Biomarkers of Asthma: Recent Patents from 2009-2011

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Abstract: This review informs on current literature on patents for biomarkers for asthma from 2009 to 2011. Variable airflow obstruction in asthma is generally triggered by gene-environment interactions that can lead to key symptoms of cough, shortness of breath, chest tightness, and wheezing. The episodic and variable degrees of airway hyper-responsiveness arise from a variety of inflammatory pathways that can make diagnosis and management difficult. Standard pulmonary function tests used for the diagnosis of asthma may fail to predict individual responses to the standard bronchodilator and corticosteroid therapies. Phenotypic predispositions can alter the severity of the asthmatic condition and treatment response. Biomarkers from sputum, exhaled gases, exhaled breath condensates, urine, serum, and broncholaveolar fluid lavage proteins are currently explored to provide objective metrics for identifying individuals at risk, provide therapy guidance, monitor disease progression and evaluate response to therapy, as a supplement to standard pulmonary function tests. Updates on the refinement of technologies, inherent limitations and benefits of these biomarkers are discussed to provide insights on how current understanding of pathologic mechanisms has been applied to provide information for addressing gaps in the diagnosis and management of asthma.

Keywords: Asthma, biomarkers, diagnosis, metabolites, proteins, small molecules.

INTRODUCTION

Asthma, a chronic disorder of the airways, affects an estimated 300 million people worldwide [1], with significant patient morbidity. It is a significant public health concern because of the growing number of cases, high healthcare and societal costs [2]. There is wide variability in the spectrum of clinical presentations and underlying mechanisms suggesting an interaction of changing environmental exposure and genetic factors in inducing the hallmark changes of airway inflammation, airway hyper-responsiveness and variable airway obstruction. Genetic, protein and metabolite profiling approaches in the form of a concentration change that correlate with the disease when compared to a control, have led to the identification of asthma biomarkers. This can provide objective parameters for supplementing traditional spirometric testing and bronchial biopsies in identifying risk in individuals, measuring response to treatment, and monitoring disease progression. This review summarizes the recent patents on asthma biomarkers from 2009-2011, analyzing key strengths and limitations, of each approach.

Biomarkers have traditionally been reserved for chemical or molecular analytes coming from various sources such as bodily fluids, e.g., blood, urine, sputum, and other tissues. These samples provide a surrogate measure that can be cheaper, less invasive, technically simpler and more available, with an acceptable sensitivity and specificity, compared with standard pulmonary function tests for the assessment of airway inflammation [3, 4]. Molecular biomarkers may be correlated with the biochemistry of disease states, often traceable to the enzymatic proteins involved in pathophysiologic and metabolic processes as well as the genes that encode for these proteins [5-7].

EXHALED BIOMARKERS

Exhaled breath is a complex mixture of low molecular weight organic and inorganic compounds. Fluctuations in the relative concentrations of these molecular components may be clinically monitored. These can also be potentially used as indices for diagnosis and therapeutic monitoring, particularly for lung and airway diseases. Some of the analytical approaches that have been applied to exhaled breath include direct measurement of expired gases, analysis of volatile organic compounds (VOC) from exhaled breath as well as analysis of volatile and non-volatile compounds in exhaled breath condensate (EBC). Exhaled breath analysis is non-invasive and thus has a distinct clinical advantage in terms of ease and convenience in sample collection [8, 9].

FeNO

The fraction of exhaled nitric oxide (FeNO) is a widely studied, non-invasive biomarker that evaluates airway inflammation in asthma. FeNO levels have been shown to correlate well with sputum eosinophil count, airway hyperre-
sponsiveness, bronchodilator response, serum IgE levels, allergen skin prick testing, asthma symptoms and lung function [10-13]. New sensing systems for gaseous biomarkers have been developed and recent patents describe instrumentation improvements. These include a photometric device using a mid-range infra-red laser as a discriminating light source for photoacoustic sensors, conversion of NO to NO$_2$ using an oxidizer unit coupled to a gas sensor, an inline preconcentrator unit with a detector array for detection of multiple gaseous analytes as well as the use of carbon nanotubes as sensor components [14-17]. These innovations address, but do not entirely eliminate, the significant interactions of the exhaled breath condensate with contaminants in the mouth (e.g. saliva, pH and bacterial elements) [4]. FeNO may have the potential to identify individuals who are likely to respond to airway inflammation [18]. While studies have pointed out differences in FeNO findings with bronchial biopsies, the Global Initiative for Asthma guideline has recognized the value of FeNO in monitoring dose adjustments of corticosteroids for the treatment of asthma. This is with the qualification that the use of FeNO has not, however, improved control nor reduced dosing of inhaled glucocorticoid treatment [19].

CARBON MONOXIDE

A recent patent by Choi et al. describes the use of carbon monoxide (CO) as a biomarker for various inflammatory pulmonary conditions related to oxidative stress, including asthma. Carbon monoxide is produced in minute but detectable amounts in the breath and is detected by a CO analyzer. An increase in CO concentration may indicate risk or a current state of oxidative stress and may be useful in the detection of the early stages of the condition [20]. The principal source of endogenous CO is heme metabolism catalyzed by hemeoxygenase (HO) enzymes, which occur as constitutive (HO-2) and inducible (HO-1) isozymes. Expression of HO-1 is highly induced by oxidative stress in inflammatory and lung cells. A limitation of analyzers, however, is that exhaled CO (eCO) may come from endogenous metabolic sources as well as airway contamination from environmental exposure. High background contamination principally from smoking presents a significant challenge for the application of eCO as a diagnostic tool [21, 22]. The marker is generally considered non-specific for asthma although its predictive value as a diagnostic marker is unclear [22].

SMALL MOLECULAR BIOMARKERS

In another patent, Van Schooten et al. empirically correlated a set of small molecular biomarkers in exhaled breath with asthma. Carbon disulfide, 1-penten-2-on, butanoic acid, benzoic acid, 3-(1-methylethyl)-benzene and C$_{13}$H$_{28}$/C$_{11}$H$_{24}$ hydrocarbons are detected by Gas Chromatography-Time of Flight-Mass Spectrometry [23]. Also, 8-isoprostane in exhaled air has been associated with small airway dysfunction in mild asthma. An increase in the concentration of these compounds among asthma patients who were not in exacerbation suggests their role as disease markers [24]. Detection of these volatile organic compounds (VOCs) was reported using a variety of methods including an electronic nose, an artificial sensor system consisting of an array of chemical sensors for VOC detection and an algorithm for pattern recognition. Findings from VOC analysis increased the predictive value of FeNO in discriminating healthy subjects from asthmatic patients for the diagnosis of asthma [25].

SERUM INFLAMMATORY BIOMARKERS

Allergens and other environmental stimuli elicit varying degrees of immune responses among individuals. Once the antigen or irritant passes and escapes the mucociliary barrier of the respiratory tract, the humoral (antibodies) and cell-mediated defense systems of the host come into action, recruiting mast cells, eosinophils, and other lymphocytes. This in turn results to the production of inflammatory mediator molecules Fig. (1) [26]. Non-specific biomarkers currently explored for asthma include detectable traces of genomic and protein markers of inflammation including eosinophil cationic proteins (ECP), eosinophil peroxidases (EPO), neutrophil elastases (NE), myeloperoxidases (MPO), matrix metalloproteinases (MMP) and monocyte chemotactic proteins (MCP) [3].

Cross-linking of the receptors initiate signaling cascades that will result in exocytosis of preformed mediators (histamine, tryptase) and production of cytokines and eicosanoids (prostaglandins, leukotrienes).

Various inflammatory mediators are known to cause constriction of bronchial smooth muscle cells. The release of histamines, leukotrienes and eosinophil granule proteins, which bind to specific receptors in the muscle cells, open calcium channels and may recruit other contractile agonists [26]. Other immunological markers are mainly cytokines such as interleukins (IL), tumor necrosis factors (TNF-) and eotaxins [3]. Small signaling molecules including increased levels of cysteinyl leukotrienes (cys-LK) have been detected and measured from various bodily fluids of asthmatic subjects [3, 5-7]. A small signaling molecule found both in serum and bronchoalveolar lavage fluid, 8-isoprostane, has been noted to be a marker for oxidative stress and antioxidant deficiency. DP2 receptors bind prostaglandin D2 molecules, released by mast cells to recruit eosinophils, basophils, and T$_H$2 cells [27]. These molecules are currently being explored not only as biomarkers in the inflammatory cascade but as potential targets for therapy [3, 5-7].

YKL-40

YKL-40, a chitinase-like protein, mediates airway inflammation in mouse models. YKL-40 levels in the serum correlate with a single nucleotide polymorphism (SNP) in the chitinase-3-like 1 gene (CHI3L1) known to increase in asthma. The rise in YKL-40 protein levels has been associated with asthma severity and thickness of the subepithelial basement membrane. Elevations of YKL-40 have been shown to correlate with blood eosinophils but not with clinical severity, control or IgE levels in a study comparing asthmatics with healthy controls (r = -.05, p = .05) [28]. These studies have pointed out a potential for YKL-40 as a biomarker for asthma and other lung disorders with bronchial hyperresponsivity or reduced lung function [29, 30].
IL-18 and Mast Cell Stability (Chymases)

IL-18 is another biomarker used to monitor treatment response against chymase activity. Alpha-chymase is a chymotrypsin-like protease expressed by mast cells. This protein degrades the extracellular matrix of the respiratory tract promoting further inflammation. Moreover, studies have shown that chymase also cleaves interleukin-18. If the chymase mechanism of action is inhibited, there should be detectable levels of IL-18 in the biological sample [31]. Significantly higher interleukin-18 levels were found in poorly controlled rather than well controlled asthma [32].

Periostin as an Anti-IL13 Surrogate Marker

Another interleukin, IL-13, is also strongly correlated with inflammatory responses in asthma [33]. The detection of IL-13 in blood and airway samples require highly-sensitive assays. IL-13 induces bronchial epithelial cells to secrete periostin, a matricellular protein which can be used as a biomarker substitute for IL-13. An anti-IL13 drug Lebrikizumab was monitored by its effect on periostin levels, as a surrogate marker for interleukin-13. A six-month course of Lebrikizumab resulted in improvements in lung function compared with a placebo group [34, 35].

BRONCHOALVEOLAR LAVAGE PROTEINS

Bronchoalveolar lavage (BAL) protein biomarkers generally have the advantage of direct sampling from the airways, yielding a variety of cellular and protein products that often correlate with the degree of inflammation in the airway [36]. Studies using BAL isolates for the identification of inflammation in asthma are generally limited by the invasive nature of the procedure, but they yield significant protein differences in segmentally challenged airways of asthmatics compared with controls. Proteins that have been identified include cytokines such as the high mobility group protein 1 (HMG-1), matrix metalloproteinases such as MMP-9 and cellular signalling proteins. These proteins have also been identified in various inflammatory responses, notably relating to cell adhesion, cell mobility, proliferation, and signal transduction [37]. Other proteins in bronchoalveolar fluid are discussed in detail.
Table 1. Summary of Current Patents on Asthma Biomarkers (2009-2011)

<table>
<thead>
<tr>
<th>Source</th>
<th>Analyte</th>
<th>Patents (Assignee)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaled air</td>
<td>FENO</td>
<td>US20107704214</td>
<td>Conversion of exhaled NO to NO₂ using an oxidation catalyst, coupled to a gas sensor unit</td>
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<td></td>
<td></td>
<td>(Siemens Aktiengessellschaft)</td>
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<tr>
<td>CO</td>
<td></td>
<td>US20107678390</td>
<td>Use of CO as a biomarker for oxidative stress or a condition/disease state to which oxidative stress is secondary</td>
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<tr>
<td></td>
<td></td>
<td>(Yale University, John Hopkins University)</td>
<td></td>
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<tr>
<td>VOC</td>
<td></td>
<td>US20097606274</td>
<td>Optical nose for detection of exhaled gases utilizing a mid-IR range laser</td>
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<td></td>
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<td>(University of Alabama)</td>
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<tr>
<td></td>
<td></td>
<td>WO2010065452</td>
<td>Breath analysis system for diagnosing asthma among other lung diseases</td>
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<td></td>
<td></td>
<td>(Tricomtech Corp.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO2009144725</td>
<td>Use of carbon nanotubes as sensing elements for biomarkers in breath samples</td>
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<td></td>
<td>(Technion Research and Development Foundation)</td>
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<tr>
<td></td>
<td></td>
<td>WO2011003922</td>
<td>Use of GC-TOF-MS to identify VOCs as biomarkers for asthma</td>
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<td>(Universiteit Maastricht)</td>
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<tr>
<td>Serum</td>
<td>YKL-40</td>
<td>US20110177963</td>
<td>CHEL1 encoding YKL-40 is associated with elevated YKL-40 levels and increased risk for developing a lung disorder, including asthma</td>
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<td>(Yale University, University of Chicago)</td>
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<tr>
<td></td>
<td></td>
<td>US20097579147</td>
<td>MSR1 gene mutation indicates increased risk for asthma among other diseases</td>
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<td></td>
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<td>(Wake Forest University)</td>
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<tr>
<td>IL-18</td>
<td></td>
<td>WO2009148958</td>
<td>IL-18 as a biomarker to assess response to treatment with drugs that inhibit chymase activity</td>
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<td></td>
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<td>(Janssen Pharmaceutica)</td>
<td></td>
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<tr>
<td>Periostin</td>
<td></td>
<td>US2011012350</td>
<td>Use of periostin as a surrogate marker for IL-13</td>
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<tr>
<td></td>
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<td>(Arron et al.)</td>
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<tr>
<td>DP2 receptors (also called CRTH2)</td>
<td></td>
<td>US20100173313</td>
<td>Not normally expressed in human neutrophils, however, neutrophils recruited in site of inflammation expressed DP2 receptors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(AMIRA Pharmaceuticals)</td>
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<tr>
<td>Bronchoalveolar lavage (BAL)</td>
<td>TGF-β1/TGF-β2</td>
<td>US20107858763</td>
<td>TGF-β induces structural changes associated with asthma</td>
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<td>(Council of Scientific &amp; Industrial Research)</td>
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<td></td>
<td>Vitamin D binding protein (DBP)</td>
<td>WO2009072749 (Soochunhyang University)</td>
<td>Elevated DBP in bronchoalveolar lavage fluid (BALF) signals bronchial asthma</td>
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<td></td>
<td>Surfactant protein D (SP-D)</td>
<td>WO2008124010 (University of Pennsylvania)</td>
<td>Presence of pro-inflammatory SP-D can be used to monitor responsiveness to corticosteroids treatment</td>
</tr>
<tr>
<td>Urine</td>
<td>Formate, hippurate, trigonellin</td>
<td>WO2011082433 (Lineagen, Inc.)</td>
<td>¹H-NMR to detect urine metabolites as markers for lung function and disease</td>
</tr>
</tbody>
</table>

TGF-β1 and TGF-β2

Ghosh et al. identified the human transforming growth factor beta 1 (TGF-β1) as an important fibrogenic and immuno- modulatory factor known to induce structural changes associated with asthma [38]. TGF-β is produced in the airways by inflammatory cells infiltrated in the bronchial mucosa, as well as by structural cells of the airway wall includ-
ing fibroblasts, epithelial, endothelial and smooth muscle cells. TGF-β1 and TGF-β2 have been shown to be increased in asthmatic airways and cells, together with evidence of increased TGF-β signaling [39, 40]. Levels of TGF-β2 have been shown to supplement the use of eNO determinations as a potential biomarker for asthma [41]. These studies point out a role for TGF not only as a potential biomarker but as a therapeutic target for modulation of airway remodeling in asthma [40].

**Vitamin D and Vitamin D Binding Protein**

It has been observed that the vitamin D binding protein (DBP), a carrier of the vitamin D sterol, is a potential biomarker for the inflammation in asthma [42]. Reduced vitamin D levels were associated with impaired lung function, increased airway hyperresponsiveness, and reduced glucocorticoid response suggesting a role of vitamin D in pro-inflammatory or immune regulatory mechanisms [43]. Elevated concentrations of vitamin D binding protein in bronchoalveolar lavage fluid (BALF) reportedly signal inflammation in bronchial asthma. Further studies have been suggested to clarify the diagnostic and therapeutic implications of vitamin D in asthma management [42, 43].

**SP-D and Steroid Response**

Surfactant protein D (SP-D), a member of the “collectin” family of lectins, has been used as a biomarker to check response to corticosteroids. SP-D is a 43 kDa glycoprotein with a structure composed of a globular head and a collagenous tail. Oxidizing agents that can trigger asthma may destroy the disulfide bridges that account for its multimeric structure, thus exposing its collagenous tail. If this happens, the tail can bind to receptors of effector cells, leading to opposing effect by encouraging the release of cytokines. Binding to viruses, bacteria, fungi, and allergens alters its multimeric structure and subsequently increases the ingestion of these proteins by macrophages [44]. SP-D binding to certain carbohydrate patterns on the surface of some pathogens helps apoptotic macrophages to recognize the pathogens. SP-D is also known to inhibit cytokine release of effector cells by the binding of its globular head to cell receptors. This triggers the signaling cascade for cytokine transcription in mast cells, basophils and eosinophils. A study conducted by Cheng et al. revealed increased SP-D concentration in alveolar lavage of asthma patients [45]. SP-D presence in asthma was noted to be a non-specific indicator of lung injury and inflammation and has potential use in evaluating the response of corticosteroid treatment for asthma [46].

**Urine Biomarkers**

A simple non-invasive and often ignored approach in asthma biomarker discovery is urine sampling. Targeted metabolite profiling of urine has recently been shown to provide suitable biomarkers for asthma. Urinary levels of bromotyrosine and F2-isoprostanes are markers of eosinophil-catalyzed oxidation and urinary lipid peroxidation, respectively [47]. McClay also used metabolomics studies to identify formate, hippurate and trigonellin among other urine metabolites as possible biomarkers for lung function and disease including asthma. These metabolites were detected and quantified using high resolution proton NMR [48]. Urinary leukotriene E4, in conjunction with exhaled nitric oxide and sputum eosinophils were noted to have potential value in monitoring conventional methods for airway control [11]. The use of these markers as potential biomarkers for asthma has yet to be established in larger studies.

**CURRENT & FUTURE DEVELOPMENTS**

The complexity of inflammatory pathways in the pathology of asthma limits the identification of suitable biomarkers for the disease. Although a number of small molecule and protein biomarkers have been identified in patents found in a search of the literature from 2009-2011, the development of metabolite signatures or fingerprints (multiple biomarkers) from metabolite profiling has yet to be explored and validated with larger studies. In addition, since metabolites are in a mixture and are often found at very low levels, the discovery of biomarkers will rely on more sensitive and selective analytical tools for the analysis of samples. Since asthma is related to a number of similar inflammatory diseases, the discovery of selective biomarkers may at best be adjuncts to conventional tests in the treatment of children with asthma and in differentiating the various phenotypes. The use of biomarkers for diagnosing specific phenotypes, monitoring and guiding therapy remains a significant challenge.

**CONFLICT OF INTEREST**

This invited review was written by the authors within the scope of their research and academic positions at the Ateneo de Manila University, Philippines and the University of Tasmania, Australia. The authors declare that they have no competing interests.

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**REFERENCES**


