Clinical and molecular phenotyping of asthma and COPD
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 10

Summary
In this thesis, we investigated the heterogeneity of asthma and COPD by performing studies in which we focused on clinical and molecular phenotyping. First, we focused on small airways dysfunction by investigating the presence and extent of small airways dysfunction in several populations, i.e. subjects with asymptomatic airway hyperresponsiveness, asthma patients, healthy non-smokers and ‘healthy’ smokers. Furthermore, we compared efficacy of inhaled corticosteroids (ICS) preferentially targeting the small airways, i.e. extrafine ICS, with non-extrafine ICS in smokers and ex-smokers with asthma and studied which clinical factors can predict a beneficial response. Second, we aimed to optimize provocation testing with adenosine, by comparing the performance of a dry powder adenosine formulation with the standard nebulized adenosine 5’ monophosphate formulation. Third, we explored molecular mechanisms underlying COPD, response to ICS treatment in asthma and remission of asthma, by studying nasal- and bronchial gene expression profiles. The main results of these studies are summarized below.

**CLINICAL PHENOTYPING**

In chapter 2, we present results of a cross-sectional study investigating the degree of small airways dysfunction (SAD) in subjects with proven airway hyperresponsiveness (AHR) but without any respiratory symptoms (asymptomatic AHR), compared to healthy controls and asthma patients. In order to assess SAD, we performed spirometry and impulse oscillometry at baseline and during a methacholine provocation test. We observed that, at baseline, subjects with asymptomatic AHR and healthy controls had less SAD as defined by lower small airway resistance ($R_{5-20}$) and higher small airway reactance ($X_{5}$) compared to asthma patients. During a methacholine provocation test, subjects with asymptomatic AHR and asthma patients both had a more severe increase in SAD compared to healthy controls. Furthermore, asthma patients had more SAD at a 20% fall of the FEV$\textsubscript{1}$ during AHR testing than subjects with asymptomatic AHR. These findings together suggest that subjects with asymptomatic AHR do not experience respiratory symptoms because they have less small airways dysfunction than asthma patients, both at baseline and after a methacholine provocation test.

Chapter 3 describes the findings of a study performed in smokers and non-smokers without respiratory symptoms, both with a normal pulmonary function. We investigated the effects of age and smoking in relation to the extent of small airways dysfunction, emphysema and parenchymal disease as detected by pulmonary CT-scans, using a novel technique called ‘parametric response mapping’. This technique allows voxel-by-voxel comparison of in- and expiratory CT-scans in order to distinguish small airways dysfunction ($\text{PRM}^{\text{SAD}}$), emphysema ($\text{PRM}^{\text{Emph}}$) and parenchymal disease ($\text{PRM}^{\text{PD}}$) based
on changes in lung density measures. We observed that \( \text{PRM}^{\text{SAD}}, \text{PRM}^{\text{Emph}} \) and \( \text{PRM}^{\text{PD}} \) significantly increase with age, and that smokers have more \( \text{PRM}^{\text{PD}} \) compared to non-smokers. Of interest, \( \text{PRM}^{\text{SAD}} \) was, independently of \( \text{PRM}^{\text{Emph}} \), associated with lower \( \text{FEV}_1/\text{FVC} \), lower \( \text{FEF}_{25-75}/\text{FVC} \) and higher \( \text{RV}/\text{TLC}\% \) predicted. We therefore speculate that early changes in pulmonary function, that may precede pulmonary pathology, are better reflected by the degree of small airways dysfunction than by the degree of emphysema on CT-scans.

Smokers with asthma have been reported to exhibit more pronounced SAD and to respond less well to treatment with inhaled corticosteroids (ICS). In Chapter 4 we report results of the OLiVIA-study: a randomized, cross-over, open-label study in which we compared effects of two-week treatment with extrafine beclomethasone (Qvar), to that of non-extrafine beclomethasone (Clenil) and fluticasone (Flixotide) in smokers and ex-smokers with asthma. Twenty-two smokers and 21 ex-smokers were included, of which 38 completed the study. We showed no significant difference in our primary outcome parameter, change in AHR, among the three treatments. Thus, we were not able to demonstrate that treatment with extrafine ICS is more effective than treatment with non-extrafine ICS in smokers and ex-smokers with asthma. This might be due to the fact that we included all smokers and ex-smokers and not exclusively those with SAD. Therefore, in a post-hoc analysis of the OLiVIA-study, presented in Chapter 5, we analyzed whether subjects with proven SAD at enrollment to the study, defined by abnormal \( R_5-R_{20}, \text{FEF}_{25-75}, \text{RV}/\text{TLC}, S_{\text{acin}} \) or \( S_{\text{cond}} \), have a better clinical response after treatment with extrafine ICS (Qvar) compared to non-extrafine ICS (Clenil and Flixotide). The results showed that subjects with SAD based on \( R_5-R_{20}, \text{FEF}_{25-75}, \text{RV}/\text{TLC} \) and \( S_{\text{acin}} \) have a similar improvement in AHR after treatment with extrafine and non-extrafine ICS. Subjects with SAD based on \( S_{\text{cond}} \) showed a higher improvement of AHR in response to non-extrafine ICS (Clenil). In our multivariate regression model, predictive factors for a better improvement of AHR were lower blood neutrophils (Qvar, Clenil and Flixotide), higher blood eosinophils (Clenil and Flixotide), younger age (Clenil) and ex-smoking status (Clenil and Flixotide). These findings provide suggestive evidence for the use of blood neutrophils and eosinophils as biomarker to guide ICS therapy in smokers and ex-smokers with asthma.

Bronchial provocation testing with inhaled adenosine, an indirect stimulus to induce airway obstruction, has been reported as a candidate biomarker for eosinophilic airway inflammation in asthma. In Chapter 6, we report results of a study among smokers and ex-smokers with asthma, in which we compared the performance of dry powder adenosine, a newly developed adenosine formulation, with the usually applied nebulized adenosine 5’ monophosphate (AMP) for bronchial provocation testing. The drawback of nebulized AMP is that not all patients reach a 20% fall in \( \text{FEV}_1 \), the definition...
of AHR, after administering the highest possible concentration. We hypothesized that dry powder adenosine, which can be administered in a higher dose, would be a more sensitive test to demonstrate AHR than nebulized AMP. We showed that 40 out of 60 participants had AHR in response to both AMP and adenosine. Ten subjects showed AHR in response to adenosine, but not to AMP. Two subjects showed AHR in response to AMP, but not to adenosine. Seven subjects did not reach a 20% fall in FEV\textsubscript{1} on either adenosine or AMP. The correlation between the provocative concentration (AMP) and dose (adenosine) inducing a 20% fall in FEV\textsubscript{1} (PC\textsubscript{20} and PD\textsubscript{20}) was high (Rho=0.799, p<0.001). Factors that significantly and independently predicted PC\textsubscript{20} and PD\textsubscript{20} were age (AMP and adenosine) and FEV\textsubscript{1} % predicted (adenosine). These results suggest that provocation testing with dry powder adenosine is more sensitive than nebulized AMP to detect AHR in asthma.

**MOLECULAR PHENOTYPING**

In the second part of this thesis, we aimed to explore molecular mechanisms involved in asthma and COPD by studying nasal gene expression in COPD, the influence of ICS on nasal gene expression in asthma and finally, changes in bronchial gene expression associated with remission of asthma.

In chapter 7, we report findings of a genome-wide gene expression analysis in which we compared nasal gene expression of 31 COPD patients with 22 controls, all current smokers. We identified 135 genes (21 up- and 114 downregulated) that were differentially expressed between COPD and controls. The gene expression profile identified in nasal epithelium, significantly overlapped with that in the bronchial epithelium in 2 independent cohorts of COPD patients and controls. Pathways associated with COPD both in nasal and bronchial epithelium included for upregulated genes: O-glycan biosynthesis and glycosphingolipid biosynthesis. For downregulated genes associated pathways were as follows: RNA degradation, DNA replication, propanoate metabolism and tight junction. A number of genes identified in this study have been previously found to be associated with features of COPD. This study demonstrates that gene expression changes related to COPD can be detected in the nasal epithelium, and that these changes reflect the changes observed in bronchial epithelium. Our study thus serves as a starting point for future studies investigating the use of nasal gene expression as a non-invasive biomarker in COPD.

In chapter 8, we studied the effects of ICS on nasal epithelial gene expression in asthma. To this end, we performed a meta-analysis of two asthma cohorts treated with ICS, the OLIVIA-study (n=39, 2-week HFA-beclomethasone, 200µg b.i.d.) and the NZRHS-study (n=28, 12-week dry powder budesonide, 400µg b.i.d.). ICS treatment changed expression
of 135 genes in the nasal epithelium significantly (76 up- and 59 downregulated). Of interest, in a subset of OLIVIA participants in which ICS were withdrawn, we found that downregulated genes after ICS treatment overlapped with upregulated genes after ICS withdrawal. ICS-induced changes in nasal gene expression were compared to ICS-induced changes in bronchial gene expression in two independent cohorts (cohort 1: n=12, 8-week budesonide 180 µg b.i.d and cohort 2: n=20, 1-week fluticasone 500µg b.i.d.), as well as in corticosteroid-treated air-liquid-interface cultures of human bronchial epithelial cells. In both analyses, we found that the ICS-induced nasal gene expression profile considerably overlapped with the corticosteroid-induced bronchial gene expression profile. These results implicate nasal epithelium as a suitable site to study the effects of ICS in asthma and we suggest further investigations to assess its potential utility in guiding ICS treatment.

Clinical remission of asthma reflects a condition in which asthma patients have outgrown their disease, i.e. asthma symptoms are absent and no asthma medication is used. In addition, complete remission of asthma further narrows this definition by requiring a normal pulmonary function, i.e. no AHR and an FEV₁ >80% predicted. In chapter 9, we explored molecular mechanisms underlying complete remission of asthma by investigating microRNA-, protein-coding RNA-, and long non-coding RNA (lncRNA) expression profiles of bronchial biopsies in subjects with current asthma, subjects in complete remission of asthma and healthy controls. We found 10 microRNAs that differentiate subjects in complete remission from subjects with asthma, while 77 microRNAs differentiate subjects in complete remission from healthy controls. To understand interaction of these microRNAs and that of other gene types, we constructed a Bayesian network to predict complete remission of asthma, which consisted of 24 microRNAs, 35 lncRNAs and 20 protein-coding RNAs. Interestingly, only microRNAs and lncRNAs, but not protein-coding RNAs, were directly connected with complete remission. Additionally, permutation analysis (in which the complete remission phenotype was swapped among individuals), confirmed that this network contained a significantly lower proportion of protein-coding RNAs than what would be expected by chance (p<0.01). Our finding emphasize that non-coding RNAs are likely contributing to remission of asthma since: 1) we find differentially expressed microRNAs between complete remission and asthma and 2) an integrative Bayesian network predicting complete remission of asthma shows that microRNAs and lncRNAs are abundantly present. These observations imply that future research should focus on unraveling the role of non-coding RNAs in complete remission of asthma.