Review

Biomarker-guided asthma management:
Science fiction or images of the imminent future?

Andriana I Papaioannou[1], Konstantinos Bartziokas[2], Stelios Loukides[1], Spyridon Papiris[1], Konstantinos Kostikas[1]

[1]2nd Respiratory Medicine Department, University of Athens Medical School, Athens, Greece;
[2]Respiratory Medicine Physician, Trikala, Greece

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Correspondence:
Andriana I. Papaioannou
2nd Respiratory Medicine Department, Chaidari, Greece
Tel.: +30 210 5831163
E-mail: papaioannouandriana@gmail.gr

ABSTRACT. OBJECTIVE: Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. Although in most asthmatic patients there is a correlation between the level of airway inflammation and the severity of symptoms, in patients with difficult to treat asthma there is often discordance between symptoms and inflammation. Clinicians need easy non-invasive and affordable biomarkers for the recognition of asthmatic inflammation in order to provide optimal and effective therapeutic interventions to their patients. DATA SOURCES: We have performed a search on PubMed using the keywords asthma therapy, biomarkers, exhaled NO, induced sputum and inflammation. STUDY SELECTIONS: We focused on papers providing results that could be useful in clinical practice. Results: In this review we focus on the treatment of asthmatic patients using biomarkers in biological samples collected using semi-invasive (serum and induced sputum) and non-invasive (exhaled breath condensate, and exhaled air) procedures. CONCLUSIONS: Asthma research is shifting from studying symptoms expression, lung function and response to medication, to cellular profile, protein analysis and genetic markers, possibly combined with clinical measures. Single biomarker approaches to phenotype asthma do not seem to be accurate and therefore studies combining multiple known biomarkers are needed. ***Pneumon 2015, 28(3):237-243.***

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The disease is characterized by bronchial hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, and cough which are associated with airway obstruction[1]. Asthma is very heterogeneous in onset, course, and response to treatment, and seems to encompass a broad collection of heterogeneous disease subtypes with different underlying pathophysiological mechanisms[2]. It is well known that in patients with refractory asthma there is great discordance between

symptoms and inflammation. This discordance is related to the fact that in some patients with severe asthmatic symptoms there is no evidence of eosinophilic inflammation whereas in other patients with little or no asthmatic symptoms there is significant eosinophilic inflammation at the time of evaluation[2]. The extreme heterogeneity is the main reason that renders the management of asthma so challenging, since a symptom-led approach would be effective only in patients with mild to moderate atopic asthma that frequently has its onset early in life, in which a concordance between inflammation and symptoms is likely to be present. Clinicians need easy non-invasive and affordable ways for the assessment of airways and/or systemic inflammation which will add on to the clinical evaluation of asthma control and provide proper and effective therapeutic interventions.

A biomarker is a substance that can be objectively measured and can serve as an indicator of a biological state (either a normal biological process, a pathogenic process or a pharmacologic response to a therapeutic intervention)[3]. Thus, any measurement that can be used to predict a patients' disease state (as a diagnostic or prognostic marker) or response to treatment can be called a biomarker. Although this category could also include measures of lung function, usually the term "biomarker" refers to chemical molecules that can be detected in biological samples. The characteristics of an "ideal" biomarker are shown in Table 1.

In asthma, biomarkers can help not only in the monitoring of inflammation and recognition of asthma control but also in the selection of therapeutic interventions and optimization of treatment. Furthermore, many of the latest biological therapeutic options of asthma (such as the use of omalizumab) are based in the measurement of a very commonly used biomarker (serum IgE levels) showing the way for the design of more personalized therapy for asthmatic patients.

TABLE 1. Characteristics of the ideal biomarker

1) Easy to use and interpret
2) Objective
3) Rapidly available
4) Reproducible
5) Good sensitivity and good specificity
6) Dynamic – rapid increases and decreases
7) Level not dependent of the underlying pathology
8) Not modified by any treatment or intervention
9) Continuous and not a discrete variable
10) Correlation with clinical severity and mortality
11) Inexpensive
12) Easily available

For the monitoring of airway diseases, including asthma, biomarkers can be measured in several biological fluids which can be obtained via invasive (i.e. bronchial or nasal biopsies, bronchoalveolar lavage), semi invasive (i.e. nasal brushing, nasal lavage, blood, induced sputum) or non-invasive methods (i.e. exhaled breath condensate, exhaled air, urine)[3]. In this review we will focus on the management of asthmatic patients using biomarkers in biological samples collected using semi invasive (serum and induced sputum) and non-invasive (exhaled breath condensate, and exhaled air) procedures.

BIOMARKERS IN THE DIAGNOSIS AND EVALUATION OF ASTHMA

Traditionally, the diagnosis of asthma is based on clinical history and presentation combined with evidence of reversibility after inhaled bronchodilators or a trial of corticosteroids. However, the presentation is not always straightforward and clinicians may need to confirm the diagnosis with objective measurements that present several limitations[4]. Spirometry results are often normal in asymptomatic asthmatics and bronchodilator reversibility testing may not constantly be evident presenting low sensitivity and specificity[5] whereas the recording of peak expiratory flow variability requires patient compliance[6] which is not always feasible. Bronchial challenge tests present higher sensitivity and specificity but require a dedicated laboratory and experienced technicians[7]. The above limitations in the use of conventional methods used in the diagnosis of asthma resulted in testing the usefulness of several biomarkers as diagnostic tools.

The most useful and standardized biomarker which has been shown to be valuable in the diagnosis of asthma is the fraction of exhaled NO (FeNO). Endogenous NO is produced from the amino acid L-arginine which is metabolized into the amino acid L-citrulline catalyzed by the enzyme NO synthase (NOS), that has three distinct isoforms (NOS 1-3).This reaction leads to the formation of several NO-related end products, including nitrotyrosine, S-nitrosothiols and nitrates[8]. Although the cellular source of the endogenous NO that can be measurable in the airways remains unclear, it is most likely the airway

epithelial cells, that constitutively express NOS 2 in normal subjects[8]. In inflammatory diseases, such as asthma, it is likely that the increased NO production is related to the induction of the NOS 2 isoform (inducible nitric oxide synthase, iNOS) in response to proinflammatory cytokines due to the increased transcription mediated by transcriptional factors such as nuclear factor kappa-B (NF-κB)[9]. NO is produced throughout the respiratory tract, secreted into the lumen of the airways and mixes with alveolar air to provide the final exhaled concentration that is characterized as FeNO. The levels of NO derived from the upper respiratory tract (200 to 1,000 ppb) and sinuses (1,000 to 30,000 ppb) are many times higher than exhaled NO measured in the lower respiratory tract (1 to 9 ppb)[8]. Corticosteroids inhibit the inflammatory induction of NOS 2 in epithelial cells and reduce exhaled NO concentrations[10]. The role of NO in bronchial mucosa may be closely related to asthmatic inflammation, since it represents a potent chemoattractant of eosinophils that may lead to vasodilatation and plasma leakage[8].

The measurement of FeNO concentration in exhaled air is a quantitative noninvasive simple and safe method of measuring airway inflammation that provides a complementary tool to other ways of assessing airways disease including asthma[11]. FeNO is known to be increased in patients with bronchial asthma and has been proved useful for the distinction of subjects having asthma from those without asthma[10,12] with a high degree of discriminatory power[13]. However, it has to be considered that FeNO is also increased in atopic subjects[14].

FeNO has been also shown to be useful as a screening tool of asthma. A recent study in a population of young adults[4] has showed that FeNO values >19 ppb presented 85.2% specificity and 52.4% sensitivity for the diagnosis of asthma. Although smoking and allergic rhinitis seem to be confounding factors, this study has concluded that FeNO values >25 ppb were characterized by specificity >90% for the diagnosis of asthma in both smokers and non-smokers[4].

Similarly, sputum eosinophils have also been shown to be able to recognize the presence of mild and moderate asthma and to differentiate it from the presence of atypical symptoms characterized as pseudo-asthma. In particular sputum differential eosinophilic count was able to diagnose asthma with 72% sensitivity and 80% specificity[5].

Regarding the type of cells in the induced sputum analysis, asthmatic patients can be divided in four different inflammatory subtypes using sputum eosinophil and neutrophil counts[15]. Subjects with a sputum neutrophil proportion >61% are classified as having neutrophilic asthma and those with an eosinophilic proportion >1% are classified as having eosinophilic asthma. Subjects who have increased both neutrophils and eosinophils are classified as having mixed granulocytic asthma and finally, those with normal levels of both neutrophils and eosinophils are classified as having paucigranulocytic asthma. It has been reported that induced sputum eosinophilia is more related to atopic symptoms[16]. Eosinophils in sputum have also been shown to correlate to the levels of FeNO[17] and it has been shown that values over 41 ppb are suggestive of sputum eosinophil count ≥3% with 65% sensitivity and 79% specificity[18].

Although the use of biomarkers in the diagnosis of asthma are characterized by poor sensitivity and specificity, FeNO and sputum eosinophilia have been shown to have a greater diagnostic accuracy for the diagnosis of asthma compared with conventional tests (such as PEF variation, PEF steroid response, FEV_1, FEV_1/FVC, and FEV_1 steroid response)[6].

BIOMARKERS IN ASTHMA MANAGEMENT

The main problem with asthma management strategies is the absence of consideration of the underlying inflammatory process, especially in cases of patients with poor correlation between symptoms and inflammation[19]. Sputum cell counts are reproducible and have known normal values and identify the presence, severity and type of inflammation. The different types of cellular inflammation recognized today (eosinophilic, neutrophilic, mixed or paucigranulocytic) have been related to known environmental causes. Eosinophilic inflammation is mainly related to exposure to allergens, or occupational chemical sensitizers, while neutrophilic inflammation is related to exposure to atmosphere pollutants, such as cigarette smoke, or viral or bacterial infections[19].

Identifying the presence and cellular features of asthma can help improving management. The identification of sputum eosinophilia provides a clue that the patient might respond to adequate corticosteroid treatment. Persistence of sputum eosinophilia despite therapy with inhaled corticosteroids, raises possible treatment compliance issues and – after exclusion of inhaler mishandling – the possibility that the patient needs higher doses of inhaled corticosteroids. Another aspect that needs to be considered in a patient with persistent sputum eosinophilia despite the use of inhaled corticosteroids is

persistent exposure to allergens. In contrast, the presence of non-eosinophilic inflammation is unlikely to respond to an increase to steroid therapy and may be associated with different environmental triggers. Furthermore, the absence of eosinophils suggest that the dose of inhaled corticosteroids can be reduced with minimal risk for recurrence of eosinophilic inflammation leading to exacerbation; in contrast, when eosinophils are in the upper normal range, a recurrence of sputum eosinophilia is likely if corticosteroids are reduced. When neutrophilic inflammation is observed, there is a great possibility of a bacterial of viral infection, always in combination with the patient's clinical presentation.

Berry et al have reported that the presence of sputum eosinophilia in asthmatic patients was associated with response to inhaled corticosteroids[20]. However, patients with sputum neutrophilia, may not respond to an increase in treatment with inhaled corticosteroids[21]. The aforementioned observations were used in clinical practice in a case reported by Gibson and co-workers[22]. This care report presented a 47 year old patient with asthma since childhood, and concordant nasal polypoids, gastroesophageal reflux and aspirin hypersensitivity. The patient used to have well-controlled asthma with the use of moderate dose budesonide and formoterol. However, in the two subsequent years the patient's asthma was completely uncontrolled despite the use of high doses of inhaled corticosteroids. HRCT of the chest was normal and for that reason the investigators decided to perform sputum cell count to the patient, in which sputum neutrophilia was revealed and this lead to specific testing that revealed *C. pneumoniae* infection. The patient was treated with macrolide antibiotics and achieved asthma control[22].

According to the above, a proposed strategy that can be used for the management of asthma based in sputum cell counts can be the following: if the patients' sputum in its initial presentation is characterized by eosinophilia, the therapy should include a proper dose of inhaled corticosteroids, and investigations of the causes of eosinophilia should be performed. If the sputum is neutrophilic, the dose of steroids should be reduced, and the cause of neutrophlia needs to be investigated (e.g. investigation for infections). An empirical course of macrolides could also be useful in this case. Finally, if the sputum cell count is normal, then again the dose of corticosteroids can be reduced and other causes of airway obstruction have to be considered (i.e. COPD, α_1-antitrypsin deficiency, bronchiolitis obliterans, vocal cord dysfunction etc.)[19]. Based on the above, it can be suggested that the various causes of loss of control or exacerbation of asthma need different therapeutic approaches and sputum cell count can help to prevent misdiagnosis and suboptimal treatment. A simplified clinical approach of asthma management based on induced sputum cell counts is shown in Table 2.

Since FeNO is related to sputum eosinophilia, it is not surprising that FeNO has also been related to response to therapy. A well-conduced early FeNO study has shown that patients with values over 47 ppb presented better

TABLE 2. Management of asthma based on sputum cell counts

Sputum cells	Therapeutic intervention

Eosinophilic inflammation (i.e. Eosinophils ≥3%)	• Provide/increase ICS
	• Exclude other causes of sputum eosinophilia (e.g. eosinophilic bronchitis, eosinophilic pneumonia and hypereosinophilic syndromes)
	• If eosinophils persist despite treatment with high dose ICS exclude
	  • Poor compliance
	  • Misuse of inhaled medication
	  • Continuous exposure to allergens

Neutrophilic inflammation (i.e. Neutrophils ≥61%)	• ICS may be reduced without risk of asthma deterioration • Search for other causes of sputum neutrophilia (e.g. infections) • Consider treatment with antibiotics (e.g. macrolides)
Normal Sputum Cell Counts	• ICS may be reduced without risk of asthma deterioration • Search of other causes of airway obstruction (e.g. α1-antitrypsin deficiency, bronchiolitis obliterans, vocal cord dysfunction etc.) • In symptomatic patients consider other treatment options (e.g. anticholinergics)

ICS: Inhaled corticosteroids.

response to inhaled corticosteroids compared to patients with lower levels[23]. In the same study, Smith and coworkers have also shown that FeNO was the best predictor of steroid response compared with conventional predictors, including peak flow variability, spirometry, bronchodilator response, and airway hyperresponsiveness[23]. In this way, FeNO measurements are helpful for the identification of patients with asthma-like symptoms who are more or less likely to benefit from corticosteroid treatment[11].

The clinical use of FeNO in the treatment of asthma has been highlighted in a guideline by the American Thoracic Society[11]. The recommendations in summary are the following:

- FeNO values <25 ppb are suggestive of non-eosinophilic inflammation or non-airway pathology, which means that this patient will probably not respond to corticosteroids and that a different pulmonary/airway disease has to be investigated[11]. In patients with known asthma and low FeNO levels one has to think of non-eosinophilic asthma, or the presence of a different cause for the symptoms[11].
- For FeNO values between 25-50 ppb a cautious interpretation is needed. The interpretation of such values depends on whether the patient is symptomatic and steroid naïve, or whether the patients' NO has increased or decreased form a previous measurement[11].
- Finally, high FeNO values (i.e. >50 ppb) in a symptomatic patient are most likely related to persistent eosinophilic inflammation, suggesting the patient might benefit from inhaled corticosteroids. In symptomatic patients with known asthma, who are already treated with inhaled corticosteroids, high FeNO values suggest that either the dose of inhaled corticosteroids is inadequate, or the patient is non-compliant to treatment. Finally, if the patient has controlled asthma using inhaled corticosteroids the physician should avoid step down therapy because there is a great risk of relapse of symptoms and loss of asthma control[11].

Several studies have used FeNO levels[24-26] or sputum eosinophilia[27,28] as a guide for alterations in the therapy of asthmatic patients. The results of a recent meta-analysis suggest that the use of FeNO as a guidance for the therapy of asthma can lead in the use of lower doses of inhaled corticosteroids without any other impact in asthma outcomes in adults; however, that was not the case in children where this strategy led to the use of increased doses of inhaled corticosteroids[29]. In contrast, the corresponding strategy using sputum eosinophilia for the adjustment of treatment has proven quite effective in reducing the number of asthma exacerbations[29]. A previous study that evaluated the different types of exacerbations according to the underlying sputum cell count has shown that this reduction refers mainly to eosinophilic exacerbations and not to non-eosinophilic ones[30].

BIOMARKERS FOR THE ASSESSMENT OF ASTHMA CONTROL

The difficulty in establishing normal values and cut-off points for FeNO[11] suggests that what may be more relevant in clinical practice is the evaluation of changes in FeNO levels and/or alterations from a personal best value. Michils and coworkers have reported that in asthmatic patients a 40% decrease in FeNO values was related with improvement of asthmatic symptoms whereas a 30% increase was related with symptoms deterioration (NPV 79% and 82% respectively)[31]. In that study the ability of FeNO to predict improvement or deterioration of asthmatic symptoms was not good in patients receiving high doses of inhaled corticosteroids[31]. In a subsequent study, the same investigators have reported that this rule seems also in asthmatics smokers, smoking representing an important confounding factor in the evaluation of FeNO values. In that group of patients a 20% decrease of FeNO was related with improvement of asthmatic symptoms whereas symptoms deterioration was related with an increase in FeNO values of at least 30% (NPV 72% and 84% respectively)[32]. In a subsequent study we reported that in patients with another known confounding factor for FeNO values, the presence of concomitant allergic rhinitis, a 40% increase in FeNO values was related to symptoms deterioration (NPV 71%)[33].

Biomarkers of eosinophilic inflammation may also predict a future loss of asthma control or exacerbation. Both FeNO and sputum eosinophils seem to be able to serve this purpose. FeNO has been found higher in patients with an asthmatic exacerbation within two weeks from their visit in an outpatient clinic (29.67±14.48 ppb vs. 12.92±5.17 ppb; p=002)[34]. In the same way, patients with recurrent asthmatic symptoms had higher levels of eosinophils in induced sputum compared with patients whose asthma remained controlled[35].

SERUM BIOMARKERS FOR NOVEL THERAPEUTIC INTERVENTIONS

Several serum biomarkers related to asthmatic inflam-

mation have been used for the development of novel therapeutic approaches in patients with asthma. The most commonly used is serum total IgE which has recently been included in the guidelines for the management of asthma[1]. Studies have shown that treatment of asthmatics with omalizumab (an anti-IgE antibody) reduced several markers of inflammation[36]. Nowadays it is suggested that by careful patient selection and dosing, and monitoring of patients following administration, omalizumab can be effectively and safely administered, and control of a high proportion of persistent severe allergic asthma cases can be successfully achieved[37]. A more recent study in a population of patients with allergic asthma not controlled on high-dose inhaled corticosteroids and long-acting β_2-agonists has shown that omalizumab treatment resulted in significantly fewer asthma exacerbations and emergency visit rates[38]. Interestingly, the reduction in exacerbations in the last study was more prominent in patients with increased levels of FeNO, blood eosinophils and serum periostin, suggesting that biomarkers may be appropriate for the selection of patients who will respond better to omalizumab[39].

Recent research has additionally focused on interleukin-5 (IL-5), a cytokine that seems to be at least in part responsible for eosinophilic inflammation. Within the bone marrow, IL-5 is the major hematopoietic responsible for terminal differentiation of human eosinophils. Mepolizumab, a monoclonal antibody against IL-5 when administered in asthmatic patients has been shown to reduce asthmatic exacerbations[40,41] and improve symptoms and health related quality of life[41]. Recent studies have also supported the possible therapeutic role of mepolizumab by showing reductions in exacerbations and improvement in asthma control[42] and a significant oral glucocorticosteroid-sparing effect[43] in patients with severe eosinophilic asthma.

Interleukin-13 is found in the airways of patients with asthma and is a significant mediator involved in airway hyperresponsiveness, inflammation, mucous metaplasia, and activation and proliferation of airway fibroblasts, which contribute to adverse airway remodeling[44]. A recent study has shown that treatment of asthmatic patients with lebrikizumab, an antibody against IL-13, significantly improved FEV_1[45]. However, a subgroup analysis of the study population revealed that this improvement in lung function was significant only in patients with increased serum levels of periostin, a surrogate biomarker of Th2 response, suggesting that periostin is a relevant biomarker for the identification of responders to anti-IL-13 therapy[45].

CONCLUSION AND FUTURE DIRECTIONS

Asthma research is shifting from studying symptoms expression, lung function and response to medication, to cellular profile, protein analysis and genetic markers, possibly combined with clinical measures. These biological parameters can be measured in different body compartments and build up to a complexity that has not yet been fully understood. From a clinical point of view, there is an almost indefinite number of possible biomarkers that can be measured in the context of asthma. Yet the clinical applicability (e.g. specificity, sensitivity and invasiveness) limits significantly that number. Noninvasive, reliable, and easily interpreted biomarkers would ideally be standard in daily clinical routine, but are currently unavailable. Single biomarker approaches to phenotype asthma are increasingly regarded to be inaccurate and outdated. In diagnosing the presence of eosinophilic inflammation for example, FeNO is a very sensitive biomarker, but not very specific. Combining FeNO with markers of eosinophilic inflammation such as the percentage of eosinophils in peripheral blood or other biomarkers would increase specificity. To test this hypothesis, studies combining multiple known biomarkers should be performed.

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