Review article

Diagnostic inflammatory markers for bronchial asthma

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Role of inflammation in the pathophysiology of bronchial asthma

Airway inflammation is a characteristic feature of asthma. It contributes significantly to many features of this disease, including airflow obstruction, bronchial hyperresponsiveness, and the initiation of the injury-repair process (remodeling) found in some patients[1].

The inflammation that occurs in asthma can be broken-down into 3 stages. First, the acute response (edema, smooth muscle contraction and increased mucus production) which is completed within a few hours. Second, a residual stimulus leads to the chronic phase (epithelial cells denudation and the influx of inflammatory cells into the airway). Finally, chronic airway remodeling (irreversible structural changes and progressive loss of pulmonary function)[2]. The mechanism that initiates airway inflammation is shown in figure (1).
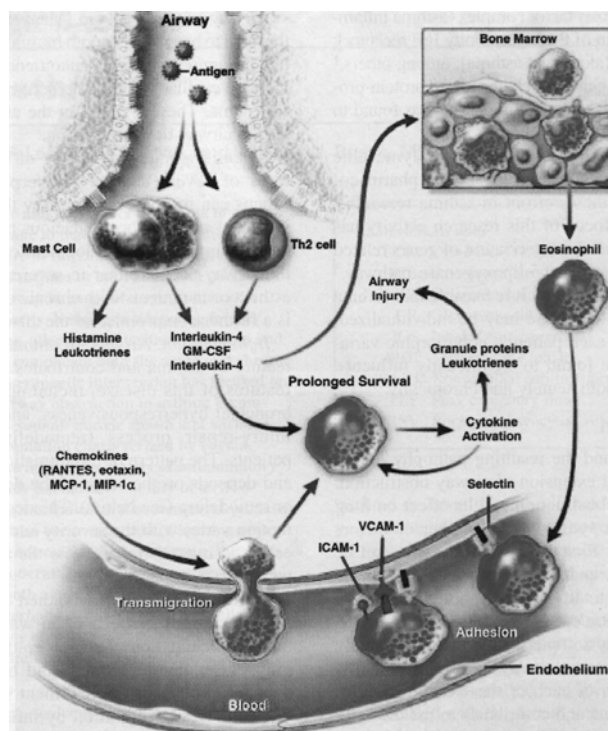
GM-CSF = granulocyte macrophage-colony stimulating factor; ICAM = Intercellular adhesion molecule; VCAM = vascular cell adhesion molecule; MCP = Monocyte chemoatractant protein; MIP = monocyte inhibitory protein.

Figure 1. The mechanism that initiates airway inflammation in bronchial asthma. In sensitized individuals antigen interaction with mast cell-bound, specific-IgE antibody results in release of preformed (histamine) and generated (leukotrienes) mediators along with cytokines [inteleukins-4 and –5 and GM-CSF]. These various compounds can induce localized inflammatory cell influx and activation through the upregulation of various chemokines and adhesion molecules and recruitment of bone marrow cells (e.g., eosinophils). Quoted from Lemanske and Busse (2003)[1].

Tests for assessing airway inflammatory markers of bronchial asthma

Ideally, a test for airway inflammation should be non-invasive, safe, simple to perform, inexpensive and should correlate with both indices of inflammation obtained using the gold standard tests (bronchoalveolar lavage "BAL" and bronchial biopsy), and other measures such as pulmonary function, disease severity and bronchial reactivity[3].

It is now possible to measure airway inflammation using non-invasive tests as induced sputum, serum measurements, exhaled breath

2

testing e.g. nitric oxide and volatile molecules in the liquid phase of breath "breath condensate" and even urinary measurements[4]. Sputum markers tend to be more sensitive than blood when assessing airway inflammation[5].

Inflammatory markers for bronchial asthma

No single parameter can accurately classify all individuals. Assessment of multiple parameters is necessary to categorize asthma clinical status accurately[6]. Inflammatory markers for bronchial asthma include:

I.   Inflammatory cells
II.   Cytokines and chemokines
III.   Lipid inflammatory mediators
IV.   Immunoglobulins (E, D)
V.   Anaphylatoxic complement C_3a, C_5a
VI.   Oxidants (including markers of exhaled breath) and antioxidants
VII.   Adhesion molecules
VIII.   Neurotrophins and neuropeptides
IX.   Endothelins
X.   Angiogenic and antiangiogenic factors
XI.   Matrix metalloproteinases
XII.   Activated protein C and plasmin system

I) Inflammatory cells

Eosinophils (number, granular proteins and apoptotic markers):
Eosinophilia in peripheral circulation and airways is a characteristic feature of asthma[7]. Hypodense eosinophils (eosinophils with lower density and greater number of immunoglobulin receptors) better reflect the severity of asthma than the mere count of peripheral blood eosinophils as they are metabolically more active and proinflammatory than the normodense eosinophils[8]. There is a high risk of exacerbation in subjects with blood eosinophilic count > 400/cubic mm[9].

Eosinophil cationic protein (one of the eosinophil granular proteins) released upon eosinophil activation has an essential role in the pathogenesis of airway hyperresponsiveness due to its cytotoxic effect on airway epithelial cells. It was detected in bronchial biopsy specimens and elevated in BAL, sputum and peripheral blood specimens[10].

Of the 4 basic eosinophil granule proteins, eosinophilic protein X (EXP) is the only one that can be accurately measured in the urine. It has been shown to correlate well with eosinophil activation and serum eosinophilic cationic protein level. Urinary EXP level reflects the presence of atopy and might be useful for monitoring the progression of allergic disease[11].

Recently, El-Gamal et al.[12] reported overexpression of sputum eosinophils and Bcl-2+ (antiapoptotic marker) eosinophil percentages in asthmatic patients with acute exacerbation than controls and demonstrated a positive correlation to disease severity.

T lymphocytes:
Numerous studies have documented elevated numbers of T cells within BAL fluid and within the epithelium and submucosa of asthmatic patients[13].
Numerous studies reported increased expression of T-helper cell activation markers as CD45RA, CD45RO, $CD45RA^+RO^+$ and CD25 in peripheral blood mononuclear cells of children with atopic asthma[14] as well as CD80 and CD86[15].

Basophils:
Blood basophil counts are often increased in asthmatic patients. Basophils have been also reported in sputum[16] and bronchial biopsy specimens[17]. Interestingly, basophils of asthmatic children showed increased spontaneous release of LTC4 during exacerbation and persisted even after subsidence of the attacks suggesting an in vivo activation state of basophils[18].

Platelets:
Platelet factor 4 and B-thromboglobulin are sensitive indices of platelet activation. They are closely related to clinical improvement, severity and response to therapy in asthmatic children[19].

Mast cells:
Increased number of mast cells has been described in the bronchial epithelium using electron microscopy and BAL of allergic patients with asthma. Increased concentration of tryptase (a marker of mast cell degranulation) has been found in BAL of asthmatics[20].

II) Cytokines and chemokines (figure 2)

The proteins synthesized and secreted by T, B and natural killer (NK) cells, with which they interact are referred to as cytokines. They are subdivided into interleukins, interferons, colony stimulating factors and other cytokines[21].

The initial indication for cytokine involvement in the pathogenesis of asthma came from studies performed in the early 1990s showing that atopic asthma was associated with local T_H2 cytokine expression (IL-3, IL-4, IL-5, and GM-CSF). They were upregulated in asthmatic patients relative to control subjects and their receptors were identified on the surface of inflammatory cells[22]. They can prolong the survival of the allergic effector cells because of delayed apoptosis[23].

Chemokines are a large group of chemotactic cytokines that have been divided into four groups,

designated CXC, CC, C and CX3C, depending on the spacing of conserved cytokines (where X is an amino acid). The CXC chemokines mainly target neutrophils and lymphocytes whereas the CC chemokines target a variety of cell types, including macrophages, eosinophils, basophils and dendritic cells[24]. Forty-seven chemokines and 18 chemokine receptors have been identified. Many of the chemokine receptors can bind more than one ligand allowing extensive overlap and redundancy of chemokine function[25].

Chemokines, particularly the eotaxin subfamily, have emerged as cytokines likely to be important in the regulation of allergic inflammation. They have an essential role in regulating leucocyte recruitment, basophil histamine release, T_H1/T_H2 function and tissue remodeling (including fibrosis and angiogenesis). Several chemokines appear to have potent effects in lung functions such as airway hyperresponsiveness[26].

The main stimuli for secretion of chemokines are the early signals elicited during innate immune responses (e.g. bacterial products, viral infection and proinflammatory cytokines such as IL-1 and TNF-α)[27]. Thus, chemokines provide an important link between early innate immune responses and adaptive immunity (by recruiting and activating T cells)[28].
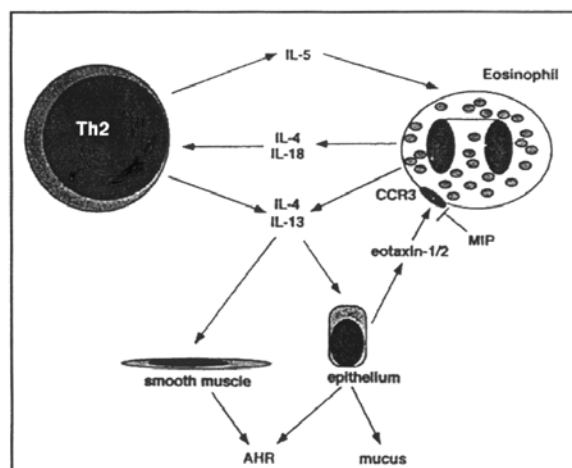
IL = Interleukin; MIP = macrophage inflammatory protein; CCR = CC chemokine receptor; AHR = airway hyperresponsiveness.

Figure 2. Chemokine/cytokine interactions in asthma (Quoted from Zimmermann et al., 2003)[26].

III) Lipid inflammatory mediators
The lipid mediators, leukotrienes (LTs), prosta-glandins and platelet-activating factor, play an important role in the complex inflammatory process of airways in bronchial asthma[29].

LTC4 and LTD4 are 1000 times more potent bronchoconstrictors than histamine. T helper type 2 cytokines upregulate LTs synthesis. LTs upregulate type 2 cytokine expression and decrease type I cytokine expression[30]. A significant increase in sputum LTs in children with asthma was demonstrated and paralleled the severity of asthma[31].

IV) Immunoglobulins (Igs)
IgE:
For IgE synthesis a complex series of interactions and signals between B and T cells is required[32]. IgE levels are often raised in allergic diseases and grossly elevated in parasitic infestations. When assessing children for presence of atopic disease, a raised IgE may aid in the diagnosis[33]. However, a significant number of allergic individuals have normal or low IgE concentrations. Indeed, low levels of serum IgE may be more useful in excluding atopic disease than elevated levels in confirming diagnosis, although patients with low IgE can have atopy. Increased total IgE levels during infancy, with no evidence of parasitic infestation, suggests the likelihood of subsequent development of atopic diseases[23].
IgD:
IgD level may be elevated in atopic diseases but there is yet no definite explanation for the high levels of IgD. Surface IgD was found to protect resting B-cells from deletion allowing them to initiate an immune reaction to antigens[34].

V) Anaphylatoxic complement C_3a, C_5a
The complement system is a vital link between innate and adaptive immunity. Complement anaphylatoxins C_3a and C_5a are potential effectors of the type 1 hypersensitivity reactions. C_3a and C_5a contribute in asthma pathogenesis. Their receptors are expressed in airway epithelial cells and smooth muscles[35,36].

VI) Oxidants and antioxidants
Many studies suggest that markers of oxidative stress (increased exposure to oxidants and/or decreased antioxidant capacities) are increased in children and adults with asthma, not only in their lungs but also in the circulation[37].
Markers in exhaled breath (nitric oxide and others)
Nitric oxide (NO) was originally recognized as an environmental pollutant that destroys the ozone layer. However, it is now recognized as an

important physiological mediator[38]. The highest NO production is from macrophages and airway epithelial cells. NO is synthesized by the oxidation of L-arginine by NO synthetase enzyme. In the lungs, NO acts as a vasodilator, a non adrenegic non-cholinergic neurotransmitter and an important mediator in the inflammatory response[39]. Exhaled NO is a non-invasive test that measures airway inflammation. It correlates with peripheral blood eosinophils and serum ECP[40].

Several other inflammatory biomarkers have been identified in exhaled air or breath condensate which include; aldehyde, glutathione, carbon monoxide, hydrogen peroxide, ethane and other volatile and non volatile compounds[41].

VII) Adhesion molecules
Leukocyte / endothelial cell adhesion molecules are essential mediators of both immune and inflammatory responses. T cell adhesion molecules are classified in three families. These are the selectins (E-selectin, L-selectin and P-selectin), immunoglobulin-like molecules (ICAM-1, ICAM-2, ICAM-3, VCAM-1, PECAM-1 and MadCAM-1) and integrins (CD29/CD49, CD18/CD11 and CD61/CD41)[42].

VIII) Neurotrophins and neuropeptides
Neurogenic inflammation may be initiated by activation of sensory nerves by inflammatory mediators, irritants, allergens and infections[43].

Neurotrophins are a family of peptides that promote survival, growth and differentiation of neurons. They may also influence the function of non-neuronal cell types, including immune cells. They include nerve growth factor, brain derived neurotrophic factor, neurotrophin 3 and neuro-trophin 4/5[44].

The traditional cellular sources of neurotrophins under physiological conditions are primarily nerve-associated cells such as glial cells, Shwann cells or fibroblasts and neurons themselves. In inflammatory processes, neurotrophins are also produced by a wide range of hematopoietic cells including mast cells, macrophages, T cells and airway epithelium[45].

Neuropeptides are small amino acid components released from sensory nerves and also from inflammatory immune cells such as monocytes, dendritic cells, eosinophils and mast cells. Neuropeptides such as substance P, neurokinin A and calcitonin gene-related peptide (CGRP) may amplify the inflammatory process through increasing plasma exudation, mucus secretion and

both recruitment and activation of inflammatory cells[46].

IX) Endothelins (ETs)
Human endothelin family comprises three 21-amino acid peptides. All three ET isopeptides are potent contractile agonists of human airway. ETs are synthesized by endothelial and epithelial cells and act in a paracrine fashion on nearby smooth muscle or connective tissue[47].

X) Angiogenic and antiangiogenic factors
Angiogenesis is regulated by a balance of angiogenic and antiangiogenic factors. Induced sputum from asthmatic subjects revealed imbalance between vascular endothelial growth factor "VEGF" (angiogenic factor) and endostatin (antiangiogenic factor) levels[48]. Angiogenesis is an essential component identified in airway remodeling of bronchial asthma[1].

XI) Matirx metalloproteinases (MMPs)
Matrix metalloproteinases are markers of extracellular matrix (ECM) degradation. MMP-2 and MMP-9 have been suggested to be the major proteolytic enzymes to induce airway inflammation and remodeling in asthma. Their levels are increased in asthmatic patients. Anti MMP therapy could be theoretically useful to prevent airway remodeling in asthma[49].

XII) Activated protein C and plasmin system
Activated protein C may protect the lung from fibrosis and airway remodeling by suppressing activation of coagulation, decreasing the secretion of inflammatory cytokines and platelet-derived growth factor, and promoting fibrinolysis. Low activated protein C activity has been observed in BAL of asthmatic patients[50].

Plasmin system plays an active role in tissue remodeling; plasmin degrades ECM, either directly or by activating MMPs. Plasmin system components are synthesized by airway cells. Inflammatory mediators affect their expression which is increased in bronchial asthma[51].

Clinical usefulness of inflammatory markers in diagnosis and treatment of bronchial asthma
Assessing airway inflammation is important for investigating the underlying mechanism, diagnosing, monitoring and treating asthma[52]. Evidence of airway inflammation (for example a raised exhaled NO or sputum eosinophilia) in

asymptomatic child may be indicative of failure to take their medications[53].

In trials of corticosteroid withdrawal, increase of sputum eosinophils preceded worsening of symptoms, deterioration of lung function and bronchial hyperresponsiveness. Increases in sputum eosinophils have also been associated with exacerbations of asthma[54]. Maintenance of sputum eosinophil count less than or equal to 3% is associated with few exacerbations, admissions and courses of oral steroids[55].

Treatment applications in asthma are based upon disease severity. Since asthma is a chronic disease whose severity may change over time, it is important to adjust treatment to appropriately match treatment requirements with disease severity. Failure to do this leads to undertreatment with the associated risk of impaired quality of life and severe exacerbations. Alternatively, over-treatment may occur, with the risk of excessive adverse effects[56].

At present, in patients with asthma, symptoms and lung function are used to monitor severity and adjust treatment. These measures are imperfectly correlated with the underlying pathophysiological processes in asthma, namely airway hyper-responsiveness and airway inflammation. Using objective measures of airway hyperresponsiveness and airway inflammation may lead to better management of asthma[57]. (Table 1).

The dream is that every patient with bronchial asthma could be characterized with respect to the profile of involving cells and mediators. Such information would provide us with a unique understanding of the underlying mechanisms of development of disease symptoms and the possibility of treating them[58]. Also, this may help in the development of new therapeutic strategies such as decreasing cytokine synthesis or release, blocking their effects by antibodies or soluble receptors as well as administration of anti-inflammatory cytokines which will undoubtedly constitute a breakthrough in the management of asthmatic patients[59]. (Figure 3).

Table 1. Inflammatory markers used more commonly to assess response to treatment in bronchial asthma.

	Marker	**Comments**
Bronchial biopsies	Cellular infiltration cytokine/TH expression Remodeling changes	Highly invasive method; direct assessment of inflammation in the target organ.
Skin and nasal biopsies	Cellular infiltration cytokine/TH expression	Alternative, less invasive methods to assess responses to systemic treatment.
BALF	Cell differential (EOS) ECP, cytokines	Invasive method. Cellular components well documented; difficulties with markers in the fluid component due to dilution factors.
Induced sputum	Cell differential (EOS) ECP, cytokines	Correlates well with biopsies and BALF data. Widely used in research but time-consuming.
Exhaled air	NO	Limited to steroid-naïve asthmatics, detected in atopic patients only.
EBC	CO, ethane, pentane	Not well documented as markers to assess treatment response
	pH, H_2O_2 and cytokines	Simple non-invasive procedures. pH seems to be the only rapid marker, whereas measuring others is still time-consuming. Repeatability, stability and use in monitoring is not well documented for all markers.
Blood	Eosinophils	Easy to measure, but reflect changes away from the target organ
	ECP, cytokines	ECP is the best eosinophil-derived marker. It correlates well with eosinophil activity in the airways.
Urine	EPX	Alternative for serum ECP; valuable tool in pediatric asthma.
	LTE_4, PGE_2	Assess the therapeutic effect of 5-lipoxygenase inhibitors, however, leukotriene levels do not respond to steroid treatment.

BALF = bronchoalveolar lavage fluid; ECP = eosinophil cationic protein; NO = nitric oxide; EBC = expired breath condensate; EPX = eosinophil peroxidase; LTE_4 = leukotriene E_4; PGF_2 = prostaglandin F_2, H_2O_2 = hydrogen peroxide, CO = carbon monoxide.

(Quoted from Gaga et al, 2003)[57].
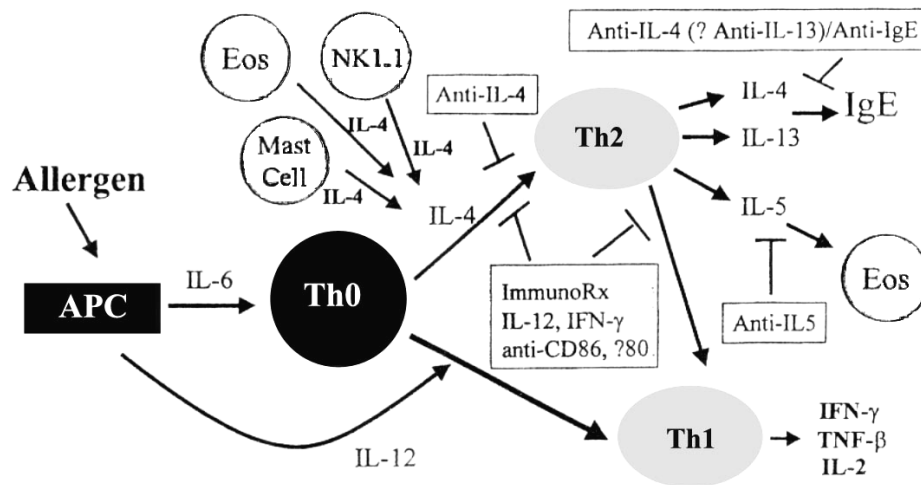
APC = antigen presenting cell; Eos = eosinophil; IL= interleukin; IFN-γ = interferon gamma;
TNF-β = tumour necrosis factor beta.

Figure 3. Important steps in the development of T_{H2}-lymphocytes and potential sites for intervention and treatment of bronchial asthma. (Quoted from Colavita et al, 2000)[60].

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