Clinical and inflammatory markers in asthma and COPD phenotyping

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Chapter 1

GENERAL INTRODUCTION AND AIMS OF THE THESIS
Introduction

Asthma is defined as a chronic inflammatory disease of the airways and is associated with variable airways obstruction, airway hyperresponsiveness and symptoms of chronic cough, dyspnea, wheeze, chest tightness and sputum production (1). The clinical diagnosis of asthma is based on variable airflow obstruction, daytime and nocturnal symptoms. The severity of asthma is based on the control of asthma, the frequency of rescue bronchodilator use and maintenance dose of inhaled corticosteroids (1).

COPD is a disease that is associated with an abnormal inflammatory response to noxious particles or gases, of which cigarette smoke is the most important (2). The clinical diagnosis of COPD is defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as the presence of not fully reversible airflow obstruction, confirmed by postbronchodilator spirometry (ratio of forced expiratory volume in 1 second to forced vital capacity: FEV\textsubscript{1}/FVC <0.70) (2).

However, it is increasingly recognized that asthma and COPD are both phenotypically heterogeneous diseases that result from complex interactions between environmental and genetic factors (3-5). In patients with asthma, the expression of the disease may vary according to age and gender, association with atopy or specific provoking factors, type of airway inflammation or severity of the disease (3;5). Recognition of specific subphenotypes may improve our understanding of pathobiology, treatment response, prognosis and the underlying genetic basis for asthma. In patients with COPD, airway neutrophilia and CD8+ T-lymphocyte infiltration have been the hallmarks of COPD infiltration, but some studies in COPD have also shown the presence of eosinophilic inflammation (6). COPD patients with eosinophilic airway inflammation present a distinct phenotype. Identification of this phenotype may have therapeutic implications. This thesis will focus on clinical phenotypes of asthma and diagnostic tests to identify inflammatory phenotypes of adult-onset airway diseases (asthma and COPD).

Phenotypes of asthma

Asthma is not a single disease, but rather a complex of multiple, separate syndromes that overlap (5). Many categories have been used to define asthma phenotypes, mostly with general or clinical criteria. A classification of asthma into allergic and non-allergic asthma is commonly used (5). Clinical or physiological phenotypes relevant to asthma include those defined by level of severity (from mild to severe) (7), the frequency of exacerbations (8) and the presence of chronic airflow limitation (9). The age at which a patient develops asthma also differentiates two asthma phenotypes (10). Childhood-onset asthma or early onset is defined by asthma symptoms that start in childhood.
Adult-onset, or late-onset, asthma is considered when asthma symptoms represent for the first time during adulthood. Despite ample evidence that asthma can develop in adults who never had any relevant respiratory symptoms as a child, not much is known about the adult-onset asthma phenotype.

**Adult-onset asthma**

About 95% of asthma patients have their first episode before the age of 6 (11). The development of asthma in childhood typically runs in families (12), is associated with allergy in most patients (13;14) and has a good prognosis with a satisfactory response to medical treatment (1). In contrast to childhood-onset asthma, less is known about the prevalence and factors associated with adult-onset asthma. Studies have shown that adult-onset asthma mainly effects women (15), has a low remission rate (16) and is less often associated with allergy and atopic diseases (17). In addition, many patients with adult-onset asthma have a poor prognosis (16), with a faster decline in lung function (18) and more severe persistent airflow limitation (9). Newly diagnosed adults with severe asthma have compromised lung function even if they have asthma of short duration (19), suggesting that significant loss of lung function occurs at or soon after the initial diagnosis. Still, adult-onset asthma is poorly investigated and underlying disease mechanisms unknown.

To better understand the disease mechanisms and to identify risk factors for asthma severity characterisation and identification of subphenotypes in a population of adults with adult-onset asthma is needed. To that end in 2009 we started the adult-onset asthma and inflammatory subphenotypes (ADONIS) study. In the first part of this study, 200 patients with longstanding adult-onset asthma as well as 200 patients who were in an early stage of adult-onset asthma were thoroughly characterized by clinical, functional and inflammatory markers and cross-sectionally evaluated. Part of the results of this evaluation is described in the present thesis.

**Airway inflammation in asthma**

Airway inflammation is heterogeneous in patients with asthma (20). Identification of the eosinophilic phenotype of asthma has important clinical and therapeutic implications since it is associated with corticosteroid responsiveness (21;22), frequent asthma exacerbations (22) and persistent airflow limitation (9). Furthermore, in patients with severe inhaled corticosteroid resistant eosinophilic asthma, a novel promising therapeutic strategy that target the eosinophil itself has been developed (23-25). Mepoluzimab, a humanised monoclonal antibody against interleukine-5, reduces the risk of asthma exacerbations (23;25).

Assessment of the eosinophilic phenotype of asthma by sputum induction is generally considered a reliable non-invasive method (26). However, in clinical practice, the
application of sputum analysis is hindered by the requirement of laboratory facilities and the duration of the analyses. Therefore different surrogate markers of eosinophilic airway inflammation have been developed. Although blood eosinophil counts and the level of exhaled nitric oxide may relate to sputum eosinophils, a study investigating the predictive level of exhaled nitric oxide (FeNO), blood eosinophils, total IgE levels and FEV₁ resulted in poor correlation with sputum eosinophils, both separately and combined (27). More recently, periostin an extracellular matrix protein that is induced by IL-4 and IL-13 was identified as a potential systemic biomarker of eosinophilic airway inflammation in asthma, showing a significant correlation with sputum eosinophils (28). Based on international STARD-guidelines (29) it is mandatory to perform external validation when assessing diagnostic or phenotypic accuracy of disease markers. This has not been done for the sputum eosinophils with the triad FeNO, blood eosinophils and serum periostin. To assess the diagnostic accuracy of these markers an external validation study was performed.

**Phenotypes of COPD**

In COPD, several clinical features can be observed including emphysema, small airway disease and asthma like characteristics such as airway hyperresponsiveness (30;31). The inflammatory response in COPD is predominantly neutrophilic, but also comprises involvement of eosinophils (32;33). Identification of the eosinophilic phenotype in patients with COPD has shown to be of clinical value. Several studies have shown a greater response to corticosteroids treatment in eosinophil predominant COPD, assessed by induced sputum analysis (34-36). However, in clinical practice, the application of sputum analysis is limited. To identify the eosinophilic phenotype in patients with COPD easy to measure diagnostic tests are urgently needed.

**Markers of airway inflammation in COPD**

*Indirect challenge testing*

Airway hyperresponsiveness (AHR) is often present in patients with COPD (37). AHR can be measured using indirect challenge tests (38). Indirect challenge tests act by the release of endogenous mediators that cause the airway smooth muscle to contract, with or without inducing microvascular leakage. Because the responses to these challenges are modified or even completely inhibited by inhaled corticosteroids, the airway response to these challenges may be a closer reflection of active airway inflammation (39). Indirect challenge tests act on intermediate pathways, such as mediator release from inflammatory cells and the release of neuropeptides. A new method of indirect challenge testing is the use of the mannitol challenge test (40). Mannitol is an osmotic stimulus that causes airway narrowing by release of bronchoconstrictor mediators such
as leukotrienes, prostaglandins and histamine \((41;42)\). The mannitol challenge test is easy to perform \((40)\), safe \((43)\) and is the first challenge test that has been FDA approved \((44)\).

**Exhaled breath analysis**

Using exhaled breath as a diagnostic test is an old concept. Already in ancient times it was recognized that breath can provide information about health and disease. For example, a fishy smell of breath could indicate liver disease, the sweet acetonic smell of breath uncontrolled diabetes, a urine-like odour kidney disease, the smell of grapes Pseudomonas infections and the smell of baked bread typhoid fever \((45)\). Exhaled breath is a mixture of nitrogen, oxygen, carbon dioxide, water and inert gases such as nitric oxide and carbon monoxide. It has been shown that exhaled breath also contains a complex mixture of volatile organic compounds \((VOCs)\) in very low concentrations \((46-48)\). There are several methods that can be applied for exhaled breath analysis. The gold standard test is considered to be gas chromatography coupled to mass spectrometry \((GC-MS)\). Using this technique, both the composition and the concentrations of components of gas mixture can be determined \((49;50)\). Electronic noses \((eNoses)\) follow a different approach, by measuring the whole spectrum of \(VOCs\) without identification of the individual components \((51)\). The great advantage of exhaled breath testing is the non-invasiveness for patients compared to the collection of induced sputum. Patients are asked to either blow in a device or a balloon and breath collection methods require no cooperation of the patients.

It is unknown whether airway hyperresponsiveness to inhaled mannitol or exhaled breath testing are related to the underlying inflammatory phenotype in patients with mild to moderate COPD. If so, these tests may reflect the type and activity of the predominant inflammatory pathways thereby facilitating management of COPD.
Aims of the present studies

• In the first study (chapter 2) we give an overview of the current literature about adult-onset asthma regarding differences between childhood-onset asthma, risk factors for development, phenotypes of adult-onset asthma and new approaches for personalized management.

• In the second study (chapter 3), we identified the clinical phenotypes of patients with longstanding adult-onset asthma.

• In the third study (chapter 4) we explored whether unsupervised non-hierarchical cluster analysis in a population of newly diagnosed adult-onset asthma revealed new adult-onset asthma phenotypes.

• In chapter 5, we assessed the frequency of chronic rhinosinusitis in newly diagnosed adult-onset asthma and evaluate whether chronic rhinosinusitis (with or without nasal polyposis) is associated with inflammation in the lower airways.

• In chapter 6 we assessed the diagnostic accuracy of FeNO, blood eosinophils and serum periostin to identify sputum eosinophils in a cohort of patients with mild to moderate asthma, and replicated our findings in a population with more severe asthma.

• The purpose of the 6th study (chapter 7) was to investigate whether airway hyper-responsiveness to inhaled mannitol was related to markers of inflammation in hypertonic saline-induced sputum, blood and exhaled air in patients with mild to moderate COPD.

• The last study (chapter 8) examined the relationship of exhaled molecular profiles measured by GC-MS, eNose and FeNO-analyzer, with markers of inflammation in induced sputum from patients with mild to moderate COPD.

• In chapter 9, data from the different studies are summarized and the implications are discussed from clinical or research perspectives.
References

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introduction


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