REVIEW

Biomarkers of Bronchial Asthma

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Summary

Asthma is a complex disease with a variable course. Efforts to identify biomarkers to predict asthma severity, the course of disease and response to treatment have not been very successful so far. Biomarker research has expanded greatly with the advancement of molecular research techniques. An ideal biomarker should be suitable to identify the disease as well the specific endotype/phenotype, useful in the monitoring of the disease and to determine the prognosis, easily to obtain with minimum discomfort or risk to the patient. An ideal biomarker should be suitable to identify the disease as well the specific endotype/phenotype, useful in the monitoring of the disease and to determine the prognosis, easily to obtain with minimum discomfort or risk to the patient - exhaled breath analysis, blood cells and serum biomarkers, sputum cells and mediators and urine metabolites could be potential biomarkers of asthma bronchiale. Unfortunately, at the moment, an ideal biomarker doesn't exist and the overlap between the biomarkers is a reality. Using panels of biomarkers could improve probably the identification of asthma endotypes in the era of precision medicine.

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Introduction

Asthma is a complex disease with multiple phenotypes (Papi *et al*. 2018). With the increasing prevalence of bronchial asthma, the continuous lack of novel therapies and inefficient disease prevention, the demand for predictive biomarkers for allergic rhinitis and asthma is steadily increasing. Following the current clinical guidelines, it is essential to identify allergen sensitization patterns as well as lung function parameters (Zissler *et al*. 2015). Besides genetic predisposition, immunological changes in the airways may provide important information for allergic disease endotypes. Biomarker research has expanded greatly with the advancement of molecular research techniques. An ideal biomarker should be suitable to identify the disease as well the specific endotype/phenotype, useful in the monitoring of the disease and to determine the prognosis, easily to obtain with minimum discomfort or risk to the patient (Fitzpatrick 2015). The aim of this study is to provide a basic overview of biomarkers in asthma.

Exhaled breath analysis

Exhaled breath condensate (EBC) is a non-invasive method of sampling the airway environment. It analyses both volatile and non-volatile compounds by saturating exhaled breath with water vapour and collecting

the condensed material. Examples of compounds collected in EBC include nitric oxide products, hydrogen peroxide, leukotrienes, and cytokines. Several components correlate with the asthma diagnosis, others with asthma severity (Medrek *et al.* 2017).

Fractional nitric oxide in the exhaled breath (FeNO) provides information about the inflammatory state of the airways (Dweik *et al.* 2011, Antosova *et al.* 2017a, Antosova *et al.* 2017b). Nitric oxide plays key roles in lung biology as bronchodilator and inflammatory mediator and is produced in the lung from nitric oxide synthases during the conversion of the amino acid L-arginine to L-citrulline. The biomarker FeNO is originated from nitric oxide production by the airway epithelium as a result of inducible nitric oxide synthase upregulation during the process of allergic inflammation (Fitzpatrick 2015, Topercerova *et al.* 2019). FeNO as a single, stand-alone biomarker might not be particularly useful and should perhaps be used as part of a more comprehensive panel (Fitzpatrick 2015). The current guidelines for the treatment of severe asthma do not recommend the use of FeNO in the routine for the management of adults and children with asthma (Chung *et al*. 2011). The measurement of exhaled volatile organic compounds by gas chromatography coupled with mass spectrometry could predict the risk for exacerbation in asthmatic children (Robroeks *et al.* 2013). These promising methods need to be standardised before a plus large implementation.

Blood cells and serum biomarkers

Blood eosinophil count is not useful for the diagnosis of asthma (GINA), but it can serve as a prognostic biomarker and predict several therapeutic responses (Eguiluz-Gracia *et al.* 2018) in asthmatic patients with type 2 inflammation.

Currently, *blood neutrophils count* is not a biomarker for asthma diagnosis (GINA).

Eosinophil cationic protein (ECP) is found in the primary matrix of the eosinophil and is released during the eosinophil degranulation. Previous data found that serum ECP is increased in adults and children with atopic asthma, associated with airway resistance and bronchospasm (Koh *et al.* 2007). It has been suggested that ECP assessment could be useful for the initiation and dose titration of inhaled corticosteroids in younger children in whom other biomarkers might be less feasible to asses (Fitzpatrick 2015), but other complementary studies are needed to validate this strategy (Tiotiu 2018).

Lipoxins have anti-inflammatory action and play an important role in chemotaxis and related signal transduction (Fitzpatrick 2015).

Periostin, an extracellular matrix protein secreted by airway epithelial cells in response to IL-13 that regulates epithelial-mesenchymal interactions (Fitzpatrick 2015), has been associated with T2-high eosinophilic asthma (Grayson *et al.* 2018). Periostin expression is increased in the asthmatic airway (Lopez-Guisa *et al.* 2012). The stability of serum periostin over disease progression in adults with asthma (without seasonal effect) (Semprini *et al.* 2018) supports its use as a biomarker for type 2-high asthma.

IgE is an immunoglobulin which mediates type 1 hypersensitivity reactions and plays a key role in the pathogenesis of allergic asthma. It binds to IgE receptors on mast cells and basophils, producing cytokines that mediate T2 responses (Medrek *et al.* 2017). Serum IgE closely correlates with the risk of asthma (Burrows *et al.* 1989).

Chitinases are hydrolases characterised by their affinity to cleave chitin that are thought to play a role in remodelling and regulation of the extracellular matrix (Fitzpatrick 2015). The chitinase-like protein YKL-40 (human cartilage glycoprotein 39) same to be an interesting biomarker for distinguishing asthma from chronic obstructive pulmonary disease (COPD) and healthy controls (James *et al.* 2016). More studies are needed to prove how useful YKL-40 is in the assessment of future asthma outcomes and risk (Tiotiu 2018).

Recent data showed that airway mucosal expression of *CCL26* (the most differentially expressed gene) is the best discriminator for type 2 inflammation (Silkoff *et al.* 2017), *serum urokinase plasminogen activated receptor* is elevated in adult patients with severe, non-atopic asthma (Portelli *et al.* 2017), and the expression of ten selected *microRNA* (HS_108.1, 112, 182.1, 240, 261.1, 3, 55.1, 91.1, has-miR-604, and has-miR-638) is higher in children with severe asthma (Midyat *et al.* 2016). *Serum high-sensitive C-reactive protein (hs-CRP)* is increased in asthmatic patients than in healthy control, in poorly controlled vs well controlled, and may represent a useful biomarker of airway inflammation in non-smoking asthmatic patients without complications, such as heart disease, hypertension, hyperlipidaemia, chronic obstructive pulmonary disease, or infection (Shimoda *et al.* 2015). Evaluation of inflammatory markers *interleukin-6 (IL-6)* and *matrix metalloproteinase-9 (MMP-9)* in serum showed higher levels in asthmatic patients vs

controls and were associated with more severe asthma (Naik *et al.* 2018). A high serum level of *IL-8* could discriminate COPD from asthma patients (Liu *et al.* 2014).

Although all advantages of serum biomarkers, it is important to remember that peripheral blood studies often do not reflect airway biology, and therefore peripheral blood biomarkers might not represent physiologic mechanisms in the airways (Grayson *et al.* 2018).

Sputum cells and mediators

Induced sputum is a non-invasive method which allows quantifying the inflammatory cell pattern in airways of asthmatic patients. To obtain samples for sputum analysis, patients nebulize 3 % saline for 20 min and the sputum expectorated over this period is centrifuged, stained, and analysed by quantifying the number of different cell types (Medrek *et al.* 2017).

Sputum quantitative cell count is the reference standard to reflect the airway inflammation in asthma. The practical advantage of sputum differential cell counts is that this method is feasible even on frozen samples (Eguiluz-Gracia *et al.* 2018). Four inflammatory phenotypes have been identified in the Severe Asthma Research Program (SARP) cohort – eosinophilic (≥2 % eosinophils in induced sputum), neutrophilic (≥40 % neutrophils), mixed granulocytic and paucigranulocytic (Moore *et al.* 2013). The presence of sputum neutrophilia is one candidate predictive biomarker for non-T2 asthma (Medrek *et al.* 2017).

Changes in sputum eosinophil count over time reflect fluctuations in clinical asthma control (Demarche *et al.* 2017). The high level of the innate lymphoid cell (Group 2 ILC) in the sputum is correlated with severe asthma suggesting these cells could be a potential novel biomarker (Cowan *et al.* 2015).

Unfortunately, despite its use as a biomarker in many clinical trials, the use of sputum cells count in daily practice has limitations. This method requires specialized training, equipment, and laboratory for processing, patient coaching and cooperation, emergency protocols and equipment, is difficult to collect (impossible in young children), not easily repeatable, and had several contraindications (Fitzpatrick 2015).

Several *sputum mediators* could be the potential biomarkers. For the diagnostic of inflammatory pattern, *sputum eosinophil peroxidase* is correlated with sputum eosinophilia (Nair *et al.* 2013). *Specific microRNAs* could

discriminate neutrophilic from eosinophilic asthma (Maes *et al.* 2016), and *neutrophil myeloperoxidase* has the potential to differentiate asthma COPD overlap syndrome from asthma (Iwamoto *et al.* 2014). As a prognostic biomarker, sputum expression of human *tumour necrosis factor-like weak inducer of apoptosis* correlates with higher severity, poor asthma control and decreased lung function in children with non-eosinophilic asthma (Kim *et al.* 2018).

Urine metabolites

Bromotyrosine is formed from post-translational modification of tyrosine protein residues by hypobromous acid produced by activated eosinophils during the process of a respiratory burst. It has many advantages as a potential biomarker given its stability and non-invasive detection in the urine. The utility of bromotyrosine in the clinical setting would probably be best when assessed as a part of a larger panel of inflammatory biomarkers (Fitzpatrick 2015).

Leukotriene E_4 is a stable and product of cysteinyl leukotriene metabolism possible to be measured non-invasively in urine samples. Urinary leukotriene E_4 (uLTE4) concentrations are increased in children with allergic asthma and adults with aspirin-exacerbated respiratory disease (Fitzpatrick 2015). Several studies suggest that uLTE4 might be an important biomarker in the selection of asthma therapy (Tiotiu 2018).

Discussion

In asthma, and particularly in severe asthma, many biomarkers have been investigated but only a few of them can be easily used in clinical practice so far (Canonica *et al.* 2018). An ideal biomarker should be suitable to identify the disease as well the specific endotype/phenotype, useful in the monitoring of the disease and to determine the prognosis, easily to obtain with minimum discomfort or risk to the patient (Eguiluz-Gracia *et al.* 2018). Unfortunately, an ideal biomarker doesn't exist and the overlap between the biomarkers is a reality at the moment. Using panels of biomarkers could probably improve the identification of asthma endotypes in the era of precision medicine (Tiotiu 2018).

In conclusion, further research and validation of emerging biomarkers are needed to define the molecular phenotype of asthma. Viewing the heterogeneity of asthma, the development of composite biomarkers from blood, urine and exhaled breath seems to be a more appropriate solution

in practice to predict therapeutic response.

Conflict of Interest

There is no conflict of interest.

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