitric oxide (FeNO) is a new method that represents an eosinophilic airway inflammation with a significant correlation with sputum eosinophilia and asthma severity instead of sputum eosinophil count that easily influenced by corticosteroid therapy. However, some reports indicated the discordance between treatment response or adjustment and FeNO levels. Serum periostin is a strong serum biomarker for eosinophilic airway inflammation and an indicator of Th2-targeted therapy (such as lebrikizumab or omalizumab) and airflow limitation. YKL-40 is associated with asthma severity and airway remodeling. In addition, genetic and metabolomic approaches have been made to determine asthma phenotypes and severity.

Summary

Biomarkers such as FeNO and serum periostin represent eosinophilic airway inflammation, together with eosinophil-derived neurotoxin and osteopontin (OPN) needed more replication studies. Periostin, YKL-40, OPN and some metabolites (choline, arginine, acetone and protectin D1) are related to asthma severity and airflow limitation.

Keywords

asthma, biomarkers, exhaled nitric oxide, metabolomics, periostin

INTRODUCTION

A clinical diagnosis of asthma is based on the symptoms such as episodic breathlessness, wheezing, cough and chest tightness; in addition, lung function (measured by spirometry or peak expiratory flow) represents a considerable part of the diagnosis [1]. However, efforts to define asthma subtypes have been made because diagnosis based on these criteria have caused clinical, physiologic and pathologic heterogeneities [2]. On cluster analysis, different clinical phenotypes were defined according to the symptoms and types of inflammations that included the early onset of atopic asthma, obese noneosinophilic asthma and benign asthma [2,3]. A definition of an asthma endotype was proposed (such as aspirin sensitive asthma, allergic bronchopulmonary mycosis, allergic asthma and severe late onset hypereosinophilic asthma) based on phenotypes and putative pathophysiologies on cluster analysis [4].

Asthma is a chronic inflammatory disorder of the airways that shows different cellular patterns [1]. Eosinophilic airway inflammation is a key

molecular parameter for the classification of asthma phenotypes or endotypes. Eosinophilic or non-eosinophilic inflammations can be documented through invasive (bronchial biopsies) and noninvasive means [induced sputum and fractional-exhaled nitric oxide (FeNO)] [5]. Distinct phenotypes and different responses to corticosteroid therapy have been reported for these two inflammations; in addition, novel asthma treatments have targeted inflammations based on phenotypes [6]. Accordingly, phenotype determination has become increasingly important. This review focuses on current knowledge in regards to biomarkers for

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KEY POINTS

- Diagnosis of asthma causes clinical, physiologic and pathologic heterogeneities, and different phenotypes have been defined. Eosinophilic inflammation is the most focused phenotype because most novel asthma treatments have targeted Th2 pathway.
- FeNO represents an eosinophilic airway inflammation with a significant correlation with sputum eosinophilia and asthma severity. However, some reports indicated the discordance between treatment response or adjustment and FeNO levels. In addition, pH and cysteinyl leukotriene level in EBC, sputum galectin-10 and eosinophil proteins in airway macrophages have been under investigation.
- Serum periostin is a strong serum biomarker for eosinophilic airway inflammation and treatment response of Th2-targeted therapy. YKL-40 is associated with asthma severity and airway remodeling. In addition, investigations using metabolomics or genomics are now in progress.

the diagnosis and determination of asthma phenotypes and endotypes.

BIOMARKERS OF CLINICAL PARAMETERS

Eosinophilic inflammation is a representative feature of asthma, and it can be recognized by the proportion of eosinophil ($\geq 3\%$) in sputum. Sputum collection is widely used for the convenient diagnosis and monitoring of asthmatic patients. The adjustment of corticosteroid doses according to sputum eosinophil percentage prevented asthma exacerbations [7,8]. However, sputum eosinophil count sometimes tends to be influenced by current treatment and not consistent. Therefore, efforts are being made to develop more simple and accurate methods to measure the severity of eosinophilic inflammation. FeNO has been regarded as next-generation examination that can replace sputum eosinophil count and be adopted in clinical practice.

Exhaled nitric oxide is one simple biological marker to measure eosinophilic inflammation in asthma. FeNO is believed to be a hallmark of eosinophilic inflammation with a significant correlation with sputum eosinophilia due to the increased FeNO level in the exhaled gas of asthma patients with T-helper type 2 (Th2)-mediated inflammation [9,10]. FeNO measurement can provide excellent disease diagnostics and predicting value for a corticosteroid response in asthma patients [11,12]. FeNO provides an independent classification of asthma severity and can identify an asthma phenotype with optimal cut off value

in severe asthma [13]. In all aspects of small airway inflammation, the evaluation of the FeNO level has been proposed with different modeling of nitric oxide dynamics that have the ability to discriminate between large and small airways [14]. Recently, it has been found that the alveolar nitric oxide level significantly correlated to asthma control status as assessed by Asthma Control Test scores; however, the level of bronchial nitric oxide was not associated with asthma control status [15[¶]]. However, there are inconsistent data regarding an adjustment of asthma treatment according to FeNO. Some discordances were noted between the level of FeNO and clinical improvement after mepolizumab treatment in refractory eosinophilic asthma [16]. In addition, the Best Adjustment Strategy for Asthma in the Long Term trial result showed that biomarker-based treatment adjustment (inhaled-corticosteroid) was not superior to adjustment based on physician assessment [17^{¶¶}]. Therefore, more longitudinal and various studies are required to clarify the clinical significance of exhaled nitric oxide in various asthma phenotypes.

Additional novel candidate biomarkers have been reported such as galectin-10 in sputum, pH and cysteinyl leukotriene level in exhaled breath condensate (EBC) and eosinophil proteins in airway macrophages. Galectin-10 is a major constituent of human eosinophils, and sputum galectin-10 showed a strong correlation to sputum eosinophil concentrations [18[¶]]. The measurement of sputum galectin-10 may be an alternative to sputum eosinophils (either alone or in combination with other biomarkers) due to galectin-10 being present only in an eosinophilic phenotype (not present in the neutrophilic type). However, further replication and validation studies are required. EBC contains a large number of mediators that reflect changes in the airway lining such as adenosine, ammonia, arachidonic acid metabolites and peptides, and can be obtained with easy and noninvasive methods. There were reports that EBC pH correlated to the sputum eosinophil count and lower in asthmatic patients than healthy controls; in addition, the level of cysteinyl leukotriene in EBC increased in severe asthma patients compared with those with mild asthma and the healthy controls [19,20]. Nonetheless, the standardization of EBC collection and multiple factors that affected the accuracy of EBC measurement remains an unsolved issue for application to real practice. Eosinophil proteins in airway macrophages are not influenced by corticosteroid therapy, and therefore can be used in determining a true noneosinophilic phenotype after a long-term corticosteroid treatment. Kulkarni *et al.* [21] found that eosinophilic cationic protein (ECP) and

eosinophilic peroxidase were increased in moderate-to-severe asthma patients compared with mild asthma and healthy control individuals after corticosteroid therapy [22]. In addition, a small radiologic study [23] reported that PET uptake rate was increased in an allergen challenged lung with linear correlation to an eosinophil count in the lavage fluid. Also, sputum hydrogen sulfide were positively correlated with neutrophilic inflammation and inversely correlated with FeNO levels [24[■]]. Although these novel measurements may be far from current clinical practice, these techniques can allow detailed studies of the relationship between airway inflammation and hyperresponsiveness that can test the anti-inflammatory effects of novel therapeutic agents.

SERUM BIOMARKERS

Recent advances in the discovery of serum biomarkers for asthma are in the field of diagnosis such as the determination of asthma phenotype or endotype as well as some that focus on eosinophilic markers. Periostin, YKL-40, osteopontin (OPN), ECP, eosinophil-derived neurotoxin (EDN) and metabolomics have been studied along with peripheral blood eosinophil counts and serum total immunoglobulin E (IgE) level. Particularly, the serum periostin level was reported as the single best predictor of airway eosinophilia if compared with serum IgE levels, blood eosinophil numbers and fraction of exhaled nitric oxide levels in adult asthmatic patients [25[■]].

Serum periostin is one of the most focused candidate biomarkers for eosinophilic asthma. Periostin was first isolated from an osteoblastic cell line and regarded as associated with subepithelial fibrosis in response to interleukin4 (IL4) and IL13 [26]. Periostin is a secreted protein composed of four tandemly lined fasciclin I domains in the middle; subsequently, periostin is suspected as an adhesion molecule and involved in subepithelial fibrosis [27]. In 2011, lebrikizumab (a monoclonal antibody to IL13) was reported to improve lung function (especially in patients with a high serum periostin level); in addition, periostin was suggested as a serum biomarker that suggested a favorable outcome in a combined treatment with lebrikizumab [28]. The other study [25[■]] demonstrated that the periostin level showed an excellent correlation with the degree of airway eosinophilia. An analysis of biomarkers in the EXTRA study [29[■]] showed that omalizumab significantly reduced exacerbation frequencies in the high serum periostin group that was comparable to the fraction of exhaled nitric oxide and blood eosinophils. Periostin was associated

with airflow limitations in adult asthmatic patients receiving inhaled corticosteroids wherein the higher periostin level group showed a greater decline in the forced expiratory volume in 1 s (FEV1) [30[■]]. These results indicate serum periostin could be a biomarker for airway eosinophilia, which would make the serum periostin level an indicator of Th2-targeted therapy (such as lebrikizumab or omalizumab) and airflow limitation.

YKL-40 is a chitinase-like protein and both chitinases and chitinase-like proteins have been reported as potential asthma biomarkers. Chitin can be degraded by two enzymes found in the human body (one is the enzymatically active chitotriosidase and the other is enzymatically inactive chitinase-like protein, YKL-40) [31]. YKL-40 is known to increase in the serum and lungs of adult asthmatic patients and is correlated to disease severity [32]. In pediatric asthmatic patients, serum YKL-40 levels were higher in the therapy-resistant asthma group than in healthy controls [33[■]]. The correlation of serum YKL-40 with disease severity and airway remodeling needs to be replicated in a larger population study that includes adults and children.

Following periostin and YKL-40, some serum biomarkers such as OPN, ECP and EDN have been under investigation. OPN is an extracellular matrix protein originally discovered in bone and is reported to be associated with the chemotaxis of eosinophil and eosinophilic airway inflammation, airway remodeling, the severity of asthma and Th1 immune responses [34,35]. OPN levels were higher in the sputum of severe asthma, and OPN-induced eosinophil chemotaxis was reported in the sputum and bronchoalveolar lavage fluid of adult asthmatic patients [35–37]. Serum ECP is a mediator secreted from activated eosinophils and regarded as a marker of eosinophilic inflammations. Asthma (together with atopic dermatitis and allergic rhinoconjunctivitis) can provoke an increased serum ECP level. Some studies reported that the measurement of ECP was useful for the diagnosis of asthma [38]. However, there are no subsequent studies in association with the ECP level and asthma phenotypes. EDN (released by eosinophils) shares several similarities with ECP in biochemical, antiviral and neurotoxic properties [39[■]]. EDN showed a good correlation with total eosinophil counts in acute pediatric asthmatic patients and a possibility as a biomarker for recurrent wheezing in infants hospitalized with respiratory syncytial virus bronchiolitis and aspirin-exacerbated respiratory disease (AERD) phenotype in adult asthmatic patients [39[■],40,41[■]].

Metabolomics targeted multiple endogenous metabolites from biological samples that can provide potential biomarkers. A nuclear magnetic resonance

Table 1. Summary of previous reports regarding biomarkers in asthmatic patients

Biomarker	High/low	Associated phenotype	Study individuals	References
Periostin	High	Eosinophilic asthma, airflow limitation	Adults	[25 [■] , 26–28, 29 [■] , 30 [■]]
YKL-40	High	Severe asthma	Adults/children	[31, 32, 33 [■]]
Osteopontin	High	Eosinophilic asthma, severe asthma	Adults	[34–37]
Eosinophil-derived neurotoxin	High	Eosinophilic asthma	Children	[39 [■] , 40]
		AERD	Adults	[41 [■]]
Choline and arginine	Low	Low FEV1	Adults	[42 [■]]
Acetone	High	Low FEV1	Adults	[42 [■]]
Protectin D1	Low	Severe asthma	Adults	[43, 44 [■]]

AERD, aspirin-exacerbated respiratory disease.

(NMR)-based metabolomic study indicated that the analysis of endogenous metabolites from the sera of adult asthmatic patients showed increased levels of methionine, glutamine, histidine and decreased levels of formate, methanol, acetate, choline, O-phosphocholine, arginine and glucose. This analysis proved an increased DNA methylation status and Th2-induced inflammation. In addition, the FEV1% predicted values correlated positively with levels of choline and arginine and negatively with acetone level. The sera from asthmatic patients with lower FEV1% predicted values showed higher levels of very low-density lipoprotein/low-density lipoprotein products that suggested asthma severity was correlated to lipid metabolism [42[■]]. In another lipidomic analysis, protectin D1 (PD1) was suggested as a biomarker for severe asthma. PD1 is a lipid mediator biosynthesized from omega-3 fatty acid docosahexaenoic acid (DHA). PD1 acted on the limitation of the polymorphonuclear cell influx and the stimulation of the clearance of apoptotic cells and monocyte's inflammatory debris; therefore, they are suggested as a potent agonist for the resolution of inflamed tissues. Exogenous supplement of PD1 attenuates eosinophilic inflammation in a murine model [43]. With these results, it is possible that PD1 may be a key mediator to exert the anti-inflammatory effects of an omega-3 fatty acid in asthmatic patients. Moreover, activated eosinophil was reported as a major source of PD1, and the biosynthesizing capacity of PD1 in eosinophils separated from severe asthmatic patients decreased compared with healthy normal controls in the presence of DHA [44[■]]. A decreased synthesizing capacity of PD1 in eosinophils may contribute to a persistent airway inflammation in severe asthmatic patients. There were inconsistent reports in the study of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine produced by methylation of arginine residues. ADMA increased in the sputum from pediatric asthmatic patients and lungs from a murine model of

allergic airway inflammation; however, it decreased in plasma from other pediatric asthmatic patients [45, 46]. More replication studies in metabolomics are encouraged to find specific phenotype applications and associations. A summary of the above candidate biomarkers is presented in Table 1 [25[■], 26–28, 29[■], 30[■], 31, 32, 33[■], 34–37, 39[■], 40, 41[■], 42[■], 43, 44[■]].

Many genetic studies in connection with asthma also contributed to a fundamental understanding of asthma pathogenesis. The findings of orosomucoid 1-like 3 (*ORMDL3*) and gasdermin-like (*GSDML*) gene variants at locus 17q21 (as markers of asthma) are well replicated in different populations such as adult-onset and childhood-onset asthma [47[■], 48]. The polymorphisms of several genes essential for a Th2-type immune response [including interleukin 1 receptor like 1 (*IL1RL1*), *IL-33*, *IL4R* and *IL-5*] were found to be associated with asthma risk and parameters such as blood eosinophil count, total IgE level and lung function [49, 50]. A number of studies have investigated asthma-related single-nucleotide polymorphisms (SNPs) in major histocompatibility genes that play a role in antigen presentation. Multiple SNPs in *HLA-DRA*, *HLA-DRB1*, *HLA-DQB1*, *HLA-DQA1* and *HLA-DQA2* were strongly associated with asthma susceptibility [51, 52[■]]. Significant evidence from asthma genetic studies has been established; however, the proposed genetic markers for asthma need to be well replicated, and the roles of these loci in asthma pathogenesis with gene–gene and gene–environment interactions should be clarified to predict asthma onset, asthma phenotypes, severity and response to treatments in clinical practice.

CONCLUSION

Many biologic agents that target Th2-mediated inflammations are being investigated for asthma treatment based on biomarkers; however, they

remain to be validated. Moreover, no molecular-targeted treatment or biomarkers are being actively investigated for other types of asthma such as neutrophilic asthma or AERD. Potential biomarkers (that are reproducible, noninvasive and uninfluenced by treatments) need to be developed, and significant efforts should be made to find reliable biomarkers that can establish a personalized treatment based on asthma phenotypes and endotypes.

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Conflicts of interest

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There were no conflicts of interest.

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