Clinical and inflammatory markers in asthma and COPD phenotyping

de Nijs, S.B.

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Chapter 9

SUMMARY AND GENERAL DISCUSSION
Summary

Both asthma and COPD are not single diseases, but rather a complex of multiple, separate syndromes that overlap. The current definitions of asthma and COPD may not reflect the phenotypes of airway diseases in the community, which may have differing disease processes, clinical features or response to medical treatment.

In asthma, the expression varies according to age of onset. While asthma that starts in early childhood is well studied, not much is known about asthma that first presents in adulthood, so called adult-onset or late-onset phenotype. Studies have shown that patients with adult-onset asthma have a poor prognosis, with a faster decline in lung function and more severe persistent airflow limitation.

In asthma and COPD, different inflammatory phenotypes can be identified. Several studies have identified an eosinophilic phenotype in both diseases, associated with different responses to medical therapy compared to the non-eosinophilic phenotype. However, most diagnostic tests to measure airway inflammation are invasive or time consuming and therefore not used in clinical practice.

This thesis focuses on phenotypes of adult-onset airway diseases (asthma and COPD) and investigates the use of new diagnostic tests to identify the eosinophilic phenotype in both conditions. The main results and conclusions from the studies in this thesis are summarized below.

Adult-onset asthma

In chapter 2, a review was written focussing on asthma that starts in adulthood. The aim of this review was to provide an overview of recent studies regarding differences between childhood-onset asthma, risk factors for development of asthma, different asthma phenotypes and new approaches for personalized asthma treatment. Based on this literature search we conclude that:

1. As compared to childhood-onset asthma, adult-onset asthma has worse prognosis and poorer response to standard asthma treatment.
2. Risk factors associated with onset of asthma in adulthood include respiratory infections, environmental sensitizers, hormonal factors, obesity and stress.
3. Using clustering techniques, two clinically well recognized adult-onset phenotypes can be distinguished. These phenotypes are associated with more severe asthma.
4. New exciting possibilities have been developed for a more targeted, phenotypic approached in patients with adult-onset asthma.
5. The underlying pathophysiological mechanisms of asthma severity and poor clinical outcome are largely unknown.
Phenotypes of adult-onset asthma

As described in chapter 2, several reports have shown that adult-onset asthma is more severe at onset, is more likely to persist and shows a faster decline in FEV₁ as compared to childhood-onset asthma. To investigate the underlying mechanisms of adult-onset asthma and to capture the complexity of the disease, we have used unsupervised clustering techniques in the later stages (chapter 3) as well as in the early stages of adult-onset asthma (chapter 4).

Therefore, 200 patients with longstanding (median asthma duration 10 years) and 200 patients with newly diagnosed (<1 year) adult-onset asthma were characterised using clinical, functional and inflammatory characteristics.

Results of cluster analysis

In chapter 3, a population of adults with longstanding adult-onset asthma, three different phenotypes could be identified. Patients in cluster 1 (n=69) consisted of patients with severe eosinophilic inflammation-predominant asthma and persistent airflow limitation despite high intensity anti-inflammatory treatment, with relatively low symptom scores. The second cluster (n=41) was characterized by obese females with frequent symptoms, high health care utilisation and low sputum eosinophils. The third cluster (n=90) consisted of patients with mild to moderate, well controlled asthma with normal lung function and low inflammatory markers.

In the early phases of adult-onset asthma (chapter 4) also three different phenotypes could be identified. The largest cluster (n=114) consisted of patients with mild asthma. The second cluster (n=45) consisted of patients with high symptom scores, high medication requirement, poor quality of life, low exhaled nitric oxide levels and high numbers of blood neutrophils. Patients in the third cluster (n=43) had lower postbronchodilator FEV₁, more air trapping and more severe airway hyperresponsiveness than the other clusters. Of these, 62% had chronic rhinosinusitis and 29% had nasal polyps. In clusters 1 and 3 asthma onset was associated with a history of respiratory infection in one-third of the patients.

In conclusion: The clusters we identified in the later stages of adult-onset asthma are not similar to those identified in the early stages which questions the stability of asthma clusters.

Early identification those patients who are prone to develop severe asthma is important for early identification with targeted therapies. In future, the predictive value of the early asthma clusters needs to be confirmed in prospective follow-up studies.

Role of chronic rhinosinusitis

Adults with severe eosinophilic asthma have a high prevalence of chronic rhinosinusitis (CRS). Whether sinus disease plays a role in the early stages of adult-onset asthma is
unknown. Therefore the aim of the study in chapter 5 was to assess the frequency of CRS (including nasal polyposis) in the early stages of adult-onset asthma, and to investigate whether CRS with or without nasal polyposis is associated with eosinophilic inflammation in the lower airways. To that end, 200 adults with newly diagnosed (< 1 year) were cross-sectionally evaluated. CRS was diagnosed according to EP3OS criteria (symptoms and sinus CT-scanning or nasal endoscopy). Lower airway inflammation was assessed by eosinophils in induced sputum and nitric oxide in exhaled air.

The results show that 54% of the patients had CRS at asthma diagnosis, of which 36% had nasal polyposis. Furthermore, sinus CT-scan score and nasal endoscopy score correlated with sputum eosinophil percentages and exhaled nitric oxide levels. Sinus CT-scan score was independently associated with sputum eosinophil percentage and exhaled nitric oxide level. Patients with nasal polyposis had the highest percentages of sputum eosinophils and exhaled nitric oxide levels. Based on these findings we can conclude that CRS and nasal polyposis are highly prevalent in newly diagnosed asthma in adults and might be an early sign of the severe eosinophilic asthma phenotype.

Diagnostic tests

In patients with asthma the identification of eosinophilic airway inflammation has important clinical and therapeutic implications since it is associated with corticosteroid responsiveness, frequent asthma exacerbations and persistent airflow limitation. Furthermore there is evidence that eosinophilic airway inflammation may also play an important role in the pathogenesis of COPD and patients with eosinophilic inflammation may represent a distinct COPD phenotype. This may have therapeutic implications. However, in clinical practice, the application of sputum analysis is hindered by the requirement of lab facilities and the duration of the analyses. Therefore, there is a need for adequate surrogate markers of eosinophilic inflammation in both airway diseases.

Systemic markers of airway inflammation in asthma

Although blood eosinophils and level of exhaled nitric oxide may relate to sputum eosinophils in patients with asthma, studies show contradictory results in predicting eosinophilic airway inflammation. More recently, serum periostin was proposed as a novel biomarker for sputum eosinophils. Therefore in chapter 6 we aimed to quantifying the mutual relationships of blood eosinophils, FeNO and serum periostin with sputum eosinophils by external validation in two independent cohorts across various severities of asthma. To that end 110 patients with mild to moderate asthma (external validation cohort) and 37 patients with moderate to severe asthma (replication cohort) were cross-sectionally evaluated. Sputum was induced for the assessment of eosinophils. In addition, blood eosinophil counts, serum periostin concentrations and FeNO were assessed.
In the external validation cohort the diagnostic accuracy, expressed as area under the curve (ROC AUC), of blood eosinophils was 89% and for FeNO level 78% to detect sputum eosinophilia ≥ 3%. Serum periostin was not able to distinguish eosinophilic from non-eosinophilic airway inflammation (ROC AUC 54%, P=0.60). When combining these three variables no improvement was seen. The diagnostic accuracy of blood eosinophils was confirmed in the replication cohort (ROC AUC 85%; p<0.001). Therefore we conclude that in patients with mild to moderate asthma as well as patients with more severe asthma blood eosinophils have the highest accuracy to identify sputum eosinophilia in asthma. This can facilitate individualised treatment and asthma management.

**Bronchial provocation tests in COPD**

Airway hyperresponsiveness (AHR) may serve as a surrogate measure of airway inflammation, since it is associated with the presence of inflammatory cells and release of mediators in the airways. In particular, this holds for indirect challenges, amongst which dry powder mannitol challenge is relatively easy to apply.

The aim of the study in chapter 7 was to investigate whether bronchial hyperresponsiveness to inhaled mannitol is related to the degree of airway inflammation in mild to moderate COPD. To that end correlations between bronchial hyperresponsiveness to mannitol and biomarkers of inflammation in exhaled breath (FeNO-level) and soluble markers of inflammation in induced sputum were investigated. In chapter 7 we found that in COPD mannitol hyperresponsiveness is associated with biomarkers of airway inflammation. Furthermore, ROC-curves showed 60% sensitivity and 100% specificity of bronchial hyperresponsiveness to inhaled mannitol for > 2.5% eosinophils in mannitol-induced sputum. The high specificity of mannitol challenge test suggests that the test is particularly suitable to exclude eosinophilic airways inflammation, which may facilitate individualized treatment in COPD.

**Exhaled breath analysis in COPD**

Exhaled air contains mixtures of gases including volatile organic compounds (VOCs) and nitric oxide (FeNO). Assessment of the profile of these volatiles by gas-chromatography and mass-spectrometry (GC-MS), nano-sensors of an electronic nose (eNose) and an NO-analyzer might lead to the discovery of novel disease specific patterns of molecular biomarkers. In chapter 8, we investigated whether the exhaled signal in mild to moderately severe COPD (GOLD-stages I-II) is related to airway inflammation. Associations between eNose and GC-MS profiles, individual VOCs and inflammatory markers in sputum were investigated. We showed that in COPD, both eNose and GC-MS breathprints were highly associated with inflammatory markers in sputum. In addition, a number of individual compounds, most derived from oxidative stress pathways, were associated with inflammatory parameters in sputum. Our results suggest that exhaled breath
molecular profiling may be used as a non-invasive and fast way to assess and monitor airway inflammation in COPD.

**General discussion**

**Conclusion**

Based on the studies described in this thesis, we conclude that adult-onset respiratory diseases (asthma and COPD) are heterogeneous conditions characterized by different clinical features and inflammatory characteristics.

The first part of the thesis focused on phenotypes of adult-onset asthma. We showed that in patients with longstanding disease, three different clinical well-recognizable phenotypes can be identified, of which two are characterized by severe asthma. One of these severe asthma phenotypes can already be detected in the early stages of the disease. Furthermore, in newly diagnosed adult-onset asthma, CRS and nasal polyposis are associated with more severe airway inflammation. Therefore, this co-morbidity could be an early sign of severe asthma developing. Finally, measuring blood eosinophils is an accurate diagnostic tool for detecting this inflammatory phenotype in patients with asthma and is easy to measure in clinical practice.

The second part of the thesis focused on new diagnostic tests to identify inflammatory phenotypes in patients with COPD. These patients might benefit from specific treatments but cannot be distinguished based on clinical characteristics. The mannitol challenge test and exhaled breath analysis are closely associated with the degree of airway inflammation. The tests are easy to perform and could be used in clinical practice to monitor airway inflammation.

**What does this thesis add to our current knowledge?**

This thesis adds important information to our current knowledge of adult-onset asthma. First, we are the first people to characterize a population of adults with longstanding as well as recent adult-onset asthma using clinical, functional and inflammatory characteristics (chapters 3 and 4). In adults with longstanding disease we identified a severe eosinophilic inflammation-predominant asthma phenotype and a phenotype characterized by obese females. These results reinforce the findings of two previous cluster analyses (1-3). In the early stages of the disease, two phenotypes associated with moderate to severe asthma were identified which were different from those mentioned above. One could speculate that the most severe of these represents the early phase of the severe eosinophilic inflammation predominant phenotype. Only limited knowledge is available about the stability of asthma phenotypes that are identified by cluster analysis over time. One study confirmed the stability of cluster-based asthma phenotypes over
1 year (4). Very recently Boudier and colleagues (5) investigated the stability of asthma phenotypes identified by a clustering approach in three large epidemiological cohorts over 10 years, but unfortunately no data is reported about the age of asthma onset. In this study the probability of remaining in the same phenotype at 10-years follow up varied from 54% to 88% across phenotypes, indicating that some asthma phenotypes are more stable over time than others. Transition towards increased asthma symptoms were more frequently observed in non-allergic phenotypes as compared to allergic phenotypes (5). In line with our results, this study supports the predictive ability of the clustering approach to identify specific phenotypes with worse long-term prognosis.

Finally, we found that already at asthma onset chronic rhinosinusitis (CRS) and nasal polyposis are associated with eosinophilic inflammation in the lower airways (chapter 4). Some previous studies have shown an association between allergic and non-allergic rhinitis and onset of asthma in adulthood (6-8), whereas others found that asthma patients with CRS reported a later onset of disease as compared to those without CRS (9). Our study is the first to indicate that adult-onset asthma, CRS and lower-airway eosinophilic inflammation, are elements of a distinct asthma phenotype that is apparent right from the start.

The second part of this thesis extends our current knowledge on the use of diagnostic tests to identify inflammatory phenotypes in patients with asthma and COPD.

In patients with mild to severe asthma, we externally validated serum periostin as a surrogate marker for sputum eosinophils (chapter 6). Interestingly, blood eosinophils and sputum eosinophils were highly correlated and exhibited the highest diagnostic accuracy, which validates previous data (26), except for a very recent report in the Journal of Allergy and Clinical Immunology (10). The discrepancy between this latter report and our study could be due to the differences in asthma population. The study by Hastie and colleagues (10) was performed in a generally young population of asthma patients with relatively high IgE levels, whereas our study consisted of older adults, with a high proportion of patients with low IgE levels.

In our study, serum periostin did not seem to be a good diagnostic marker for sputum eosinophils. This is in contrast to a previous study that investigated the relationship between sputum eosinophils and all three markers (11) and demonstrated the highest diagnostic accuracy for serum periostin. Again, the discrepancy between this study and our results might be explained by the differences in asthma population. Serum periostin is generated by airway epithelial cells in response to IL-13 and is identified as a marker of Th2-related airway inflammation in asthma patients with high IgE levels (12). Therefore it could be speculated that periostin is only a marker of eosinophilic airway inflammation in patients with allergic asthma.
In patients with mild-to-moderate COPD, we investigated whether mannitol challenge and exhaled breath analysis could be used to identify eosinophilic airway inflammation. First, we found a relationship between hyperresponsiveness to inhaled mannitol and markers of airway inflammation in sputum (chapter 7). While the performance of the mannitol challenge test has been extensively studied in patients with asthma (13-16), studies in patients with COPD are limited. Leuppi and colleagues (17) demonstrated in a proof of concept study that mannitol challenge might also be useful in identifying COPD patients who will most likely benefit from inhaled corticosteroids. Recently, 68 patients with mild to moderate COPD were randomized in a double-blind manner to receive inhaled budesonide or placebo for 3 months (18). In patients who showed airway hyperresponsiveness to mannitol, budesonide was more frequently associated with improved quality of life compared with the placebo. A positive mannitol challenge test was also associated with a favourable effect on budesonide on airway hyperresponsiveness. These studies extend our observations and suggest that the mannitol challenge appears to provide valuable information on the inflammatory profile in patients with COPD. Secondly, we were the first to assess the relationship and estimate the performance of molecular profiles of volatile biomarkers in exhaled air and markers of airway inflammation in sputum (chapter 8). The present results extend findings from earlier studies in which COPD could be distinguished from asthma using exhaled breath analysis by eNose (19) and from healthy smoking and non-smoking controls by gas chromatography and mass spectrometry (20;21). In these studies, however, the relationships between exhaled volatile organic compound patterns and the accompanying inflammatory profiles were not examined.

Interpretation of the results in terms of mechanism

There are several underlying mechanisms which can explain the adult-onset asthma phenotypes described in this thesis. First, we identified an adult-onset obese female phenotype in patients with longstanding adult-onset asthma (chapter 3). The mechanistic basis for the interaction between obesity and asthma and the role of female gender has been subject of debate and speculation since its earliest description (22). One of the hypotheses included that, not obesity itself, but the obesity-associated metabolic syndrome would explain this interaction. Studies also suggested that also other elements of the metabolic syndrome such as dyslipidemia, insulin resistance, elevated blood pressure, and proinflammatory or procoagulant state were associated with asthma (23). Recently, Assad and colleagues provided evidence that the interaction of obesity, female gender and asthma are clearly not explained by elements of the metabolic syndrome (24). It is thought that endocrine pathways, including the hypothalamic-putuitary-gonadal, leptinergic, and serotonergic pathways, are more likely to play a role in the onset of asthma in obese adults (25). Secondly, we identified a severe eosinophilic inflammation-
predominant adult-onset asthma phenotype in patients with longstanding adult-onset asthma (chapter 3). The mechanism of the eosinophil infiltration of the bronchial mucosa is believed to be driven by the secretion of interleukins by T\textsubscript{H}2-cells when stimulated with antigen (26) which activates immunoglobulin E. IgE is captured by immunoglobulin receptors present on inflammatory cells including mast cells and eosinophils, which release toxic inflammatory molecules that elicit airway obstruction (27). The antigens stimulating the T\textsubscript{H}2-cells in non-atopic asthma are not yet known, although it has been suggested that the immunologic response could be caused by undefined allergens (28) or autoimmunity triggered by viruses (29). Thirdly, we observed that patients with recent adult-onset asthma and CRS represent a separate, more severe inflammatory asthma phenotype (chapter 5). It is still unknown whether inflammation of the upper and lower airways is causally related. One factor that may contribute to the onset of asthma in the early stages of adult-onset asthma is sensitization to enterotoxin of Staphylococcal aureus, a pathogen that often colonizes the sinuses in patients with CRS (30;31). This might lead to an “intrinsic” IgE response, with subsequent eosinophilic inflammation of the airway mucosa, as has been suggested recently (32). Another explanation may be related to the expression of transforming growth factor-beta 1 (TGF-\textbeta1) in both the upper and lower airways (33;34). TGF-\textbeta1 has been demonstrated as representing a master switch in inflammation and remodelling processes and this could provide a key for understanding the onset and persistence of mucosal inflammation and remodelling in paranasal sinuses and bronchi (34). Finally, infections might play an important role in the development of adult-onset asthma viral or bacterial respiratory infections, as described in chapter 4. It is thought that age-related altered antigen presentation and decreased specific antibody responses may lead to subtle immune deficiencies that may allow respiratory infections to provoke injury to the airways. This in turn may set up a vicious cycle of an ongoing inflammatory process leading to asthma (35). Another explanation is that respiratory pathogens may act as triggers of asthma onset to other factors, such as environmental exposures. Cluster 1 and cluster 3 were both characterized by asthma onset after a viral or bacterial respiratory infection in one-third of the patients. Of interest, cluster 1 had mild, well-controlled asthma with low medication requirement and normal lung function; whereas cluster 3 had more severe airway hyperresponsiveness, relatively low FEV\textsubscript{1}, higher medication requirements and higher incidence of CRS with or without nasal polyps. This suggests that respiratory infections in adults can either lead to mild post-infectious wheeze or to more extensive and persistent inflammatory disease of the upper and lower airways.

The results of the second part of this thesis show that surrogate markers of airway inflammation can be used to identify inflammatory phenotypes in asthma and COPD. These results contain no biological surprises. First, the correlation between blood and
sputum eosinophils in patients with mild to severe asthma was to be expected (chapter 6). Eosinophils are produced in the bone marrow and in the event of inflammation the formation is amplified and the eosinophils traffic into inflammatory sites, all under the influence of a number of cytokines, such as IL-5 (36). Secondly, the correlation between airway hyperresponsiveness to mannitol and eosinophilic airway inflammation in patients with mild to moderate COPD is not surprising (chapter 7). This could be explained by the fact that mannitol is an osmotic stimulus that causes airway narrowing by releasing bronchoconstrictor mediators such as leukotrienes, prostaglandins and histamine (37;38). The source of these mediators is likely to be mast cells and eosinophils in the airways as both these cell types release mediators in vitro in response to mannitol (39-41). Finally, exhaled breath profiling using the quantitative method GC-MS can both identify the type and the activation of inflammation (eosinophilic vs neutrophilic), whereas multi-compound breath profiling using nano-sensor pattern recognition by eNose appears to be more suitable for detecting activation of inflammatory cells, especially in COPD GOLD I (chapter 8). It is tempting to speculate that the inflammatory drive and activity in early stages of COPD are reflected differentially or more prominently in exhaled breath than in more advanced stages of COPD. This agrees with the findings by Pierrou et al, who observed a peak in oxidative stress gene expression in mild COPD as compared to more severe stages of the disease (42).

What are the strengths and weakness of this thesis?

The studies described in this thesis may have some strengths and weakness. The strength of these studies (chapter 3-8) is that all patients were well characterized using subjective and objective criteria according to international guidelines (43;44). This included the presence of symptoms, variable airway obstruction or bronchial hyperresponsiveness for patients with asthma, and symptoms, fixed airway obstruction and smoking history for patients with COPD. Secondly, all asthma patients (chapter 3-6) as well as all COPD patients (chapter 7 and 8) were derived from a clinical population rather than an epidemiological one, in order to strengthen the applicability of our results. Finally, particular attention was paid to methodological aspects such as design and methods. For example, for the cluster analysis, we performed factor analyses on clinical, functional and inflammatory parameters to objectively determine the number of clusters as well as the variables to be used in the cluster analysis. Furthermore, we also analyzed whether non-response to participating in the study (chapter 4 and 5) biased our results, which was not the case.

Nevertheless, our studies do have their limitations. First, we were unable to obtain adequate sputum samples in all patients with asthma and COPD. Secondly, the time of asthma-onset was defined by time of doctors’ diagnosis, which may not have been fully accurate in some patients with poor perception of dyspnea (chapter 3). However,
if airway disease was present during childhood, it was apparently not severe enough to be remembered. The reported year of asthma onset does seem fairly accurate (45). Finally, some asthma patients with adult-onset asthma described in chapter 3, 4 and 5 may have had mixed asthma/COPD since smokers and ex smokers were allowed to participate in the study, provided that they had normal diffusion capacity and at least \( \geq 12\% \) reversibility. However, excluding all patients with a smoking history would have reduced the relevance of our findings since smoking is an important risk factor for the development of asthma (46).

**Clinical implications**

The recognition of clinical and inflammatory phenotypes in patients with asthma and COPD furthers our understanding of the underlying mechanism, leading to improved disease management and treatment response. Furthermore, early identification of patients who are prone to develop severe asthma is important for early intervention with targeted therapies. This chapter discusses the clinical implications of the study results.

**Clinical phenotypes**

An obesity-associated adult-onset asthma phenotype can be identified by calculating the body mass-index (*chapter 3*). However, for the clinician it is important to realize that this asthma phenotype is not associated with eosinophilic airway inflammation and responsiveness to inhaled corticosteroids. Furthermore, doctors need to be aware of comorbidity that might provoke or worsen asthma control, in particular gastroesophageal reflux (23). When treating these patients, reducing overweight might be more effective than anti-inflammatory medication.

In patients with recent adult-onset asthma, CRS was presented most frequently in the two clusters with moderate-severe asthma (*chapter 4*). CRS (including nasal polyposis) comprises clinical features that are assessed by symptoms of CRS confirmed by sinus ct-scanning or nasal endoscopy abnormalities (47). Moreover, in this thesis we demonstrated that in newly diagnosed adult-onset asthma, CRS and nasal polyposis was an underdiagnosed and therefore undertreated condition (*chapter 5*). However for the clinician, it is important to know whether CRS and nasal polyposis exist, because medical or surgical treatment of CRS and nasal polyposis may improve asthma control (48). Furthermore we demonstrated that in the early stages of adult-onset asthma, CRS is already associated with the more severe eosinophilic inflammatory asthma phenotype. In line with this observation, in the two clusters associated with less-controlled asthma, CRS was present in about 65\% of the patients with newly diagnosed adult-onset asthma. Therefore patients with CRS at asthma diagnosis should be followed more intensively because this might be an early sign of developing severe asthma.
Severe eosinophilic inflammation-predominant adult-onset asthma was identified in patients with longstanding adult-onset asthma (chapter 3). This phenotype was characterized by persistent airflow limitation, despite treatment with medium to high doses of ICS, and in 26% of the cases combined with maintenance OCS. Since patients with this asthma phenotype experienced few asthma symptoms, a management approach based on symptoms may not be effective. For these patients, a promising new strategy is to target the eosinophil itself. Mepoluzimab, a humanised monoclonal antibody against interleukine 5, reduces the risk of asthma exacerbations in patients with severe therapy resistant, eosinophilic asthma (49-51). Furthermore, patients with characteristics of this severe asthma phenotype might be identified in the early stages of the disease (chapter 4). In contrast to the obesity-related phenotype and upper airway diseases, this severe asthma phenotype cannot be identified by the physician based on clinical characteristics. For this, accessible diagnostic tests are needed in clinical practice that can be used as surrogates for sputum inflammatory cell measurements in asthma as well as in COPD.

Inflammatory phenotypes

The identification of eosinophilic airway inflammation has important clinical and therapeutic implications since it is associated with corticosteroid responsiveness (52-55), frequent asthma exacerbations (54;56) and persistent airflow limitation (57). Furthermore, the most severe asthma phenotype identified by cluster analysis was characterised by persistent eosinophilic airway inflammation, despite corticosteroid treatment (chapter 3). There is evidence that this group of patients benefit novel treatments (51;58;59). Until now, the only way to assess airway inflammation is by direct airway sampling through sputum induction, which is technically challenging and often impractical.

Blood eosinophils are readily available in medical institutions and the risk to patients is minimal. We demonstrated that blood eosinophils are a highly effective surrogate marker for sputum eosinophils in patients with mild to moderate asthma (chapter 6). Blood eosinophil count may help the clinician to better monitor anti-inflammatory treatment in patients with the severe eosinophilic asthma phenotype.

In chapters 7 and 8 we demonstrated that in COPD, the mannitol challenge test and exhaled breath profiling may help the clinician to identify patients with eosinophilic airway inflammation and who will benefit from inhaled steroid therapy (56). This COPD phenotype cannot be distinguished from other patients with COPD on clinical grounds or lung function criteria. The advantage of the mannitol challenge test is a standardized and easy-to-perform challenge test, so this test could be part of daily clinical practice. The advantage of exhaled breath testing is the non-invasiveness for patients. Patients
only need to blow in a balloon device. Therefore, both tests may qualify as a feasible diagnostic test in monitoring anti-inflammatory therapy in COPD.

**Future directions**

It is becoming increasingly clear that asthma is a complex disease made up of number of disease variants with different underlying pathophysiologies. The first part of this thesis described clinical phenotypes of adult-onset asthma. Although the phenotypes we identified are clinical relevant, in terms of presentation, triggers and treatment response they do not necessary relate to or give any insight into the underlying disease processes. An endotype is a subtype of a disease, which is defined by a distinct functional or pathobiological mechanism (60). Until now, such endotypes have not been identified, although some investigators have proposed a few asthma endotypes on the basis on their clinical phenotypes and putative pathophysiology (61). At present, there is no evidence that these proposed endotypes are defined by specific molecular pathways. More advanced approaches such as systems biology relying on multiple disciplines including genomic, proteomic, and metabolomics are therefore needed to understand mechanisms and identify the real asthma endotypes. Before we can start endotyping adult-onset asthma, interesting questions for future studies would be:

- Which pathophysiological mechanisms are involved in the development of adult-onset asthma after a viral or bacterial respiratory infection?
- What is the role of obesity in the development of severe adult-onset asthma?
- Do chronic rhinosinusitis and nasal polyps at asthma onset predict a more severe course of the disease with worse long-term prognosis?
- Are the adult-onset asthma phenotypes identified at onset of the disease stable over time?
- What is the natural history of adult-onset asthma?

To that end, large-prospective follow-up studies of patients with adult-onset asthma are needed, especially in those patients who are in an early stage of their disease.

Adult-onset airway diseases (asthma and COPD) are both characterized by airway inflammation. In the second part of this thesis, we have attempted to validate and identify diagnostic markers of disease activity and therapeutic response. Unlike newly developed drugs and therapies, there is no formal standard for acceptance of new diagnostic tests into routine care (62). Therefore effort should be put into careful validation of diagnostic tests following current guidelines (63). We identified blood eosinophils as the best marker to predict the presence of eosinophilic airway inflammation in patients with mild to severe asthma. However, further studies are needed to investigate whether
blood eosinophils can be used as a unique marker of inflammation to predict and 
monitor response to asthma treatment. We also identified a correlation between airway 
hyperresponsiveness to mannitol and airway inflammation in patients with COPD, but 
this connection needs further investigation with a randomized controlled study to test 
whether the mannitol challenge test can adequately predict the responsiveness to 
inhaled corticosteroids treatment (55;56;64). Although we found eNose breathprints 
to show promising results for estimating inflammatory activity in the airways of pa-
tients with mild to moderate COPD, many issues must be resolved before these eNose 
breathprints can be applied in daily clinical practice. Currently, the biggest limitation 
to progress in the field of exhaled breath diagnostics is the lack of sensors that can be 
produced identically in large quantities. Furthermore, guideless on the standardization 
of breath collection, sampling and interpretation of test results need to be formulated.

Final remark
Taken together, this thesis provides novel insights in the underlying pathobiology of 
different phenotypes of adult-onset asthma and COPD, and provides evidence that sur-
rogate markers can be used to identify the type of airway inflammation in patients with 
these conditions. This thesis thereby contributes to a better disease understanding and 
provides clinicians with directions for personalized management in asthma and COPD.
References


Summary and general discussion


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