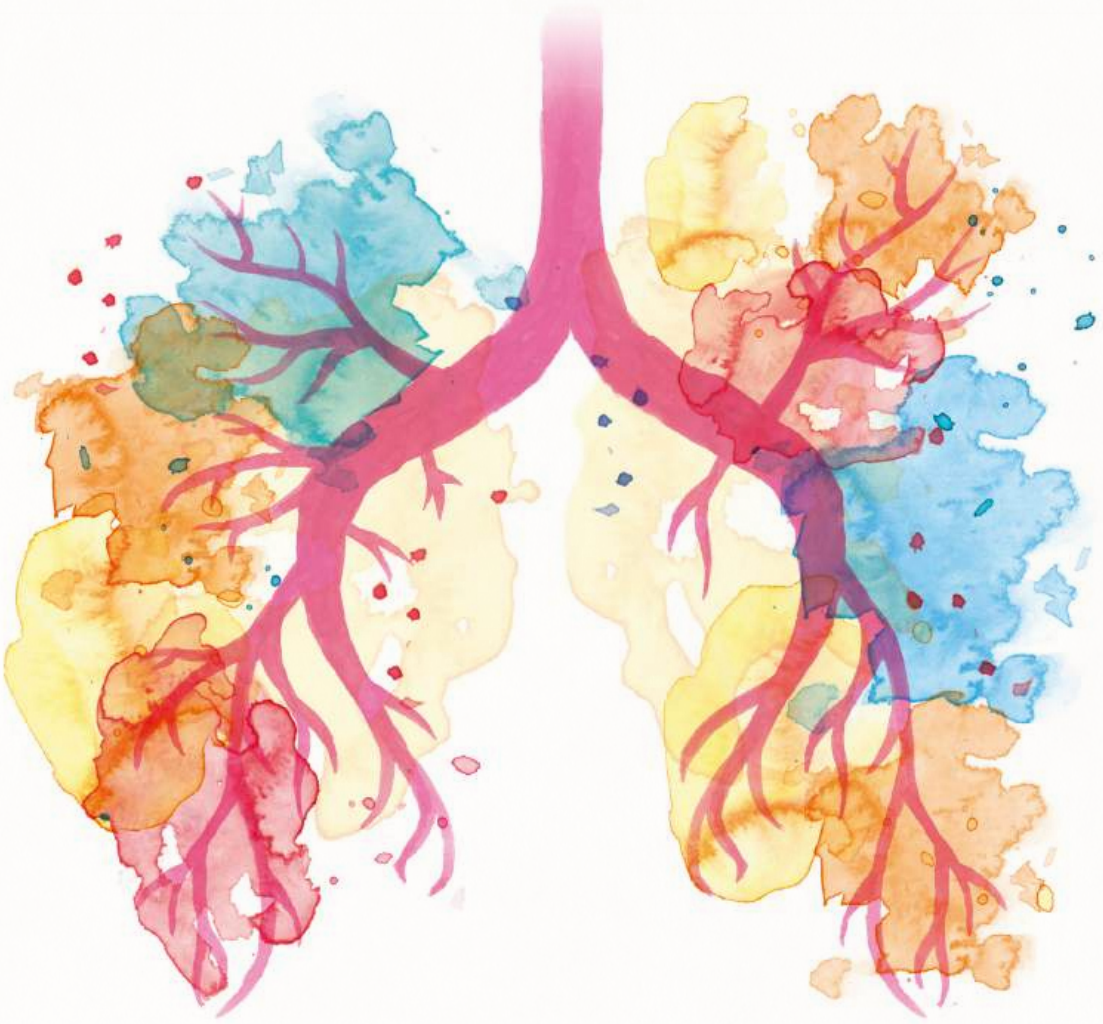
Whispers of Respiration

Factors underlying phenotypic heterogeneity in COPD and asthma



Cathelijne van Zelst

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Whispers of Respiration
Factors underlying phenotypic heterogeneity in COPD and asthma

Factoren die de fenotypische variatie van COPD en astma beïnvloeden

Thesis

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Chapter 1

General introduction

RISK FACTORS FOR COPD

Chronic Obstructive Pulmonary Disease (COPD) is characterized by irreversible airflow limitation, and its prevalence rises with age. Currently, approximately 10 percent of individuals aged 40 years or older are affected by COPD. In the Netherlands the prevalence of COPD in 2022 is 835,000, with projections suggesting an increase due to the compounding effects of climate change and an aging population (1, 2). Climate change contributes to COPD prevalence through exposure to fumes and dust. Most recent estimates suggest that ~50% of the risk of developing COPD may be related to air pollution (3, 4). In lifetime non-smokers, air pollution is the leading known risk for COPD (4). Tobacco products are responsible for most of the disease burden worldwide. The threshold for developing COPD varies among individuals, based on quantity and duration of cigarette smoke, whereby also genetic factors play a role (5). Early-life infections and indoor and outdoor pollution can increase the risk of developing COPD (6, 7). Indoor air pollution may also result from the use of biomass fuels in inefficient and poorly ventilated stoves for cooking and heating in low-income countries, and from the use of gas for cooking and heating without extractor fans in middle-income and high-income countries (6). Also, overweight, insulin resistance, and autosomal recessive inherited alpha-1 antitrypsin deficiency are associated with COPD (6, 8). Hobbs et al. identified 22 genetic loci that elevate the risk of developing the disease when exposed to one or more environmental risk factors within a specific time window (9). However, COPD genetics is certainly much more complex, as currently 1,150 genetic associations - with varied levels of supporting evidence - have been identified in over 100 genetic studies (10). COPD is a multifaceted condition influenced by various risk factors that can interact over the course of one's life. For instance, not all smokers develop COPD, and at least 20-30% of people with COPD have never smoked (6).

RISK FACTORS FOR ASTHMA

Asthma is a heterogeneous disease characterized by persistent inflammation of the airways, typically exhibiting chronic features. It manifests through a history of respiratory symptoms, including wheezing, shortness of breath, chest tightness, and cough, which may fluctuate in both frequency and intensity. The condition is defined by the variability in expiratory airflow limitation (11). The diagnosis of asthma relies on a comprehensive assessment encompassing symptoms, clinical evaluation, and pulmonary function tests. This prevalent lung disease affects individuals across the age spectrum, impacting both children and adults. In children aged 0 to 4 years, the prevalence of asthma was reported to be 3.8%, increasing to 8.1% in the age group of 5 to 11 years, and further to 9.9% in individuals aged 12 to 27 years. Among adults, the overall prevalence is estimated to be 7.7%, with a notably higher incidence in women at 9.8%, compared to men at 5.5% (12). Patients with severe asthma are defined as

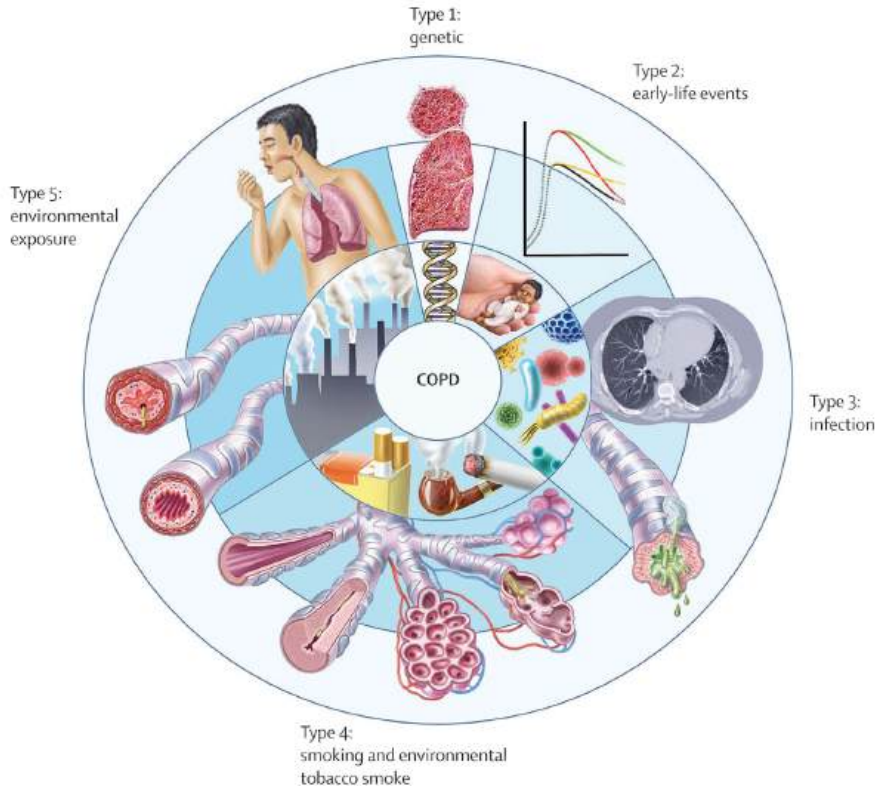

Figure 1. Proposed classification of COPD according to major risk factors. The five proposed types are related to genetics, early-life events, infections, exposure to tobacco smoke, and environmental exposures. We remain cognizant, however, that individuals are prone to multiple exposures throughout life, which could cause additive or interactive damage to lung health. COPD=chronic obstructive pulmonary disease.

patients with a confirmed diagnosis of asthma who require treatment with high dose inhaled glucocorticoids, plus a second controller and/or systemic glucocorticoids for 50 percent or more of the year to prevent asthma from becoming "uncontrolled" (13).

Risk factors associated with the development of asthma are genetic susceptibility (14) exposure to allergens (15), younger maternal age (age <30 years) (16), maternal diet or vitamin D deficiency during pregnancy (17), prematurity and pre-eclampsia. Additionally, there is a correlation between atopy, allergen-specific IgE antibodies and airway hyperresponsiveness. The microbiome also plays a role in the development of allergen sensitization and asthma. Childhood respiratory infections, exposure to air pollution, and obesity can further elevate the prevalence of asthma (12).

DUTCH AND BRITISH HYPOTHESES

As early as 1960, Orie and Sluiter attempted to conceptualize obstructive airway disease not as distinct illnesses but rather as varying manifestations of a single disease entity—chronic nonspecific lung disease (CNSLD) (18). This perspective, later named the Dutch Hypothesis, poses that both endogenous (host) and exogenous (environmental) factors contribute to the pathogenesis of asthma, chronic bronchitis, and emphysema (19). Host factors manifest clinically in a non-uniform manner and are influenced by interactions with other characteristics, such as age or exposure to allergens, infections, and air pollution. While there is indirect evidence suggesting a potential link between smoking and asthma, it's crucial to acknowledge that not all young adults exposed to cigarette smoke will develop asthma. Similarly, not every smoker will progress to COPD. These findings imply that the long-term impact of smoking, when considered alongside an individual's genetic predisposition, may be influenced by the nature and duration of the exposure to smoking. Following the publication by Orie and colleagues, pulmonologists sought to characterize the clinical features of patients with obstructive airway disease rather than categorizing the disease itself (18). In 1991, the Dutch hypothesis faced opposition from researchers in the United Kingdom and the United States. This opposing perspective, known as the British hypothesis, asserted that asthma and COPD were separate diseases with distinct causal mechanisms (20). The proponents of the British hypothesis argued that shared features between the two conditions should not automatically suggest a common pathogenesis (20).

COPD PHENOTYPES

Originally, COPD was subdivided in two phenotypes: the "pink puffer" and "blue bloater" (21). The "pink puffer" phenotype is characterized by cachexia, low levels of FEV1 and hyperinflation, while the "blue bloater" phenotype involves mild dyspnoea, cyanosis and obesity.

Now, our understanding of COPD encompasses various underlying pathological mechanisms that significantly impact prognosis and the effectiveness of treatments. A clinical phenotype refers to a single or collection of disease characteristics that distinguish individuals with COPD and are linked to crucial clinical outcomes, including symptoms, exacerbations, treatment response, disease progression, or mortality (22, 23). Hierarchical clustering through principal component analysis (PCA) is a well-established method for identifying clusters. Numerous efforts have been made to establish a meaningful classification of COPD patient phenotypes (24). Recent research has identified etioendophenotypes for COPD exacerbations (ECOPD) (25). Etiotype is defined by the primary inciting insult triggering the exacerbation, endotype by the underlying pathophysiologic mechanism associated with the exacerbation, and

phenotype by the clinical characteristics of the exacerbation. Examples of phenotypes include the frequency and severity of exacerbations, while etiotypes encompass viral, bacterial, environmental, and multimorbidity factors. Endotypes involve innate immunity, T1-, T2-, and T17 inflammation (25). The definition of exacerbations has evolved over the years and was recently proposed by the Lancet Commission for COPD to be: "an increase in cough, dyspnea or sputum production and at least one of an increase in airflow limitation or ventilation heterogeneity, an increase in airway or systemic inflammation, or evidence of bacterial or viral infection, in the absence of evidence of acute cardiac ischemia, congestive heart failure, or pulmonary embolism (25)." In heterogeneous conditions such as COPD exacerbations, the presence of these varied etioendophenotypes can offer a comprehensive clinical overview, potentially signalling the necessity for adjustments in maintenance therapy to mitigate the likelihood of future exacerbations. COPD treatment begins with a long-acting beta2 agonist (LABA) inhaler or a long-acting muscarinic antagonist (LAMA) inhaler. If patients experience worsening symptoms, as indicated by disease burden questionnaires, or have two exacerbations or one hospitalization, both inhalers will be prescribed. Inhaled corticosteroids (ICS) are only considered if blood eosinophil levels exceed 300 (26).

ASTHMA PHENOTYPES

Nowadays, asthma is seen as highly heterogeneous and includes many different observable characteristics (phenotypes) and underlying mechanisms (endotypes) (27). Examining this diversity contributes to our comprehension of disease pathogenesis and the development of novel therapeutic approaches. Eosinophilic asthma, commonly known as high type 2 high ($T2^{high}$) immunity, is prevalent in approximately 50% of adults with asthma. Atopy is evident in 50-60% of both adult and paediatric asthma cases. Its prevalence is higher in severe asthma among children and adults who experienced childhood-onset rather than late-onset disease. While non-eosinophilic asthma has been observed in both adults and children, it remains insufficiently understood. The mechanisms driving eosinophilic inflammation and non-eosinophilic asthma, whether allergy-dependent or independent, can undergo changes over time, resulting in mixed granulocytic inflammation. Non-allergic asthma may manifest at any age, with a higher prevalence among women, particularly those dealing with obesity (28). Late-onset asthma is a broad term encompassing onset from as early as 12 years of age to as late as over 65 years, often escaping proper diagnosis. Late-onset asthma tends to be non-atopic, more severe, and linked to a swifter decline in lung function. Adult-onset phenotypes encompass asthma in obese females with persistent airflow limitation, non-atopic eosinophilic predominant asthma accompanied by persistent airflow limitation, mild atopic asthma, and asthma in smokers (29). Asthma treatment starts with inhaled corticosteroids (ICS) as needed. If symptoms worsen, the treatment is

stepped up. In Step 2, patients are advised to use ICS daily. In Step 3, a low-dose ICS-LABA combination is recommended. Step 4 involves a medium-dose ICS-LABA. In Step 5, patients use a high-dose ICS-LABA, with add-on therapies considered for further therapeutic evaluation (anti-IgE, anti-IL5, anti-IL4 etc) (30).

ASTHMA-COPD OVERLAP

The term Asthma-COPD overlap refers to patients with functional and clinical features of both asthma and COPD, including persistent airflow limitations (27). ACO describes a set of clinical features rather than defining a singular entity (31). The preference for the term ACO over asthma-COPD overlap syndrome (ACOS) arises from the absence of a single disease or "syndrome," and to date, no universally accepted definition of ACO has been established. In 2020, even the GOLD Strategy update abandoned the use of the term "asthma-COPD overlap," arguing that asthma and COPD are distinct disorders that may share common features like eosinophilia or some degree of reversibility (32). Among individuals experiencing ACO symptoms, the quality of life, frequency of exacerbations, hospitalizations, and mortality are more severe compared to those with asthma or COPD alone (33). Studies indicate that ACO patients are more likely to be female, have a higher body mass index (BMI), lower socioeconomic status, and lower education levels than patients with COPD (34). Furthermore, the prevalence of asthma-COPD overlap tends to increase with age, possibly because aging is related to increased years of smoking (33). Finally, also shared genetic loci between COPD and asthma have been identified (10, 35).

IMMUNOLOGY OF COPD AND ASTHMA

T helper subsets and their innate counterparts. Inflammatory cytokines play a critical role in orchestrating and perpetuating the chronic airway inflammation in both COPD and asthma. Different adaptive immune responses each have specific contributions of CD4+ T helper subsets. Type 1 responses, driven by Th1 cells producing interferon g (IFNg), are generally considered responses to viruses or intracellular bacteria. Type 2 responses, driven by Th2 cells that secrete IL-4, IL-5, and IL-13, are critical for host defence against helminths, but also involved in allergies. Type 3 responses, driven by Th17 cells producing IL-17 and IL-22, are crucial for the response to extracellular bacteria. In addition, regulatory T cells (Tregs) have anti-inflammatory properties.

Next to T helper cells, innate lymphoid cells (ILCs) are important sources of inflammatory cytokines. The ILCs are newly discovered non-B/non-T lymphocyte types and play diverse roles in mucosal inflammation and tissue repair. Activated by stress and cytokine signals within tissues (36), they are classified as ILC1, ILC2 and ILC3 based on signature transcription factors and cytokine profiles mirroring those of T helper (Th) cell subsets. On the other hand, NK cells can be regarded as innate counterparts of cytotoxic CD8+ T cells. The assumption is that the three ILC groups derive from a

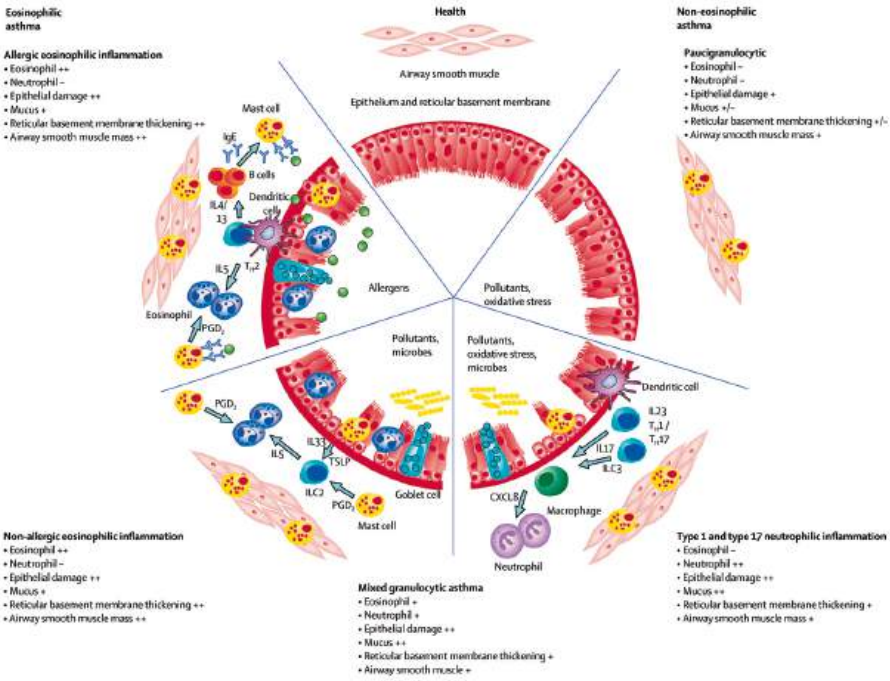

Figure 2. Mechanisms and characteristic pathological features of asthma immunopathology. Features are divided into eosinophilic (allergic and non-allergic), non-eosinophilic (neutrophilic type 1 and type 17 and paucigranulocytic), and mixed granulocytic inflammation. IL=interleukin. TH=T helper. PDG2=prostaglandin D2. TSLP=thymic stromal lymphopoietin. ILC2=type 2 innate lymphoid cells. CXCL8=C-X-C motif chemokine ligand 8. ILC2=type 3 innate lymphoid cells

common precursor, the ILC progenitor (ILCP), also found in peripheral blood (37, 38). While mainly present in tissues, they are not strictly tissue-resident and can migrate to peripheral organs. Their phenotypes and functions are influenced by the local tissue microenvironment and cytokines. ILC1 are often associated with immune responses against intracellular pathogens and tumour cells and produce IFNγ (39). ILC2s respond to helminth infections, are critically involved in allergic responses, producing cytokines such as IL-4, IL-5, and IL-13, as well as tissue repair because they have the capacity to produce amphiregulin. Similar to ILC1s, they are not strictly tissue-resident and can – under particular circumstances - migrate to various peripheral organs. ILC3s are associated with maintaining gut homeostasis and protecting against intestinal infections, producing IL-22 and IL-17. ILC3s are heterogeneous and contain lymphoid tissue inducer (LTi) cells, which play a key role in the development of lymphoid tissues (40). Primarily tissue-resident, especially in mucosal tissues, they play a crucial role

in the local immune response (36, 38, 41-44). Regulatory ILCs (ILCreg) have been discovered in the gut and express IL-10 and TGF-β. While their involvement in asthma has been documented, their role in COPD has not yet been reported (40). ILC3 and LTi cells play a role in the development of induced bronchus-associated lymphoid tissue (iBALT), which is the site of ILC localisation in COPD lungs and can be a feature of advanced COPD (45).

Immunology of COPD. The chronic inflammation in the lung of COPD patients is characterized by accumulation and activation of macrophages and neutrophils (46). The lung exhibits an increased presence of CD8+ T lymphocytes, which outnumber CD4+ T cells. In patients who do not respond to inhaled corticosteroids, there is a predominance of increased markers of T17-driven inflammation in the airways (47). In COPD, there is an elevated presence of ILCs, especially ILC1 and ILC3. Studies have shown elevated levels of ILC1 and ILC3 in COPD lungs, with ILC2 cells displaying plasticity toward IFNγ-producing ILC1 cells during viral infection in COPD patients (48-50). Positive correlations were found between the numbers of ILC1s (IFNγ producers) in the circulation and disease severity, as defined by decreased forced expiratory volume in 1 second (FEV1) and increased frequency of exacerbations (50). Increased ILC1 frequencies in COPD lungs correlated with smoking and the severity of respiratory symptoms (48, 51). In mice exposed to smoke, proportions of ILC1s and ILC3s were increased, while ILC2s showed a decrease (52). However, conflicting reports exist, for example the finding of an increase in both ILC1s and ILC2s in broncho-alveolar lavage in mice exposed to cigarette smoke (51). Although evidence was provided that ILC3 levels are elevated in donor COPD lungs and smoker lungs in comparison with controls (49, 53, 54), this cannot be correlated with peripheral blood since circulating ILC3 are essentially absent (38).

Immunology of asthma. Eosinophilic asthma primarily manifests as a Th2-related condition. Key features of type 2 immunity involve eosinophilic inflammation, allergen-specific immunoglobulin E (IgE) production by plasma cells, mast cell activation, goblet cell hyperplasia and tissue remodelling. The orchestration of these processes is governed by signature cytokines, namely interleukin-4 (IL-4), IL-5, IL-9, and IL-13, which are produced by both innate and adaptive immune cells (55). For asthma there is evidence that ILC2s are increased or more active in peripheral blood and sputum. Because of this association, these cells are thought to contribute to the induction of asthma symptoms, implicating them as drivers of eosinophilic airway inflammation (56-58). The presence of the CD45RO isoform by inflammatory ILC2s in blood is linked to reduced sensitivity to steroids and is positively correlated with the severity of asthma (55). In obesity-related asthma patients increased levels of ILC3s were found in the

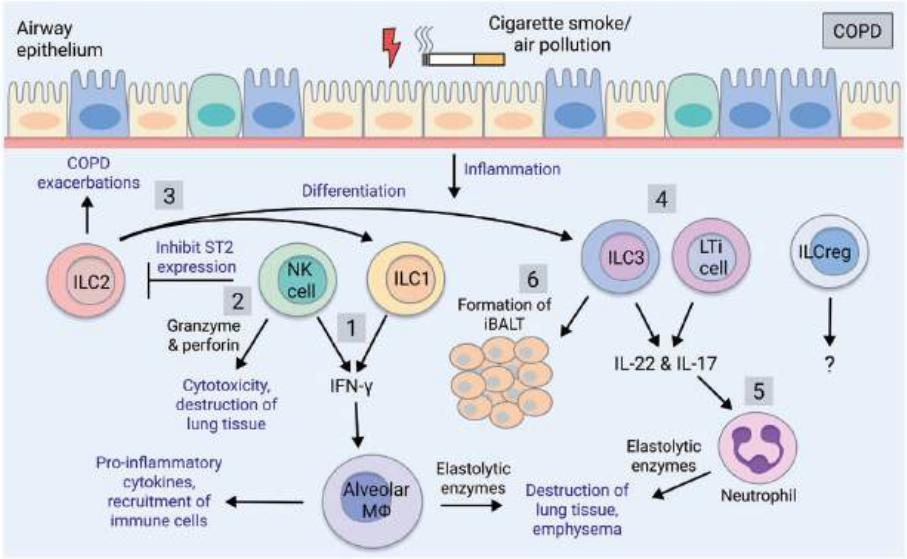

Figure 3. ILC involvement in COPD. COPD is caused by cigarette smoking and insults such as air pollutants. COPD patients exhibit increases in group 1 and group 3 ILCs, which correlate with severity and exacerbations, whereas ILC2 numbers are reduced. 1) ILC1 and NK cells produce the pro-inflammatory cytokine IFNγ, which activates alveolar macrophages causing the release of inflammatory mediators. Macrophages secrete proteases (MMPs, cathepsins) inducing the destruction of the lung parenchyma thereby contributing to emphysema. 2) NK cell cytotoxic activity through secretion of granzyme and perforin induces death of lung tissue, furthering emphysema. NK cells also inhibit the production of ILC2 through downregulation of their ST2 receptor. 3) ILC2s promote Th2 inflammation during COPD exacerbations or differentiate into ILC1-like cells in the presence of IL-1b and IL-12 during lung inflammation. They potentially also differentiate into ILC3s. 4) ILC3 and LTi cells produce IL-17 and IL-22, which are elevated in COPD patients, driving pathogenesis. 5) IL-17 induces the maturation and recruitment of neutrophils, which are expanded in COPD patients, and via their release of proteases (neutrophil elastase, cathepsin G, proteinase-3), contribute to mucus secretion and alveolar destruction. 6) ILC3 and LTi cells contribute to the formation of induced bronchus-associated lymphoid tissue (iBALT), which is a feature of advanced COPD (5) and is the site of ILC localisation in COPD lungs. ILCregs are yet to be understood in the regulation of COPD pathogenesis.

broncho-alveolar lavage (59). Certain patients exhibit neutrophil-predominant disease, involving the release of cytokines from T helper 1 cells, T helper 17 cells, or type 3 innate lymphoid cells (ILC3), alongside macrophage activation and the release of neutrophil chemokines (60).

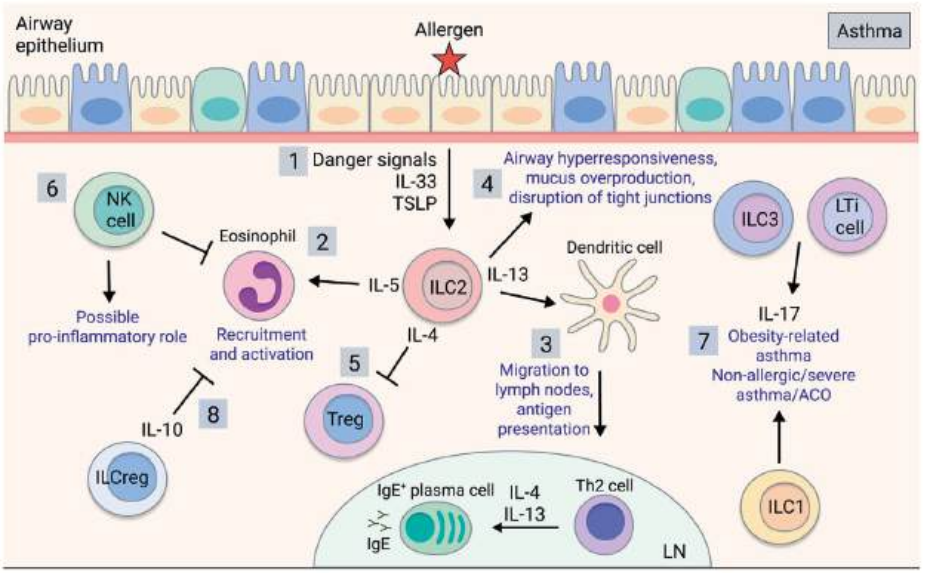

Figure 4. ILC involvement in asthma. Upon allergen detection by airway epithelium, 1) ILC2s are activated by signals released by the airway epithelial cells and other activated immune cells, producing type 2 cytokines such as IL-4, IL-5, and IL-13 in allergic asthma. 2) IL-5 is key for eosinophil recruitment and activation in the lung and 3) IL-13 mediates dendritic cell migration to the lymph nodes, promoting T cell differentiation into effector Th2 cells, which mediate B cell class switching and IgE production. 4) ILC2-derived IL-13 also acts on the airway epithelium to induce airway hyperresponsiveness, mucus overproduction and disruption of barrier integrity. 5) ILC2-derived IL-4 may potentially inhibit Treg production in asthma. 6) NK cells play an ambiguous role in asthma with both disease-driving and disease-modulatory activity shown. 7) ILC3s/LTi cells and possibly ILC1s contribute to obesity-related asthma and potentially nonallergic, severe asthma or ACO through production of IL-17. 8) ILCregs may regulate asthma by inhibiting eosinophil recruitment through IL-10.

BEHAVIOR AND SELF-MANAGEMENT SUPPORT

Currently, non-pharmaceutical treatments, combined with improved self-management skills, are pivotal in the care of patients with asthma and COPD. Behavioral change is crucial throughout the entire spectrum of chronic disease management, spanning from prevention and progression to rehabilitation and even advanced care planning (61). These self-management strategies empower patients to adjust their behavior and effectively cope with their illnesses (62). According to a recent Delphi process, an international panel of COPD self-management experts published the following perspective on self-management interventions: "A COPD self-management intervention is structured yet personalized and often multi-component, with the goal of motivating,

engaging, and supporting patients to positively adapt their health behaviors and develop skills to better manage their disease (63).” Digital platforms, such as eHealth platforms, have the potential to facilitate widespread education on self-management (64). These platforms eliminate limitations related to accessibility, including issues such as geography, finances, and transportation. eHealth platforms could enable the transfer of monitoring and treatment from the hospital to the home, potentially easing the burden on hospital staff and diminishing the requirement for extra employees. However, the drawback of these platforms could lie in the significant carbon footprint associated with storage and transmission of data from remote monitoring systems to the hospital. Therefore, the crucial factor is the overall net benefit of these interventions.

Utilizing self-management questionnaires focused on assessing the quality of life can enhance the understanding of patients’ needs. The severity and treatment approach for both asthma and COPD relies on the individually experienced burden of disease and preference of the patient (11, 65). Various questionnaires designed to evaluate disease burden have been developed for this purpose. Notably, the Asthma Control Questionnaire (ACQ) and the Clinical COPD Questionnaire (CCQ) were specifically created to gauge the disease burden in patients with asthma and COPD, respectively (66, 67). These questionnaires, known for their brevity and practicality, are routinely employed in primary, secondary, and tertiary care settings in the Netherlands (68, 69). Another well-known questionnaire to measure quality of life is the St. George Respiratory Questionnaire (70). This questionnaire was utilized to validate both the ACQ and CCQ, both of which are briefer compared to the St. George Respiratory Questionnaire. To measure severity of symptoms, the modified Medical Research Council (mMRC) scale and the COPD Assessment Test (CAT) are used (62, 71, 72). In GOLD 2017 the spirometric GOLD I-IV, based on FEV1, and the CAT, mMRC and number of exacerbations in the last year were combined in four groups, called ABCD groups. Pharmacotherapy recommendations per ABCD subgroup were introduced for COPD patients (65). In 2024 subgroup C and D were merged into a single group called E to highlight the clinical relevance of exacerbations (73). Gold classes might contribute to forming phenotypes (22) and were integrated with etioendotype data of COPD patients (50). Currently, it is uncertain whether combining results from these questionnaires could effectively distinguish COPD patients from asthma patients or if a single combined questionnaire could be utilized for both diseases. Additionally, it is unclear whether we can differentiate COPD patients from asthma patients based on combined etioendophenotype data.

AIMS AND OUTLINE OF THE THESIS

The overall aim of this thesis is to investigate the heterogeneity of COPD and asthma, and explore the role of general risk factors, immunological differences and behavioural aspects. Asthma and COPD are both obstructive airway diseases characterized by dyspnea, cough and wheezing. However, both diseases have a different etiology. Asthma is a characterized by a variable airflow limitation and by hyperresponsiveness to a variety of stimuli. COPD patients have persistent postbronchodilator airflow limitation. Some patients have symptoms of both asthma and COPD, however no single definition has emerged for this group of patients yet.

In the introduction (**Chapter 1**) we summarize current knowledge of the risk factors of COPD and asthma, phenotypes of the diseases, heterogeneity in the pathogenesis of COPD and asthma and overlap in clinical and immunological features of COPD and asthma. A better understanding of both diseases, specifically the role of immunology and behavioural aspects, will ultimately stimulate the development of more effective treatment options in obstructive airway disease. We addressed the following six research questions per aim.

Clinical heterogeneity in COPD calls for the development of specific treatment strategies for individual patients. Does a comprehensive clustering that also includes behavioural variables impact improve stratification of COPD patients? To answer this question, we first applied a PCA to investigate if a reported clustering based on clinical phenotypes could be reproduced in an independent cohort. We added behaviour variables as potential risk factors and formed new clusters to search for clinical stability of the phenotypes (**Chapter 2**).

Adults with a high BMI have an increased risk of developing asthma, but the underlying mechanisms are not fully understood. Are high levels of triglycerides a potential risk factor for asthma? We compared lipid levels and neutrophils and eosinophils in patients with asthma and the controls with a wide BMI range (17.8-63.8 kg/m²). Multivariable logistic regression was used to analyse the data **(Chapter 3)**.

It appears possible to distinguish between asthma and COPD by examining elevated levels of ILC1s, which are linked to severe COPD, or increased levels of ILC2s, which are specifically associated with asthma. However, it remains unclear whether the inflammatory profiles of ILC1s and ILC2s vary between COPD and asthma. Are there discrepancies in the frequencies of peripheral blood ILC subsets and their expression of inflammatory cell surface markers among COPD patients, asthma patients, and control groups? With a PCA, we aggregated endotype data to identify distinct immunological characteristics among asthma, COPD, smoking controls, and non-smoking controls (**Chapter 4**).

While the quantity of ILC1s correlates with COPD severity as measured by FEV1 and exacerbations, a thorough examination of ILC subsets in relation to clinical outcomes has not yet been reported. Is it possible to identify specific immunological profiles for clinically different COPD patients, based on disease burden, age, BMI, FEV1 and diffusion capacity? To address this query, we employed principal component analyses and multiple analyses to create immunological clusters and clinical clusters, which we superimposed in search of any overlap (**Chapter 5**).

Although COPD and asthma exhibit significant clinical overlap, distinct questionnaires are utilized to assess disease burden for each condition, respectively the Clinical COPD Questionnaire (CCQ) and the Asthma Control Questionnaire (ACQ). Therefore, we investigated whether it is feasible to develop a unified questionnaire capable of evaluating disease burden in asthma, COPD and asthma- COPD overlap (ACO) cases? We proposed a novel questionnaire for obstructive airway disease, derived from merged data using PCA of the two existing questionnaires. We replicated this selection process in three new cohorts. This new instrument was validated by calculating a Pearson correlation coefficient test with the Asthma Quality of Life Questionnaire (AQLQ) in asthma and COPD Assessment Test (CAT) in COPD (**Chapter 6**).

eHealth platforms have the potential to aid in self-management for COPD patients, yet the influence of healthcare professionals on patient adherence to such platforms remains unexplored. These findings are crucial given the emphasis on self-management and the increasing adoption of remote patient monitoring and management. Do patients more frequently utilize eHealth platforms when using them independently or in a blended care setting, involving healthcare professionals? To assess platform usage, we employed propensity score matching and conducted adjusted Poisson regression analysis on CCQ submission rates. (**Chapter 7**).

Finally, we integrated the presented findings, offering an evaluation of the significance of immunological variances, behavioural factors, and overall risk elements in COPD and asthma from a clinical standpoint (**Chapter 8**).

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Chapter 2

Stratification of COPD patients towards personalized medicine: reproduction and formation of clusters

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ABSTRACT

Background

The global initiative for chronic obstructive lung disease (GOLD) 2020 emphasizes that there is only a weak correlation between FEV_1, symptoms and impairment of the health status of patients with chronic obstructive pulmonary disease (COPD). Various studies aimed to identify COPD phenotypes by cluster analyses, but behavioral aspects besides smoking were rarely included.

Methods

The aims of the study were to investigate whether (i) clustering analyses are in line with the classification into GOLD ABCD groups; (ii) clustering according to Burgel PR et al. (ERJ. 2010; 36-531-539) can be reproduced in a real-world COPD cohort; and (iii) addition of new behavioral variables alters the clustering outcome.
Principal component and hierarchical cluster analyses were applied to real-world clinical data of COPD patients newly referred to secondary care (n=155). We investigated if the obtained clusters paralleled GOLD ABCD subgroups and determined the impact of adding several variables, including quality of life (QOL), fatigue, satisfaction relationship, air trapping, steps per day and activities of daily living, on clustering.

Results

Using the appropriate corresponding variables, we identified clusters that largely reflected the GOLD ABCD groups, but we could not reproduce Burgel's clinical phenotypes. Adding six new variables resulted in the formation of four new clusters that mainly differed from each other in the following parameters: number of steps per day, activities of daily living and QOL.

Conclusion

We could not reproduce previously identified clinical COPD phenotypes in an independent population of COPD patients. Our findings therefore indicate that COPD phenotypes based on cluster analysis may not be a suitable basis for treatment strategies for individual patients.

INTRODUCTION

The severity of chronic obstructive pulmonary disease (COPD) is defined by forced expiratory volume in 1 second (FEV_1), divided into four stages of severity (Global initiative for chronic obstructive lung disease; GOLD I-IV) (1). Nonetheless, the heterogeneity of the clinical presentation and disease development among patients within the same GOLD stage is substantial (2). GOLD 2011 introduced the ABCD assessment tool to classify stable COPD patients on the basis of airflow limitation, number of exacerbations per year and questionnaires to measure the severity of symptoms: modified Medical Research Council (mMRC) scale and COPD Assessment Test (CAT) (1). This approach is more comprehensive than airflow limitation alone, but it is still based on a limited number of variables. In GOLD 2017 a refinement of the ABCD assessment tool was suggested, which separated the spirometric GOLD I-IV grades from the ABCD groups and introduced pharmacotherapy recommendations per ABCD group.

An alternative method facilitating subgroup-specific treatment might be established by the identification of phenotypes on the basis of prognostic, demographic, clinical, pathophysiological or therapeutic characteristics. Han et al. proposed the following definition of phenotypes in the context of COPD: “a single or combination of disease attributes that describe differences between individuals with COPD as they relate to clinically meaningful outcomes” (3).

Phenotyping can be aided by using descriptive statistics, such as cluster analysis to identify separate patient groups according to preselected variables (4). With regards to these variables, patients within a certain cluster are more similar to each other than to patients in different clusters (5). The identification of coherent clusters may lead to the recognition of phenotypes, which could be a valuable step towards tailored treatment strategies per subgroup.

Several attempts have been made to develop a useful classification of phenotypes of COPD patients (4). To be potentially useful in clinical practice, the identity of the defined clusters needs to be confirmed in different, independent cohorts of COPD patients, but to the best of our knowledge such replication studies have not been performed yet. Burgel et al. (6) performed more extensive phenotyping of COPD patients based on the clinical variables age, cumulative smoking, airflow limitation, body mass index (BMI), exacerbations per year, dyspnea, health status and depressive symptoms. Hereby, four clinical phenotypes were defined: phenotype 1 were relatively young subjects (median 58 [IQR 55-63] years old) with predominantly severe to very severe respiratory disease, frequent exacerbations and low BMI; phenotype 2 were older patients (median 68 [IQR 60-74] years old) with mild symptoms; phenotype 3 were younger subjects (median 59 [IQR 50-65] years old) with moderate to severe airflow limitation. In the fourth phenotype older patients (median 72.5 [IQR 67-77] years old)

with moderate to severe airflow limitation were included. Compared to phenotype 3, these patients had a higher prevalence of depressive symptoms, higher BMI and more severe dyspnea. Patients with comparable FEV_1 were assigned to different phenotypes (6). Longitudinal 2-year follow-up showed that phenotype 2 is associated with a very low risk of mortality and that patients with phenotype 1 had the highest mortality rates and died at a younger age (7).

Moreover, in the current literature, cluster classifications are largely based on clinical variables, while behavioral variables are rarely used. Whereas most attention is drawn to smoking behavior (6, 8), other behavioral aspects such as coping, physical activity and quality of life (QOL) are not included. Nevertheless, these are important variables because they influence the impact of self-management interventions and can interfere with active participation (9). For example, a high rate of physical activity is known to increase shortness of breath and therefore it is avoided by most COPD patients. On the other hand, in the long run physical exercise in COPD is associated with a reduction of shortness of breath (10). Shortness of breath during physical activity can be mechanically influenced by air trapping, which makes this an interesting physiological parameter to add to phenotyping (11).

The aim of this study is threefold. First, to investigate whether the results of our cluster analyses match the ABCD groups defined by the GOLD criteria, which are either based on CAT and exacerbation frequency or on mMRC and exacerbation frequency. Second, to address whether the four COPD phenotypes previously identified by Burgel et al. can be reproduced by cluster analysis in another real-world COPD cohort. Third, to determine whether the addition of six new variables: QOL, fatigue, satisfaction relationship, air trapping and steps per day and activities of daily living improves the classification into distinct subgroups.

METHODS

Study design

We performed three independent cluster analyses of COPD patient characteristics. In the first analysis, we aimed to identify clusters that corresponded with the ABCD groups (1). Secondly, we investigated the reproducibility of Burgel's clusters in our study population (6). Thirdly, we added six new variables, QOL, fatigue, satisfaction relationship, air trapping and steps per day and activities of daily living to the parameter of the second analysis.

Setting and participants

Data were part of a registry study of patients with asthma and COPD, who were newly referred to the Franciscus Gasthuis and Vlietland Hospital in Rotterdam, the Netherlands, a center of excellence for asthma and COPD. All referred COPD patients (n= 155) who completed a previously published (12) comprehensive assessment during the period December 2012 till December 2017 were included. The diagnosis of COPD was based on an assessment by a pulmonologist and confirmed by spirometry (FEV1 / forced vital capacity (FVC) <0.7). In this study, we used pseudonymized assessment data. Ethics approval for this study was waived by the Institutional Research Board of the Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands, because routinely collected health care data were used after pseudonymization.

Data collection

The following variables were collected for all patients:
Lung function. FEV_1, FVC, and static and dynamic hyperinflation were performed according to the ATS/ ERS taskforce "standardization of spirometry" (13, 14). Values for post-bronchodilation dynamic hyperinflation were measured by metronome-paced tachypnea after bronchodilation (400µg of inhaled salbutamol) (15). Lower levels of air trapping (dynamic hyperinflation after bronchodilation measured in liters decreasing inspiratory capacity) reflect poor outcome. All tests were performed with the Vmax Sensor Medics Viasys, type 6200 Encore.
Pack years. A pack year is defined as twenty cigarettes smoked per day for one year.
Body mass index (BMI): BMI is defined as the body mass divided by the square of the body height, expressed in units kg/m^2 (16).
Exacerbations. The number of antibiotic courses and/or systemic steroids for their respiratory disease in the previous year (0, 1, 2 or 3+).
Symptoms and health status. Modified Medical Research Council (mMRC) is a five-item questionnaire to score the dyspnea of COPD patients (17, 18) (**Table 1**). The Clinical COPD Questionnaire (CCQ) is a ten-item questionnaire about symptom severity (19). A higher score indicates a worse health status. The minimal clinically important difference is 0.4. (20) The Beck Depression Inventory for primary care (BDI-PC) was used to score symptoms of depression independently of physical function (21, 22).
BOD-score. BOD-score includes the variables BMI, airflow obstruction and dyspnea in COPD (**Table 1**). Higher BOD scores for indicate a greater risk of death (23).
Physical activity. Physical activity was measured by an activity tracker (McRoberts© Triaxial accelerometer) during one week. The mean number of steps in 24h over 7 days was used for analyses.

ABCD groups. Group 'A' includes patients with mMRC 0-1 or COPD Assessment Test (CAT) <10 and 0-1 exacerbation per year; group 'B' includes patients with mMRC ≥ 2 or CAT ≥ 10 and 0-1 exacerbation per year; group 'C' includes patients with ≥ 2 exacerbations or ≥ 1 exacerbation leading to hospital admission with mMRC 0-1 or CAT < 10 and group 'D' includes patients with ≥ 2 exacerbations or ≥ 1 exacerbation leading to hospital admission with mMRC ≥ 2 or CAT ≥ 10. (1)

Nijmegen Clinical Screening Instrument (NCSI). Four NCSI domains were included; QOL, satisfaction relationship, behavioral impairment (termed activities of daily living) and fatigue (24). The minimum and maximum scores are shown in **Table 1.**

Table 1. Scoring range of questionnaires

Variables	Scoring range *)
mMRC dyspnoea score	1-5
CCQ total score	0-6
BDI-PC total score	0-21
BOD score	0-7
NCSI quality of life	1-101.6
NCSI satisfaction relationship	2-10
NCSI activities of daily living	0-135.5
NCSI fatigue	8-56

*) Higher scores reflect poor condition.

Analyses

Following the methodology applied by Burgel et al (6), we used Principal Component Analysis (PCA) and Ward's hierarchical cluster analysis. In Ward's method, the analysis starts with each subject forming its own cluster (25). Step by step, the number of clusters is reduced until all subjects are in one cluster. In each step, the two most similar clusters from the previous steps are combined, based on the variables that are have been selected to describe the clusters. These two clusters are selected in such a way that the total of the variances of all variables within the new clusters is as small as possible. Before clusters were formed, PCA was used to reduce the number of variables by replacing them by newly created uncorrelated variables ('components') with minimal information loss (26).

Dendrograms were used to graphically represent the hierarchical relationship between the clusters and the distance between them. The resulting cluster solutions were described and compared to determine the optimal number of clusters. All statistical analyses were performed using Stata/SE 15.1. Following Burgel et al. (6), variables were standardized (i.e. using Z-score or PCA) before they were included in the cluster analysis and categorical or dichotomous variables were expressed numerically.

PCA were performed to reduce interaction between the variables included in the cluster analyses. Components with an eigenvalue >1 were used.

In the first cluster analysis, in which we aimed to match ABCD groups, two times two variables (exacerbations per year and mMRC versus exacerbations per year and CAT) were used. We used two types of symptom questionnaires (CAT and mMRC) as a marker of disease burden, because GOLD uses either one of these to form the ABCD groups.

In the second cluster analysis, in which we investigated whether the four clinical phenotypes of Burgel could be reproduced, the following eight variables were used: age, packyears, FEV_1, BMI, mMRC, CCQ, BDI-PC and the number of exacerbations per year. Given the availability of data, CCQ analysis was used instead of the St. George Respiratory Questionnaire (27), and BDI-PC instead of the Hospital Anxiety and Depression Scale (28). For comparative purposes in Figure 3, we projected the CCQ and BDI-PC scores of our study onto the SGRQ and HADS (Burgel's study) as follows. We divided the 50^{th}, 25^{th} and 75^{th} percentiles of the CCQ and BDI-PC by the score range (which is 6 and 21 respectively), and multiplied this with the score range of the SGRQ and HADS which is 100 and 42 respectively.

In the third cluster analysis, six new variables, NCSI QOL, NCSI satisfaction relationship, NCSI fatigue, air trapping, steps per day and NCSI activities of daily living were added (**Figure 1**).

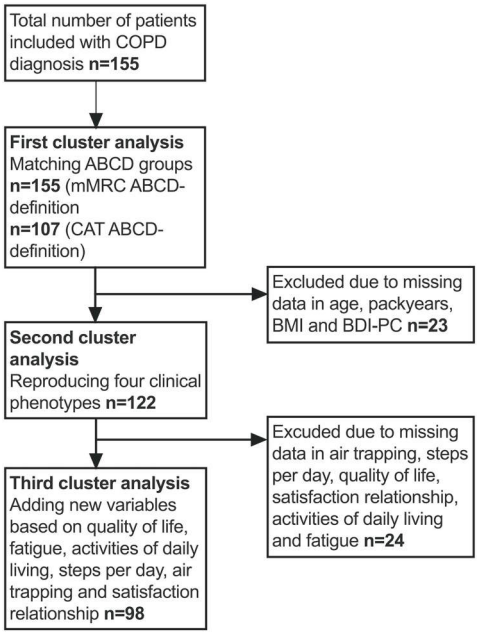

Figure 1. Flowchart of patient enrollment in the different cluster analyses.

RESULTS

Patient characteristics and ABCD classification

COPD Patients were divided into ABCD groups, based on the mMRC definition (n=155) or the CAT definition (n=107) (Shown in **Table 2** and **Suppl. Table 1**, respectively).

Using the mMRC-based classification, patients in group A (n=61) were ~53% male with a median age of ~62y [IQR 54-68y] and scored best on CCQ total score, BOD score, QOL, activities of daily living and fatigue. Patients in group B (n=37) were ~62% male with a median age of ~66y [IQR 60-71y]. All four GOLD stages of airflow limitation were represented in group B, ~11% of the patients were classified in GOLD stage I and ~6% in GOLD stage IV. In group C (n=31), the patients were ~55% female with a median age of ~62y [IQR 55-67y]. They had the lowest number of smoked pack years (PY) with a median value of 28 [IQR 19-50 PY], lowest depression score and most steps per day (median value 5743 [IQR 4473-6904]). Patients in group D were ~58% female with a median age of ~61y [IQR 52-70y]. All four GOLD stages of airflow limitation were represented in group D with ~12% GOLD stage I and ~24% stage IV. They scored worst on CCQ, depressions score, BOD score, QOL, activities of daily living and fatigue.

Using the CAT-based definition, patients in group A (n=15) were ~60% female with a median age of ~63 [IQR 59-68y]. They had the lowest FEV_1 with a median value ~49% of predicted [IQR 42-66] and only GOLD stage II and III were represented, resp. ~53% and ~47%. Patients in group B (n=51) were ~67% male with a median age of ~64 [56-68y]. They scored worst on activities of daily living, QOL, satisfaction relationship and had the highest number of PY with a median value of ~62 [IQR 51-74 PY]. The four GOLD stages of airflow limitation were represented in group B with ~18% GOLD stage I and ~4% stage IV. Patients in group C (n=2) included one male and one female, both GOLD stage II. Patients in group D (n=39) were ~54% female with a median age of ~62y [IQR 55-69y] and scored worst on CCQ total score, depression score and fatigue. The four GOLD stages of airflow limitation were represented in group D with ~21% GOLD stage I and ~15% stage IV.

First cluster analysis: relation to ABCD groups

We performed cluster analysis based on exacerbation numbers in conjunction with mMRC (n=155 patients) or CAT values (available in n=107 patients).

Using the mMRC scale questionnaires, four clusters (n=37, n=22, n=42, and n=54) were identified, which showed only limited parallels with the ABCD groups (**Table 2; Figure 2**). For the individual clusters, the largest contributing fraction of one of the ABCD groups was ~59-87%. By contrast, the four CAT-based clusters displayed a high

level of similarity with the ABCD groups: in each of the four clusters ~90-100% of the patients were classified as a single ABCD group (**Figure 2**, **Suppl. Table 1**): in clusters 1, 3 and 4 all patients fit in group D (CAT ≥10 and exacerbations ≥2), group A (CAT <10 and exacerbations <2) and group B (CAT ≥10 and exacerbations <2), respectively. Only for cluster 2 we found patients classified in two different ABCD groups: group D (~ 90%) and group C (~10%; (CAT <10 and exacerbations ≥2).

Table 2. Cluster analysis (ABCD groups) using the variables exacerbation number and mMRC

Cluster	**Group A**	**Group B**	**Group C**	**Group D**
Number	61	37	31	26
				Cluster number
1	0 ***)**	0	24 (65)	13 (35)
2	0	9 (41)	0	13 (59)
3	14 (33)	28 (67)	0	0
4	47 (87)	0	7 (13)	0
				Variables used in clustering**)
Exacerbations p/y	0 [0-1]	1 [0-1]	2 [2-3]	3 [3-3]
mMRC dyspnoea score	1 [0-1]	2 [2-3]	1 [0-1]	3 [2-4]
				Other patient and disease characteristics
Male/ female %	53/47	62/38	45/55	42/58
Age in years	62 [54-68]	66 [60-71]	62 [55-67]	61 [52-70]
Smoked PY	40 [21-50]	40 [25-63]	28 [19-50]	33 [26-55]
FEV1 % pred	62 [48-73]	52 [43-65]	63 [43-69]	54 [30-70]
GOLD stage %	15	11	23	12
1	59	50	52	44
2	26	33	22	20
3	0	6	3	24
4				
BMI kg/m²	26 [21-30]	31 [22-36]	27 [24-29]	24 [20-30]
CCQ total score	1.4 [0.8-2.5]	2.7 [1.9-3.3]	2.1 [1.6-2.7]	3.2 [2.7-3.9]
CAT	13 [9-18]	21 [16-24]	18 [13-23]	27 [21-28]
BDI-PC total score	1 [1-2]	1 [1-4]	1 [0-4]	3 [2-6]
BOD score	1 [1-3]	4 [3-5]	2 [1-3]	5 [4-7]
Steps per day	5261 [3863-8260]	4042 [2466-5733]	5743 [4473-6904]	3936 [2226-5777]
NCSI quality of life	13 [7-21]	20 [11-28]	14 [6-28]	32 [22-42]
NCSI satisfaction relationship	3 [2-4]	3 [2-5]	2 [2-4]	5 [3-6]
NCSI activities of daily living	8 [3-17]	22 [14-27]	12 [5-24]	28 [13-40]
NCSI fatigue	35 [28-41]	42 [38-48]	43 [35-49]	44 [38-51]

*) Data are presented as N (%) or median [25-75 interquartile], unless otherwise stated. PY: packyears, FEV1 % pred: Forced Expiratory Volume in 1 second percentage predicted, BMI: Body Mass Index, mMRC: Modified Medical Research Council, CCQ: Clinical COPD Questionnaire, BDI-PC: Beck Depression Inventory for primary care. *Definition of group A: mMRC<2 and exacerbations <2, group B: mMRC>=2 and exacerbations <2, group C: mMRC<2 and exacerbations>=2, group D: mMRC>=2 and exacerbations>=2

**) Hierarchical clustering is performed based on two variables: Exacerbation per/year and mMRC.

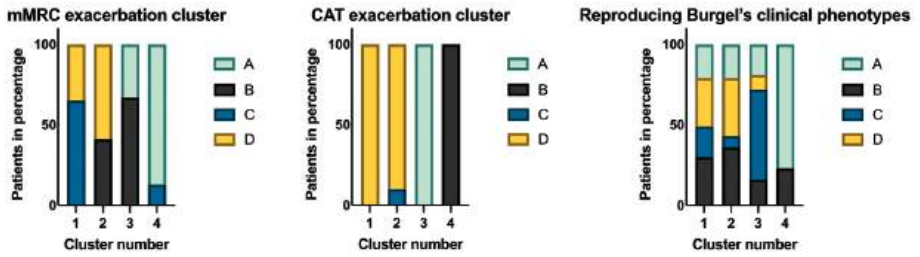

Figure 2. Overview of the cluster analyses categorized in ABCD groups. The three different cluster analyses (resp. used variables: mMRC and exacerbation frequency, CAT and exacerbation frequency and Burgels eight clinical variables) are shown categorized in group A, B, C and D. The definition of the ABCD group in the reproduction phenotype is based on mMRC. Definition ABCD groups: Group 'A' includes patients with mMRC 0-1 or CAT <10 and 0-1 exacerbation per year; group 'B' includes patients with mMRC ≥ 2 or CAT ≥ 10 and 0-1 exacerbation per year; group 'C' includes patients with ≥ 2 exacerbations or ≥ 1 exacerbation leading to hospital admission with mMRC 0-1 or CAT < 10 and group 'D' includes patients with ≥ 2 exacerbations or ≥ 1 exacerbation leading to hospital admission with mMRC ≥ 2 or CAT ≥ 10; mMRC: modified Medical Research Council; CAT: COPD Assessment Test.

Second cluster analysis: reproducing four clinical phenotypes

In the second analysis we aimed to reproduce Burgel's four clinical phenotypes and included 122 COPD patients from whom a complete set of the preselected variables (see Materials and Methods), was available (**Figure 1**).

The PCA transformed the eight original variables (see **Suppl. Table 2**) into independent components, the first four of which contained ~71% of the information and had an eigenvalue>1, which indicated that they contained more information than the average of the replaced variables. To PC1 the variables CCQ total score, BDI-PC, mMRC and exacerbations contributed the most. PC2 was predominantly based on age and BMI. PC3 reflected FEV1%pred and BMI and was independent of mMRC and number of exacerbations. Finally, PC4 was correlated with numbers of exacerbation and inversely correlated with smoking pack years and BDI-PC (see **Suppl. Table 2**).

Based on these four components, the four-cluster solution consisted of the following clusters: reproduction cluster 1 (n=37) with ~54% female and median age of ~64y [IQR 57-71y]. These patients had the lowest FEV1 % predicted ~39 [IQR 30-52] and lowest value of BMI ~21 [IQR 19-25]; reproduction cluster 2 (n=14) with 71% male and median age ~55 [IQR 48-61], had the highest number of PY ~50 [IQR 30-66] and scored worst on depression score, CCQ and QOL. Reproduction cluster 3 (n=32) was ~53% female with median age ~65 [58-72]. They had the lowest number of PY: ~22 [IQR 5-35] and the most exacerbations per year ~2 [IQR 1-3 PY]. Reproduction cluster

4 (n=39) with ~59% male and median age ~63 [57-68], scored best on mMRC, CCQ, exacerbations per year, depression score and activities of daily living.

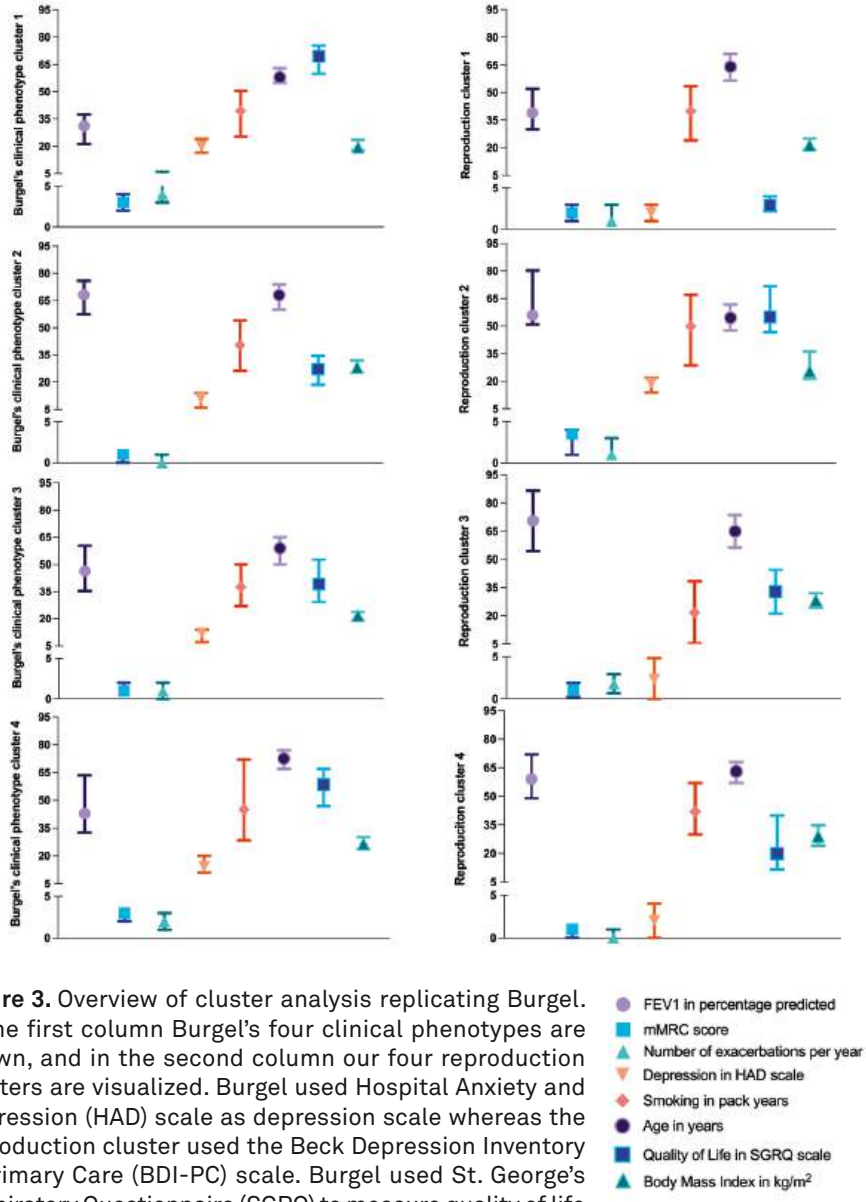

Figure 3. Overview of cluster analysis replicating Burgel. In the first column Burgel's four clinical phenotypes are shown, and in the second column our four reproduction clusters are visualized. Burgel used Hospital Anxiety and Depression (HAD) scale as depression scale whereas the reproduction cluster used the Beck Depression Inventory – Primary Care (BDI-PC) scale. Burgel used St. George's Respiratory Questionnaire (SGRQ) to measure quality of life (QOL) and the reproduction cluster used CCQ. To improve optical comparison between the two cohorts, the BDI-PC median [IQR] of the replication clusters are re-calculated in the range of the HAD scale and the CCQ median [IQR] re-calculated in the range of the SGRQ. Re-calculation is outlined in the Methods section.

Only one of our four clusters was comparable with one of the phenotypes of Burgel: reproduction cluster three appeared to be similar to phenotype two of Burgel et al. (6). The patient groups had a similar median age (~65y [IQR 58-72y] vs. 68y [IQR 60-74y]), mMRC score (1 [IQR 0.5-1.5] vs. 1 [0-1]), and BMI value (28 [25-31] vs 28.1 [25.2-31.9]). None of the three other clusters were comparable with the phenotypes of Burgel et al. All levels of mMRC and BOD scores were present across our groups. Our clusters were mainly separated by PY, BMI and depression scale (**Table 3; Figure 3**). None of the four clusters we identified matched with any of the ABCD groups (**Figure 2**).

Table 3. Cluster analysis reproducing four clinical phenotypes

	Reproduction cluster 1	**Reproduction cluster 2**	**Reproduction cluster 3**	**Reproduction cluster 4**
Number	37	14	32	39
				Variables used in clustering *)
Age in years	64 [57-71] **)	55 [48-61]	65 [58-71.5]	63 [57-68]
Smoked PY	40 [25-53]	50 [30-66]	22 [5-35]	42 [30-57]
FEV1 % pred	39 [30-52]	56 [51-79]	68 [62-80]	59 [49-72]
BMI kg/m²	21 [19-25]	26 [21-35]	28 [25-31]	29 [24-35]
mMRC dyspnoea score	2 [1-3]	3.5 [1-4]	1 [0.5-1.5]	1 [0-1]
CCQ total score	2.5 [2-3.2]	3.3 [2.8-4.3]	1.9 [1.3-2.7]	1.2 [0.7-2.4]
BDI-PC total score	1 [1-4]	9.5 [7-11]	1 [0-2.5]	1 [0-2]
Exacerbations p/y	1 [1-3]	1 [1-3]	2 [1-3]	0 [0-1]
			Other patient and disease characteristics	
Male/ female %	46/54	71/29	47/53	59/41
FVC% pred	87 [72-101]	89 [77-105]	99 [91-115]	93 [75-108]
GOLD stage %	0	21	25	10
1	35	64	66	64
2	46	7	9	26
3	19	7	0	0
4				
BOD score	4 [4-5]	5 [1-5]	2 [1-2]	2 [1-3]
Steps per day	5283 [3165-7116]	3032 [2277-3936]	5037 3416-6797]	5041 [3371-8371]
NCSI quality of life	19 [11-31]	57 [41-63]	14 [6-24]	12 [6-23]
NCSI satisfaction relationship	3 [2-5]	6 [4-7]	2 [2-3]	3 [2-4]
NCSI activities of daily living	22 [12 -30]	18 [9-28]	17 [5-29]	12 [3-18]
NCSI shortness of breath	12 [10-17]	16 [12-20]	10 [8-15]	10 [7-13]
NCSI fatigue	42 [37-47]	49 [38-55]	39 [32-50]	38 [29-45]
				Main corresponding ABCD groups
A	8 (21)	3 (21)	6 (19)	30 (77)
B	11 (30)	5 (36)	5 (16)	9 (23)
C	7 (19)	1 (7)	18 (56)	0 (0)
D	11 (30)	5 (36)	3 (9)	0 (0)

*) Hierarchical clustering is performed based on PCA of eight variables: age, packyears, FEV_1 % pred, BMI, mMRC, CCQ, BDI-PC, exacerbation per year.
**) Data are presented as N (%) or median [25-75 interquartile], unless otherwise stated. PY: packyears, FEV1 % pred: Forced Expiratory Volume in 1 second percentage predicted, FVC: Forced Vital Capacity, BMI: Body Mass Index, MMRC: Modified Medical Research Council, CCQ: Clinical COPD Questionnaire, BDI-PC: Beck Depression Inventory for primary care. Definition of group A: mMRC<2 and exacerbations <2, group B: mMRC>=2 and exacerbations <2, group C: mMRC<2 and exacerbations>=2, group D: mMRC>=2 and exacerbations>=2

Third cluster analysis: addition of six new variables based on behaviour

In the third cluster analysis, 98 patients with COPD were selected for which 14 variables were available: in addition to the eight variables described above (**Table 2**) we included the following six variables: QOL, fatigue, satisfaction relationship, air trapping, steps per day, and activities of daily living (**Figure 1**). The first five components of the PCA contained ~68.5% of the information (eigenvalue >1). Correlations between these five components and the fourteen variables are shown in **Suppl. Table 3**. Second, Ward's cluster analysis was performed, resulting in the identification of four new clusters (n=22, n=26, n=24, n=26) that mainly differed from each other in the number of steps per day, NCSI QOL and NCSI activities of daily living (**Table 4, Figure 4**).

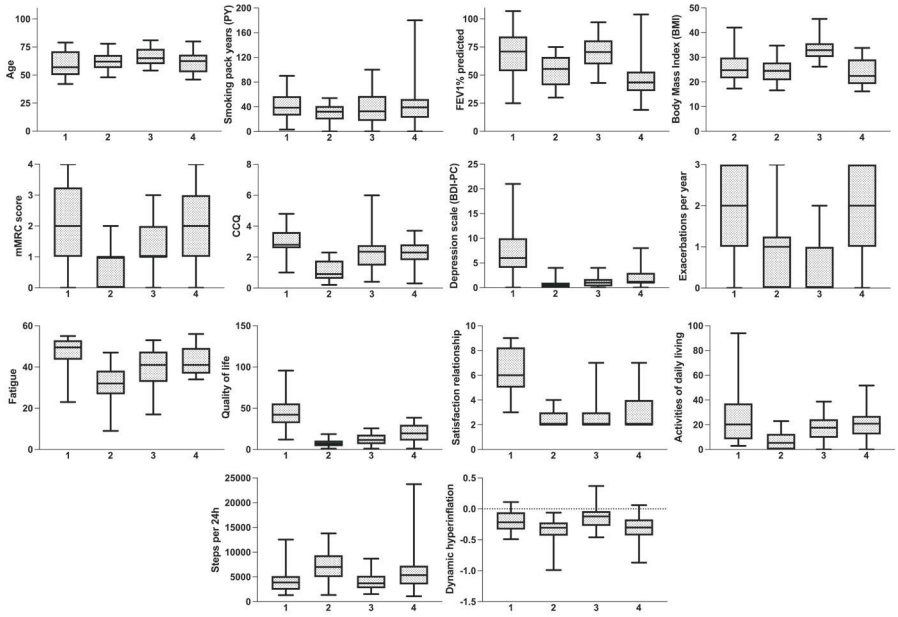

Figure 4. Overview of cluster analysis behavioral variables included. Six new variables; NCSI QOL, NCSI satisfaction relationship, activities of daily living, fatigue, air trapping and steps per day were added to the eight clinical variables of Burgel. These added variables altered the clusters substantially and led to the formation of four clusters that mainly differed from each other on non-physiological parameters. The four behavioral clusters are represented in the X-axis.

The variables age, PY, mMRC and NCSI satisfaction relationship and NCSI fatigue largely overlapped across the four clusters. Cluster 1 was defined by worse scores on NCSI QOL, NCSI satisfaction relationship, NCSI activities of daily living and NCSI fatigue. Cluster 2 was defined by the best scores on CCQ, depression scale, NCSI QOL,

NCSI activities of daily living and most median steps per day 7010 [IQR 5063-9343]. Cluster 3 was characterized by the highest BMI values (33 [IQR 30-36]), low levels of NCSI QOL 11.5 [6.7-17.9] but the lowest levels of dynamic hyperinflation measured in liters decreasing inspiratory capacity after bronchodilation (-0.12 [IQR -0.27- -0.04L]). Cluster 4 was defined by the highest level of NCSI activities of daily living (20.9 [12.3-26.0]). The new clusters with extra variables were not comparable to the clusters from the second cluster analysis reproducing clinical phenotypes.

Table 4. Cluster analysis behavior variables included

Cluster	**1**	**2**	**3**	**4**
Number	22	26	24	26
Variables used in clustering *)				
Age in years	57 [50-71] **)	62 [57-68]	65 [60.5-73]	63 [53-68]
Smoked PY	39 [26-57]	32 [20-40]	33 [19-55]	39 [23-50]
FEV1 % pred	71 [54-84]	56 [42-66]	71 [60-80]	44 [36-53]
Dynamic hyperinflation post (air trapping)	-0.22 [-0.31- -0.06]	-0.31 [-0.41- -0.22]	-0.12 [-0.27 – -0.04]	-0.3 [-0.43- -0.17]
BMI kg/m²	25 [23-29]	25 [21-28]	33 [30-36]	22 [19-29]
MMRC dyspnoea score	2 [1-3]	1 [0-1]	1 [1-2]	2 [1-3]
CCQ total score	2.8 [2.6-3.6]	0.9 [0.6-1.7]	2.4 [1.5-2.8]	2.3 [1.8-2.8]
BDI-PC total score	6 [4-10]	0.5 [0-1]	1 [0.5-1.5]	1 [1-3]
Exacerbations p/y	2 [1-3]	0.5 [0-1]	1 [0.5-1.5]	1 [1-3]
Steps per day	3885 [2442-5147]	7010 [5063-9343]	3727.5 [2781-5066]	5361 [3516-7221]
NCSI quality of life	42.1 [32.1-55.6]	7.4 [4.4-10.4]	11.5 [6.7-17.9]	19.6 [11-29.7]
NCSI satisfaction relationship	6 [5-8]	2 [2-3]	2 [2-3]	2 [2-4]
NCSI activities of daily living	20.3 [8.8-36.9]	5.4 [0-12.7]	17.6 [10.5-23.5]	20.9 [12.3-26.0]
NCSI fatigue	49.5 [44-53]	32 [27-38]	41 [33.5-47]	41 [37-49]
Other patient and disease characteristics				
Male/ female %	55/45	38/62	67/33	46/54
FVC% pred	0.895 [0.83-1.05]	1.03 [0.87-1.11]	0.9 [0.84-1.01]	0.96 [0.76-1.04]
GOLD stage %	27	0	25	7
1	59	62	67	31
2	9	38	8	46
3	4	0	0	15
4				
BOD score	2 [1-5]	2 [1-3]	2 [1-3]	4 [3-5]

*) Hierarchical clustering is performed based 5 components with an eigenvalue >1 with variables: age, packyears, FEV_1 % pred, BMI, mMRC, CCQ, BDI-PC, number of exacerbations per year, steps per day, QoL, satisfaction relationship, activities of daily living, fatigue, and dynamic hyperinflation. **) Data are presented as N (%) or median [25-75 interquartile], unless otherwise stated. PY: packyears, FEV1 % pred: Forced Expiratory Volume in 1 second percentage predicted, FVC: Forced Vital Capacity, BMI: Body Mass Index, MMRC: Modified Medical Research Council, CCQ: Clinical COPD Questionnaire, BDI-PC: Beck Depression Inventory for primary care. BOD: Body mass index, airflow Obstruction and Dyspnea score.

DISCUSSION

In a real-world COPD cohort, we were essentially able to identify the GOLD ABCD groups based on CAT, but only one of the four clinical phenotypes described by Burgel et al. (6). The addition of the six new variables QOL, fatigue, satisfaction relationship, air trapping, steps per day and activities of daily living to the clinical variables, resulted in the formation of four new clusters that did not match the original clinical phenotypes. The new clusters mainly differed in QOL and physical activity, while the previously formed clusters based on clinical variables were very heterogeneous in satisfaction relationship, fatigue, QOL, air trapping, steps per day and activities of daily living.

Burgel et al. suggested it is important to apply PCA and cluster methodology to other COPD cohorts to examine whether similar, or different COPD phenotypes can be identified in different populations. (6). In our cohort, we first attempted to reproduce clinical COPD phenotypes before we added new variables. At first, we clustered based on ABCD GOLD criteria. We noticed that CAT provided a better prediction of the ABCD groups than mMRC, which may be explained by the fact that CAT is a more extensive questionnaire for dyspnea. Second, when we aimed to reproduce the clinical COPD phenotypes as identified by Burgel et al. (6), our clusters were mostly separated by PY, BMI and depression scale. These variables are very different from those that differentiated the original clusters in the report by Burgel et al. (age, airflow limitation and symptoms). It remains unclear why we could not reproduce the clusters of Burgel et al., in our study. Possible explanations include (i) unmeasured confounding factors, (ii) greater heterogeneity in the COPD population, or (iii) the use of slightly different questionnaires.

Not only Burgel et al. used cluster analysis to identify clinical COPD phenotypes. Cluster analysis was previously applied to predict the first acute COPD exacerbation (29); clusters were identified on the basis of lung function assessment (30) and comorbidity clusters related to inflammatory markers were formed (31). Although these studies all use similar cluster methods, the clinical variables that were used differed. Clustering on exacerbation type or comorbidities can have clinical value when the clusters are reproducible or correlate with applicable longitudinal data. A longitudinal study based on comorbidity clusters (31) was performed, to associate the changes in exercise performance and health status after pulmonary rehabilitation (32). This study showed that none of the comorbidity clusters influence the likelihood of clinically meaningful change in exercise performance and health status following pulmonary rehabilitation. The authors conclude that comorbidities in COPD patients should not preclude patients from following pulmonary rehabilitation. Clustering on lung function assessment resulted in seven different clusters (30). However, based on health status these clusters could not be differentiated from each other because of small differences in mMRC and CCQ (30). These cluster analyses illustrate the heterogeneity across individual COPD

cohorts and the complexity of the identification of COPD phenotypes. In another study the variables used were comparable with our study; COPD phenotypes were clustered according to levels of physical activity, body composition, health related quality of life (HRQoL) and sedentary behavior (33). Three groups were identified. Phenotype 1 was more physically active and less sedentary compared with phenotype 2 and 3. Phenotype 2 was older and phenotype 3 had worse HRQoL and body composition. Lung function did not differ across the three phenotypes. These results are in line with our behavioral clustering results **(Table 2)**. However, inclusion of these variables in the cluster analysis changed the previously formed clusters based on the clinical variables of Burgel **(Table 3)**, which demonstrates that these clusters are not stable. A study in a COPD population discriminating on asthma, emphysema and chronic bronchitis symptoms, the main phenotypes were recognized by easy to obtain clinical characteristics such as smoke exposure and questionnaires on complaints (34). In parallel to Burgel's study, we also excluded patients diagnosed with asthma-COPD overlap syndrome, in order to prevent clustering based on smoke exposure and symptom severity. We used the study of Burgel et al. as a reference to reproduce clinical phenotypes, because of a good matching with our clinical variables and because these phenotypes focus on treatable traits instead of future risk factors (35).

The first strength of the study is that we used the same method to form the hierarchical clusters as Burgel did. Second, as we did not use specific exclusion criteria for our real-world COPD patient cohort, our results are expected to have a good external validity for patients in secondary care. All patients with COPD were diagnosed by a pulmonologist. The data we collected were routinely available in daily practice.

Some limitations need to be mentioned. It remains challenging to directly compare our clustering analysis to the clustering by Burgel. First, although the cluster methodology was identical, we did not have exactly the same variables as Burgel et al. We used two different questionnaires: the Clinical COPD Questionnaire (CCQ) instead of the St. George Respiratory Questionnaire (SGRQ), and the Beck Depression Inventory (BDI-PC) instead of the Hospital Anxiety and Depression Scale (HADS). However, the two questionnaires to measure depression – HADS and BDI-PC– are highly correlated (21, 28). The CCQ and SGRQ are disease-specific questionnaires that measure shortness of breath, amongst others, and are also highly correlated (19, 27, 36). Compared to the CCQ, the SGRQ is more extensive and includes QOL-related questions. It is possible that these differences in the questionnaires explain the inability to reproduce the clinical clusters, however we would expect more similarity between the clusters because the residual six variables were identical. A second limitation is the small sample size. In the second analysis, 8 variables were used for clustering in a sample of 122 patients, compared to 322 patients in the study of Burgel et al. (6). Only patients with a complete set of variables could be included in our cluster analyses, which resulted in a small

sample size. Phenotyping based on cluster analysis may improve when the number of included patients increase or more suitable variables are added. Third, there may be critical differences between the two cohorts. We included ~48% females, whilst Burgel et al. included ~23% females. Perez et al. showed that female COPD patients are younger, have lower pack-years, higher FEV1%, lower BMI and exacerbate more often (37). Moreover, the clinical characteristics of the population of COPD patients may well differ across different medical centers. Newly formed clinical phenotypes need longitudinal validation to determine how they are associated with important clinical outcomes of disease progression or mortality, before conclusions on their clinical relevance can be drawn (3).

In our study, clinical characteristics were used in an attempt to identify clusters as a step towards tailored treatment strategies per subgroup of COPD patients. The inability to reproduce earlier reported clusters in our real-world COPD population questions the relevance of clustering approaches for clinical practice. Clinical practice seems to call for personalized medicine (38), given the heterogeneity of the COPD population even within clusters. Individual patient characteristics should be the main focus to improve clinical outcomes and minimize unnecessary side effects for individual patients with COPD (39). In this context, it may be more productive to develop personalized medicine approaches based on treatable traits (40, 41), than on clinical phenotypic characteristics.

CONCLUSION

In this study we used statistical clusteranalyses in a real-world COPD cohort to identify subgroups of patients. Hereby, patients could be divided into clusters that largely reflected the GOLD ABCD groups. By contrast, we could not reproduce the four clinical phenotypes identified by Burgel et al. in our cohort on the basis of a series of 8 variables that were essentially the same as those used by Burgel et al. (6) The addition of six new variables, air trapping, steps per day, QOL, satisfaction relationship, activities of daily living and fatigue, altered the clusters substantially and led to the formation of four clusters that were separated mainly by these behavioral parameters. We conclude that heterogeneity in the COPD population calls for a personalized medicine approach that is not based on the stratification of patients into subgroups but rather on individual characteristics.

SUPPLEMENTARY

Suppl. Table 1. Cluster analysis (ABCD groups), using the variables exacerbation number and CAT

	Group A	Group B	Group C	Group D
Number	**15**	**51**	**2**	**39**
Cluster numbers				
1	0 *)	0	0	21 (100)
2	0	0	2 (100)	18 (90)
3	15 (100)	0	0	0
4	0	51 (100)	0	0
*Variables used in clustering**)*				
Exacerbations p/y	0 [0-1]	0 [0-1]	3 [2-3]	3 [2-3]
CAT	7 [4-9]	19 [15-23]	2 [0-3]	22 [16-26]
Other patient and disease characteristics				
Male/ female %	40/60	67/33	50/50	46/54
Age in years	63 [59-68]	64 [56-68]	68 [64-71]	62 [55-69]
Smoking PY	34 [20-45]	44 [29-58]	10 [0-20]	32 [23-50]
FEV1 % pred	49 [42-66]	62 [51-74]	70 [69-70]	56 [35-68]
GOLD stage %	0	18	0	21
1	53	55	100	41
2	47	23	0	23
3	0	4	0	15
4				
BMI kg/m^2	23 [21-25]	29 [23-35]	27 [25-29]	26 [21-29]
CCQ total score	0.7 [0.6-1.1]	2.4 [1.6-2.8]	0.6 [0.4-0.8]	2.5 [1.8-3.3]
mMRC	0 [0-1]	1 [1-2]	0.5 [0-1]	1 [1-3]
BDI-PC total score	1 [0-2]	1 [1-4]	0 [0-0]	3 [1-6]
Steps per day	6832 [5063-9522]	4115 [2605-5723]	8230 [2658-13801]	5147 [4016-6772]
BOD score	2 [1-3]	3 [1-4]	0.5 [0-1]	3 [2-5]
NCSI quality of life	10 [6-18]	20 [10-32]	3 [1-5]	20 [12-34]
NCSI satisfaction relationship	2 [2-3]	3 [2-6]	2 [2-2]	3 [2-5]
NCSI activities of daily living	7 [0-13]	16 [5-25]	3 [0-5]	13 [8-27]
NCSI fatigue	29 [26-35]	41 [35-46]	24 [9-38]	44 [36-50]

*) Data are presented as N (%) or median [25-75 interquartile], unless otherwise stated. PY: packyears, FEV1 % pred: Forced Expiratory Volume in 1 second percentage predicted, BMI: Body Mass Index, MMRC: Modified Medical Research Council, CCQ: Clinical COPD Questionnaire, BDI-PC: Beck Depression Inventory for primary care. *Definition of group A: CAT<10 and exacerbations <2, group B: CAT>=10 and exacerbations <2, group C: CAT<10 and exacerbations>=2, group D: CAT>=10 and exacerbations>=2

**) Hierarchical clustering is performed based on two variables: Exacerbation per/year and CAT.

Suppl. Table 2. Principal component analysis of clinical variables

	Principal component			
	PC1 *)	**PC2 *)**	**PC3 *)**	**PC4 *)**
Age	-0.1487	0.6463	-0.0719	0.3544
Smoking (PYs)	0.1966	0.2990	-0.1077	-0.6987
FEV1% pred	-0.1512	-0.2199	0.7503	0.1070
BMI	-0.0467	0.4748	0.6053	-0.1287
BDI-I-PC	0.4949	-0.2667	0.2037	-0.2378
mMRC	0.4832	0.3527	-0.0004	0.1873
Exacerbations p/y	0.3525	-0.1388	-0.0255	0.5092
CCQ total	0.5582	0.0657	0.1084	0.0859

*) Contribution of principal component: resp. 26%, 17%, 15%, 13%.

Suppl. Table 3. Principal component analysis behavior variables included

	Principal component				
	PC1 *)	**PC2 *)**	**PC3 *)**	**PC4 *)**	**PC5 *)**
Age	-0.1335	0.4328	-0.1464	0.0452	-0.0779
Smoked (PYs)	0.1165	-0.0492	-0.1913	-0.5311	0.5024
FEV1% pred	0.0401	0.1079	0.6542	0.1215	0.0655
BMI	-0.0251	0.4665	0.2403	-0.2532	0.2315
BDI-I-PC	0.4480	-0.1823	0.0788	-0.1307	-0.2345
mMRC	0.2813	0.3172	-0.3111	0.0981	-0.0024
Exacerbations p/y	0.2077	-0.0783	-0.1391	0.5359	0.1493
CCQ total	0.3377	0.1639	-0.0066	0.0955	0.0743
Dynamic hyperinflation post	0.0093	0.3296	0.4517	0.1404	-0.0365
Steps per day	-0.1638	-0.3649	0.1061	0.4228	0.3896
NCSI quality of life	0.4620	-0.1795	0.1323	-0.1003	-0.1467
NCSI satisfaction relationship	0.3685	-0.1956	0.2317	-0.1356	-0.0147
NCSI activities of daily living	0.2944	0.2999	-0.2173	0.2753	-0.1457
NCSI fatigue	0.2579	0.1092	-0.0133	0.1058	0.6362

*) Contribution of principal component: resp. 26%, 14%, 11%, 10%, 7.5%.

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Chapter 3

Association between elevated serum triglycerides and asthma in patients with obesity: an explorative study.

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ABSTRACT

Background

Adults with a high BMI have an increased risk of developing asthma. To explore the impact of increased lipids on the presence of asthma, this study investigated the relationship between lipid levels and inflammatory markers in asthma patients and controls with obesity.

Objective

We hypothesized: higher lipid levels are more prevalent in obese asthma patients.

Methods

In this explorative cohort study 96 asthma patients and 45 controls were included. All patients participated in one of three asthma studies; two of these studies included only patients with obesity. Asthma diagnosis was defined by the presence of typical clinical symptoms, reversible airway obstruction (+12% improvement in FEV1 after bronchodilator), or bronchial hyperreactivity (PC20 < 8mg/ml), or a FeNO > 50 ppb. We compared lipid levels and neutrophils and eosinophils in patients with asthma and controls with a wide BMI range [17.8-63.8 kg/m^2]. Multivariable logistic regression was used to analyze the data.

Results

Serum triglycerides were statistically significant higher in obese asthma patients, adjusted for BMI, blood eosinophils and statin use (OR 2.56, 95% CI 1.34- 4.88*; p*=0.004*)*. *Inclusion or exclusion of LABA and ICS users led to comparable adjusted ORs for blood triglyceride and blood eosinophils levels.*

Conclusion

Elevated serum triglycerides are associated with the presence of asthma in obese patients. This indicates that elevated triglycerides might be a yet unrecognized trait that contributes to the development of asthma. The precise cause and effect of these high triglyceride levels in asthma patients with obesity are not determined in this study.

Trial numbers NL4262, NL3056 and NCT03278561.

INTRODUCTION

Asthma is a chronic heterogeneous disease characterized by bronchial hyperresponsiveness and airway inflammation. Increased BMI in adults is known to be related to the risk of asthma development (1). A meta-analysis of 300,000 asthma patients showed the odds ratio of asthma was 1.38 in the overweight group and 1.92 in the obese group compared to the normal weight group (2). Epidemiological studies distinguish two phenotypes of obese asthma: 1) late-onset asthma (asthma symptoms started after the age of 18 years), characterized by low markers of allergic inflammation, and improvement of lung function after weight loss, "asthma because of obesity", and 2) early-onset asthma (symptoms started before the age of 18) characterized by higher markers of allergic inflammation "asthma complicated by obesity" (3). Body composition and inflammation independently play a role in decreasing lung function in obese asthma patients, so it is hypothesized that systemic inflammation may play a role in initiating asthma symptoms in obese patients (4). However, the pathophysiological link between systemic inflammation, lipids and asthma is still unclear. To explore the impact of increased lipids on the presence of asthma, we compared lipid levels and neutrophils and eosinophils in patients with asthma and controls with a wide BMI range. We corrected for inhaled corticosteroids (ICS) to determine any effect of ICS on body weight and lipid levels. We hypothesized that obese asthma would be associated with higher lipid levels.

MATERIALS AND METHODS

Our design was a cross-sectional study. Anonymized data were obtained from three clinical studies conducted in the Franciscus Gasthuis and Vlietland Hospital, Rotterdam, the Netherlands (NL4262, NL3056 and NCT03278561 executed between 2011 and 2018). The Medical Ethics Committees United (MEC-U, formerly tWoR) approved the three studies and all patients signed informed consent (NL25637.101.08, NL46602.101.13, NL60437.101.17).

Included were all asthma patients (no distinction is made in late-onset or early-onset asthma) and non-asthma subjects (controls) and all were non-smokers or (former) smokers with a smoking history of less than 10 pack years or smoking less than 10 cigarettes a day. Asthma diagnosis was based on the presence of typical clinical symptoms, reversible airway obstruction (+12% improvement in FEV1 after bronchodilator), or bronchial hyperreactivity (PC20 < 8mg/ml), or a FeNO > 50 ppb (5). All patients of the three studies were included to minimize selection bias. Two of the three studies included only patients with obesity. Inclusion criterion of one study was BMI >30 kg/m^2 and the other BMI >35kg/m^2. In the third study, BMI was no inclusion criterion. Controls did not have a history of asthma or any other chronic lung disease.

BMI was not an inclusion criterion. Data of venous blood samples were used to analyze systemic inflammation and non-fasting lipid profiles.

Having asthma was the dependent variable. First, univariate logistic regression was used for preselection of candidate covariables ($p<0.3$). Multiple logistic backwards stepwise logistic regression was used to adjust the relationship between asthma, serum triglycerides and all other preselected variables. To avoid overestimation, a maximum of five variables was included (about 10% of sample size). A p-value of <0.05 two-sided was considered as statistically significant.

Inhaled corticosteroids (ICS) and Long Acting Beta2-Agonist (LABA) were not included in the univariate logistic regression because the use of these medicines is a consequence of the asthma diagnosis and not a cause. Differences in triglyceride levels between asthma patients who used LABA and/or ICS and who did not use maintenance medication was tested with a second multiple logistic backwards stepwise logistic regression. The impact of ICS and LABA was evaluated by stratified multiple logistic regression: the impact of serum triglyceride levels on presence of asthma including the users of LABA and ICS vs. the impact of serum triglyceride levels on presence of asthma excluding the users of LABA and ICS.

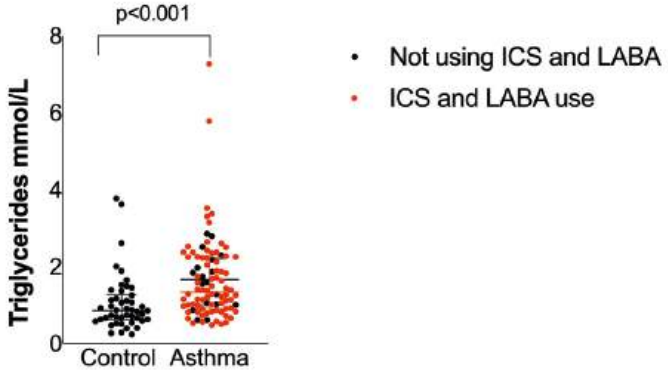

Figure 1. Triglyceride levels and ICS and LABA use in asthma and controls

Table 1. Characteristics of the study population

Variables	*Asthma*	*Control*	*P-value*	*Asthma and no ICS and LABA use*	*Asthma with ICS and LABA use*
Numbers	96	45		19	77
Sex males N (%)	23 (24)	13 (29)	0.58	6 (32)	17 (22)
Age in years (II)	41.0 [30.0-50.0]	36.5 [26.0-44.5]	0.02	30.0 [25.0-42.3]	44.0 [31.0-54.0]
BMI in kg/m² (II)	35.9 [30.9-42.9]	41.7 [32.5-47.3]	0.06	44.5 [40.9-46.5]	33.2 [30.1-40.1]
BMI category N (%) <30 kg/m²	17 (18)	11 (24)		-	17 (22)
30-40 kg/m²	43 (45)	6 (13)		3 (16)	40 (52)
>40 kg/m²	36 (38)	28 (62)		16 (84)	20 (26)
Smoking status N (%) Never smoked	67 (70)	34 (76)	0.68	12 (63)	55 (72)
Ex-smoker	19 (20)	8 (18)		2 (11)	17 (22)
Active smoker	10 (10)	3 (6)		5 (26)	4 (6)
Statin use N (%)	4 (4)	6 (13)	0.08	0 (0)	4 (5)
FEV_1 post % (I)	91.61 ± 14.51	102.11 ± 12.75	<0.001	92.39 ± 11.00	91.04 ± 15.10
Neutrophils absolute x 10^9/L (II)	4.4 [3.2-5.6]	4.0 [3.2-5.4]	0.55	4.65 [3.6-5.7]	4.20 [3.0-5.4]
Eosinophils absolute x 10^7/L (II)	200 [100-300]	100 [100-200]	0.02	150 [100-230]	200 [100-320]
LDL-cholesterol mmol/L (I)	3.04 ± 0.90	3.07 ± 0.84	0.81	3.02 ± 0.93	3.05 ± 0.90
HDL-cholesterol mmol/L (II)	1.30 [1.1-1.5]	1.30 [1.2-1.5]	0.68	1.15 [1.00-1.30]	1.4 [1.1-1.6]
Total cholesterol mmol/L (I)	5.12 ± 1.04	4.95 ± 0.99	0.57	4.98 ± 1.08	5.1 ± 1.03
Triglycerides in mmol/L (II)	1.40 [0.93-2.25]	0.86 [0.63-1.31]	<0.001	1.67 [1.02-2.22]	1.29 [0.90-2.23]
LABA use N (%)	48 (50)	0 (0)	<0.001	0 (0)	48 (63)
ICS use N (%)	77 (80)	0 (0)	<0.001	0 (0)	77 (100)
High dose ICS (>800 µg)	6 (6)	-		-	6 (8)
Medium dose ICS (400-800 µg)	40 (42)	-		-	40 (52)
Low dose ICS (<400 µg)	13 (13)	-		-	13 (17)
Unknown dose	18 (19)	-		-	18 (23)
No ICS use	19 (20)	-		-	-

(I) mean±SD in normal distribution, (II) median [iqr Q1-Q3] used in non-normal distribution N: numbers, SD: standard deviation, iqr: interquartile range. BMI: body mass index, FEV1: Forced Expiratory volume in 1 second, LDL-cholesterol: Low Density Lipoprotein cholesterol, HDL-cholesterol: High Density Lipoprotein cholesterol, ICS: inhaled corticosteroids, LABA: Long Acting Beta2-Agonist.

Table 2. Univariate pre-adjusted and multivariate post-adjusted outcome for asthma

	All asthma patients included in the analyses				Asthma without ICS and LABA	
	Univariate pre-adjusted		Multivariable post-adjusted P>0.3 selected for exclusion		Multivariable post-adjusted	
Variables	**OR, 95% CI**	**P-value**	**OR, 95% CI**	**P-value**	**OR, 95% CI**	**P-value**
Age	1.042, 1.010-1.075	0.010	1.038, 0.997-1.080	0.069	0.976, 0.911-1.046	0.490
Sex	1.250, 0.565-2.768	0.582	-	-	-	-
BMI	0.970, 0.934-1.007	0.113	0.957, 0.914-1.001	0.054	1.043, 0.961-1.133	0.315
Statin use	0.289, 0.077-1.081	0.065	0.118, 0.025-0.552	0.007	-	-
Smoking		0.381	-	-	-	-
HDL-cholesterol	0.843, 0.288-2.468	0.755	-	-	-	-
LDL-cholesterol	0.949, 0.628-1.434	0.804	-	-	-	-
Triglyceride	2.769, 1.517-5.053	0.001	2.558, 1.340-4.881	0.004	3.013, 1.295-7.010	0.010
Total cholesterol	1.109, 0.780-1.576	0.566	-	-	-	-
Eosinophils absolute	1.036, 1.007-1.066	0.016	1.039, 1.006-1.073	0.021	1.025, 0.978-1.073	0.301
Neutrophils absolute	1.110, 0.893-1.379	0.346	-	-	-	-

OR: Odds ratio, CI: Confidence interval, BMI: body mass index, LDL-cholesterol: Low Density Lipoprotein cholesterol, HDL-cholesterol: High Density Lipoprotein cholesterol. In the subgroup Asthma without ICS and LABA none of the patients used statins.

RESULTS

A total of 96 patients with asthma and 45 controls were included. Patient characteristics are shown in **table 1**. Patients with asthma were significantly older, had a lower FEV_1 and had significantly higher serum levels of triglycerides and eosinophils, compared to controls. The median BMI in asthma was 35.9 kg/m^2 vs. 41.7 kg/m^2 in controls.

Table 2 shows the results of the multivariable post-adjusted logistic regression. After adjusting for age, BMI, and statin use, higher blood triglyceride levels as well as higher blood eosinophil levels were significant factors associated with an increased risk for presence of asthma (adjusted *OR 2.56, 95% CI 1.34- 4.88,* p= *0.004;* and adjusted OR 1.04 with 95% CI *1.01-1.07*, p=0.021).

Table 1 also shows the patient characteristics of the asthma groups with and without ICS and LABA use. Patients without LABA and ICS had higher BMI, were younger and did not use statins. Excluding LABA and ICS users showed the following results; higher blood triglyceride levels (*OR 3.01, 95% CI 1.30- 7.01*; p= *0.010)* and higher blood eosinophils (OR 1.03, 95% CI *0.98-1.07;* p=0.021) were associated with presence of asthma

adjusted for age and BMI. (**Figure 2)** Inclusion or exclusion of LABA and ICS users led to comparable adjusted ORs for blood triglyceride and blood eosinophils levels. **Figure 1** shows the differences in triglyceride levels in controls and asthma patients, with or without ICS and/or LABA use.

DISCUSSION

In this study lipid levels and inflammatory markers in asthma patients with a wide BMI range were compared to controls within the same BMI range. We have demonstrated that levels of triglycerides were statistically significant elevated in asthma patients with obesity compared to controls with obesity, after adjusting *for BMI, statin use and eosinophils.*

Our results are in line with Ko et al. (6), in which a relation between triglycerides/HDL ratio and asthma prevalence in non-obese asthma patients is suggested, despite the fact that results were not adjusted for sex and other confounders.

There is limited literature on the relationship between triglycerides and asthma available. Lu et al. (7) did not find a difference in triglyceride levels in serum in children and adolescents with asthma compared to controls. Su et al. (8) studied the association between lipid profile and the prevalence of asthma in a meta-analysis. Levels of LDL-cholesterol and total cholesterol were significantly higher in patients with asthma compared to non-asthmatic patients (p<0.001 and p=0.002). Triglycerides were not associated with the presence of asthma (p=0.503). The population analyzed in this meta-analysis clearly differed from our study. In this study of Su et al. also children

were included, the mean BMI of the cohort was between 16.1 and 28.2 kg/m^2 and only in 1 of the 16 studies non-fasting serum lipid levels were analyzed. Vinding et al. (9) studied triglyceride levels in children with age 5-7 years old and with a mean BMI of 16.09 kg/m^2. This cohort is not completely comparable to our cohort since our cohort consists of adults only. The result was that triglyceride levels were not statistically significant elevated in asthma patients (p=0.61). Fang et al. (10) compared obese asthmatic children to non-obese asthmatic children. There were no significant differences in the levels of triglycerides, LDL-cholesterol and total cholesterol between the groups. Controls were not included in this study.

In this study, eosinophils and triglycerides were both statistically significant elevated in asthma patients with obesity compared to controls with obesity, adjusted for BMI and age. Moussa et al. (11) did a similar observation in patients with metabolic syndrome, who had increased eosinophils in subcutaneous adipose tissue. To diagnose metabolic syndrome three out of five ATP III criteria must be fulfilled, one of which being elevated serum triglycerides (cut-off 1.7 mmol/L) (12).

Our study has the following limitations: in this cross-sectional study the controls and asthma patients were mainly women with asthma, which may have influenced the results. However, this is in line with the literature showing that adult asthma is more common in women (13). In addition, it should be noted that the control group had a higher median BMI compared to asthma patients (41.7 kg/m^2 vs. 35.9 kg/m^2), and that the distribution of BMI in our cohort was skewed to the right. Based on a post hoc sample size calculation, the relatively small sample size is sufficient to support the conclusion. Assuming an estimated OR of 2.558 (see Table 2), an asthma/control ratio of 2.13 (see Table 1), an expected prevalence of the outcome in the control group of 25%, a 95%CI of the estimated OR (see Table 2) and a relative precision of 55%, 44 control patients and 94 asthma patients needed to be included in our analysis to obtain a statistically significant result. It is unlikely that using inhaled corticosteroids and long acting B-2 agonist (LABA) seriously affected the association between triglycerides and asthma, as Table 2 shows. Moreover, the doses of ICS and LABA were low. Patients using maintenance oral steroids were not included in this study. However, occasional use of oral steroids, which are the treatment for asthma exacerbations, are not taken into account. The use of occasional oral steroids can not be ruled out as a possible explanation for the elevated triglyceride levels in asthma patients. Thereby, Deng et al. showed visceral obesity increases the risk of an exacerbation with 1.55 for moderate exacerbations and 2.25 for severe exacerbations, compared to patients without visceral obesity (14). However, oral steroids used for asthma exacerbations are prescribed for a short term (5-7 days). Thereby, asthma exacerbations are overall not common. In the study of Suruki et al. 430,000 asthma patients were included with a follow-up time of 12 months, in which respectively 12.5% had at least one exacerbation (15).

Including the effect of visceral obesity on exacerbations, asthma patients would use oral corticosteroids only 10-14 days a years. This should have a minimal effect on our study outcome.

The period and reason of statin use was undescribed in our cohort. Also, statins were used only in a limited number of subjects and asthmatics were less likely to be statin users than the controls. Still, the risk of bias is low since excluding all statin users from the data analysis did not influence the odds ratio of triglycerides in asthma patients (OR = 2.81).

CONCLUSION

This study shows elevated levels of triglycerides in asthma patients with obesity, compared to controls with obesity and independently of ICS and LABA use. This may suggest that elevated triglycerides are a yet unrecognized trait that contributes to the development of asthma, especially since triglycerides are not elevated in obese controls without asthma. The precise cause and effect of these high triglyceride levels in asthma patients with obesity are not determined in this study. Future studies in obese asthma should include a larger study population comprising at least three sufficiently large groups; 1) asthmatics with obesity, 2) asthmatics without obesity and 3) controls with obesity in order to clarify the role of triglycerides in asthma and to ascertain triglyceride as a possible therapeutic target in asthma.

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Chapter 4

Aberrant characteristics of peripheral blood innate lymphoid cells in COPD, independent of smoking history

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ABSTRACT

Background

Distinguishing asthma and chronic obstructive pulmonary disease (COPD) can pose challenges in clinical practice. Increased group 1 innate lymphoid cells (ILC1s) have been found in the lungs and peripheral blood of COPD patients, while asthma is associated with elevated levels of ILC2s. However, it's unclear whether the inflammatory characteristics of ILC1s and ILC2s differ between COPD and asthma. This study aims to compare peripheral blood ILC subsets and their expression of inflammatory markers in COPD patients, asthma patients, and controls.

Methods

The study utilized multi-color flow cytometry to analyze peripheral blood ILC populations in clinically stable COPD patients (n=38), asthma patients (n=37), smoking (n=19) and non-smoking (n=16) controls.

Results

Proportions of peripheral blood inflammatory CD4+ ILC1s were significantly higher in COPD patients than in asthma. Proportions of CD4- ILC1s were increased in COPD patients compared to asthma patients and smoking controls. Frequencies of CD117- ILC2s were significantly reduced in COPD patients compared with asthma patients. In contrast, the fraction of inflammatory CD45RO+ cells within the CD117- ILC2 population was significantly increased. Principal component analyses showed that combined features of the circulating ILC compartment separated COPD patients from asthma patients and both control groups.

Conclusion

Our in-depth characterization of ILC1 and ILC2 populations in peripheral blood revealed significant differences in their phenotypes between COPD and asthma patients and smoking or non-smoking controls. These findings suggest a role for both ILC subsets in COPD disease pathology, independent of smoking history, and may have implications for patient stratification and therapy development.

Trial registration NCT03278561, www.clinicaltrials.gov, and NL8286, trialregister.nl

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a heterogeneous progressive lung disease with persistent airflow obstruction and respiratory symptoms, mostly caused by tobacco smoking or pollution exposure (1). Because not all smokers develop COPD, it is thought that the etiology of COPD involves a combination of cellular damage by inhaled particulate matter and genetic, early-life risk and social factors. Pathological alterations in COPD include epithelial cell reprogramming in combination with immune and tissue remodeling responses, leading to the development of emphysema (2, 3).

Recent evidence supports a role for natural killer (NK) cells and innate lymphoid cells (ILCs) in the pathogenesis of COPD, although the mechanisms involved are still elusive (4, 5). The NK cell population is increased in the lungs of COPD patients in comparison with non-smoking controls (4). ILC subsets have diverse roles in mucosal inflammation and tissue repair and are activated by cytokines and stress signals (6, 7). Based on cytokine production and transcription factor expression, ILCs are classified into group 1 ILC (ILC1), ILC2 and ILC3, mirroring the profiles of T helper (Th) cell subsets (8). Although ILC precursors (ILCp), ILC1 and ILC2 can be found in human peripheral blood, evidence was provided that under homeostatic conditions ILCs are tissue resident, whereby ILC precursors differentiate locally as a mechanism of maintenance, maturation and tissue adaptation. (6, 7, 9-12).

In peripheral blood of COPD patients increased ILC1s and decreased ILC2s were observed, compared with smoking and non-smoking controls (13). Moreover, ILC1s were positively associated with disease severity as defined by GOLD classification, forced expiratory volume in 1 second (FEV1)) or average number of exacerbations per year. In contrast, the frequency of circulating ILC2s in patients with COPD was negatively associated with disease severity (13). For asthma there is evidence that ILC2s are increased or more active in peripheral blood and sputum. Because of this association, these cells are thought to contribute to the induction of eosinophilic airway inflammation (14-16).

Recently, subsets of ILC1 and ILC2 were described with specific characteristics regarding inflammatory phenotype or cellular plasticity. Roan et al. (17) identified a distinct CD4+ ILC1 subset, containing potent producers of TNF-α, GM-CSF and IL-2, which was increased in patients with the autoimmune disease systemic sclerosis. We identified steroid-resistant CD45RO^{+} inflammatory ILC2s that were elevated in blood from patients with severe asthma (18). In addition, c-kit (CD117) expression on a subpopulation of ILC2s was associated with functional plasticity (19, 20).

Given the evidence that ILCs are involved in the pathophysiology of both COPD and asthma, in this report we aimed to compare the inflammatory phenotype of the ILC1 and ILC2 subsets in patients with COPD and asthma. Particularly, because the distinction between the two conditions may be difficult in clinical practice and

coexistence of asthma and COPD in some patients. In our experiments, we used smoking and non-smoking healthy individuals as controls. We investigated peripheral blood ILCs for the expression of markers associated with plasticity or inflammatory phenotypes, including CD4 and CD45RO on ILC1s and c-kit (CD117) and CD45RO on ILC2s, with the primary objective to identifying disease-specific inflammatory ILC profiles in individuals with COPD compared to those with asthma.

MATERIAL AND METHODS

Study design

In this prospective cross-sectional study design, we investigated ILC populations in peripheral blood of patients with COPD and patients with asthma. We included two controls groups: smoking controls and non-smoking controls (**Figure 1**).

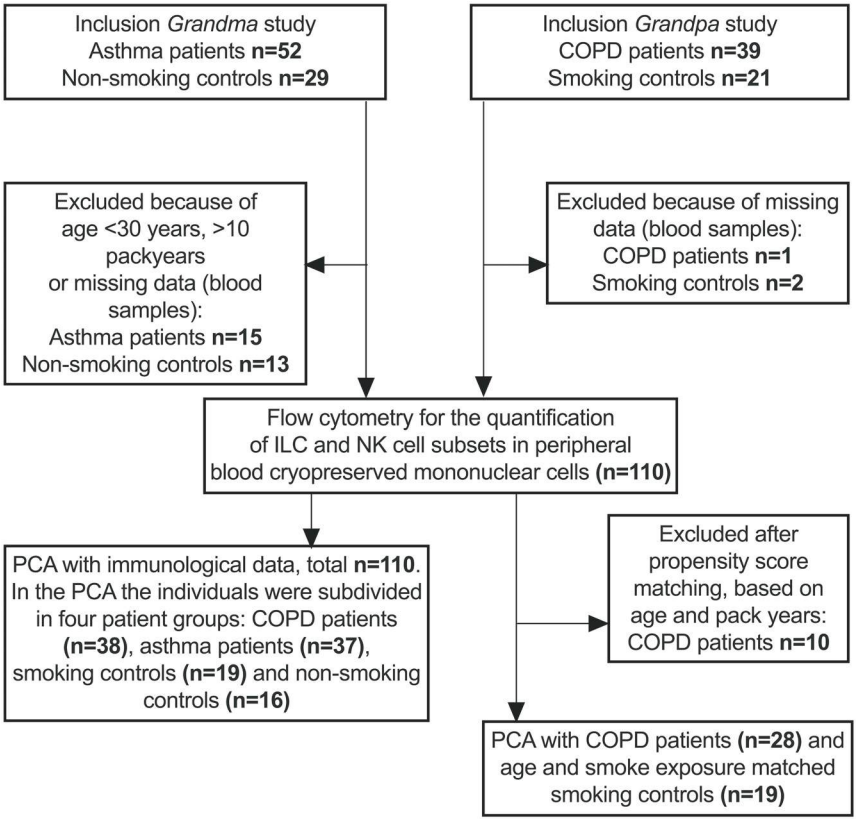


Figure 1. Patient enrolment. Details of the Grandma and Grandpa studies are given in Materials and Methods. PCA, principal component analysis.

Participants

Peripheral blood was obtained from patients and controls included in two clinical studies conducted in the Franciscus Gasthuis and Vlietland Hospital Rotterdam, the Netherlands (NCT03278561 and NL8286, executed between 2017 and 2021). The Medical Ethics Committees United (MEC-U) approved the studies, and all participants provided a written informed consent. The *Grandpa-study,* had the following inclusion criteria: patient with COPD or (ex)-smoking individual with age >40 year and >10 smoked pack years (20 daily cigarettes for one year). COPD patients were diagnosed by a pulmonologist based on the presence of airway obstruction, measured by FEV1 divided by the Forced Vital Capacity (FVC) < 0.7; FEV_1 and FVC measurements were performed according to the ATS/ERS taskforce "standardization of spirometry", with the Vmax Sensor Medics Viasys, type 6200 Encore (21). All COPD patients were clinically stable for >6 weeks and did not use a high dose of oral corticosteroids 6 weeks before participation. Severe COPD and asthma patients who used a daily maintenance dose of 5mg prednisone were eligible for inclusion in the study. Patients with features of both asthma and COPD were excluded. A second cohort, the *Grandma-study*, served as a disease group for comparison. The study also included never- or ex-smoking individuals with <10 smoked pack years. The (ex)-smoking control individuals included had no (reversible) airway obstruction. Detailed inclusion and exclusion criteria of this study have been described previously (18, 22-24).

Clinical data collection

Clinical data were collected specifically for this prospective cross-sectional study design. All patients underwent spirometry and venous blood sampling and completes questionnaires about quality of life and symptoms. Collected patient characteristics included age, sex, body mass index (BMI), smoking status, number of pack years, medication use, medical history, family history and start of symptoms.

Flow cytometry analysis

Peripheral blood mononuclear cells (PBMCs) were isolated from patient or control blood draws, using standard procedures (22), and ILC subsets were characterized by flow cytometry, as previously described (18). Briefly, cells were stained with fluorochrome-labeled antibodies (**Suppl. Table 1**) for 30 min at 4°C in PBS (Gibco) containing 0.5% BSA (Sigma Aldrich) and 2 mM EDTA (Sigma Aldrich). After washing with PBS, a life/dead marker was stained for 15 min at 4°C in PBS. Data were acquired with a Symphony A5

flow cytometer (BD Biosciences, Erembodegem, Belgium) and analyzed using FlowJo 10.8.1 software (Tree Star Inc, Ashland, Ore).

Statistical analyses

Principal component analysis (PCA) was performed using R and R-Studio (R-Studio Server Version 1.4.1717) and the packages FactoMineR and Factorextra, as described previously (25). The number of dimensions to be interpreted were determined by the R package FactoInvestigate. In comparisons of four groups, the Kruskal-Wallis test with Bonferroni correction to account for multiple testing was employed. In cases involving two groups, we utilized the Mann-Whitney U test. To provide the best representation of the data variability, dimensions with an inertia higher than the inertia obtained by a random distribution were considered. After the formation of dimensions by PCA, the diagnoses (COPD, asthma, smoking control or non-smoking control) were labelled and statistical significance was calculated per dimensions by Kruskall-Wallis test with Bonferroni correction for multiple testing in IBM SPSS Statistics (Version 28.0.0.0 (190)). P-values <0.05 were considered statistically significant. In a sub-analysis of smoking individuals with COPD and without COPD (dependent), a propensity score matching by logistic regression was used to reduce the bias of pack years and age (predictors). Probabilities were estimated, ranging from 0 to 1, for each patient in the study population. After propensity score matching a second PCA was performed.

RESULTS

Patient characteristics

From a total of 141 individuals of the COPD and asthma cohorts described in the Materials and Methods section, 110 patients met the inclusion criteria for our study (See for enrolment and selection of patients: **Figure 1**). Characteristics of the COPD patients (n=38), asthma patients (n=37), smoking (n=19) and non-smoking (n=16) controls are shown in **Table 1**. Quantification of blood leukocyte differentiation showed significantly increased proportions of neutrophils, lymphocytes and monocytes in COPD patients in comparison with asthma patients (**Suppl. Table 2**). Neutrophils were also significantly increased in smoking controls in comparison with asthma patients. Eosinophils did not differ between the groups.

Table 1. Characteristics of COPD and asthma patients and controls.

	COPD N=38	Asthma N=37	Smoking controls N=19	Non-smoking controls N=16	P value¶
Sex, female (%)	23 (60.5) §	21 (56.8)	9 (47.4)	8 (50)	0.729
Age, year	60 [56-68]*	58 [44-64]	60 [45-67]	48.5 [40-64.5]*	0.042
BMI, kg/m²	25.1 [22.2-30.0]	27.6 [24.7-31.9]	26.3 [24.6-29.5]	27.3 [23.9-30.5]	0.216
Smoking status					0.020
Never smoker	0	28 (75.7)	0	13 (81.3)	
Ex-smoker	17 (44.7)	9 (24.3)	12 (63.2)	3 (18.8)	
Current smoker	21 (55.3) ‡	0	7 (36.8) ‡	0	
Pack years	33 [27-49]	0 [0-5]	19 [13-30]	0 [0-0]	<0.001#
FEV_1 post % pred	48 [31-63]	95 [80.5-106]	100 [86-105]	103.5 [94.3-109.3]	<0.001
FEV_1/FVC post % pred	46.0 [35.7-56.7]	77 [70-85.5]	73.9 [70.2-81.1]	84 [77.3-103.5]	<0.001
Exacerbations last year in N (%)					0.087
0 exacerbations	19 (50)	21 (56.8)	100 (19)	100 (16)	
1 exacerbation	10 (26)	7 (18.9)	0	0	
>1 exacerbations	9 (24)	9 (24.3)	0	0	
Medication					<0.001
SABA use, N (%)	13 (33.3)	7 (18.9)	0	0	
SAMA use, N (%)	5 (12.8)	3 (8.1)	0	0	
LABA use, N (%)	32 (82.1)	38 (97.4)	0	0	
LAMA use, N (%)	34 (87.2)	6 (16.2)	0	0	
ICS use N (%)	26 (66.6)	39 (100)	0	0	

§ Data are presented as N (%) or median [75-25 interquartile], unless otherwise stated.
¶ Statistically significant differences between COPD patients, asthma patients and any of the control groups, adjusted by the Bonferroni correction for multiple testing.
* For age, there was a significant difference between COPD and the non-smoking control groups.
‡ For current smoking, there was a significant difference between COPD and smoking controls.
# Although there were significant differences in pack years across the four groups, the difference between COPD and smoking control group was not significant (p=0.674).
BMI: Body Mass Index, FEV1 % pred: Forced Expiratory Volume in 1 second percentage predicted, FVC: Forced Vital Capacity, SABA: Short-Acting Beta Agonist, SAMA: Short Acting Muscarinic Antagonist LABA: Long-Acting Beta Agonist, LAMA: Long Acting Muscarinic antagonist, ICS: Inhaled. Corticosteroids.

COPD patients show increased $CD4^+$ and $CD4^-$ ILC1s and reduced $CD117^-$ ILC2s in peripheral blood compared to asthma patients

To characterize the populations of NK cells and ILCs in peripheral blood of the four groups of patients and controls, we analyzed peripheral blood mononuclear cell (PBMC) fractions by flow cytometry. We stained PBMCs with a rich cocktail of antibodies against lineage markers to exclude lineage-positive cells, and quantified NK cells

using CD56 and ILC subsets using CD127, CD117 and CRTH2 (See **Suppl. Figure 1** for gating strategy).

NK cells are generally divided into a mainly cytokine-producing $CD56^{bright}$ and a mainly cytolytic $CD56^{dim}$ subset (26). There were no significant differences between COPD and asthma patients in the proportions of $CD56^{bright}$ NK cells within the lymphocyte gate. However, COPD patients showed significantly reduced proportions of $CD56^{bright}$ NK cells in comparison with non-smoking controls (**Figure 2A**). $CD56^{dim}$ NK cells were not different across the four groups.

The proportions of total ILCs were significantly lower in COPD patients in comparison with asthma and the two control groups (**Figure 2A**). In COPD patients the frequency of $CD4^+$ ILC1s within the total ILC population appeared to be higher compared with all three other groups, although significance was only reached for the comparison with asthma patients (**Figure 2B**). The frequency of $CD4^-$ ILC1s was also higher in COPD patients than in smoking controls and asthma patients (**Figure 2B**).

The proportions of $CD117^+$ ILC2s, which display a high level of plasticity towards ILC1 and ILC3 (19, 20), were similar across the four groups of patients and controls (**Figure 2B**). In contrast, the subset of $CD117^-$ ILC2s was significantly reduced in COPD patients, compared with asthma patients, but not when compared with controls (**Figure 2B**). Finally, the proportions of ILC precursors (ILCP: $CD117^+CRTH2^-$ ILCs, (9)) did not differ between the four groups.

Taken together, these analyses revealed various significant differences in the frequencies of NK cells and ILCs in peripheral blood between COPD patients, asthma patients and controls. As summarized in **Suppl. Figure 2**, within the total population of circulating ILCs of COPD patients, both $CD4^+$ and $CD4^-$ ILC1 were elevated and $CD117^-$ ILC2s were decreased, when compared to asthma patients. Proportions of CD4- ILC1s in COPD patients were also significantly higher than in smoking controls.

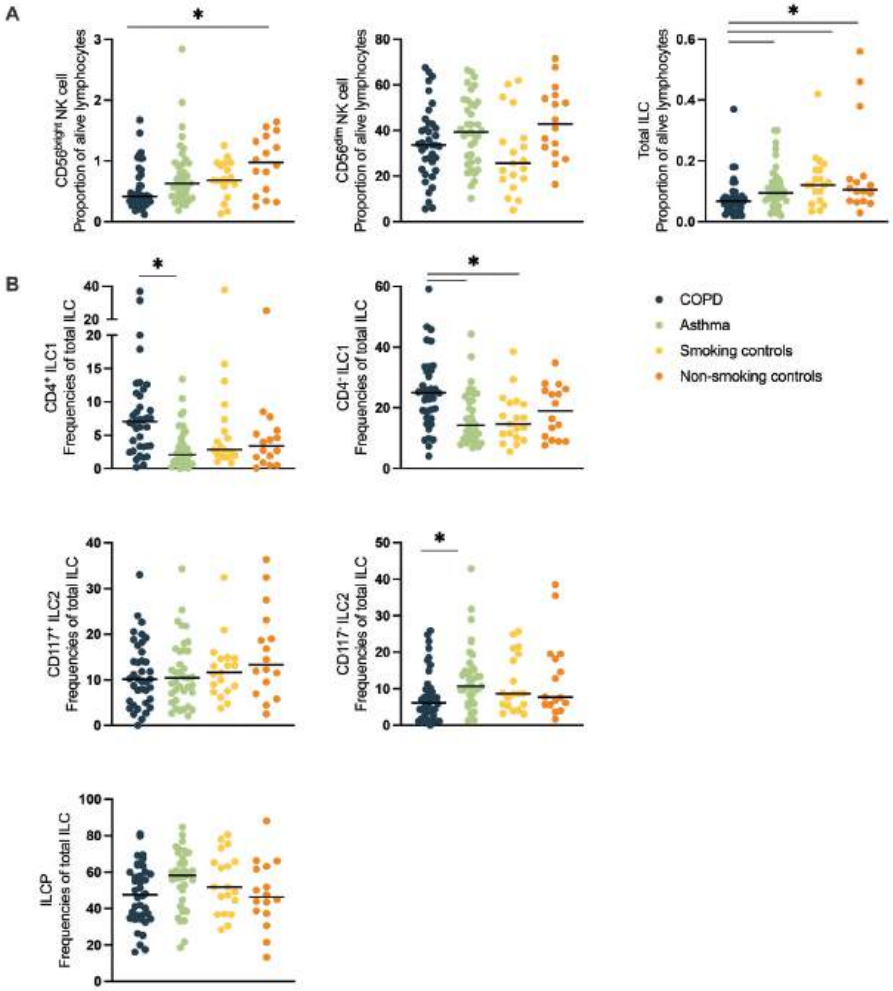


Figure 2. Aberrant frequencies of innate lymphoid cells (ILC) in peripheral blood of COPD patients. (A,B) Subsets of peripheral blood ILCs of COPD patients, asthma patients, smoking and non-smoking controls, as determined by flow cytometry. A) Proportions of natural killer (NK) cells and total ILCs within the alive lymphocyte population. B) Frequencies of the indicated ILC subsets within the total ILC population. Dots represent an individual patient or control, as indicated. Horizontal bars represent median values. Statistical analyses were performed using Kruskal-Wallis test combined with a Bonferroni correction for multiple testing, * $p<0.05$.

COPD patients show a shift from CD45RA$^+$ to CD45RO$^+$ cells in CD4$^-$ ILC1s and CD117$^-$ ILC2s

Since we recently showed that CD45RO$^+$ ILC2s have an inflammatory phenotype and are increased in the circulation of patients with severe, uncontrolled asthma (18), we decided to analyze surface expression of the CD45RA and CD45RO isoforms, both on ILC1s and ILC2s.

The frequencies of CD45RA$^+$ and CD45RO$^+$ cells within the CD4$^+$ ILC1 population were variable and did not differ significantly across the four groups (**Figure 3A**). In contrast, a significant decrease in the fraction of CD45RA$^+$ cells was seen within the CD4$^-$ ILC1 population of COPD patients, when compared with asthma patients. Conversely, we observed a parallel increase in the proportion of CD45RO$^+$ CD4$^-$ ILC1, although this did not reach statistical significance **(Figure 3A)**.

COPD patients displayed decreased proportions of CD45RA$^+$ cells and increased proportions of CD45RO$^+$ cells in the CD117$^-$ ILC2 population, which was significant when compared to patients with asthma **(Figure 3B)**. Next to our aim to compare the inflammatory phenotypes of asthma and COPD, we conducted additional analyses to compare COPD patients directly with the smoking controls that did not develop COPD. Using a Mann-Whitney U test to compare the two groups, we observed that COPD patients had significantly lower proportions of CD45RA+CD117+ ILC2s (p=0.005) and CD45RA+CD117- ILC2s (P=0.006) than smoking controls (Figure 3B). Furthermore, a trend was observed that inflammatory CD45RO+ ILC2s were more prevalent in patients with COPD, but this did not reach significance.

Correlation analysis showed that COPD patients with elevated proportions of CD45RO$^+$ cells within the population of CD117$^-$ ILC2s also had increased proportions of CD45RO$^+$ within CD117$^+$ ILC2s or CD4$^-$ ILC1s, but these correlations were weak **(Suppl. Figure 3).** We did not find correlations between proportions, subsets or phenotypes of ILC1s or ILC2s and clinical parameters such as FEV1, GOLD-stage or the number of exacerbations in the previous year.

In summary, particularly when compared with patients with asthma, both CD4$^-$ ILC1 and CD117$^-$ ILC2 from COPD patients manifested a shift from surface CD45RA$^+$ to CD45RO$^+$ expression, which for ILC2s was shown to be associated with an inflammatory phenotype (18).

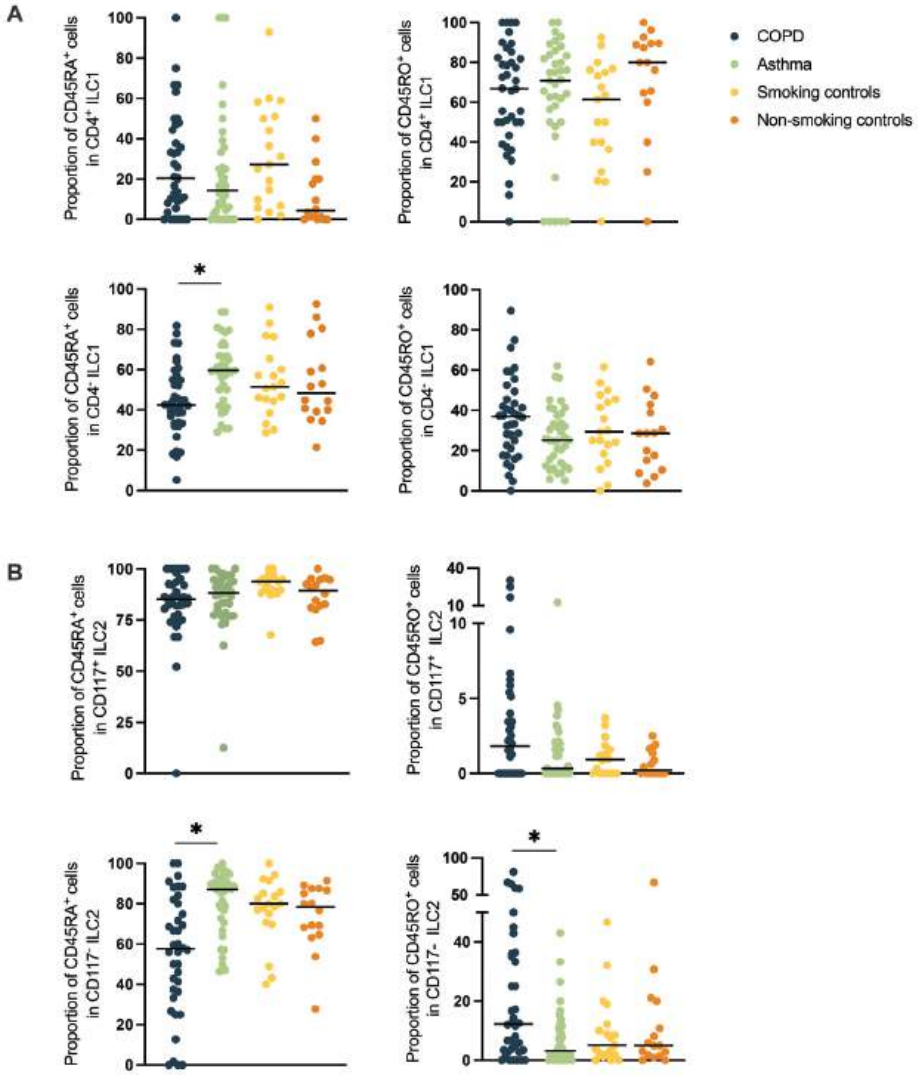


Figure 3. Aberrant phenotype of innate lymphoid cells (ILC) in peripheral blood of COPD patients. (A,B) Distribution of CD45RA+ and CD45RO+ subsets of ILCs in peripheral blood of COPD patients, asthma patients, smoking and non-smoking controls, as determined by flow cytometry A) within $CD4^+$ ILC1 and $CD4^-$ ILC1 subsets; and B) within $CD117^+$ ILC2 and $CD117^-$ ILC2 subsets. Dots represent an individual patient or control, as indicated. Horizontal bars represent median values. Statistical analyses were performed using Kruskal-Wallis test combined with a Bonferroni correction for multiple testing, * $p<0.05$.

Principal component analysis separates COPD patients from asthma patients on the basis of ILC1 and ILC2 characteristics

To obtain a more comprehensive overview of the differences between ILC subsets across the four patient and control groups, we performed a dimensionality reduction by principal component analysis (PCA) (**Figure 4A**). Hereby, we included frequencies and phenotypes of ILC subsets, as well as peripheral blood leucocyte differentiation parameters. The distribution over dimension 1 (Dim.1) and Dim.2 was non-random, which was not due to age, sex and smoking status (**Suppl. Figure 4**). COPD patients were significantly separated from asthma patients, based on both Dim.1 and Dim.2 (with 18.7% and 11.7% of variance, respectively) (**Figure 4B**). Dim.1 showed a significant difference between COPD patients and all the other groups. COPD patients and smoking controls could not be separated based on Dim.2, implying that Dim 2 was more related to smoking than to the disease. Likewise, Dim 2 did not separate asthma patients from non-smoking controls. Whereas the subsets of $CD117^-$ ILC2s and $CD4^-$ ILC1s contributed most to Dim.1, Dim.2 was dominated by the absolute numbers of leucocytes, neutrophils and the subsets of $CD4^+$ ILC1s (**Figure 4C)**.

In summary, this PCA revealed that COPD patients were significantly separated from asthma patients, smoking controls and non-smoking controls (independent of age or sex) on the basis of the phenotype of $CD117^-$ ILC2s and $CD4^-$ and $CD4^+$ ILC1s. The contribution of NK cells, $CD117^+$ILC2s and ILCP to the separation of COPD patients was limited.

ILC subsets or phenotype characteristics do not separate ex-smokers from current smokers

To allow for a comparison of COPD patients and smoking controls with similar smoke exposure and age, a propensity score matching was performed. Upon exclusion of 10 COPD patients (**Figure 1**), there were no significant differences between the COPD patients (n=28) and smoking controls (n=19) in sex, age, pack years and current smoking status (**Suppl. Table 3**).

When we performed a PCA using these individuals, we observed that the distribution over Dim.1 and Dim.2 was independent of sex and age **(Suppl. Figure 5)**. Interestingly, our immune cell parameters separated ex-smokers from current smokers in Dim.2 (**Suppl. Figure 6AB**). In Dim.1 the frequency values of various ILC1 subsets were dominant; Dim.2 was dominated by absolute leucocyte numbers, and frequencies of neutrophils as well as frequencies of total ILCs (**Suppl. Figure 6C)**. Although values for COPD patients and controls tended to be different in Dim.1, significance was not reached, likely due to small groups sizes **(Suppl. Figure 6)**.

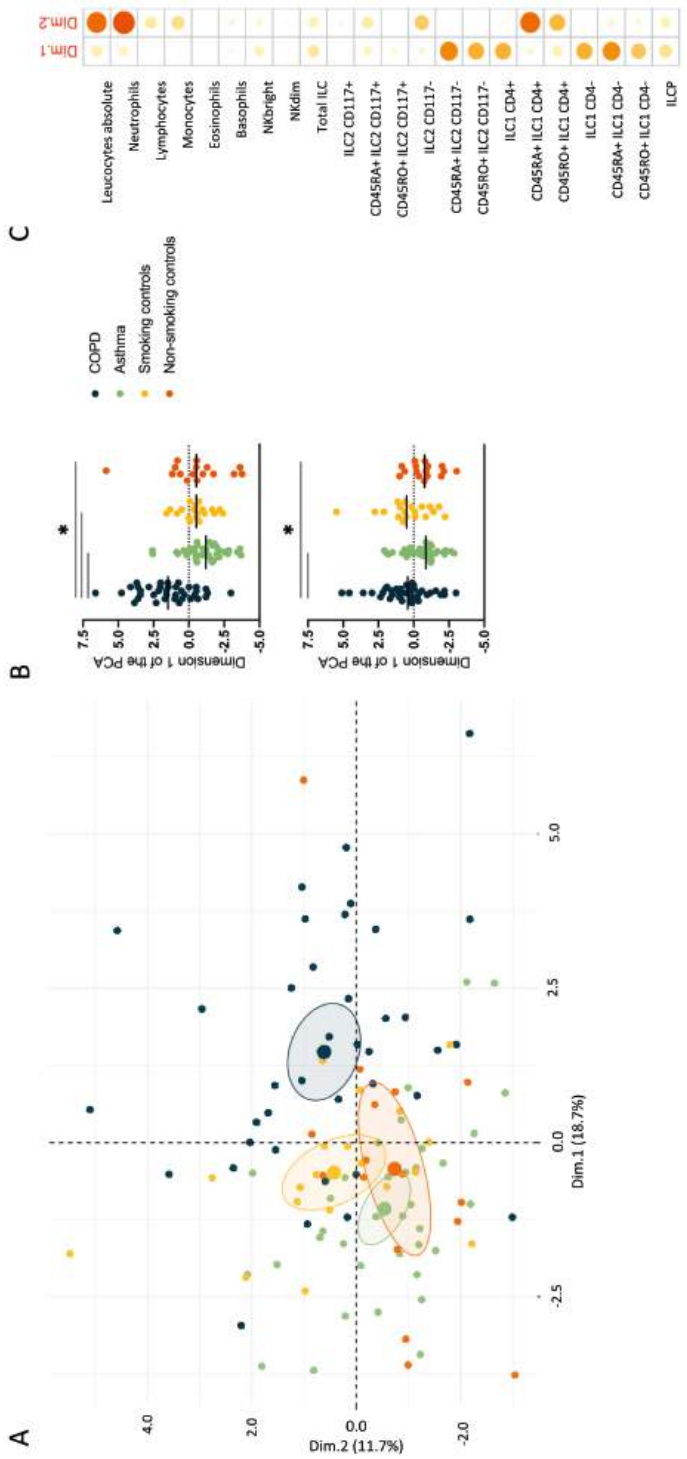


Figure 4. Principal component analysis distinguishes COPD patients from control groups.
Principal component analysis (PCA) of frequencies and phenotype of peripheral blood ILC subpopulations and leucocyte differentiation in peripheral blood of COPD patients, asthma patients, smoking and non-smoking controls. **A)** PCA in which each symbol point represents an individual patient or control **B)** Dimension 1 (Dim.1) and Dimension 2 (Dim.2) of PCA from the indicated patients and control groups. **C)** Relative contribution (as shown by size and orange color range of dots) of the indicated ILCs and leucocyte subgroups in Dim.1 and Dim.2 of the PCA. Statistical analyses were performed using Kruskal–Wallis test combined with a Bonferroni correction for multiple testing,* p<0.05.

In summary, our analysis revealed that frequencies of neutrophils and total ILCs, as well as absolute leucocyte numbers separated ex-smokers and current smokers, but parameters of ILC subset phenotype or subsets did not.

DISCUSSION

In this translational study we investigated whether the frequency or inflammatory phenotype of peripheral blood ILC subsets differ between COPD patients and asthma, smoking controls and non-smoking controls, when adjusted for age, sex, smoking status and pack years. We observed that COPD patients had higher frequencies of both $CD4^+$ and $CD4^-$ ILC1s within the total ILC population when compared with controls, which reached significance for the comparison between COPD and asthma patients. In addition, proportions of $CD117^-$ ILC2s, representing the most mature population of ILC2s (19, 20), were significantly reduced. Both $CD4^-$ ILC1s and $CD117^-$ ILC2s manifested a shift from CD45RA to CD45RO surface expression, which for ILC2s was shown to be associated with an inflammatory phenotype in severe asthma (18). Finally, PCA separated COPD patients from smoking and non-smoking controls and asthma patients. Hereby, mainly the $CD4^-$ and $CD4^+$ ILC subsets as well as $CD117^-$ ILC2s contributed to the variance in the first two dimensions, and not so much the ILC precursors or the more plastic $CD117^+$ subpopulation of ILC2s.

Our results are in line with Silver et al., who also found elevated proportions of ILC1 and reduced proportions of ILC2 in COPD patients, which were associated with disease severity (27). However, we did not observe correlations between proportions, subsets or phenotype of ILCs in COPD patients and key clinical parameters. The relative frequencies of ILC1 and ILC2 subsets in the circulation op COPD patients seem to reflect local changes in the lung. It was reported that ILC1 frequencies are increased in the lungs of COPD patients and correlate with smoking and severity of respiratory symptoms (28, 29). Also, proportions of pulmonary ILC2s were lower in patients with severe, GOLD stage IV, COPD than in GOLD stage I or II patients or healthy controls (28) and the frequency of $IL\text{-}13^+$ ILC was positively correlated with FEV1 (29). Accordingly, it was shown in mice that whereas proportions of ILC1s and ILC3s in the lung increased following smoke exposure, ILC2s showed a decrease (30). Nevertheless, Blomme et al. reported an increase in both ILC1s and ILC2s in broncho-alveolar lavage in mice exposed to cigarette smoke (29). Although evidence was provided that ILC3 levels are elevated in donor COPD lungs and smoker lungs in comparison with controls (31-33), this cannot be correlated with peripheral blood since circulating ILC3 are essentially absent (9).

We found that the frequency of $CD117^-$ ILC2s in COPD patients was lower than in asthma patients, but that $CD117^+$ ILC2s were comparable. This implies that particularly the more committed $CD117^-$ ILC2s with a greater potential to produce type 2 cytokines

are maintained, rather than the more immature and plastic CD117$^+$ ILC2 (19, 20). Our finding of elevated frequencies within the ILC2 population of CD45RO$^+$ inflammatory cells, which were associated with corticosteroid resistance in asthma patients (18), may suggest that these cells are also involved in steroid-resistance in COPD patients.

Although CD4+ ILC1s were recognized as a distinct ILC1 subset increased in systemic sclerosis it is of note that there are concerns about the identity of ILC1s, which may reflect technical issues related to contaminating T cells (17, 19, 34). To minimize contamination of ILC1, we included antibodies to CD3, TCRαβ and TCRγδ in our lineage marker cocktail. In line with Roan et al. we found that ~%70 of the CD$^+$4 ILC1s were CD45RO$^+$ and ~30% of CD4$^-$ ILC1 were CD45RO$^+$ (17). Literature on the inflammatory characteristics of CD45RO$^+$ ILC1 is currently lacking. Nevertheless, the finding of increased CD4$^+$ ILC1s in systemic sclerosis suggest that CD4$^+$ ILC1s, of which a majority expresses CD45RO, are pro-inflammatory in nature. It is attractive to speculate that the relative shift from CD45RA$^+$ to CD45RO$^-$ CD4$^-$ ILC1s in COPD patients, compared to asthma patients, may point to a pro-inflammatory role of these cells in COPD. Future experiments should show the functional implications of surface CD4 and CD45RO expression on ILC1s.

Our study has some limitations. Firstly, we quantified the proportions of ILCs and not their functional properties, such as cytokine production. This is also relevant in the light of conflicting data regarding the identity of ILC1s (as described above; Simoni and Newell Immunity 2017) and the recent identification of unconventional CRTH2- ILC2s (35). Secondly, it remains unknown how the phenotype or activation status of peripheral blood ILCs is linked to pathophysiological effects of these cell populations in the lungs, particularly since ILCs are known to adopt tissue-specific functional phenotypes (36) and ILCs are thought to be largely tissue-resident cells (9, 10). Thirdly, our study is cross-sectional and it would be interesting to investigate ILC dynamics over time and during disease exacerbations. Finally, our analyses were explorative with small sample sizes and need to be confirmed in larger, well-defined cohorts of patients.

In conclusion, we provide evidence that COPD patients can be distinguished from asthma patients, as well as smoking and non-smoking controls, by analyzing the composition and phenotypic characteristics of the circulating ILC1 and ILC2 compartments. Although the frequencies of mature CD117$^-$ ILC2s were reduced in COPD patients, compared with asthma patients, the fraction of inflammatory CD45RO$^+$ cells within this ILC2 population was significantly increased. These observations suggest that not only ILC1s (13), but also ILC2s may play a role in COPD pathogenesis. Our detailed characterization of ILC subsets also allowed us to differentiate between COPD inflammation and smoking effects, which may have implications for patient stratification. Further experiments in larger patient cohorts should show whether the shift from CD45RA to CD45RO in ILC1s and ILC2s in COPD is linked to steroid resistance or disease exacerbation, which may prove useful for therapeutic strategies.

SUPPLEMENTARY

Supplementary Table 1. Antibodies used in flow cytometry analyses.

Antigen	Fluorchrome	Clone	Manufactorer
TCRab	FITC	IP26	Biolegend
TCRgd	FITC	B1	Becton Dickinson
CD14	FITC	61D3	Life Technologies
CD19	FITC	HIB19	Becton Dickinson
CD16	FITC	3G8	Becton Dickinson
CD94	FITC	DX22	Biolegend
FCeRI	FITC	AER-37	Life Technologies
CD56	PE	TULY56	Life Technologies
CD45RA	PE TxR	MEM-56	Invitrogen
NKp44	PCP	P44-8	Biolegend
CRTH2	PE-Cy7	BM16	Biolegend
CD117	BV421	104D2	Biolegend
LD	BV510	-	Life Technologies
CD25	BV605	BC96	Biolegend
CD3	BV711	UCHT1	Becton Dickinson
CD4	BV786	SK3	Becton Dickinson
CD127	APC	eBioRDR5	Life Technologies
CD45RO	APC-Cy7	UCHL-1	Biolegend
CD5	AF700	UCHT-2	Becton Dickinson
NHS	-	-	Biolegend

Supplementary Table 2. Baseline Leucocyte differentiation in COPD and asthma patients and controls.

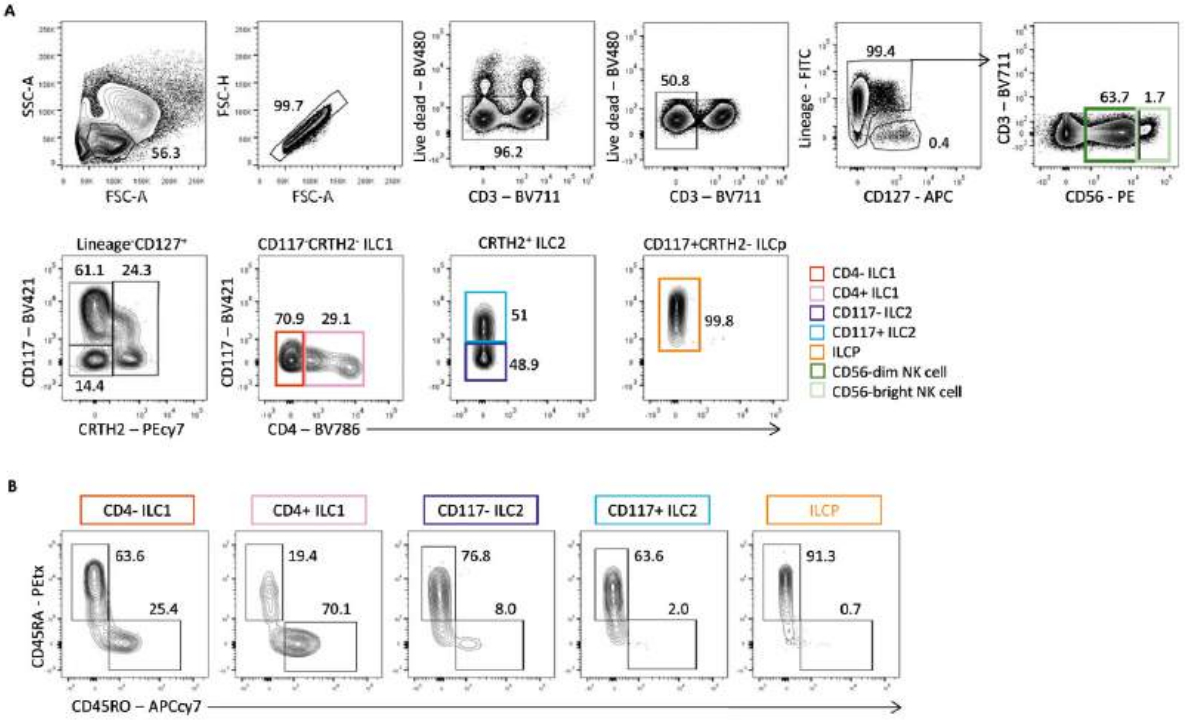
	COPD N=38	**Asthma N=37**	**Smoking controls N=19**	**Non-smoking controls N=16**	**P value**
Neutrophils#	5.4 [3.8-6.7] §*	3.2 [2.7-4.1]* ¶	4.5 [3.4-4.7] ¶	3.7 [3.0-.5.0]	<0.001
Lymphocytes#	2.1 [1.8-2.5]*	1.6 [1.3-2.1]*	2.0 [1.4-2.4]	1.9 [1.6-2.2]	0.004
Monocytes#	0.7 [0.6-0.9]* ¶	0.5 [0.4-0.6] ¶	0.6 [0.5-0.8]	0.5 [0.4-0.7]*	<0.001
Eosinophils#	0.2 [0.1-0.2]	0.2 [0.1-0.3]	0.2 [0.1-0.2]	[0.1 [0.1-0.2]	0.061
Basophils#	0.1 [0.0-0.1]	0.0 [0.0-0.1]	0.1 [0.0-0.1]	0.1[0.0-0.1]	0.448

§ Data are presented as median [25-75 interquartile], * or ¶ statistically significant differences ($p<0.05$) between patients and control groups, adjusted by the Bonferroni correction for multiple testing. # Values are presented in 10*^9/L

Supplementary Table 3. Patient characteristics of subanalysis after propensity score matching.

	COPD N=28	Smoking controls N=19	P value
Baseline clinical characteristics			
Gender, female (%)	16 (57) §	9 (47.4)	0.359
Age, years	59 [51.5-65.8]	60 [45-67]	0.845
BMI, kg/m^2	24.5 [21.7-28.7]	26.3 [24.6-29.5]	0.069
Pack years	32 [19.3-34.8]	19 [13-30]	0.086
Smoking status			0.108
Never smoker	0	0	
Ex-smoker	11 (39)	12 (63.2)	
Current smoker	17 (61)	7 (36.8)	

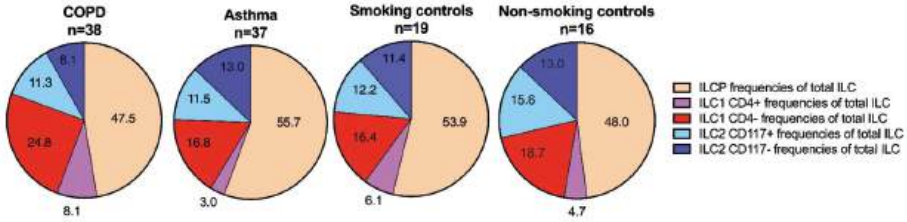
Patient characteristics of subanalysis after propensity score matching, n=10 COPD patients were excluded based on pack years and age. § Data are presented as N (%) or median [25-75 interquartile], unless otherwise stated. BMI: Body Mass Index.



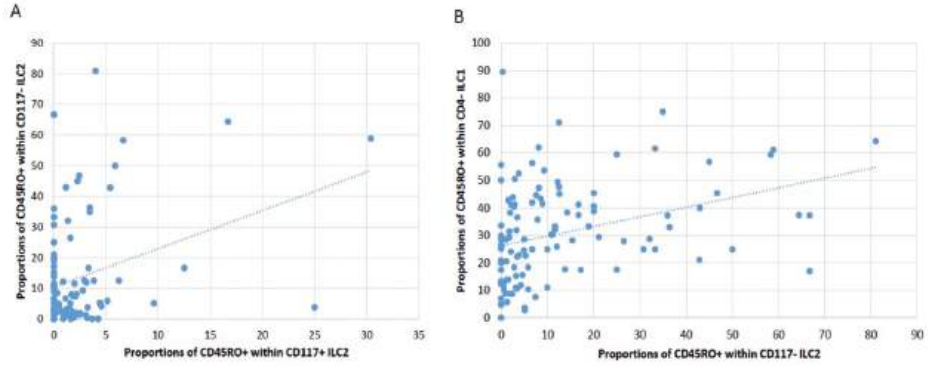
Supplementary Figure 1. Gating strategy for ILC subsets in peripheral blood

a) Representative flow cytometry gating strategy used for the quantification of ILC and NK cell subsets in cryopreserved peripheral blood mononuclear cells (PBMCs) from healthy donors and patients. PBMCs were stained with antibodies against lineage markers (CD14, CD16, CD19, CD94, FcRεR1a, RCRαβ, TCRγδ) and indicated ILC/ NK cell-specific markers.

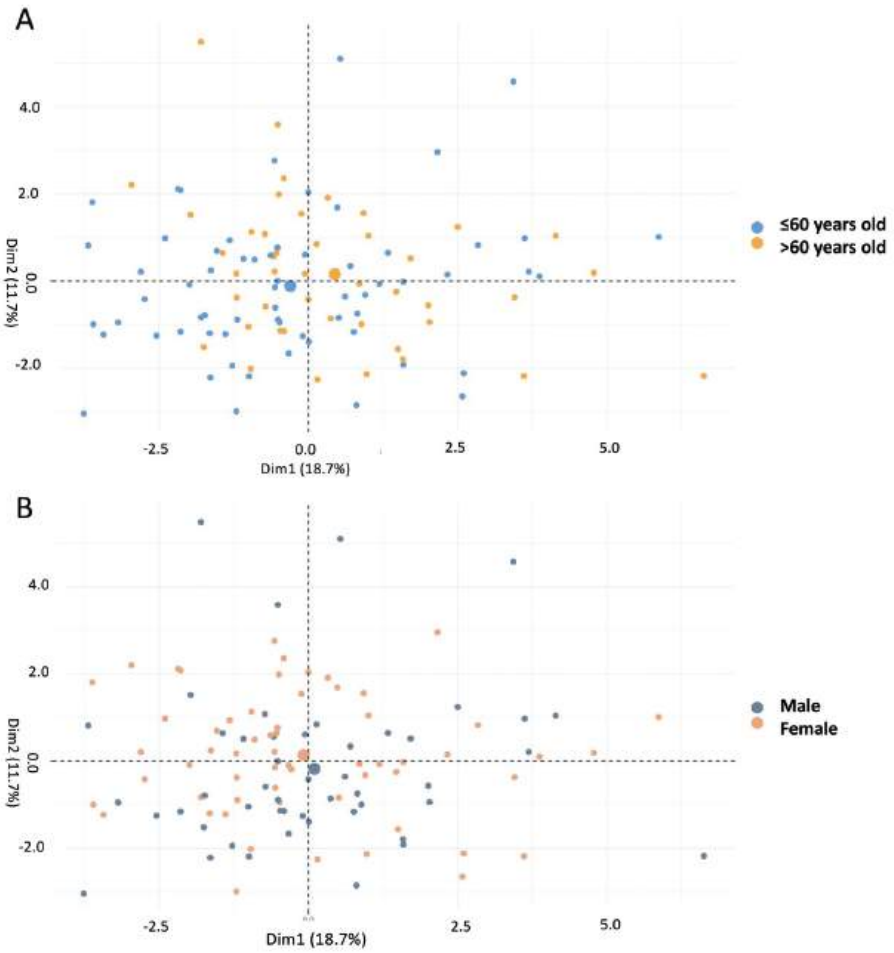
b) Representative flow cytometry analysis fo CD45RA and CD45RO surface expression on the indicated ILC subsets as gated in panel A. Antibodies used for flow cytometry are given in Suppl. Table 1.



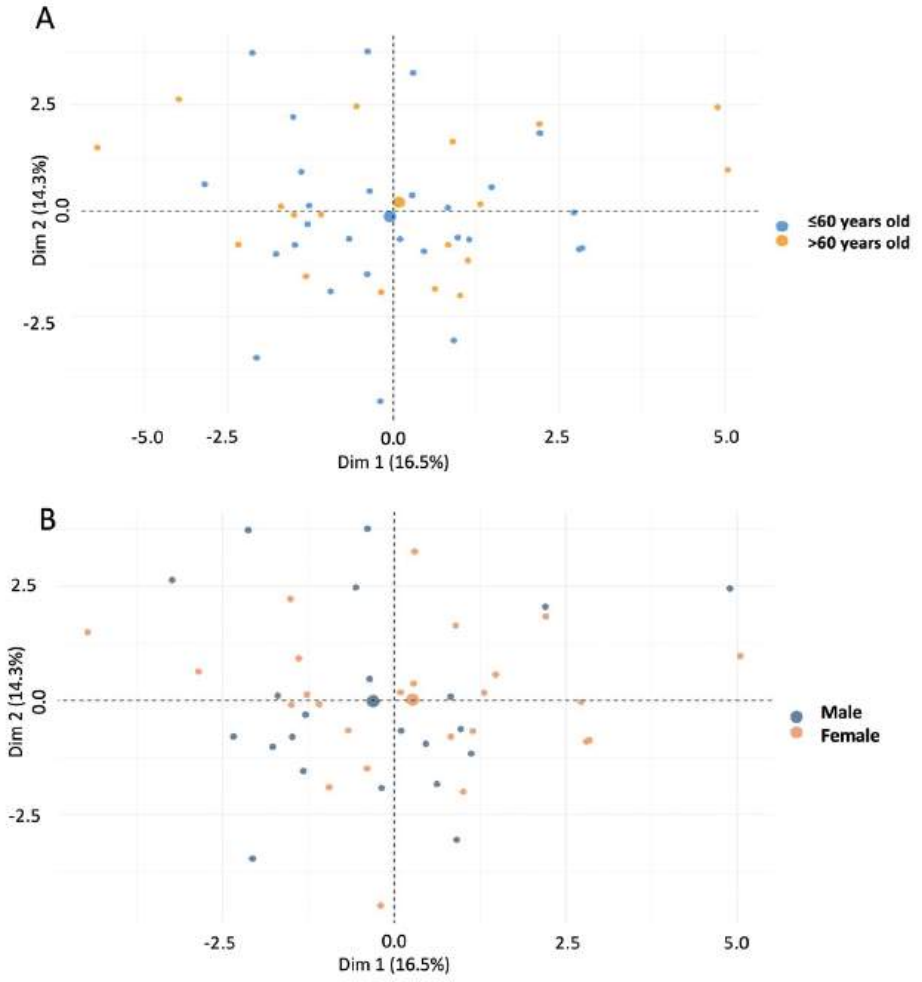
Supplementary Figure 2. Distribution of ILC subpopulations is altered in COPD. Pie charts showing mean values of the indicated ILC subsets as proportions of total ILCs present in peripheral blood mononuclear cell fractions of the indicated groups of patients and controls, as determined by multi-color flow cytometry.



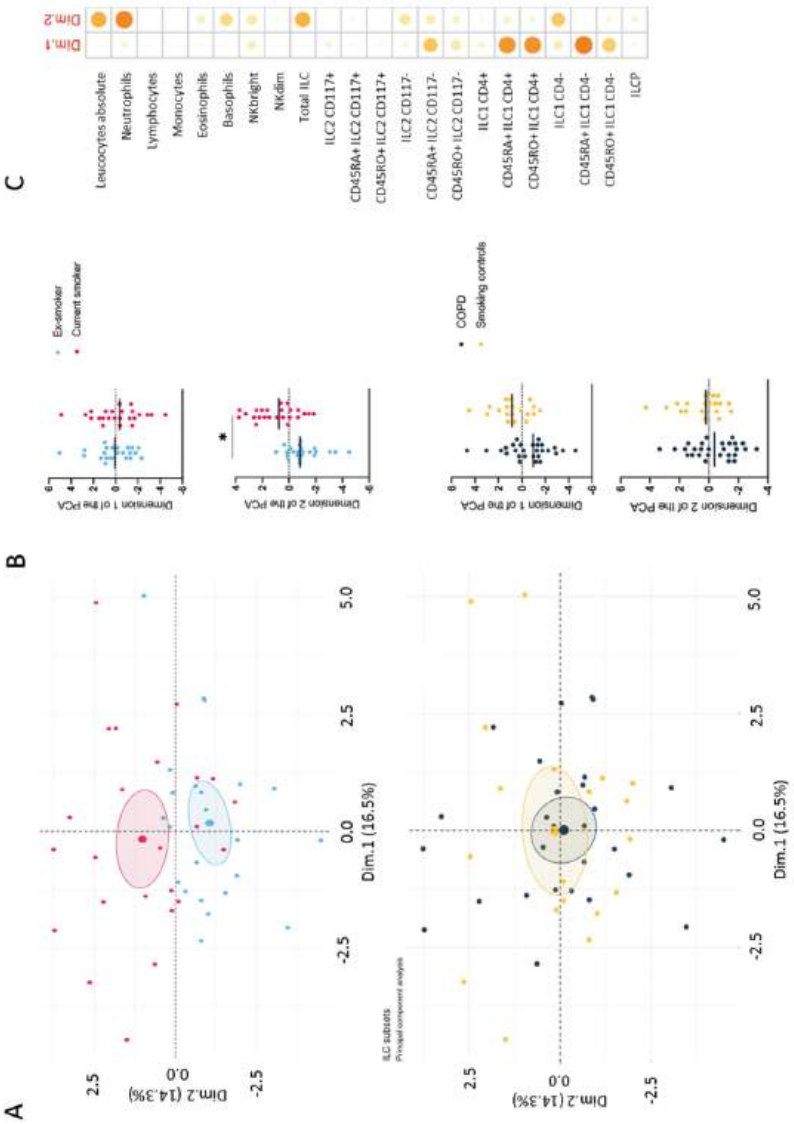
Supplementary Figure 3. Correlations of CD45RO+ cells within ILC subsets in peripheral blood. A) Correlations between proportions of CD45RO+ cells within CD117+ and CD117- ILC2s (R2=0.094, p=0.001) B) Correlations between proportions of CD45RO+ cells within CD117- ILC2s and CD4- ILC1s (R2=0.085, p=0.002).



Supplementary Figure 4. Principal component analysis does nog separate age/ sex groups on the basis of peripheral blood ILC characteristics or leucocyte differentiation. Principal component analysis (PCA) or frequencies and phenotype of peripheral blood ILC subpopulations and leucocyte differentiations in peripheral blood of COPD patients, non-smoking and smoking controls and asthma patients, seperated in two age **(A)** or sex **(B)** groups.



Supplementary Figure 5. Principal component analysis does not separate age/ sex groups on the basis of peripheral blood ILC characteristics or leucocyte differentiation of ex-smokers or current smokers.
Principal component analysis (PCA) of frequencies and phenotype of peripheral blood ILC subpopulations and leucocyte differentiation in peripheral blood of ex-smokers and current smokers, searated in two age **(A)** or sex **(B)** groups.



Supplementary Figure 6. Principal component analysis does not reveal significant differences between COPD patients and current smokers. Principal component analysis (PCA) of frequencies and phenotype of peripheral blood ILC subpopulations and leucocyte differentiation in peripheral blood COPD patients and smoking controls. To allow for a comparison of COPD patients and smoking controls with similar smoke exposure and age (Suppl. Figure 5), a propensity score mathcing was performed and 10 COPD patients were excluded, compared to PCA in Figure 4 (see text). A) PCA in which each sumbol points represents as individual COPD patients and control. B) Dimension 1 (Dim.1) and DImension 2 (Dim.2) of PCA from the indicated smoking group; the relative contribution of ILC and leucocyte subgroups in DIm.1 and DIm.2 of the PCA is given in Figure 5C. Statistical analyses were performed using Kruskal-Wallis test combined with a Bonferroni correction for multiple testing, * p<0.05. C) Relative contribution (as shown by size and orange color range of dots) of the indicated ILCs and leucocyte subgroups in Dim.1 and Dim.2 of the PCA.

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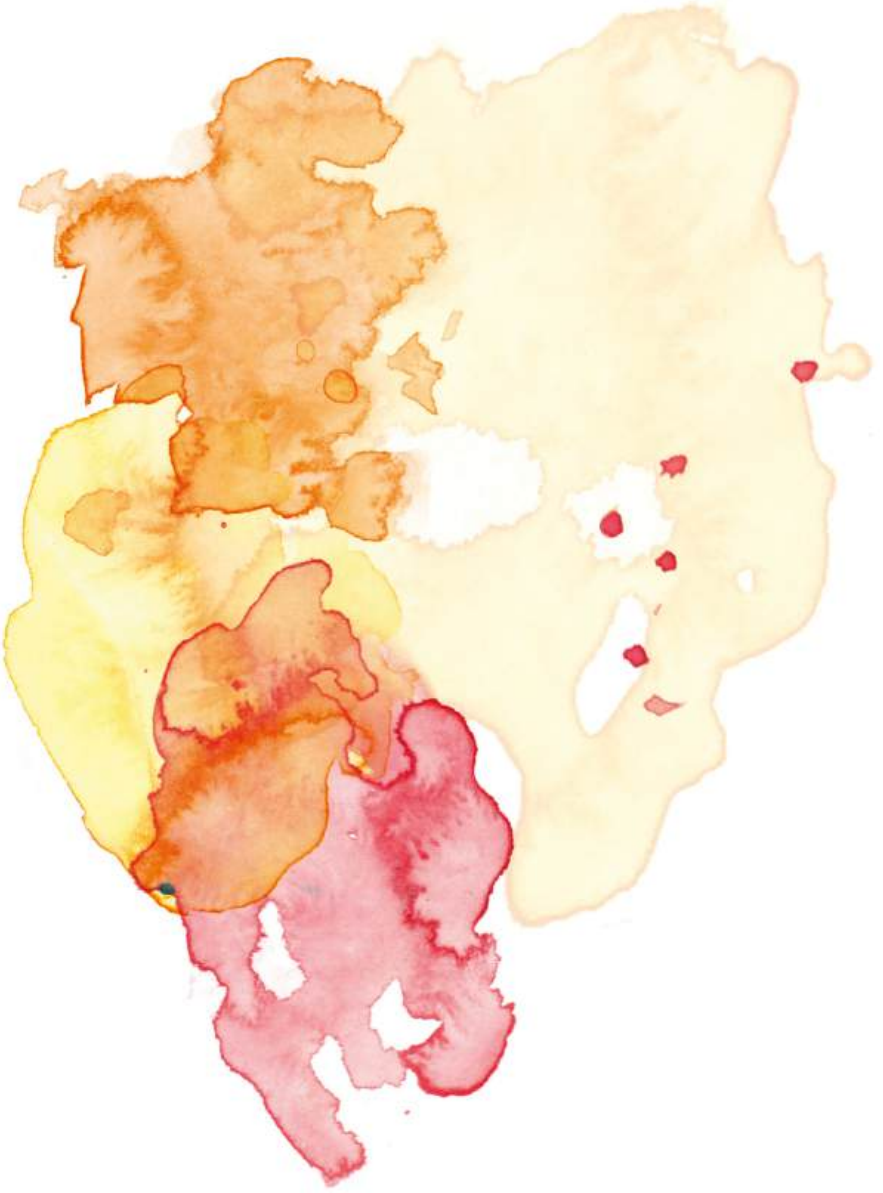

Chapter 5

Peripheral blood innate lymphoid cell profiles of COPD patients reflect their clinical phenotype as identified by cluster analysis

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ABSTRACT

Introduction

Chronic obstructive pulmonary disease (COPD) has a heterogeneous clinical and immunological presentation. Although proportions of group 1 innate lymphoid cells (ILC1) in peripheral blood were shown to correlate with COPD severity, no comprehensive analysis of ILC subsets in relation to clinical outcomes has been performed yet. Our aim was to explore the relationship between clinical phenotype clusters and ILC subsets in COPD.

Methods

Thirty-four patients with COPD were included in this cross-sectional study. Forced Expiratory Volume in 1 second (FEV1), Diffusion Capacity for Carbon monOxide (DLCOc), COPD Assessment Test (CAT) and body mass index (BMI) values were collected. Clinical clusters were defined, ILC populations in peripheral blood were quantified and characterized by flow cytometry. Data were analyzed by principal component.

Results

First, we identified three clinical clusters. C1 (n=12) had low FEV1 and BMI values and the highest disease burden, as determined by CAT. C2 (n=11) comprised older patients with increased BMI and the highest number of pack years. C3 (n=11) had the highest FEV1 values and fewer exacerbations. Based on leukocyte differentiation and phenotypic characterization of ILC subsets, two immunological clusters of COPD patients were identified. Remarkably, C1, representing the most severe COPD patient group, was associated with reduced frequencies of circulating ILCs with a potentially inflammatory phenotype.

Conclusion

The finding that clinical clusters of COPD patients were strongly associated with peripheral blood immunophenotype and ILC profile suggest a role for ILCs in disease course and may have relevance for the development of personalized therapies.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous disease characterized by chronic lung inflammation that causes air flow obstruction. COPD imposes a high disease burden, marked by recurring exacerbations that result in frequent hospital visits (1). With an increasingly ageing population and chronic exposure to particulate matter, the prevalence of COPD is expected to further increase. To reduce disease burden and healthcare costs for COPD, it is important to develop new treatment modalities.

COPD has various underlying pathological mechanisms, which affect prognosis and treatment efficacy. Airway abnormalities in COPD are associated with distinct genetic loci, environmental exposure, and clinical manifestations (1). Therefore, personalized medicine focusing on phenotype and the identification of treatable traits is critical for effective treatment of COPD (2). A clinical phenotype is defined as a set of disease characteristics that can be used to distinguish individuals with COPD and can be associated with relevant clinical outcome, such as symptoms, exacerbations, treatment response, disease progression or death (3, 4). In this context, hierarchical clustering analysis has revealed correlations between exacerbation-prone COPD patients with severe airflow limitations and high levels of both airway and systemic inflammation (5). The chronic inflammation in the lungs of COPD patients is generally characterized by enhanced activation and increased numbers of macrophages and granulocytes, predominantly neutrophils (6). Moreover, the lungs exhibit an increased presence of $CD8^+$ T lymphocytes, which outnumber $CD4^+$ T cells (7).

Recently, an elevated presence of circulating innate lymphoid cell (ILC) subsets was reported in COPD (8-10). ILCs are relatively recently discovered cell types of non-B/non-T lymphocytes with diverse roles in mucosal inflammation and tissue repair (11). ILCs are classified as ILC1, ILC2 and ILC3, based on signature transcription factor expression and cytokines secretion profiles, which mirror those of T helper (Th) cell subsets. It is assumed that the three ILC groups are derived from a common precursor, referred to as the ILC progenitor (ILCP), which is also present in peripheral blood (12, 13). Different studies have shown elevated levels of ILC1s and ILC3s in COPD lungs and plasticity of ILC2s towards IFNγ-producing ILC1s during viral infection in COPD patients (9, 14). Silver et al. found a positive correlation of disease severity, as defined by decreased forced expiratory volume in 1 second (FEV1), with the numbers of circulating ILC1s (8). A negative correlation was observed with the numbers of ILC2s, which are characterized by IL-5 and IL-13 production and have been implicated in allergic asthma (8, 15). Recently, we identified a relative shift from $CD45RA^+$ to $CD45RO^+$ ILC1s and ILC2s in COPD patients (10). Notably, we previously reported that $CD45RO^+$ ILC2s constitute an inflammatory, steroid-resistant ILC2 subset (16).

However, to the best of our knowledge, a comprehensive cluster analysis that includes ILC subsets and clinical characteristics has not yet been conducted in COPD patients. In this study, we aim to identify COPD phenotypes based on various clinical parameters, such as age, pack years, exacerbation rate and lung function data. In addition, we explore the relationship between these clinical phenotypes and immune cells – in particular the ILC compartment – in peripheral blood.

MATERIALS AND METHODS

Study design

In this prospectively designed cross-sectional study, we identified patient clusters based on their clinical phenotype and explored their relationship with the main peripheral blood leukocyte populations, as well as NK cells and ILC subset characteristics (10).

Participants

Pseudonymized data were obtained from a clinical study conducted in the Franciscus Gasthuis and Vlietland Hospital Rotterdam, the Netherlands, executed between 2019 and 2021. The Medical Ethics Committees United (MEC-U) approved the studies and all participants provided a written informed consent. Next to clinical data, peripheral blood was collected from patients as well as sex- and age-matched smoking and non-smoking healthy controls (HCs) (10). COPD patients were diagnosed based on the presence of airway obstruction, measured by FEV1 divided by the Forced Vital Capacity (FVC) < 0.7. All COPD patients were ex- or current smokers with more than ten pack years. Patients were clinically stable for more than six weeks and received only low dose oral steroids (max 5mg per day) were allowed.

Clinical data collection

We collected twelve clinical characteristics of the COPD patients: age in years, sex, pack years, smoking status, exacerbation frequency in the previous twelve months, body mass index (BMI), COPD Assessment Test (CAT), Medical Research Council dyspnea (MRC), Forced Expiratory Volume in 1 second (FEV1), Forced Vital Capacity (FER), Residual Volume (RV) within Total Lung Capacity (TLC) and Diffusing Capacity for Carbon monoxide (DLCOc).

Analysis of peripheral blood

Within the leukocyte differentiation analysis of peripheral blood, we determined the number of leukocytes, neutrophils, lymphocytes, eosinophils, basophils and monocytes, using Beckman Coulter equipment (DxH 800, Fabia and Immage 800). As we reported previously, peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density separation using standard procedures and stained with fluorochrome-labeled antibodies to distinguish ILC subset by flow cytometry (10, 16) Lineage-negative cells were defined by the absence of the following cell surface markers: CD14, CD16, CD19, CD94, FCεRI and TCRαβ and TCRγδ. Antibody information is presented in **Suppl. Table 1.** Data were acquired with a FACS Symphony A5 cytometer using FACS Diva software 9.1 (BD Biosciences, Erembodegem, Belgium) and analyzed using FlowJo 10.8.1 (Tree Star Inc, Ashland, Ore).

Statistical analyses

The dimension reduction methods Principal Component Analysis (PCA) and Multiple Factor Analysis (MFA) were performed in R (R studio Server Version 1.4.1717) and the packages FactoMineR and Factorextra, as described previously (17). The number of dimensions to be interpreted were determined by the R package FactoInvestigate. Statistical significance was calculated using the non-parametric Kruskall-Wallis test with Bonferroni correction for multiple testing - or alternatively by Mann-Whitney U tests for evaluation of two parameters - in IBM SPSS Statistics (Version 28.0.0.0 (190)). Median scores were used for all data sets. P-values <0.05 were considered statistically significant.

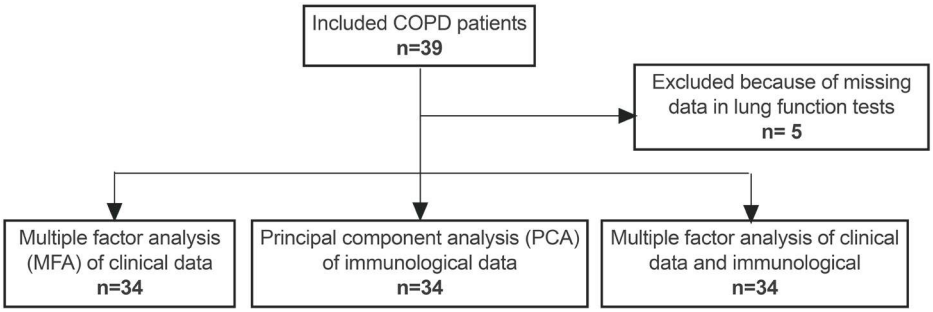


Figure 1. Patient enrolment.
In total 39 COPD patients were included (of which five were excluded because of missing data in lung function tests). Two multiple factor analyses (MFA) and one principal component analysis were performed with 34 patients.

RESULTS

COPD patient characteristics and the identification of three clinical clusters

The patient enrolment is illustrated in **Figure 1**. A total of 39 COPD patients were included in the study, and for 34 patients the data were complete for all clinical characteristics. Based on twelve clinical variables, three clinical clusters were identified by multiple factor analysis (MFA) (**Table 1; Figure 2A**). The distribution over the first dimension (Dim1) and second dimension (Dim2) was non-random. This was not due to sex or smoking status (**Suppl. Figure 1**).

Clinical cluster 1 (C1) was characterized by median age of 59.5 years, median pack years of 30.5 and median FEV1 of ~30.0. Cluster 2 (C2) by median age of 68 years, median pack years of 55, and median FEV1 of ~52.0 and Cluster 3 (C3) by median age of 58 years, median pack years of 32, and median FEV1 of ~62.0 (**Table 1**). When these data were clustered by MFA, Dim1 and Dim2 contained ~29% and ~22% of variance, respectively. In Dim1 significant differences were observed between clinical clusters C1 and C3 and between C2 and C3. In Dim2, significant differences were found between clinical clusters C1 and C2 and between C2 and C3 **(Figure 2B)**. Dim1 was dominated by CAT, FEV1 and MRC and Dim2 by age, pack years and BMI **(Figure 2C)**. C1 with the lowest FEV1, also had the highest level of disease burden in terms of MRC and CAT and the lowest proportion of patients without exacerbations over the last year (75%) (**Table 1**). Patients within C2, which were relatively older and had the highest number of pack years, were essentially overweight. C3 consists of patients with milder disease, given the higher FEV1 values and fewer exacerbations.

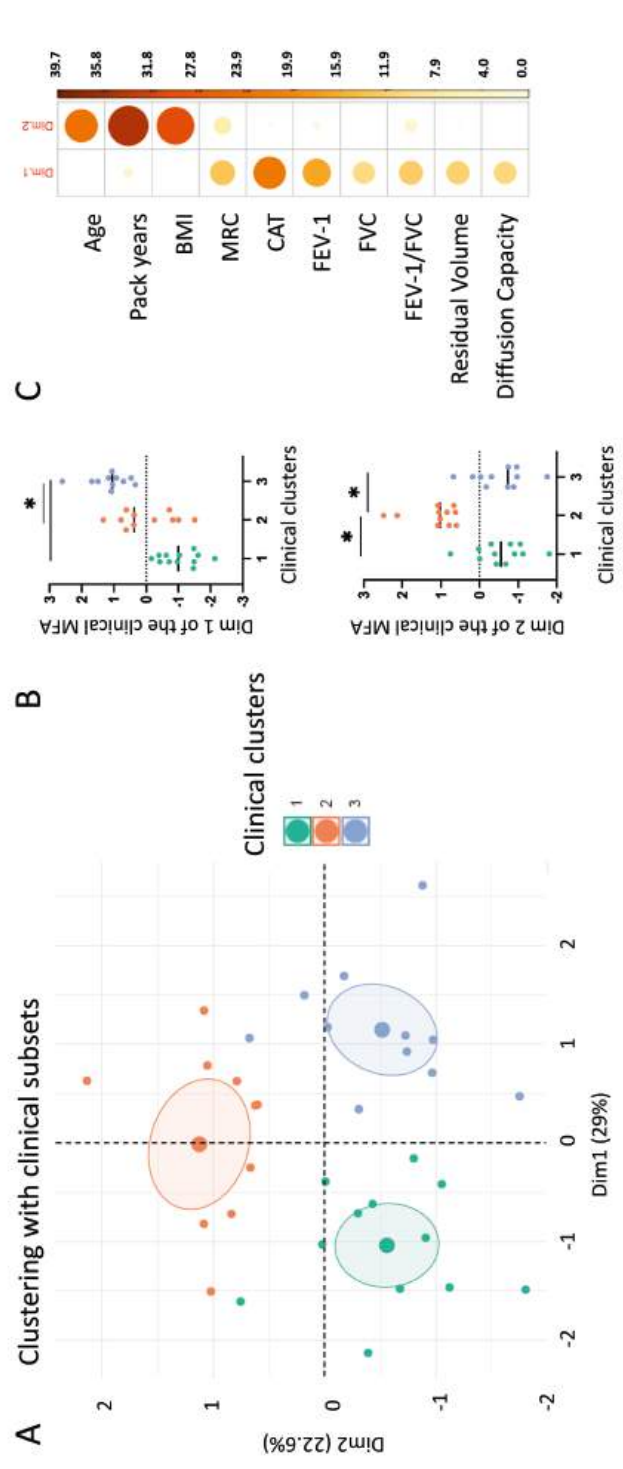


Figure 2. Hierarchical cluster analyses of clinical parameters.
A) Dimensions 1 and 2 (Dim1 and Dim2) of a multiple factor analysis (MFA) of COPD patients, based on the clinical parameters shown in panel C. **B)** Statistical analysis of clinical clusters defined by PCA. The X-axis represents clinical clusters. Y-axis represents Dim1 and Dim 2 values of the clinical MFA mentioned in A). Dots represent individual patients. Horizontal bars represent median values. Statistical analyses were performed using Kruskal-Wallis test combined with a Bonferroni correction for multiple testing, * $p<0.05$. **C)** Relative contribution (as shown by size and orange color range of dots) of the indicated clinical parameter in Dim1 and Dim2 of the MFA.

Table 1. Patient characteristics of the clinical clusters C1, C2 and C3.

	Clinical cluster 1 N=12	Clinical cluster 2 N=11	Clinical cluster 3 N=11	P-value
*Variables used in clustering **				
Female	6 (50) **	7 (63.6)	7 (63.6)	0.742
Current smokers	7 (58.3)	4 (36.4)	7 (63.6)	0.395
Zero exacerbations last year	3 (25)	7 (63.6)	9 (81.8)	0.052
Age	59.50 [49.25–65.50]	68.00 [65.00–74.00]	58.00[51.00–65.00]	0.005
Pack Years	30.50[23.75–34.50]	55.00 [49.00–60.00]	32.00[17.00–38.00]	<0.001
BMI	23.73[21.74–29.92]	28.69[25.13–31.00]	25.09[20.62–26.96]	0.055
MRC	3.5 [2.25-4]	3 [3-4]	2 [1-3]	0.025
CAT	21 [18-29]	12 [11-21]	13 [9-18]	0.004
FEV1 pre %pred	30.00[26.00-44.00]	52.00[30.00–63.00]	62.00[52.00–80.00]	<0.001
FVC %pred	70.50[65.00-85.50]	83.00[75.00–96.00]	104.00[92.00–110.00]	<0.001
FEV1/FVC in %	29.69[22.55–44.72]	43.64[31.00-58.28]	46.59[43.77–59.19]	0.008
RV/TLC in %	59.95[52.11–67.72]	51.76[44.88–59.30]	42.24[34.44–49.32]	0.002
DLCOc % pred	40.00[34.00–46.75]	58.00[43.00–66.00]	69.00[47.00–72.00]	0.014

*) Hierarchical clustering is performed based on 2 components with an eigenvalue >1 with variables: sex, current smokers, exacerbations previous year, age, packyears, BMI, MRC, CAT, FEV1 % pred, FVC% pred, FEV1/ FVC in %, RV/TLC in % DLCOc in % **) Data are presented as N (%) or median [25-75 interquartile]. Statistical significance was calculated by Kruskall-Wallis test with Bonferroni correction. BMI: Body Mass Index, MRC: Medical Research Council, CAT: COPD assessment Test, FEV1 % pred: Forced Expiratory Volume in 1 second percentage predicted, FVC: Forced Vital Capacity, RV: residual volume, TLC: Total Lung Capacity, DLCOc: Diffusion capacity of the Lungs for carbon monoxide.

Identification of two immunological clusters based on peripheral blood leukocyte composition and ILC subset phenotypes

We performed a PCA of total blood counts for various myeloid and lymphoid cell populations, as well as characteristics of NK and ILC subsets, which we reported previously (10). Hereby, NK^{dim} and NK^{bright} cells were gated based on CD56 expression. Total ILCs were first gated as lineage-negative cells positive for CD127/IL-7R; ILC subsets and precursors were subsequently identified by subset-specific markers (**Suppl. Figure 2).** We included additional markers associated with ILC plasticity (CD117/c-kit) or an inflammatory phenotype ($CD4^+$ and $CD45RO^+/CD45RA^-$) (**Suppl. Figure 2**) (16, 18-20).

Based on these immunological parameters, two clusters of COPD patients were identified, leukocyte clusters L1 and L2, which showed a statistically significant difference for both Dim1 and Dim2 (**Table 2; Figure 3AB**). Hereby, ILC1 subpopulations contributed most to Dim1; absolute numbers of myeloid cell populations, including monocytes and basophils, contributed most to Dim2 **(Figure 3C)**. The parameters of leukocyte counts, ILC subsets, and ILC phenotype in the two separate clusters L1 and

L2 are shown in **Table 2**. The two clusters differed significantly in a range of parameters, particularly in the numbers of myeloid cells and subpopulation characteristics of ILC1s. L2 had significantly higher peripheral blood counts for monocytes, neutrophils, basophils and eosinophils, compared with L1. Moreover, in L2 the ILC1s showed significantly lower frequencies of $CD45RO^+$ cells both in the $CD4^+$ and in the $CD4^-$ subpopulation, suggesting a less inflammatory phenotype of these cells. For mature c-kit^- ILC2s the reduction of the frequencies of $CD45RO^+$ cells in cluster L2 compared to cluster L1 almost reached statistical significance (5.51 and 21.1, respectively; p=0.051). In summary, L2 was characterized by higher myeloid cell counts but fewer inflammatory ILCs in the circulation, compared with L1.

Next, we superimposed the three clinical clusters of COPD patients onto the leukocyte clustering based on immunological subsets (**Figure 3D**). These clinical clusters showed a non-random distribution, because we found that clinical cluster C1 (with the most severe phenotype) was underrepresented in immunological cluster L1 and overrepresented in immunological cluster L2 (**Figure 3D-E**). In fact, the majority of immunological cluster L2 consisted of patients from clinical cluster C1 (**Figure 3E**). Clinical cluster C1 showed somewhat higher average values for Dim1 and Dim2 than C2 and C3, but these differences were not statistically significant (**Suppl. Figure 3A**).

To further explore the relation between clinical and immunological clusters, we first investigated the individual ILC parameter values across the three clinical clusters. We found that C1 had significantly lower proportions of $CD45RO^+$ cells within the population of $CD4^-$ ILC1, when compared to C2 (**Figure 4A**). Other immunological parameters exhibited negligible differences across the clinical clusters. Conversely, we compared clinical parameter values between the two immunological clusters and noticed multiple significant differences **(Figure 4B).** Immunological cluster L2, with higher myeloid cell counts but reduced frequencies of $CD45RO^+$ ILCs, had increased levels of MRC, CAT and RV/TLC and decreased levels of FEV1(% predicted) and FEV-1/FVC. Interestingly, all of these differences were indicative of a more severe COPD state of patients within immunological cluster L2.

In summary, based on peripheral blood leukocyte differentiation and ILC subset phenotype, we were able to identify two immunological clusters. Hereby, immunological cluster L2 was associated with higher myeloid cell counts and a lower proportion of $CD45RO^+$ ILCs, and showed a substantial overlap with clinical cluster C1 with a more severe disease phenotype.

Table 2. Two patient clusters, L1 and L2, based on myeloid and lymphoid cell counts and NK cell ILC subsets.

	Immunological cluster L1[†] N=20	**Immunological cluster L2[†] N=14**	**P-value**
*Variables used in clustering *)*			
Leukocytes *10^9	7.50 [5.97 - 8.85] ***)*	9.65 [7.95 - 11.43]	0.010
Neutrophils *10^9	4.05 [3.58 - 5.43]	6.65 [5.95 - 7.92]	<0.001
Lymphocytes *10^9	2.00 [1.67 - 2.40]	2.45 [1.72 - 2.58]	0.436
Monocytes *10^9	0.60 [0.50 - 0.72]	0.85 [0.62 - 1.00]	0.014
Eosinophils *10^9	0.12 [0.09 - 0.17]	0.19 [0.14 - 0.38]	0.012
Basophils *10^9	0.05 [0.04 - 0.07]	0.08 [0.07 - 0.10]	0.006
Neutrophils/ Lymphocytes ratio	2.01 [1.67-2.53]	2.84 [2.14-3.80]	0.043
NK^{bright} within alive lymphocytes	0.38 [0.27 - 0.59]	0.64 [0.44 - 1.02]	0.036
NK^{dim} within alive lymphocytes	29.60 [16.75 - 43.58]	36.750 [32.93 - 40.85]	0.217
Total ILC within alive lymphocytes	0.071 [0.0428 - 0.0963]	0.0635 [0.044 - 0.0745]	0.341
CD117+ ILC2 of total ILC	7.85 [3.84 - 10.60]	15.1000 [10.07 - 19.10]	0.006
CD45 RA+ of CD117+ ILC2	86.05 [73.48 - 92.85]	83.950 [82.25 - 88.18]	0.666
CD45 RO+ of CD117+ ILC2	1.00 [0.00 - 4.35]	2.29 [0.28 - 4.71]	0.522
CD117- ILC2 within total ILC	6.97 [3.54 - 12.20]	6.455 [3.12 - 7.68]	0.545
CD45 RA+ of CD117- ILC2	49.45 [25.00 - 66.70]	72.15 [47.00 - 86.68]	0.077
CD45 RO+ of CD117- ILC2	21.10 [10.37 - 48.33]	5.51 [2.94 - 21.90]	0.051
CD4+ ILC1 within total ILC	7.24 [5.33 - 9.73]	4.49 [3.27 - 8.51]	0.545
CD45 RA+ of CD4+ ILC1	8.575 [0.00 - 20.88]	41.10 [28.95 - 59.98]	<0.001
CD45 RO+ of CD4+ ILC1	80.40 [69.53 - 91.35]	46.60 [33.98- 50.75]	<0.001
CD4- ILC1 within total ILC	25.65 [19.03- 33.50]	22.70 [16.48 - 25.18]	0.359
CD45 RA+ of CD4- ILC1	38.90 [24.88 - 44.58]	49.45 [39.63 - 70.00]	0.007
CD45 RO+ of CD4- ILC1	39.15 [32.83 - 56.55]	26.4500 [18.55 - 37.13]	0.017
ILCP within total ILC	48.500 [33.45-60.85]	49.750 [36.40-60.10]	0.877

*) Hierarchical clustering is performed based on 2 components with an eigenvalue >1. **) Data are presented as median values [25-75 interquartile]. Statistical significance was calculated by Mann- Whitney U tests. NK: Natural Killer cell, ILC: innate lymphoid

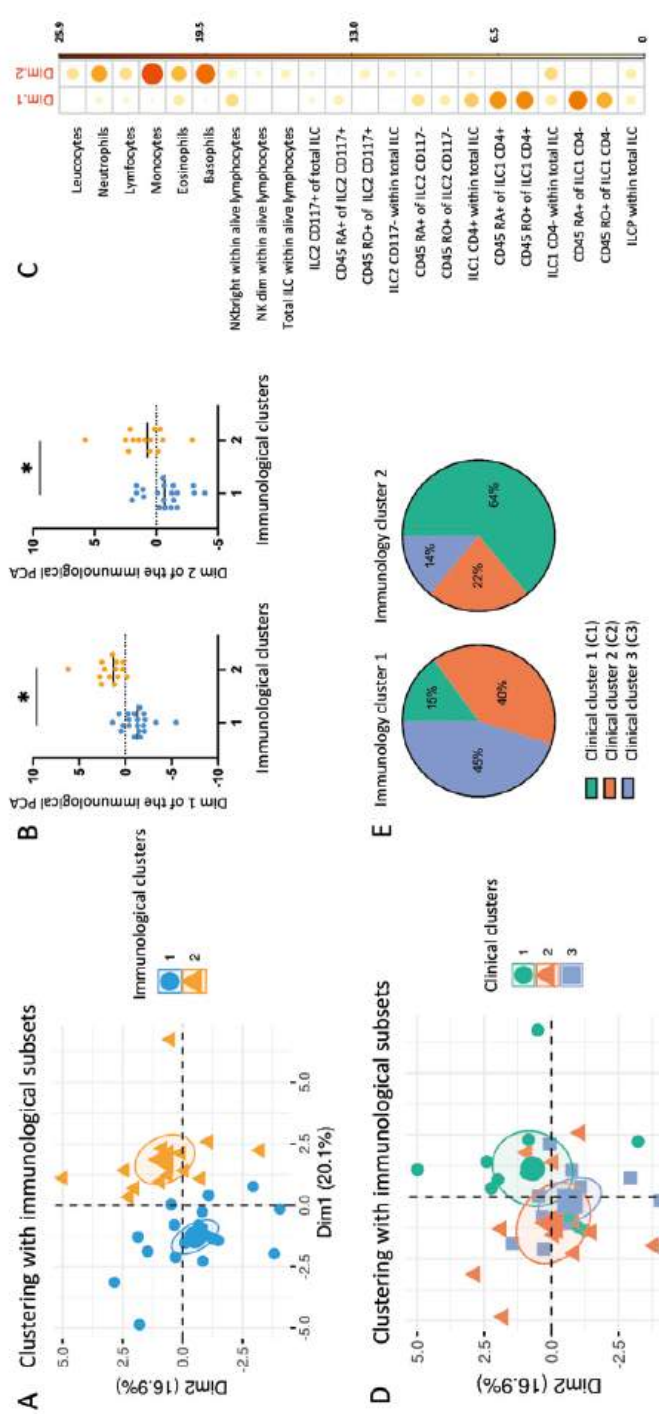


Figure 3. Hierarchical cluster analyses of immunological parameters.
A) Dimensions 1 and 2 (Dim1 and Dim2) of a principal component analysis (PCA) of COPD patients, based on the leukocyte parameters shown in panel C. **B)** Statistical analysis of immunological clusters defined by PCA. The X-axis represent immunological clusters. Y-axis represent Dim1 and Dim2 of the clinical PCA mentioned in A). Dots represent an individual patient per dimension. Horizontal bars represent median values. Statistical analyses were performed using Mann-Whitney U tests, * p<0.05. **C)** Relative contribution (as shown by size and orange color range of dots) of the indicated immunological parameter in Dim1 and Dim2 of the PCA. **D)** The clinical clusters of Figure 2A were used to search for overlap with the immunological profile (Figure 3A). **E)** Pie charts of immunological clusters L1 and L2, showing the distribution of patients of clusters C1, C2 and C3.

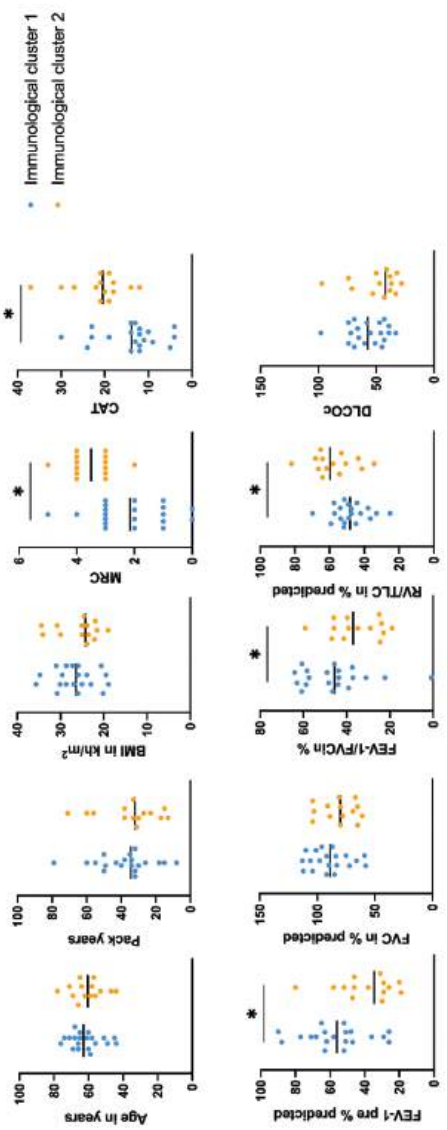


Figure 4. The impact of immunological and clinical parameters on the immunological- and clinical clusters.
A) Frequencies of CD45RO$^+$ cells within the population of CD4$^-$ ILC1 in the indicated clinical clusters. Dots represent individual patients and median values are indicated by horizontal bars. Statistical analyses were performed using Kruskal-Wallis test combined with a Bonferroni correction for multiple testing, * p<0.05. **B)** Values for the indicated clinical parameters in the immunological clusters L1 and L2. Dots represent individual patients and median values are indicated by horizontal bars. Statistical analyses were performed using Mann-Whitney U tests. Significant differences between the immunological clusters L1 and L2 are shown; * p<0.05. BMI: Body Mass Index, MRC: Medical Research Council, CAT: COPD assessment Test, FEV1 % pred: Forced Expiratory Volume in 1 second percentage predicted, FVC: Forced Vital Capacity, RV: residual volume, TLC: Total Lung Capacity, DLCOc: Diffusion capacity of the Lungs for carbon monoxide.

COPD patients within the clinical clusters share characteristics in their immunological profiles

Finally, we performed an integrated MFA with the combined data of clinical and immunological parameters (**Tables 1 and 2**). The distribution of the patients over Dim1 and Dim2 was not due to sex or smoking status (**Suppl. Figure 4**). We first superimposed the three clinical clusters of COPD patients on this MFA (**Figure 5A**). Whereas in Dim1 cluster C1 was significantly separated from clusters C2 and C3, in Dim2 cluster C2 was significantly separated from clusters C1 and C3 (**Figure 5B**). In this MFA, clinical parameters, including MRC, CAT and lung function, as well as immunological parameters contributed mostly to Dim1. Age, pack years and BMI contributed mostly to Dim2 (**Figure 5C**).

Conversely, when we superimposed the two immunological clusters onto the MFA, we found a robust separation of L1 and L2 in Dim1 (**Figure 5D**). Dim2 did not significantly differ between the two immunological clusters, which would be consistent with the strong contribution of age, pack years and BMI, but not of immunological parameters, to this dimension (**Figure 5C-E**). Comparison of **Figure 5A and Figure 5D** revealed a substantial overlap between clinical cluster C1 and immunological cluster L2, and that COPD patients from clinical clusters C2 and C3 mostly ended up together in immunological cluster L1.

Taken together, these findings indicate an association between the clinical traits of COPD patients and their peripheral blood immunophenotype defined by total leukocyte and ILC characteristics. In particular, our PCA an MFA indicated that more severe COPD (clinical cluster C1) is associated with a reduced proportion of $CD45RO^+$ ILC1s and ILC2s in the circulation (immunological cluster L2).

DISCUSSION

In this translational study we performed unbiased clustering of clinical COPD phenotypes based on various clinical parameters, such as age, pack years, exacerbation rate and lung function. We identified three clinical phenotypes and two immunological clusters. When the clinical and immunological clusters were merged in a single clustering analysis, there was an overlap between clinical cluster C1, denoting severe COPD patients, and immunological cluster L2, characterized by elevated myeloid cell levels and reduced frequencies of potentially inflammatory $CD45RO^+$ cells within the ILC1 population. This overlap implies an association between these two phenotypes.

The clinical clusters that were identified correspond to well-established patterns mentioned in the literature, often referred to as the “pink puffer” and “blue bloater” phenotypes in COPD (21). Clinical cluster 1 aligns with the “pink puffer” phenotype, characterized by cachexia, low levels of FEV1 and hyperinflation, while clinical cluster

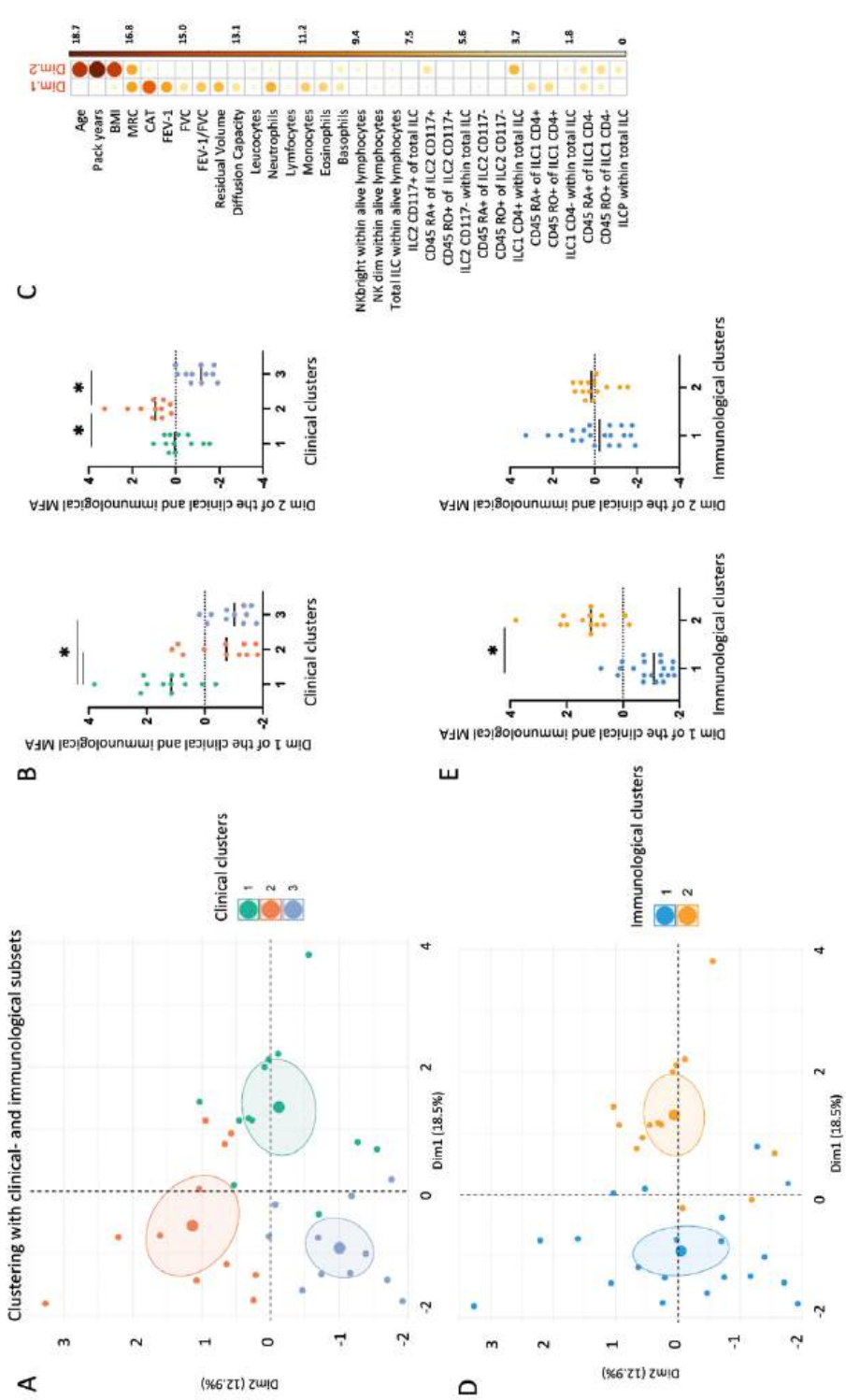

A
Clustering with clinical- and immunological subsets
Clinical clusters
1
2
3
Dim2 (12.9%)
Dim1 (18.5%)
B
Dim 1 of the clinical and immunological MFA
Clinical clusters
C
Dim.1
Dim.2
18.7
16.8
15.0
13.1
11.2
9.4
7.5
5.6
3.7
1.8
0
Age
Pack years
BMI
MRC
CAT
FEV-1
FVC
FEV-1/FVC
Residual Volume
Diffusion Capacity
Leucocytes
Neutrophils
Lymfocytes
Monocytes
Eosinophils
Basophils
NKbright within alive lymphocytes
NK dim within alive lymphocytes
Total ILC within alive lymphocytes
ILC2 CD117+ of total ILC
CD45 RA+ of ILC2 CD117+
CD45 RO+ of ILC2 CD117+
ILC2 CD117- within total ILC
CD45 RA+ of ILC2 CD117-
CD45 RO+ of ILC2 CD117-
ILC1 CD4+ within total ILC
CD45 RA+ of ILC1 CD4+
CD45 RO+ of ILC1 CD4+
ILC1 CD4- within total ILC
CD45 RA+ of ILC1 CD4-
CD45 RO+ of ILC1 CD4-
ILCP within total ILC
Dim 2 of the clinical and immunological MFA
Clinical clusters
D
Immunological clusters
1
2
Dim2 (12.9%)
Dim1 (18.5%)
E
Dim 1 of the clinical and immunological MFA
Immunological clusters
Dim 2 of the clinical and immunological MFA
Immunological clusters

Figure 5. Hierarchical cluster analyses of clinical and immunological parameters.
A) Dimensions 1 and 2 (Dim1 and Dim2) of a multiple factor analysis (MFA) of COPD patients, based on both clinical and leukocyte parameters shown in panel C. The clinical clusters C1, C2 and C3, as defined in Table 1 and Figure 2, are superimposed. **B)** Values of Dim1 and Dim2 of the clinical and immunological MFA for clinical clusters C1, C2 and C3. Dots represent individual patients and median values are shown as horizontal bars. Statistical analyses were performed using a Kruskal-Wallis test combined with a Bonferroni correction for multiple testing, * $p<0.05$. **C)** Relative contribution (as shown by size and orange color range of dots) of the indicated clinical and immunological parameters in Dim1 and Dim2 of the MFA. **D)** MFA of panel A, whereby the immunological clusters L1 and L2 are superimposed. **E)** Values of Dim1 and Dim2 of the clinical and immunological MFA for Immunological clusters L1 and L2. Dots represent individual patients and median values are shown as horizontal bars. Statistical analyses were performed using Mann-Whitney U test, * $p<0.05$.

2 corresponds to the "blue bloater" phenotype of mild dyspnea, cyanosis and obesity. Cluster 3 represents COPD patients with milder symptoms. The finding that these well-known phenotypes are evident in our clusters underlines the robustness of our data.

In our dataset, we noted elevated neutrophil counts in patients with severe COPD, in line with existing literature that highlights an association between increased neutrophil-to-lymphocyte ratios and 90-day mortality in COPD patients during exacerbations (22-24). Furthermore, we observed heightened proportions of eosinophils in patients with severe COPD. Increased eosinophils in COPD are a well-recognized occurrence and have been proposed as a novel COPD phenotype that is associated with a type-2 inflammatory profile also observed in patients with asthma (25). Type 2 cytokines IL-4, IL-5 and IL-13 and inflammatory cells, including eosinophils, are linked to airway remodeling and the deterioration of lung parenchyma, which are prevalent characteristics of COPD (26). Notably, eosinophils could be useful in predicting response to anti-IL-5 treatment (6).

In our analysis, the clinical cluster C1 containing severe COPD patients showed an association with reduced frequencies of CD45RO+ ILC1s. Given the evidence that $CD45RO^+$ ILCs are pro-inflammatory (10, 16) this suggests that severe COPD patients exhibit a limited immunological response in their ILC1s compared to those with less severe COPD. This observation would align with the finding of decreased capacity of $CD8^+$ T-cells to produce IFN-γ or TNFα among COPD patients in GOLD IV stage, in contrast to those in GOLD II and III stages (7). Alternatively, it is conceivable that in patients with severe COPD (and high myeloid cell counts) $CD45RO^+$ ILC1 abundance is low in the circulation since these cells accumulate in the lung. The relationship between the activation status of circulating ILC1s and their counterparts in the lung should be an important subject for future studies. It is attractive to speculate that in the early stage of COPD, there is enhanced inflammation marked by increased levels of $CD45RO^+$ ILC1s and IFN-γ/TNFα-expressing $CD8^+$ T cells. This inflammation may have a broader systemic impact and contribute causally to COPD development. As

COPD advances to the GOLD IV stage, there is a rise in airway destruction leading to exacerbations. The original primary inflammation subsides, giving way to exacerbation-driven inflammation characterized by increased neutrophil/lymphocyte ratio in response to viral infections.

A key strength of this work lies in the successful classification of COPD patients into distinct clinical clusters, despite the relatively small sample size of 34 individuals with COPD. This may be attributed to the comprehensive range of clinical characteristics available for each COPD patient. Secondly, our analysis revealed that variables such as sex and current smoking were evenly distributed among the clinical clusters. This indicates that these variables did not significantly influence the formation of the clinical clusters.

There are certain limitations that should be acknowledged. First, we focused on quantifying the proportions of ILCs and their subsets rather than exploring their functional attributes, such as cytokine production, which poses challenges due to the their very low cell numbers in the circulation. Nevertheless, in our analyses, we concentrated on surface expression of $CD45RO^+$ cells and CD4, which may serve as pro-inflammatory markers, thereby shedding light on the functionality of the ILCs (16, 18). Secondly, the connection between the phenotype or activation status of peripheral blood ILCs and their pathophysiological impact within the lungs remains unexplored. This is particularly pertinent considering that ILCs tend to adopt functional phenotypes specific to different tissues (27), and they are often considered to be primarily resident cells within tissues (13, 28). Future experiments should shed light on the specific roles and effects of these surface markers CD4 and CD45RO in ILC1 function and their contribution to the pathophysiology of COPD. Finally, our finding of a correlation between the clinical COPD phenotype and the inflammatory profile of circulating ILCs needs to be confirmed in an independent and larger cohort of COPD patients.

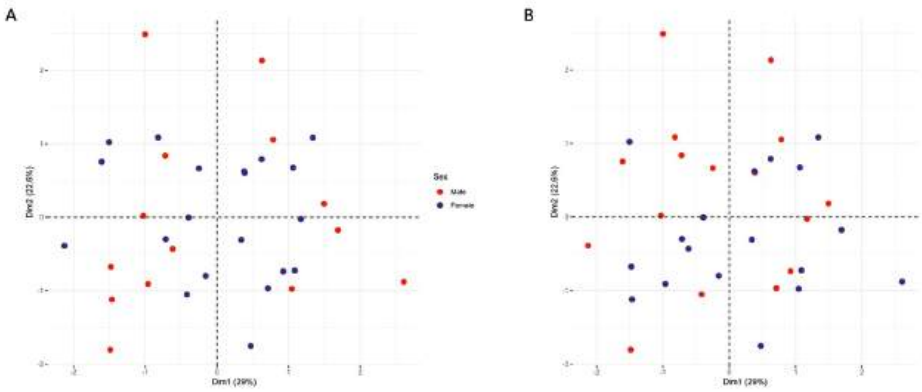
CONCLUSION

In this cross-sectional study with translational implications, we delineated three distinct clinical phenotypes within the COPD population. These phenotypes were accompanied by corresponding immunological profiles, as evidenced by consistent patterns in lymphoid and myeloid cell counts, as well as the presence and phenotype of ILC subsets within peripheral blood. COPD patients with high disease burden were associated with reduced frequencies of potentially inflammatory ILC1s, suggesting that severe COPD patients exhibit a limited immunological response in their circulating ILC1s compared to those with less severe COPD. These findings would support the involvement of ILCs in the trajectory of the disease. This insight could hold relevance for the identification of biomarkers and the exploration of novel personalized therapies.

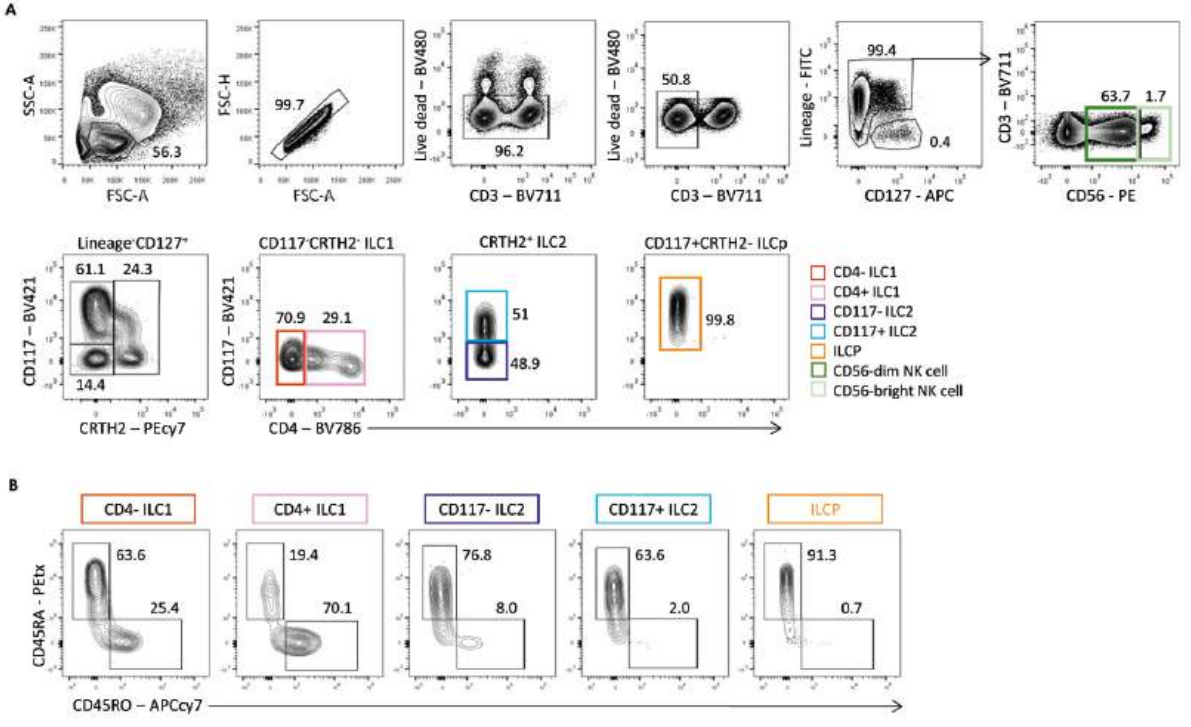
SUPPLEMENTARY

Suppl. Table 1. Antibody information FACS analyses

Antigen	Fluorchrome	Clone	Manufactorer
TCRab	FITC	IP26	Biolegend
TCRgd	FITC	B1	Becton Dickinson
CD14	FITC	61D3	Life Technologies
CD19	FITC	HIB19	Becton Dickinson
CD16	FITC	3G8	Becton Dickinson
CD94	FITC	DX22	Biolegend
FCeRI	FITC	AER-37	Life Technologies
CD56	PE	TULY56	Life Technologies
CD45RA	PE TxR	MEM-56	Invitrogen
NKp44	PCP	P44-8	Biolegend
CRTH2	PE-Cy7	BM16	Biolegend
CD117	BV421	104D2	Biolegend
LD	BV510	-	Life Technologies
CD25	BV605	BC96	Biolegend
CD3	BV711	UCHT1	Becton Dickinson
CD4	BV786	SK3	Becton Dickinson
CD127	APC	eBioRDR5	Life Technologies
CD45RO	APC-Cy7	UCHL-1	Biolegend
CD5	AF700	UCHT-2	Becton Dickinson
NHS	-	-	Biolegend



Supplementary Figure 1. Multiple Factor Analysis of clinical parameters of 34 patients with COPD. **A)** Male and female distribution within the clinical clusters. **B)** Current smoker or ex-smoker distribution within the clinical clusters.



Supplementary Figure 2. Gating strategy for ILC subsets in peripheral blood

a) Representative flow cytometry gating strategy used for the quantification of ILC and NK cell subsets in cryopreserved peripheral blood mononuclear cells (PBMCs) from healthy donors and patients. PBMCs were stained with antibodies against lineage markers (CD14, CD16, CD19, CD94, FcRεR1a, RCRαβ, TCRγδ) and indicated ILC/ NK cell-specific markers.

b) Representative flow cytometry analysis fo CD45RA and CD45RO surface expression on the indicated ILC subsets as gated in panel A. Antibodies used for flow cytometry are given in Suppl. Table 1.

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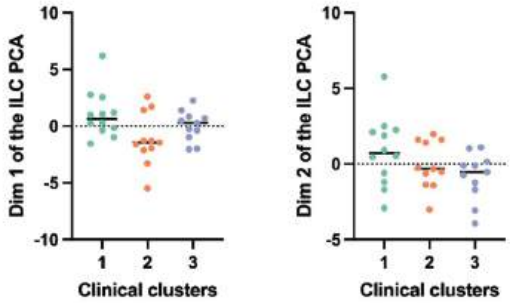
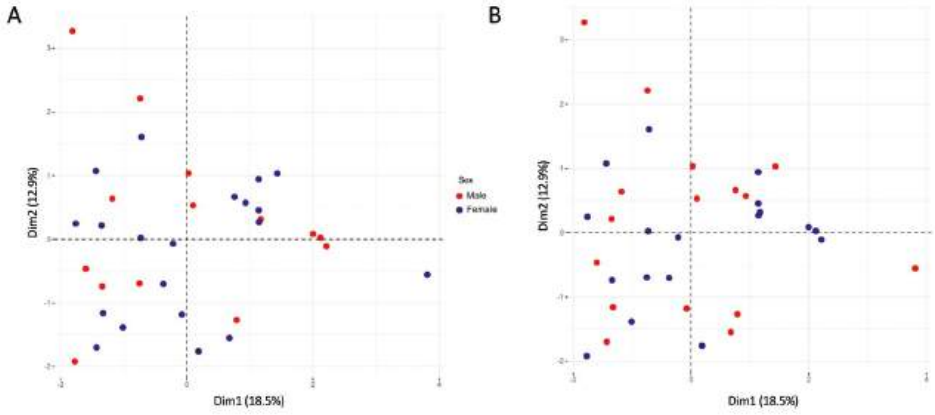


Supplementary Figure 3. Principal component analysis of immunological parameters in the three identified clinical clusters.
Dimension 1 (Dim1) and Dim2 are based on immunological variables (Figure 3A). The clinical clusters of Figure 2A were used to search for overlap in the merged clinical and immunological data (Figure 3A). Dots represent an individual patient per dimension. Horizontal bars represent median values. Statistical analyses were performed using Kruskal-Wallis test combined with Bonferroni correction for multiple testing.



Supplementary Figure 4. Distribution of male, female, current smoker and ex-smoker. **A)** Male and female distribution within the plot with clinical and immunological data (FIgure 5). **B)** Current smoker or ex-smoker distribution within the plot with clinical and immunological data (Figure 5).

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Chapter 6

Measuring burden of disease in both asthma and COPD by merging the ACQ and CCQ: less is more?

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ABSTRACT

Introduction

There is considerable overlap in symptoms of asthma and COPD, and both diseases can co-exist in one patient. The asthma control questionnaire (ACQ) and clinical COPD questionnaire (CCQ) were developed to assess disease burden in respectively asthma or COPD. A single practical tool for both diseases is still lacking.

Objective

To explore the possibility of creating a new questionnaire to assess disease burden in obstructive lung disease by integrating and reducing questions of the ACQ and CCQ

Methods

Data of patients with asthma or COPD were collected from a secondary care center. Patients completed ACQ and CCQ on the same day. Linear regression was used to test correlations. Principal Component Analysis (PCA) was used for item reduction. Data were reproduced in a secondary cohort of patients with asthma-COPD overlap (ACO) and in a primary care cohort.

Results

252 patients with asthma and 96 with COPD were included in the development cohort. Correlation between ACQ and CCQ in asthma was R=0.82, and in COPD R=0.83. PCA determined a selection of 9 questions. Reproduction in secondary care data of ACO patients (n=53) and primary care data (asthma n=1110, COPD n=1041, ACO=355) showed similar correlations and PCA determined a similar selection of questions as in the development cohort.

Conclusion

ACQ and CCQ are strongly correlated in asthma, COPD and ACO. PCA determined a selection of nine questions of the ACQ and CCQ: working title ‘the Obstructive Lung Disease Questionnaire’. This implicates that this pragmatic set of questions might be sufficient to assess disease burden in obstructive lung disease.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) and asthma are both obstructive pulmonary diseases with a high disease burden. Although the diagnoses of COPD and asthma are clearly defined, the symptoms and clinical manifestations of COPD and asthma show considerable overlap, e.g. wheeze, dyspnea, and cough. Additionally, some patients seem to have both diseases: the Global Initiative for Chronic Obstructive Lung Disease (GOLD) and the Global Initiative for Asthma (GINA) previously described this condition as asthma-COPD overlap (ACO) (1). In today's context, there exists some controversy surrounding this term, with the current terminology often describing this group as COPD patients with asthma features or asthma patients with COPD features. Nevertheless, some studies suggest that the prevalence of ACO can be as high as 25% in COPD patients and 31% in asthma patients; this means that a substantial part of the COPD and asthma population have features of both diseases (2, 3). Furthermore, the prevalence of ACO increases with age, so it might be expected that this group will be even larger in the future due to the aging population (4).

The asthma control questionnaire (ACQ) and clinical COPD questionnaire (CCQ) were developed to assess the disease burden in patients with respectively asthma or COPD (5, 6). The ACQ and CCQ are short, practical, and are used regularly in primary, secondary, and tertiary care in the Netherlands (7, 8). Because of the similarities in clinical features in asthma and COPD, the diagnosis may not be clear in the beginning, and the diagnostic process can take several consultations. During this initial period both the ACQ and the CCQ have to be completed to assess disease burden, resulting in extra work for patients and health care professionals (9, 10). Moreover, patients with features of both diseases were excluded in the development and validation of the ACQ and CCQ, so these questionnaires may be less appropriate for patients with ACO (5, 6). A single, practical questionnaire for both diseases is needed to improve the assessment of disease burden in this substantial proportion of the asthma and COPD population and might also be useful in patients with asthma or COPD only.

The aim of our study was to explore the possibility of creating a single questionnaire for assessment of the disease burden in asthma, COPD, and ACO. We hypothesize that this new approach, containing a selection of questions from the ACQ and CCQ, could be used to assess disease burden and quality of life in asthma, COPD and ACO.

METHODS

Study design

In this study, retrospective cross-sectional cohort analyses were performed in asthma and COPD patients treated in a secondary care cohort. In the first analysis, we aimed to compare two disease burden questionnaires and tested the correlation between the questionnaires. Patients completed both the ACQ and the CCQ on the same day, and these were compared separately in the asthma group and COPD group. Secondly, we selected questions of the ACQ and CCQ based on data reduction of the two questionnaires in a development cohort. Thirdly, we reproduced this selection process by data reduction in three reproduction cohorts: 1) a secondary cohort of patients with ACO; 2) a primary care cohort with asthma or COPD; and 3) a primary care cohort with ACO patients. Patients with ACO in the secondary cohort were not included in the development cohort, because this was a small patient group. The questions for this potential new questionnaire were selected by comparing and combining the results of the development cohort and the results of the three reproduction cohorts. Finally, we took a first step in testing the validity and reliability of this new potential questionnaire.

Setting and participants

Development cohort: asthma and COPD in secondary cohort

Data were part of a registry study of patients with asthma or COPD. Patients were newly referred to the Franciscus Gasthuis and Vlietland in Rotterdam, the Netherlands, a center of excellence for asthma and COPD care. Diagnosis was confirmed by a pulmonologist with special interest in obstructive lung disease during a previously published comprehensive assessment (11). Patients were included between December 2012 and December 2017. Both ACQ and CCQ had to be completed on the same day. Asthma diagnosis was based on the presence of typical clinical symptoms, reversible airway obstruction (+12% and 200ml improvement in FEV_1 after bronchodilator) or bronchial hyperreactivity (PC20 < 8mg/ml) or a FeNO > 50 ppb (12). The diagnosis of COPD was based on an assessment by a pulmonologist and confirmed by spirometry (post-bronchodilator forced expiratory volume (FEV_1) / forced vital capacity (FVC) <0.7) (13). In this study, we used pseudonymized data. Ethics approval for this study was waived by the Institutional Research Board of the Franciscus Gasthuis & Vlietland, Rotterdam, the Netherlands (identification number 2017-084,) because routinely collected health care data were used after pseudonymization.

Reproduction cohorts: ACO in secondary cohort, and asthma, COPD and ACO in primary cohort

The process of selecting questions from the ACQ and CCQ to form the new questionnaire was reproduced in three separate cohorts: 1) ACO group in secondary care dataset; 2) asthma and COPD group from the primary care dataset; 3) ACO group from the primary care dataset. Patients who exhibited characteristics of both asthma and COPD, as determined by the pulmonologist during assessment at the secondary care center, were enrolled in the first reproduction cohort. Data of the second and third cohort were part of a registry study of patients with asthma, COPD or ACO, who were diagnosed by Star-Shl, a diagnostic center for primary care in Rotterdam. Diagnoses of COPD and asthma were confirmed by spirometry and by a general practitioner or pulmonologist with special interest in obstructive lung diseases, using the same criteria as the development cohort (12-14). ACO patients were analyzed separately, because this patient group was smaller and not-clearly defined compared to the asthma and COPD groups. Data was pseudonymized and ethics approval for this study was waived by Star-Shl in line with the waiver procedure for the secondary care cohort.

Data collection

The following variables were collected for all patients:

Lung function. FEV_1 and FER (FEV_1/ FVC) were performed according to the ATS/ ERS taskforce "standardization of spirometry". All tests in the secondary cohort were performed with the Vmax Sensor Medics Viasys, type 6200 Encore (15). In the primary cohort (Star-Shl), all spirometry studies were performed with the Welch Allyn Cardioperfect spirometer.

Clinical COPD Questionnaire (CCQ). This is a ten-item questionnaire about symptom severity in the past seven days and health-related quality of life. CCQ total score ranges from 0 to 6, where a higher score indicates a worse health status (6). The minimal clinically important difference of the CCQ is 0.4. (16). CCQ-1 is a question about shortness of breath at rest and CCQ-2 about shortness of breath during physical activities. CCQ-3 is about concerns of getting a cold or breathing getting worse, CCQ-4 about depressive feelings due to breathing problems, CCQ-5 about coughing, and CCQ-6 about production of phlegm. The four last questions are about limitations during activities because of breathing problems: limitations during strenuous physical activities (CCQ-7), moderate physical activities (CCQ-8), daily activities at home (CCQ-9) or social activities (CCQ-10).

Asthma Control Questionnaire (ACQ). This questionnaire assesses average symptom severity and control in asthma in the past week. ACQ total score ranges from 0 to 6, where a higher score indicates a worse disease control. (5). We used the five-item

questionnaire, according to the preference of the GINA guideline (10). ACQ-1 is a question about frequency of wakening due to asthma symptoms, ACQ-2 about severity of symptoms during wakening, ACQ-3 about limitation during activities, ACQ-4 about severity of shortness of breath, and ACQ-5 is about the frequency of wheezing. The minimal clinically important difference is 0.5 (17). The ACQ and CCQ share a comparable scoring system and partially similar format since the original developers collaborated in designing these questionnaires. (5, 6).

To study the construct validity of the subset of questions that resulted from the Principal Component Analysis (PCA), we administrated the *COPD Assessment Test (CAT)* in COPD patients and the *Asthma Quality of Life Questionnaire (AQLQ)* in asthma patients. A higher score in the eight-item questionnaire CAT reflects a worse outcome and in the 32-item questionnaire AQLQ a lower score reflects more impairment (18, 19).

Statistical analyses

At first, linear regression was used to test for correlation between the ACQ and CCQ. The Pearson correlation coefficient was calculated separately for the asthma, COPD, and ACO patients in both the secondary and primary care cohort.

Secondly, Principal Component Analysis (PCA) was used to identify the questions of the ACQ and CCQ that could be used to develop the new questionnaire. Based on the correlation between the individual questions, PCA reduced the number of questions by replacing them by newly created variables ('components') with minimal information loss. This method was used to identify trends in the answers of the questionnaires and understand what these answers have in common. To develop a component an eigenvalue >1 was used. Oblimin with rotation, converged in 25 iterations, was used with method Kaiser Normalization. The component included questions that met correlation cut-off values of 0.7 and -0.7. The question with the highest correlation in the component was chosen as its identifier. To ensure there was no cross-talking between components, the identifying question was examined to see if any other variable had a loading of more than 0.4 on the same component. If this occurred, the question could not be used as the component's identifier (20). Questions that were not related to any of the components were added individually to the final questionnaire to ensure that valuable information from those questions was not lost. Alpha Cronbach's was used to measure internal consistency of the new questionnaire. Third, three reproduction cohorts were used to repeat this process of selecting the questions by PCA. The same conditions of the PCA were investigated: Eigenvalue >1, cut-off values of 0.7 and Oblimin with rotation, converged in 25 iterations, with method Kaiser Normalization.

Fourth and final, first steps were initiated to test the validity and reliability of this prospective new questionnaire. New correlation plots were performed in asthma and COPD separately to test the correlation between the new questionnaire and the golden standard: the ACQ in asthma patients and the CCQ in COPD patients. Furthermore, we calculated a Pearson correlation coefficient test to study the construct validity of the new selection of questions with the AQLQ in asthma and CAT in COPD. All statistical analyses were performed using IBM SPSS Statistics, version 28.0.0.0 (190).

RESULTS

Patient characteristics

Development cohort

In total 814 patients were considered in the secondary care cohort. Patients were excluded for this study because of missing data (n=307) or when CCQ and ACQ were not completed on the same day (n=106). So, in total 252 asthma patients and 97 COPD patients were included in the development cohort, **Figure 1**. In the asthma group 162 (64.3%) were female and in the COPD group 42 (43.8%), **Table 1**. The median age [IQR] of the asthma patients was 48.5 years old [38.3-59.0] and in COPD 63.0 years old [55.0-70.0]. There were more active smokers in the COPD group, compared to the asthma group (52.1% vs. 12.7%, <0.001). The median FEV-1 post bronchodilator percentage predicted [IQR] was higher in asthma patients compared to the COPD patients (93% [79-104] vs. 66% [52-82]). The items of the ACQ and CCQ differed significantly between asthma and COPD in the questions ACQ 1, ACQ 2, CCQ 1 and CCQ 6, **Suppl. Figure 1.** Median scores of ACQ 2 and CCQ 1 were higher in asthma patients, compared to COPD patients and ACQ 1 and CCQ 6 scores were elevated in COPD patients.

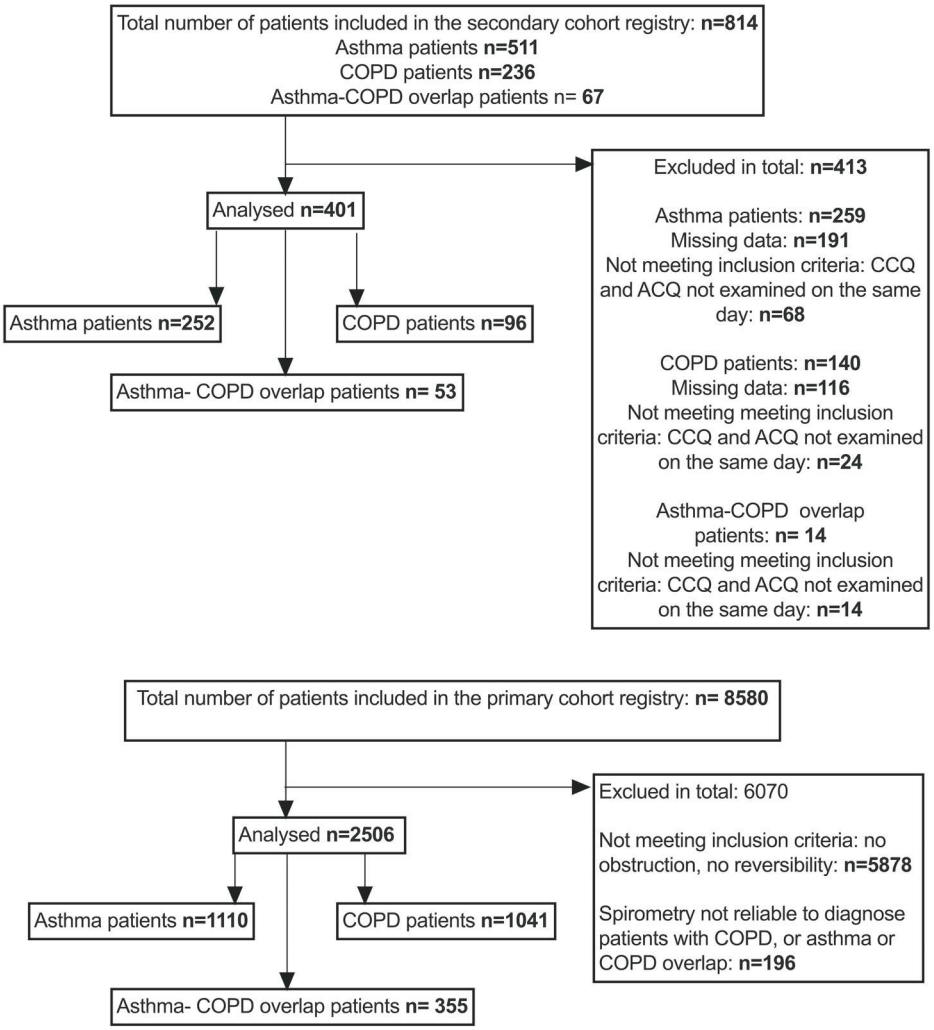


Figure 1. Patient enrolment of secondary cohort and primary cohort

Reproduction cohorts

The first reproduction cohort included 53 ACO patients from the secondary care data, **Figure 1**. In this patient group 22 (41.5%) were female and 19 (35.8%) were active smokers. The median age was 61 [55-68], **Suppl. Table 1.** In total 1110 asthma patients and 1041 COPD patients of the primary care cohort were included in the second cohort, **Figure 1**. In this primary care cohort, the patients in the COPD patient group were older (64 y [57-71] vs. 43 y [28-56]), and more men (52.4% vs. 44.3%) and active smokers were included compared to the asthma patient group (49% vs. 20%), **Table 1.** The third and final reproduction cohort included 355 ACO patients of the primary care dataset. In

this group 165 (46.5%) were female and 148 (41.7%) were active smokers. The median age was 59 [50-68], mean FEV-1 post bronchodilator percentage predicted was 74.4 [61.4-84.6] and median FER was 60.7 [53.5-65.6], **Suppl. Table 1.**

Table 1. Patient characteristics in secondary and primary cohort. BMI: Body Mass Index in kg/m^2. FEV-1: Forced Expiratory Volume in 1 second.

	Secondary care cohort (n= 348)		Primary care cohort (n= 2151)	
Characteristics	**Asthma (n=252)**	**COPD (n=96)**	**Asthma (n=1110)**	**COPD (n=1041)**
Female sex, n(%)	162 (64.3)	42 (43.8)	629 (56.7)	496 (47.6)
Age, median [IQR]	48.5 [38.3-59.0]	63.0 [55.0-70.0]	43 [28-56]	64 [57-71]
BMI, median [IQR]	28.0 [24.4-32.4]	26.6 [21.6-29.5]	26.8 [23.5-30.6]	26.2 [23.5-29.4]
Active smokers, n(%)	32 (12.7)	50 (52.1)	223 (20)	510 (49)
FEV-1 pre percentage predicted, median [IQR]	83 [69-95]	62 [50-75]	79 [69-90]	67 [52-81]
FEV-1 post percentage predicted, median [IQR]	93 [79-104]	66 [52-82]	91 [82-101]	71 [56-84]
Tiffeneau, median [IQR]	72[64-79]	52 [42-63]	76 [71-82]	58 [48-65]

Correlation between the ACQ and CCQ

In the secondary care cohort, the Pearson correlation coefficient between the ACQ and CCQ was 0.82 in asthma patients, 0.83 in COPD patients, and 0.83 in ACO patients. In the primary care cohort, R was 0.81 in asthma patients, 0.80 in COPD patients and 0.81 in ACO patients, **Figure 2.**

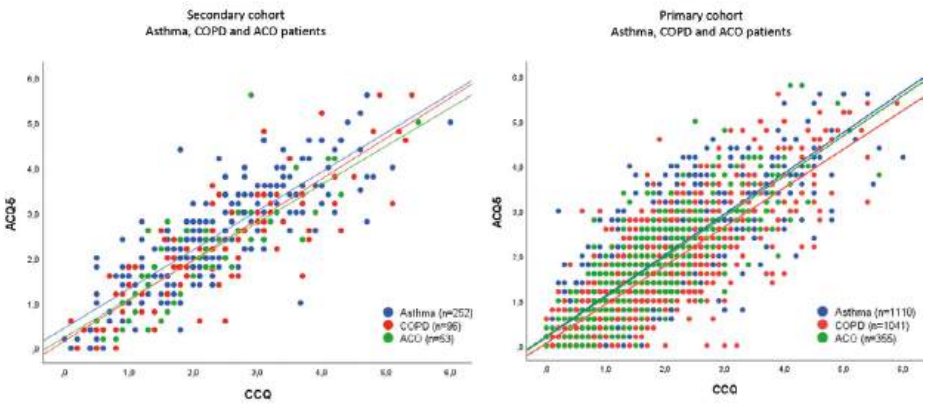


Figure 2. Correlation plot showing the correlation between the ACQ and CCQ in secondary cohort (left) and in primary cohort (right). In the secondary cohort, the Pearson correlation coefficient (R-score) was 0.82 in asthma patients, 0.83 in COPD patients, and 0.83 in ACO patients. In the primary cohort, the R-score was 0.81 in asthma patients, 0.80 in COPD patients and 0.81 in ACO patients

Selecting questions of the ACQ and CCQ in the development cohort

Principal Component Analysis (PCA) was performed to integrate the ACQ and CCQ and reduce the number of questions. Three components were formed based on correlations between questions. Component 1 consists of: CCQ 7, CCQ 8, CCQ 2, CCQ 9 and ACQ 3. Component 2 consists of CCQ 6 and CCQ 5 and component 3 consists of CCQ 3, CCQ 4, ACQ 2. The CCQ 10, ACQ 4, ACQ 5, CCQ 1 and ACQ 1 were not correlated with the other items and therefore not included in a component, **Figure 3**. This process of data reduction resulted in the selection of eight required questions: one question was selected for each component (CCQ 7 in component 1, CCQ 6 in component 2, CCQ 3 in component 3); and the residual questions (CCQ 10, ACQ 4, ACQ 5, CCQ 1 and ACQ 1).

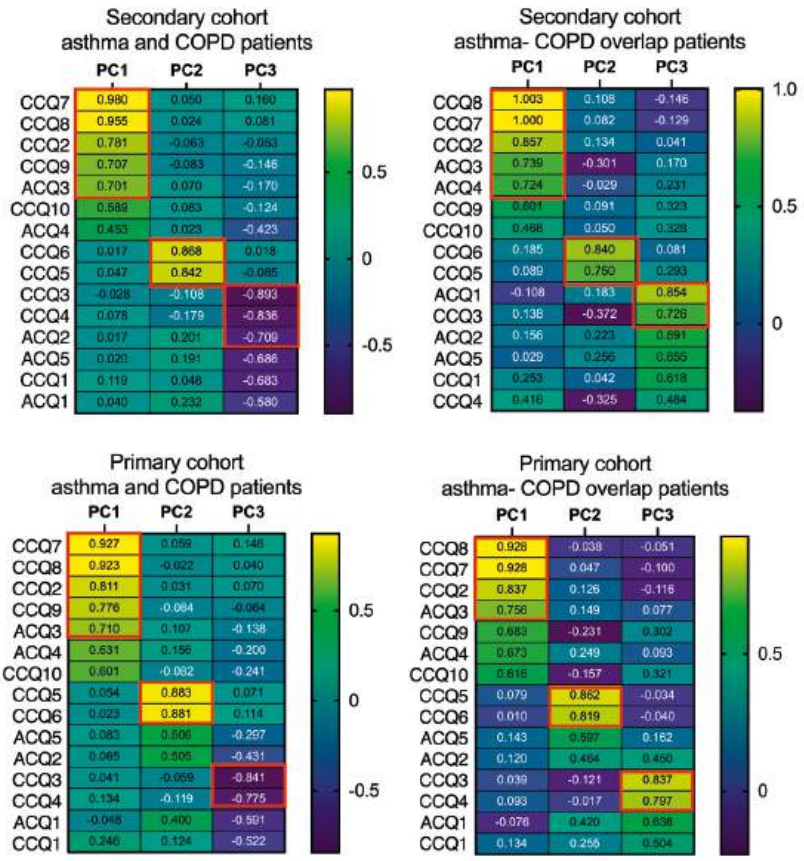


Figure 3. Results of Principal component analyses in secondary cohort and primary cohort, in asthma, COPD and asthma-COPD overlap patients. The red box contains the questions of that particular component. PC1 = component 1; PC2 = component 2; PC3 = component 3.

Reproduction of selection procedure in three cohorts

The selection procedure of the questions was repeated in three reproduction cohorts: 1) the ACO patients of the secondary cohort; 2) asthma and COPD patients of in the primary care cohort; 3) the ACO patients of the primary care cohort. As in the development cohort, the answers of ACQ and CCQ questions were combined into one dataset and questions were reduced by PCA.

The PCA of the data of the ACO patients in the secondary dataset showed similar results as the development cohort with a few exceptions. Component 1 also consists of CCQ 7, CCQ 8, CCQ 2, and ACQ 3; however, it contains the ACQ 4 instead of the CCQ 9. Component 2 in this reproduction cohort is identical to component 2 in the development cohort. Component 3 also consist of the CCQ 3, but contains the ACQ 1 instead of the CCQ 4 and ACQ 2, **Figure 3**.

In the primary care data of the patients with asthma or COPD, the PCA resulted in similar components as in the development cohort; except for ACQ 2 which had a lower correlation value for component 2 in primary care in comparison with secondary care (0.505 vs. 0.709). Component 1 consists of resp.: CCQ 7, CCQ 8, CCQ 2, CCQ 9 and ACQ 3. Component 2 consists of CCQ 6 and CCQ 5 and component 3 consists of CCQ 3, CCQ 4. The residual questions were ACQ 1, ACQ 2. ACQ 4, ACQ 5, CCQ 1 and CCQ 10, **Figure 3**.

The PCA with data of the asthma-COPD overlap patient group in the primary care data yielded similar results. Component 1 consists of resp.: CCQ 8, CCQ 7, CCQ 2, CCQ 9 and ACQ 3. Component 2 consists of CCQ 6 and CCQ 5 and Component 3 consists of CCQ 3, CCQ 4. The residual questions were ACQ 1, ACQ-2. ACQ 4, ACQ 5, CCQ 1 and CCQ 10, **Figure 3**.

Development of the Obstructive Lung Disease Questionnaire

Combining the results of the four PCA's resulted in a selection of 9 questions. For each component, the question with the highest correlation in the development cohort was included in our selection. Component 1, containing the CCQ 7, CCQ 8, CCQ 2, CCQ 9 and ACQ 3, the CCQ-7 was selected as the identifying question of this component. These five questions were about complaints during physical activity; more specifically: shortness of breath during physical exercise (CCQ 2), limitations because of intense physical activity (CCQ 7), or moderate physical activity (CCQ 8), or daily activities (CCQ 9), or limitations because of activity (ACQ 3).

The CCQ 6 was selected from component 2. Both questions in component 2 are about coughing: CCQ 5 is about the amount of coughing and CCQ 6 is about the amount of sputum during coughing. CCQ 3 was selected as the identifying question of component 3, which contained the CCQ 3 and CCQ 4. CCQ 3 is a question about feeling

concerned and the CCQ 4 about feeling depressed because of respiratory complaints. ACQ 2 was also statistically correlated with CCQ 3 and CCQ 4 in the development cohort. However, the ACQ 2 was not included in component 3 in any of the reproduction cohorts. Therefore, the ACQ 2 was not merged with the CCQ 3 and CCQ 4. The ACQ 1, ACQ 4, ACQ 5, CCQ 1 and CCQ 10 were not statistically correlated to any of the components. These questions were included in the final selection. This process resulted in a 9-item questionnaire with a 6 point scale with working title ''the Obstructive Lung Disease Questionnaire (OLD-Q)''. ''Asthma'' was replaced for ''obstructive lung disease'' to make the questionnaire applicable for all patients, **Table 2.**

Table 2. Working title ''Obstructive Lung Disease Questionnaire'' as potential new tool for measuring disease burden in obstructive lung disease
Circle the number of the response that best describes how you have been during the past week

1. On average, during the past week, how often were you woken by your obstructive lung disease during the night? *ACQ 1*	0. Never 1. Hardly ever 2. A few minutes 3. Several times 4. Many times 5. A great many times 6. Unable to sleep because of obstructive lung disease
2. On average, during the past week, how bad were your obstructive lung disease symptoms when you woke up in the morning? *ACQ 2*	0. No symptoms 1. Very mild symptoms 2. Mild symptoms 3. Moderate symptoms 4. Quite severe symptoms 5. Severe symptoms 6. Very severe symptom
3. In general, during the past week, how much shortness of breath did you experience because of your obstructive lung disease? *ACQ 4*	0. None 1. A very little 2. A little 3. A moderate amount 4. Quite a lot 5. A great deal 6. A very great deal
4. In general, during the past week, how much of the time did you wheeze? *ACQ 5*	0. Not at all 1. Hardly any of the time 2. A little of the time 3. A moderate amount of the time 4. A lot of the time 5. Most of the time 6. All the time

5. On average, during the past week, how often did you feel short of breath at rest? *CCQ 1*	0. Never 1. Hardly ever 2. A few times 3. Several times 4. Many times 5. A great many times 6. Almost all the time
6.On average, during the past week, how often did you feel concerned about getting a cold or your breathing getting worse? *CCQ 3*	0. Never 1. Hardly ever 2. A few times 3. Several times 4. Many times 5. A great many times 6. Almost all the time
7.In general, during the past week, how much of the time did you produce phlegm? *CCQ 6*	0. Never 1. Hardly ever 2. A few times 3. Several times 4. Many times 5. A great many times 6. Almost all the time
8. On average, during the past week, how limited were you in these activities because of your breathing problems: strenuous physical activities (such as climbing stairs, hurrying, doing sports)? *CCQ 7*	0. Not limited at all 1. Very slightly limited 2. Slightly limited 3. Moderately limited 4. Very limited 5. Extremely limited 6. Totally limited/ or unable to do
9 On average, during the past week, how limited were you in these activities because of your breathing problems: social activities (such as talking, being with children, visiting friends/relatives) *CCQ 10*	0. Not limited at all 1. Very slightly limited 2. Slightly limited 3. Moderately limited 4. Very limited 5. Extremely limited 6. Totally limited/ or unable to do

Validity and reliability of the Obstructive Lung Disease Questionnaire

In asthma patients, the correlation coefficient of the ACQ and total score of OLD-Q was 0.93 in the secondary cohort and 0.94 in the primary cohort. The correlation coefficient between the CCQ and the OLD-Q in COPD patients was 0.94 in the secondary cohort and 0.93 in the primary cohort, **Figure 4**. The correlation coefficient in COPD (n=61) between CAT and OLD-Q was 0.723 and between CCQ and CAT was 0.731. The correlation coefficient in asthma (n=197) between AQLQ and OLD-Q was -0.686 and for AQLQ and

ACQ total -0.652. The Cronbach's alpha of the OLD-Q in the secondary care was for asthma: 0.877, for COPD patients: 0.885, and for ACO patients: 0.884. In primary care, the Crohnbach's alpha was for asthma patients 0.867, for COPD 0.858 and for ACO 0.858.

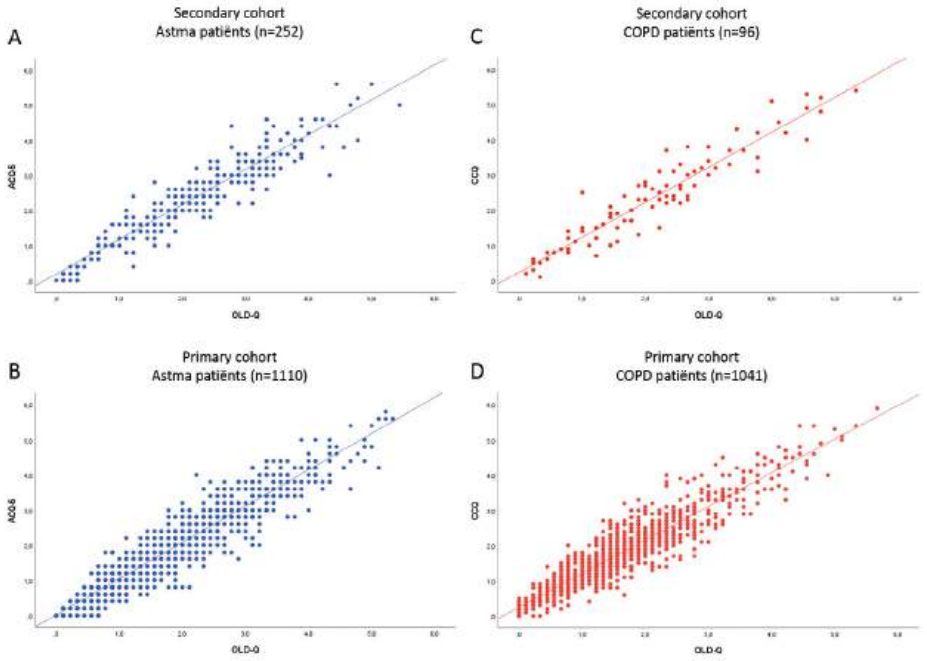


Figure 4. Correlation plot showing the correlation between the ACQ and the OLD-Q in asthma patients in secondary cohort (A) and primary cohort (B) and the correlation between the CCQ and the OLD-Q in COPD patients in secondary cohort (C) and primary cohort (D). The Pearson correlation coefficient (R-score) was respectively 0.93 (A), 0.94 (B), 0.94 (C), and 0.93 (D).

DISCUSSION

In this study, we searched for statistical and clinical overlap in questions contained in the asthma control questionnaire and the clinical COPD questionnaire in asthma, COPD and ACO in a primary and secondary cohort. This new approach could increase the efficacy of the assessment of the disease burden in asthma and COPD by merging the ACQ and CCQ based on statistical correlations. Our study explored the possibility for creating a new questionnaire and showed that a combination of nine questions of the ACQ and CCQ may be sufficient to assess disease burden in obstructive lung disease. This 9-item Obstructive Lung Disease Questionnaire, was strongly correlated to respectively the ACQ in asthma patients and the CCQ in COPD patients.

During the selection procedure of the questions, in addition to selecting one question from each component, various other factors were also taken into consideration. In the development cohort, the ACQ-2 was correlated to the CCQ-3 and CCQ-4 in component 3. Initially, this question about dyspnoea in the morning seems clinically unrelated to questions about feeling concerned or depressed. However, a systematic review showed that the burden of COPD is more severe in the morning and that these symptoms are associated with a lower quality of life (21). Nevertheless, the components of the three reproduction cohorts did not contain the ACQ 2. Therefore, the ACQ-2 was not merged with the CCQ-3 and CCQ-4. In total, six questions were not related to any of the components in the primary or secondary cohort. These questions were included in our selection, because removing them would result in loss of information. Moreover, the CCQ and ACQ were originally formulated by expert opinions for COPD and asthma separately. The intention of this research was not to remove questions, but to merge questions with statistical overlap to be useful for patients with asthma, COPD and asthma-COPD overlap.

To our knowledge, this study is the first to attempt to integrate and reduce the ACQ and CCQ to develop a pragmatic set of questions to assess disease burden in obstructive lung disease in daily practice. Modifying questionnaires to extend their applicability to a broader patient group is feasible, as shown in a recent study that transformed the COPD Assessment Test (CAT) into the Chronic Airways Assessment Test (CAAT). The CAAT seems applicable in both COPD and asthma (22). Also, the Assessment of Burden of Chronic Obstructive Pulmonary Disease (ABC)-scale was created as an adaptation of the CCQ by adding extra domains (23). Later, the ABC-tool was extended to the Assessment of Burden of Chronic Conditions (ABCC)-tool, adding information on comorbidity (24). The ABC- and ABCC-tool were created for another purpose than the OLD-Q. Whereas the OLD-Q is created to monitor disease burden in daily practice, the ABC- and ABCC-tool are used for an in-depth assessment of a patient to help healthcare-professionals to formulate a personalized treatment plan together with their patient (23, 24). Some alternative questionnaires exist for evaluating the quality of life or disease burden in respiratory disease. The Quality-of-life for Respiratory Illness Questionnaire (QoL-RIQ), for instance, is a questionnaire for assessment of disease burden and is validated in both asthma and COPD. However, this questionnaire is not practical due to the large number of questions (25). For that reason, the reduced ten-item version RIQMON-10 was developed (26). The Respiratory Symptoms Questionnaire (RSQ) is another questionnaire for assessment of respiratory symptoms regardless of a specific diagnosis. Similar to the OLD-Q, the RSQ was developed as a practical four-item tool. However, the development of the RSQ was based on the GINA and GOLD guidelines (9, 10), whereas our selection of questions is based on data and statistical analysis of two still acclaimed questionnaires (27). The RIQMON-10 and RSQ are not

commonly used in daily care. Whereas the ACQ and CCQ are frequently used, we expect that the selection of questions in the OLD-Q are familiar to both healthcare professionals and patients, so it should be more easily adopted into daily practice.

Our study has a number of significant strengths and some limitations. The first strength of the study is that the ACQ and CCQ were collected on the same day in a well-defined primary and secondary cohort. The second strength is the methodology: we developed the OLD-Q by merging two well-known questionnaires for two diseases that show clinical and physiological overlap. The ACQ and CCQ were developed based on expert opinion, validated, and are widely used in primary, secondary and secondary care. Furthermore, the ACQ and CCQ are self-administered, so health care professionals do not influence the results. We examined the relationship within the group of questions by PCA and consequently reduced questions. This statistical analysis can visualize correlations between questions in large numbers of patients, which is impossible for a clinician to observe. By developing the questionnaire in a secondary care cohort and reproducing the results in a primary cohort, we assume that this new questionnaire could be used in primary and secondary care. Third, the OLD-Q showed a very strong correlation with the ACQ in asthma patients and in CCQ in COPD patients. Fourth and final, with a high value of Cronbach's alpha, we showed that the scale of the outcome is reliable with internal consistency.

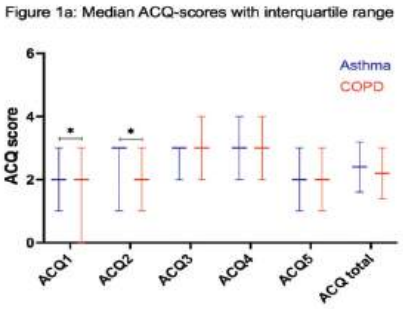
This study has some limitations. First, the COPD group was relatively small in the secondary cohort (n=92). Second, the PCA of the secondary cohort with ACO patients showed some other results compared to the development cohort and to the other three reproduction cohorts, most likely because this patient group was small compared to the other patients group. Therefore, these results were be interpreted with caution. Third, we investigated the correlation between the OLD-Q and the original CCQ and ACQ as a gold standard, either for COPD patients or asthmatics. The ACQ covers a greater number of aspects related to disease control, while the CCQ places a stronger focus on quality of life. One could argue that we should also validate the OLD-Q with another disease burden questionnaire, such as the St. George Respiratory Questionnaire (SGRQ), which is validated for both asthmatics and COPD patients. However, significant differences are unlikely since our results show a similar correlation between the OLD-Q and the AQLQ in asthma, or the CAT in COPD, to the correlations previously documented in the literature between the ACQ and AQLQ or CCQ and CAT (28). Fourth, the PCA showed that it is statistically possible to reduce the number of questions without interfering with the total score. This reduction may have resulted in the loss of clinically relevant information, for example to differentiate in exercise ability. However, this selection of questions is developed for a particular purpose: that is monitoring disease burden in daily care, and not as an in-depth assessment of the patient.

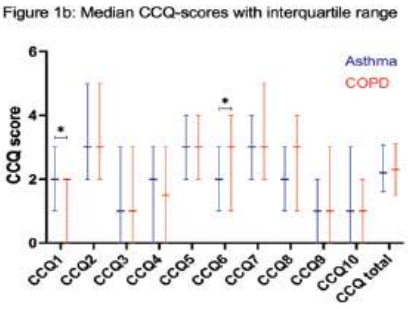
In this study, we explored the possibility of developing a new questionnaire by using the ACQ and CCQ. However, several crucial steps are yet to be completed in the development process. The OLD-Q should be tested for convergent and divergent validity, differential and linearity of item response, item response characteristics and cognitive debriefing (5, 6). Furthermore, in this study the questions used in the OLD-Q have been presented in the context of the ACQ and CCQ, so previous questions may have influenced the responses to subsequent questions. To further validate the OLD-Q as a new questionnaire, the questions should be presented in the correct order. This will help ensure that responses are not biased by previous questions and that the questionnaire is reliable and valid. A prospective study in a real-world population with asthma, COPD and ACO is warranted to validate the OLD-Q with the SGQR at two different time points to confirm validity and investigate the test-retest reliability of this pragmatic set of questions.

CONCLUSION

This potential new practical disease burden questionnaire is clinically relevant considering the similar outcomes for both primary care and secondary care. These results suggest that the OLD-Q could be used early in the diagnostic process. In this way patients need to answer fewer questions than in the current situation, which is time-efficient for both patients and health professionals.

SUPPLEMENTARY





Suppl. Figure 1a and 1b Median ACQ-scores and CCQ-scores with IQR in development cohort (asthma and COPD patients in secondary data base)
*Statistically significant difference between asthma and COPD patients in ACQ1 (p-value 0,019), ACQ2 (p-value 0,011), CCQ1 (p-value 0,002) and CCQ6 (p-value 0,01) scores, calculated by Mann-Whitney U test

Suppl. Table 1. Patient Characteristics of Asthma COPD overlap patient group in secondary and primary care.

Characteristics *Asthma-COPD Overlap*	**Secondary care cohort (n=53)**	**Primary care cohort (n=355)**
Female sex, n(%)	22 (41.5)	165 (46.5)
Age, median [IQR]	61 [55-67.5]	59 [50-68]
BMI, median [IQR]	26.4 [23.8-30.2]	27.2 [24.0-30.6]
Active smokers, n(%)	19 (35.8)	148 (41.7)
FEV-1 pre percentage predicted, median [IQR]	62 [51-72]	64.8 [52.5-75.7]
FEV-1 post percentage predicted, median [IQR]	69 [58-79]	74.4 [61.4-84.6]
Tiffeneau, median [IQR]	54 [43-63]	60.7 [53.5-65.6]

Suppl Table 2. Questions of the ACQ and CCQ that were not included in the OLD-Q

ACQ 3 In general, during the past week, how limited were you in your activities because of your asthma?	0. Not limited at all 1. Very slightly limited 2. Slightly limited 3. Moderately limited 4. Very limited 5. Extremely limited 6. 6 Totally limited
CCQ 2 On average, during the past week, how often did you feel short of breath doing physical activities?	0. Never 1. Hardly ever 2. A few times 3. Several times 4. Many times 5. A great many times 6. Almost all the time
CCQ 4 On average, during the past week, how often did you feel depressed (down) because of your breathing problems?	0. Never 1. Hardly ever 2. A few times 3. Several times 4. Many times 5. A great many times 6. Almost all the time
CCQ 5 In general, during the past week, how much of the time did you cough?	0. Never 1. Hardly ever 2. A few times 3. Several times 4. Many times 5. A great many times 6. Almost all the time
CCQ 8 On average, during the past week, how limited were you in these activities because of your breathing problems: moderate physical activities?	0. Not limited at all 1. Very slightly limited 2. Slightly limited 3. Moderately limited 4. Very limited 5. Extremely limited 6. Totally limited/ or unable to do
CCQ 9 On average, during the past week, how limited were you in these activities because of your breathing problems: daily activities at home (such as dressing, washing yourself)?	0. Not limited at all 1. Very slightly limited 2. Slightly limited 3. Moderately limited 4. Very limited 5. Extremely limited 6. Totally limited/ or unable to do

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Chapter 7

The impact of the involvement of a healthcare professional on the usage of an eHealth platform: a retrospective observational COPD study

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Respiratory Research 2021

ABSTRACT

Background

Ehealth platforms, since the outbreak of COVID-19 more important than ever, can support self-management in patients with Chronic Obstructive Pulmonary Disease (COPD). The aim of this observational study is to explore the impact of healthcare professional involvement on the adherence of patients to an eHealth platforms. We evaluated the usage of an eHealth platform by patients who used the platform individually compared with patients in a blended setting, where healthcare professionals were involved.

Methods

In this observational cohort study, log data from September 2011 until January 2018 were extracted from the eHealth platform Curavista. Patients with COPD who completed at least one Clinical COPD Questionnaire (CCQ) were included for analyses (n=299). In 57% (n=171) of the patients, the eHealth platform was used in a blended setting, either in hospital (n=128) or primary care (n=29). To compare usage of the platform between patients who used the platform independently or with a healthcare professional, we applied propensity score matching and performed adjusted Poisson regression analysis on CCQ-submission rate.

Results

Using the eHealth platform in a blended setting was associated with a 3.25 higher CCQ-submission rate compared to patients using the eHealth platform independently. Within the blended setting, the CCQ-submission rate was 1.83 higher in the hospital care group than in the primary care group.

Conclusion

It is shown that COPD patients used the platform more frequently in a blended care setting compared to patients who used the eHealth platform independently, adjusted for age, sex and disease burden. Blended care seems essential for adherence to eHealth programs in COPD, which in turn may improve self-management.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a chronic airway disease characterized by an irreversible airway obstruction. (1) As one of the leading causes of chronic morbidity worldwide, it ranks fourth on the worldwide list of Disability Adjusted Life Years (DALY). To alleviate this burden, digital health support may be a potential solution.

Currently, non-pharmaceutical treatments reinforced by improved self-management skills, are key in treating COPD patients. Self-management strategies empower patients to change and influence their behavior to manage disease more effectively. (1) Based on a recent Delphi process, an international panel of COPD self-management experts published the following on self-management interventions: "A COPD self-management intervention is structured but personalized and often multi-component, with goals of motivating, engaging and supporting the patients to positively adapt their health behavior(s) and develop skills to better manage their disease". (2) Self-management strategies lead to a significant increase in quality of life, six-minute walk test, self-efficacy, reduced duration of exacerbations and hospitalizations, and decreased healthcare costs. (3-6) Spruit et al. also discuss the critical role of behavioral change in pulmonary rehabilitation in chronic disease management. He describe the major barrier to participation is accessibility. (7) Access may be limited by geography, finance, transport, culture and logistics. eHealth platforms may solve this problem. They can facilitate education of self-management on a large scale and may lead to behavioral change. (8)

eHealth is an upcoming term in the Public Health sector, containing a set of different concepts, including health, technology and commerce. (9) Shaw et al. described three prominent but overlapping domains of eHealth: 1) monitoring, tracking and informing patients; 2) digital technologies used for communication between healthcare professional and patient; 3) collecting, managing and using health data. (10) An eHealth platform can be used by patients themselves on an individual basis or in a blended care setting. Using an eHealth platform in a blended care setting means online collaboration between patient and healthcare professional. Especially in the COPD population, mostly vulnerable, relatively old and often lower educated, this blended care setting may increase platform adherence. The impact of collaboration with a healthcare professional in self-management programs has been studied previously. In 2012, Fan et al. reported an increase in mortality in patients that pursued a comprehensive care management program (CCMP). The CCMP intervention consisted of individual weekly sessions using an educational booklet with an overview of COPD topics (e.g. respiratory symptoms and self-initiation of an antibiotic or prednisone for an exacerbation) with a call from a case manager once per month. It is discussed that the high mortality is possibly due to the lack of collaboration between the healthcare

professional and the patient. (11) A quantitative study on perceptions and behaviors related to self-management diaries for asthma and COPD, showed positive effects on disease coping by regonizing exacerbations and adjusting medications. However, patients experienced practical barriers to integrating the diaries in their daily life. (12) Usage of a self-management programme in routine care can improve self-efficacy over time in COPD patients. (13) An increase in the use of a COPD eHealth platform in blended care setting was seen in a controlled study. (14) However, until now, no real-life data on this is available. It is necessary to achieve long-term eHealth platform adherence for COPD patients, to improve insight in their symptoms, and potentially improve their self-management abilities. To stimulate self-management in COPD patients, it is necessary that these patients recognize the severity of their symptoms. To achieve this, it is essential to observe their COPD symptoms for a longer period. More insight in symptom-fluctuation over time by closer monitoring of COPD patients improves patient empowerment and thus health status. There are different possibilities to observe COPD symptoms, such as measuring blood oxygen levels, observe forced expiratory volume in one second and by filling in the COPD questionnaires such as the Clinical COPD Questionnaire.

The aim of this observational study is to compare the usage of an eHealth platform between patients who use the platform in a blended setting to those who use it individually. We hypothesize that patients use the platform more frequently in the blended setting, which indicates an improved adherence.

METHODS

Study design, setting, and participants

Real-life data of the eHealth platform Curavista (www.curavista.health) were used. Curavista is an open online certified (NEN7510, ISO 27001, CE class I MDD) eHealth platform. Currently, the platform hosts 80 different modules for managing health in chronic diseases, for example COPD, Diabetes Mellitus and Parkinson. Modules contain forms, questionnaires, e-consultations, monitoring and self-management programs. Individuals can use this platform independently or with the assistance of their healthcare professional. For the current study, data from September 2011 until January 2018 were extracted. In the current study analyses, we included all people who: (1) signed up for the COPD module (2) gave informed consent to analyze their data and (3) entered an age of ≥ 18 years old. People could delete their records at all times without giving a reason. Only patients that had at least one complete Clinical COPD Questionnaire (CCQ) on the Curavista platform were included for analyses. The CCQ is a questionnaire for COPD patients to score the severity of their symptoms. (15) The

questionnaire consists of 10 items with 7 answer possibilities (0, no symptoms or no limitations; 6, severe symptoms or limitations). Total score ranges from 0-60, a higher score indicates a worse health status. (16)

There were several ways patients could be notified about the platform. Firstly, patients were able to find the platform through advertising by the Lung Foundation Netherlands, and use the platform on their own without assistance of a healthcare professional. The Lung Foundation Netherlands is a large platform active in the Netherlands. On this platform patients can find information about pulmonary diseases and healthcare professionals can be informed about research projects. Further, volunteers of the Lung Foundation advertise for conventions and patient meetings. Secondly, patients could be invited by their healthcare professionals (i.e. a medical doctor or a respiratory nurse) in primary or hospital care and use the platform in a blended care setting. For all patients, with or without healthcare professional involved in the platform, it was possible to fill in a CCQ daily; they all received a reminder to do so every three months.

Curavista COPD module

The COPD module contains Patient Reported Outcome Measures (PROMs), including the Clinical COPD Questionnaire (CCQ), Medical Research Council Dyspnoea (MRC), Assessment of Burden of COPD (ABC)-tool, exacerbation plan, information about inhalation techniques and eConsult. This COPD module is validated in 2015. (12) The COPD module was at first designed to be used in a blended care setting together with a healthcare professional, during consultation or for remote monitoring. Later, it became possible for people to use the system independently as well. The role of the healthcare professional in the blended care setting, in both primary and hospital care, is to check and discuss the results of the included PROM's with the patient. The time in between these regular consults varies per individual. In general, a higher burden of disease leads to more consultations. The healthcare professionals are trained for motivational interviewing. Two examples of the platform are shown in **Figure 1** and **Figure 2**; a frequent submitter and a non-frequent submitter. CCQ scores > 2 mean not stable and are colored red, orange included CCQ score 1 and 2 and is not entirely stable. Green is a CCQ score <1 and refers to a stable COPD state. These cut-off points are commonly used in primary care. (17)

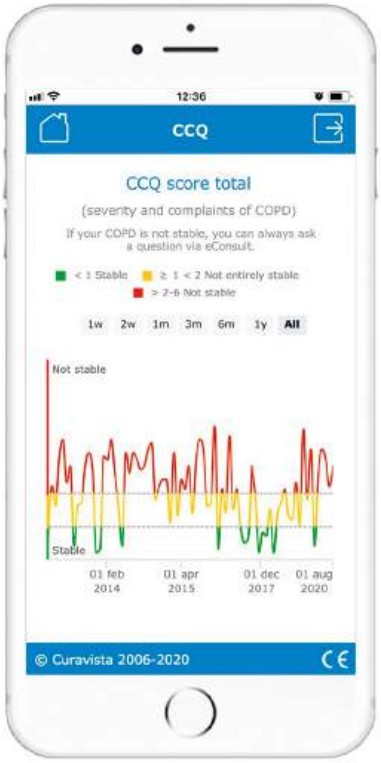

Figure 1. Example of a frequent submitter.
CCQ scores > 2 is coloured red, orange included CCQ score 1 and 2 and green is a CCQ score <1.

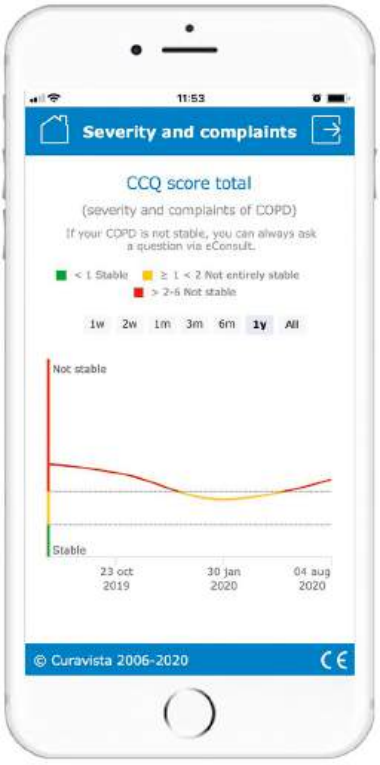

Figure 2. Example of a non-frequent submitter.
CCQ scores > 2 is coloured red, orange included CCQ score 1 and 2 and green is a CCQ score <1.

Data collection

All variables were extracted from the eHealth platform Curavista. CCQ submissions were used to operationalize usage of the platform. The date of the first CCQ-submission was used as starting point for using the eHealth platform. Data collection included age, sex, symptom scores at baseline and at the end of participation (first and last CCQ score) and participation length (number of days between date data extraction and date first CCQ-submission). The two groups of patients, i.e. patients who used the eHealth platform independently and those who used it in a blended care setting, naturally emerged from their registration. The outcome of the analysis was CCQ-submission rate as an indicator of the extent to which the eHealth platform was used. This research was declared as outside the scope of the Medical Research Involving Human Subjects

Act. Patients' informed consent was obtained to use their data for non-commercial anonymous analyses and they were able to delete their records at all times.

Analyses

Patients whose data were incomplete were excluded from the analyses. Propensity score matching was used to reduce the bias in estimating the effect of CCQ-submission rates, in patients using the eHealth platform with or without healthcare professional. For this propensity score matching, a logistic regression was used. For this analysis, using the platform in a blended care setting was the dependent variable and the characterstics of the patient (age, sex and score of first CCQ-submission) were predictors. Probabilities were estimated, ranging from 0-1, for each patient in the study population. Due to the propensity score matching, n=44 patients were excluded for analyses. For descriptive data (age, participation score of first CCQ submission and score of last CCQ submission), normally distributed continuous variables were reported as means with standard deviations, non-normally distributed continuous variables as medians with 25th and 75th interquartile ranges (IQRs) and categorical variables as numbers with percentages. Patients who used the platform with a healthcare professional were compared with patients who used the platform independently, using Chi-square test for categorical data, Mann Whitney- U tests for non-normally distributed continuous variables and unpaired T-test for normally distributed continuous variables. A p-value of <0.05 was considered statistically significant.

Multivariate Poisson regression analyses were used to compare CCQ-submission rates between the group using the eHealth platform independently and the group using the platform in a blended care setting. Patients with two or more CCQ-submissions were included in these analyses. In the Poisson regression model, the group using the eHealth platform independently was used as reference group. Participating in a blended setting vs. independent use was included as an independent variable, CCQ-submission rate was used as dependent variable. The duration of participation (log-transformed) in days was used as offset variable. To reduce confounding, we included sex, age and CCQ score at first completion as covariates. Furthermore, we performed a second multivariate Poisson regression analysis to estimate the impact of type of healthcare setting (hospital vs. primary care setting) in the blended care group. In this analysis, the group using the eHealth platform in primary care was used as reference group. SPSS version 25.0 was used for all analyses.

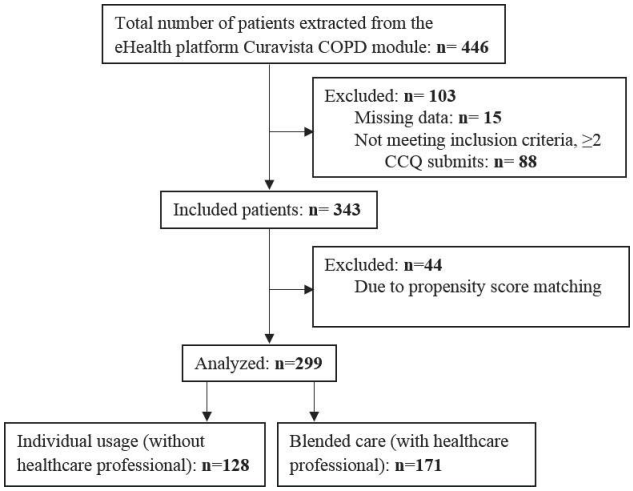

Figure 3. Flowchart of patient enrollment process

RESULTS

A total of 299 patients were included in this study; 57% (n=171) used the platform in collaboration with a healthcare professional ("blended care group") and 43% (n=128) independently ("independent user group"). Healthcare professionals worked either in hospital (n=142) or in primary care (n=29). Missing patient data included age (n=14) or sex (n=1) (**Figure 3**). The median [IQR] age of the patients in the blended care group and in the independent user group were comparable 65.8 [60.5-72.0] vs. 66.5 [58.9-73.2], p=0.939, (**Table 1**).

The median [IQR] scores of the first CCQ were significantly lower in patients in the blended care group compared to the patients in the independent user group (3.0 [2.0-4.0] vs. (4.0 [3.0-5.0], p<0.001). The last CCQ-submission showed a similar trend in patients in the blended care group vs. independent user group (3.0 [2.0-4.1] vs. (3.7 [2.2-5.0], p=0.071). The difference in number of CCQ submissions of the eHealth platform between the patients in the blended care group vs. independent user group is shown in **Figure 4a** and **Figure 4b**. In the group without healthcare professional 54% remained stable after intervention, where stable is defined as a change in CCQ between -0.4 and 0.4. In the blended care group 45% remained stable (data not shown).

In total 211 patients were included in the Poisson analyses. In the crude Poisson analysis blended care was associated with a 3.00 (95% CI: 2.79-3.23, p<0.001) higher CCQ-submission rate compared to patients who used the platform independent (**Table 2**). In the adjusted analysis, the CCQ-submission rate was 3.25 (95% CI: 3.01-3.50, p<0.001) higher for patients in a blended care setting compared to patients who used

Table 1. Characteristics of the study population at baseline after propensity score matching, with and without healthcare professional

	Without healthcare professional (n=128)	**With healthcare professional(n=171)**	**p-value**
Male N (%)	**72 (56.3)**	**79 (46.2)**	**0.085**
Age Y median [IQR]	**66.5 [58.9-73.2]**	**65.8 [60.5-72.0]**	**0.939**
Score of first CCQ submission [IQR]	4.0 [3.0-5.0]	3.0 [2.0-4.0]	***<0.001***
Score of last CCQ submission [IQR]	3.7 [2.2-5.0]	3.0 [2.0-4.1]	0.071

Median (IQR Q1-Q3) used in non-normal distribution N: numbers, IQR: interquartile range 25th - 75th percentile.

Table 2. Difference in number of CCQ-submission rates

	Aim 1[a]		
Model	**Rate; Exp (B)**	**95% CI**	**p-value**
1[b]	3.001	2.792-3.226	**<0.001**
2[c]	3.245	3.009-3.500	**<0.001**

a. Difference in number of CCQ-submission rates in patients participating independently or in a blended care setting (independently group ref)
b. Crude (unadjusted) analysis
c. Analysis adjusted for sex, age and score of first CCQ-submission

the platform independently. The adjusted analyses of the blended care group showed a 1.83 (95% CI: 1.66-2.01, p<0.001) higher CCQ-submission rate in the hospital care group compared to the primary care group.

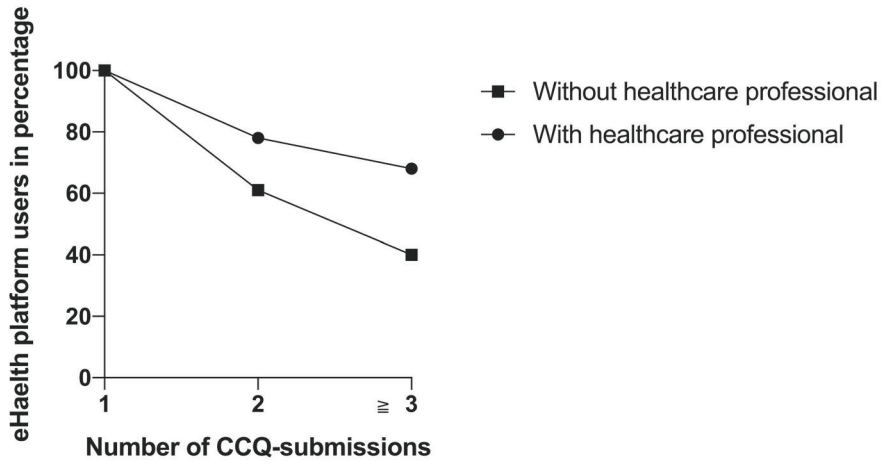

Figure 4a. Number of CCQ-submissions in users with and without healthcare professional.

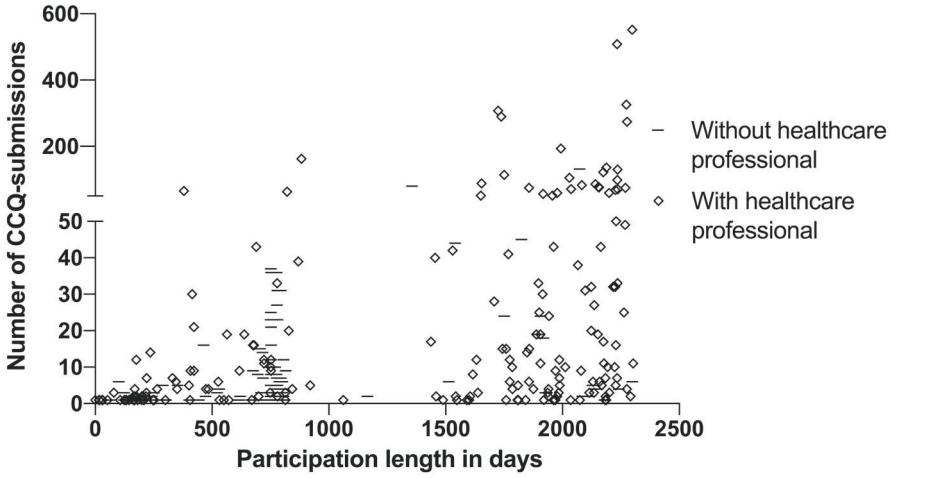

Figure 4b. Number of CCQ-submissions and participation length in days in users with and without healthcare professional.

DISCUSSION

In this real-life study, we showed that COPD patients used an eHealth platform more frequent in a blended care setting compared to patients who used the platform independently, adjusted for sex, age and burden of disease. Our results imply that blended care results in higher usage of an eHealth platform. This confirms the crucial role of the healthcare provider in applying blended care and thus involving the patient in the e-program.

Our results confirm a prospective controlled study of Talboom-Kamp et al. (14), in which the visits of an eHealth platform among a COPD patient group with high assistance and low assistance in primary care were compared. The high-assistance group used the self-management platform statistically significant more frequent than the low-assistance group. We reported a higher usage of the eHealth platform in a blended care setting.

Literature on CCQ monitoring on blended care is not conclusive on the assumption that it improves the health status of COPD patients. An increase in mortality in a new comprehensive care management program has been shown, possibly due to the lack of collaboration between the healthcare professional and the patient. (11) In the field of psychology, improvement in adequate behavior is seen in a blended-care setting. For example, internet-based cognitive behavior therapy for symptoms of depression and anxiety was more effective with the support of a professional, than internet-based intervention without professional support. (18) A positive effect of blended care is also visible in the treatment of panic disorders: internet administrated self-help plus

minimal therapist contact via e-mail had the same effect as traditional individual cognitive behavior therapy. (19) In pulmonology, an improvement of the subscale CCQ symptom score was found after participating in an eHealth platform for a prospective COPD study in a blended care setting. (20) In a meta-analysis about the effectiveness of eHealth intervention in Obstructive Sleep Apnea (OSAS) no difference was found between studies using eHealth as an add-on to care as usual and studies using eHealth as a replacement of care as usual. (21) The nightly use of CPAP (adherence) increased, with eHealth interventions compared to care as usual, regardless of the involvement of a healthcare professional. The different purposes of the eHealth platforms in blended care setting may explain the differences in outcomes. It is proven that nightly use of CPAP during three months improves self-reported sleepiness after four hours of use every night. (22) We speculate it is easier to achieve adherence when patients experience a direct effect on their symptoms. In this study, we did not have data to investigate if a better adherence to the eHealth platform led to changes in COPD (self-) management, and whether that- in turn- led to changes in health status.

A strength of the study is taking the score of the first CCQ-submission into account, which makes it possible to conclude that patients, irrespective of disease burden, use the eHealth platform more frequently in a blended care setting. Secondly, to reduce bias, propensity score matching and adjustment on sex, age and first CCQ score were used in these analyses. These two methods are complementary and best used in combination. Third, the time between the first and last CCQ submission was taken into account, so we adjusted for a potential impact of length of participation on the results. Fourth, by using real-life data, we were able to generate results that are more likely to be externally valid. In Randomized Control Trials (RCTs) only a small and highly selected fraction of the real-life population is used, because of the strict inclusion criteria. Herland et al. (23) have presented that in case of strict COPD criteria (Obstructive lung disease and FEV1< 70% of predicted normal, >15 pack-years and absence of atopy), only 17% of the COPD population would be included in a clinical trial. Travers et al. (24) suggests only 5% of the COPD patients meet the inclusion criteria for major RCTs, which implies limited external validity. By using real-life data, this study emphasizes the conclusion of Talboom-Kamp et al. (14) in which the high-assistance group used the self-management platform significantly more frequent than the low-assistance group. Fifth, this study has evaluated the effectiveness of an eHealth platform, in contrast to most COPD eHealth platforms, which have not been evaluated. (25)

One of the limitations of this study is the limited information available about outcomes in study participants. In addition, it was not possible to compare the submission per healthcare professional because of a lack of data. However, our main result is not affected by this limitation because using the eHealth platform with a healthcare professional, reveals higher CCQ-submission rates. An extraction date

was used; at that time all data of the platform was downloaded for the analyses. If patients signed up close to the extraction date, they had had a shorter follow-up time and consequently less CCQs could have been submitted. Most presumably, this has a minor impact on the results as we included time as an offset in the Poisson analyses and patients were included in a time-range of seven years.

A second limitation of the study is that participants without a healthcare professional could potentially sign up for the COPD module without a COPD diagnosis of a pulmonologist or following the GOLD criteria. (1) However, because the eHealth platform was only offered to the general public via the website of the Lung Foundation Netherlands, it is unlikely participants without COPD would find this platform and submit at least two CCQs. The CCQ score of the patients without healthcare professional is higher, which indicates more complaints. Rennard (26) showed that only 15-20% of cigarette smokers visit a physician with symptoms. In the remaining 80-85% of the patients, lung function is usually abnormal. Thereby, in this study CCQ-submission rate was used as surrogate marker of usage of the eHealth platform. However, CCQ-submission rate might underestimate the actual usage of the platform, because other functions were not included such as usage of eConsult, submission of the MRC or usage of the knowledge base. Yet, it was possible to visit the platform without filling in a CCQ, but for example do an eConsult or submit the MRC questionnaire. A third limitation is: it was not possible to measure a direct (behavior) or indirect (QoL and exacerbations) effect on self-management, because of a lack of data before and after usage of the eHealth platform. (27) Thereby, an effect on the quality of life is dependent on different variables and possibly not an isolated effect of the eHealth platform. We can only suggest an improvement of self-management because of higher CCQ-submission rates and therefore adherence. Attrition, which is defined as participants stopping usage and/or being lost to follow up, is also an important topic in eHealth. This is important since many eHealth applications report high numbers of dropouts and non-users. (28) It is interesting to define the group in which this application works and to define the dropping out group. Because of a lack of data we were not able to look into attrition, but we expect in a blended care setting not only higher usage but also longer usage because of the guidance of a healthcare professional. A fourth limitation is the inability to make a distinction between submissions by mobile or by desktop. If patients who submitted in 2011 were more likely to be non-adherent, this effect can be explained by the absence of the technical improvements that made the eHealth platform more user-friendly in later years.

Filling in questionnaires and submitting does not directly have an effect on the COPD symptoms, which makes it hard to achieve improved adherence to an eHealth platform. We speculate it is more relevant for patients to submit CCQs when they know a healthcare professional is involved and can act on a severe score of the CCQ.

Our study shows a positive effect on usage of an eHealth platform in a blended care setting, we believe that this might in turn have a positive effect on self-management.

CONCLUSION

This study presents a higher usage of the eHealth platform in a blended care setting, adjusted for sex, age and disease burden. Blended care seems essential for adherence to eHealth programs in COPD and thereby may support self-management. Further research into the usage of eHealth platforms in a blended care setting is needed to demonstrate an improvement in self-management.

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Chapter 8

General discussion

In this thesis, we conducted studies to explore the heterogeneity of COPD. To achieve this, we identified clinical and immunological phenotypes of COPD patients. Additionally, we observed differences in immunological and behavioural aspects when compared to asthma patients, healthy smoking and non-smoking controls. Considering the heterogeneity of COPD, we explored whether identifying specific risk factors and treatable traits is essential for enabling personalized treatment in COPD patients.

In **Chapter 2**, we found that previously reported patient clusters of Burgel et al. (1), based on clinical phenotypes, could not be replicated in a different cohort. Furthermore, when we incorporated behavioural variables as potential risk factors and created new clusters, the resulting phenotypes lacked stability. **Chapter 3** revealed elevated lipid levels in patients with asthma and obesity compared to healthy individuals with obesity. In **Chapter 4**, we discovered that characteristics of the circulating Innate Lymphoid Cell (ILC) compartment distinguished COPD patients from both smoking and non-smoking controls, as well as from asthma patients. The findings in **Chapter 5** demonstrated strong associations between clinical COPD clusters, based on patients from a COPD Center of excellence, and the peripheral blood immunophenotype and ILC profile. **Chapter 6** involved the integration and refinement of the Asthma Control Questionnaire (ACQ) and COPD Control Questionnaire (CCQ) into a single, concise questionnaire applicable to both asthma and COPD. Finally, in **Chapter 7**, we found that COPD patients engaged more with an eHealth platform in a blended care setting involving healthcare professionals, compared to when they used the platform on an individual basis.

Based on the findings of this thesis, I will discuss three main COPD topics. First, I will address the **risk factors in general**. Second, I will examine the **inflammatory determinants**. Third, I will explore the **behavioural aspects**. In the concluding section, I will discuss the clinical implications and future perspectives.

RISK FACTORS IN GENERAL

In **Chapter 2**, we highlighted the difficulty of replicating clinical clusters of COPD (2). Introducing new variables—such as air trapping, daily step count, quality of life, relationship satisfaction, activities of daily living, and fatigue—significantly altered the clusters, leading to the identification of four distinct groups primarily based on these behavioural parameters. This study emphasizes that traditional risk factors (age, pack years smoked, annual exacerbations, and BMI) are insufficient to fully explain variations in disease burden, severity, and response. The diversity within the COPD patient population suggests that a comprehensive approach, beyond simple patient subgrouping, is necessary to account for individual characteristics. Neither the small

sample size nor variations in baseline patient characteristics—both noted as study limitations—can explain the formation of new clusters when behavioral variables are introduced. This shift in clustering may only be explained by adding new variables related to patient characteristics. Consequently, this thesis explores new risk factors for COPD, including cardiovascular diseases, immunological differences related to ILCs, and behavioural differences assessed through disease burden questionnaires and an eHealth platform. Given the overlap in symptoms between COPD and asthma, we also examined similarities and differences in risk factors, immunological characteristics and behavioural aspects between these two diseases.

In literature, a connection is described between pulmonary and cardiovascular diseases. COPD is even mentioned as a risk factor for cardiovascular diseases (3). Moreover, 30 percent of individuals with COPD exhibit the presence of metabolic syndrome (MetS) (4). Additionally, systemic inflammation is identified as a contributor to the development of MetS in COPD (5). COPD patients with MetS often experience a more severe form of the disease, marked by increased dyspnea, lower FEV1, and a greater need for medication (particularly inhalation corticosteroids) to manage the condition (6).

In asthma, obesity represents a distinct phenotype and endotype, resulting in a more severe disease course that is less responsive to conventional therapies. Notably, significant weight loss has been demonstrated to enhance asthma control (7). Research indicates that obese individuals with asthma exhibit a respiratory metabolic profile markedly different from those solely affected by asthma or obesity (8). In our investigation (**Chapter 3**), asthma patients with obesity exhibited higher serum triglyceride levels compared to obese controls, even after adjusting for BMI, statin use, and eosinophil counts (9). This finding suggests that elevated triglycerides may be an overlooked trait contributing to asthma development. A larger population-based study identified a link between MetS and asthma (10). MetS is defined by the presence of three of the following five traits: abdominal obesity; serum triglycerides; serum high- density lipoprotein (HDL) cholesterol; hypertension or increased fasting plasma glucose or treatment for these last four conditions. This study linked MetS with asthma, emphasizing waist circumference and high glucose levels as key factors, however with limited evidence for elevated triglycerides, reduced high-density lipoprotein cholesterol, or hypertension (10). The difference in outcomes between this study and ours could be attributed to the smaller sample size of our study, although a post hoc sample size calculation indicates that the sample size was adequate to support our conclusion. Moreover, asthma diagnoses in our study were made by a pulmonologist, rather than relying on pharmacy records, and adjustments were made for statin use, inhaled corticosteroids (ICS), and long-acting beta-agonists (LABA), factors that were not accounted for in the larger population-based study (10). A different study showed

that adult-onset asthma, but not childhood onset asthma is associated with the metabolic syndrome, independent from body mass index (11). Adult-onset asthma patients had significantly increased serum IL-6 and leptin-adiponectin in comparison with childhood onset asthma patients (11). Ethnic variations in the asthma-obesity association have not been documented to date, however maternal obesity during pregnancy has been linked to an increased incidence of asthma in offspring (12). In contrast, the likelihood of developing cardiovascular disease is elevated in adults experiencing severe and poorly managed asthma in comparison with milder and better controlled asthma (13). In our data, we did not distinguish between adult-onset and childhood-onset asthma. Instead, we included patients from an asthma specialty center, where individuals often seek a second opinion due to severe and poorly managed asthma. As a result, the inclusion of these asthma patients may introduce a bias toward a higher risk of metabolic syndrome (13).

Four possible links between asthma and obesity are reported. At first, studies showed increased levels of TNF-alpha in high-fat diet mice and decreased levels in the lungs after weight loss. In addition, blocking TNF-alpha has shown improvements in airway hyperresponsiveness in an obese-asthma model (14). Second, it has been shown that increased levels of leptin in obese mice lead to an exacerbation of allergic asthma and increase airway hyperresponsiveness. Conversely, adiponectin, an adipokine, has anti-inflammatory properties and mitigates obesity-related effects. Third, inverse associations between BMI and exhaled nitric oxide (eNO) were found in asthma patients. The obese-asthma phenotype typically shows resistance to corticosteroids due to low or even absence of T2-high inflammation. However, dexamethasone has been found to impact nitric oxide (NO) metabolism, reducing inducible nitric oxide synthase expression in the lungs, suggesting involvement of NO metabolism in the obese-asthma phenotype (15). Fourth, the accumulation of adipose tissue around the rib cage alters the inflation/deflation pressure balance, reducing functional residual capacity (FRC) (16). A substantial waist circumference may amplify resistance in small airways, diminish functional residual capacity, consequently reducing lung compliance, and inducing compression atelectasis (17) (7). This suggests that the hip-waist ratio, rather than BMI, should be used as an indicator for developing asthma.

Some of these described links between asthma and obesity are also mentioned in the pathogenesis of COPD. First, abdominal obesity in individuals in COPD stages I and II is a reliable indicator of impaired lung function (18, 19). Second, TNF-alpha is proposed as a potential instigator of COPD (18), and its levels are elevated in stable COPD (20). In mice, increased TNF-alpha is responsible for 70% of cigarette smoke-induced emphysema (21). Additionally, TNF-alpha can contribute to systemic insulin resistance by facilitating the release of fatty acids from adipose tissue into the bloodstream, affecting tissues such as muscle and liver. Insulin resistance is the

cause of diabetes mellitus type 2, which is a risk factor for MetS. TNF-alpha also has the capacity to stimulate the production and release of IL-6 and IL-8 from adipocytes, while promoting the synthesis of leptin from adipose tissue (22). Third, elevated levels of circulating leptin have been correlated with impaired lung function, which is linked to COPD (23). On the contrary, adiponectin exhibits anti-inflammatory properties, reducing the production of TNF-alpha and inhibiting IL-6 production. Consequently, adiponectin is inversely associated with both smoking and the incidence of diabetes (22).

The connection between metabolic syndrome, obesity, cardiovascular disease, TNF-alpha, asthma and COPD has not yet been fully explored, but existing literature suggests it holds potential. We will highlight this potential risk factor in suggestions for future research.

We aim to understand why certain individuals develop COPD while others, with similar pack-year histories, do not. Agusti et al. describes the identification of abnormal lung development and accelerated decline in lung function with age (24). They suggest that while 4-12% of the general population experiences poor lung development, some catch up by age 15, while others remain below normal, predisposing them to premature death due to factors such as smoking exposure. Conversely, some individuals experience supranormal lung development, which may offer protection against COPD development, even with smoking exposure. (24). However, this remains theoretical, as there is a lack of longitudinal data tracking lung function from childhood to adulthood up to the age of 40, which would help identify the onset of COPD symptoms. Studies from childhood to adolescent suggest that muscle mass is positively associated with lung function measures, while fat mass is negatively associated (25). Nevertheless, evidence suggests that COPD mortality rates are more closely linked to spirometric restriction (measured as low FVC) rather than obstruction, particularly in those under 60 years old (26).

INFLAMMATORY DETERMINANTS

Another hypothesis contributing to the rapid decline in lung function observed in smokers with emphysema, may be dysregulated or systemic autoimmune inflammatory disorders (27). Studies on mice lacking T and B cells indicate that acute exposure to smoke is adequate to induce emphysema, yet the scenario is more intricate in humans (28). Our focus lies on delineating the immunological distinctions among COPD, asthma and smoking controls, as well as disparities across various stages of COPD (**Chapter 4** and **Chapter 5)**.

Before the discovery of ILCs in 2010, it was thought that CD4+ T cells are the major lymphocyte population that produce pro-inflammatory cytokines, including IFN-γ, IL-4, IL-5, IL-13, and IL-17, which are central to the pathogenesis of asthma and COPD. It is known that emphysema in COPD is marked by the expression of Th1- and Th17-

associated chemokine receptors on memory CD4+ T cells, (27). While tobacco triggers elastase secretion from innate immune cells, chronic and progressive emphysema stem from lymphocyte activation (27). Even after smoking cessation there is a persisting Th1/Th17 predominance (27). In smokers with COPD, the lung parenchyma and bronchoalveolar lavage show a reduced presence of Regulatory T-cells (Tregs), which are essential for immune regulation, preventing autoimmunity, and suppressing inflammation, compared to individuals without COPD (29). The decreased levels of Tregs also have a direct correlation with lung function in COPD patients (30). Exacerbations of COPD triggered by respiratory infections may incite Th1/Th17 processes, which can further complicate the immunological landscape (31). The role of B cells and their autoreactive antibodies in COPD is explored but revealed no significant differences in anti-elastin antibody titers between COPD subjects and smokers without COPD (31).

Since the discovery of the ILCs a picture is emerging in which also ILCs may significantly contribute to the production of cytokines, mirroring the T- helper cell cytokine production, and contribute to the development of COPD and asthma. The ILC family includes NK-cells, ILC1, ILC2, ILC3 and progenitor ILC. The ILCs are not activated in an antigen-specific fashion but by signals derived from cell damage or cellular activation, e.g. of lung epithelial cells. Previous studies have highlighted the association between ILC1 frequencies and both smoking habits and the severity of respiratory symptoms in COPD (32-34). Additionally, research has shown that in peripheral blood the proportion of pulmonary ILC2s is lower in patients with severe COPD (GOLD stage IV) compared to those with less severe stages (GOLD stage I or II) or healthy individuals (33) and there is a positive correlation between the frequency of IL-13+ ILC and FEV1 (34). The literature lacks information on the phenotypes or activity of ILC1. Therefore, we conducted two comprehensive studies in COPD patients and used the findings to explore correlations with lung function and smoking status (**Chapter 4** and **Chapter 5**).

In the first study we found a separate immunological profile for COPD patients compared to the three control groups (**Chapter 4)** (35). Our findings demonstrated the potential to differentiate COPD patients from asthma patients, as well as smoking and non-smoking controls, by examining the composition and phenotypic features of circulating ILC1 and ILC2 compartments. Hereby, mainly the $CD4^-$ and $CD4^+$ ILC1 subsets as well as $CD117^-$ ILC2s contributed to the variance, and not so much the ILC precursors or the more plastic $CD117^+$ subpopulation of ILC2s (35). COPD patients had significantly higher frequencies of both $CD4^+$ and $CD4^-$ ILC1s within the total ILC population when compared with asthma. These results are in line with literature (36), describing increased ILC1s and decreased ILC2 in COPD, both of which correlated with disease severity. A possible explanation for this correlation may be the production of TNF-alpha and IFN-gamma by ILC1s, which is associated with COPD severity

(20). However, infliximab which is an anti-TNF therapy, did not show improvement in symptoms or lung function in COPD patients after six months treatment (37). This suggests that TNF-alpha could severe as a biomarker for severe COPD, but it cannot be utilized as a therapeutic strategy to alleviate the disease's burden. Also, researchers found signs of plasticity of ILC2 into ILC1, which may be an active process in the lungs of COPD patients (38). In our thesis, $CD4^-$ ILC1s manifested a shift from $CD45RA^+$ to $CD45RO^+$ surface expression in COPD, which for ILC2s was shown to be associated with an inflammatory phenotype in severe asthma and resistance to corticosteroids (39). However, there is currently limited literature on the inflammatory characteristics of CD45RO+ ILC1s. It is intriguing to speculate that the relative shift from $CD45RA^+$ to $CD45RO^-$ $CD4^-$ ILC1s in COPD patients, compared to asthma patients, might indicate a pro-inflammatory role of these cells in COPD and resistance to steroid treatment.

While in this thesis the frequencies of mature CD117- ILC2s were diminished in COPD patients compared to asthma patients, there was a notable increase in the fraction of inflammatory CD45RO+ cells within this ILC2 population. This subtype has been linked to an inflammatory phenotype in severe asthma and resistance to corticosteroids (39). The relatively elevated presence of this asthma-associated ILC2 subtype in COPD patients suggests a possible link to COPD pathogenesis and may provide insight into corticosteroid resistance in these individuals. Additionally, this inflammatory ILC2 subtype may play a role in the eosinophilic form of COPD, marked by type 2 immunity with elevated levels of IL-4, IL-5, and IL-13. Although we did not measure eosinophils or levels of IL-4, IL-5, and IL-13 in our cohort, this would be a valuable direction for future research. It is known that anti-IL-5 therapy, which targets eosinophilic inflammation, can reduce exacerbations in COPD patients. This effect is more pronounced in those with higher baseline blood eosinophil counts (37).

We could also differentiate COPD inflammation from smoking effects, based on their immunological profile. Frequencies of neutrophils, total ILC and absolute leukocyte numbers in blood separated ex-smokers and current smokers, but parameters of ILC subset phenotype or subsets did not.

In **Chapter 5** we successfully identified three distinct clinical phenotypes within the COPD population. These phenotypes were accompanied by unique immunological profiles, characterized by consistent patterns in lymphoid and myeloid cell counts, as well as the presence and phenotype of ILC subsets in peripheral blood. Among COPD patients with a high disease burden, reduced frequencies of potentially inflammatory ILC1s were observed, suggesting that severe COPD patients may exhibit a diminished immunological response in their circulating ILC1s compared to those with milder forms of the disease. It is conceivable that in severe COPD patients, whose myeloid cell counts are elevated, the abundance of CD45RO+ ILC1s in circulation is low due to their accumulation in the lung tissue. Speculatively, in the early stages of COPD, heightened

inflammation characterized by increased levels of CD45RO+ ILC1s and IFN-γ/TNFα-expressing CD8+ T cells may be present. This inflammation could potentially have broader systemic implications and contribute causally to the development of COPD. As COPD progresses to the GOLD IV stage, airway destruction escalates in response to viral infection, which may lead to exacerbations. The original primary inflammation decreases, giving rise to exacerbation-driven inflammation typified by an increased neutrophil/lymphocyte ratio in response to viral infections. Measuring low levels of CD45RO+ ILC1s in the peripheral blood may be explained by the migration of cells to the lungs in advanced COPD. This hypothesis is illustrated in **Figure 1**.

These two studies have some limitations, including a small sample size and its cross-sectional design. Investigating ILC dynamics over time and during disease exacerbations would be valuable. Additionally, concerns exist about the identity of ILC1s, which could be influenced by potential contamination of T-cells. To reduce this risk, we included antibodies against CD3, TCRαβ, and TCRγδ in our lineage marker cocktail (40-42).

In conclusion, based on blood immunological profiles, we successfully distinguished COPD patients from asthma patients, as well as from smoking and non-smoking controls. Our findings indicate that ILC1 and ILC2 cells play a role in COPD pathogenesis. We were unable to differentiate between asthma, smoking and non-smoking controls. However, we did observe differences in the immunological profile between current smokers and ex-smokers, regardless of COPD status. Our analyses reveal distinct immunological profiles between COPD and asthma, indicating that they are separate diseases with unique immunological profiles. Furthermore, our findings suggest that smoking individuals without COPD follow a distinct immunological pathway, particularly concerning ILCs, compared to COPD patients. These findings offer additional insight into why not all smokers develop COPD. It appears that distinct immunological profiles may predict the development of specific diseases. In some individuals, dysfunctional immune responses to harmful stimuli including cigarette smoke could lead to hyperactivation of the immune system, resulting in emphysematous destruction and autoimmunity. In others, immune mechanisms might suppress COPD development, though they may potentially increase the risk of cancer (43).

Future studies should explore the functional significance of surface CD45RO+ expression on ILC1s. It remains unclear whether this molecule is linked to the activation status of ILC1s, as was shown for ILC2s. Additionally, understanding the mechanistic relationship between CD45RO+ expression and the activation status of both ILC1s and ILC2s is crucial. To address this, it would be valuable to investigate whether ILCs increase in the lungs in advanced COPD, as we hypothesized (**Figure 1**). Bronchoalveolar lavage samples from COPD patients undergoing bronchoscopy for diagnostic purposes, or lung tissue from transplantation surgery, could provide more

relevant insights compared to measuring ILCs in peripheral blood, as we did. While ILC3s are suggested to play a role in COPD (44), these cells are tissue-resident, with limited numbers detectable in peripheral blood (45), which is why we did not focus on ILC3s in peripheral blood. Since we were searching for a potential biomarker, peripheral blood remains a useful medium to study ILCs, given its relative ease of collection and measurement.

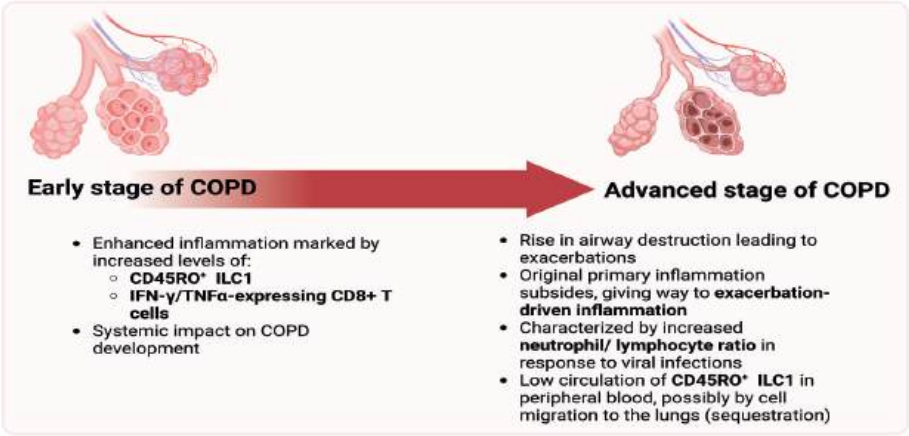

Figure 1. Speculation of immunological process during COPD progression, based on the results of this thesis. Figure is created with BioRender.com

BEHAVIOURAL ASPECTS

In this thesis the behavioural aspects investigated include the self-reported burden of disease and submission of these disease burden questionnaires to an eHealth platform. In **Chapter 6**, we illustrated the feasibility of combining disease burden questionnaires for COPD, asthma, and asthma- COPD overlap (ACO) in both primary and secondary care (46). Our study included a correlation analysis of the ACQ and CCQ, revealing a Pearson correlation coefficient exceeding 0.80 across all three diseases, indicating the interchangeability of questionnaires tailored exclusively for obstructive diseases. This potential new practical disease burden questionnaire could be used early in the diagnostic process and is less time-consuming for both patient and health professional. The current limitations are the lack of data on convergent and divergent validity, differential and linearity test of item response and cognitive debriefing of the obstructive lung disease questionnaire (OLD-Q) questionnaire.

Although we didn't directly compare disease burden between COPD and asthma in our study, existing literature suggests similar disease burden in eosinophilic and non-eosinophilic airway diseases regardless of the diagnosis COPD or asthma,

characterized by diminished health-related quality of life (HRQoL) and a high frequency of exacerbations in the past year (47). Additionally, the burden of disease appears comparable across eosinophilic severe asthma, COPD, and ACO.

Patients with "undiagnosed" asthma and COPD face a greater symptom burden and a lower health-related quality of life than patients with diagnosed asthma and COPD, indicating that early detection and treatment could be beneficial (48). In this study, "undiagnosed" patients were identified through a case-finding process that began with random phone calls with participants with breathing problems in the last 6 months, but were not diagnosed with any lung disease. This was followed by a COPD and asthma screening questionnaire and, ultimately, spirometry testing. Patients newly diagnosed with asthma or COPD through this method were classified as having "undiagnosed" asthma or COPD (48). Another group which endure greater symptom burdens, poorer quality of life, and more frequent and severe respiratory exacerbations includes individuals with ACO (49, 50). The underlying pathophysiological mechanisms of ACO are still a topic of ongoing debate (49). While emerging evidence implicates environmental and inhalational exposures in its pathogenesis among patients with pre-existing airway diseases, biomarker profiling and genetic analyses suggest ACO may constitute a heterogeneous condition with definable characteristics (49). Early-life factors, including childhood-onset asthma, may interact to elevate the risk of airflow obstruction later in life. However, therapeutic options for the ACO population have historically been excluded from clinical trials, resulting in a lack of robust, evidence-based recommendations beyond first-line inhaler therapies (49).

Our research aims to encourage the adoption of the OLD-Q. We demonstrated that it is feasible to combine disease burden questionnaires for COPD, asthma and ACO, allowing for the efficient monitoring of disease burden in routine clinical practice without requiring a lengthy patient assessment. This streamlined approach reduces the number of questions patients need to answer compared to the current standard, offering benefits for both patients and healthcare providers.

In **Chapter 7** we highlight the importance of integrating digital and personal services by healthcare professionals to enhance adherence to eHealth programs and potentially improve self-management strategies (51). Utilizing eHealth programs presents a promising avenue for improving self-management and health outcomes among patients grappling with somatic illnesses such as COPD and asthma. These platforms facilitate access to healthcare services from the comfort of one's home while upholding rigorous standards of care (52). Effective implementation of telemonitoring hinges on personalized approaches, adaptability – especially in low socioeconomic classes- and services attuned to local needs (53). Studies suggest that telemonitoring holds particular promise for patients grappling with severe symptoms,

frequent exacerbations, multiple health conditions, and limited community support. Factors as self-efficacy, mastery and readiness to change are targets involved in the aim to optimize the routine care offered by self-management program (54). Notably, telemonitoring interventions for asthma and COPD have demonstrated heightened efficacy when supplemented with educational components addressing various facets of self-management (55). Nevertheless, in the Netherlands, despite the availability of numerous self-care websites and mobile apps, only a handful of these programs have demonstrated effectiveness in improving quality of life or reducing hospitalizations, based on preliminary studies (54, 56, 57).

Another limitation of eHealth programs is the large number of patients who are non-adherent (51). Adherence is generally defined as "the extent to which a person's behaviour – taking medication, following a diet, and/or executing lifestyle changes, corresponds with agreed recommendations from a healthcare provider" (58). In literature, two forms of non-adherence are delineated: unintentional and intentional non-adherence. Intentional non-adherence is often motivated by alternative health beliefs or a lack of persuasion by medical advice. On the other hand, unintentional non-adherence stems from misunderstandings of patient instructions, complex treatment plans, or the absence of routines or reminders necessary for consistent medication usage. Addressing intentional non-adherence is particularly challenging, requiring trust in both the advice given and the advisor, as well as multifaceted psycho-behavioural interventions. (59). Patients who intentionally do not adhere to treatment may also be less likely to use an eHealth platform, whether or not they receive help from a healthcare professional. To ensure these patients do not fall through the cracks in the digital future, it is essential to identify this group during the implementation of eHealth programs. Factors contributing to unintentional non-adherence may include lower self-efficacy, lower mastery, and lower readiness to change, which affect the optimization of routine care provided by self-management programs (54). Insights from studies on medication adherence in COPD could be valuable. Although adherence in COPD is notably lower compared to other diseases, patients are well aware of the negative consequences associated with poor adherence to prescribed treatments. (60). Factors influencing treatment adherence in COPD include age, sex, education, smoking status and socioeconomic factors. (61). Patients who had lower knowledge about COPD medications and disease management were more likely to be in the low-adherence group (62). However, higher education – defined as more than 10 years of school education was associated with reduced adherence to maintenance medication as well (63). A possible explanation could be that highly educated individuals may choose to rely more on their own judgment and, consequently, adjust dosages to perceived symptoms rather than simply follow a fixed-dosage regime. Additionally, highly educated individuals may have greater concerns about the side effects of

long-term medication use. More severe dyspnea and cardiac comorbidity also lower adherence to COPD exacerbation action plans (64).

As the population ages and the number of healthcare professionals declines, a transition to digital healthcare becomes essential. There is an urgent need to create a nationwide open-source platform that features thoroughly evaluated eHealth applications, benefiting both patients and healthcare providers to improve the standard of COPD care. Involvement and training for healthcare professionals are crucial, as eHealth platforms should ultimately make interactions more time-efficient compared to face-to-face contact. To facilitate this shift, further research is required to identify both intentional and unintentional non-adherence groups. These individuals should either be encouraged to engage with eHealth platforms or, if necessary, receive alternative support through hospital or home visits. It is vital to ensure that these patients are not neglected due to digitalization. While eHealth platforms can offer benefits such as a sense of mastery for some patients, those with limited literacy or other barriers may not experience the same advantages.

When addressing behavioural aspects in the treatment of COPD and asthma, it may not be necessary to differentiate between the two diseases, as adherence is largely determined by individual patient needs. This approach contrasts with the previous described mechanistic differences, such as risk factors and immunological impacts, between the two conditions. Employing motivational interviewing and tailored approaches based on each patient's unique challenges is in my opinion the most effective strategy for promoting adherence. Conducting interviews to assess the suitability of eHealth platforms is essential, paying attention to factors such as literacy skills, digital equipment, and digital skill. If it is determined that a significant number of patients benefits from eHealth programs, establishing a comprehensive nationwide open-source platform for both patients and healthcare providers will be crucial.

CLINICAL IMPLICATIONS

Based on the findings of this thesis, I would like to highlight the clinical implications. First, in **Chapter 2**, we concluded that the heterogeneity within COPD populations calls for a personalized medicine approach, focusing on individual patient characteristics rather than simply stratifying based on clinical features. However, in **Chapters 4** and **Chapter 5**, clustering based on immunological data proved useful. We identified distinct immunological profiles for COPD compared to asthma and smoking and non-smoking controls, as well as differences between severe and less severe COPD patients. Based on these finding we conclude that clustering of disease mechanisms can provide meaningful insights for treatment, but an individual approach that considers socio-economic and behavioural aspects is necessary for successful treatment. In **Chapter 6**, using the same clustering method as in Chapters 2, 4, and 5, we found that

the two questionnaires used to measure disease burden in both asthma and COPD could be combined into a single, shorter questionnaire. Cluster methods appear to be useful for analyzing large datasets where variables are mutually correlated, such as immunological data or disease burden questionnaires. However, for clinical characteristics, which are mostly independent characteristics, it is necessary to correlate the clinical clusters with mechanistic features of the disease pathogenesis or longitudinal data such as mortality of exacerbation frequency to make it useful in the clinic.

Second, there should be an increased emphasis on new treatable traits, such as behavioural aspects, with particular attention to treatment adherence. Given the complex nature of adherence, it is recognized that no single intervention or strategy can effectively improve it on its own. A collaborative effort among all stakeholders—including government authorities, patient organizations, scientific societies, and others—is crucial to developing a comprehensive action plan tailored to the complexities of individual patients. To enhance adherence through self-management and increase insight into illness, eHealth platforms can be a valuable tool. In **Chapter 7**, we recommend the use of eHealth platforms in collaboration with healthcare professionals, a model known as blended care. eHealth platforms can be useful for uploading disease burden questionnaires and teaching patients when to seek help for exacerbations. When introducing an eHealth platform to patients, it is important to provide support and consider their literacy and digital skills. The goal is encouraging to engage with eHealth platforms or, if necessary, receive alternative support through hospital or home visits. It is vital to ensure that these patients are not overlooked due to digitalization. On the other hand, digitalization might be cost-effective, requiring fewer healthcare professionals, and eHealth programs can support patient self-management (51).

Third, in **Chapter 6**, we combined two disease burden questionnaires—the ACQ for asthma and the CCQ for COPD. We demonstrated that a single, practical questionnaire, the OLD-Q, can effectively measure disease burden for obstructive diseases. We recommend using this new questionnaire for all obstructive diseases, as it is validated for asthma, COPD, and ACO in both primary and secondary care. This tool is less time-consuming for both patients and healthcare professionals. To diagnose a patient with COPD or asthma, we adhere to the strict criteria established by GOLD for COPD and GINA for asthma, which are primarily based on lung function tests. The OLD-Q requires extra validation against the St George's Respiratory Questionnaire at two different time points to confirm its validity and assess the test-retest reliability of this practical set of questions.

Fourth, in **Chapter 4,** we advise to treat asthma and COPD as separate diseases with unique immunological profiles. It appears that distinct immunological profiles may predict the development of specific diseases. Our findings suggest that smoking

individuals without COPD follow a distinct immunological pathway, particularly concerning ILCs, compared to COPD patients. These findings offer additional insight into why not all smokers develop COPD. In this thesis we make a distinction in considering COPD and asthma as separated diseases (Chapter 4) or as one disease (Chapter 2 and 6). This is based on pathophysiological mechanisms such as identified immunological differences, or behavioural differences such as adherence and disease burden. Patients with COPD and asthma are not as different in terms of disease burden.

Finally, in **Chapter 3**, we emphasize the significance of comorbidity in asthma and COPD. Both conditions are associated with cardiovascular diseases, including metabolic syndrome. COPD is recognized as a risk factor in cardiovascular risk management by general practitioners. According to the literature and findings from this thesis, we identify seven risk factors for COPD: smoking, genetics, early life events, behavioural factors, infections, environmental exposure, and immunological factors (**Figure 2**). The risk factors that overlap with those for asthma include genetics, early life events, behavioural factors, infections, environmental exposure, and immunological factors.

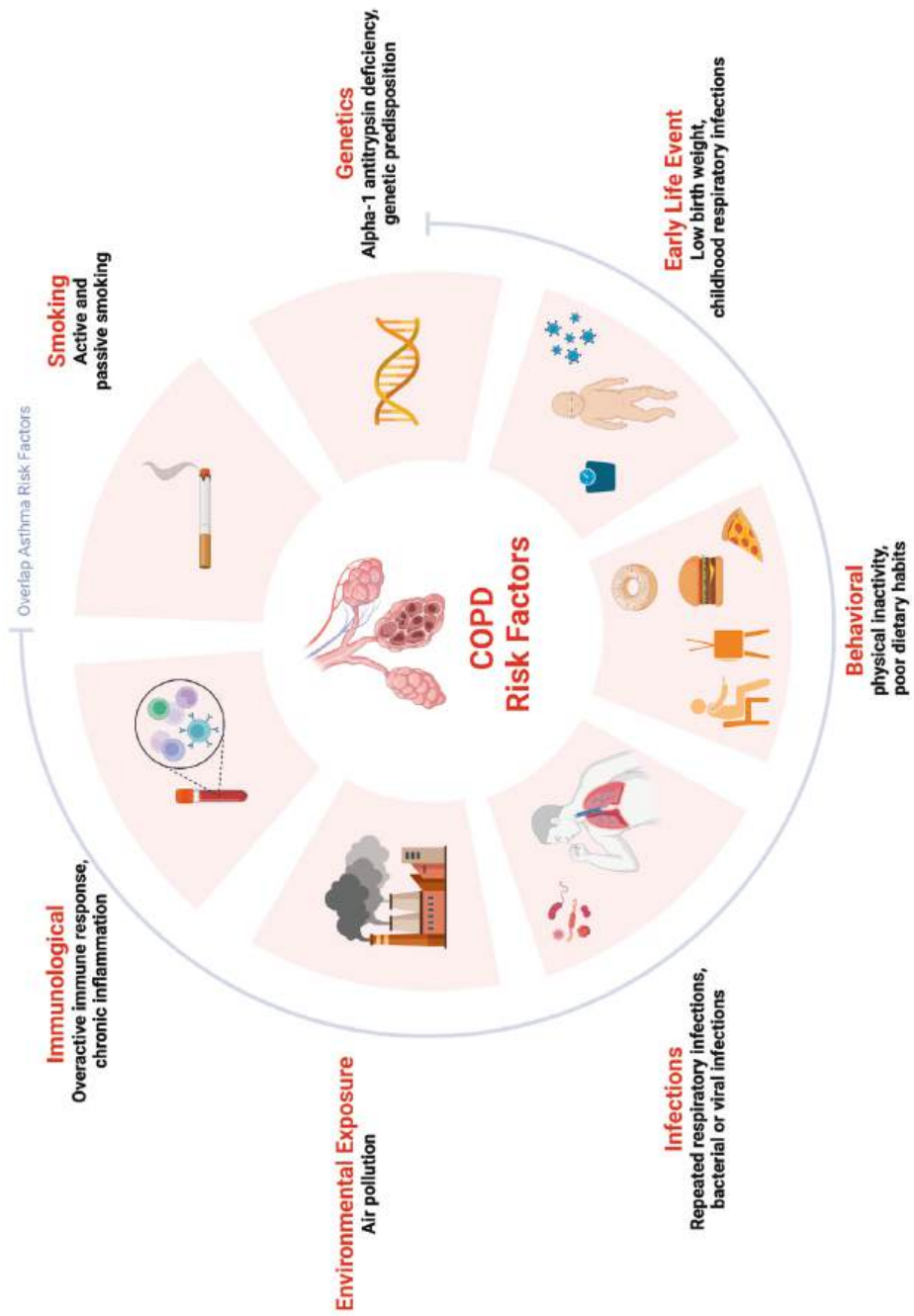

Figure 2. COPD Risk Factors and overlap in asthma risk factors. We included two risk factors for COPD based on this thesis; immunology and behavioural risk factors. The other risk factors of this figure is based on Stolz et al. Lancet 2022 (64). Figure is created with BioRender.com

SUGGESTIONS FOR FUTURE RESEARCH

The discovery of ILC1 and ILC2, along with the expression of surface CD45RO, in the immunology of both COPD and asthma might offer potential for identifying biomarkers and developing novel personalized therapeutic approaches.

- *Development of novel personalized therapeutic approaches in COPD.*

To advance this, it is essential to determine the functional significance of CD45RO+ expression on ILC1s. It remains unclear whether this molecule is linked to the activation status of ILC1s, as it is in ILC2s. Additionally, understanding the mechanistic connection between CD45RO and the activation status of both ILC1s and ILC2s is crucial. We hypothesized that the lower levels of CD45RO+ ILC1s in advanced COPD, compared to early COPD, could be due to their migration to the lungs. The next step in developing a novel therapeutic approach would be to phenotypically characterizes ILC1s in the lungs and correlate their presence with disease severity. Lung tissue from transplant patients or bronchoalveolar lavage samples from individuals with COPD could provide valuable insights for this purpose. Additionally, it would be valuable to identify the cytokines produced by ILCs and examine their correlation with disease severity.

- *Identifying biomarkers for COPD treatment.*

Previous research has shown a correlation between corticosteroid resistance and CD45RO+ ILC2s in asthma (39) making it worthwhile to investigate this association in COPD as well. Understanding this relationship can inform personalized treatment: if we identify a patient as corticosteroid-resistant, we can avoid prescribing prednisone, reduce side effects, and consider alternative treatment strategies. To identify potential biomarkers, it is essential to explore a broad range of cells, including B and T cells as well as granulocytes. This approach can help uncover correlations and differentiate them from ILCs, which may produce similar cytokines.

- *Metabolic Syndrome as a risk factor for COPD and asthma and role of TNF-alpha.*

This thesis highlighted elevated triglyceride levels in asthma patients with obesity compared to healthy individuals with obesity. Literature indicates a connection between cardiovascular diseases and both asthma and COPD, with obesity representing a distinct entity in asthma. TNF-alpha plays a role in both conditions. Blocking TNF-alpha has been shown to improve airway hyperresponsiveness in an obese-asthma model. In COPD, increased TNF-alpha levels are correlated with cigarette smoke-induced emphysema. Additionally, TNF-alpha may contribute to systemic insulin resistance by promoting the release of fatty acids from adipose tissue into the bloodstream, which affects tissues such as muscle and liver. Further research into TNF-alpha in COPD

patients with obesity, including differences in COPD severity, and comparisons with asthma patients with obesity and healthy controls with obesity, would enhance our understanding of the pathogenesis of COPD. COPD is recognized as a risk factor for cardiovascular disease; however, the role of metabolic syndrome as a risk factor for COPD has not yet been established.

- *Validation Obstructive Lung Disease Questionnaire (OLD-Q).*

This single, practical questionnaire can measure disease burden for patients with COPD, asthma and Asthma- COPD Overlap. This tool is practical and more time-efficient for both patients and healthcare professionals. Further validation of the OLD-Q against the St George's Respiratory Questionnaire at two distinct time points is required to confirm its validity and evaluate the test-retest reliability of this streamlined set of questions.

- *Identifying non-adherent patients for eHealth platform use and understanding their self-management needs.*

As highlighted in this thesis, the behavioural aspect is a key factor in the treatment of COPD. Providing tailored treatment that meets the specific needs of each patient is essential for the patient and healthcare professional. With the ongoing shift towards digitalization in medical treatments, eHealth platforms can play a vital and cost-effective role. We demonstrated that a blended care approach improved adherence to the use of eHealth platforms. However, it is important to remain vigilant about patients who are non-adherent. Identifying this group is the first step, which can be followed by using motivational interviewing to enhance their awareness and understanding of COPD management, as well as to discuss their perspectives on the role of healthcare professionals throughout the disease trajectory.

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Chapter 9

Summary

SUMMARY

Chronic Obstructive Pulmonary Disease (COPD) is a condition with irreversible airflow limitation, affecting around 10% of people over 40, with prevalence expected to rise due to aging and climate change. Air pollution is a significant risk factor, accounting for up to 50% of COPD risk, while tobacco smoke remains the primary global risk factor. Genetic predispositions, early-life infections obesity and insulin resistance also contribute to COPD development. COPD is multifaceted, though 20-30% of COPD patients have never smoked. Asthma, characterized by fluctuating airway inflammation and airflow limitation, affects both children and adults with varying severity and is influenced by genetic factors, allergens, maternal age, and early-life factors. The Dutch and British hypotheses offer opposing views on whether asthma and COPD are manifestations of a single disease or separate conditions. The Dutch hypothesis considers both obstructive airway diseases as a single disease entity, while the British hypothesis argues that asthma and COPD are distinct disease with different causal mechanisms. The concept of Asthma-COPD Overlap (ACO) refers to patients displaying features of both diseases, but there is ongoing debate about its definition and significance. This discussion has led to a focus on identifying specific observable characteristics or manifestations of these diseases, known as phenotypes. Recent research has uncovered various clinical, etiological, and pathophysiological phenotypes for COPD. Similarly, asthma exhibits a range of phenotypes, including eosinophilic and non-eosinophilic types, which are influenced by genetic and environmental factors. Immune responses in both diseases involve cytokine production of T helper cell subsets and innate lymphoid cells (ILCs), which play a role in chronic inflammation and disease progression. Behavioral and self-management strategies, including digital platforms and burden of disease questionnaires, are crucial for effective disease management and improving patient outcomes in both diseases. In this thesis we explored general risk factors, inflammatory determinants and behavioral aspects in COPD and asthma.

In **Chapter 2**, we assessed the applicability of COPD phenotypes previously defined by Burgel et al. in our patient population. We conducted cluster analyses consistent with the classification of COPD severity, defined as GOLD ABCD, and explored whether adding behavioral variables would alter the clustering results. Our results showed that the clusters we identified generally corresponded to the GOLD ABCD groups, but we could not replicate Burgel's phenotypes. Additionally, the inclusion of behavioral variables (such as quality of life, fatigue, and daily activities) resulted in four distinct clusters, which varied primarily in daily steps, activities of daily living, and quality of life. The study indicates that previously defined COPD phenotypes might not be effective for customizing individualized treatment strategies and that behavioral factors also contribute to COPD risk.

In **Chapter 3** we examined the link between lipid levels and inflammation in asthma patients with obesity in comparison with healthy patients with obesity. We demonstrated that serum triglycerides were significantly higher in asthma patients with obesity, even after adjusting for BMI, eosinophil levels, and statin use. We also corrected for the use of inhaled corticosteroids. This suggests that elevated triglycerides may contribute to asthma development in obese individuals, though further research is needed to confirm this.

After identifying behavioral aspects and lipid levels as potential risk factors for COPD and asthma, respectively, we searched for differences in Innate Lymphoid Cells (ILCs) between COPD, asthma and (non-) smoking controls in **Chapter 4**. ILCs are a non-B/non-T lymphocytes that often serve as an important innate source of inflammatory cytokines and be categorized in three different subsets, ILC1, ILC2 and ILC3, on the basis of their cytokine profile. Previous research suggests elevated IFNγ+ ILC1s in COPD and IL-5/IL-13^{+} ILC2s in asthma, but the specific inflammatory profiles of these ILC subsets remained unclear. With multi-color flow-cytometry we found higher levels of inflammatory CD4^{+} and CD4^{-} ILC1s in peripheral blood of COPD compared to asthma patients and (non-) smoking controls. Furthermore, in COPD patients a decrease in CD117^{-} ILC2s was observed, but an increase in inflammatory CD45RO^{+} cells within this subset. Principal component analysis (PCA) highlighted that these ILC features distinguished COPD patients from controls and asthma patients. We concluded that significant differences in ILC1 and ILC2 phenotypes between COPD and asthma patients suggest a role for these cells in COPD pathology, independent of smoking history.

We conducted an in-dept analysis in **Chapter 5** to explore the relationship between clinical phenotypes of COPD patients and ILCs. We assessed lung function, symptom severity, pack years, age, BMI and used flow cytometry to analyze ILC populations in peripheral blood. Three clinical clusters were identified: Cluster 1 had the lowest FEV1, BMI, and highest disease burden; Cluster 2 included older, high-BMI patients with the highest number of pack years; Cluster 3 had the highest FEV1 and fewer exacerbations. Two distinct immunological clusters emerged, with the most severe COPD group (Cluster 1) showing a less inflammatory ILC phenotype, identified by lower levels of CD45RO+ frequencies of CD4^{-} ILC1s. The strong association between clinical clusters and ILC profiles in COPD suggests that ILCs play a role in disease progression.

After the inflammatory determinants we dived further into overlapping symptoms between COPD and asthma. In **Chapter 6** we aimed to develop a unified questionnaire to assess disease burden in both asthma and COPD. Currently, separate tools (the Asthma Control Questionnaire (ACQ) and Clinical COPD Quesitonnaire (CCQ)), exist for each condition, but a single tool is lacking. We included asthma and COPD patients who completed both ACQ and CCQ on the same day and reduced the number of questions

by PCA. We also included ACO patients and validated our findings in a secondary cohort. We showed strong correlations between ACQ and CCQ in asthma and COPD and identified nine key questions forming the basis for a new "Obstructive Lung Disease Questionnaire" to assess disease burden in both conditions. We conclude that this new questionnaire is suitable for early use in the diagnostic process for both COPD and asthma and is more concise than current tools, making it time-efficient for both patient and healthcare professional.

To complete the holistic perspective on COPD, **Chapter 7** examines how healthcare professional involvement impacts patient's adherence to an eHealth platform. The study compared usage rates of CCQ between patients who used the platform individually and those who used it in a blended setting, where healthcare professionals were involved. The results revealed that patients using the platform in a blended setting had a 3.25 times higher CCQ submission rate compared to those using it independently. Additionally, within the blended setting, patients in hospital care had a 1.83 times higher CCQ submission rate than those in primary care. This study highlights the importance of the healthcare professional to help the COPD patients for effective use of eHealth programs and improved self-management.

In **Chapter 8**, we contextualize the results of this thesis within the latest insights and discuss their implications for future research and clinical practice. Firstly, while clustering methods effectively analyze large datasets with correlated variables, their clinical application requires linking these clusters to disease mechanisms. Clustering disease mechanisms can offer valuable insights for treatment; however, an individualized approach that takes into account socio-economic and behavioral factors is essential for successful outcomes. Secondly, further research into ILC1 and ILC2, and the expression of surface CD45RO, is necessary as these cells offer potential for identifying biomarkers and developing personalized therapies for asthma and COPD. Thirdly, the study underscores the significance of comorbidity, highlighting potential risk factors between COPD and asthma, such as cardiovascular issues and metabolic syndrome, and calls for further investigation into the role of TNF-alpha. Fourthly, the OLD-Q, a new combined questionnaire, simplifies disease burden assessment for COPD, asthma, and ACO. Lastly, we recommend incorporating eHealth platforms into a blended care model to enhance treatment adherence and provide support for patients with diverse digital skills.

Appendices

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Chronische obstructieve longziekte (COPD) is een aandoening met onomkeerbare luchtwegbeperking die ongeveer 10% van de mensen ouder dan 40 jaar treft. Door vergrijzing en klimaatverandering zal de prevalentie naar verwachting stijgen. Luchtwegvervuiling is een belangrijke risicofactor en kan tot 50% van het COPD risico verklaren, terwijl tabaksrook wereldwijd de belangrijkste risicofactor blijft. Genetische aanleg, infecties op jonge leeftijd, obesitas en insuline resistentie dragen ook bij aan de ontwikkeling van COPD. COPD kenmerkt zich als een heterogene en complexe ziekte, gezien ook 20-30% van de COPD patiënten nooit heeft gerookt. Astma, gekenmerkt door luchtweginflammatie en reversibele luchtwegobstructie, treft zowel kinderen als volwassenen. De ernst verschilt per individu en wordt beïnvloed door genetische factoren, blootstelling aan allergenen, de leeftijd waarop moeder zwanger werd en factoren in de vroege levensfase.

De 'Nederlandse' en 'Britse' hypothesen bieden tegenstrijdige standpunten over de vraag of astma en COPD uitingen zijn van één ziekte of afzonderlijke aandoeningen. De Nederlandse hypothese beschouwt beide obstructieve longziekten als één ziekte entiteit, terwijl de Britse hypothese stelt dat astma en COPD aparte ziekten zijn met verschillende oorzaken. Het concept van Astma-COPD Overlap (ACO) verwijst naar patiënten met kenmerken van beide ziekten, maar er is nog steeds discussie over de definitie en betekenis ervan. Deze discussie heeft geleid tot een focus op het identificeren van specifieke waarneembare kenmerken of manifestaties van deze ziekten, bekend als fenotypes. Recent onderzoek heeft verschillende klinische, etiologische en pathofysiologische fenotypes voor COPD ontdekt. Astma vertoont ook een reeks fenotypes, waaronder eosinofiele en niet-eosinofiele typen, die worden beïnvloed door genetische en omgevingsfactoren. De immuunrespons bij beide ziekten omvatten cytokineproductie door T-helpercel-subtypen en zogenaamde innate lymphoïd cells (ILCs), die een rol spelen in chronische ontsteking en ziekteprogressie. Gedrags- en zelfmanagementstrategieën, waaronder digitale platforms en vragenlijsten over ziektelast, zijn cruciaal voor effectief ziektebeheer en verbetering van patiëntuitkomsten bij beide ziekten. In dit proefschrift hebben we algemene risicofactoren, inflammatoire determinanten en gedragsaspecten bij COPD en astma onderzocht.

In **Hoofdstuk 2** beoordeelden we de toepasbaarheid van onderscheidende COPD-fenotypes in onze patiëntpopulatie. Deze fenotypes zijn eerder gedefinieerd door Burgel *et al.* We hebben clusteranalyses uitgevoerd in overeenstemming met de classificatie op grond van de ernst van de ziekte, gedefinieerd als GOLD ABCD, en hebben onderzocht of het toevoegen van gedragsvariabelen de clustervorming zou beïnvloeden. Onze resultaten tonen aan dat de geïdentificeerde clusters over het algemeen overeenkomen met de GOLD ABCD-groepen, maar we kunnen de

fenotypes van Burgel et al. niet reproduceren. Bovendien resulteert de toevoeging van gedragsvariabelen (zoals kwaliteit van leven, vermoeidheid en dagelijkse activiteiten) in vier verschillende clusters, die voornamelijk verschilden in dagelijkse stappen, dagelijkse activiteiten en kwaliteit van leven. De studie suggereert dat eerder gedefinieerde COPD-fenotypes mogelijk niet effectief zijn voor gepersonaliseerde behandelstrategieën en dat gedragsfactoren ook een belangrijke bijdragen vormen in het fenotyperen van COPD patiënten.

In **Hoofdstuk 3** hebben we het verband tussen lipidewaarden en ontstekingswaarden bij astma patiënten met obesitas vergeleken met gezonde patiënten met obesitas. We hebben hierbij gevonden dat serumniveaus van triglyceriden significant hoger waren bij astmapatiënten met obesitas, ook na correctie voor gewicht (body mass index, BMI), eosinofiel waarden in het bloed en statine gebruik. Dit suggereert dat verhoogde triglyceriden mogelijk bijdragen aan astmaontwikkeling bij obese personen, hoewel verder onderzoek nodig is om dit te bevestigen.

Na het identificeren van gedragsaspecten en lipidenwaarden als potentiële risicofactoren voor respectievelijk COPD en astma, hebben we in **Hoofdstuk 4** gezocht naar verschillen in ILCs tussen patiënten met COPD en astma en (niet-) rokende controlepersonen. Eerdere studies suggereerden verhoogde interferon-γ producerende ILC1s bij COPD en interleukine-5 en -13 positieve ILC2s bij astma, maar de specifieke ontstekingsprofielen van deze ILC subsets waren vooralsnog onduidelijk. Met behulp van multi-color flowcytometrie technologie hebben we hogere waarden van inflammatoire CD4+ en CD4- ILC1s in perifeer bloed van COPD-patiënten gevonden, in vergelijking met astma patiënten en (niet-)rokende controles. Verder hebben bij COPD-patiënten een afname van CD117- ILC2s waargenomen en een toename van inflammatoire CD45RO+ cellen binnen deze specifieke subset. Op de verkregen data sets hebben we een zogenaamde principal component analyse (PCA) uitgevoerd. Dit is een wiskundige multivariate analysemethode om een grote hoeveelheid gegevens te beschrijven met als doel ze eenvoudiger te maken (datareductie), zonder dat essentiële informatie verloren gaat. De PCA toonde aan dat deze ILC-kenmerken COPD patiënten onderscheiden van controles en astma patiënten. We hebben uit deze analyse geconcludeerd dat de gevonden significante verschillen in ILC1- en ILC2-fenotypes tussen COPD- en astma patiënten suggereren dat deze cellen een rol spelen in de COPD pathologie, onafhankelijk van de rookgeschiedenis.

In **Hoofdstuk 5** hebben we een diepte-analyse uitgevoerd om de relatie tussen klinische fenotypes van COPD patiënten en ILCs te onderzoeken. We hebben gegevens over longfunctie zoals FEV1 (*forced expiratory volume in 1 second)*, symptomen, rookhistorie in 'rookjaren', leeftijd en BMI verkregen en flowcytometrie gebruikt om ILC-populaties in perifeer bloed te analyseren. Drie klinische clusters werden geïdentificeerd: Cluster 1 had de laagste FEV1, BMI en hoogste ziektelast;

Cluster 2 omvatte oudere, hoge-BMI-patiënten met de meeste rookjaren; Cluster 3 had de hoogste FEV1 en minder exacerbaties (periodes van sterke toename van ziektesymptomen). Twee onderscheidende immunologische clusters zijn hierbij naar voren gekomen, waarbij de ernstigste COPD-groep (Cluster 1) een minder inflammatoir ILC-fenotype vertoonde, gekenmerkt door lagere percentages van CD45RO+ cellen binnen de populatie van CD4- ILC1s. De sterke associatie tussen klinische clusters en ILC-profielen bij COPD suggereert dat ILCs een rol spelen in ziekteprogressie.

Na de inflammatoire determinanten hebben we de overlappende symptomen tussen COPD en astma in detail onderzocht. In **Hoofdstuk 6** hebben we geprobeerd een uniforme vragenlijst te ontwikkelen om de ziektelast bij zowel astma als COPD te beoordelen. Momenteel bestaan er afzonderlijke instrumenten, de *Asthma Control Questionnaire* (ACQ) en de *Clinical COPD Questionnaire* (CCQ), maar één enkel hulpmiddel voor beide ziekten ontbreekt. We hebben astma- en COPD-patiënten geïncludeerd, die op dezelfde dag zowel de ACQ als de CCQ invulden, en hebben met behulp van PCA onderzocht of het aantal vragen kon worden verminderd. We hebben uit deze analyse kunnen concluderen dat een nieuwe, uniforme vragenlijst, de *Obstructive Lung Disease Questionnaire* (OLD-Q), geschikt is voor vroeg gebruik in het diagnostische proces voor zowel COPD als astma, en bovendien tijdsefficiënt is voor zowel patiënt als zorgverlener.

Om tot een meer holistische perspectief op COPD te komen, hebben we in **Hoofdstuk 7** onderzocht hoe betrokkenheid van zorgverleners de therapietrouw van patiënten ten aanzien van een eHealth-platform beïnvloedt. In deze studie hebben we de CCQ gebruiksfrequentie vergeleken tussen patiënten die het platform individueel gebruikten en degenen die dit deden in een gezamenlijke setting met zorgverleners. De verkregen resultaten tonen aan dat patiënten die het platform in een gezamenlijke setting gebruikten 3,25 keer hogere CCQ-indieningsfrequenties hadden in vergelijking met degenen die het platform zelfstandig gebruikten. Daarnaast hadden patiënten in ziekenhuizen binnen de gezamenlijke setting de CCQ 1,83 keer vaker ingediend dan patiënten in de eerstelijnszorg. Deze studie benadrukt het belang van zorgverleners voor effectief gebruik van eHealth-programma's en verbeterd zelfmanagement bij COPD patiënten.

In **Hoofdstuk 8** hebben we de resultaten van dit proefschrift in de context van de nieuwste inzichten geplaatst en de implicaties voor toekomstig onderzoek en de klinische praktijk besproken. Ten eerste kunnen clusteringmethoden effectief worden gebruikt om grote datasets met samenhangende variabelen te analyseren, maar de klinische toepassing vereist een koppeling van deze clusters aan ziekteprocessen. Het clusteren van ziekteprocessen kan waardevolle inzichten bieden voor behandelingen; een gepersonaliseerde aanpak die rekening houdt met sociaaleconomische en gedragsfactoren is echter essentieel voor succesvolle uitkomsten. Ten tweede is verder

onderzoek nodig naar ILC1- en ILC2-cellen en de expressie van het oppervlakte-eiwit CD45RO. Deze cellen bieden namelijk potentieel voor het identificeren van biomarkers en het ontwikkelen van gepersonaliseerde therapieën voor astma en COPD. Ten derde benadrukt de studie het belang van co-morbiditeiten door te wijzen op potentiële risicofactoren voor COPD en astma, zoals cardiovasculaire problemen en metabool syndroom, en roept het op tot verder onderzoek naar de rol van de ontstekingsmediator tumornecrosefactor α. Ten vierde vereenvoudigt de OLD-Q, een nieuwe gecombineerde vragenlijst, de beoordeling van ziektelast bij COPD, astma en ACO. Tot slot raden we aan om eHealth-platforms toe te passen in een gezamenlijke setting met zorgverleners om de therapietrouw te verbeteren en patiënten met diverse digitale vaardigheden te ondersteunen.

Appendices

Publications

PUBLICATIONS

Cuperus LJA, Bult L, **van Zelst CM**, van den Brink WJ, Kamstra KRJM, van den Broek TJ, van den Eijnden MAC, Panditha P, In 't Veen JCCM, Braunstahl GJ. Wearable technology for detection of COPD exacerbations: feasibility of the Health Patch. **ERJ Open Res. 2024 Nov 25;10(6):00396-2024.**

Cuperus LJA*, **van Zelst CM***, Kerstjens HAM, Hendriks RW, Rutten-van Molken MPMH, Muilwijk-Kroes JB, Braunstahl GJ, In 't Veen JCCM. Measuring burden of disease in both asthma and COPD by merging the ACQ and CCQ: less is more? **NPJ Prim Care Respir Med. 2024 May 3;34(1):8**. * Shared first author

van Zelst CM, In 't Veen JCCM, Krabbendam L, de Boer GM, de Bruijn MJW, van Nimwegen M, van der Ploeg EK, van Uden D, Stadhouders R, Tramper-Stranders GA, Hendriks RW, Braunstahl GJ. Aberrant characteristics of peripheral blood innate lymphoid cells in COPD, independent of smoking history. **ERJ Open Res. 2024 Feb 19;10(1):00652-2023.**

Thelen JC, **van Zelst CM,** van Brummelen SE, Rauh S, In 't Veen JCCM, Kappen JH, Braunstahl GJ. Efficacy and safety of dupilumab as add-on therapy for patients with severe asthma: A real-world Dutch cohort study. **Respir Med. 2023 Jan;206:107058.**

van Zelst CM, Goossens LMA, Witte JA, Braunstahl GJ, Hendriks RW, Rutten-van Molken MPMH, Veen JCCMI. Stratification of COPD patients towards personalized medicine: reproduction and formation of clusters. **Respir Res. 2022 Dec 9;23(1):336.**

de Boer GM, Tramper-Stranders GA, Houweling L, **van Zelst CM**, Pouw N, Verhoeven GT, Boxma-de Klerk BM, In 't Veen JCCM, van Rossum EFC, Hendriks RW, Braunstahl GJ. Adult but not childhood onset asthma is associated with the metabolic syndrome, independent from body mass index. **Respir Med. 2021 Nov;188:106603.**

de Boer GM, Braunstahl GJ, van der Ploeg EK, **van Zelst CM**, van Bruggen A, Epping G, van Nimwegen M, Verhoeven G, Birnie E, Boxma-de Klerk BM, de Bruijn MJW, Stadhouders R, Hendriks RW, Tramper-Stranders GA. Bacterial lysate add-on therapy to reduce exacerbations in severe asthma: A double-blind placebo-controlled trial. **Clin Exp Allergy. 2021 Sep;51(9):1172-1184.**

Deenstra DD, van Helvoort HAC, Djamin RS, **van Zelst C**, In't Veen JCCM, Antons JC, Spruit MA, van 't Hul AJ. Prevalence of hyperventilation in patients with asthma. **J Asthma. 2021 Aug 6:1-8.**

van Zelst CM, de Boer GM, Türk Y, van Huisstede A, In't Veen JCCM, Birnie E, Boxma-de Klerk BM, Tramper-Stranders GA, Braunstahl GJ. Association between elevated serum triglycerides and asthma in patients with obesity: An explorative study. **Allergy Asthma Proc. 2021 May 1;42(3):e71-e76.**

van Zelst CM, Kasteleyn MJ, van Noort EMJ, Rutten-van Molken MPMH, Braunstahl GJ, Chavannes NH, In 't Veen JCCM. The impact of the involvement of a healthcare professional on the usage of an eHealth platform: a retrospective observational COPD study. **Respir Res. 2021 Mar 21;22(1):88**

van Zelst CM*, Janssen ML*, Pouw N, Birnie E, Castro Cabezas M, Braunstahl GJ. Analyses of abdominal adiposity and metabolic syndrome as risk factors for respiratory distress in COVID-19. **BMJ Open Respir Res. 2020 Dec;7(1):e000792.** * *Shared first author*

Appendices

Portfolio

PHD PORTFOLIO

ERASMUS UNIVERSITY ROTTERDAM

Cathelijne Marianne van Zelst

Description Required	Organizer	EC
ERS Paris International Congress 2018 Poster presentation (2018)		0.30
Wetenschapsdag Franciscus Gasthuis and Vlietland 2018 Poster presentation (2018)		0.30
NRS Young Investigator Symposium 2018 (2018)		0.30
Wetenschapsdag 2019 – poster jury (2019)		0.30
Erasmus MC - Basic Introduction Course on SPSS (2019)	Dr. R.A. Gruters	1.00
Erasmus MC - CC02 Biostatistical Methods I: Basic Principles (2019)	Prof. Dr. M.G.M. Hunink	5.70
NRS Young Investigator Symposium 2019 (2019)		0.30
GCP WMO (2019)	Examination Board EMWO	1.50
Bomen over COPD (2019)		0.60
EGSL - Academic Integrity (2020)	Dr. S. van de Vathorst	0.30
Scientific Integrity (2020)	Erasmus MC Graduate School	0.30
ERS Vienna virtual 2020, 2 poster presentations (2020)		0.60
ERS Madrid International Congress 2019 (2020)		0.30
Wetenschapsdag 2020 Gasthuis and Vlietland, poster presentation (2020)		0.30
Advanced Immunology course (2020)		3.00
Scientific writing course (2020)	Erasmus MC graduate school	1.50
Biostatistiek AUMC (2021)		0.60
Member of “Zin in Zorg’ team in Franciscus Gasthuis and Vlietland (2021)		2.00
Peer-support for junior residents in Franciscus Gasthuis & Vlietland (2021)		2.00
Member ‘Arts-assistenten vereniging’ in Franciscus Gasthuis & Vlietland (2022)		2.00
Weekly Journal Club Pulmonology Franciscus (2022)		2.00
Monday morning research meeting EMC (2022)		1.00
ERS Barcelona International Congress 2022 2 poster presentations and 1 oral (2022)		1.00
Wetenschapsdag Franciscus Gasthuis en Vlietland poster and oral presentation 2022 (2022)		1.00
Workshop omgaan met grensoverschrijdend gedrag (2023)	Onderzoek en Ontwikkeling Franciscus	0.30
GCP herregistratie (2023)		0.30
Vooropleiding interne geneeskunde, cursus farmacologie en water en zout (2024)		2.00

Optional

Master Research Student (2022)		**2.00**
Teach the Teacher II (2022)	**Sanne Ruseler**	**0.30**
Total EC		------ + **33.10**

Appendices

Dankwoord

DANKWOORD

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Appendices

About the author

ABOUT THE AUTHOR

Cathelijne Marianne van Zelst was born on August 21, 1992, in Leiderdorp, the Netherlands. At the age of 11, she and her family moved to Groningen. After completing high school, she studied biomedical sciences at the Rijksuniversiteit Groningen for a year before beginning her medical studies in Groningen in 2011. She completed her clinical rotations in Isala Zwolle. Since August 2017, she has been affiliated with the Franciscus Gasthuis and Vlietland in Rotterdam, initially for a research internship (chapter 3 of this thesis) and later as a medical doctor (ANIOS) in the pulmonary department. After a year of clinical practice, she started her PhD track in 2019 in collaboration with Erasmus MC, while also working evening, night, and weekend shifts at the pulmonary clinic. In September 2022 she started her residency pulmonology at Reinier de Graaf in Delft, working in the internal medicine, cardiology, and ICU departments. In 2024, she began her specialization as a pulmonologist at the Franciscus Gasthuis and Vlietland in Rotterdam.

In her free time, Cathelijne enjoys spending time with family and friends. She is married to Ruben Renkema and they have a son, Remi (born 2024).