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Biomarkers for Diagnosis and Management of Eosinophilic Asthma

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Abstract

Asthma is a complex chronic airway disease with several distinct phenotypes characterized by different immunopathological pathways, clinical presentation, physiology, comorbidities, biomarker of allergic inflammation and response to treatment. Approximately 10% of patients with asthma have severe refractory disease, which is difficult to control on high doses of inhaled corticosteroids and long-acting beta2-agonists. About 50% of these individuals suffer from eosinophilic asthma. Eosinophilic asthma is a phenotype of asthma that is severe and persistent, with frequent exacerbations. It is associated with comorbidities such as chronic rhinosinusitis and nasal polyps. Laboratory findings include high sputum and blood eosinophil counts, high serum levels of perisotin and dipeptidyl petiptidase-4; and high levels of fractional exhaled nitric oxide. The T helper 2 cytokines, Interleukin-5 (IL-5), IL-4, IL-13, IL-25 and Thymic Stromal Lymphopoietin (TSLP), play a very important role in the pathogenesis of eosinophilic asthma. They are responsible for eosinophilic airway inflammation, hyper responsiveness and airway remodeling. Biomarkers such as sputum and blood eosinophil counts, fractional expired nitric oxide, serum periostin, dipeptidyl peptidae-4 and osteopontin are currently been used to diagnose eosinophilic asthma. This permits personalized therapies targeted at the inflammatory cytokines. Patients with steroid-resistant eosinophilic asthma respond favorably to biologics targeted against IGE (omulizumab), IL-5 (mepolizumab, reslizumab and benralimab), IL-4/13 (dupilumab) and TSLP (tezepemumab). Biologics are effective in achieving disease control, reducing exacerbations, improve the quality of life and have the advantage of steroid-sparing.

Keywords: Eosinophilic asthma; Biomarkers; Interleukins; Monoclonal antibodies

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Introduction

Asthma is a complex chronic airway disease with several distinct phenotypes characterized by different immunopathological pathways, clinical presentation, physiology, comorbidities, biomarker of allergic inflammation and response to treatment [1-4]. It has now become common practice to phenotype asthma for precision and targeted treatment, because asthmatic patients respond to the standard treatment differently [5]. There are several proposed distinct clinical phenotypes of asthma, such as childhood-onset allergic asthma, adult-onset eosinophilic asthma, neutrophilic asthma, paucigranulocytic asthma, Exercise-Induced Asthma (EIA), obesity-related asthma and Aspirin-Exacerbated Respiratory Disease (AERD) [6-13]. Eosinophilic asthma is a severe, persistent phenotype of asthma characterized by recurrent exacerbations, hospitalizations and poor response to the standard treatment, including high doses of Inhaled Corticosteroids (ICS), Long-Acting β2-Agonists (LABAs) and/or other modifier [6,8,10,14-16]. Patients with eosinophilic asthma are among the 5% to 10% of patients classified as having severe refractory asthma [10-12]. Guidelines on the definition, evaluation and treatment of severe refractory asthma are discussed in detail by the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Health Organization (WHO) [14-16].

Eosinophilic Asthma

Eosinophilic asthma is one of the well-defined clinical phenotypes of asthma [17-19,20]. Eosinophilic asthma is mostly observed in adult asthmatic patients after 20 years or later, although it may occur in children [19,20]. It is a severe and persistent disease, with frequent exacerbations, worse quality of life and has a poor prognosis [18-22]. Eosinophilic asthma is associated with more urgent visits to emergence rooms, hospitalizations and intubations and a history of a near-fatal asthma in about 23% of the patients [23,21]. Patients with eosinophilic asthma experience persistent airflow limitation, air trapping [24] and severe symptoms despite the use of high-dose Inhaled

Table 1: Clinical and diagnostic features of neutrophilic asthma.

Late on-set, most cases after 12 years
More atopic than neutrophilic asthma
More severe exacerbations compared to neutrophilic asthma
Comorbidities: chronic rhinosinusitis, nasal polyps, AERD, EIA
Sputum eosinophil count >2%-3%
High FeNO >30 ppb
High periostin levels - indicator of IL-13 inflammatory activity
High dipeptidyl peptidase-4 level >250 ng/L - indicator of IL-4 and IL-13 inflammatory responses
Subepithelial basement membrane thickness - indicator of IL-13 and IL-25 inflammatory responses
Severe airway obstruction (low FEV1)
Hyper responsiveness to methacholine bronchoprovocation tests
Corticosteroid responsiveness
Good response to biologics

Abbreviations: IL: Interleukin; FeNO: Fractional exhaled Nitric Oxide; ppb: parts per billion; FEV1: Forced Expired Volume in 1 sec; AERD: Aspirin-Exacerbated Respiratory Disease; EIA: Exercise-Induced Asthma

Corticosteroids (ICS) and Long-Acting Beta-Agonists (LABAs) [21,22,25]. They have frequent exacerbations and are often dependent on Oral Corticosteroids (OCS), but benefit from the targeted anti-interleukin biologics. Eosinophilic asthma is mostly associated with chronic rhinosinusitis and nasal polyps [26] and aspirin-exacerbated respiratory disease [27] which requires appropriate treatment in order to achieve adequate asthma control. The clinical and diagnostic characteristics of eosinophilic asthma are summarized in Table 1.

This subgroup of patients imparts a disproportionate pharmaco-economic burden, because the disease is very expensive to diagnose and treat, due frequent hospital visits, intubations and cost of the newly introduced targeted biologics [28,29].

Th2 Cytokines and Eosinophilic Asthma

The pathogenesis of eosinophilic asthma is complex. It involves imbalance between T-helper type 2 (Th2) lymphocytes and Th1 lymphocyte-driven airway inflammation, switching the balance towards the Th2 pathway [30-33]. Th2-driven eosinophilic inflammation leads to abnormal production of Th2 cytokines from Th2 cells and innate lymphoid cells [34,35]. The Th2 cytokines implicated in the pathogenesis of eosinonophilic asthma include interleukin-5 (IL-5), IL-4, IL-13, IL-25, IL-33 and Thymic Stromal Lymphopoitein (TSLP) [36-38]. Interleukin- IL-3, IL-4, IL-9, IL-13 are responsible for activation of mast cells and eosinophils [36]. Interleukin-5 stimulates production, proliferation and differentiation of eosinophils from myeloid progenitor cells in the bone marrow [39,40]. It also aids in the extrusion of eosinophils from the marrow. Peripherally, IL-5 participates in the terminal maturation of the eosinophil in the circulation. IL-5 is also important in recruitment and activation of eosinophils in the lungs and for eosinophil survival [36,40].

The "alarmin" cytokines, such as IL-25, IL-33 and TSLP, are also involved in orchestrating eosinophilic airway inflammation, hyper responsiveness, subepithelial fibrosis and airway remodeling [41,42]. Most important, is that, Th2 cytokines stimulates Th2 cells and other immunologic cells to produce more Th2 cytokines, chemokines and adhesion molecules which cause further recruitment and activation of eosinophils, basophils and mast cells. Activated mast cells and eosinophils further generate inflammatory mediators which perpetuate and amplify the airway inflammatory process and cause severe bronchospasm.

Eosinophil Mediators

Activated eosinophils either *via* allergic and non-allergic pathways, can undergo autolysis and release an array eosinophil-specific granule found in the extracellular DNA traps [43]. The most predominant bio-active mediators in the granules are the four cytotoxic cationic proteins, such as Major Basic Protein (MBP), Eosinophil Cationic Protein (ECP), Eosinophil-Derived Neurotoxin (EDN), Eosinophil-Derived Peroxide (EDPX) and Reactive Oxygen Species (ROS) [44-48]. Major basic protein, ECP and EDPX are toxic to a number of cells, including airway epithelial cells [47,48] and may contribute to airway inflammation, hyper responsiveness and airway remodeling. Eosinophil-derived neurotoxin is toxic to nerves [49], whereas eosinophil-derived peroxidase produces reactive oxygen species and reactive nitrogen intermediates which promote oxidative stress in tissue, causing cell death by apoptosis and necrosis [44].

Eosinophils also release a plethora of inflammatory mediators, including lipid-derived mediators, namely cysteinyl leukotrienes, prostaglandins, thromboxane and Platelet-Activating Factor (PAF) [50], chemokines [51,52], cytokines [51,53] and growth factors [54]. An endless list of cytokines synthesized and released by eosinophils include several Th2 and ILC2 cytokines, such as IL-3, IL-4, IL-5, IL-9, IL-13, IL-15, IL-23, IL-25, IL-33, GM-CSF, TNF-α and GM-CSF (Table 2). The above mediators and those secreted by Th2 cell act synergistically to cause and orchestrate eosinophilic airway inflammation and airway remodeling. Table 2 summarized mechanisms of bronchoconstriction in patients with eosinophilic asthma, which can also lead to production of measurable biomarkers.

The biomarker FeNO is produced by airway epithelial cells by inducible nitric oxide synthase upregulation during eosinophilic airway inflammation and can aid in the diagnosis of eosinophilic asthma. Interleukin-4 and Il-13 induce the expression of biomarkers, such as periostin and DPP-4 which can be measured in serum and assist in the differential diagnosis of eosinophilic asthma [38].

Biomarkers for the Diagnosis of Eosinophilic Asthma

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes,

Table 2: Mechanisms of airflow obstruction in patients with eosinophilic asthma.

Airway eosinophil recruitment, migration and activation
Release of eosinophilic cationic proteins
Release of Th2 cytokines, chemokines, and adhesion molecules
Airway epithelial damage and further release of cytokines
Mucus gland hyperplasia and hypersecretion
Release of growth factors
Airway hyper responsiveness
Subepithelial fibrosis
Airway smooth muscle proliferation
Airway remodeling
Corticosteroid resistance

pathogenic processes, or pharmacologic responses to a therapeutic intervention [55]. They are measurable substances used to examine organ function and other aspects of health [56]. There are several different phenotypes of asthma which respond differently to specific treatment, such as corticosteroids and the newly introduced biologics. Identification of specific biomarker for different phenotypes of asthma can provide information about the pathophysiology of the phenotype and lead possibility to a more targeted therapy or "precision medicine" [57]. Precision medicine recognizes that patients with different types of asthma have diverse immunopathological mechanisms, biomarkers and response to treatment, including biotherapeutics [58].

Biologics for the treatment of asthma are expensive; therefore attempts at identifying specific and easily obtainable biomarkers can help predict clinical responsiveness and are critical for precision targeted treatment [58]. In the diagnosis and management of patients with severe refractory asthma, biomarkers have been recommended to assess optimum ICS maintenance therapy, determine treatment adherence, guide selection of targeted therapies and predict and assess response to treatment [59]. Specific biomarkers useful for the diagnosis of eosinophilic asthma, include sputum and blood eosinophil counts, Fractional Exhaled Nitric Oxide (FeNO), serum periostin, Dipeptidyl Peptidase-4 (DPP-4) and osteopontin [58,60-65]. Table 3 shows the list readily available biomarkers used for the diagnosis of eosinophilic asthma in primary health care.

Sputum Eosinophil Count

Sputum eosinophil counts are usually calculated from induced sputum or Bronchoalvelolar (BAL) fluid and are expressed as a percentage of eosinophils from the total inflammatory cells [11]. The normal range is about 1% to 2% [66-69] and values greater than ≥ 3% indicate airway eosinophilia. Measurement of esinophils in induced sputum [62-65,69] and BAL fluid [70], has been shown to be a reliable biomarker of airway eosinophilic inflammation. Indeed, induced sputum is considered as the gold standard non-invasive method for assessing airway inflammation in asthma to identify inflammatory phenotypes [70]. The ERS/ATS guidelines suggest that induced sputum can be used in the management of severe asthma in specialized centers with a dedicated laboratory with experience in sputum induction technique [71].

Increased sputum eosinophil counts (≥3%) is associated with exacerbations in patients with severe asthma [72] and correlate partially with FeNO and blood eosinophil numbers [73]. Hastie et al. [74] in the Belgian severe asthma registry have shown a significant correlation between sputum eosinophil levels and blood eosinophil counts.

Table 3: Biomarkers for the diagnosis, and monitoring patients with eosinophilic asthma.

Induced sputum count
Bronchoalveolar Lavage (BAL) fluid cytometry
Fractional expired Nitric oxide (FeNO)
Serum periostin
Serum Dipeptidyl Dipeptidase-4 (DPP-4)
Serum osteopontin

Sputum eosinophil counts have been used to guide step-up/step-down treatment with corticosteroids and is a useful predictor of response to treatment with inhaled corticosteroids [75]. Increase in sputum eosinophil count after a stepwise reduction or discontinuation of ICS may be predictive of asthma exacerbation [76,77].

Sputum counts can also be useful in guiding response to anti-IgE [78] and interleukin antagonists in patients with eosinophilic asthma [79]. Anti-IL-5 monoclonal antibodies, such as mepolizumab [80,81], and reslizumab, [82] decreased exacerbations and improved quality of life in patients with sputum eosinophilia greater than 3%. Similarly, dupilumab, a monoclonal antibody against IL-4Rα that modulates the IL-4/13 inflammatory pathway improved asthma control and lung function in asthmatic patients with sputum eosinophilia (≥ 3%) or blood eosinophil count (≥ 300 cells·μL-1) [83]. In In summary, induced sputum count has been very useful in the diagnosis of eosinophilic asthma and in monitoring response to ICS and interleukin antagonists; and in the follow-up of the patients. The list of approved biologics and in clinical trials is shown in Table 4.

Blood Eosinophil Count

Blood eosinophil count is an established biomarker of severe eosinophilic asthma [84,85]. Patient with eosinophilic asthma have a raised peripheral blood eosinophilia (≥ 300 cells/μL^{-1}; 0.300×10^9); and other biomarkers, including high expression of Th2 cytokines (Il-4, IL5 and IL-13) [86]. High blood eosinophil counts correlate with poor asthma control, increased risk of exacerbations and re-hospitalization [84,87-89]. Patients with eosinophilic asthma have severe airflow obstruction and an enhanced longitudinal decline in lung function [90,91]. They exhibiting both local and systemic eosinophilic inflammation and have more severe asthma reflected

Table 4: Monoclonal antibodies, and interleukin receptor antagonists, and their target.

Agent	Target	Stage of Development
Omalizumab	IgE	Marketed 2003
Mepolizumab	IL-5	Marketed 2015
Reslizumab	IL-5	Marketed 2016
Benralizumab	IL-5R	Marketed 2017
Dupilumab	IL-4α (IL-4/IL-13)	Marketed 2018
Tezepelumab	TSLP	Marketed 2018
Pitrakinra	IL-4α (IL-4/IL-13)	II
Lebrikizumab	IL-13	III
Tralokinumab	IL-13	III
Brodalumab	IL-17RA	II
Secukinumab	IL-17A	II
Fezakinumab	IL-22	II

by lower baseline pulmonary function (FEV1) [92]. They have poor asthma control and quality of life; and have a greater number of exacerbations in the year preceding measurement of blood eosinophil count and FeNO [84,87]. Price et al. [84] analysis of the Optimum Patient Care Database (OPCRD) in the UK, patients with a high blood eosinophil count (≥ 0.300 × 10^9/μL) and higher FeNO (≥ 50 ppb) were four-times more likely to have severe exacerbations compared with patients with low eosinophil count and low FeNO [84]. Therefore, combination of blood eosinophil count and FeNO may be even a stronger marker of exacerbation risk compared with individual biomarker [84]. Both markers may be required to select patients with poor sensitivity to ICS who may require a more targeted, personalized approach of therapy [84]. Konradsen et al. [93] have also reported that pediatrics patients with blood eosinophilia (≥ 300 cells/μL^{-1}) have more severe asthma, more exacerbations, lower FEV1/FVC ratios and airway hyper responsiveness.

There is a high correlation between blood eosinophil levels and sputum eosinophil counts and both exhibit the highest accuracy in the diagnosis of eosinophilic asthma [74,94]. Blood eosinophil count has been recommended by the ERS/ATS as a surrogate biomarker of airway eosinophilia, because quantification is much simpler, inexpensive and requires fewer resources [95]. It can serve as a prognostic biomarker and predict response to several therapeutic interventions in asthmatic patients with type 2 inflammation [96,97].

A baseline blood eosinophil threshold of 150 cells/μL^{-1} or greater or a historical blood eosinophil threshold of ≥ 300 cells/μL^{-1} or greater will allow selection of patients who are more likely to achieve clinically significant improvement with treatment with corticosteroids and anti-interleukin monoclonal antibodies (IL-4, IL-5, IL-13 and TSLP) [97].

Blood eosinophil counts can also predict responsiveness to ICS in atopic children with blood eosinophilia (≥ 300 cells/μL^{-1}) [98]. It has shown to monitor the response to corticosteroid because the adjustment of dose to maintain blood eosinophil (≥ 200 cells/μL^{-1}) was successful in preventing exacerbations, improved asthma and resulted in less prednisone use [98]. It can also be used to guide step-up/step-down treatment with corticosteroids [99].

Baseline blood eosinophil count is useful as a biomarker to stratify patients for treatment with interleukin monoclonal antibodies, such as anti-IL-4 antibodies for mepolizumab [100,101] and reslizumab [102] anti-IL-5 receptor antibody for benralizumab [103] and anti-IL-4 receptor antibody for dupilumab [104]. The cut-off of blood eosinophils count is 300 cells/μL^{-1} for most biologics, except the reslizumab (400 cells/μL^{-1}) [102]. There is a range of responses at different blood eosinophil levels and patients with higher blood eosinophil levels (≥ 300 cells/μL^{-1}) tending to have a better response to treatment [101,102]. In most clinical trials, patients with eosinophilic asthma showed improvement in asthma control, rate of exacerbation, pulmonary function (FEV1) and quality of life.

Blood eosinophilia is a superior assessment for detection of airway eosinophilia in patients with asthma and can be used to stratify the different asthma phenotypes, for targeted therapy with interleukin antagonists [105-107]. Although blood eosinophil count is easy to obtain and correlates well with sputum eosinophilia, the optimal threshold has yet to be standardized. Furthermore, eosinophil levels may be elevated due to co-existing conditions such as hypereosinophilic syndromes and parasitic infestations, such as helminthiases, schistosomiasis and filariases [38].

Fractional Exhaled Nitic Oxide

Nitric oxide plays an important role in lung immunophysiology. It is a bronchodilator and an inflammatory mediator. Gas phase nitric oxide is produced in the lung by Nitric Oxide Synthase (NOS) during the conversion of the amino acid L-arginine to L-citrulline [108]. The biomarker Fractional Exhaled Nitric Oxide (FeNO) is produced by airway epithelial cells by inducible nitric oxide synthase upregulation during allergic inflammation [108]. The levels of FeNO reflect indirectly the inflammatory responses in the airways [109,110]. FeNO is a useful method for indirectly assessment of eosinophilic airway inflammation in adults [109,111-113] and in children [114].

In patients with eosinophilic asthma, FeNO levels correlates with airway hyper responsiveness and the risk of severe exacerbation [113,115,116]. FeNO concentration greater than 50 ppb is a marker for eosinophilic airway inflammation and predicts the likelihood to respond to corticosteroids [109,117]. Moreover, high levels of FeNO concentration are considered a risk for exacerbations and poor disease control in adult patients treated with ICS [113,118-120] and may identify patients with poor response to ICSs who may benefit from targeted personalized biotherapeutics.

Although both FeNO concentration and blood eosinophil count are elevated in patients with eosinophilic asthma, they only show modest correlation reflecting different activation of the Th2-driven inflammatory pathways [113,121]. Price et al. [113] using patients' data from the Optimum Patient Care Database (OPCRD) in the UK, 122 have reported that patients with higher FeNO (≥ 50 ppb) and a high blood eosinophil count (≥ 300 cells/μL^{-1}) were four-times more likely to have had severe exacerbations compared with patients with low FeNO (<25 ppb) and low eosinophil levels (≥ 300 cells/μL^{-1}) in the year preceding the FeNO readings. They have demonstrated that the combination of high blood eosinophil count and FeNO may be even a stronger marker of exacerbation risk in patients with eosinophilic asthma compared with individual biomarkers. Because of the variations in the measurements of FeNO, the American Thoracic Society (ATS) suggests that it should be used to complement other biomarkers in the diagnosis of eosinophilic asthma.

The ATS cutoff of FeNO is commonly used in clinical practice [109]. The high FeNO cutoff has been set at ≥ 50 ppb and the low cutoff at <25 ppb [109,117,118]. The ATS recommend FeNO thresholds of 25 ppb to 50 ppb in adults and 20 ppb to 35 ppb in children and the results should to be interpreted with caution and with reference to the clinical context [109].

Fractional exhaled nitric oxide can be used to diagnose steroid-responsive disease and guide asthma management in routine care [109,119]. FeNO can also be used to stratify patients who are more likely to respond to interleukin monoclonal antibodies. Patients with an FeNO level ≥ 50 ppb have been shown to have a positive response to mepolizumab[100] and to benralizumab [123].

Fractional exhaled nitric oxide is a useful surrogate biomarker of eosinophilic airway inflammation and offers the advantage of being non-invasive and easy to obtain [124]. The National Institute for Health and Care Excellence [125] and the British Thoracic Society [126], recommend FeNO measurements to guide diagnosis and treatment of eosinophilic asthma. In Great Britain primary care practices, FeNO monitoring is being used to guide decision on ICS

usage or step-up therapy. In addition, the 2019 Global Initiative for Asthma strategy report recommends the use of FeNO and/or blood eosinophil count to determine the phenotype of asthma and for selection of biologics for personalized guided treatment [127]. Thus, composite, non-invasive biomarkers, such as FeNO and easily obtainable blood eosinophil count may provide insight into a patient's risk of exacerbations as well as guide asthma treatment [113,119].

Serum Periostin

Periostin, also termed as osteoblast-specific factor 2, is an Extracellular Matrix (ECM) protein belonging to the fascilin 1 family, [128-131] with a molecular weight of about 90-kDa [131,132]. Periostin acts as a matrix protein involved in cell activation by binding to its specific receptors and several integrins (av β3, av β5, a4β6 and aMβ2) [133,134], which are promigratory periostin receptors [134]. Periostin has been implicated in many multisystem diseases [32,135-138] and cancer [139,140].

Periostin is mainly produced from the basolateral membranes of airway epithelial cells [141] and to a lesser extent from the lung fibroblasts [131,132,141] and its secretion is stimulated by IL-13 and IL-4. Periostin plays an important role in the pathogenesis of allergic inflammation, especially Th2-driven eosinophilic asthma [130,131,133]. Several studies have reported high serum levels of periostin in patients with eosinophilic asthma compared to health control subjects [141-144] and the increase in periostin levels correlated to the gradual decline in lung function (FEV1) [143,144].

Noteworthy, serum periostin levels have been suggested as surrogate markers of Th2-driven eosinophilic airway inflammation [145-150]. In the BOBCA study of several biomarkers in eosinophilic asthma, Jia et al. [147] reported that, periostin was the best predictor of airway eosinophilia in patients with severe asthma that was uncontrolled despite maximal ICS treatment. Serum periostin levels show good correlations with blood or sputum eosinophilia [149] and with FENO. Serum periostin levels can be used as a composite marker to identify severe, steroid-insensitive asthma [150].

Dipetidyl Peptidase-4

Dipeptidyl Peptidase-4 (DPP-4) also known as cluster differentiation antigen 26 is a glycoprotein with a molecular weight of about 110,000 and composed of 766 amino acid residues [151]. It is a serine exopeptidase belonging to the S9B family that cleaves the X-proline dipeptides from the N-terminal of polypeptides such as chemokines, neuropeptides and peptide hormones [152,153]. DPP-4 is expressed in the membranes of specialized cells in the liver, kidney, spleen, pancreas and lungs [154]. It is strongly expressed in adipocytes, endothelial cells, epithelial cells, 4 and various immune cells including macrophages, T cell, B cells, dendritic cells and Invariant Natural Killer Cells (INKT) cells [151,154-156].

A catalytic active form of DPP-4 is detectable in body fluids and can be measured in serum and used as a biomarker for the diagnosis of eosinophilic asthma and other diseases [156]. The enzyme degrades several chemokines, neuropeptides and hormones which expand its physiological and immunopathological actions [152,153]. Apart for its role in participating in the pathogenesis of eosinophilic asthma, it is implicated in several diseases and disorders, including cardiovascular disease [157], several cancers [158], obesity and type 2 diabetes mellitus [152,159,160].

Upregulation of DPP-4 may regulate immunological pathways implicated in asthma by inactivating chemokines and growth factors involved in the pathogenesis of asthma. Similar to periostin, DPP-4 is induced by IL-13 and other profibrotic agents and can be measured in serum, making it a potential biomarker to guide IL-13 mAb therapy in patients with eosinophilic asthma [161]. Brightling et al. [162] in phase 2b tralokinumab clinical trial, have reported that increased serum DPP-4 levels predict a beneficial response to tralokinumab, an anti-IL-13 mAb, in terms of alleviating symptoms, reducing exacerbations and improving pulmonary function. James et al. [163] have shown that serum DPP-4 levels did not correlate with FEV1, FeNO, blood or sputum eosinonils or IgE in asthmatic patients from the U-BIOPRED and BIOAIR studies. This may indicate that periostin is associated with other Th2 pathways in the pathogenesis of eosinophilic asthma. Serum periostin may be suitable as a composite biomarker in addition to other markers of airway eosinophilia.

Streicher et al. [164] have provided a method of treating IL-13 mediated diseases or disorders comprising administration of anti-IL-13 antagonists. They recommend initiation of anti-IL-13 biologics in patients whose blood DPP-4 is above threshold (>250 ng/ml) in patients with eosinophilic asthma and other IL-13 mediated diseases [165].

The potential of DPP-4 in the pathogenesis of eosinophilic asthma is of clinical importance because of the increasing use of DPP-4 inhibitors, such as alogliptin, saxagliptin, sitagliptin and vildagliptin in the management of patients with type 2 diabetes mellitus, some of whom may have concomitant asthma. Colice et al. [166] reported that there was no difference in asthma control between asthmatic patients initiated on DPP-4 inhibitors and patient not on the inhibitors. We recommend that, patients with type 2 diabetes mellitus, metabolic syndrome and cardiovascular disease coexisting with asthma, should have their serum DPP-4 levels determined before initiating DPP-4 inhibitors.

Osteopontin

Osteopontin (OPN) also known as early T lymphocyte activation 1 is a 44-kD extracellular phosphorylated acidic matrix glycoprotein, which binds to proteins and several types of matrix collagen [167,168]. It is involved both in Th1 [168] and Th2 [169] immunological responses and is implicated in several physiological and pathological conditions.

Osteopontin is produced by several types of immune cells, such as macrophages, mast cells, neutrophils, eosinophils, T cells and natural killer cells [170-172]. It is also produced by structural cells, such as epithelial cells, fibroblasts and Airway Smooth Muscle (ASM) cells [173]. Osteopontin functions as a multifunctional cytokine and is expressed by several cells, such as epithelial cells, macrophages, ASM cells and T cells [174,175].

Osteopontin plays an important role in recruitment and migration of eosinophils into the asthmatic airways [172,176]. It is also responsible for the migration and degranulation of mast cells in the airways [170]. OPN is involved in pulmonary fibrosis by regulating the extracellular matrix protein interactions and by modulating Transforming Growth Factor-1β (TGF-β) and Metalloproteinase (MMP) expression [168,177,178]. It plays an important role in inducing the expression of cytokines which are involved in airway inflammation and tissue repair in lung diseases [175,179].

In murine model of asthma, Kohan et al. [180] have shown that OPN may modulate lung fibroblasts phenotype by direct

activation of these cells, or *via* an indirect effect that involves altered airway inflammation and the expression of mediators, such as TGF-β1, MMP-2 and Th2 cytokines, namely IL-13 and IL-4. These proinflammatory mediators are known to promote subepithelial fibrosis, airway remodeling and severe eosinophilic asthma.

Samitas et al. [181] have reported that BAL fluid and serum OPN levels were significantly increased in patient with mild-to-severe asthma; and OPN expression was up-regulated in epithelial cells, myofibroblasts, vascular smooth muscle cells, mast cells and T lymphocytes. Their study also revealed that OPN expression in bronchial biopsies correlated with reticular basement membrane thickness and was more pronounced in patients with severe asthma compared to mild-to-moderate asthma and healthy controls. Subepithelial basement membrane thickness was inversely correlated with lung function (FEV1), thus indicating severe airflow obstruction [181].

Demimpoura et al. [174] have demonstrated significantly higher levels of induced sputum OPN in patients with severe refractory asthma than in those with mild-to-moderate asthma and healthy subjects. Sputum OPN levels correlated with serum levels of profibrotic cytokines, such as IL-13 and TGF-β1 which are associated with intense inflammation; fibroblasts and ASM cells proliferation; leading to subepithelial fibrosis and airway remodeling [174].

Similarly, other studies have reported significantly higher serum OPN levels in adult patients with asthma, compared to healthy controls [143,176,181,182]. Kanemitsu et al. [143] have reported that high levels of osteopontin and periostin were associated with a gradual decline in lung function over 20 years.

The above studies indicate the importance of oesteopontin in promoting the expression of cytokines and growth factors which lead to subepithelial fibrosis, ASM cell proliferation and airway remodeling. Osteopontin has a diagnostic, prognostic and therapeutic potential in the management of patients with eosinophilic asthma [177], particularly when used with other backbone biomarkers, such as induced sputum and blood eosinophil counts.

Demimpoura et al. [174] have shown that subepithelial cells in bronchial tissue express more OPN in patients with severe refractory asthma compared to patients with mild-to-moderate asthma or healthy controls. Similarly, the subepithelial basement membranes were significantly thicker in patients with severe asthma compared to mild-to moderate asthma and healthy controls. Subepithelial basement membrane thickness was inversely correlated with lung function (FEV1), thus indicating severe airflow obstruction. Demimpoura et al. have also demonstrated significantly higher levels of induced sputum OPN in patients with severe refractory asthma than in those with mild-to-moderate asthma and healthy subjects. Sputum OPN levels correlated with serum levels of profibrotic cytokines, such as IL-13 and TGF-β1 which are associated with intense inflammation; fibroblasts and ASM cells proliferation, which lead to subepithelial fibrosis, airway remodeling and severe steroid-resistant eosinophilic asthma [174].

Silila some studies have also reported an increase in serum OPN levels in adult patients with asthma, compared to healthy controls [143,176,182]. Kanemitsu et al. [143] have reported that high levels of osteopontin and periostin are associated with a gradual decline in lung function over 20 years. Osteopontin has a diagnostic, prognostic and therapeutic potential [177], particularly when used with other backbone biomarkers of eosinophilic asthma, such as induced sputum and blood eosinophil counts.

Conclusion

Eosinophilic asthma is a phenotype of asthma that is severe and persistent, with frequent exacerbations and hospitalizations. Laboratory findings reveal high sputum and blood eosinophil counts, high serum levels of perisotin and dipeptidyl petiptidase-4; and elevated levels of fractional exhaled nitric oxide. Th2 cytokines, such as IL-5, IL-4, IL-13, IL-25 and thymic stromal Lymphopoitein, play a key role in the pathogenesis of eosinophilic asthma. They are responsible for eosinophilic airway inflammation, hyper responsiveness and airway remodeling. Biomarkers such as sputum and blood eosinophil counts, fractional expired nitric oxide, serum periostin, dipeptidyl peptidae-4 and osteopontin are currently been used to diagnose and guide therapy in patients with eosinophilic asthma. This has modernized the use of biologic therapies targeted at the inflammatory cytokines. Patients with steroid-resistant eosinophilic asthma respond favorably to monoclonal antibodies targeted against Ig-E (omulizumab), IL-5 (mepolizumab, reslizumab and benralimab), IL-4/13 (dupilumab) and TSLP (tezepemumab). Biologics are effective in achieving disease control, reducing exacerbations, improve the quality of life and have the advantage of being steroid-sparing.

References

1. The Global Asthma Network. The Global Asthma Report 2014. 2018.
2. Masoli M, Fabian D, Holt S, Beasley R. Global Initiative for Asthma (GINA) Program. The global burden of asthma: Executive summary of the GINA dissemination committee report. Allergy. 2004;59(5):469-78.
3. National Asthma Education and Prevention Program. Expert Panel Report 3 (EPR 3): Guidelines for the diagnosis and management of asthma - a summary report 2007. J Allergy Clin Immunol. 2007;120(5 Suppl):S94-S138.
4. Asher MI, Montefort S, Bjorksten B, KW Lai C, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phase one and three repeat multi-country cross-sectional surveys. Lancet. 2006;368:733-43.
5. Chung KF. Asthma phenotyping: A necessity for improved therapeutic precision and new targeted therapies. J Intern Med 2016;279(2):192-204.
6. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudue JB, Gibbs RL. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med. 1999;160(3):1001-8.
7. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology. 2006;11(1):54-61.
8. Moore W, Bleecker E, Curren-Everett D, Erzurum S, Ameredes BT, Bachier L, et al. National heart, lung, and blood institute's severe asthma research program. Characterization of the severe asthma phenotypes by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. J Allergy Clin Immunol. 2007;119(2):405-413.
9. Anderson GP. Endotypying asthma: New insights into key pathogenic mechanism in a heterogenous disease. Lancet. 2008;372:1107-19.
10. Moore WC, Meyer DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2010;181(4):315-23.
11. Wenzel SE. Asthma phenotypes: The evolution from clinical to molecular

approach. Nat Med. 2012;18(5):716-25.

12. Siroux V, González JR, Bouzigon E, Curjuric I, Boudier A, Imboden M, et al. Genetic heterogenicity of asthma phenotypes identified by a clustering approach. Eur Resp J. 2014;43(2):439-52.

13. Sutherland ER, Basagana X, Boudier A, Goleva E, King TS, Lehman E, et al. Cluster analysis of obesity and asthma. Phenotypes. PLoS One. 2012;7(5).e36631.

14. Proceeding of the ATS workshop on refractory asthma: Current understanding, recommendations, and unanswered questions. American Thoracic Society. Am J Respir Crit Care Med. 2000;162(6):2341-51.

15. Chung K, Godard P, Adelroth E, Ayres J, Barnes N, Barnes P, et al. Difficult/therapy-resistant asthma: The need for integrated approach to define clinical phenotypes, evaluate risk factors, understand pathophysiology and find novel therapies. Eur Respir J. 1999;13:1198-208.

16. Bousquet J, Mantzouranis E, Cruz AA, Aït-Khaled N, Baena-Cagnani CE, Bleecker ER, et al. Uniform definition of asthma severity, control, and exacerbations: Document presented for the World Health Organization Consultation on Severe Asthma. J Allergy Clin Immunol. 2010;126:926-38.

17. Pavord ID. Eosinophilic phenotypes of airway disease. Ann Am Thorac Soc. 2013;10:S143-S9.

18. Buhl R, Humbert M, Bjermer L, Chanez P, Heaney LG, Pavord I, et al. Severe eosinophilic asthma: A roadmap to consensus. Eur Respir J. 2017;49(5):1700634.

19. Miranda C, Busacker A, Balzar S, Trudeau J, Wenzel SE. Distinguishing severe asthma phenotypes: Role of onset and eosinophilic inflammation. J Allergy Clin Immunol. 2004;113:101-08.

20. de Groot JC, ten Brinke A, Bel EHD. Management of patients with eosinophilic asthma: A new era begins. ERJ Open Res. 2015;1(1):00024-2015.

21. Wenzel SE, Fahy J, Irvin C, Peters S, Spector S, Szefler S, et al. (Writing committee). Proceeding of the ATS on refractory asthma: Current understanding, recommendations, and unanswered questions. Am J Respir Crit Care Med. 2000;162(6):2341-51.

22. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014;43:343-73.

23. Moore W, Bleecker E, Curren-Everett D, Erzurum S, Emeredes BT, Bachier L, et al. National heart, lung, and blood institute's severe asthma research program. Characterization of the severe asthma phenotypes by the national heart, lung, and blood institute's severe asthma research program. J Allergy Clin Immunol. 2007:119(2):405-13.

24. ten Brinke A, Zinderman AH, Sterk PJ. Factors associated with persistent airflow limitation in severe asthma. Am J Respir Crit Care Med. 2014;113:19-24.

25. Price D, Fletcher M, van der Molen T. Asthma control and management in 8,000 European patients: The Recognise Asthma and Link to Symptoms and Experience (REALISE) survey. NP J Prim Care Respir Med. 2014;24:14009.

26. de Groot JC, Storm H, Amelink M, de Nijs SB, Eichom E, Reitsm BH, et al. Clinical profile of patients with adult-onset eosinophilic asthma. ERJ Open Res. 2018;2:00100-2015.

27. Szczeklik A, Stevenson DD. Aspirin-induced asthma: Advances in pathogenesis, diagnosis and management. J Allergy Clin Immunol. 2003;111:913-21.

28. Department of Health. NHS atlas of variation in healthcare for people with respiratory disease. Department of Health. 17 July, 2011.

29. O'Neill S, Sweeney J, Patterson CC, Menzies-Gow A, Niven R, Adel Mansur H, et al. The cost of treating severe refractory asthma in the UK: An economical analysis from the British Thoracic Society Difficult Asthma Registry. Thorax. 2015;70:376-78.

30. Kim HY, DeKruyff RH, Umetsu DT. The main path of asthma: Phenotypes shaped by innate and adaptive immunity. Nat Immunol. 2010;11:577-584.

31. Moore WC, Fitzpatrick M, Li X, Hastie AT, Huashi Li, Deborah Meyers A. Clinical heterogenicity in the severe asthma research program. Ann Am Thorac Soc. 2013;10:S118-24.

32. Siroux V, Gonzalez JR, Bouzigon E, Curjuric I, Boudier A, Imboden M, et al. Genetic heterogenicity of asthma phenotypes identified by a clustering approach. Eur Resp J. 2014;43:439-452.

33. Wenzel SE. Asthma: Defining of persistent adult phenotypes. Lancet. 2006;368(9537):804-13.

34. Schleich FN, Chevremont A, Paulus V, Henket M, Manise M, Seidel L, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. Eur Respir J. 2014;44(1):97-108.

35. Chung KF. Targeting the interleukin pathway in the treatment of asthma. Lancet. 2015;386:1086-96.

36. Barnes PJ, Chung KF, Page CP. Inflammatory mediators in asthma: An update. Pharmacol Rev. 1998;50(4):515-96.

37. Barnes PJ. Th2 cytokines and asthma: An introduction. Respir Res. 2001;2(2):64-5.

38. Syabbalo N. Clinical features and management of eosinophilic asthma. J Respir Dis Treat. 2020;1:105.

39. Clutterbuck E, Hirst E, Sanderson C. Human Interleukin-5 (IL-5) regulates the production in bone marrow cultures: Comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. Blood. 1989;73(6):1504-12.

40. Kouro T, Takatsu L. IL-5- and eosinophil-mediated inflammation: from discovery to therapy. Int Immun. 2009;21(12):1303-9.

41. Sharkhuu T, Matthael KL, Forbes E, Mahalingam S, Hogan SP, Hansbrow PM. Mechanism of Interlekin-25 (IL-17E)-induced pulmonary inflammation and airway hyper-reactivity. Clin Exp Allergy. 2006;36(12):1575-83.

42. Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. J Allergy Clin Immunol. 2013;132(3):676-85.

43. Lambrecht BN, Hamad H. Immunology of asthma. Immunol. 2015;16(1):45-6.

44. Gleich GJ. Mechanisms of eosinophil-associated inflammation. J Allergy Clin Immunol. 2000:105(4):651-63.

45. Trulson A, Byström J, Engström A, Larsson R, Venge P. The functional heterogeneity of eosinophilic cationic protein determined by a gene polymorphism and post-transitional modifications. Clin Exp Allergy. 2007;37(2):208-18.

46. Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, et al. Eosinophils: Biological properties and roles in health and disease. Clin Exp Allergy. 2008;38(5):709-50.

47. Gleich GJ. Eosinophil granule proteins and bronchial asthma. Annu Rev Med. 1993;44:85:101.

48. Young JD, Peterson CG, Venge P, Cohn ZA. Mechanisms of membrane damage by human eosinophil cationic protein. Nature. 1986;321(6070):613-6.

49. Rosenberg HF. Eosinophil-derived neurotoxin/RNase 2: Connecting the past, the present and the future. Curr Pharm Biotechnol. 2008;9(3):135-40.

50. Bandeira-Melo C, Bozza PT, Weller PF. The cellular biology of eosinophil eicosanoid formation and function. J Allergy Clin Immunol. 2002;109(3):393-400.

51. Giembycz MA, Lindsay MA. Pharmacology of the eosinophil. Pharmacol Rev. 1999;51:213-340.

52. Ying S, Meng Q, Zeibecoglou R, Robinson DS, Macfarlane A, Humbert M, et al. Eosinophil chemotactic chemokines (eotaxin-2, RANTES, Monocyte Chemoattractant Protein-3 (MCP-3), and MCP-4, and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. J Immunol. 1999;163(11):6321-9.

53. Spencer LA, Szela CT, Perez SA, Kirchhoffer CL, Neves JS, Radke AL, et al. Human eosinophils constitutively express multiple Th1, Th2 and immunoregulatory cytokines that are secreted rapidly and differentially. J Leukoc Biol. 2009;85(1):117-23.

54. Horiuchi T, Weller PF. Expression of vascular endothelial growth factor by human eosinophils: Upregulation by granulocyte macrophage colony-stimulating factor and interleukin-5. Am J Respir Cell Mol Biol. 1997;17(1):70-7.

55. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;63(3):89-95.

56. Lapraz ZJC, Hedayat KM, Pauly P. Endobiogeny: A global approach to biology (part2 of 2). Global Adv Health Med. 2013;2:33-44.

57. Frank R, Hargreave R. Clinical biomarkers in drug discovery and development. Nat Rev Drug Discov. 2003;2:566-80.

58. Casale TB. Biologics and biomarkers for asthma, urticaria, and nasal polyposis. J Allergy Clin Immunol. 2017;139(5):1411-21.

59. Fricker M, Heaney LG, Upham JW. Can biomarkers help us hit targets in difficult-to treat asthma? Respirology. 2017;22(3):430-2.

60. Wan XC, Woodruff PG. Biomarkers in severe asthma. Immunol Allergy Clin North Am. 2016;36(3):547-57.

61. Barminski G, Crossley M, Turcanu V. Novel biomarkers for asthma stratification and personalized therapy. Expert Rev Mol Diagn. 2015;15:415-30.

62. Schleich F, Sophie D, Renauld L. Biomarkers in the management of difficult asthma. Curr Top Med Chem. 2016;16(14):1561-73.

63. Berry A, Busse WW. Biomarkers in asthmatic patients: Has their time come to direct treatment? J Allergy Clin Immunol. 2016;137(5):1317-24.

64. Kim H, Ellis AK, Fischer D, Noseworthy M, Olivenstein R, Chapman KR, et al. Asthma biomarkers in the age of biologics. Allergy Asthma Clin Immunol. 2017;13:48.

65. Tiotiu A. Biomarker in asthma: state of the art. Asthma Res Pract. 2018;4:10.

66. Reddel HK, Taylor DR, Bateman ED, Boulet L-P, Boushey HA, Busse WW, et al. An official American thoracic society/European respiratory society statement: Asthma control and exacerbations. Am J Respir Crit Care Med. 2009;180(1):59-99.

67. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. Am J Respir Crit Care Med. 2000;161(2 Pt 1):475-8.

68. Spanevello A, Confaloneieri M, Sulatto F, Romano F, Balzano G, Miglioni GB, et al. Induced sputum cellularity. Am J Respir Crit Care Med. 2000;162(3):1172-74.

69. Fitzpatrick AM. Biomarkers of asthma and allergic airway disease. Ann Allergy Asthma Immunol. 2015;115:335-40.

70. Simpson JL, Scott R, Boyles MJ, Gibson PG. Inflammatory subtypes in asthma: Assessed and identification using sputum. Respirology. 2006;11(1):54-61.

71. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014;43:343-73.

72. Kupczyk M, ten Brinke A, Sterk PJ, Bel EH, Papi A, Chanez P, et al. Frequent exacerbations - distinct phenotype of severe asthma. Clin Exp Allergy: J Brit Soc Allergy Clin Immunol. 2014;44(2):212-21.

73. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, et al. External validation of blood Eososinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. Thorax. 2015;70(2)115-20.

74. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li H, Peters SP, et al. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. J Allergy Clin Immunol. 2010;125:1028-36.

75. Cowan DC, Taylor DR, Peterson LE, Cowan JO, Palmay R, Williamson A, et al. Biomarker-based asthma phenotypes of corticosteroid response. J Allergy Clin Immunol. 2015;135(4):877-83.

76. Leuppi JD, Salome CM, Jenkins CR, Anderson SD, Xuan W, Marks GB, et al. Predictive markers of asthma exacerbation during stepwise dose reduction of inhaled corticosteroids. Am J Respir Crit Care Med. 2001;163(2):406-12.

77. Deykins A, Lazarus SC, Fahy JV, Wechsler ME, Boushey HA, Chinchilli VM, et al. Sputum eosinophil count predict asthma control after discontinuation of inhaled corticosteroids. Am J Respir Crit Care Med. 2015;115(4):720-7.

78. Busse W, Spector S, Rosen K, Wang Y, Alpan O. High eosinophil count: A potential biomarker for assessing successful omalizumab treatment effects. J Allergy Clin Immunol. 2013;132(2):485-6.

79. Medrek SK, Parulekar AD, Hanania NA. Predictive biomarkers for asthma therapy. Curr Allergy Asthma Rep. 2017;17(10):69.

80. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): A multicentre, double-blind, placebo-controlled trial. Lancet. 2012;380(9842):651-9.

81. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med. 2009;360(10):973-84.

82. Castro M, Mathur S, Hargreave F, Boulet L-P, Xie F, Young J, et al. Reslizumab for poorly controlled, eosinophilic asthma: A randomized, placebo-controlled study. Am J Respir Crit Care Med. 2011;184(10):1125-32.

83. Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, et al. Dupilumab in persistent asthma with elevated eosinophil levels. N Engl J Med. 2013;368(26):2455-66.

84. Price DB, Bosnic-Anticevech S, Pavord ID, Roche N, Halpin DMG, Bjermer L, et al. Association of elevated fractional exhaled nitric oxide concentration and blood eosinophil count with severe asthma exacerbations. Clin Transl Allergy. 2019;9:41.

85. Karz LE, Gleich GJ, Hartley BF, Yancey SW, Ortega HG. Blood eosinophil count is a useful biomarker to identify patients with severe eosinophilic asthma. Ann Am Thor Soc. 2014;11(4):531-6.

86. Woodruff PG, Khashayar R, Lazarus SC, Janson S, Avila P, Boushey HA, et al. Relationship between airway inflammation, hyper responsiveness, and obstruction in asthma. J Allergy Clin Immunol. 2001;108(5):753-8.

87. Price DP, Rigazioa A, Campbell JD, Bleecker ER, Corrigan CJ, Thomas M, et al. Blood eosinophil count and prospective annual asthma disease burden: A UK cohort study. Lancet Respir Med. 2015;3(11):849-58.

88. Tran TN, Khatry DB, Ke X, Ward CK, Gossage D. High blood eosinophil count is associated with more frequent asthma attacks in asthma patients. Ann Allergy Asthma Immunol. 2014;113(1):19-24.

89. Kerkhof M, Tran TN, van den Berge M, Brusselle GP, Gopalan G,

Jones RCM, et al. Association between blood eosinophil count and risk of readmission for patients with asthma: Historical cohort study. PLoS ONE. 2018;13:e020114.

90. Hancox RJ, Pavord ID, Sears MR. Associations between blood eosinophils and decline in lung function among adults with and without asthma. Eur Respir J. 2018;51(4):1702536.

91. Nadif R, Siroux V, Cryszczyn MP, Ravault C, Pison C, Pin I, et al. Heterogeneity of asthma according to blood inflammatory pattern. Thorax. 2009;64(5):374-80.

92. Schleich FN, Chevremont A, Paulus VH, Henket M, Manise M, Seidel L, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. Eur Respir J. 2014;44(1):97-108.

93. Konradsen JR, Skantz E, Nordlund B, Lidegran M, James A, Ano J, et al. Predicting asthma morbidity in children using proposed markers of Th2-type inflammation. Pediatr Allergy Immunol. 2015;26:772-9.

94. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJM, Bel EH, et al. External validation of blood eosinophils in asthma. Thorax. 2015;70(2):115-20.

95. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014;43:343-73.

96. Eguiluz-Gracia I, Tay TR, Hew M, Escribese MM, Barker D, O'Hehir RE, et al. Recent developments and highlights in biomarker in allergic diseases and asthma. Allergy. 2018;73(12):2290-305.

97. Yancey SW, Keene ON, Alders FC, Ortega H, Bates S, Bleecker ER, et al. Biomarkers for eosinophilic asthma. J Allergy Clin Immunol. 2017;140(6):1509-18.

98. Fitzpatrick AM. Severe asthma in children: Lessons learned and future directions. Allergy Clin Immunol Pract. 2016;4(1):11-9.

99. Wark PA, McDonald VM, Gibson PG. Adjusting prednisone using blood eosinophils reduces exacerbations and improves asthma control in difficult patients with asthma. Respirology. 2015;20(8):1282-4.

100. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe asthma (DREAM): A multicenter, parallel, double-blind, randomsed, placebo-controlled, phase 3 trial. Lancet. 2012;380(9842):651-9.

101. Ortega HG, Yancey SW, Mayer B, Gunsoy NB, Keene ON, Bleecker ER, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: A secondary analysis of the DREAM and MENSA studies. Lancet Respir Med. 2016;4(7):549-56.

102. Bjemer L, Lemiere C, Maspero J, Weiss S, Zangrilli J, Germinaro M. Reslizumab for inadequately controlled asthma with elevated eosinophil levels: A randomized phase 3 study. Chest. 2016;150(4):89-98.

103. Bleecker ER, FitzGerald JM, Chanez P, Papi A, Weinstein SF, Barker P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β2-agonists (SIROCCO): A randomised, multicentre, placebo-controlled phase 3 trial. Lancet. 2016;388(10056):2115-27.

104. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab efficacy and safety in moderate-to-severe uncontrolled asthma. N Engl J Med. 2018;378(26):2486-96.

105. Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Sterk PJ, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: A systemic review and meta-analysis. Lancet Respir Med. 2015;3(4):290-300.

106. Zhang X-Y, Simpson JL, Powell H, Yang IA, Upham JW, Reynolds PN, et al. Full blood count parameters for the detection of asthma inflammatory phenotypes. Clin Exp Allergy. 2014;44(9):1137-45.

107. Westerhof GA, Korevaar DA, Amelink M, de Nijs SB, de Groot JC, Wang J, et al. Biomarkers to identify sputum eosinophilia in different asthma phenotypes. Eur Respir J. 2015;46(3):688-98.

108. Fitzpatrick AM. Biomarkers of asthma and allergic diseases. Ann Allergy Asthma Immunol. 2015;115(5):335-40.

109. Dweik RA, Boggs PB, Erzurum SC, Irwin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: Interpretation of exhaled nitric oxide level (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184(5):602-15.

110. Lane C, Knight D, Burge SS, Franklin P, Horak F, Legg J, et al. Epithelial inducible nitric oxide synthase activity is the major determinant of nitric oxide concentration in exhaled breath. Thorax. 2004;59(9):757-60.

111. Maleba M, Ragnoli B, Raaeli A, Tantucci C. Usefulness of exhaled nitric oxide and sputum eosinophils in the long-term control of eosinophilic asthma. Chest. 2008;134(4):733-9.

112. Gao J, Wu F. Association between fractional exhaled nitric oxide, sputum induction and peripheral blood eosinophil in uncontrolled asthma. Allergy Asthma Clin Immunol. 2018;14:21.

113. Price DB, Bosnic-Anticevich S, Pavord ID, Roche N, Halpin DMG, Bjermer L, et al. Association of elevated fractional exhaled nitric oxide concentration and blood eosinophil count with severe asthma exacerbations. Clin Transl Allergy. 2019;9:41.

114. Warke TJ, Fitch PS, Brown V, Taylor R, Lyons JDM, Ennis M, et al. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. Thorax. 2002;57(5):383-7.

115. Dupont LJ, Rochette F, Demedts MG, Verleden GM. Exhaled nitric oxide correlates with airway hyper responsiveness in steroid-naïve patients with mild asthma. Am J Respir Crit Care Med. 1998;157(3 pt 1):894-8.

116. Konradsen JR, Skantz E, Nordlund B, Lidegran M, James A, Ono J, et al. Predicting asthma morbidity in children using proposed markers of Th2-type inflammation. Pediatr Allergy Immunol. 2015;26:772-9.

117. Price DB, Buhl R, Chan A, Freeman D, Gardner E, Godley C, et al. Fractional exhaled nitric oxide as a predictor to inhaled corticosteroids in patients with non-specific respiratory symptoms and insignificant bronchodilator reversibility: A randomized controlled trial. Lancet Respir Med. 2018;6(1):29-39.

118. Gelb AF, Flyn Taylor C, Shuar CM, Gutierrez C, Zamel N. Role of spirometry and exhaled nitric oxide to predict exacerbations in treated asthmatics. Chest. 2006;129(6):1492-9.

119. Price D, Ryan D, Burden A, Ziegenweidt JV, Gould S, Freeman D, et al. Using fractional exhaled nitric oxide (FeNO) to diagnose steroid-responsive disease and guide asthma management in routine care. Clin Transl Allergy. 2013;3(1):37.

120. Zeiger RS, Schatz M, Zhang F, Crawford WW, Kaplan MS, Roth RM, et al. Elevated exhaled nitric oxide is a clinical indicator of future uncontrolled asthma in asthmatic patients on inhaled corticosteroids. J Allergy Clin Immunol. 2011;128(2):412-4.

121. Malinovschi A, Fon Seca JA, Jacinto T, Alving K, Janson C. Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma in National Health and Nutrition Examination Survey subjects. J Allergy Clin Immunol. 2013;132(4):821-27.e1-5.

122. Optimum Patient Care. Anonymised Data Ethics & Protocol Transparency (ADEPT) committee.

123. Castro M, Wenzel SE, Bleecker ER, Pizzichini E, Kunal P, Bussse WW, et al. Benralizumab, an anti-interleukin receptor monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: A phase 2b randomized dose-ranging study. Lancet Respir Med. 2014;2(11):879-90.

124. Sandrini A, Taylor DR, Thomas PS, Yates DH. Fractional exhaled nitric oxide in asthma: An update. Respirology. 2010;15(1):57-70.

125. Asthma: Diagnosis, monitoring and chronic asthma management. National Institute for Health and Care Excellence. NICE Guidelines. 2017.

126. Scottish Intercollegiate Network: British guidelines on the management of asthma. British Thoracic Society. 2016.

127. Global strategy for asthma management and prevention. Global Initiative for Asthma. 2019.

128. Takeshita S, Kikumo R, Tezuka K, Amann E. Osteoblast factor-2: Cloning of a putative bone adhesion protein with homology with insect protein fasciclin 1. Bioch J. 1993;294(Pt 1):271-8.

129. Conway SJ, Izuhara K, Kudo Y, Litvin J, Markwald R, Ouyang G, et al. The role of periostin in tissue remodeling across health and disease. Cell Mol Life Sci. 2014;71(7):1279-88.

130. Izuhara K, Arima K, Ohta S, Suzuki D, Inamitsu M, Yamamoto K. Periostin in allergic inflammation. Allergol Int. 2014;63(2):143-51.

131. Izuhara K, Matsumoto H, Ohta S, Ono J, Arima K, Ogawa M. Recent developments regarding periostin in bronchial asthma. Allergol Int. 2015;64(Suppl):S3-10.

132. Morra L, Reschsteiner M, Casagrande S, von Teichman A, Schraml P, Moch H, et al. Characteristerization of periostin isoform pattern in non-small cell lung cancer. Lung Cancer. 2012;76(2):183-90.

133. Izuhara K, Conway S, Moore BB, Matsumoto H, Holweg CTJ, Mathews JG, et al. Role of periostin in respiratory disorders. Am J Respir Crit Care Med. 2016;193(9):949-56.

134. Johansson MW, Annis DS, Mosher DF. Alpha(M)beta(2) integrin-mediated adhesion and motility of IL5 stimulated eosinnophils on periostin. Am J Respir Cell Mol Biol. 2013;48(4):503-10.

135. Mael-Ainin M, Abed A, Conway SJ, Dussaule JC, Chatziantoniou C. Inhibition of periostin expression protects against the development of renal inflammation and fibrosis. J Am Soc Nephrol. 2014;25:1724-36.

136. Arima K, Ohta S, Tagaki A, Shiraishi H, Masuoka M, Ontsuka K, et al. Periostin contributes to epidermal hyperplasia in psoriasis common to atopic dermatitis. Allergol Int. 2015;64(1):41-8.

137. Huang Y, Yiu W, Xiao H, Maitikabili A, Lin Q, Wu T, et al. Matricellular periostin contributes to hepatic inflammation and fibrosis. Am J Pathol. 2015;185(3):786-97.

138. Uchida M, Shiraishi H, Ohta S, Arima K, Taniguchi K, Suzuki S, et al. Periostin, a matrix protein plays a role in the induction of cytokines in pulmonary fibrosis. Am J Respir Cel Mol Biol. 2012;46:677-86.

139. Hong LZ, Wei XW, Chen JF, Shi Y. Overexpression of periostin predict poor prognosis in non-small cell lung cancer. Oncol Lett. 2013;6(6):1595-1603.

140. Ishiba T, Nagahawa T, Sato T, Ishikawa T, Uetake H, Sugihara K, et al. Periostin suppression induces decorin secretion leading to reduced breast cancer cell motility and invasion. Sci Rep. 2014;4:7069.

141. Takashima K, Meguro K, Kawashima H, Kashiwakuma D, Kagami SI, Ohta S, et al. Serum periostin levels serve as both eosinophilic airway inflammation and fixed airflow limitation in well-controlled asthmatics. J Asthma. 2019;56(3):236-43.

142. Matsuka K, Kabata H, Fukunaga K, Suzuki Y, Masaki K, Mochimaru T, et al. Phenotype of asthma related to high serum periostin levels. Allergol Int. 2015;64(2):175-80.

143. Kanemitsu Y, Ito I, Niimi A, Izuhara K, Ohta S, Ono J, et al. Ostepontin and periostin are associated with a 20-year decline of pulmonary function in patients with asthma. Am J Respir Crit Care Med. 2014;190(4):472-4.

144. Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. J Allergy Clin Immunol. 2013;132(2):305-12.

145. Nair P, Kraft M. Serum periostin as a marker of eosinophilic inflammation. J Allergy Clin Immunol. 2012;130(3):655-6.

146. Izuhara K, Ohta S, Ono J. Using periostin as a biomarker in the treatment of asthma. Allergy Asthma Immunol Res. 2016;8(6):491-8.

147. Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, et al. Bronchoscopic exploration research study of biomarkers in corticosteroid-refractory asthma (BOBCA) study group. Periostin is a systemic biomarkers of eosinophilic airway inflammation in asthma patients. J Allergy Clin Immunol. 2012;130:647-654.

148. Matsumoto H. Serum periostin: A novel biomarker for asthma management. Allergol Int. 2014;63(2):153-60.

149. Li W, Gao P, Zhi Y, Xu W, Wu Y, Jin J, et al. Periostin: Its role in asthma and its potential as a diagnostic or therapeutic target. Repir Res. 2017;16:57.

150. Nagasaki T, Matsumoto H, Kanemitsu Y, Izuhara K, Tohda Y, Horiguchi T, et al. Using inhaled nitric oxide and serum periostin as composite marker to identify severe/steroid-insensitive asthma. Am J Respir Crit Care Med. 2014;190(12):1449-52.

151. Misumu Y, Hayashi Y, Arakawa F, Ikehara Y. Molecular cloning and sequence analysis of human dipeptidyl peptidase IV, a serine protease on the cell surface. Biochim Biophys Acta. 1992;1131:333-6.

152. Lambier A-M, Durinx C, Sharpé S, De Meester I. Dipeptidyl dipeptidase IV from bench to bedside: An update on structure properties, function, and clinical aspects of the enzyme DPP IV. Crit Rec Clin Lab Sci. 2003;40(3):209-94.

153. Nieto-Fontarigo JJ, González-Barcala JF, José S, Arias P, Nogueira M, Salgado FJ. CD26 and asthma: A comprehensive review. Clin Rev Allergy Immunol. 2019;56(2):139-60.

154. Gorrell MD. Dipeptidyl dipeptidase IV and related enzymes in cell biology and liver disorders. Clin Sci (Lond). 2005;108(4):277-92.

155. Lun SWM, Wong VW, Ko FWS, Hui DS, Lam CW. Increased expression of plasma CD4+ T lymphocyte costimulatory molecule CD26 in adults with allergic asthma. Clin Immunol. 2007;27(4):430-7.

156. Lettau M, Dietz M, Vollmars S, Armbrust F, Peters C, Dang TM, et al. Degranulation of human cytotoxic lymphocytes is a major source of proteolytic active soluble CD26/PDPP4. Cell Mol Life Sci. 2020;77(4):751-64.

157. Pack M. Do DPP-4 inhibitors cause heart failure events by promoting adrenergically mediated cardiotoxicity? clues from laboratory models and clinical trials. Cir Res. 2018;122(7):928-32.

158. Enz N, Vliegen G, De Meester I, Jungraithmayr W. CD26/DPP-4 - A potential biomarker and target for cancer therapy. Pharmacol Ther. 2019;198:135-59.

159. Zhou Y, Yang L, Zhou Z. Dipeptidyl peptidase-4: Multitarget drugs, not only antidiabetes drugs. J Diabetes. 2014;6(1):21-9.

160. Wang X, Zhang P, Huang G, Yang L, Zhou Z. Dipeptidyl Dipeptidase-4 (DPP-4) inhibitors: Promising new agents for autoimmune diabetes. Clin Exp Med. 2018;18(4):473-80.

161. Zhang T, Urbanek C, Burchard EG, Chu H, Seibold MA. The asthma biomarker dipeptidyl peptidase 4 (dpp4) is IL-13 inducible in the airway epithelial cells and inhibits rhinovirus infection. In: Presented at the American Thoracic Society. 2014;189:A4875.

162. Brightling CE, Chanez P, Leigh R, O'Byrne PM, Korn S, She D, et al. Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: A randomized, double-blind, placebo-controlled phase 2b trial. Lancet Respir Med. 2015;3(9):692-701.

163. James S, Kolmert J, Knowle S, Dahlen S-L. Late-breakfast abstract: Lack of association between circulating dipeptidyl peptidase-4 and other biomarkers of asthma: Data from U-BIOPRED and BIOAIR. Eur Respir

J. 2016;18:PA1095.

164. Streicher K, Yao Y, Ranade K, Liang M, Vainshtein I, Piper E, et al. Dipeptidyl peptidase (DPP-4/CD26) as a peripheral biomarker of IL-13 activation in asthmatic lung. EP3099323A1. European Patent Office. 2016-12-07.

165. Kim SH, Choi H, Yoon MG, Park HS. Dipeptidyl peptidase 10 as a genetic biomarker for aspirin-exacerbated respiratory disease phenotype. Ann Allergy Asthma Immunol. 2015;114(3):208-13.

166. Colice G, Price D, Gerhardsson de Vendier M, Rabon-Stith K, Ambrose C, et al. The effect of DPP-4 inhibitors on asthma control: An administrative database study to evaluate a potential pathophysiological relationship. Pragmat Obs Res. 2017;8:231-40.

167. Butler WT. Structural and functional domain of osteopontin. Ann NY Acad Sci. 1995;760:6-11.

168. Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Jansson M, Zawaideh S, et al. Eta-1 (osteopontin) an early component of type-1 (cell-mediated) immunity. Science. 2000;287(5454):860-4.

169. Xanthou G, Alissafi T, Semitekolou M, Somoes DC, Economidous E, Gaga M, et al. Osteopontin has a crucial role in allergic airway disease through regulation of dendritic cell subsets. Nat Med. 2007;13(5):570-8.

170. Nakasaka A, Matsue H, Matsushima H, Aoki R, Nakamura Y, Kambe N, et al. Osteopontin is produced by mast cells and affects IgE-mediated degranulation and migration of mast cells. Eur Respir J. 2008;38(2):489-99.

171. Koh A, da Silva AP, Bansal AK, Sun C, Lee H, Glogauer M, et al. Role of osteopontin in neutrophil function. Immunology. 2007;122(4):466-75.

172. Puxeddu I, Berkman N, Ribatti D, Bader R, Haitchi HM, Howarth PH, et al. Osteopontin is expressed and functional in eosinophils. Allergy. 2010;65(2):168-74.

173. Samatas K, Zervas E, Vittorakis S, Semitekolou M, Alissafi T, Bossios A, et al. Osteopontin expression and relation to disease severity in human asthma. Eur Respir J. 2011;37:331-41.

174. Demimpoura V, Bakakos P, Tseliou E, Bessa V, Hillas G, Simoe DCM, et al. Increased levels of osteopontin in sputum supernatant in severe refractory asthma. Thorax. 2010; 65(9):782-6.

175. Lenga Y, Koh A, Perera AS, McCulloch CA, Sodak J, Zohar R. Osteopontin expression is required for myoblast differentiation. Cir Res. 2008;102(3):319-27.

176. Takahashi A, Kurokawa M, Konno S, Ito K, Kon S, Ashino S, et al. Osteopontin is involved in migration of eosinophils in asthma. Clin Exp Allergy. 2009;39(8):1152-9.

177. O'Regan A. The role of osteopontin in lung disease. Cytokine Growth Factor Rev. 2003;14(6):479-88.

178. Berman JS, Serlin D, Li X, Whitley G, Hayes J, Rishikof DC. Altered bleomycin-induced lung fibrosis in osteopontin-deficient mice. Am J Physiol Lung Cell Mol Physiol. 2004;286(6); L1311-8.

179. Denhardt DT, Noda M, O'Regan AW, Pavlin D, Berman JS. Osteopontin as a means to cope with environmental insults: Regulation of inflammation, tissue remodeling, and survival. J Clin Invest. 2001;107(9):1055-61.

180. Kohan M, Breuer R, Berkman N. Osteopontin induces airway remodeling and lung fibroblast activation in a murine model of asthma. Am J Respir Cell Mol Biol. 2008;41(3): 290-6.

181. Samitas K, Zevrva E, Vittorakis S, Semitekolou M, Alissafi T, Gogos H, et al. Osteopontin expression and relation to disease severity in human asthma. Eur Respir J. 2011;37(2):331-41.

182. Zhao J-J, Lang L, Zhao F-Q, Shi S-M, Tan P. Osteopontin levels are elevated in patients with asthma. J In Med Res. 2011;39(4):1402-07.