Novel views on endotyping asthma, its remission, and COPD

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Chapter 12

Summary

In this thesis, we provide an overview of the pathophysiology of asthma remission and the asthma-obesity syndrome, and present novel techniques for endotyping asthma, pheno- and endotyping remission of asthma, endotyping COPD, and analyzing airway remodeling. The main results of the chapters are summarized below.

In **chapter 2**, an overview of what is known about clinical and complete asthma remission is presented. Despite the fact that the definition of asthma remission is a complex issue and varies greatly between studies, some clinical features have been reproducibly observed to be associated with asthma remission: younger age of onset, mild asthma at onset, male sex, higher baseline lung function, less bronchial hyperresponsiveness at baseline, lower blood eosinophils and IgE at baseline, lower number of allergens with a positive skin prick test, absence of allergic environmental factors (e.g. pets in household), absence of specific comorbidities (i.e. nasal polyps, eczema, atopy or rhinitis), lack of/absence of a history of pneumonia, a negative family history of asthma and atopy, and cessation of smoking. In subjects with asthma remission, the levels of inflammatory markers were lower, especially in complete asthma remission. However, Broekema *et al.* stated that both clinical and complete asthma remission subjects still had a degree of airway remodeling. The most important consideration of this chapter is that the pathophysiological state of complete asthma remission yields more scientific interest than that of clinical remission, since this strict phenotype has higher potential to elucidate biological pathways of the molecular and cellular mechanisms that hold the potential for future therapeutic intervention aimed a inducing asthma remission in patients with persistent asthma.

In **chapter 3** we further elaborate on remission of asthma. By following a cohort of asthmatic children, we were able to calculate the prevalence of clinical and complete asthma remission at age 25 and 49. We showed that this long-time, persistent remission of asthma does occur, but the prevalence was only 11% of the individuals who had childhood-onset asthma. Moreover, persistent asthma remission was more likely in subjects with complete compared to clinical asthma remission at age 25.

In **chapter 4,** we analyzed the differences in amounts of exhaled particles in asthmatics, subjects with clinical- or complete remission, and healthy controls from the *exploring Asthma ReMission by Single-cell TRansciptiONal sequencinG* (ARMSTRONG) study, using the

Particles of Exhaled Air (PExA) device. We hypothesized that the number of exhaled particles is reduced in asthmatics and subjects with clinical asthma remission due to the ongoing small airways dysfunction. Indeed, the mass of exhaled particles in asthmatics proved to be significantly lower compared to healthy controls (P=0.009) and subjects with complete asthma remission (P=0.028). Additionally, subjects with clinical remission had significantly lower exhaled particle mass than healthy individuals (P=0.018). We also correlated the mass of PExA particles in nanogram per liter (ng/L) in exhaled air by the participants with both small- and large airways disease parameters. PExA mass was significantly associated with a variety of airway parameters, such as small airways-associated lung function parameters (i.e. $FEF_{25-75\%}$), lung function reversibility to salbutamol, bronchial hyperresponsiveness (i.e. PC_{20} methacholine and adeno-5-monophosphate slope), small airways resistance (i.e. impulse oscillometry R_5-R_{20} resistance and AX reactance), hyperinflation (i.e. body plethysmography RV % predicted and RV/TLC ratio), and conductive airway nitrogen clearance (i.e. multiple breath nitrogen wash-out S_{cond}). Stepwise multiple regression analysis showed that the small airways disease-associated parameter S_{cond} was the only independent factor associated with PExA mass. These results suggest that better large and small airways function is linked to higher PExA mass.

A comparison of small airways function and inflammatory cell count in healthy subjects, subjects in clinical- and complete asthma remission, and asthmatics is presented in **Chapter 5**. We found evidence that individuals with clinical asthma remission still have a degree of small airways disease and inflammatory markers, while complete asthma remission subjects are clinically similar to healthy controls. Again, we propose that in order to elucidate the pathogenesis of true asthma remission, studying complete asthma remission is the most promising approach. To explore this in detail, further work is needed to establish histological differences such as other remodeling and bronchial inflammatory parameters, and provide a cellular landscape of human lung tissue of clinical and complete asthma remission subjects at the single-cell level.

In **chapter 6,** we evaluate a recently published model predicting more than 80% of the asthma remission cases in young adulthood, by testing its performance in our Dutch asthma remission cohorts. The model from the *Childhood Asthma Management Program* (CAMP) study for predicting asthma remission later in life consisted of the

following characteristics: asthmatic children having no pulmonary obstruction as reflected by FEV_1/FVC% ≥85%, no severe bronchial hyperresponsiveness defined as PC_{20} methacholine ≥1mg/ml, and low blood eosinophils (<500 cells/μL). In concordance with the findings of the CAMP authors, children in our cohorts had a significantly higher FEV_1, FEV_1/IVC%, and PC_{20} threshold and significantly lower blood eosinophils in the asthma remission group compared with the persistent asthma group. Even though the cohorts had similar characteristics, the clinical asthma remission rates of our subjects and CAMP cohort were 10.0% versus 26.1%, while the model predicted future development of asthma remission in 40.0% versus 82.6% respectively. The FEV_1/FVC% measured in the CAMP trial was overall higher than in ours, probably contributing to the discordance. We showed that the proposed model has predictive value in the CAMP cohort, but would be insufficient in our cohort. Therefore, we propose that biomarkers that are associated with pathways inducing complete asthma remission should be incorporated in such models to achieve a better predictive power.

In **Chapter 7** we review the relation between asthma and obesity. There is evidence that obesity increases the risk for asthma development and enhances the difficulty of gaining control of asthma symptoms. Even though the mechanisms underlying the link between asthma and obesity are not well elucidated, we point out several pheno- and endotypical features: first, obesity reduces the expiratory reserve volume (ERV) and slightly increases bronchial hyperresponsiveness. Second, asthma with obesity is associated with a higher neutrophil count than asthma in non-obese patients. Third, obesity is linked to various comorbidities that could aggravate asthma symptoms and which could lead to their persistence. And finally, corticosteroid therapy is less effective in obese asthmatics, which would consequently contribute to the worsening of asthma control. To conclude, this review addressed the relevance and complexity of the asthma-obesity phenotype combination, including current therapeutic strategies to treat patients affected by this syndrome. Future studies should focus on exploring the pathogenic relation of asthma and obesity, in order to find novel treatment options.

In **Chapter 8** we present our exploration of the cellular landscape of the healthy lung, and of the asthmatic airways by single-cell RNA-sequencing (scRNA-Seq). We describe the transcriptomic profile of lung-resident structural and inflammatory cells and their interaction in healthy lung tissues from several tissue sources: nasal brush, bronchial

biopsy and brush, lung resection specimens, and transplant donor lungs. In addition, we profile airway wall biopsies from six patients with asthma and six healthy controls from the ARMSTRONG-study. We identified a tissue-resident $CD4^+$ T-cell subset with transcriptomic features of both (circulating) central memory T cells and tissue-resident memory T cells. Second, by comparing the cellular composition of the airway wall between asthmatics and healthy volunteers, we identify novel epithelial cell states, asthma-associated changes in the composition of the airway wall and predict large changes in cell-cell communication in the airway wall in asthma. Summarized, **chapter 8** generates novel insights into the epithelial cell changes and transcriptomic-defined communication patterns between immune- and structural cells of the airways that underlie asthmatic airway inflammation and remodeling.

In **Chapter 9** we focus on detecting and quantifying airway remodeling using OCT imaging. We paired histological and immunohistochemical stainings on sections of 36 tissue samples from the airways with ex-vivo obtained OCT images of the exact same physical location, derived from five lobectomy specimens. The histological sections were stained with Picosirius Red (total collagen), Masson's Trichrome (blue color: total collagen and bone), anti-collagen A1 antibody (collagen type 1 A1), Verhoeff's (elastin) and anti-fibronectin antibody (fibronectin) to quantify the ECM component area and intensity in the airway wall. All of the ECM component areas were positively correlated with the paired OCT areas, while total collagen, Masson's Trichrome blue (marking e.g. total collagen), and collagen A1 mean intensity correlated with OCT intensity as well.

Chapter 10 assessed the potential of serum periostin, a promising biomarker in the asthma field, to predict disease severity and inhaled corticosteroid (ICS) responsiveness in patients with COPD. Baseline serum periostin was associated with cross-sectional and longitudinal features, including levels of inflammatory cells in three compartments (i.e. blood, sputum and bronchial biopsies) and properties of bronchial extracellular matrix (ECM) components in COPD patients from the *Groningen and Leiden Universities Corticosteroids in Obstructive Lung Disease* (GLUCOLD) study. Smoking and former-smoking COPD patients had significantly higher serum periostin values compared to healthy smoking controls. Nevertheless, the periostin levels did not predict ICS responsiveness in COPD: there was no correlation with improvement in lung function (FEV_1% predicted), decrease in hyperinflation (RV), or COPD Control Questionnaire score after either 6 or

30 months of ICS therapy. Finally, no correlation was found between serum periostin and change in ECM component area or density after 30 months of ICS or placebo treated COPD patients. Overall, we show that measuring serum periostin has little clinical relevance in COPD.

In **Chapter 11**, we demonstrated the use of transcriptional profile clustering of bronchial biopsies to predict long-term clinical outcome of COPD patients, which was originally introduced in asthma research in 2014. COPD- and asthma phenotypes have been frequently associated with specific gene expression signatures. The other way around, associating transcriptional clusters from relevant tissue samples with asthma phenotypes, has been studied less extensively. By using a COPD-associated gene signature from an American bronchial brush cohort, we performed unsupervised clustering of our own COPD cohort. The number of bronchial RNA clusters was determined by an algorithm that calculated the least transcriptional consensus between these clusters. Consequently, the GLUCOLD-enrolled COPD patients were divided into two clusters: COPD Associated Gene Expression #1 (CAGE1) and CAGE2 subjects. The latter group had significantly higher lymphocyte percentage in sputum and T-cells in bronchial biopsies, compared to CAGE1 subjects. But most importantly, CAGE2 subjects had more rapid lung function decline between 0.5 and 7.5 years compared to the CAGE1 cluster. We concluded that this gene expression signature enables us to identify a COPD phenotype with a more rapid lung function decline.