

EDITORIAL

The usefulness of inflammatory markers in monitoring treatment responses in asthma

In clinical practice, monitoring of asthmatic patients is usually based on symptom scores, use of rescue medication and measurement of lung function and peak expiratory flow rate (PEFR). However, asthma is a chronic inflammatory disease of the airways and there is a need to assess and monitor this inflammation through the use of markers such as cells and mediators that are directly involved in the inflammatory reaction. Vigorous research effort is invested in defining the best marker for the assessment of inflammation (Table 1). The markers must be non-invasive, reproducible, easy to measure serially and cost-effective. Furthermore, they should mirror the degree of inflammation accurately and be sensitive to the effects of treatment or allergen exposure and avoidance.

In this issue of the journal, Polosa et al. present the results of a double-blind specific immunotherapy (SIT) trial in non-asthmatic subjects with allergic rhinitis. The authors assess treatment responses through the use of symptom and medication score, changes in the level of bronchial hyper-responsiveness (BHR) to methacholine and adenosine monophosphate (AMP) as well as the presence of eosinophils and bronchial epithelial cells in induced sputum. In these atopic rhinitic patients and during a 2-year period, the measurement of BHR using AMP is shown to be the most sensitive marker of inflammatory changes due to seasonal variations and the effect of treatment [1].

BHR to a variety of bronchoconstrictor stimuli is a crucial feature of asthma. It has been shown to be a good marker of inflammation that correlates well with asthma severity [2]. Methacholine, a direct stimulus of bronchoconstriction, is currently the gold standard for the assessment of BHR [2, 3], as it is more sensitive than exercise or cold air challenge and produces fewer side-effects than histamine. In addition, a fairly recent study showed that, compared to symptom scores, PEFR and forced expiratory volume in 1 s (FEV₁), BHR measurement using methacholine challenge was the most sensitive predictor of an asthma exacerbation and the most sensitive marker to adjust anti-inflammatory treatment [4]. Studies have shown that treatment with inhaled corticosteroids (ICS) [2, 3] as well as SIT [5, 6] reduces methacholine-induced BHR in subjects with allergic rhinitis and asthma. Therefore, BHR is a very useful surrogate marker of airway inflammation, and currently methacholine challenge seems to be the recommended test method. On the other hand, AMP is an indirect stimulus of airway narrowing and exerts its effect through mast cell degranulation and probably also through activation of neuronal reflexes [7]. There are studies showing that asthmatics and atopic individuals are very sensitive to AMP challenge whereas non-atopic subjects are not [7, 8].

Additionally, ICS treatment seems to have a significantly greater effect on AMP-induced airway responsiveness than on methacholine-induced BHR [9]. This measurement may therefore be the test of choice in monitoring treatment responses in atopic and asthmatic patients, and the study by Polosa et al. [1] adds further support to the use of AMP.

BHR, however, is an indirect method of assessment of airway inflammation and remodelling [2, 3, 10]. In order to assess and monitor inflammation directly, cells and mediators involved in the asthmatic reaction can be measured in lung tissue or fluids. For research purposes and to elucidate mechanisms of inflammation, the time course of the asthmatic response and responses to treatment, bronchoscopy with local sampling through biopsies or bronchoalveolar lavage fluid (BALF) is an invaluable tool. Analysis of biopsies and BALF has shown that asthma is characterized by Th2 CD4⁺ and eosinophilic inflammation as well as remodelling changes [11]. Steroid treatment attenuates this inflammatory response [11, 12]. When the effects of systemic treatment such as oral medication or SIT are examined, less invasive biopsies such as skin or nasal biopsies can be taken and used to monitor inflammation changes as they reflect the inflammatory changes of the lower airways and the allergen-induced late-phase reaction [13–15]. Nasal and skin biopsies have confirmed the attenuating effect of steroids in the allergen-induced late-phase inflammatory reaction [14] and have shown that SIT enhances Th1 cytokine responses, thus indicating SIT's possible mechanism of immunomodulation [15, 16]. However, these measurements cannot be performed serially and, in clinical practice, sampling of more easily accessible media such as blood, sputum, urine and exhaled air are reliable and practical alternative methods for non-invasive assessment of inflammation.

Sputum induction is a non-invasive means to monitor inflammation, it samples the lower airways more directly than other body fluids and it is considered a good alternative to fiberoptic bronchoscopy. Measurement of cells such as the eosinophil is relatively straightforward and correlates well with data from bronchial biopsies and BALF [17]. Following allergen challenge and during the pollen season, increased eosinophil counts and increased eosinophilic cationic protein (ECP) and IL-5 levels have been found in induced sputum [17, 18], reflecting the allergen-induced late-phase response. Data presented in the current issue suggest that *Parietaria* pollen SIT did not result in modifications of sputum eosinophils [1]. However, previous studies have shown that SIT decreases inflammatory cells and mediators in induced sputum as well as airway hyper-responsiveness, possibly by inhibiting both T cell- and mast-cell-dependent allergen-

specific pathways [19]. Both ICS and oral steroids modulate sputum eosinophils as well as inflammatory mediators [20] and this effect is dose-dependent. A decrease in sputum eosinophils is also observed with other anti-inflammatory agents, such as theophylline and anti-leukotrienes, but their effectiveness is not as potent as that observed with steroids [21]. Furthermore, combination therapy with steroids and β_2 -agonists decreases sputum eosinophils more significantly than monotherapy with steroids [21].

Measuring inflammatory mediators in the blood offers an alternative way to appreciate the type and extent of airway inflammation and assess the effects of therapeutic intervention. The number of blood eosinophils is shown to respond to ICS and allergen immunotherapy [22, 23]. Moreover, serum ECP, a marker of eosinophil activation, correlates well with clinical symptoms and lung function parameters, while its levels decline in response to anti-inflammatory therapy in asthmatic patients [24]. In addition, it has been demonstrated that, using serum ECP, reduction of steroid dosing can be performed successfully [25]. Other circulating markers have also been proposed, including sIL-2r, sICAM1, sVCAM1 and various cytokines. However, these have not been extensively evaluated as markers of treatment response.

Measurement of inflammatory mediators in urine has distinct advantages and urinary mediators have been used extensively in clinical and investigative monitoring of asthmatic patients. Urinary eosinophil peroxidase (EPX) offers a less invasive alternative marker to serum ECP in monitoring eosinophilic inflammation. Increased urinary EPX levels are found in symptomatic atopic asthmatic patients, whereas decreased levels are shown after anti-inflammatory treatment [26]. EPX seems to be more useful in the monitoring of

paediatric asthmatic patients [27]. Other mediators that can be measured in urine are eicosanoid metabolites such as leukotriene LTE_4 and prostaglandin PGF_2 . Urinary LTE_4 is a useful tool to assess ongoing activity of the 5-lipoxygenase pathway products and to assess the therapeutic effect of 5-lipoxygenase inhibitors [28]. However, leukotriene levels do not respond to steroid treatment and studies with ICS failed to detect any effect in urinary LTE_4 excretion [29]. LTE_4 and PGF_2 have been shown to increase significantly during the late response to allergen challenge [30] and to be significantly reduced after allergen avoidance [31].

More recently, markers in exhaled air have been introduced in the assessment and monitoring of asthmatic and rhinitic patients. Nitric oxide (NO) is the most extensively studied exhaled marker and increased levels of exhaled NO have been widely documented in patients with asthma [32]. Nevertheless, NO is not diagnostic for the disease and its value is limited to steroid-naïve asthmatics mainly. Exhaled NO levels are significantly increased during the late phase of allergen challenge; this increase is detected in atopic but not non-atopic asthmatics and is associated with sensitization to perennial allergens [33, 34]. In addition, exhaled NO levels may be an early non-invasive marker of airway inflammation in asymptomatic atopic subjects [35]. Short-acting β_2 -agonists have no effect on exhaled NO levels [36], while treatment with both oral and inhaled steroids results in a reduction of these levels [32]. Low doses of inhaled corticosteroids are sufficient to control exhaled NO levels in mild to moderate asthma, whereas no effect has been shown in patients with severe disease, even when high doses of both oral and inhaled corticosteroids are used [37]. Treatment with leukotriene antagonists has also been shown to result in a reduction of

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

	Marker	Comments
Lung function and BHR	Spirometry PEFR variation BHR	Commonly used in clinical practice, easy to assess Methacholine: ERS and ATS 'gold standard' AMP: perhaps preferable in atopic asthmatics
Bronchial biopsies	Cellular infiltration cytokine/Th expression Remodelling changes	Highly invasive method, direct assessment of inflammation in the target organ. Serial biopsies assess time course and modulation changes
Skin and nasal biopsies	Cellular infiltration cytokine/Th expression	Alternative, less invasive methods to assess responses to systemic treatment
BALF	Cell differential (EOS) ECP cytokines	Invasive method. Cellular component well documented, difficulties with markers in the fluid component due to dilution factors
Induced sputum	Cell differential (EOS) ECP, cytokines	Correlates well with biopsies and BALF data. Widely used in research but time-consuming.
Exhaled air	NO	Limited to steroid-naïve asthmatics, detected in atopic but not non-atopic patients
EBC	CO, ethane, pentane pH, H_2O_2 and cytokines	Not well documented as markers to assess treatment response Simple non-invasive procedure. pH seems to be the only rapid marker, whereas measuring others is still time-consuming. Repeatability, stability and use in monitoring not well documented for all markers
Blood	Eosinophils ECP cytokines	Easy to measure, but reflect changes away from the target organ The best eosinophil-derived marker correlates well with eosinophil activity in the airways. Valuable tool in paediatric asthma
Urine	EPX LTE_4 PGE_2	Alternative for serum ECP, valuable tool in paediatric asthma Assess the therapeutic effect of 5-lipoxygenase inhibitors; however, leukotriene levels do not respond to steroid treatment

BHR = bronchial hyper-responsiveness; PEFR = peak expiratory flow rate; ATS = American Thoracic Society; AMP = adenosine monophosphate; BALF = bronchoalveolar lavage fluid; ECP = eosinophil cationic protein; NO = nitric oxide; CO = carbon monoxide; EBC = expired breath condensate; EPX = eosinophil peroxidase; LTE_4 = leukotriene E_4 ; PGF_2 = prostaglandin F_2 .

exhaled NO levels in asthma, albeit minor compared to that of steroids, and to prevent NO level increase during steroid withdrawal [38]. Increased levels of other gases like carbon monoxide (CO), ethane and pentane have been observed in the exhaled air of steroid-naïve asthmatic patients [39]. Exhaled CO is reported to be elevated during both the early and late allergen-induced asthmatic reactions and nasal CO levels increase in parallel [39]. Nevertheless, several other studies do not confirm these data about CO, reporting that exhaled CO in asthmatics is not higher than that in control individuals, decreases immediately after allergen challenge and returns to baseline levels during the late asthmatic response [40].

Another approach to assess airway inflammation is the use of expired breath condensate (EBC), a simple non-invasive procedure to detect non-volatile mediators. EBC is acquired when cooling or freezing the exhaled air. EBC from asthmatic patients is characterized by increased concentrations of markers expressing oxidative stress, leukotrienes, products of NO metabolism and cytokines [39]. However, the measurement of such markers still remains a time-consuming procedure and recently published data suggest that EBC pH is the only rapid marker, which reflects the underlying inflammatory process as assessed by differential cell counts from induced sputum [41]. Anti-inflammatory treatment, particularly ICS, seems to affect the levels of all markers in ECB significantly [39]. Atopy does not influence concentrations of markers expressing oxidative stress, indicating that atopy is probably not a contributing factor in the overall oxidative stress procedure [42].

In conclusion, several invasive and non-invasive biomarkers have been examined as markers of airway inflammation, in the diagnosis and follow-up of asthma and atopic diseases. However, it is not yet clear as to which outcome measure (inflammatory markers, lung function or symptoms) should be used to guide anti-inflammatory treatment. This difficulty arises because, as we have mentioned several times, these outcome measures do not always correlate [4, 11, 34] and respond to different degrees and over different time spans to the use of therapeutic modulation [1, 19, 29]. Furthermore, it is important to determine which marker to measure in each medium and for each specific treatment and to develop quick and low-cost tests. Therefore, large long-term studies focused on the relationship between indices of inflammation, and treatment plans are needed to define the optimum biomarker for each different treatment. Furthermore, the procedures used to measure these markers should be made rapid and relatively low-cost. At the moment, available data support the use of BHR and sputum eosinophils as the best biomarkers to guide treatment in the majority of asthmatic patients. However, BHR and sputum induction require patient and personnel time so that, although they can be used in research settings, their repetitive use in the regular follow-up of asthmatic patients does not seem feasible yet.

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