Biomarkers for Diagnosis and Management of Eosinophilic Asthma

Nightingale Syabbalo*
Department of Medicine and Physiology, Copperbelt University, Kitwe, Zambia

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 peptidae-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 perisotin, 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 (omalizumab), 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

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 emergency 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: ForcedExpired 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 pharmacoeconomic 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 eosinophilic asthma include interleukin-5 (IL-5), IL-4, IL-13, IL-25, IL-33 and Thymic Stromal Lymphopoietin (TSLP) [36-38]. Interleukin-1IL-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 stimulate 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 Neutrophil (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 neutrotoxin 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 perioxidin 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,
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 Bronchoalveolar (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 eosinophils 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.

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-4Ra 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 × 109); 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 rehospitalization [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 2:** Mechanisms of airflow obstruction in patients with eosinophilic asthma.

<table>
<thead>
<tr>
<th>Mechanisms</th>
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<tr>
<td>Airway eosinophil recruitment, migration and activation</td>
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<tr>
<td>Release of eosinophilic cationic proteins</td>
</tr>
<tr>
<td>Release of Th2 cytokines, chemokines, and adhesion molecules</td>
</tr>
<tr>
<td>Airway epithelial damage and further release of cytokines</td>
</tr>
<tr>
<td>Mucus gland hyperplasia and hypersecretion</td>
</tr>
<tr>
<td>Release of growth factors</td>
</tr>
<tr>
<td>Airway hyper responsiveness</td>
</tr>
<tr>
<td>Subepithelial fibrosis</td>
</tr>
<tr>
<td>Airway smooth muscle proliferation</td>
</tr>
<tr>
<td>Airway remodeling</td>
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<td>Corticosteroid resistance</td>
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**Table 3:** Biomarkers for the diagnosis, and monitoring patients with eosinophilic asthma.

<table>
<thead>
<tr>
<th>Biomarker</th>
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<tbody>
<tr>
<td>Induced sputum count</td>
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<tr>
<td>Bronchoalveolar Lavage (BAL) fluid cytometry</td>
</tr>
<tr>
<td>Fractional expired Nitric Oxide (FeNO)</td>
</tr>
<tr>
<td>Serum periostin</td>
</tr>
<tr>
<td>Serum Dipeptyl Dipeptidase-4 (DPP-4)</td>
</tr>
<tr>
<td>Serum osteopontin</td>
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<table>
<thead>
<tr>
<th>Blood Eosinophil Count</th>
</tr>
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<tbody>
<tr>
<td>Counts</td>
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</table>

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

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Stage of Development</th>
</tr>
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<tbody>
<tr>
<td>Omalizumab</td>
<td>IgE</td>
<td>Marketed 2003</td>
</tr>
<tr>
<td>Mepolizumab</td>
<td>IL-5</td>
<td>Marketed 2015</td>
</tr>
<tr>
<td>Reslizumab</td>
<td>IL-5</td>
<td>Marketed 2016</td>
</tr>
<tr>
<td>Benralizumab</td>
<td>IL-5R</td>
<td>Marketed 2017</td>
</tr>
<tr>
<td>Dupilumab</td>
<td>IL-4α (IL-4/IL-13)</td>
<td>Marketed 2018</td>
</tr>
<tr>
<td>Tezepelumab</td>
<td>TSLP</td>
<td>Marketed 2018</td>
</tr>
<tr>
<td>Pitrakinra</td>
<td>IL-4α (IL-4/IL-13)</td>
<td>II</td>
</tr>
<tr>
<td>Lebrikizumab</td>
<td>IL-13</td>
<td>III</td>
</tr>
<tr>
<td>Tralokinumab</td>
<td>IL-13</td>
<td>III</td>
</tr>
<tr>
<td>Brodalumab</td>
<td>IL-17RA</td>
<td>II</td>
</tr>
<tr>
<td>Secukinumab</td>
<td>IL-17A</td>
<td>II</td>
</tr>
<tr>
<td>Fezakinumab</td>
<td>IL-22</td>
<td>II</td>
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</table>
Blood eosinophil counts can also predict responsiveness to ICS in atopic children with blood eosinophilia (≥ 300 cells/µL) [98]. 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].

Blood eosinophil counts can also predict responsiveness to ICS in asthmatic patients with blood eosinophilia (≥ 300 cells/µL) [98]. It has shown to monitor the response to corticosteroid because the adjustment of dose to maintain blood eosinophil (≥ 200 cells/µL) 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 for most biologics, except the reslizumab (400 cells/µL) [102]. There is a range of responses at different blood eosinophil levels and patients with higher blood eosinophil levels (≥ 300 cells/µL) tends 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 Nitric 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) 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) 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 ≥ 25 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 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 fascinil 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].

**Dipeptidyl 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 tralokimunab clinical trial, have reported that increased serum DPP-4 levels predict a beneficial response to tralokimunab, 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 eosinophils 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-β (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].

Deimimpoura 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 osteopontin 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 of eosinophilic asthma, such as induced sputum and blood eosinophil counts.

Deimimpoura 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. Deimimpoura 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].

Sillia 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 perisinostin and dipeptidyl pettidipase-4; and elevated levels of fractional exhaled nitric oxide. Th2 cytokines, such as IL-5, IL-4, IL-13, IL-25 and thymic stromal Lymphopoetin, 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 perisinostin, dipeptidyl peptidase-4 and osteopontin are currently being 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 (omalizumab), 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.

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