Metabolomics in Severe Asthma

Subjects: Allergy

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Precision medicine refers to the tailoring of therapeutic strategies to the individual characteristics of each patient; thus, it could be a new approach for the management of severe asthma that considers individual variability in genes, environmental exposure, and lifestyle. Metabolomics is the systematic study of low molecular weight (bio)chemicals in a given biological system and offers a powerful approach to biomarker discovery and elucidating disease mechanisms. In this point of view, metabolomics could play a key role in targeting precision medicine.

Keywords: asthma ; metabolomics ; microbiota

1. Introduction

Asthma is a chronic respiratory disease characterized by shortness of breath, airway inflammation, and airway hyperresponsiveness. It is a heterogeneous, multifaceted disease with variable severity and treatment response. For patients with severe asthma who experience poor symptom control and/or frequent asthma exacerbations, the addition of biological therapies to controller medication is becoming the new standard of care [1]. The introduction of novel treatments with biologics targeting type 2 inflammation pathways urges the development of clinical decision-making tools to guide therapy based on underlying asthma endotypes driving the disease in an individual patient. Severe asthma is driven by heterogeneous molecular mechanisms. Nevertheless, the response rates reported in phase III regulatory trials make it highly desirable to identify biomarkers able to predict response to these biological treatments in severe asthma patients [2]. The omic approach could represent a useful and very interesting tool to better phenotype severe asthma patients and then target biological therapy. However, multi-omics projects in asthma are challenging in terms of costs and require computational and human resources. Therefore, their success requires the coordination and collaboration of diverse research groups from different disciplines in an international multicenter approach. Due to the demanding nature, multi-omics projects in asthma are scarce but have proven value in the comprehensive evaluation of molecular processes in asthma pathogenesis [3]. Metabolomics is the systematic study of low molecular weight (bio)chemicals in a given biological system [4] and offers a powerful approach to biomarker discovery and elucidating disease mechanisms; inter- and intra-study irreproducibility often arise from technical inconsistency, absent metabolite inclusion criteria, inappropriate or overtrained chemometric analyses and poor reporting standards [5].

2. Metabolomics, Microbiota, and Biomarkers in Severe Asthma

2.1. Strategies in Multi-Omics Research

The local and systemic responses are highly activated in asthma, and multiplexed analysis indicates a broad range of inflammatory mediators and responses that are better understood but still largely unsolved [6].

In the last decade, the understanding of the immune response of asthma and the integration of systems biology approaches (genomics, methylomics, transcriptomics, proteomics, metabolomics, etc.) has led to the identification of disease clusters and asthma endotypes [7].

Disease clusters are potentially linked to disease markers and tailored treatment targets by pathobiological mechanisms.

Metabolomics systematically studies the variety of endogenous metabolites (e.g., small molecules as amino acids, carbohydrates, lipids, nucleotides, and organic acids) in biological specimens (e.g., blood, serum, exhaled breath and urine, cells, and tissue) and represents a comprehensive assessment of ongoing biological processes in states of health and disease. The main biological pathways that can be mapped by metabolomics are an immune response, inflammation, and signaling; metabolism of amino acids, sugars, bile acids, steroids, and lipids; oxidative stress, redox balance, and hypoxia; energy homeostasis; and DNA methylation [8].

The heightened immune response is linked to a shift in tissue metabolism as a result of inflammation-driven recruitment of inflammatory cells, such as neutrophils and monocytes. At sites of inflammation, there are metabolic changes due to an increased nutrient, energy, and oxygen demand to accomplish cell migration, phagocytosis, and other processes [9]. In addition to altered cellular metabolism, extracellular pathways can generate biologically active molecules capable of initiating and modulating inflammatory responses. There is a growing body of evidence that microbiota plays a pivotal role in the modulation of amino acid and lipid metabolism, which exert a strong effect on the immune system [10].

2.2. Asthma

In the last decade, an increasing interest in metabolomics research applied to asthma has been observed. Metabolomics profiling of asthmatic patients could serve both as a diagnostic tool and as a biomarker. Currently, there is a limited number of studies concerning the discrimination of asthmatic patients from healthy controls using liquid or gas chromatography-mass spectrometry (LC-MS or GC-MS) and nuclear magnetic resonance (NMR)-based metabolomics in several biofluids such as serum, urine, and volatile organic compounds (VOCs) in exhaled breath and exhaled breath condensate (EBC) [11].

In general, exhaled VOCs provide a composite biomarker signal based on pattern recognition profiles that seem correlated with eosinophils both in blood and in alveolar fluid [12].

According to the same concept of exhaled VOCs, eNose may be used as a predictor of poor asthma control [13], showing higher sensitivity than FeNO or sputum eosinophilia in the prediction of clinical efficacy of systemic corticosteroids. However, further validation studies are needed; therefore, this method cannot be considered at the time of clinical use.

Recently, several urinary metabolites have been detected as possible biomarkers in asthma, considering that they are noninvasive, easily assessed, and allow multiple analyses. Urinary leukotriene E4 (LTE4), prostaglandins D2 (PGD2), and bromotyrosine, for example, are currently investigated, but their role as biomarkers is still limited to research studies and requires further evaluation [14].

Among metabolites, fatty acids play an essential role in the development and resolution of inflammatory pathways relevant to the pathophysiology of asthma; therefore, they represent an interesting class of mediators under investigation in this field. Some lipid mediators promote inflammation, whereas others are involved in the resolution phase, even if it has been documented that the same mediator might be proinflammatory in one disease or tissue and it can be anti-inflammatory in another. Most of the lipid mediators that regulate inflammation are metabolites derived from omega-6 (n-6) or omega-3 (n-3) fatty acids, including arachidonic acid (AA; 20:4n-6), linoleic acid (LA; 18:2n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3), as is the case for the AA-derived prostaglandin (PG) E2. The main AA-derived mediators of inflammation in asthma are PGs and cysteinyl leukotrienes (CysLTs). There are many other eicosanoids that have been implicated; however, their roles remain somewhat controversial compared with PGs and leukotrienes (LTs) [15].

In this scenario, metabolomics appears to be a promising strategy to increase our phenotyping and endotyping abilities of asthmatic patients. Considering this also changes in diet have the potential role in modifying the anti-inflammatory/proinflammatory balance, and this can allow us to deepen new intervention strategies.

Understanding the metabolic implications of chronic inflammatory processes is, therefore, a crucial need.

Two analytical approaches are employed in metabolomics, untargeted and targeted. Untargeted metabolomics measures a broad range of metabolites in a biological sample, giving an advantage for the investigation of the complex interaction between metabolites from multiple pathways in a holistic, hypothesis-free analysis of (potentially) all the metabolites present in the analyzed sample [16].

The untargeted approach also allows the discovery of novel metabolites; however, it produces a bulk of data that is difficult to analyze. Untargeted approaches require protocols for unbiased data coverage, validation, and quality control of the obtained results and resources to characterize newly identified metabolites.

Targeted metabolomics is based on the focused quantification of predefined known groups of metabolites. This method has several advantages over untargeted metabolomics, including higher sensitivity and selectivity as the metabolites captured are based on previous experiments and libraries of specific metabolites, enzymes and kinetics, and biochemical pathways. In targeted metabolomics, optimized protocols for sample preparation can decrease the number of high-

abundance molecules that may interfere, and novel associations between metabolites can be analyzed in the context of a specific illness.

To generate a metabolomic profile, spectroscopic and spectrometric techniques are used, such as high-field nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS), and separation techniques coupled to mass spectrometric detection, such as High-performance liquid chromatography (HPLC) , ultra-HPLC (UHPLC), gas chromatography (GC), capillary electrophoresis (CE), 2D chromatography, and supercritical fluid chromatography. The matrix of the sample, the concentration and properties of the metabolites, and the quantity of sample drive the choice of a given method [4].

NMR is reproducible and non-destructive for the molecules, can be automatized, results in fast analyses, and provides the opportunity to simultaneously measure many types of small molecules from the metabolome, with detection limits in the order of μM or nM [4]. A drawback is that NMR has poor sensitivity as compared to MS, and the NMR spectra can produce overlapping signals among metabolites rendering quantification unfeasible with low-abundant metabolites. MS is based on the separation of molecules of a biofluid (or other biological sample) and their fragments by their mass-to-charge ratio. This technique has a higher sensitivity, better reproducibility, and selectivity than NMR. LC-MS and GC-MS platforms require minimal sampling compared to the wide collection of metabolic fluctuations that can be assessed. The main drawbacks include the costs to run a laboratory compared to NMR and the reduced levels of fragmentation in LC-derived ionization [4].

In contrast to NMR, the detectability of a metabolite in MS depends on the ionization efficiency that is influenced by the sample composition and by analyte separation before the mass analyzer. The coupling of different platforms with different ionization and/or chromatographic strategies has been recently applied to detect untargeted metabolomic signatures in food allergy and asthma [4][17].

In the last couple of years, the challenge of personalized medicine entered the field of respiratory medicine and asthma and drove the first identification of endotypes, each linked to different biological mechanisms that have been partially elucidated in their inherent complexity [18].

2.2.1. Chronic Rhinosinusitis (CRS)

Based on metabolomics studies, the metabolic fingerprinting strategy, metabolic profiling, and quantitative targeted metabolomics are identified as useful in CRS development.

Multiple clinical studies have reported dysregulated fatty acid metabolism in severe asthma and aspirin-exacerbated respiratory diseases. Miyata et al. [19] focused on the metabolic regulation of cysLTs in inflammatory eosinophils, a major cellular source of these mediators, and explained how fatty acid metabolism of eosinophils could be regulated in asthma and aspirin-exacerbated respiratory diseases (AERD), which are often complicated with eosinophilic rhinosinusitis. Further integrated analysis identified type 2 cytokines or microbial components as inducers of fatty acid metabolic abnormality, suggesting the importance of the tissue environment of nasal polyps to affect cellular inflammatory characteristics. Recent clinical trials reported the usefulness of antibody drugs for type 2 cytokines to improve disease outcomes of chronic eosinophilic rhinosinusitis. [20].

2.2.2. Food Allergy and Atopic Dermatitis

Metabolomics studies of atopic children focused on the metabolism of different pathways, including tyrosine and tryptophan, lipids, Polyunsaturated fatty acids (PUFAs), Short-chain fatty acids (SCFAs), and bile acids, to investigate the potential role of these metabolites for discriminating between health and atopic diseases and for identifying different endotypes of atopic disease.

The pattern of SCFAs is altered as the maturation of the gut microbiome takes place until the age of approximately 3 to 4 years [21]. Assfalg et al. [22] investigated urine samples from infants with AD, and their analysis indicated increased 2-hydroxyburyrate in infants with asthmatic disease compared to healthy control.

Huang et al. [23] found higher levels of tryptophan and indolelactic acid in children with AD with elevated specific-IgE compared to those with normal IgE and HCs, respectively, while these metabolites did not differ among children with AD and normal IgE levels compared to HCs.

2.3. Biomarker Research in Type 2 Severe Asthma

Useful biomarkers in respiratory disease can be obtained by several different types of clinical samples, such as bronchoscopic samples, induced sputum, blood, urine, and exhaled gases with several potential applications in the management of SA such as understanding biology, ameliorating diagnosis, screening, assessment of severity, control, or prognosis of patients and in the identification of endotypes. Moreover, there is a growing application in clinical trials and safety monitoring by identifying pharmacodynamic or predictive-of-response biomarkers, and a variety of response outcomes is used in the different clinical trials, as well as a huge range of potential predicting factors.

Principal biomarkers in T2 asthma and their common site of sampling are summarized in **Table 1**.

Table 1. Principal biomarkers in T2 asthma and their common site of sampling in clinical use.

Site of Sampling	Biomarker
Peripheral blood	**Eosinophils**
	ECP
	EDN
	IgE
	Periostin
	DPP-4
Sputum	**Eosinophils**
	EPX
Exhaled breath	**FeNO**

Abbreviations: eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), immunoglobulin E (IgE), dipeptidyl peptidase-4 (DPP-4), eosinophil peroxide (EPX), fractional exhaled nitric oxide (FeNO).

Eosinophilia, both in blood and in sputum, is likely to be one of the most studied and used biomarkers in T2 severe asthma.

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