

Tissue-Based and Bronchoalveolar Lavage–Based Biomarkers in Asthma

Sally E. Wenzel, MD

KEYWORDS

• Tissue-based biomarkers • Bronchoalveolar lavage–based biomarkers • Asthma

KEY POINTS

- There are no tissue or bronchoalveolar lavage biomarkers that are specific for asthma in general.
- A biomarker is defined as a cell or compound of interest that has been measured in at least 2 different studies, which is associated with disease, its severity, phenotype, or a specific process associated with that disease.
- Recent efforts to phenotype asthma are strongly linked with the evolution of biomarkers specific to that phenotype.

INTRODUCTION

Asthma is increasingly understood to be a disease consisting of multiple different phenotypes. This heterogeneous disease presents a diagnostic and treatment challenge, especially in moderate to severe asthmatics who continue to be poorly controlled despite high-dose systemic or inhaled steroids. It is likely that previous studies, which have not accounted for this heterogeneity, have limited the ability to identify single biomarkers. As of 2012, there are no tissue or bronchoalveolar lavage (BAL) biomarkers that are specific for asthma in general. In this article, tissue and BAL biomarkers of asthma are evaluated from their use in asthma in the context of the phenotype that they may best represent. For purposes of this article, a biomarker is defined as a cell or compound of interest that has been measured in at least 2 different studies, which is associated with disease, its severity, phenotype, or a specific process (forced expiratory volume in 1 second) associated with that disease. It is hoped that studies that better link biomarkers to specific phenotypes will eventually improve the ability to evaluate genetic features, diagnose, measure progression, and tailor treatments. Although some biomarkers may only be associated

Division of Pulmonary Allergy and Critical Care Medicine, Department of Medicine, Asthma Institute, University of Pittsburgh Medical Center, Northwest 931 Montefiore Hospital, 3459 Fifth Avenue, Pittsburgh, PA 15213, USA

E-mail address: wenzelse@upmc.edu

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with disease, it is also likely that some may be mechanistically involved. Some of these biomarkers may then also become targets for specific treatment.

BIOMARKERS FOR EOSINOPHILIC ASTHMA

Tissue eosinophils have been studied in the greatest detail and most studies support that eosinophils in the tissue are generally increased in asthma (Table 1).¹ For many years, it was believed that eosinophils were a specific biomarker for asthma, but more recently, many studies using tissue and sputum samples have shown that eosinophils are present only in a percentage of patients with asthma, with estimates between 40% and 60% of asthmatics.^{2–4} This situation has led to an increasingly accepted concept that asthma, defined as reversible airflow limitation or bronchial hyperresponsiveness with accompanying clinical symptoms, can be divided into those with and without eosinophils. Eosinophils are a biomarker of the eosinophilic phenotype. This phenotype seems to identify a group of patients with more symptomatic disease, more risk of exacerbations, and, in patients with severe asthma, a higher risk of near fatal events. Only a few tissue studies have sorted patients with asthma by their eosinophil numbers and no longitudinal studies have evaluated the change in tissue eosinophils over time. Several published studies have compared tissue and BAL eosinophils; however, a recent study found modest concordance between biopsy and sputum eosinophils, with sputum identifying more eosinophilic patients.⁵ Because bronchoscopy is never likely to be performed at short intervals in patients, or in relation to exacerbations, the prospective ability of tissue eosinophils to guide and monitor therapy is limited. However, sputum eosinophilia seems to be a reasonable surrogate.^{6,7}

In severe asthma, the presence of tissue eosinophils has been extensively evaluated in a cross-sectional manner. In this subgroup, there is persistent eosinophilia in tissue despite high-dose oral and inhaled steroids. At least 1 study suggested that tissue eosinophilia is more prevalent in late-onset rather than early-onset disease, consistent with the greater likelihood for aspirin-sensitive (or related hypereosinophilic) subtypes of asthma to occur later in life.^{3,8} However, persistent tissue eosinophilia can be seen in asthma that begins at any age. In the tissue, the eosinophilic phenotype has been defined as those patients with tissue eosinophils greater than 2 times the standard deviation of the mean number measured in normal individuals.^{2,3,9}

Considerable effort has been spent to identify factors that contribute to the tissue eosinophils. However, few studies have consistently observed relationships between tissue cytokine, chemokine, or lipid mediator levels and eosinophils. Only one of these factors, interleukin 5 (IL-5), has been specifically inhibited in humans and shown to lower tissue eosinophil levels.¹⁰ IL-5 plays a crucial role in eosinophil recruitment,

Table 1 Biomarkers associated with phenotypic types or aspects of asthma	
Phenotypes	Biomarkers
Eosinophils	IL-4, IL-5, IL-13, eotaxins, 15-HETE, 15-LO, CysLTs
Neutrophils	IL-8, LTB4, MMP-9
Paucigranulocytic	Smooth muscle, mast cells in smooth muscle
Remodeling	SBM, collagens, tenascin, TGF-β, chymase-positive mast cells

Abbreviations: CysLT, cysteinyl leukotrienes; HETE, hydroxyeicosatetraenoic acid; IL, interleukin; LO, lipoxygenase; MMP, matrix metalloproteinase; SBM, subepithelial basement membrane; TGF, transforming growth factor.

expansion, and survival in numerous murine models of asthma.^{11,12} In addition, it promotes differentiation of eosinophil precursors in the bone marrow. During eosinophil development, there is upregulation of the IL-5 receptor on CD34+ cells and this population increases in bone marrow, blood, and sputum 24 hours after inhaled allergen challenge.^{13,14} A recent study reported that 10 mg of inhaled IL-5 had no effect on tissue eosinophils of asthmatics, but in normal individuals, a significant increase in tissue eosinophils occurred. Despite the lack of increase in tissue eosinophils, there was a dramatic decrease in eosinophil progenitor cells in the asthmatic but not in normal individuals. The reasons for these differences are not clear.¹⁵ IL-5 has also been shown to increase in BAL fluid after segmental allergen challenge, but the IL-5 receptor seems to be shed from eosinophils found in BAL fluid, suggesting that eosinophils may become less responsive to IL-5 over time/with maturation.^{16,17} Because IL-5 is believed to be produced primarily by lymphocytes, evaluating IL-5 protein in tissue is difficult. IL-5 messenger RNA (mRNA) is increased in bronchial biopsies of asthmatic patients, and has been suggested to correlate with disease severity.^{18,19} Although early studies of all-comer asthmatics not subgrouped by eosinophilia were negative, 3 studies have recently been published that confirm the importance of IL-5 to asthmatic exacerbations, but only in a subgroup with blood or sputum eosinophilia.^{20–24} In one of those studies, although the impact on blood eosinophils was profound, the effect on tissue (and sputum) eosinophils was less dramatic.²²

Another factor closely linked to tissue eosinophils is the family of eosinophilic chemokines known as eotaxins. There are 3 identified eotaxins (1, 2, and 3), which do not share a similar structure but which all activate the CCR3 receptor present primarily on eosinophils. In contrast to IL-5, eotaxins are produced by structural cells, such as fibroblasts and epithelial cells, making eotaxins ideal candidate biomarkers to identify tissue (as opposed to luminal) eosinophils. Eotaxin promotes eosinophil tissue migration and has been shown to be increased both at the mRNA and protein level in the bronchial mucosa in asthmatics compared with nonasthmatic individuals.^{25,26} Eotaxins are known to be strongly upregulated by Th2 cytokines, such as IL-4 and IL-13, which have also been linked to asthma.^{27,28} Studies have shown Th2 cytokines IL-4 and IL-5 are increased in BAL fluid.²⁹ After segmental allergen challenge, IL-4 remained increased for at least 2 weeks and had a dose-dependent effect on α -smooth muscle actin and collagen III synthesis by human lung fibroblasts.³⁰

Cysteinyl leukotrienes have also been found to be increased in BAL, urine, sputum, and exhaled breath condensate in asthmatic patients.^{31–34} They act through the cysteinyl leukotriene 1 receptor, primarily, although LTE4 may preferentially activate a different receptor, the P2Y2 receptor, potentially explaining the increased tissue eosinophils seen after LTE4 inhalation.^{35,36} Montelukast and zafirlukast are leukotriene receptor antagonists that selectively block the cysteinyl leukotriene 1 receptor.³⁷ These medications have been shown to decrease blood, sputum, and BAL eosinophils.^{38,39} Moreover, the use of leukotriene receptor antagonists also decreased blood eosinophils in the pediatric population.⁴⁰

Although its role in eosinophilic inflammation and asthma remains unclear, the eicosanoid 15-hydroxyeicosatetraenoic acid (15-HETE) and its enzyme, 15-lipoxygenase (LO), have also been reported to be increased in asthma, and particularly in severe eosinophilic asthma in both BAL and tissue (**Fig. 1**).⁴¹ 15-HETE is also increased in wheezing children. 15-LO is upregulated by IL-4 and IL-13 in various cell types.^{42,43} Clinical relevance for this increase in 15-LO1 is suggested by the observations that 15-LO and its product are critical for the generation of MUC5AC, a mucin believed to be of key importance to asthma.^{44,45}

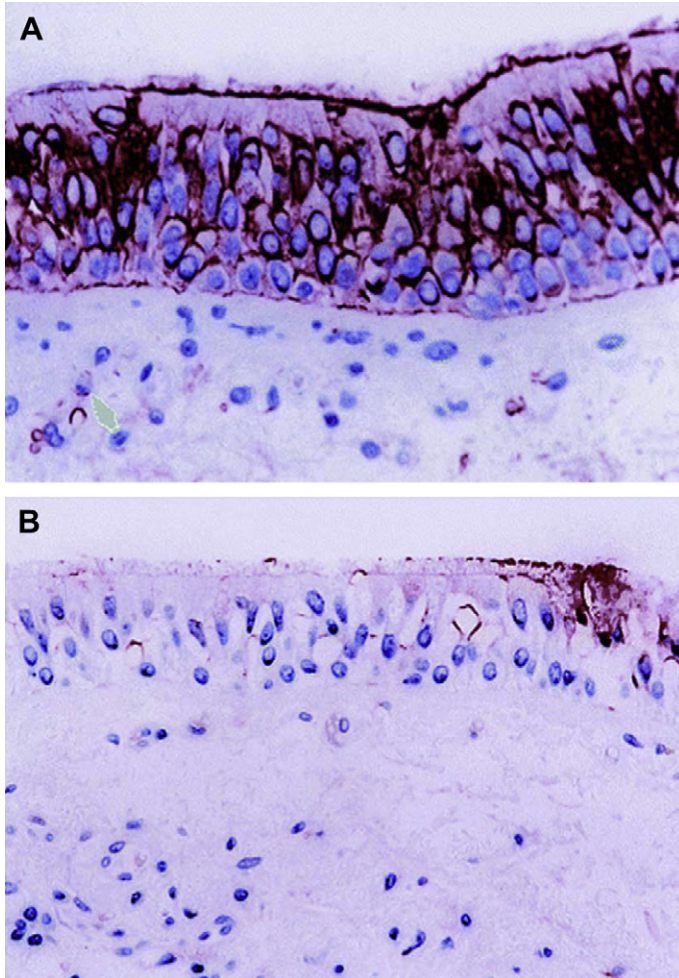


Fig. 1. Representative photomicrographs of 15-LO immunostaining in an eosinophil-positive severe asthmatic (A) and a normal individual. (B) Bronchial epithelial cells and inflammatory cells in the submucosa were stained for 15-LO (15-LO immunostaining, original magnification x400). (From Chu HW, Balzar S, Westcott JY, et al. Expression and activation of 15-lipoxygenase pathway in severe asthma: relationship to eosinophilic phenotype and collagen deposition. Clin Exp Allergy 2002;32:1561; with permission.)

BIOMARKERS FOR NEUTROPHILIC ASTHMA

Although numerous candidate tissue biomarkers have been identified for the eosinophilic phenotype of asthma, biomarkers for noneosinophilic asthma are not so well understood (see [Table 1](#)). Noneosinophilic asthma has been proposed to consist of 2 different subtypes: neutrophilic and paucigranulocytic,⁴⁶ and generally fall under the category of Th2-Lo asthma.⁴⁷ Of these subtypes, neutrophilic asthma has been associated with a more severe phenotype of asthma.^{48,49} Sputum analysis of neutrophils more consistently discriminates severe asthma from milder asthma than tissue eosinophils. Sputum can also be followed serially over time. This neutrophilic type

of asthma has been described in relation to increases in several proneutrophilic factors, including LTB₄ and IL-8. LTB₄ is a lipid mediator produced from arachidonic acid by the 5-LO enzyme. LTB₄ has been noted to be increased in BAL fluid in relation to neutrophils in severe asthma, as well as in less severe patients with nocturnal asthma, LTB₄ was also associated with eosinophilic inflammation.⁵⁰ Higher levels of LTB₄ are also found in wheezing children defined as those with 2 episodes of wheezing or wheezing greater than or equal to 2 months in a 6-month period.⁵¹ IL-8 is a potent neutrophilic chemokine produced by a variety of cell types, which in addition to being associated with neutrophilic asthma, has been suggested to increase with increasing severity of disease.⁴⁹ IL-8 in BAL fluid was significantly higher than serum and also higher in patients with asthma compared with patients with chronic obstructive pulmonary disease.⁵² In contrast, in children, IL-8 levels in BAL were found to be similar to normal controls.⁵³

Increased tissue and BAL neutrophils have also been linked to increases in matrix metalloproteinase-9 (MMP-9) in tissue and BAL fluid.^{54,55} MMP-9 has been shown to be increased in the BAL in both adult and pediatric asthmatic patients.^{56–58} MMP-9 exists in many forms, but needs to be activated before its gelatinolytic enzymatic properties are present. In most studies, most MMP-9 is in the inactive or pro form.⁵⁹ However, in severe asthma, there is an increase in the high-molecular-weight form of MMP-9, which is present when the MMP-9 is linked to a neutrophilic protein. This high-molecular-weight form is also less likely to be inhibited by inhibitors of MMPs.⁵⁹ Submucosal expression of MMP-9 has been reported to correlate with subepithelial thickness of the subepithelial basement membrane (SBM), as measured by collagen III expression, suggesting it may contribute in airway remodeling.⁶⁰ In addition, MMP-9 has been shown to be present within the subbasement membrane and to correlate with the level of neutrophils in the tissue, and the numbers of cells expressing transforming growth factor β (TGF- β). Both neutrophils and MMP-9 levels are also correlated with worsening forced expiratory volume in 1 second.^{49,54}

Neutrophilic inflammation has also been proposed to be associated with activation of Th17 pathways. However, the tissue data to support this proposition in humans are still modest, including studies that suggest no relationship of IL-17 with neutrophilic inflammation.^{61,62}

BIOMARKERS FOR PAUCIGRANULOCYTIC ASTHMA

Not all asthmatic patients have measurable eosinophilic or neutrophilic inflammation in tissue or BAL fluid (see **Table 1**). The mechanisms by which asthma exists in individuals with paucigranulocytic asthma are not clear; however, several possible explanations may exist. First, the patient may have undergone a degree of remodeling in their airways such that inflammation is no longer the primary cause for their disease. These patients may have more persistent airflow limitation and less variability in their disease.⁴ Specific or reproducible biomarkers of remodeling in tissue are not yet available. It has been suggested that the amount of smooth muscle present in the tissue and the size of the smooth muscle cells are increased with increasing severity of asthma, and that these changes exist independent of inflammation.⁶³ However, these studies have not been replicated. Another group has reported mast cells to be increased in bronchial smooth muscle bundles and that this increase is specific to asthma.⁶⁴

Because tissue inflammation is traditionally measured in proximal airways, as opposed to the distal lung, it is also possible that distal lung inflammation exists and persists in patients with little tissue inflammation in the proximal lung. Few studies

have used distal lung tissue. However, mast cells, particularly chymase-positive mast cells, are increased in the distal lung in patients with severe asthma and correlate positively with lung function, suggesting that they may be protective in severe asthma.⁶⁵ The chymase-positive mast cell has also been shown to be increased in asthmatics and associated with increase in vessels and vascular endothelial growth factor expression.⁶⁶

BIOMARKERS FOR REMODELING

Perhaps the greatest attention has been paid to the thickness of the SBM (see **Table 1**). Although it is clear that the SBM is a reasonable biomarker for asthma, the data to suggest that it is a biomarker for severity are less clear.^{2,67–69} In addition, the thickness of the SBM has been reported to be higher in those with active eosinophilic inflammation as opposed to those with no or minimal eosinophilic inflammation.² The SBM is made up of a variety of different collagens (primarily I, III, and V), and numerous other extracellular matrix glycoproteins, including tenascin, lumican, decorin, and others. Tenascin expression was shown to be increased 24 hours after allergen inhalation challenge, returning to baseline levels at 7 days. In contrast, procollagen I and procollagen III expression was increased at 7 days after challenge.⁷⁰ Although the mechanisms for the increase in airway smooth muscle and SBM in asthma remain poorly understood, TGF- β has been suggested in several studies to play a critical role.^{2,3,71,72} Increases in TGF- β can induce fibroblast proliferation and collagen synthesis. In severe asthma, TGF- β 2 in particular has been associated with increased fibrosis.^{72,73} Anti-IL-5 was shown to decrease matrix deposition in the SBM, while decreasing the levels of TGF- β in the tissue likely through an effect on eosinophils.⁷⁴

SUMMARY

Although there are as yet no biomarkers in lavage or lung tissue of sufficient validity to diagnose and manage asthma in general, recent efforts to phenotype asthma are strongly linked with the evolution of biomarkers specific to that phenotype. Because of the difficulty in obtaining these bronchoscopic specimens, it is unlikely that any factor in these compartments will achieve sufficient reproducibility or predictive value over time to be widely accepted as a clinically useful biomarker. However, efforts to compare tissue and lavage biomarkers with other, less invasive measures of sputum, serum, or exhaled breath may lead to better support for these systems. Sputum eosinophils are perhaps the best example, with tissue and lavage eosinophils studied first, but validated as a biomarker of poor asthma control, asthma exacerbations, and steroid responsiveness in the less invasive sputum studies. Further evaluation and understanding of these biomarkers in relation to phenotype, genotype, and less invasive measures that can be repeated over time should greatly enhance the use of these bronchoscopic findings.

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