REVIEW ARTICLE

Airway Inflammatory Biomarker: Could It Tailor the Right Medications for the Right Asthmatic Patient?

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ABSTRACT

Asthma is a heterogeneous disease, in which asthmatic patients present with different clinical phenotypes, variable endotypes, and different response to asthma medicines. Thus, we are faced with an asthma paradox; asthma is diagnosed subjectively by clinical history and treated with biologically active drugs. To solve this paradox, we need objective airway biomarkers to tailor the proper medications to the proper patient. Biomarkers should have one or more of the following characteristics: 1) could differentiate poor symptoms perceivers from over-perceivers, 2) could predict disease activity and hence disease outcome, 3) could clarify asthma phenotype responders from non-responders, and finally 4) could characterize different clinical asthma phenotypes. Therefore, we have conducted a review of literature trying to apply those four parameters to different airway inflammatory biomarkers. We found that FeNO fulfilled the four proposed clinical parameters of airway inflammatory biomarkers whereas; serum periostin was the single best systemic biomarker of airway luminal and tissue eosinophilia in severe uncontrolled TH2 asthma phenotype. Thus, this may be considered a trial towards tailoring the proper medication to the proper patient. However, application of biomarkers in clinical practice requires easier and cheaper techniques together with standardized methods for sample collection and analysis.

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INTRODUCTION

Asthma is a heterogeneous and a genetically determined disease with different presentations, disease progression, and responses to therapy. Chronic airway inflammation that occurs in asthma is characterized by episodic reversible airway obstruction that variably presents with cough, wheezing, shortness of breath, or chest tightness (1).

Asthma Pathogenesis

Asthma pathogenesis is complex and varies across clinical endotypes. Multifaceted interactions between genetic, epigenetic, and environmental factors predispose patients to develop a number of dysfunctional immunologic regulatory patterns, which in turn dictate the presentation of clinical endotypes (2).

Several T cell subsets are important in asthma (Figure 1). Traditionally, TH2 cells have been thought to predominate, with characteristic raised levels of interleukin (IL)-4, IL-5, and IL-13. IL-4 and IL-13 promote inflammation (through signaling to eosinophils and B cells) and remodeling (through signaling to fibroblasts, airway smooth muscle, dendritic cells, and epithelial cells) (3). IL-5 is crucial for B cell survival and maturation and for stimulation of eosinophils. The biology of the eosinophil is complex, and the effects of its secreted products are diverse. Recruitment of eosinophils is mediated by IL-13, histamine, prostaglandin type 2, and eotaxins (through the CCR3 receptor). In addition to releasing toxic granular proteins, such as eosinophilic cationic protein (ECP) and eosinophil derived neurotoxin, eosinophils secrete dozens of cytokines and chemokines, which promote inflammation through the TH2 pathway and airway epithelial damage (4).

Some patients with asthma show a predominance of TH1 cells. This pattern can develop under the influence of IL-18 and interferon-γ (IFN-γ) and is characterized by further production of IFN-γ (5). IL-12 is a key cytokine involved in regulating the balance between TH1 and TH2 cells by promoting TH1 response. Together with its impact on invariant natural killer T, it plays a major role in asthma pathogenesis of (6). TH17 cells, which are CD4 positive T cells that express IL-17, also play a role in a subset of patients with asthma. TH17 pathway results in neutrophil activation and recruitment as the primary effector cells (7). TH9 cells are CD4 positive T cells that secrete IL-9. Frequency of TH9 cells are raised in individuals with atopy, and these cells promote allergic responses, probably through activation of mast cells (8). T regulatory cells, characterized by secretion of transforming growth factor-β (TGF-B) and IL-10, are thought to be important because of their role in blunting atopic responses (9).

Airway Remodeling

Airway remodeling refers to a constellation of structural changes in asthma including epithelial injury, increased thickness of the basement membrane, increased volume of airway smooth muscle, goblet cell metaplasia, and increased airway angiogenesis and lymphangiogenesis (10). Epithelial cells demonstrate rapid repair mechanisms and initiate signal cascades central to asthma in response to several stimuli. This process is mediated at least in part by epithelial growth factor. Abnormal repair processes and decreased barrier function have also been demonstrated (11).

Basement membrane thickening in children does not correlate with TH2 cytokine profile but may be due to changes in connective tissue (12). Increased airways smooth muscle mass is mediated in part by the release of cysteinyl leukotriene (CysLTs) from eosinophils (19). The increase in smooth muscle mass is associated with increases in growth factors including TGF-β1 and platelet derived growth factor (13).

Goblet cell metaplasia is another important structural change that occurs in asthma. It has been observed in models of TH2 driven asthma, but is not a feature of TH1 models of asthma (21). It seems to be dependent on the actions of the epidermal growth factor receptor as well as IL-13 and may be inhibited by IFN-γ (14).
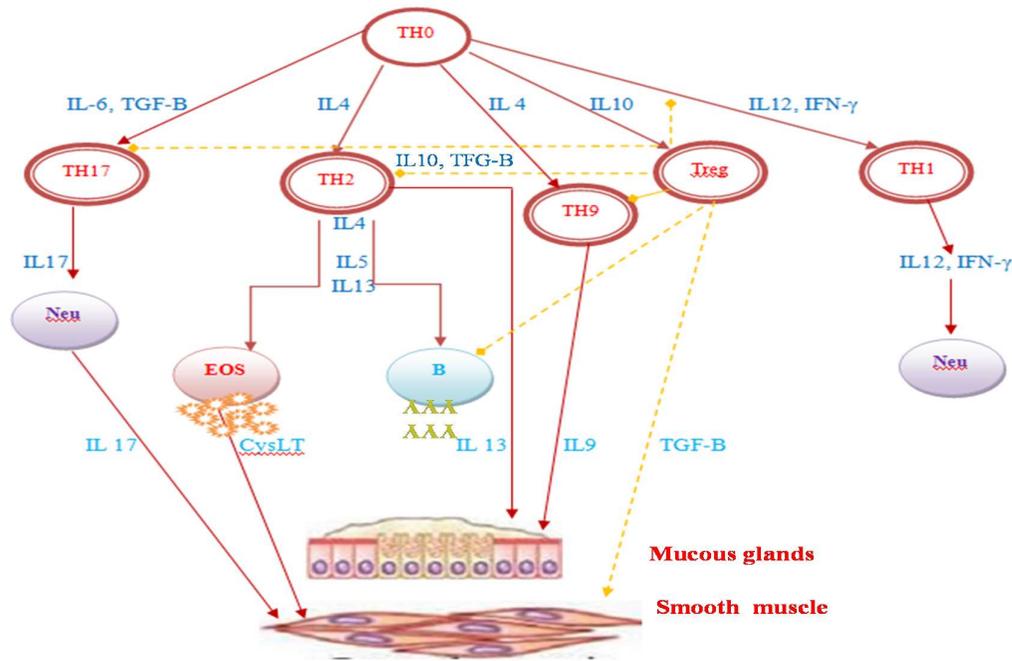

Figure 1. Different T-cell subtypes are involved in the pathogenesis of asthma and airway remodeling. T regulatory cell act to blunt the inflammatory response. EOS; eosinophils, CysLTN; cysteinyl leukotriene, Neu;neutrophils, TGF-B; transforming growth factor-B, Treg; T regulatory cell .

Asthma Impact

Asthma has a high prevalence worldwide with increasing mortality and high health care cost burden (15). Care of children with asthma is often inadequate due to lack of significant impairment in baseline forced expiratory volume in 1 second (FEV1) values even when symptoms are severe, the reluctance to prescribe corticosteroids because of actual or perceived associated morbidities in children together with heterogeneity of asthma. Therefore, guidelines based management strategies do not work equally well in all

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patients (16). To decrease the impact of asthma through treatment directed towards specific groups of patients, multiple inflammatory profiles and multiple phenotypic clusters of patients with asthma have been identified, although precise definitions of these clusters remain elusive (17). About 5% of the patients with asthma require individualized strategies for the optimum control of their disease. Thus, the success of the individualized strategies depends on accurate phenotyping with the help of biomarkers (26). Thus, quantifiable noninvasive biomarkers that are informative for assessing asthma pathogenesis and control in a specific patient will be of clinical utility in designing successful personalized treatment plans (18).

What is a Biomarker?

The National Institutes of Health (NIH, USA) defines a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (19). The ability to diagnose or treat a disease by measuring a biological molecule from a noninvasive source, such as blood, urine, or exhaled breath, has a great advantage over traditional pathological techniques because direct access to diseased tissue is no more required (20).

Asthma Biomarkers should Fulfill One or More of the Following Characteristics (Table 1):

1. A biomarker that monitor perception of asthma symptoms as we have poor perceivers and over perceivers. Although asthma diagnosis is based mainly on clinical basis using history taking and physical examination (1), patient's perception of asthma symptoms vary from one to another and factors influencing perception of these symptoms are numerous and are not well established (20).
2. A biomarker that monitors the disease activity and hence, could predict the clinical outcome.
3. A biomarker that monitors responders and non-responders phenotypes of asthma i.e. define patients that will gain the greatest benefits of asthma medicines.
4. A biomarker that could characterize different clinical asthma phenotypes.

Different Types of Biomarkers:

Exhaled biomarkers
   a) Fractional exhaled nitric oxide (FeNO)
   b) Exhaled breath condensate (EBC)

2. Urine leukotriene E4

3. Serum biomarkers
   a) Peripheral Eosinophils
   b) ECP
   c) Immunoglobulin (Ig) E
   d) Serum periostin
   e) Complement (C)3, C4
   f) Cytokines

4. Induced sputum

FeNO

FeNO is a non-invasive biomarker which isuseful in prediction of asthma development, recognition of specific asthma phenotypes like the eosinophilic phenotype, amelioration of asthma diagnosis and management in selected populations and prediction of efficacy of standard corticosteroid and biologic therapy. In addition, FeNO assessment may also be useful in patients with allergic rhinitis in order to detect eosinophilic bronchial inflammation in subjects at risk of asthma diagnosis (21).

Values of Using FeNO as an Airway Inflammatory Biomarker:

  i.   Monitoring the perception of asthma symptoms
  ii.   Monitoring the disease activity and the clinical outcome
  iii. Monitoring responder and non-responder asthma phenotypes
  iv.   Characterizing clinical asthma phenotype

i.   Monitoring the perception of asthma symptoms:
FeNO could be considered as a screening tool for asthma. FeNO level above certain cutoff levels may be a helpful adjunct for the diagnosis of asthma after exclusion of other clinical conditions associated with elevated FeNO (22). In previous clinic-based studies, the sensitivity and specificity of FeNO measurements were considered acceptable for the diagnosis of asthma. In particular, the diagnostic accuracy of FeNO in differentiating asthmatics from healthy subjects is significantly higher than lung function tests. However, it is important to remember that low FeNO values do not exclude the diagnosis of asthma because FeNO levels are normal especially in non-atopic subjects (23). Moreover, in children for whom a symptom report may be more vague (cough, exercise intolerance), a low FeNO may help to avoid the misdiagnosis of asthma and the use of costly, unnecessary treatments (24).

ii.   Monitoring the disease activity and the clinical outcome:
Monitoring of asthma control based on symptoms and normal FEV1 might result in under-treatment because persistent airway inflammation does not necessarily manifest itself as symptoms or lung malfunction. High FeNO levels in the absence of symptoms or lung function abnormalities raise the possibility of active eosinophilic airway inflammation and the likelihood of deterioration in asthma control. Thus, in cases of silent airway inflammation, monitoring with FeNO measurements might potentially allow for better assessment of disease activity and monitoring of anti-inflammatory treatment (24). Further, a number of previous studies have examined the role for FeNO in identifying patients at risk for future development of asthma exacerbation. The available data suggested that FeNO is useful in identifying patients at risk for future impairment or for developing loss of asthma control during reduction/cessation of inhaled corticosteroid (ICS) treatment (25).

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Table 1. Clinical values of airway inflammatory biomarkers.

Clinical Applications	Airway Inflammatory Biomarkers									
	FeNO	EBC	Urinary leukotriene E4	Peripheral Eosinophilia	ECP	IgE	Serum Periostin	C3,C4	Cytokines	Induced Sputum
Perception of Asthma Symptoms	+	-	-	-	-	-	-	-	-	-
Disease Activity	+	+	+	-	+	+	+	+	+	-
Responder Phenotype	+	+	+	+	+	-	+	+	-	+
Clinical Phenotype	+	-	+	+	+	+	-	+	+	-

FeNO, Fractional exhaled nitric oxide; EBC, Exhaled breath condensate; ECP, Eosinophilic cationic protein.

FeNO fulfilled the five proposed clinical values of airway inflammatory biomarkers whereas,

iii. Monitoring responder and non-responder asthma phenotypes:

FeNO could assess the potential response to ICS and could help distinguish patients with corticosteroid-responsive asthma from those whose asthma is unlikely to be corticosteroid responsive (26). Also, it is useful in monitoring the effectiveness of biologic treatments like omalizumab (27). High FeNO asthma phenotype (FeNO 0.05 > 35 ppb) was characterized by greatest airway reactivity, airflow limitation, hyperinflation, sputum eosinophilia and levels of symptoms (39). However, low FeNO levels (< 25 ppb) in an asymptomatic individual can indicate that ICS dose may be reduced or even withdrawn. Furthermore, high FeNO levels at the time of loss of asthma control and decrease of FeNO after treatment with corticosteroids imply that FeNO is useful not only in predicting asthma exacerbation but in monitoring the response to the treatment (28).

iv. Characterizing phenotypes of clinical asthma:

Recently, asthma phenotype recognized as "shortness of breath" was found to be associated with significant increase in FeNO values when compared to the cough phenotype. Also, it showed more frequent association with allergic rhinitis and atopic dermatitis compared to the other clinical phenotypes which could highlight the feature of allergic march in this phenotype (28).

Exhaled breath condensate (EBC)

EBC analysis is an increasingly used and promising method in research, as a wide number of inflammatory mediators can be measured in EBC. It is a simple, well tolerated and safe method, even in children with severe asthma, and is feasible in 100% of children over 4 years old. However, due to methodological issues and the lack of clinical trials of EBC in the monitoring of asthma, EBC does not play a role in the monitoring of asthma in children (29).
   a) EBC pH
   b) Asymmetric dimethyl arginine (ADMA)
   c) Purine derivatives
   d) Chemokines
   e) Prostaglandins
   f) Others

a) EBC pH
- *Values of using* **EBC** *pH as an airway inflammatory biomarker:*
   i.   Monitoring the disease activity and the clinical outcome
   ii. Monitoring responder and non-responder asthma phenotypes

   i.   Monitoring the disease activity and the clinical outcome:
       Low EBC pH may be a potential biomarker to identify asthma patients and exacerbations of asthma, but its clinical utility is controversial (30).

   ii. Monitoring responder and non-responder asthma phenotypes:
       In Severe Asthma Research Program (SARP) study of 572 subjects with stable asthma, a subgroup of asthmatics (low pH phenotype) was characterized by

prominently low FeNO, low FEV1, high body mass index, low levels of airway eosinophils, and gastric esophageal reflux symptoms. Due to the fact that most nebulized treatments are in acidic solutions, this subgroup might benefit from less acidic therapy, and might even experience improvement with inhaled base (44). Thus, EBC pH could be used to guide the therapy of asthma.

b) ADMA
A product of post-translational methylation of L-arginine that competes with L-arginine for binding to nitric oxide synthase (NOS). It increases oxidative stress, and airway epithelial cells injury in asthma (32).

    i.   Monitoring the perception of asthma symptoms
Children with asthma have elevated sputum and EBC levels of ADMA and decreased L-arginine:ADMA ratios (33). Elevated ADMA results in increased collagen deposition, airways remodeling, and increased methacholine responsiveness. In murine models, it results in increased total respiratory and airways resistance (33).

    ii.   Characterizing clinical asthma phenotype
Obese adults with late onset asthma phenotype have lower L-arginine/ADMA ratio. This low ratio results in greater airway epithelial NOS uncoupling and lower FeNO levels. Also, it is significantly associated with increased frequency of respiratory symptoms, poorer asthma-related quality of life and reduced lung volumes. This could lead to new phenotype-specific therapeutic options. L-arginine supplementations may prevent ADMA mediated NOS uncoupling in obese with late onset asthma (35).
Although L-arginine supplementation has been shown to induce modest improvements in lung function (50), this level of clinical effectiveness may be due to its extensive first-pass intestinal and hepatic metabolism, which may limit achieving adequate therapeutic levels. L-Citrulline has been shown to more effectively raise L-arginine levels and could therefore present an attractive alternative supplementation (36).

c) Purine derivatives
Extracellular purines are biologically active molecules including ATP and its metabolites – have been shown to function as danger signals in response to a variety of noxious stimuli (37).
    i.   Biomarkers that monitor the disease activity
Airway concentrations of adenosine are elevated in stable asthmatic subjects and are sensitive to exacerbations and effective treatment (38). Adenosine can be further metabolized to hypoxanthine and uric acid, compounds that have also been linked to responses to oxidative stress, with uric acid (UA) a known activator of inflammasomes (39).
UA was found to initiate and amplify Th2 cell immunity in asthmatic patients and mice (40). Furthermore, strategies targeting inhibiting UA synthesis with allopurinol or the UA degrading enzyme-uricase led to adecrease in proTh2 cytokines production, lung inflammation, repair, and fibrosis. These results suggest that reducing endogenously released UA may be a novel therapeutic approach to control chronic airway inflammation by extenuating Th2 cell immunity (41).

Serum UA is significantly elevated during exacerbation of acute asthma than healthy control and the more severe the exacerbation, the higher the serum uric acid levels (42).

d) Chemokines:
Airway eosinophilia is considered a central event in the pathogenesis of asthma. Eotaxin and RANTES play a key role in selective eosinophil accumulation in the airways and, subsequently, their activation and degranulation (43).

- **Eotaxin**
  i. Monitoring the perception of asthma symptoms
Eotaxin levels were higher in bronchoalveolar lavage fluid, sputum and EBC obtained from asthmatics than in healthy controls (44).
  ii. Monitoring responder and non-responders asthma phenotypes
Eotaxin-1 levels were elevated in patients with unstable ICS-treated asthma compared with stable ICS-treated asthma and steroid-naïve asthma patients with a significant correlation between the concentrations of eotaxin-1 in EBC and FeNO. Eotaxin-1 levels in sputum and blood were negatively correlated with the lung function as measured by FEV_1% predicted (44).

- **RANTES**
  i. A biomarker that monitors the disease activity and hence, could predict the clinical outcome.
RANTES levels in EBC are higher in asthmatic patients than in healthy controls. In patients with unstable asthma, levels of RANTES are significantly higher than in steroid-naïve and ICS-treated patients with stable asthma. Concentrations of RANTES in EBC significantly correlate with FeNO levels (45). This agrees with Teran *et al.* who suggested that ICS-treated mild-to-moderate asthmatics have significant lower levels of RANTES compared to steroid-naïve mild allergic asthma patients. This could suggest the beneficial effect of ICS-treatment in down-regulation of RANTES in the airways. RANTES expression was significantly correlated with the percentage of FEV_1 and airway resistance values (46).

e) Prostaglandins
The 8-iso-prostaglandin (PG) F 2α (8-isoprostane/ 8-ISO) is a stable oxidative stress marker, formed non-enzymatically by oxidation of arachidonic acid (47).
  i. A biomarker that monitors the disease activity and hence, could predict the clinical outcome
8-ISO levels are increased in EBC of asthmatic patients and correlate with disease severity, without being suppressed by corticosteroid treatment (48).
  ii. Biomarkers that could characterize different clinical asthma phenotypes
8-ISO should also reflect non-eosinophilic inflammation and involve small airways function, thus suggesting that it may be used complementarily to spirometry for the monitoring of patients with asthma (49). 8-ISO is elevated in the EBC of patients with occupational asthma both before and after methacholine challenge. In this asthma phenotype, initial 8-ISO in the plasma negatively correlated with FEV_1 and with methacholine PD20 (50).

f) Others
Eicosanoids as pro-inflammatory Leukotriene B4 (LTB4) and anti-inflammatory Lipoxin A4 (LXA4) are increased in asthmatics versus healthy subjects and LXA4/LTB4 ratio dramatically decreases in EBC in correlation with severity of asthma (51). High levels of nitrogen reactive species (NO, NO_2^-, NO_3^-) and endogenous

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reactive oxygen species (superoxide, hydrogen peroxide, hydroxyl radical) provided evidence for pathologic oxidizing processes in asthma and are indicative of airways oxidative and nitrosative stress (52).

Biomarkers in Urine

The CysLTs are central mediators in asthma. They are synthesized from arachidonic acid through a 5-lipoxygenase (5-LO) pathway and are mainly generated by many inflammatory cells, particularly eosinophils, mast cells and macrophages. The CysLTs include LTC4, LTD4, and LTE4. LTE4 is the stable end product of this pathway and can be measured (53).

Values of using* LTE4 *as an airway inflammatory biomarker:
  i.   Monitoring the disease activity and the clinical outcome
  ii.   Monitoring responders and non-responders asthma phenotypes
  iii.   Characterizing clinical asthma phenotypes

i.   Monitoring the disease activity and the clinical outcome

Urine LTE4 has been validated as a biomarker of CysLTs overactivity. LTE4 levels increase with acute asthma attacks and with aspirin-exacerbated respiratory disease, and decrease with CysLTs synthesis blockade but not with corticosteroids (17).

ii.   Monitoring responder and non-responder asthma phenotypes

Inhibitors of the CysLTs receptor are in clinical use for asthma treatment. However, many patients with asthma are not effectively treated with leukotriene receptor antagonists. Further, 5-LO inhibition can cause hepatotoxicity. Therefore, identification of asthma subgroups in which these specific anti-leukotriene agents may be effective has been a focus of genetic studies in asthma. Genetic testing for 5-LO gen polymorphisms has been proposed as a screening tool for identifying responsive subpopulations (54). Additionally, higher LTE(4)/FeNO ratios predict a better response to montelukast than ICS therapy in children with mild-to-moderate asthma (55).

Although the use of therapies in the leukotriene pathway are valuable, lack of widespread availability of clinical testing for LTE4 limits the use of this biomarker in optimizing asthma therapies (17).

iii.   Characterizing clinical asthma phenotypes

Serum Biomarkers

Serum biomarkers are simple and noninvasive and can be used to identify different asthma phenotypes and help selection of specific asthma therapy.

They include:

  a) Peripheral Eosinophilia
  b) Eosinophilic cationic protein
  c) IgE
  d) Serum periostin
  e) Complement C3,C4
  f) Cytokines

a) *Peripheral Eosinophilia*

Eosinophils preferentially accumulate in the sites of allergic inflammation and are believed to play important roles in the pathophysiology of asthma through the release of a variety of inflammatory mediators, including major basic protein (MBP), CysLTs, radical oxygen species, and cytokines (58).

Values of using **peripheral eosinophilia as an airway inflammatory biomarker**
  i.   Monitoring responder and non-responder asthma phenotypes
  ii.   A biomarker that could characterize different clinical asthma phenotypes

i. Monitoring responder and non-responder asthma phenotypes
A direct correlation exists between blood eosinophil counts and symptom scores, while there is an inverse correlation with FEV_1 in both children and adults. Mechanistically, it indicates a high Th2 phenotype and predicts responsiveness to steroids and anti-IL-5 therapy (15).
However, the use of circulating eosinophil count as a biomarker of clinical efficacy of asthma treatment is debatable, since treatment with an anti-IL-5 blocking antibody (mepolizumab) completely inhibits the circulating eosinophils but has no effect on clinical parameters, probably because only a modest decrease in lung eosinophils was achieved (59).

ii. A biomarker that could characterize different clinical asthma phenotypes:
Miranda *et al.* distinguished early-onset severe asthma patients, characterized by allergen sensitivity, allergic symptoms, eosinophilia and higher serum immunoglobulin E (IgE) levels, and late-onset severe asthma subjects, with lower lung function than early-onset ones, despite a shorter duration of illness and significantly more symptoms if presenting persistent eosinophils at onset (60). The cough with shortness of breath clinical asthma phenotype has significant increase in peripheral eosinophilic percentage compared to the cough group. It significantly decreases after treatment with fluticason but not montelukast (15).

b) *Eosinophilic Cationic Protein (ECP)*

Eosinophilic cationic protein (ECP) is one of the four major cationic granule proteins released by activated eosinophils. It is found in many body fluids but serum and sputum sampling are the most established to date. It is presently the most widely used clinical marker of eosinophil activity in asthma (61).

Values of using ECP as an airway inflammatory biomarker:
  i. A biomarker that monitors the disease activity and hence, could predict the clinical outcome
  ii. Monitoring responders and non-responders asthma phenotypes
  iii. A biomarker that could characterize different clinical asthma phenotypes

i. A biomarker that monitor the disease activity and hence, could predict the clinical outcome:

ECP reflects airway inflammation and not hyper-responsiveness. It rises during acute asthma exacerbation rather than during clinical remission. In assessing asthma severity, it is more sensitive than the eosinophil count in blood and sputum. However, it is not useful as a diagnostic tool for asthma because of its low specificity as it is raised in other atopic diseases and infections (62).

ii. Monitoring responders and non-responders asthma phenotypes

ECP can be used as a guide to anti-inflammatory treatment of asthma. Several studies supported the close relationship between ECP and use of corticosteroids, suggesting that ECP can be used as a marker of compliance to oral and ICS therapy and in guiding the tapering of ICS in stabilized asthmatics (63). ECP may be useful in assessing the level of asthma control and hence in refining asthma management as it is found to be significantly higher in partially controlled asthmatics compared with healthy control children as well as controlled cases (64).

iii. A biomarker that could characterize different clinical asthma phenotypes

Serum ECP and peripheral eosinophilic percentage were found to be significantly increased in asthmatic patients presented with cough and whom in turn responded significantly to treatment with montelukast alone (65). This could highlight their role in predicting clinical asthma phenotype and its specific therapy.

c) *Serum IgE*

IgE is the class of antibody that is responsible for mediating hypersensitivity reactions, such as allergic asthma, by binding to high-affinity receptors on the surface of mast cells and basophils and triggering activation of these cells on contact with allergen (66).

i. A biomarker that monitor the disease activity and hence, could predict the clinical outcome.

The levels of total IgE and allergen-specific IgE in serum are both biomarkers for defining phenotype of asthmatic patients. The presence of allergen specific IgE by skin prick test is a biomarker for atopic asthma (55). Asthma severity in children was associated with the serum level of IgE to dust mite; findings which suggested that specific IgE may be useful in identifying children who are at risk for severe asthma symptoms (67).

ii. Characterizing different clinical asthma phenotypes

Mean values of total serum IgE and eosinophilic percentage were reported to be significantly higher in wheezy infants with positive family history of asthma and atopy and those who had not been breast-fed, compared with those without asthma risk factors (68). Also, Serum IgE was found to be significantly increased in asthmatic patients presented with shortness of breath compared to other clinical phenotypes and this clinical phenotype showed a significant response to treatment with fluticasone alone (65). This highlights the role of serum IgE with other biomarkers in identifying specific asthma phenotypes and in initiation of specific anti-asthma medications.

d) *Serum periostin*

Periostin, which is an IL-4 and IL-13 inducible protein produced by airway epithelium, is a systemic biomarker of airway eosinophilia in asthma and can be used in patient

selection for emerging asthma therapeutics targeting Th2 inflammation (55). It has the ability to bind fibronectin, tenascin-C, collagen I, III and V and contributes to the process of subepithelial fibrosis in asthma patients (69).

i. A biomarker that monitors the disease activity and hence, could predict the clinical outcome
ii. Monitoring responder and non-responder asthma phenotypes
iii. A biomarker that could characterize different clinical asthma phenotypes

i. A biomarker that monitor the disease activity and hence, could predict the clinical outcome

Kanemitsu *et al.* found that high serum periostin concentration (≥95 ng/ml) is the unique biomarker, among several serum markers, associated with the greater annual decline in FEV_1 (at least 30 ml/year) (70).

ii. Periostin as a biomarker that differentiates responder and non-responder phenotypes of asthma:

A recent clinical trial has shown that patients with pretreatment higher levels of periostin respond to lebrikizumab treatment (anti-IL-13 monoclonal antibody) (16). Also, Hanania *et al.* found that the high serum periostin group had a greater decreased exacerbation rate after omalizumab treatment compared to low serum periostin group (71).

iii. A biomarker that could characterize different clinical asthma phenotypes

Serum periostin can be considered a systemic biomarker related to Th2-high asthma because it is a signature molecule associated to higher airway hyper-reactivity, serum IgE, eosinophilic inflammation, subepithelial fibrosis, compared to Th2-low asthma. Serum periostin is a promising biomarker for two main reasons. First, it easily moves from inflamed tissues to blood so its serum concentrations reflects its local production in lesions induced by Th2-type immune responses (72). Moreover, its basal serum levels are physiologically relatively low (~50 ng/ml) compared to other extracellular matrix proteins such as fibronectin or vitronectin. So, the Bronchoscopic Exploratory Research Study of Biomarkers in Corticosteroid-refractory Asthma (BOBCAT), identified serum periostin as the single best systemic biomarker of airway luminal and tissue eosinophilia in severe uncontrolled asthmatics. Adopting 25 ng/ml serum periostin as an arbitrary cut-off, eosinophil-low and eosinophil-high patients are effectively differentiated, with a positive predicted value of 93%. This study evidenced the superiority of serum periostin for predicting sputum and tissue eosinophilia, compared to blood eosinophils, IgE levels, and FeNO (73).

e) Complement 3, Complement 4 (C3, C4)
Values of using C3, C4 as airway inflammatory biomarkers:
i. A biomarker that could characterize different clinical asthma phenotypes
ii. A biomarker that monitor the disease activity and hence, could predict the clinical outcome.

i. A biomarker that could characterize different clinical asthma phenotypes

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Some studies concluded that the presence of elevated C3 and/or C4 complement components could represent a biomarker for diagnosis of intermittent atopic asthma (74)**.**

ii.   A biomarker that monitors the disease activity and hence, could predict the clinical outcome

Some studies have reported increased plasma complement level especially C3 in patients with severe asthma with correlation between asthma severity and serum C3 (75).

f) *Cytokines:*

Cytokines are extracellular signaling proteins produced by many different cell types including immune cells like T lymphocytes. They play an integral role in the coordination and persistence of inflammatory process in chronic inflammation of the airways in asthma (76).

Values of using cytokines as airway inflammatory biomarkers:

i.   A biomarker that monitors the disease activity and hence, could predict the clinical outcome.
ii. A biomarker that could characterize different clinical asthma phenotypes

i.   A biomarker that monitors the disease activity and hence, could predict the clinical outcome

Circulating markers that have been found to be raised in asthmatics include IL-4 and IL-5, and the CCR4 ligand TARC. Plasma concentrations of MCP-4 are elevated in asthmatics and further increased during an asthma exacerbation (76). Strong inverse relationship between FEV1 values and serum IL-9 exists (28).

ii.   A biomarker that could characterize different clinical asthma phenotypes

A significant difference was found in serum IL-2R in different clinical asthma phenotypes being significantly higher in shortness of breath group in comparison to cough and wheezy groups. Further, the cough with shortness of breath asthma phenotype showed significant increase in serum IL-9 compared to cough phenotype (41).

Induced Sputum

Sputum induction is a relatively safe and noninvasive procedure that can be successfully performed in adults and children with asthma and provide a rich source of biomarkers (55).

i. Induced sputum as a biomarker that monitors responder and non-responder phenotypes of asthma

Sputum eosinophil count acts as a key marker of asthma severity and steroid responsiveness. The identification of sputum eosinophilia currently has the greatest clinical value as it supports the diagnosis of asthma, predicts a favorable response to corticosteroids and can therefore guide treatment. In asthma management, protocols aimed at normalizing sputum eosinophil count have markedly reduced exacerbations without an overall increase in therapy. In addition, a recent study concluded that

approximately half of patients with mild-to-moderate asthma have persistently noneosinophilic disease, a phenotype that responds suboptimally to currently available anti-inflammatory therapy (56). In addition, Cyst-LT have been found in sputum supernatant and montelukast markedly inhibited the eosinophilicchemotaxis among asthmatics (55).

The Current Guidelines

There are no consensus guidelines from the large respiratory societies that advocate for use of biomarkers in guiding asthma management. NHLBI National Asthma Education Prevention Program states that "biomarkers, such as sputum eosinophils and FeNO, are increasingly used in clinical research and will require further evaluation in adults and children before they can be recommended as a clinical tool for routine asthma management" (77). Also, Global Strategy for Asthma Management and Prevention (GINA) does not make a recommendation about measurement of sputum eosinophilia or FeNO (78). On the other hand, the British Thoracic Society (BTS) and Scottish Intercollegiate Guidelines Network (SIGN) guidelines recommend clinicians "consider monitoring induced sputum eosinophil counts to guide steroid treatment" in patients with "difficult asthma" (79). More precisely, International ERS/ATS Guidelines suggest that in adults with severe asthma, treatment should be guided by clinical criteria and sputum eosinophil counts in centers experienced in using this technique rather than by clinical criteria alone. While using regular FeNO measurements as the basis for adjusting the dose of ICS therapy is not recommended (80).

Future Research

It has been estimated that 5-10% of patients can't achieve symptom control despite adequate environmental control, proper prescription of ICS and long acting β agonists, proper drug adherence and administration technique. Moreover, current asthma drugs generally do not reverse or slow down most of the long term remodeling changes that occur in various cell types in the airway.As new asthma drugs emergeincluding ultra long acting β agonists and modulators of IL-4, IL-5, IL-13, and IL-17 pathways, specific biomarkers which help clinicians to choose between different biologic therapies is a raw area for future research.The huge number of proteins involved in asthma pathogenesis makes discovery and clinical application of asthma biomarkers a difficult mission. Data analysis from genomic and proteomic profiling studies may help new biomarkers research studies. Application of biomarkers in clinical practice requires easier and cheaper techniques which could carry out these biomarkers into daily practice. Standardized methods for sample collection and analysis taking in consideration the change in biomarkers' concentrations according to the inflammatory condition of the patient and the disease-associated processes should be established.

Conclusion

In conclusion, the heterogeneity of airway diseases especially asthma makes the development of airway biomarkers essential and challenging. Airway biomarkers are objectively measured tools that could be helpful in monitoring the perception of asthma

symptoms, the disease activity, responder and non-responder asthma phenotypes, beneficial and adverse effects of asthma medicines, and finally characterizing different clinical asthma phenotypes. Nevertheless, FeNO is the most useful asthma biomarker that can be applicable in all the previously mentioned parameters. Sputum eosinophil count could be used in clinical practice to guide ICS therapy. Serum periostin is the single best systemic biomarker of airway luminal and tissue eosinophilia in severe uncontrolled TH2 asthma phenotype. Thus, better understanding and description of airway biomarkers is likely to ameliorate the management of asthma and other airway diseases especially if accurately employed.

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