Novel views on endotyping asthma, its remission, and COPD

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Chapter 13

Discussion and
future perspectives

The discussion and future perspectives of this thesis are subdivided in five themes: asthma remission, small airways dysfunction, the asthma-obesity relationship, airway wall remodeling, and endotyping of COPD. Suggestions for future studies are delineated at the end of the paragraphs. Finally, the discussion and future perspectives are summarized in the conclusion.

13.1 Asthma remission

This thesis summarizes what is known about clinical and complete asthma remission. Apart from additional phenotyping of these phenomena, various chapters emphasize the need for further endotyping. As an example, in **chapter 6** it is shown that the *Childhood Asthma Management Program* (CAMP) prediction model lacks biomarkers that are associated with cellular or molecular pathways linked to the induction of asthma remission, which might contribute to the low predictive power to determine asthma remission in the Dutch cohorts.

To predict and determine persistent remission of asthma, further endotyping is needed. Endotyping of asthma remission can be achieved via multiple strategies: defining endotypes based on structural features, or on molecular features that are collectively referred to as –omics, such as transcriptomics, metabolomics, and proteomics [1,2]. Future approaches to characterize complete asthma remission through various -omic modalities (genomics and microbiomics are highlighted in **chapter 2**), will be discussed below.

Structural endotyping

Exploration of the disease activity by visualizing structure and location of airways affected by asthma can be performed by quantifying high-resolution computed tomography (HR-CT) parameters [3,4], by assessing airway remodeling using optical coherence tomography (OCT) of the airway walls [5], or by analyzing the architecture of airway wall histology sections [6]. Current HR-CT analyses are able to quantify the degree of airtrapping, airway thickness and lumen diameter in asthma patients [7]. One increasingly used method for quantifying HR-CT images is parametric response mapping (PRM) [3,4], which we also tested in **chapter 4 & 5** (PRM-defined small airways disease is discussed in *paragraph 13.2*). In the *exploring Asthma ReMission by Single-cell TRansciptiONal sequencinG* (ARMSTRONG) study, we observed a significantly lower PRM-defined interstitial lung density in subjects with complete asthma remission than in persistent asthmatics. Additionally, these HR-CT analyses revealed a ventilation-shift towards the upper lobe in asthmatics, which was not seen in individuals in complete asthma remission and healthy individuals. Ultimately, automated HR-CT programs could be easily incorporated to quantify asthma activity, absence, or treatment response. The evaluation of OCT imaging linked to airway remodeling is discussed in *paragraph 13.4*.

Transcriptomic endotyping

Gene expression profiling [8–11] allows to create gene signatures that we can subsequently link to a phenotype, as demonstrated in **chapter 11**. Various studies have aimed to define endotypes of asthma by transcriptomics in blood [12], sputum [8,11], bronchial epithelium [10], and bronchial biopsies [13]. However, studies focusing on the genetic background of complete asthma remission are scarce [14,15], and no study to date has reported transcriptomic profiling of lung tissue samples in complete remission patients. Unfortunately, complete asthma remission is a unique phenotype representing only a small proportion of all asthmatics [16,17], thereby limiting the possibilities for recruiting the numbers of participants needed for large transcriptomic studies required to realize this kind of endotyping.

In **chapter 8**, we demonstrate a novel technique in transcriptomic endotyping, so-called single-cell RNA sequencing (scRNA-seq) [18,19]. scRNA-Seq has a major advantage compared to previously described genome wide transcriptomic profiling on complex tissue samples such as airway wall biopsies: it enables identification of transcriptionally divergent cell subsets within a certain cell type. We index-sorted blood samples and bronchial biopsies of persistent asthmatics and healthy controls to obtain a 200 to 300 $CD4^+$ T-cells per donor, and performed single-cell transcriptomic profiling by SmartSeq2 on these cells [20]. The second method, the *10X Genomics Chromium* microfluidics platform, can be used to process larger numbers of cells, allowing us to analyze 2,000 to 5,000 single cells from a suspension of all cells contained in the bronchial biopsy [18]. In contrast to the FACS-sorted $CD4^+$ T cells analyzed by SmartSeq2 scRNA-Seq, the cell types in 10X Genomics scRNA-Seq datasets are primarily categorized by their transcriptomic profile. With both SmartSeq2 and 10X, we had the ability to define cell

types through unsupervised clustering based on transcriptome similarity [21,22]. In the near future, we will implement these and novel scRNA-seq methods on blood samples, bronchial and nasal brushes, and bronchial biopsies of subjects with clinical or complete asthma remission in an attempt to characterize the biology of the airway wall structural cells in asthma remission patients.

Several issues need to be resolved in order to make scRNA-Seq more accessible for clinical practice. First, due to the high dimensionality of transcriptional data of so many cells, it is very difficult to conveniently visualize molecularly and clinically relevant cell types [21]. Future research should focus on lifting "the curse of dimensionality"[23], by introducing new analysis methods that integrate biologically linked –omics. Second, scRNA-Seq needs to become cheaper than the current €3,600 to €8,900 per sample [24]. By becoming less expensive, more institutes will invest in scRNA-Seq projects, and more samples can be processed to increase statistical power and scientific impact. And last, a challenge for scRNA-Seq studies is generating a high-quality single-cell suspension, which needs to be from fresh tissue free from both RNA degradation and transcriptional stress responses [25]. A promising technique to tackle these technical demands, is to analyze the samples via single-nucleus instead of single-cell RNA-sequencing. For future studies, we could perform single-nucleus RNA-sequencing on our extensively characterized ARMSTRONG dataset and extend the subjects numbers by adding frozen biopsy samples of older asthma datasets as well. To realize the full potential of these novel technologies also in a clinical setting, the international *Human Cell Atlas* consortium aims to establish optimized workflows and analysis pipelines, share best practices and establish a reference dataset describing all cell types, states and their interactions in all organs of the healthy human body [26].

Epigenomic endotyping

Epigenetic factors (e.g. microRNAs and DNA methylation) are expected to have a significant impact on the cellular and molecular interactions driving asthma [27]. Epigenetic signatures from tissue samples integrate information from genetic makeup, cell type composition and programming due to environmental factors accumulated during the life course, thereby holding great promises as potential biomarkers for disease progression or therapy response. In concordance with this, epigenetic factors may very well be relevant for understanding complete asthma remission. Boudewijn *et al.* searched for underlying mechanisms of asthma remission by investigating bronchial microRNA expression [28], and found that complete asthma remission had a distinct bronchial microRNA expression profile compared to persistent asthmatics and healthy participants. They found differential expression of microRNAs such as *mir-320d*, which is associated with anti-inflammatory effects. By understanding how microRNAs like *mir-320d* interact with protein-coding RNAs that are responsible for these anti-inflammatory effects, it would bring us one step closer to revealing the pathways driving the induction of asthma remission.

Proteomic endotyping

Analysis of the proteins which mediate the pathogenesis of asthma, can be applied on various samples, such as sputum [29,30], bronchoalveolar lavage fluid [31–33], bronchial biopsies [34], but also particles in exhaled air [35]. Analyses in exhaled air have shown that albumin, and surfactant proteins can be collected by the (Particles of Exhaled Air) PExA device and analyzed using ELISA [35–37]. Proteins like surfactant, an immunomodulator involved in innate immune recognition and regulation of surface tension [33], could be produced insufficiently or in an altered form thereby affecting asthma severity [33]. For instance, Soares *et al.* demonstrated that topological data analysis of albumin and surfactant was able to non-invasively identify a small airways disease phenotype in asthma [35]. One important limitation that needs to be addressed when implementing this approach to endotype complete asthma remission is the difference between the number of exhaled particles in diseased and healthy, which is thought to be elicited by blockage of the smaller airways in the asthmatics (see *paragraph 13.2*). Future studies should focus on the discovery of exhaled, aberrant proteins linked to asthma, absence of which could be associated with complete asthma remission.

Metabolomic endotyping

Analyzing biochemical molecules derived from metabolic processes in a disease [1,38], is a strategic approach called metabolic endotyping. Profiling of exhaled biochemical molecules is a concept that is applied in the electronic nose, called "breathprints" [39,40]. Since the electronic nose is able to differentiate healthy controls from asthmatics [41], it is plausible that the exhaled breathprints of complete asthma remission subjects are different from persistent asthmatics and clinical asthma remission subjects as well. Future studies should explore this approach, since it is non-invasive, cheap, and

has potential to work as an early biomarker, for instance of those subjects that could discontinue medication.

13.2 Small airways dysfunction

Abnormalities in small airways, defined as the airways with an internal diameter of less than 2 mm, contribute to the clinical expression of asthma [42]: their dysfunction is associated with reduced asthma control, higher number of exacerbations, more severe bronchial hyperresponsiveness, and exercise-induced asthma [42,43]. Two research questions are addressed in this thesis with regard to small airways: one, whether abnormalities in the mass of exhaled particles reflects small airways function. Two, whether there is persistent small airway dysfunction in clinical- and complete asthma remission subjects.

Various tests are available to assess small airways disease [44,45], and we propose measuring exhaled particles with the PExA device as a novel technique. In **chapter 4**, we correlated the amount of PExA particles in nanogram per liter, exhaled by the participants with both small- and large airways disease parameters. Apart from Soares *et al.*, who found a positive correlation between the PExA mass per exhalation and R_5-R_{20} airway resistance (R=0.257, P<0.05) [35], no other studies investigated associations between PExA mass and small airways dysfunction. We find… All in all, our data suggest that the total exhaled particle mass could potentially be used as a tool to assess small airways dysfunction. However, there are some limitations to this technique. First, the PExA device does not distinguish the origin of exhaled particles, and does not reveal the location of airway blockage that causes the observed decrease in PExA mass. Figure 2 illustrates the hypothesis of the origin of PExA particles [46]. Basically, the concept is that the exhaled particles are generated whenever the airway re-opens an area of aerosol-producing airway tract. In line herewith, it is plausible that the number of small, but also that of large airways is negatively correlated with the exhaled particle mass, since both would result in a smaller lining tract area. Even though age, length and gender were not associated with exhaled PExA mass, we cannot rule out that obstruction of the larger airway areas has more effect on the measured PExA particles that of the smaller airways. Other factors could have effect on the exhaled particle mass, such as season

[47], airway wall remodeling (including airway wall elasticity), comorbidity, and many other factors.

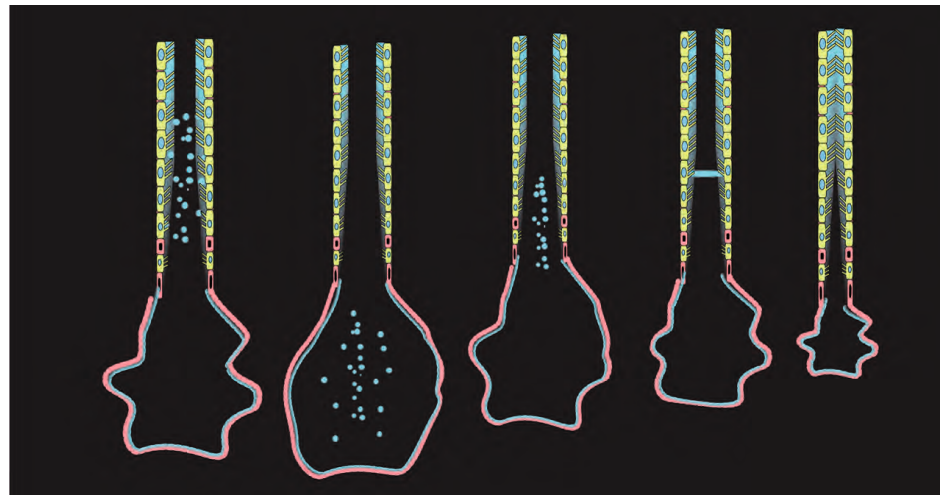

Figure 2: Schematic illustration of the airway reopening concept. When airways close, opposing airway walls get in contact creating a plug of respiratory tract lining fluid. As the airway walls distend during inspiration, forming a meniscus that finally breaks and generate particles. Figure of Bake B, et al. Respir Res. 2019 Jan 11;20(1):8 is distributed under the terms of the Creative Commons Attribution 4.0 International License.

Second, differences in breathing manoeuvres may affect the mass of exhaled particles. Morawski *et al.* studied exhaled particle concentrations and size distributions in healthy volunteers, while performing different types of breathing manoeuvres, such as whispering, voiced expiration, and coughing [48]. In agreement with a previous study [49], their data confirmed that air velocity and vibration of vocal cords affect the mass of exhaled particles. Hence, the individual airflow limitation of each patient or the instructions provided to the patient during measurement could alter the mass of PExA particles.

Even when taking these limitations into account, it is likely that measuring the exhaled PExA mass relates to both large and small airways function. As already highlighted in *paragraph 13.1*, future PExA studies should focus on proteomics and – if possible – transcriptomics in exhaled air.

Our main findings in **chapter 5** were that subjects with complete asthma remission had significantly lower inflammatory markers, and better large and small airways function. Moreover, complete asthma remission and healthy controls were indistinguishable on these parameters. In our opinion, these findings demonstrate that complete asthma remission is the closest to the healthy condition, meaning that in a sense these patients were spontaneously cured, while clinical asthma remission is similar to the definition "sub-clinical asthma". Steinbacher *et al.* investigated the multiple breath nitrogen washout (MBNW) parameters, that are thought to reflect small airway function, in children who were into asthma remission for more than one year, and either normoresponsive or hyperresponsive to cold air challenge [50]. In the hyperresponsive children, there was a significant increase and subsequent decrease in the MBNW parameters after cold air challenge, and salbutamol inhalation, respectively. This was not the case in normoresponsive children. The MBNW response illustrates the presence of small airways dysfunction in subjects with asthma remission who still appear to react on exogenous triggers. This is in concordance to our findings, since clinical asthma remission subjects, practically all having a positive provocation test (i.e. exogenous trigger), had higher MBNW S_{cond} and S_{cond} and worse small airways function.

There is one important limitation in the assessment of small airways dysfunction in clinical and complete asthma remission: it is challenging to evaluate the history of asthma severity, let alone the history of small airways disease. All individuals with asthma remission described in **chapter 4 & 5**, had a diagnosis of asthma, confirmed by documented spirometry and provocation tests before the age of 20. Again, remission subjects had early-onset asthma, and were 40 – 65 year at enrollment for the ARMSTRONG study. It is a delicate process to find the diagnoses in old charts, because these documents are frequently stored remotely or even destroyed due to rules and regulations. The limited availability of medical tests and statuses hindered the estimation of asthma severity at onset. Additionally, tests to assess small airways function were not performed at the time of diagnosis of these asthma patients. Therefore, we can only assume that individuals that are into complete asthma remission nowadays had a significant degree of small airways disease at asthma-onset.

As already put forward, there is no gold standard to determine small airway dysfunction. Future studies will need to introduce novel or improve current methods to do so, e.g.

by visualizing airtrapping, calibrating localized airway resistance, or measuring small airways-specific metabolites and gases. An example of an upcoming technique is the quantification of functional small airways disease reflected by HR-CT PRM (PRM ^{fsad}) [4] (figure 3).

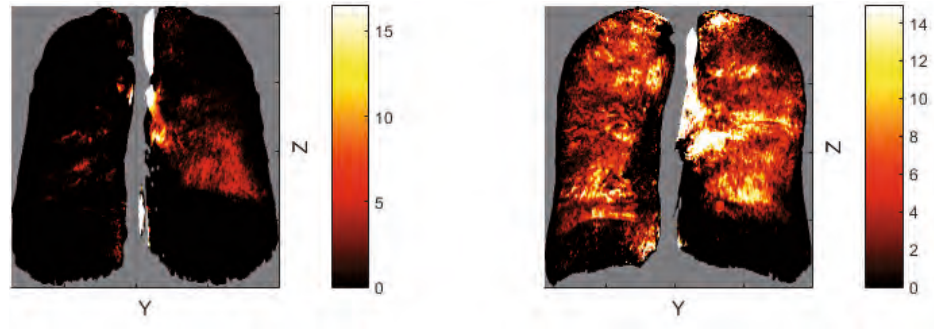

Figure 3: examples of parametric response mapping functional small airways disease (PRM ^{fsad}) quantified by inspiration-expiration HR-CT. Left pair of lungs are from a subject with complete asthma remission, right pair from an asthmatic. Degree of color intensity resembles percentage of PRM ^{fsad}.

Unlike our study, a larger study found a significantly higher PRM ^{fsad} in asthmatics than healthy controls [51]. Within asthma, a higher PRM ^{fsad} was associated with an eosinophil high (>300 cells per µl) group, while a lower PRM ^{fsad} was associated with low eosinophil counts (<150 cells) [52]. In our own cohort of healthy never- and former-smokers, more PRM ^{fsad} was found to be associated with higher RV/TLC- and lower $FEF_{25-75\%}$/FVC ratios [53], indicating early pulmonary alterations in lungs without subjects having symptoms. Applying the PRM algorithms to HR-CTs should be easily feasible.

In contrast to HR-CT, thoracic magnetic resonance imaging (MRI) has historically played a minor part in respiratory medicine, because of inferior spatial and temporal resolution of lung tissue compared to HR-CT [54,55]. HR-CT excels in determining lung morphology but relies on indirect signs in images of obstructive pulmonary disease [55]. Therefore, functional MRI combining with inhaled hyperpolarized noble gases such as helium-3 and xenon-129 has been put forward as a potential method to integrate lung morphology with small airways function and gas transfer within the lungs [54–57]. It would be of interest to associate hyperpolarized MRI parameters with asthma disease severity levels, and correlate it with small airways parameters in healthy and asthmatics.

13.3 Asthma-obesity relationship

The asthma-obesity relationship is a poorly understood, and the phenotype is difficult-to-treat. A major issue in obese asthmatics is the over-use of corticosteroids [58]. A distinct endotypical process presumably determines this phenotype, making it less responsive to ICS. Patients are therefore more likely to receiveimmunosuppressants. To fight the combined chronic condition, one intervention attracts attention as an effective treatment: bariatric surgery [59–61]. In addition to improvement of asthma control, bariatric surgery has been shown to improve small and large airway function and bronchial hyperresponsiveness. Although bariatric surgery is considered to be a potent intervention to treat obesity (and related comorbidities) and reduce mortality [62], post-operative complications are well known and common [63]. The overall complications rate is around 17% with a re-operation rate of 7% [64]. The 30-day rate of death among patients who underwent gastric bypass is around 0.3% [65]. But when successful, it has major beneficial consequences for the patient [62]. Thus, future improvements of bariatric surgery, decreasing the complication rate, would be of interest for patients with severe asthma and morbid obesity that are eligible for this type of intervention.

It is well-known that the early- and late-onset asthma phenotypes have distinct clinical and genetic features [66]. In line with this, it is conceivable that this also applies specifically to obese astmatics [59,67]. For instance, childhood-onset obesity with late-onset asthma might well have a distinct pathogenesis from late-onset obesity with childhood-onset asthma. The findings of the *Severe Asthma Research Program* are in accordance with this idea, since the authors found that obese subjects with early-onset asthma had more airway obstruction, bronchial hyperresponsiveness, and more oral steroid use per year, or intensive care unit admissions due to asthma, compared to their obese peers with late-onset asthma [68]. These results highlight the need to understand the interaction between both chronic conditions, in order to develop tailored treatment regimens. As a speculation, patients with early-onset asthma and late-onset obesity might be more responsive to weight reduction, while subjects with asthma and obesity since childhood might be less susceptible, due to their chronic exposition to the metabolic syndrome or oxidative stress.

Either way, treating overweight in asthma is relevant to control the latter condition; the metabolic syndrome is thought to up-regulate pathways contributing to asthma [69], via inflammatory cytokines such as adipokines [70,71]. Future therapy to resolve the asthma-obesity syndrome will presumably focus on pathways decreasing leptin [72], increasing adiponectin [73], and on further development of bariatric surgery.

13.4 Airway wall remodeling

Obstructive pulmonary diseases are characterized by structural airway remodeling, including alterations in the extracellular matrix (ECM) [6,74]. To date, two diagnostic tools are used to assess airway remodeling: HR-CT of the chest, and immunohistochemistry in bronchial biopsies [5,75], both having their drawbacks. A novel technique, OCT, generates infrared-based cross-sectional images of the airway wall [76], with potential to visualize airway remodeling. Some studies investigating OCT imaging have quantified collagen deposition, a parameter of airway wall remodeling, in skin [77], ovarian tissue [78], and primary lung tumors [5]. However, no study has linked OCT images to other ECM components within a healthy airway wall. Exploring the potential of OCT to analyze airway remodeling is of interest for at least two reasons: first, by using remodeling parameters (e.g. localized or overall airway wall infrared intensity area), early stages of pulmonary obstructive conditions could be detected [79]. And second, airway wall remodeling could be correlated more directly to effects of treatment [80].

In **chapter 9,** we demonstrated the use of OCT imaging to analyze airway wall remodeling. To our knowledge, this is the first study demonstrating that OCT imaging enables us to detect and quantify ECM collagen deposition in the airway wall, without the need of extracting and processing bronchial biopsies. By measuring the thickness [5,79], but also the ECM content of the airway wall, we hope to further explore the potential of OCT to analyze airway remodeling in asthma and COPD. Between 2016 and 2019, OCT imaging has been performed in several airway branches of never-smoking and former-smoking healthy controls, persistent asthmatics, past-smoking GOLD I or II COPD patients, subjects with clinical-, and complete asthma remission. Together with OCT airway wall images of severe asthmatics, collected by colleagues from the departments of Respiratory Medicine and Biomedical Engineering & Physics,

Amsterdam University Medical Center, we hope to compare the OCT images of asthma- and COPD severity groups with healthy controls. With this data, we can analyze whether there is a degree of airway remodeling in individuals with asthma, clinical- and complete asthma remission, and whether remodeling has ceased in complete asthma remission subjects. Additionally, we can relate the OCT-defined airway remodeling parameters with the degree of fixed airflow obstruction in asthmatics, and with scRNA-seq inflammatory cell types and proportions. These future projects would increase our knowledge on the molecular mechanisms behind airway wall remodeling, which would in turn give insight on how to reverse it.

13.5 Endotyping COPD

There is consensus that asthma and COPD share features [81], such as epidemiological and clinical characteristics. The theory of pathophysiological overlap, is known as the "Dutch hypothesis", proposed by Orie and colleagues in 1961 [82]. In line with this, it is of interest to test biomarkers and transcriptional profiles that are used in asthma research, on COPD patients and explore their clinical relevance.

We tested the clinical relevance of serum periostin in COPD patients in **chapter 10**, yet this biomarker did not reflect TH2-driven inflammation, airway remodeling, longitudinal FEV_1 decline, and inhaled corticosteroid (ICS) treatment responsiveness. We found two other studies focusing on the use/application of periostin in COPD patients and its predictive value for treatment response [83,84]. Even though these studies linked serum periostin to clinical outcome, their design or outcome restricted their persuasiveness: Park *et al.* studied 130 COPD patients before and after three months of ICS/long-acting β agonist treatment and found that a combination of high periostin level and blood eosinophil count was able to predict FEV_1 improvement. However, patients with high periostin and blood eosinophils who received this therapy, already had a higher bronchodilator response at baseline [84]. Therefore, the FEV_1 improvement might have been due to long-acting β agonists in patients with documented reversibility alone. The second study measured serum periostin in COPD patients admitted for a COPD exacerbation, as well as differences in death during follow-up, exacerbation-rate after discharge, or hospitalizations between low and high periostin (i.e. <25 ng/ml and

≥25 ng/ml respectively) [83]. Based on the current knowledge, it is unlikely that serum periostin will be integrated in future COPD clinical decision-making.

In **chapter 11,** we demonstrated that transcriptional profile (i.e. COPD Associated Gene Expression #1 (CAGE1) and CAGE2) was able to identify clusters of COPD patients that are distinguished on clinical features. Patients categorized into the CAGE2 cluster had significantly higher lymphocyte percentage in sputum and T-cells in bronchial biopsies, less ICS responsiveness, and more rapid lung function decline compared to CAGE1 COPD patients. Generating and implementing a gene signature such as CAGE2, may be useful as a biomarker for lung function decline and response to ICS. Blood or sputum sampling would be more convenient and more patient friendly compared to extracting bronchial biopsies. Nevertheless, if the CAGE2 signature truly predicts rapid lung function decline, a more invasive diagnostic method such as bronchoscopy could be justified when externally validated in a prospective study. Ultimately, harmonizing the multiple levels of –omics is key towards the development of biomarkers for personalized prognosis and treatment response.

13.6 Conclusion

The chapters presented in this thesis provide an overview of what is known about asthma remission, further characterize of complete asthma remission, elaborate on the asthma-obesity complexity, apply cutting edge techniques to endotype asthma and COPD as well as novel devices to analyze airway remodeling and small airways dysfunction.

Various conclusions have been made:

I. In order to elucidate the pathophysiological state of asthma remission, future studies should focus on complete asthma remission, since this phenomenon is likely to yield superior prognostic and scientific impact. This is of interest, since elucidation of the pathophysiology of asthma remission could potentially lead to new treatment options for asthma.
II. To clearly predict asthma remission later in life, we need to integrate biomarkers with clinical features at asthma-onset.
III. Measuring particles of exhaled air correlates with large, and indirectly, small airways parameters, in asthmatics, clinical-, complete asthma remission subjects, and healthy controls.
IV. Transcriptomic bronchial cell typing (e.g. single-cell RNA-sequencing) characterizes the landscape of lung-resident structural and inflammatory cells and their interactions, enabling us to identify differences in proportions and transcriptional output of cells between asthmatics and healthy.
V. Optical coherence tomography enables us to quantify extracellular matrix components in the airway wall, such as collagen. This now allows for future studies 'in vivo' to explore the clinical characteristics and the underlying pathobiology related to airway remodeling in asthma and asthma remission.
VI. The asthma-obesity syndrome is a common combination of diseases with its own distinct pathophysiological processes.
VII. There will presumably be no room for serum periostin in COPD clinical decision-making.
VIII. Transcriptomic profiling can be implemented as a biomarker for COPD patient prognosis.

Unavoidably, aforementioned conclusions lead to new questions and recommendations for future studies. These recommendations include:

I. To expand our knowledge on asthma remission by implementing single-cell RNA-sequencing on blood samples, bronchial- and nasal brushes, and bronchial biopsies of subjects with clinical and complete asthma remission, while comparing to asthmatics and healthy controls.
II. To test single-nucleus RNA-sequencing in the *ARMSTRONG* study. This method enables sequencing of frozen biopsy samples of former datasets, consequently extending the number of subjects.
III. To analyze exhaled, aberrant proteins linked to asthma, instead of merely counting exhaled particles.
IV. To compare metabolomic breathprints of various asthma severities.
V. To introduce novel methods in small airways disease-phenotypes in asthma, which enable visualization of airtrapping and gas exchange, such as functional MRI.
VI. To study the effects of leptin and adiponectin in the asthma-obesity syndrome. Specifying the eligibility for bariatric surgery in patients with severe asthma and morbid obesity, in order to treat this phenotype more safely.
VII. To analyze the presence of airway remodeling – defined by optical coherence tomography and histological parameters - in complete and clinical asthma remission, compared to asthmatics and healthy controls.
VIII. To correlate optical coherence tomography-defined airway wall remodeling parameters with both fixed airway obstruction and single-cell RNA sequencing inflammatory cell types or proportions.

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