Biomarker discovery for asthma phenotyping: From gene expression to the clinic
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CHAPTER 1

General Introduction
Asthma Phenotypes

Asthma is highly complex with respect to pathophysiology and response to treatment. Only recently, asthma was defined as a syndrome (1;2), and by characterization multiple asthma phenotypes were recognized (3-6). So far, the phenotypic characterization of asthma has been limited to clinical symptoms, age of onset, lung function, gender, medication use, general inflammatory markers, body mass index (BMI), nasal polyps and sinusitis. Since the comorbidity of the upper airway diseases in asthma are highly prevalent, upper respiratory tract symptoms are included when studying asthma phenotypes. According to epidemiological studies, the majority of patients with asthma have concomitant allergic rhinitis and the presence of allergic rhinitis is an independent risk factor for development of asthma (7). This frequent co-existence of allergic rhinitis and asthma has been referred to as “united airways disease”, suggesting a single inflammatory process that causes both upper and lower airway diseases (8). A better molecular understanding of this biological complex disease will allow for discovery of various disease phenotypes and the mechanisms driving the inflammatory processes involved.

Unmet Needs

Eventually, asthma profiling will lead to phenotypic targeted treatment, one of the major unmet needs for asthma. Previous studies have shown that sputum eosinophils are predictive of treatment responsiveness and disease outcome (9). Because of the limitations of sputum induction, several studies have tried to find alternative markers for sputum eosinophils. Blood differential cell counts, fractional exhaled nitric oxide (FENO) and serum periostin have been considered in various studies as surrogate markers to diagnose airway eosinophilia, even though a recent meta-analysis was still inconclusive (10). Recently, blood eosinophil count was successfully used to identify patients with eosinophilic asthma responsive to anti-IL-5 treatment (11;12). Furthermore, periostin was demonstrated to be a predictive biomarker for the effectiveness of anti-IL-13 therapy (13). This suggests that inflammatory biomarkers should be used in asthma management. Unfortunately, limited progress has been made in the discovery of new useful clinical biomarkers and further validation of existing biomarkers is needed (14).

A second unmet need is prevention and improved treatment of asthma exacerbations. The continuous occurrence of acute exacerbations despite optimal asthma management imposes considerable morbidity on patients and is a major economic burden (15). The most common cause of asthma exacerbations are viral respiratory tract infections. The upper airway is the first line of defence, being constantly exposed to various viruses, bacteria, and allergens. In case of a viral upper respiratory tract infection, patients with
asthma have increased risk of developing a lower respiratory tract infection with more severe symptoms as compared to the same type of infection in healthy individuals (16). To identify new targets for prevention and therapy, studies are needed to increase our understanding of the mechanisms of virus induced asthma exacerbations. A recent trial showed inhaled IFN-β as potential treatment for deterioration of asthma symptoms caused by respiratory viruses. This therapy is based on studies examining bronchial epithelial cells from patients with asthma having an impaired interferon production in response to viruses (17-19). More clinical studies are needed to further our understanding and identify new targets.

BIOMARKER DISCOVERY

To increase the understanding of asthma and discover new biomarkers, knowledge of the molecular mechanisms involved is required. By now, high-throughput omics technologies are available to quantify gene expression (transcriptomics), proteins (proteomics), lipids (lipidomics) along with other metabolites (metabolomics) in blood, urine and exhaled air (breathomics). In general, biomarker studies can either analyse specific predefined biomarkers, or use an unbiased approach which is more statistically based to allow for the discovery of new biomarkers. An experimental design using omics technology often involves a hypothesis-generating component, studying large comprehensive datasets for new insights or diagnostics in disease instead of reducing explanations to specific identified targets. In this thesis, we apply both transcriptomics analysis and breathomics analysis to increase our understanding and search for surrogate biomarkers. We use microarrays for high-throughput quantification of RNA transcripts because this is the present state-of-the-art method for biomarker discovery. Transcriptomics analysis can be used to study gene expression of samples composed of an isolated cell type or samples of heterogeneous cellular composition; however, analysing mixed cell types will make interpretation of results difficult. Breathomics was more recently introduced and may offer a noninvasive easy-to-use method capturing composite molecular signatures in exhaled air. Using these methods will allow further characterization of complex diseases such as asthma, and will potentially offer biomarker discovery (20).

VALIDATION

Validation is an essential part of modern research, including validation of techniques or tools, statistical analysis and populations. When identifying or testing biomarkers to diagnose or phenotype disease, validation of all these elements are required and
Once markers have been identified, external validation is essential. Several international guidelines have been published to improve the quality and standardize the reporting of studies. The guidelines on STAndards for the Reporting of Diagnostic accuracy studies (STARD) were initiated to improve the reporting of studies on diagnostic accuracy (21), and the recently published Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis (TRIPOD) initiative help to improve reporting of prediction model studies (22).

In this thesis, we firstly review several elements of validation for transcriptomics analysis and breathomics analysis. Next, we use various methods to validate elements of the clinical studies, which are further discussed in the final chapter.

**OBJECTIVES OF THIS THESIS**

1. To review and compare transcriptomics by microarrays and next-generation RNA sequencing.
2. To review breathomics as a non-invasive tool.
3. To define the gene expression profiles of upper and lower airway epithelial cells of the same individuals with and without upper or lower airways disease.
4. To examine the impact of allergic rhinitis with or without allergic asthma on the gene expression profiles of the upper and lower airway epithelium.
5. To define the gene expression profiles of upper and lower airway epithelial cells of the same individuals with and without upper or lower airways disease after stimulation with double-stranded RNA (dsRNA), a surrogate marker for viruses.
6. To examine the impact of allergic rhinitis with or without allergic asthma on the gene expression profiles of the upper and lower airway epithelium after stimulation with dsRNA.
7. To quantify the mutual relationships of blood eosinophils, exhaled nitric oxide (FE_{NO}) and serum periostin with sputum eosinophils by external validation in two independent cohorts of patients with mild to severe asthma.
8. To study and validate the relationship of breathprints analysed by a composite electronic nose (eNose) platform with sputum eosinophils in patients with mild to severe asthma.
OUTLINE OF THIS THESIS

Chapter 2 addresses the concept of transcriptomics analysis by microarrays and next-generation RNA sequencing, including the strengths and limitations, and reviews breathomics as a patient-friendly method.

In chapter 3 the transcriptomic profiles of isolated upper and lower airway epithelial cells are compared between patients with allergic rhinitis with or without concomitant asthma and healthy individuals. Chapter 4 describes the effect of dsRNA on the transcriptomic profiles of airway epithelial cells by comparing gene expression profiles of poly(I:C)-induced nasal and bronchial epithelial cells of patients with allergic rhinitis with or without concomitant asthma and healthy individuals.

In chapter 5 blood eosinophils, FE_{NO} and serum periostin are analysed as surrogate biomarkers for sputum eosinophils by external validation in two independent cohorts of patients with various severities of asthma. Furthermore, in chapter 6 breathprints obtained by a composite eNose are validated to predict sputum eosinophilia in asthma.

Finally, in chapter 7 the main findings of this thesis and research implications are summarized and discussed.
REFERENCES


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