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Biomarkers in Asthma: A Real Hope to Better Manage Asthma

Serpil C. Erzurum^a and Benjamin M. Gaston^b

^aProfessor and Chair, Department of Pathobiology, Lerner Research Institute, and the Respiratory Institute, Cleveland Clinic, Cleveland Clinic, Cleveland, USA

^bProfessor, Department of Pediatric Pulmonary Medicine, University of Virginia School of Medicine, Charlottesville, Virginia, USA

SYNOPSIS

Diagnosis and treatment of asthma are currently based on assessment of patient symptoms and physiologic tests of airway reactivity. The morbidities and costs associated with the over and/or under treatment of this common disease, as well as the growing numbers of biologically-specific targeted strategies for therapy, provide a rationale for development of biomarkers to evaluate the presence and type of inflammation in individuals with asthma in order to optimize treatment plans. Research over the past decade has identified an array of biochemical and cellular biomarkers, which reflect the heterogeneous and multiple mechanistic pathways that may lead to asthma. These mechanistic biomarkers offer hope for optimal design of therapies targeting the specific pathways that lead to inflammation. This article provides an overview of blood, urine and airway biomarkers, summarizes the pathologic pathways that they signify, and begins to describe the utility of biomarkers in the future care of patients with asthma.

Keywords

asthma; biomarkers; asthma management

THE CLINICAL NEED FOR BIOMARKERS TO INFORM THE CARE OF PATIENTS WITH ASTHMA

Asthma is defined as reversible airflow obstruction in the setting of airway inflammation. Asthma prevalence increased dramatically in the period between 1970 and 2000, with more than 22 million people—of whom over 4.8 million are children—now living with asthma in the U.S.^{1,2} The increase of asthma has been variously ascribed to improved hygiene worldwide, acetaminophen use, increased exposure to allergens and pollution and/or

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Corresponding Authors: Benjamin M. Gaston, MD, Department of Pediatric Pulmonary Medicine, University of Virginia School of Medicine, Box 800386, UVA HSC, Charlottesville, VA 22908, Phone: 434-924-1820, FAX: 434-982-8388, BMG3G@virginia.edu. Serpil C. Erzurum, MD, Department of Pathobiology, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Ave/NC2, Cleveland Clinic, Cleveland, Ohio, 44195, Phone: 216-445-5764, Fax: 216-636-0104, erzurus@ccf.org.

Conflict of Interests

Dr. Erzurum: None

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increased transmission of respiratory viruses³. This epidemic has occurred against a backdrop of a variety of genetic, biochemical and immunological host characteristics that substantially affect asthma phenotype.

Currently, standard clinical practice relies on patient history of symptoms and the measure of bronchial obstruction and reactivity, which are surrogates of the inflammatory and biochemical processes that give rise to the inflammation underlying all asthma⁴. For example, the phenotype of severe asthma, which comprises up to 5% of asthma patients, is based on a compilation of criteria most important of which is the documentation of the lack of clinical treatment response^{5,6}. Asthma treatment is directed equally toward reversing bronchoconstriction and treating airway inflammation. Common, non-invasive measures of airflow are usually able to quantitate the efficacy of the treatment of bronchoconstriction in adults. However, commonly available methods do not precisely measure the biology of inflammation underlying the bronchoconstriction. Further, the care of children with asthma is often inadequate due to the lack of bronchial obstruction on lung function tests even when symptoms are severe, and the reluctance to prescribe corticosteroids due to actual or perceived associated morbidities in children⁷. Thus, quantifiable non-invasive biomarkers that are informative for asthma control, and optimally for assessing the pathobiologic pathways leading to the chronic airway inflammation in a specific patient, will be of clinical utility in designing successful personalized treatment plans.

Based upon this rationale, the National Institutes of Health (NIH) and the Agency for Health Care Quality Research (AHRQ) have launched efforts to promote the use of biomarkers in clinical studies of new therapies of asthma and ultimately in the evaluation of routine clinical care. A recent National Heart, Lung and Blood Institute (NHLBI) report identifies biomarkers, most of which assess atopic inflammation, such as the multiallergen screen, sputum and blood eosinophil numbers, serum IgE, exhaled nitric oxide (F_ENO), and urine leukotrienes⁸. While there is a tendency for asthma to be particularly problematic in patients with allergies, in excess of 40% of some populations suffer from allergies, allergic diathesis genes do not seem to be uniquely associated with asthma^{2,6,9}. Thus, whatever is driving the asthma epidemic, the asthma syndrome has affected a tremendous spectrum of individuals with diverse immunological and biochemical responses^{9,10}. This heterogeneity in the human population has resulted in a heterogeneity among asthma phenotypes^{10–12}. Therefore, biomarker tests have been recently developed and extended to identify and quantitate specific pathways of inflammation in order to identify specific asthma phenotypes—particularly those amenable to biologically-based anti-inflammatory therapy. The benefit of a non-invasive biomarker in assessment of therapeutic strategies is clear; the alternative is inspection and biopsy of the airway using invasive bronchoscopy¹³.

The mechanism-based biomarker approach avoids the limitations that occur with unbiased genotyping and phenotyping approaches¹⁰. For example, unbiased genetic analyses did not reveal a unifying asthma gene. Rather, asthma susceptibility genes are manifest in populations dependent upon environmental exposures, such as second hand cigarette smoke¹⁴. Similarly, unbiased asthma clinical phenotypes, while clearly revealing the heterogeneity of asthma¹⁵, require association to underlying pathobiologic mechanisms for clinically meaningful use. In a large asthma population of nonsevere and severe asthma, nonbiased hierarchical cluster analysis of clinical variables, such as age of asthma onset and duration, gender, race, lung functions, atopy, and questionnaire data, identified three phenotype clusters that contained the most severe asthma patients¹⁵. Similar cluster analyses of children confirmed heterogeneity in childhood severe asthma¹⁶. This variety supports that there will be a range of underlying biochemical and immunologic disorders in asthma. Thus, an informed, biochemical and pathophysiological approach is most likely to lead directly to

clinical applications. Here, we describe biomarkers and their potential use to stratify asthma patients into medically meaningful unique asthma phenotypes [Table 1].

THE EOSINOPHILIC OR TH2 HIGH INFLAMMATION PHENOTYPE: SPUTUM EOSINOPHILS, URINE BROMOTYROSINE AND PERIOSTIN

Atopic status was one of the first ways by which asthma phenotyping was performed, *i.e.* classification of asthma as extrinsic allergic or intrinsic nonallergic⁹. Allergic asthma is common and documentation of this phenotype has been helpful for avoidance of allergen triggers and consideration of immunologic based therapies. Classification as atopic asthma, which is typified by interleukin (IL)-4, IL-5 and IL-13 cytokines, has traditionally used standard clinical tests, including circulating numbers of eosinophils and total and allergen-specific IgE. These biomarkers are used in planning immunotherapy and anti-IgE therapy. In extension of these biomarkers, several groups describe that the number of eosinophils in sputum is closely related to airway obstruction and hyperresponsiveness^{17–19}. Exciting early data suggested that sputum eosinophils predict asthma control and loss of control, particularly in children who suffer predominantly with atopic asthma^{20,21}. The presence of more than 2% eosinophils in sputum has been used to define the eosinophilic or atopic asthma phenotype, which is also usually corticosteroid responsive²². A recent study of severe asthma validates that sputum eosinophils can identify individuals with poor asthma control, and greater healthcare utilization²³, however the test requires sputum induction, specialized processing of the sputum sample, as well as an experienced cytotechnologist for accurate counting, all of which have limited the use of sputum eosinophils in general clinical care. More recently, Woodruff *et al.* further identified a TH2-high inflammation phenotype on the basis of combination of biomarkers, which include the presence of high serum IgE (> 100 ng/ml), blood eosinophilia (> 0.14×10^9 eosinophils/L), and high sputum eosinophils²⁴. The use of microarrays has also identified a TH2-high blood biomarker, *i.e.* periostin, an IL-13 inducible protein produced by the airway epithelium. The use of periostin as a biomarker of the TH2-high phenotype to select patients that benefitted the most from treatment with an inhibitor of IL-13²⁵ provided a proof of concept that biomarkers may be used to stratify patients for biologic-based therapies. Conversely, the data also validate the important point that a substantial number of asthma patients have non-TH2 predominant inflammation.

A high sputum eosinophil count correlates with a high fractional excretion of NO [FENO] in the exhaled breath, which is often suggested as a biomarker of inflammation. However, it turns out that low FENO is a much better predictor of a non-eosinophilic phenotype than a high FENO is for eosinophilic phenotype. Hence, FENO should be considered as a unique metabolic biomarker of asthma, as discussed later. On the other hand, eosinophils generate high levels of reactive oxygen species²⁶, and the eosinophil peroxidase is unique in its ability to convert hydrogen peroxide to hypobromous acid^{27,28}, which oxidizes tyrosines to bromotyrosine (BrY) (Figure 1). BrY is highly stable and can be measured noninvasively in urine as a biomarker highly specific for eosinophil activation^{29,30}. Because BrY is a biomarker of eosinophil activation, its levels increase dramatically following experimental or clinical asthma exacerbations²⁹ and in early studies, urine BrY of asthmatic children appears highly correlated to asthma control and predicts risk of asthma exacerbations³¹. Studies are needed to evaluate the relationship of BrY and periostin in the TH2-high phenotype, but given that BrY is unrelated to IgE or blood eosinophils, these biomarkers may be non-redundant and possibly complementary in predicting asthma phenotypes for clinical treatments.

A subpopulation of asthma patients has increased activity of the enzyme, S-Nitrosoglutathione (GSNO) reductase^{32–34} (Figure 1). GSNO is an endogenous nitric oxide

synthase (NOS) product that causes cGMP-independent relaxation of human airway smooth muscle^{35,36}. It can directly prevent actin-myosin interaction³⁷, and it can S-nitrosylate G protein-coupled kinase 2 (GRK2), preventing β_2 adrenergic agonist stimulation-induced tachyphylaxis³⁸. GSNO is broken down *in vivo* by GSNO reductase^{32–34,39}. Antigen-sensitized mice deficient in GSNO reductase are protected from methacholine-induced increased airway resistance. Further, mice deficient in GSNO reductase are prevented from having pulmonary tachyphylaxis to isoproterenol³³. In children with asthmatic respiratory failure, GSNO reductase activity is upregulated and GSNO levels are profoundly low³². This creates a situation in which there is an endogenous airway smooth muscle relaxant that is deficient in the asthmatic airway; and this deficiency also exacerbates refractoriness to β_2 agonist-based treatment. Indeed, gain of function SNPs in GSNO reductase are associated with refractory asthma in certain subpopulations. Biopsies with increased GSNO reductase expression are anatomically associated with areas of poor airflow, as analyzed by hyperpolarized xenon or helium imaging. Thus, GSNO reductase is an important mechanism in the origins of a reactive asthma phenotype, but bronchoalveolar lavage studies show that GSNO reductase activity is not increased in all asthma patients³⁴. Further, there is essentially no difference between stable, ambulatory severe and non-severe asthma with regard to GSNO reductase activity (unpublished observation). How can we identify this important subpopulation with high GSNO reductase activity in clinic in order to target therapy with S-nitrosoglutathione replacement and/or S-nitrosoglutathione reductase inhibition? The importance of this question is highlighted by recent evidence that chronic, excessive inhibition of S-nitrosoglutathione reductase has the potential to be associated with the development of cancer in patients exposed to chronic nitrosative stress⁴⁰. We envision the following clinical paradigm to approach this type of question. First, the clinical disease phenotype needs to be identified. What are the clinical characteristics of a patient with increased airway GSNO reductase activity? This can be done by defining the phenotype of patients who are identified by biochemical analysis of bronchoscopic samples. It can also be done through SNP-wide analysis of phenotype (SWAP). Next, biomarkers that validate the biochemical abnormality should be developed. In the example of GSNO reductase, there are at least two possible biomarkers. GSNO reductase also serves as an S-formylglutathione dehydrogenase^{41,42}. The product of the latter enzymatic activity is formic acid, which can be measured in breath condensate. Levels are high in a subpopulation of asthma patients, and these appear to be the same patients as those with high GSNO reductase activity. This hypothesis requires validation. Another approach is challenge testing. In cystic fibrosis, GSNO replacement is associated with an increase of FENO⁴³. The faster the rate of GSNO breakdown, the faster the decline of FENO after GSNO inhalation. This principle can likely be applied to asthma, as well. A challenge with GSNO or a GSNO precursor is associated with an increase in FENO. The decay rate of FENO after GSNO inhalation can be used as a surrogate for airway GSNO reductase activity. Indeed, FENO will likely be used as a readout in challenge testing for a number of subpopulations, including those with low pH^{36,44}, those with high levels of eosinophils and those with high levels of GSNO reductase activity. We believe that the challenge test may serve as a useful paradigm for identifying treatable subpopulations.

THE REDUCING-OXIDIZING [REDOX] IMBALANCE PHENOTYPE: LIPID OXIDATION AND LOSS OF ANTIOXIDANT SUPEROXIDE DISMUTASE

Recruitment and activation of inflammatory cells, both eosinophils and neutrophils, causes a respiratory burst in the airways that produces reactive oxygen species and reactive nitrogen species^{45–48}. Certain of these species can damage proteins via specific enzyme-catalyzed oxidations or nonspecific oxidation of susceptible molecules. For example, eosinophil peroxidase and neutrophil myeloperoxidase cause halogenation of tyrosine residues,

bromination and chlorination, respectively, and the halogenated products serve as molecular fingerprints of eosinophilic or neutrophilic inflammation. On the other hand, peroxidation of membrane lipids occurs spontaneously and results in the F₂-isoprostanes (F₂IsoPs), *i.e.* 8-epi-PGF_{2α} and its metabolite 2,3-dinor-8-epi-PGF_{2α}. These structurally stable products are renally excreted, making them quantifiable non-invasive biomarkers of nonspecific redox imbalance and inflammation^{49–51}. In support of an increase of nonspecific oxidation, urine F₂IsoPs are higher in asthma patients than healthy controls, and urine levels rise upon an allergen-induced asthma exacerbation^{49,52,53}. Biomarkers of enzyme-catalyzed oxidation pathways, such as urine BrY, and nonspecific oxidation pathways, such as F₂IsoPs, allow multiple opportunities to monitor asthma control and plan appropriate treatments. For example, stepping up corticosteroid therapy may be warranted in those individuals with high urine BrY, but may be less helpful in those individuals with low or normal BrY and high urine F₂IsoPs. In the latter case, neutrophilic inflammation or environmental oxidant-mediated inflammation might be suspect.

Nonspecific oxidation events also indicate a relative inadequacy of protective antioxidants. A wide array of antioxidants are present in the airways⁵⁴, but oxidative and nitrosative stress can overwhelm these defenses, resulting in redox imbalance and oxidative injury^{10,48}. The airway contains non-enzymatic antioxidants, such as glutathione, and enzymatic antioxidants, such as superoxide dismutases (SOD) and catalase (Figure 1). Asthmatic airways have increased total glutathione levels but loss of SOD and catalase enzymic activities⁴⁸. Interestingly, the loss of SOD activity is found in the airways and serum of asthma patients allowing the serum SOD to be used as a biomarker of redox imbalance^{48,55–60}. SOD activity is inversely related to airway reactivity and airflow obstruction, with higher levels of SOD related to lower airway reactivity and better airflow⁶⁰. Murine models of asthma confirm that SOD plays a mechanistic role in airway hyper-responsiveness and inflammation, *e.g.* SOD transgenic mice have less allergen-induced airway inflammation and reactivity in comparison to wild type mice⁶¹. In patients with asthma and in the murine model of asthma, SOD activity loss is related in part to the oxidation of the MnSOD protein and is linked to epithelial apoptosis and airway remodeling^{60,62}. Interestingly, low levels of serum SOD activity was an independent biomarker for airflow obstruction in severe asthma nonresponsive to corticosteroids⁵⁹. Furthermore, second hand smoke exposure is associated with poorer lung functions and lower levels of serum SOD activity⁵⁸. These findings suggest that strategies aimed at restoration of normal redox balance, perhaps through the use of SOD-mimetics, may help those subjects with non-eosinophilic inflammation in whom corticosteroids may have little impact on asthma control.

THE LOW PH PHENOTYPE

During acute exacerbations of asthma, breath condensate pH is decreased⁶³. This is associated with decreased serum and airway activity of glutaminase—activated downstream of Th1 cytokines associated with viral asthma exacerbations—preventing airway buffering^{63,64}. Decreased airway pH promotes ciliary dysfunction, mucus hypersecretion and cough^{63–65}. Recent evidence suggests that this decreased airway pH might be successfully treated with inhaled buffer^{36,44}. That said, most stable asthmatics in day-to-day practice do not have decreased pH⁶⁶. In the NHLBI Severe Asthma Research Program (SARP) study of 572 stable asthmatic subjects, only 7% had breath condensate pH < 6.5 at baseline. This group was characterized by prominently low FENO, low FEV₁, high BMI, low levels of airway eosinophils, and gastric esophageal reflux symptoms along with other features. Because most nebulized treatments are in acidic solutions, this subpopulation of outpatient asthmatics with decreased airway pH might benefit from less acidic therapy; indeed, they might improve with inhaled base.

In order to identify these patients, initial medical suspicion should be on the basis of the phenotype described above. Patients who have a characteristic phenotype can be then diagnosed by biomarker analysis. Because nitrite has a pKa of 3.6—and, once protonated, dissociates to form NO—increasing airway pH decreases FENO. Therefore, inhaled buffer followed by serial FENO measures can be used to diagnose the low pH phenotype^{36,44}. Those patients with a decrease in FENO after inhaled buffer should be those who will improve with inhaled base or glutamine supplement as a targeted therapy.

AIRWAY REMODELING PHENOTYPE: AIRWAY ANGIOGENIC BIOMARKERS

Increased number of blood vessels is universally found in asthmatic airway remodeling of children and adults^{67,68}. The mouse model of asthma suggests that the switch to a pro-angiogenic airway and neovascularization occurs early and well in advance of eosinophilic inflammation⁶⁹. This suggests that angiogenesis participates in the genesis of asthma. In fact, several lines of evidence indicate that angiogenesis and chronic inflammation are mutually supportive⁷⁰. Inflammatory cells produce many pro-angiogenic factors in asthmatic lungs⁷¹, chief among which is vascular endothelial growth factor (VEGF). VEGF levels are increased in asthma bronchoalveolar lavage fluid, and related to blood vessel numbers in the mucosa^{69,71}. High VEGF levels in sputum are associated with airflow obstruction, and levels of VEGF increase in sputum during an asthma attack⁶⁷. Likewise in model systems, allergen or virus-induced inflammation increases airway VEGF levels^{72,73}. In support of a mechanistic role, VEGF over-expression in airways of transgenic mice leads to an asthma-like phenotype⁷⁴. Mast cells and eosinophils can produce high levels of pro-angiogenic factors, including VEGF^{75,76}. Pro-angiogenic factors such as VEGF cause neovascularization in large part through effects on myeloid progenitor cell proliferation and mobilization from the bone marrow. Cell surface markers CD34 and CD133 define the subset of pro-angiogenic myeloid progenitors^{77,78}, and can thus easily be enumerated by flow cytometric analysis. Studies identify high levels of CD34⁺CD133⁺ cells in blood of asthmatic patients and that the levels correlate with airflow obstruction. Intriguingly, this same population of CD34⁺CD133⁺ cells also contain the mast cell progenitor. Further work is needed to determine if quantitation of circulating CD34⁺CD133⁺ cells can serve as a biomarker of airway remodeling and/or mast cell numbers and types in asthma.

THE ARGININE/NO PHENOTYPE: FENO, ARGINASE, METHYLARGININES

Measure of NO in the exhaled breath has been labeled as a sensitive and reliable biomarker of airway inflammation in adults and children^{79–84}. On this basis, FENO was approved by the United States Food and Drug Administration for the purpose of evaluating anti-inflammatory treatment responses of asthmatic patients⁸⁴. However, FENO exhibits a broad range of values in asthmatic patients⁸⁵. In asthma patients, FENO is useful to identify those patients characterized by the greatest airflow obstruction and most frequent utilization of emergency care.

Inflammation, as characteristic in asthmatic airways, results in the expression of the inducible NO synthase (iNOS)^{86–88}. iNOS is expressed in many airway cells, but is prominent in the airway epithelial cells, where it catalyzes the conversion of L-arginine into NO and L-citrulline^{86,87} (Figure 1). The synthesized gaseous NO may be measured noninvasively in the exhaled breath as a biomarker of chronic airway inflammation induction of the iNOS^{79,80,86}. Eosinophils are not necessary for iNOS production of NO, and TH2 cytokines are not essential for iNOS expression. Hence, FENO does not uniformly track with airway eosinophils, urine BrY or periostin. While studies suggest that monitoring anti-inflammatory therapy by FENO is useful, it does not necessarily diminish the rate of

asthma exacerbations^{81,89,90}. This limitation is likely due to the fact that not all inflammatory processes are reflected by FENO measurements^{91, 8,92}. This supports the concept that biomarkers of asthma may be more informative when used in combination with challenge testing *i.e.*, FENO measures with inhalation of base, or in combination with other biomarkers such as urine BrY levels. In this context, while FENO, urine BrY, and urine F₂IsoP each are useful as a biomarker of asthma, pooled together they serve as a highly sensitive and specific biomarker panel for diagnosis of asthma⁹³.

Although a multitude of studies focus on the meaning of high FENO in asthma^{29,62,86,94–97}, asthma patients may often have values within the normal range (<25 ppb). Patients with low to normal FENO values usually do not have eosinophilic inflammation, but may still have signs of inflammation and remodeling. Low FENO is helpful in that it suggests a phenotype that is less responsive to corticosteroids, preventing excessive medication that may have substantial morbidity. The low FENO patients may suffer from non-TH2 inflammation and/or abnormalities in arginine metabolism and/or increased oxidative consumption of NO in the airway. Additionally, FENO may be low due to low levels of GSNO in the airways due to catabolism by higher than normal levels of GSNO reductase. Chronic loss of airway nitrite in the context of low airway pH leads to low baseline FENO in the low pH phenotype.

Asthmatics often have greater metabolism of arginine via iNOS and the arginase enzymes, which identifies a subpopulation of asthma as a disease of increased arginine catabolism⁹⁸ (Figure 1). High levels of arginase activity in asthma are often associated with increased levels of methylarginines, which are endogenous NOS inhibitors. This suggests that methylarginines may impact arginine availability as a substrate for iNOS^{97,99,100}. Serum arginase increases during acute asthma exacerbations and decreases with improved asthma control, and is inversely related to airflow^{97,98}. Studies indicate that arginase expression and activity is increased by allergen-induced gene activation in asthma, and TH2 cytokines^{86,99–105}. On the other hand, oxidative stress can decrease the metabolism of methylarginines through effects on dimethylarginine dimethylaminohydrolase (DDAH)¹⁰⁶. This diminished metabolism of methylarginines may block iNOS and contribute to low FENO in some asthmatic patients, particularly in severe corticosteroid-resistant asthma. Serum arginase also provides insight into mechanisms of airway remodeling¹⁰⁷. Arginase regulates polyamine synthesis, which is required for DNA synthesis in cell proliferation^{108,109}. Arginase also regulates precursors for the synthesis of proline, required for collagen production^{110,111}. Thus, serum arginase and FENO are unique biomarkers of inflammation in severe asthma and independent of the eosinophilic phenotype. Increased arginase is associated with low FENO in adults with severe asthma⁹⁸. Decreased SOD activity is also associated with low FENO in adults with severe asthma, suggesting an oxidative consumption of NO that may lead to low normal values of FENO⁹⁸ (Figure 1).

THE LEUKOTRIENE PHENOTYPE: LTE₄, LIPOXINS AND PROTECTANTS

Endogenous lipid mediators can help to maintain tissue homeostasis, yet they can also contribute to inflammation and bronchoconstriction¹¹². Leukotrienes are examples of potent pro-inflammatory and bronchoconstricting agents. Inhibitors of the cysteinyl leukotriene receptor and of the upstream cysteinyl leukotriene synthetic enzyme, 5-lipoxygenase, are in clinical use for asthma treatment¹¹³. However, many asthma patients are not effectively treated with leukotriene receptor antagonists. Further, 5-LO inhibition can cause hepatotoxicity. Therefore, identification of asthma subpopulations for whom these specific, anti-leukotriene agents be effective has for some time been a focus of genetic studies in asthma. Genetic testing for polymorphisms and 5-lipoxygenase has also been proposed as a screening tool for identification of responsive subpopulations^{52,114}. Additionally,

biomarkers have been sought—including urinary leukotrienes and FE_{NO} —to try to identify those subjects who would be most responsive^{115,116}.

Urine LTE_4 has been validated as a biomarker of cysteinyl leukotriene overactivity. Levels of LTE_4 increase with acute asthma attacks and with aspirin exacerbated respiratory disease, and decrease with cysteinyl leukotriene synthesis blockade but not with corticosteroids^{117–124}. Although the use of therapies in the leukotriene pathway are valuable, lack of widespread availability of clinical testing for LTE_4 limit the use of this biomarker in optimizing asthma therapies. Specific lipid mediators can also prevent airway inflammation. For example, lipoxins have anti-inflammatory properties affecting epithelial cells, airway leukocytes and the pulmonary endothelium. They inhibit eosinophil and neutrophil migration into the airway, and block the cytotoxicity of natural killer cells^{112,125}. The dysregulation and site of effect of lipoxins and other potentially beneficial lipid mediators (such as resolvins and protectins) in asthma is complex^{112,126}. Lipoxin A4 levels are decreased in the bronchoalveolar lavage of patients with severe asthma, and lipoxin synthesis is inhibited in the circulation of many severe asthmatics^{127,128}. Decreased levels of lipoxin A4 in exhaled breath condensate have been proposed as a biomarker of lipoxin-pathway abnormalities severe asthma. Levels of protectin D1 are similarly decreased in breath condensate during acute asthma exacerbations¹²⁹. The identification of these biomarkers may permit targeted pharmacological interventions for specific patients: they might ultimately be used to predict whether interventions can benefit, fail to benefit, or even harm patients based on their lipid mediator phenotypes. It is increasingly apparent that the use of blood, sputum and/or breath condensate biomarker analysis will be critically important in tailoring specific pharmacotherapy for specific asthma patients.

ADDITIONAL CONSIDERATIONS FOR FUTURE STUDY

Biological understanding is improving for several risk factors for severe asthma. These include sex, race, obesity and environmental tobacco exposure. Severe asthma is more prevalent in women after puberty^{130,131}. Obesity is associated with asthma severity in adult-onset disease⁶. The greater prevalence of severe asthma among obese women may be related to menstrual cycle effects on circulating $CD34^+ CD133^+$ cells¹³⁰ or adipose-related factors^{132,133}. Additionally, circulating chitinase-like protein (YKL-40) levels are higher among patients with severe asthma in several cohorts, including SARP¹³⁴. Asthma is characterized by air trapping^{6,15,135}. Although in the early stages, CT studies may allow measurement of airways^{136,137} and parenchyma¹³⁷ that can serve as biomarkers of airway remodeling and airtrapping phenotypes. In studies of severe asthmatics, CT-determinations of air trapping were associated with severity of disease^{138,139}. New applications of molecular imaging promise the ability to track specific mechanistic biomarkers to specific structures using co-registered CT images, opening the door to imaging biomarkers in clinical practice. Biomarkers for severe asthma symptoms are likely to emerge from these studies.

SUMMARY

Asthma occurs in individuals with a broad range of different inflammatory and biochemical phenotypes. Most of these phenotypes have the potential to be targeted with specific treatments. Targeted treatment of the underlying disease process has the potential to be corticosteroid-sparing, particularly in severe asthma. Many biomarkers are being developed to identify these specific phenotypes noninvasively. This development is grounded in a medically meaningful paradigm in which an underlying pathophysiology is suspected based on clinical presentation, a biomarker(s) test is used to confirm the cause/diagnosis, and a targeted treatment is provided.

In this article, we have discussed several phenotypes of asthma, and there are almost certainly more phenotypes associated with additional underlying processes. We anticipate that each pathophysiology-based phenotype will ultimately be diagnosed by a defining biomarker, a panel of biomarkers and/or biomarker challenge test. Phenotypes may overlap in specific patients and may change over time. Hence, patients will likely profit from repetitive testing in order to define asthma pathophysiological phenotype(s) and to tailor therapy. Indeed, targeted therapy, particularly for severe patients, based on interpretation of biomarker profiles may ultimately be the role of the asthma specialist. We believe that this approach will provide a clear path forward to improve treatment and minimize adverse effects.

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References

1. Yawn BP, Brennum SK, Allen-Ramey FC, et al. Assessment of asthma severity and asthma control in children. *Pediatrics*. 2006; 118:322–9. [PubMed: 16818581]
2. NHLBI. Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma Full Report. 2007.
3. Wenzel S, Holgate ST. The mouse trap: It still yields few answers in asthma. *Am J Respir Crit Care Med*. 2006; 174:1173–6. discussion 6–8. [PubMed: 17110654]
4. Masoli M, Fabian D, Holt S, et al. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy*. 2004; 59:469–78. [PubMed: 15080825]
5. Busse WW, Banks-Schlegel S, Wenzel SE. Pathophysiology of severe asthma. *J Allergy Clin Immunol*. 2000; 106:1033–42. [PubMed: 11112883]
6. Moore WC, Bleecker ER, Curran-Everett D, et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol*. 2007; 119:405–13. [PubMed: 17291857]
7. Paull K, Covar R, Jain N, Gelfand EW, et al. Do NHLBI lung function criteria apply to children? A cross-sectional evaluation of childhood asthma at National Jewish Medical and Research Center, 1999–2002. *Pediatric Pulmonology*. 2005; 39:311–7. [PubMed: 15678505]
8. ATS Workshop Proceedings: Exhaled nitric oxide and nitric oxide oxidative metabolism in exhaled breath condensate: Executive summary. *Am J Respir Crit Care Med*. 2006; 173:811–3. [PubMed: 16556701]
9. Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet*. 2006; 368:804–13. [PubMed: 16935691]
10. Gaston B. The biochemistry of asthma. *Biochim Biophys Acta*. 2011; 1810:1017–24. [PubMed: 21718756]
11. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet*. 2008; 372:1107–19. [PubMed: 18805339]
12. Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet*. 2000; 356:2144–8. [PubMed: 11191542]
13. Pavord ID, Shaw DE, Gibson PG, et al. Inflammometry to assess airway diseases. *Lancet*. 2008; 372:1017–9. [PubMed: 18805315]

14. Meyers DA, Postma DS, Stine OC, et al. Genome screen for asthma and bronchial hyperresponsiveness: interactions with passive smoke exposure. *J Allergy Clin Immunol.* 2005; 115:1169–75. [PubMed: 15940130]
15. Moore WC, Meyers DA, Wenzel SE, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med.* 2010; 181:315–23. [PubMed: 19892860]
16. Fitzpatrick AM, Teague WG, Meyers DA, et al. Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. *J Allergy Clin Immunol.* 2010; 127:382–9. e1–13. [PubMed: 21195471]
17. Woodruff PG, Khashayar R, Lazarus SC, et al. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. *J Allergy Clin Immunol.* 2001; 108:753–8. [PubMed: 11692100]
18. Polosa R, Renaud L, Cacciola R, et al. Sputum eosinophilia is more closely associated with airway responsiveness to bradykinin than methacholine in asthma. *Eur Respir J.* 1998; 12:551–6. [PubMed: 9762778]
19. Louis R, Lau LC, Bron AO, et al. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med.* 2000; 161:9–16. [PubMed: 10619791]
20. Covar RA, Spahn JD, Martin RJ, et al. Safety and application of induced sputum analysis in childhood asthma. *J Allergy Clin Immunol.* 2004; 114:575–82. [PubMed: 15356559]
21. Gibson PG. Use of induced sputum to examine airway inflammation in childhood asthma. *J Allergy Clin Immunol.* 1998; 102:S100–1. [PubMed: 9819317]
22. Berry M, Morgan A, Shaw DE, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax.* 2007; 62:1043–9. [PubMed: 17356056]
23. Hastie AT, Moore WC, Meyers DA, et al. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. *J Allergy Clin Immunol.* 2010; 125:1028–36. e13. [PubMed: 20398920]
24. Woodruff PG, Modrek B, Choy DF, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med.* 2009; 180:388–95. [PubMed: 19483109]
25. Corren J, Lemanske RF, Hanania NA, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med.* 2011; 365:1088–98. [PubMed: 21812663]
26. MacPherson JC, Comhair SA, Erzurum SC, et al. Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma: characterization of pathways available to eosinophils for generating reactive nitrogen species. *J Immunol.* 2001; 166:5763–72. [PubMed: 11313420]
27. Aldridge RE, Chan T, van Dalen CJ, et al. Eosinophil peroxidase produces hypobromous acid in the airways of stable asthmatics. *Free Radic Biol Med.* 2002; 33:847–56. [PubMed: 12208372]
28. Erpenbeck VJ, Hohlfeld JM, Petschallies J, et al. Local release of eosinophil peroxidase following segmental allergen provocation in asthma. *Clin Exp Allergy.* 2003; 33:331–6. [PubMed: 12614447]
29. Wu W, Chen Y, d'Avignon A, et al. 3-Bromotyrosine and 3,5-dibromotyrosine are major products of protein oxidation by eosinophil peroxidase: potential markers for eosinophil-dependent tissue injury in vivo. *Biochemistry.* 1999; 38:3538–48. [PubMed: 10090740]
30. Mita H, Higashi N, Taniguchi M, et al. Urinary 3-bromotyrosine and 3-chlorotyrosine concentrations in asthmatic patients: lack of increase in 3-bromotyrosine concentration in urine and plasma proteins in aspirin-induced asthma after intravenous aspirin challenge. *Clin Exp Allergy.* 2004; 34:931–8. [PubMed: 15196282]
31. Wedes SH, Wu W, Comhair SA, et al. Urinary bromotyrosine measures asthma control and predicts asthma exacerbations in children. *J Pediatr.* 2011; 159:248–55. e1. [PubMed: 21392781]
32. Gaston B, Sears S, Woods J, et al. Bronchodilator S-nitrosothiol deficiency in asthmatic respiratory failure. *Lancet.* 1998; 351:1317–9. [PubMed: 9643794]
33. Que LG, Liu L, Yan Y, et al. Protection from experimental asthma by an endogenous bronchodilator. *Science.* 2005; 308:1618–21. [PubMed: 15919956]
34. Que LG, Yang Z, Stamler JS, et al. S-nitrosoglutathione reductase: an important regulator in human asthma. *Am J Respir Crit Care Med.* 2009; 180:226–31. [PubMed: 19395503]

35. Gaston B, Reilly J, Drazen JM, et al. Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. *Proc Natl Acad Sci USA*. 1993; 90:10957–61. [PubMed: 8248198]
36. Gaston B, Kelly R, Urban P, et al. Buffering airway acid decreases exhaled nitric oxide in asthma. *J Allergy Clin Immunol*. 2006; 118:817–22. [PubMed: 17030232]
37. Evangelista AM, Rao VS, Filo AR, et al. Direct regulation of striated muscle myosins by nitric oxide and endogenous nitrosothiols. *PloS One*. 2010; 5:e11209. [PubMed: 20585450]
38. Whalen EJ, Foster MW, Matsumoto A, et al. Regulation of beta-adrenergic receptor signaling by S-nitrosylation of G-protein-coupled receptor kinase 2. *Cell*. 2007; 129:511–22. [PubMed: 17482545]
39. Fang K, Johns R, Macdonald T, et al. S-nitrosoglutathione breakdown prevents airway smooth muscle relaxation in the guinea pig. *Am J Physiol*. 2000; 279:L716–21.
40. Marozkina NV, Wei C, Yemen S, et al. S-nitrosoglutathione reductase in human lung cancer. *Am J Respir Cell Mol Biol*. 2011; 46:63–70. [PubMed: 21816964]
41. Jensen DE, Belka GK, Du Bois GC. S-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. *Biochem J*. 1998; 331 (Pt 2):659–68. [PubMed: 9531510]
42. Greenwald R, Fitzpatrick AM, Gaston B, et al. Breath formate is a marker of airway S-nitrosothiol depletion in severe asthma. *PloS One*. 2010; 5:e11919. [PubMed: 20689836]
43. Snyder AH, McPherson ME, Hunt JF, et al. Acute effects of aerosolized S-nitrosoglutathione in cystic fibrosis. *Am J Respir Crit Care Med*. 2002; 165:922–6. [PubMed: 11934715]
44. Shin HW, Shelley DA, Henderson EM, et al. Airway nitric oxide release is reduced after PBS inhalation in asthma. *J Appl Physiol*. 2007; 102:1028–33. [PubMed: 17110506]
45. Caramori G, Papi A. Oxidants and asthma. *Thorax*. 2004; 59:170–3. [PubMed: 14760161]
46. Mak JC, Chan-Yeung MM. Reactive oxidant species in asthma. *Curr Opin Pulm Med*. 2006; 12:7–11. [PubMed: 16357572]
47. Andreadis AA, Hazen SL, Comhair SA, et al. Oxidative and nitrosative events in asthma. *Free Rad Biol Med*. 2003; 35:213–25. [PubMed: 12885584]
48. Comhair SA, Erzurum SC. Redox control of asthma: molecular mechanisms and therapeutic opportunities. *Antioxid Redox Signal*. 2010; 12:93–124. [PubMed: 19634987]
49. Dworski R, Murray JJ, Roberts LJ 2nd, et al. Allergen-induced synthesis of F(2)-isoprostanes in atopic asthmatics. Evidence for oxidant stress. *Am J Respir Crit Care Med*. 1999; 160:1947–51. [PubMed: 10588611]
50. Morrow JD, Roberts LJ. The isoprostanes: their role as an index of oxidant stress status in human pulmonary disease. *Am J Respir Crit Care Med*. 2002; 166:S25–30. [PubMed: 12471085]
51. Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. *Eur Respir J*. 2003; 21:177–86. [PubMed: 12570126]
52. Tse SM, Tantisira K, Weiss ST. The pharmacogenetics and pharmacogenomics of asthma therapy. *Pharmacogen J*. 2011; 11:383–92.
53. Dworski R, Sheller JR. Urinary mediators and asthma. *Clin Exp Allergy*. 1998; 28:1309–12. [PubMed: 9824399]
54. Heffner JE, Repine JE. Pulmonary strategies of antioxidant defense. *Am Rev Respir Dis*. 1989; 140:531–54. [PubMed: 2669581]
55. De Raeve HR, Thunnissen FB, Kaneko FT, et al. Decreased Cu,Zn-SOD activity in asthmatic airway epithelium: correction by inhaled corticosteroid in vivo. *Am J Physiol*. 1997; 272:L148–54. [PubMed: 9038914]
56. Comhair SA, Bhathena PR, Dweik RA, et al. Rapid loss of superoxide dismutase activity during antigen-induced asthmatic response. *Lancet*. 2000; 355:624. [PubMed: 10696986]
57. Comhair SA, Erzurum SC. Antioxidant responses to oxidant-mediated lung diseases. *Am J Physiol*. 2002; 283:L246–55.
58. Comhair SA, Gaston BM, Ricci KS, et al. Detrimental effects of environmental tobacco smoke in relation to asthma severity. *PloS one*. 2011; 6:e18574. [PubMed: 21572527]

59. Comhair SA, Ricci KS, Arroliga M, et al. Correlation of systemic superoxide dismutase deficiency to airflow obstruction in asthma. *Am J Respir Crit Care Med*. 2005; 172:306–13. [PubMed: 15883124]
60. Comhair SA, Xu W, Ghosh S, et al. Superoxide dismutase inactivation in pathophysiology of asthmatic airway remodeling and reactivity. *Am J Pathol*. 2005; 166:663–74. [PubMed: 15743779]
61. Larsen GL, White CW, Takeda K, et al. Mice that overexpress Cu/Zn superoxide dismutase are resistant to allergen-induced changes in airway control. *Am J Physiol*. 2000; 279:L350–9.
62. Ghosh S, Janocha AJ, Aronica MA, et al. Nitrotyrosine proteome survey in asthma identifies oxidative mechanism of catalase inactivation. *J Immunol*. 2006; 176:5587–97. [PubMed: 16622028]
63. Hunt JF, Erwin E, Palmer L, et al. Expression and activity of pH-regulatory glutaminase in the human airway epithelium. *Am J Respir Crit Care Med*. 2002; 165:101–7. [PubMed: 11779738]
64. Carraro S, Doherty J, Zaman K, et al. S-nitrosothiols regulate cell-surface pH buffering by airway epithelial cells during the human immune response to rhinovirus. *Am J Physiol*. 2006; 290:L827–32.
65. Hunt JF, Fang K, Malik R, et al. Endogenous airway acidification. Implications for asthma pathophysiology. *Am J Respir Crit Care Med*. 2000; 161:694–9. [PubMed: 10712309]
66. Liu L, Teague WG, Erzurum S, et al. Determinants of exhaled breath condensate pH in a large population with asthma. *Chest*. 2011; 139:328–36. [PubMed: 20966042]
67. Barbato A, Turato G, Baraldo S, et al. Epithelial damage and angiogenesis in the airways of children with asthma. *Am J Respir Crit Care Med*. 2006; 174:975–81. [PubMed: 16917118]
68. Hashimoto M, Tanaka H, Abe S. Quantitative analysis of bronchial wall vascularity in the medium and small airways of patients with asthma and COPD. *Chest*. 2005; 127:965–72. [PubMed: 15764783]
69. Asosingh K, Swaidani S, Aronica M, et al. Th1- and Th2-dependent endothelial progenitor cell recruitment and angiogenic switch in asthma. *J Immunol*. 2007; 178:6482–94. [PubMed: 17475878]
70. Jackson JR, Seed MP, Kircher CH, et al. The codependence of angiogenesis and chronic inflammation. *Faseb J*. 1997; 11:457–65. [PubMed: 9194526]
71. Chetta A, Zanini A, Foresi A, et al. Vascular endothelial growth factor up-regulation and bronchial wall remodelling in asthma. *Clin Exp Allergy*. 2005; 35:1437–42. [PubMed: 16297139]
72. Psarras S, Volonaki E, Skevaki CL, et al. Vascular endothelial growth factor-mediated induction of angiogenesis by human rhinoviruses. *J Allergy Clin Immunol*. 2006; 117:291–7. [PubMed: 16461129]
73. Avdalovic MV, Putney LF, Schelegle ES, et al. Vascular remodeling is airway generation-specific in a primate model of chronic asthma. *Am J Respir Crit Care Med*. 2006; 174:1069–76. [PubMed: 16931639]
74. Lee CG, Link H, Baluk P, et al. Vascular endothelial growth factor (VEGF) induces remodeling and enhances TH2-mediated sensitization and inflammation in the lung. *Nature Med*. 2004; 10:1095–103. [PubMed: 15378055]
75. Puxeddu I, Alian A, Piliponsky AM, et al. Human peripheral blood eosinophils induce angiogenesis. *Int J Biochem Cell Biol*. 2005; 37:628–36. [PubMed: 15618019]
76. Ribatti D, Crivellato E, Roccaro AM, et al. Mast cell contribution to angiogenesis related to tumour progression. *Clin Exp Allergy*. 2004; 34:1660–4. [PubMed: 15544587]
77. Prater DN, Case J, Ingram DA, et al. Working hypothesis to redefine endothelial progenitor cells. *Leukemia*. 2007; 21:1141–9. [PubMed: 17392816]
78. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science (New York, NY)*. 1997; 275:964–7.
79. Kharitonov SA, Yates D, Robbins RA, et al. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet*. 1994; 343:133–5. [PubMed: 7904001]
80. Ricciardolo FL, Gaston B, Hunt J. Acid stress in the pathology of asthma. *J Allergy Clin Immunol*. 2004; 113:610–9. [PubMed: 15100663]

81. Smith AD, Cowan JO, Brassett KP, et al. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *New Engl J Med*. 2005; 352:2163–73. [PubMed: 15914548]
82. Dinakar C. Exhaled nitric oxide in pediatric asthma. *Curr Allergy Asthma Rep*. 2009; 9:30–7. [PubMed: 19063822]
83. Nordvall SL, Janson C, Kalm-Stephens P, et al. Exhaled nitric oxide in a population-based study of asthma and allergy in schoolchildren. *Allergy*. 2005; 60:469–75. [PubMed: 15727578]
84. Silkoff PE, Carlson M, Bourke T, et al. The Aerocrine exhaled nitric oxide monitoring system NIOX is cleared by the US Food and Drug Administration for monitoring therapy in asthma. *J Allergy Clin Immunol*. 2004; 114:1241–56. [PubMed: 15536442]
85. Dweik RA, Sorkness RL, Wenzel S, et al. Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med*. 2010; 181:1033–41. [PubMed: 20133930]
86. Guo FH, Comhair SA, Zheng S, et al. Molecular mechanisms of increased nitric oxide (NO) in asthma: evidence for transcriptional and post-translational regulation of NO synthesis. *J Immunol*. 2000; 164:5970–80. [PubMed: 10820280]
87. Guo FH, Erzurum SC. Characterization of inducible nitric oxide synthase expression in human airway epithelium. *Environ Health Perspect*. 1998; 106 (Suppl 5):1119–24. [PubMed: 9788885]
88. Guo FH, Uetani K, Haque SJ, et al. Interferon gamma and interleukin 4 stimulate prolonged expression of inducible nitric oxide synthase in human airway epithelium through synthesis of soluble mediators. *J Clin Invest*. 1997; 100:829–38. [PubMed: 9259582]
89. Shaw DE, Berry MA, Thomas M, et al. The use of exhaled nitric oxide to guide asthma management: a randomized controlled trial. *Am J Respir Crit Care Med*. 2007; 176:231–7. [PubMed: 17496226]
90. Szeffler SJ, Mitchell H, Sorkness CA, et al. Management of asthma based on exhaled nitric oxide in addition to guideline-based treatment for inner-city adolescents and young adults: a randomised controlled trial. *Lancet*. 2008; 372:1065–72. [PubMed: 18805335]
91. Khatri SB, Hammel J, Kavuru MS, et al. Temporal association of nitric oxide levels and airflow in asthma after whole lung allergen challenge. *J Appl Physiol*. 2003; 95:436–40. discussion 5. [PubMed: 12576414]
92. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. *Am J Respir Crit Care Med*. 2000; 162:2341–51. [PubMed: 11112161]
93. Wedes SH, Khatri SB, Zhang R, et al. Noninvasive markers of airway inflammation in asthma. *Clin Transl Sci*. 2009; 2:112–7. [PubMed: 20234847]
94. Nelson BV, Sears S, Woods J, et al. Exhaled nitric oxide as a marker for childhood asthma. *J Pediatr*. 1997; 130:423–7. [PubMed: 9063418]
95. Ceylan E, Aksoy N, Gencer M, et al. Evaluation of oxidative-antioxidative status and the L-arginine-nitric oxide pathway in asthmatic patients. *Respir Med*. 2005; 99:871–6. [PubMed: 15939249]
96. Li H, Romieu I, Sienra-Monge JJ, et al. Genetic polymorphisms in arginase I and II and childhood asthma and atopy. *J Allergy Clin Immunol*. 2006; 117:119–26. [PubMed: 16387594]
97. Morris CR, Poljakovic M, Lavrishia L, et al. Decreased arginine bioavailability and increased serum arginase activity in asthma. *Am J Respir Crit Care Med*. 2004; 170:148–53. [PubMed: 15070820]
98. Lara A, Khatri SB, Wang Z, et al. Alterations of the arginine metabolome in asthma. *Am J Respir Crit Care Med*. 2008; 178:673–81. [PubMed: 18635886]
99. Zimmermann N, King NE, Laporte J, et al. Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis. *J Clin Invest*. 2003; 111:1863–74. [PubMed: 12813022]
100. Zimmermann N, Rothenberg ME. The arginine-arginase balance in asthma and lung inflammation. *Euro J Pharmacol*. 2006; 533:253–62.
101. Maarsingh H, Leusink J, Bos IS, et al. Arginase strongly impairs neuronal nitric oxide-mediated airway smooth muscle relaxation in allergic asthma. *Respir Res*. 2006; 7:6. [PubMed: 16409620]

102. King NE, Rothenberg ME, Zimmermann N. Arginine in asthma and lung inflammation. *J Nutr.* 2004; 134:2830S–6S. discussion 53S. [PubMed: 15465795]
103. Corraliza IM, Soler G, Eichmann K, et al. Arginase induction by suppressors of nitric oxide synthesis (IL-4, IL-10 and PGE2) in murine bone-marrow-derived macrophages. *Biochem Biophys Res Commun.* 1995; 206:667–73. [PubMed: 7530004]
104. Modolell M, Corraliza IM, Link F, et al. Reciprocal regulation of the nitric oxide synthase/arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. *Eur J Immunol.* 1995; 25:1101–4. [PubMed: 7537672]
105. Mistry SK, Zheng M, Rouse BT, et al. Induction of arginases I and II in cornea during herpes simplex virus infection. *Virus Res.* 2001; 73:177–82. [PubMed: 11172921]
106. Ito A, Tsao PS, Adimoolam S, et al. Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation.* 1999; 99:3092–5. [PubMed: 10377069]
107. Cohen L, EX, Tarsi J, et al. Epithelial cell proliferation contributes to airway remodeling in severe asthma. *Am J Respir Crit Care Med.* 2007; 176:138–45. [PubMed: 17463414]
108. Li H, Meininger CJ, Hawker JR Jr, et al. Regulatory role of arginase I and II in nitric oxide, polyamine, and proline syntheses in endothelial cells. *Am J Physiol Endocrinol Metab.* 2001; 280:E75–82. [PubMed: 11120661]
109. Janne J, Alhonen L, Leinonen P. Polyamines: from molecular biology to clinical applications. *Ann Med.* 1991; 23:241–59. [PubMed: 1930914]
110. Albina JE, Abate JA, Mastrofrancesco B. Role of ornithine as a proline precursor in healing wounds. *J Surg Res.* 1993; 55:97–102. [PubMed: 8105150]
111. Kershenovich D, Fierro FJ, Rojkind M. The relationship between the free pool of proline and collagen content in human liver cirrhosis. *J Clin Invest.* 1970; 49:2246–9. [PubMed: 5480851]
112. Haworth O, Levy BD. Endogenous lipid mediators in the resolution of airway inflammation. *Eur Respir J.* 2007; 30:980–92. [PubMed: 17978156]
113. O'Byrne PM, Israel E, Drazen JM. Antileukotrienes in the treatment of asthma. *Ann Intern Med.* 1997; 127:472–80. [PubMed: 9313005]
114. Kalayci O, Birben E, Sackesen C, et al. ALOX5 promoter genotype, asthma severity and LTC production by eosinophils. *Allergy.* 2006; 61:97–103. [PubMed: 16364163]
115. Montuschi P, Mondino C, Koch P, et al. Effects of montelukast treatment and withdrawal on fractional exhaled nitric oxide and lung function in children with asthma. *Chest.* 2007; 132:1876–81. [PubMed: 18079221]
116. Rabinovitch N, Graber NJ, Chinchilli VM, et al. Urinary leukotriene E4/exhaled nitric oxide ratio and montelukast response in childhood asthma. *J Allergy Clin Immunol.* 2010; 126:545–51. e1–4. [PubMed: 20816189]
117. Daffern PJ, Muilenburg D, Hugli TE, et al. Association of urinary leukotriene E4 excretion during aspirin challenges with severity of respiratory responses. *J Allergy Clin Immunol.* 1999; 104:559–64. [PubMed: 10482828]
118. O'Shaughnessy KM, Wellings R, Gillies B, et al. Differential effects of fluticasone propionate on allergen-evoked bronchoconstriction and increased urinary leukotriene E4 excretion. *Am Rev Respir Dis.* 1993; 147:1472–6. [PubMed: 8389108]
119. O'Sullivan S, Roquet A, Dahlen B, et al. Urinary excretion of inflammatory mediators during allergen-induced early and late phase asthmatic reactions. *Clin Exp Allergy.* 1998; 28:1332–9. [PubMed: 9824404]
120. Green SA, Malice MP, Tanaka W, et al. Increase in urinary leukotriene LTE4 levels in acute asthma: correlation with airflow limitation. *Thorax.* 2004; 59:100–4. [PubMed: 14760145]
121. Liu MC, Dube LM, Lancaster J. Acute and chronic effects of a 5-lipoxygenase inhibitor in asthma: a 6-month randomized multicenter trial. Zileuton Study Group. *J Allergy Clin Immunol.* 1996; 98:859–71. [PubMed: 8939149]
122. Wenzel SE, Trudeau JB, Kaminsky DA, et al. Effect of 5-lipoxygenase inhibition on bronchoconstriction and airway inflammation in nocturnal asthma. *Am J Respir Crit Care Med.* 1995; 152:897–905. [PubMed: 7663802]

123. Pavord ID, Ward R, Woltmann G, et al. Induced sputum eicosanoid concentrations in asthma. *Am J Respir Crit Care Med.* 1999; 160:1905–9. [PubMed: 10588604]
124. Miranda C, Busacker A, Balzar S, et al. Distinguishing severe asthma phenotypes: role of age at onset and eosinophilic inflammation. *J Allergy Clin Immunol.* 2004; 113:101–8. [PubMed: 14713914]
125. Levy BD. Lipoxins and lipoxin analogs in asthma. *Prostaglandins Leukot Essent Fatty Acids.* 2005; 73:231–7. [PubMed: 16046112]
126. Haworth O, Cernadas M, Yang R, et al. Resolvin E1 regulates interleukin 23, interferon-gamma and lipoxin A4 to promote the resolution of allergic airway inflammation. *Nature Immunol.* 2008; 9:873–9. [PubMed: 18568027]
127. Planaguma A, Kazani S, Marigowda G, et al. Airway lipoxin A4 generation and lipoxin A4 receptor expression are decreased in severe asthma. *Am J Respir Crit Care Med.* 2008; 178:574–82. [PubMed: 18583575]
128. Levy BD, Bonnans C, Silverman ES, et al. Diminished lipoxin biosynthesis in severe asthma. *Am J Respir Crit Care Med.* 2005; 172:824–30. [PubMed: 15961693]
129. Levy BD, Kohli P, Gotlinger K, et al. Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness. *J Immunol.* 2007; 178:496–502. [PubMed: 17182589]
130. Farha S, Asosingh K, Laskowski D, et al. Effects of the menstrual cycle on lung function variables in women with asthma. *Am J Respir Crit Care Med.* 2009; 180:304–10. [PubMed: 19520904]
131. Tantisira KG, Colvin R, Tonascia J, et al. Airway responsiveness in mild to moderate childhood asthma: sex influences on the natural history. *Am J Respir Crit Care Med.* 2008; 178:325–31. [PubMed: 18420965]
132. Holguin F, Bleecker ER, Busse WW, et al. Obesity and asthma: an association modified by age of asthma onset. *J Allergy Clin Immunol.* 2011; 127:1486–93. e2. [PubMed: 21624618]
133. Holguin F, Fitzpatrick A. Obesity, asthma, and oxidative stress. *J Appl Physiol.* 2010; 108:754–9. [PubMed: 19926826]
134. Chupp GL, Lee CG, Jarjour N, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. *New Engl J Med.* 2007; 357:2016–27. [PubMed: 18003958]
135. Sorkness RL, Bleecker ER, Busse WW, et al. Lung function in adults with stable but severe asthma: air trapping and incomplete reversal of obstruction with bronchodilation. *J Appl Physiol.* 2008; 104:394–403. [PubMed: 17991792]
136. Little SA, Sproule MW, Cowan MD, et al. High resolution computed tomographic assessment of airway wall thickness in chronic asthma: reproducibility and relationship with lung function and severity. *Thorax.* 2002; 57:247–53. [PubMed: 11867830]
137. Mitsunobu F, Ashida K, Hosaki Y, et al. Decreased computed tomographic lung density during exacerbation of asthma. *Eur Respir J.* 2003; 22:106–12. [PubMed: 12882459]
138. Busacker A, Newell JD Jr, Keefe T, et al. A multivariate analysis of risk factors for the air-trapping asthmatic phenotype as measured by quantitative CT analysis. *Chest.* 2009; 135:48–56. [PubMed: 18689585]
139. de Lange EE, Altes TA, Patrie JT, et al. Evaluation of asthma with hyperpolarized helium-3 MRI: correlation with clinical severity and spirometry. *Chest.* 2006; 130:1055–62. [PubMed: 17035438]

Key Points

1. Diagnosis and treatment of asthma are currently based on assessment of patient symptoms and physiologic tests of airway reactivity.
2. This article provides an overview of blood, urine and airway biomarkers, summarizes the pathologic pathways that they signify, and begins to describe the utility of biomarkers in the future care of patients with asthma.

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FIGURE 1. Biomarkers of specific and nonspecific pathophysiologic pathways in asthma. Environmental exposures trigger and/or amplify underlying pathophysiology
See text for details.

TABLE 1

BIOMARKERS OF ASTHMA

Biomarker Phenotype	Pathogenetic Pathway(s)	Implication(s) for Mechanistic Management
<i>In Blood:</i>		
Eosinophils *	Atopic; excessive TH2 pathways activation	Immunotherapy depending on clinical signs and symptoms of asthma
IgE *	Atopic; excessive TH2 pathways activation	Immunotherapy depending on clinical signs and symptoms of asthma
Allergen specific IgE *	Atopic; excessive TH2 pathways activation	Avoidance of allergens, Immunotherapy, anti-IgE therapies
Periostin	Interleukin (IL)-13 driven asthma (TH2 pathway gene)	May benefit from biological blockade of IL-4/IL-13 receptors
Superoxide Dismutase (SOD)	Oxidative and nitrative inflammation and injury leading to reducing-oxidizing (redox) imbalance and loss of SOD activity	Associated with greater airflow obstruction and bronchial hyper-reactivity; evaluate environmental oxidative exposures such as second hand smoke and air pollutants; may benefit from redox regulation in the future
CD34 ⁺ CD133 ⁺ Progenitor Cells	Circulating myeloid progenitors are increased in asthma, and increase further with exacerbations; differentiate into proangiogenic monocytic cells and mast cells in the airway	Promote remodeling and inflammation in the airway; anti-cKIT directed therapies to decrease bone marrow precursors
<i>Airway Derived:</i>		
Sputum Eosinophils	Atopic asthma; excessive TH2 pathways activation	Avoidance of allergens, Immunotherapy, anti-IgE therapies
Exhaled NO (FENO) *	<i>High:</i> Excessive inducible NO Synthase (iNOS); when used with GSNO challenge may indicate greater GSNO reductase, or low pH	Risk for exacerbation; may need to step up anti-inflammatory therapies, <i>e.g.</i> corticosteroids decrease iNOS
	<i>Low or normal:</i> limited arginine bioavailability due to consumption by arginases (activated by TH2 cytokines) or competition by methylarginines [when dimethylarginine dimethylamino hydrolase (DDAH) activity is low]	Metabolic abnormalities in arginine metabolism
Exhaled breath condensate (EBC) pH and formate	Airway acidification due to infection, low airway glutaminase, or when formate is high, greater activity of GSNO reductase	Buffered solutions and/or glutamine inhalation
Ethyl Nitrite Challenge and measure of FENO	High GSNO reductase in the airway, low levels of GSNO (loss of bronchodilator response)	Therapies to block GSNO reductase or supplement beneficial S-nitrosothiols in the airways
<i>In urine:</i>		
Bromotyrosine (BrY)	Activation of eosinophil peroxidase for specific bromination pathways	Unstable asthma, predicts exacerbations, particularly in children; step up of anti-inflammatory therapy, <i>i.e.</i> corticosteroids
Leukotriene (LTE) 4	Cysteinyl leukotriene pathway overactivity	Aspirin avoidance and/or desensitization; leukotriene receptor or 5-Lipoxygenase inhibition
F2 Isoprostanes (F2IsoP)	Nonspecific peroxidation of membrane lipids to generate F2IsoP (8-epi-PGF2alpha and 2,3-dinor-8-epi-PGF2alpha) by excessive generation of reactive oxygen species	Nonspecific inflammation by neutrophils or eosinophils; evaluate environmental oxidative exposures such as second hand smoke and air pollutants; may benefit from leukotriene receptor or 5-Lipoxygenase inhibition

* Currently readily available to general practitioners and approved for asthma evaluation, others are not generally available, emerging and/or experimental.