Breath by breath - Unveiling Early Small Airway Changes with Novel Diagnostics

Nasr, Abir

2025

Document Version:
Publisher's PDF, also known as Version of record

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Unveiling Early Small Airway Changes with Novel Diagnostics

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Printed by Media-Tryck, Lund 2025 NORDIC SWAN ECOLABEL 3041 0903

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Chronic obstructive pulmonary disease (COPD) and asthma both involve inflammation and structural changes in the peripheral airways, which are not adequately detected by current standard diagnostic tools. This thesis has focused on exploring small airway impairments in the peripheral regions of the lungs using less commonly applied methods, including respiratory oscillometry and V/P SPECT. The studies have applied advanced analytical approaches and related these findings to symptoms and conventional lung function tests. Overall, the work presented in this thesis demonstrates that advanced physiological and imaging techniques can reveal small airway impairments that remain undetected by conventional diagnostics, thereby contributing to a deeper understanding of obstructive airway diseases.

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2025:108
ISBN 978-91-8021-761-3
ISSN 1652-8220

Breath by breath

Unveiling Early Small Airway Changes with Novel Diagnostics

Abir Nasr

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 15th of October at 13.00 in Segerfalk Hall, Department of Clinical Sciences, Sölvegatan 17, 223 62 Lund, Sweden

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Organization: LUND UNIVERSITY

Document name: DOCTRORAL DISSERTATION **Date of issue** 2025-10-15

Author(s): Abir Nasr **Sponsoring organization:**

Title and subtitle: Breath by breath, Unveiling Early Small Airway Changes with Novel Diagnostics

Abstract:
Chronic obstructive pulmonary disease (COPD) and asthma both involve inflammation and structural changes in the peripheral airways that are not detectable with current standard tools such as spirometry. Accurate and sensitive assessment of lung function is crucial for early diagnosis, monitoring disease progression, and guiding appropriate treatment.

The overall aim of this thesis was to investigate alternative and less explored methods for detecting early or subtle dysfunction in small airway physiology in individuals with COPD, asthma, and healthy smokers, and to compare their diagnostic value with established pulmonary function tests (PFTs).

In Papers I and II, more sophisticated measures of respiratory oscillometry were investigated in different settings in COPD and asthma. Paper III evaluated comprehensive PFTs, including respiratory oscillometry, in never-smokers with COPD. Paper IV explored the severity of airway obstruction and preserved lung function assessed by ventilation/perfusion (V/P) SPECT in young and older healthy smokers and subjects with COPD.

In Paper I, within-breath measures of respiratory oscillometry, reflecting expiratory flow limitation, were most prominent in parameters measuring peripheral resistance and reactance. Paper II demonstrated that higher baseline inspiratory resistance, primarily reflecting peripheral airways, was an indicator of a positive mannitol challenge test in asthma. Paper III provided further evidence that COPD in never-smokers presents with mild pathophysiological changes and constitutes a distinct phenotype affecting the small airways. Paper IV identified increased ventilation impairment in a subgroup of young healthy smokers despite normal spirometry. In the older cohort, most variables indicated impaired lung function, with higher obstruction grades observed in the COPD group.

In conclusion, respiratory oscillometry is a promising technique for assessing peripheral airway function, and its clinical utility should be further explored alongside conventional methods. V/P SPECT is a sensitive imaging modality that can detect ventilation heterogeneities earlier in the disease process than standard lung function tests.

Key words: Peripheral airways, COPD, asthma, respiratory oscillometry, resistance, reactance, V/P SPECT, symptoms

Language English, Swedish **Number of pages**:80

ISSN and key title: 1652-8220 **ISBN:** 978-91-8021-761-3

Signature Date 2025-09-10

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ISBN 978-91-8021-761-3
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2025

In loving memory of my father, and with deep gratitude to my mother and family for their endless love and support

Every harvest begins with a seed

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I. Expiratory and inspiratory resistance and reactance from respiratory oscillometry defining expiratory flow limitation in obstructive lung diseases.

Nasr, A., Papapostolou, G., Jarenbäck, L., Romberg, K., Tunsäter, A., Ankerst, J., Bjermer, L., & Tufvesson, E. (2024). Expiratory and inspiratory resistance and reactance from respiratory oscillometry defining expiratory flow limitation in obstructive lung diseases. *Clinical physiology and functional imaging*, *44*(6), 426–435. https://doi.org/10.1111/cpf.12895

II. Airway hyperresponsiveness to mannitol in relation to inspiratory and expiratory resistance in subjects with asthma, COPD and healthy smokers

Nasr, A., Papapostolou, G., Jarenbäck, L., Romberg, K., Tunsäter, A., Ankerst, J., Bjermer, L., & Tufvesson, E. (2025). Airway hyperresponsiveness to mannitol in relation to inspiratory and expiratory resistance in subjects with asthma, COPD, and healthy smokers. *European clinical respiratory journal*, *12*(1), 2546677. https://doi.org/10.1080/20018525.2025.2546677

III. Understanding lung function in never-smokers with COPD and Airway obstruction over the range of FEV_1 in comparison to controls

Anders Andersson, Abir Nasr, Finn Radner, Anders Blomberg, Jonas Erjefält, Petra Jacobson, Christer Janson, Andrei Malinovschi, Lennart Persson, Pernilla Sönnerfors, Åsa Wheelock, Magnus Sköld, Leif Bjermer, Ellen Tufvesson
Manuscript

IV. Ventilation/Perfusion SPECT for Assessing Lung Function Deficiencies in Young and Older Healthy Smokers and subjects with COPD

Abir Nasr, Linnea Jarenbäck, Marika Bajc, Jaro Ankerst, Leif Bjermer, Ellen Tufvesson
Manuscript

Other Papers

1. Papapostolou, G., Nasr, A., Jarenbäck, L., Romberg, K., Tunsäter, A., Ankerst, J., Bjermer, L., & Tufvesson, E. (2025). Peripheral Airway Obstruction in Association with Symptoms and Quality of Life in Asthma. *Journal of asthma and allergy*, *18*, 491–505. https://doi.org/10.2147/JAA.S497218
2. Backer, V., Klein, D. K., Bodtger, U., Romberg, K., Porsbjerg, C., Erjefält, J. S., Kristiansen, K., Xu, R., Silberbrandt, A., Frøssing, L., Hvidtfeldt, M., Obling, N., Jarenbäck, L., Nasr, A., Tufvesson, E., Mori, M., Winther-Jensen, M., Karlsson, L., Nihlén, U., Veje Flintegaard, T., … Bjermer, L. (2020). Clinical characteristics of the BREATHE cohort - a real-life study on patients with asthma and COPD. *European clinical respiratory journal*, *7*(1), 1736934. https://doi.org/10.1080/20018525.2020.1736934.
3. Nasr, A., Lindqvist, A., & Bajc, M. (2017). Ventilation defect typical for COPD is frequent among patients suspected for pulmonary embolism but does not prevent the diagnosis of PE by VP SPECT. *EC Pulmonol Respir Med*, *4*, 85-91.

Populärvetenskaplig sammanfattning

Kroniskt obstruktiv lungsjukdom (KOL) är en av våra största folksjukdomar och en ledande orsak till död globalt. I Sverige har cirka 8–10 % av befolkningen KOL, och en stor andel är fortfarande odiagnostiserade. Den främsta riskfaktorn är rökning, men även luftföroreningar och genetiska faktorer kan bidra till sjukdomsutvecklingen. KOL är en kronisk inflammatorisk lungsjukdom som ofta börjar i de små luftvägarna i lungornas perifera delar. Dessa små luftvägar kan även vara påverkade vid astma, särskilt vid svårare former av sjukdomen.

Spirometri är det vanligaste testet för att mäta lungfunktion, men metoden fångar främst förändringar i de stora och medelstora luftvägarna. Tidiga förändringar i de små luftvägarna kan därför lätt missas. I avsaknad av enkla och lättillgängliga metoder för att mäta funktionen i de små luftvägarna undersökte vi en mindre använd metod – respiratorisk oscillometri – som snabbt och enkelt mäter luftvägarnas motstånd och elasticitet.

Ett välkänt fenomen hos patienter med KOL är att luftvägarna tenderar att kollapsa vid snabb och kraftig utandning, och i mer avancerade stadier även vid normal utandning. I vår första studie undersökte vi om detta fenomen kunde mätas som en större skillnad mellan in- och utandning i parametrar som speglar de små luftvägarna. Vi jämförde patienter med KOL och astma samt friska rökare och friska aldrig-rökare. Som väntat hade KOL-patienterna större skillnader än både astmapatienterna och de friska aldrig-rökarna. Ett särskilt intressant fynd var att friska rökare uppvisade mönster liknande KOL-patienterna, trots att deras lungfunktion bedömdes som normal med spirometri. Dessutom hängde dessa mätvärden ihop med patienternas symtom.

I den andra studien kombinerade vi oscillometri med ett test som mäter hur reaktiva luftvägarna är genom att andas in mannitol. En tredjedel av astmapatienterna, hälften av KOL-patienterna och flera av de friska rökarna reagerade positivt på testet. Vid analys av in- och utandning separat såg vi att astmapatienter som reagerade positivt hade högre värden i vissa oscillometrimått vid inandning. Dessa mått skulle i framtiden kunna användas för att identifiera astmapatienter med särskilt känsliga luftvägar.

I den tredje studien undersökte vi en grupp icke-rökare med KOL, baserat på en låg FEV_1/FVC-kvot (<0,7). Denna grupp utgör cirka 20–40 % av alla KOL-patienter och förväntas bli vanligare när rökningen minskar i västvärlden. De har ofta exkluderats från kliniska studier, vilket gör att kunskapen om dem är begränsad. Vi jämförde dessa patienter med friska icke-rökare och inkluderade även en grupp med låg spirometrikvot på grund av stora lungvolymer. Resultaten visade att icke-rökande KOL-patienter hade mildare nedsättning av lungfunktionen och bevarade lungvolymer och diffusionskapacitet, men tecken på påverkan i de små luftvägarna. Personer med stora lungvolymer hade ingen patologisk påverkan.

I den fjärde studien använde vi lungscintigrafi (V/P SPECT) för att bedöma graden av luftvägsobstruktion och hur stor del av lungans ventilation och perfusion som var bevarad. Vi jämförde yngre och äldre rökare med icke-rökare och patienter med KOL. Hos de yngre hade fler rökare tecken på ventilationstörningar än icke-rökarna, trots normal spirometri. Hos de äldre kunde lungscintigrafin tydligt klassificera obstruktionsgraden vid KOL och resultaten stämde väl överens med övriga lungfunktionsmätningar. Inga signifikanta skillnader sågs mellan äldre rökare och äldre icke-rökare, vilket kan tyda på att de inkluderade rökarna tillhörde en friskare grupp.

Sammanfattningsvis visar våra studier att både respiratorisk oscillometri och lungscintigrafi kan ge viktig och kompletterande information vid utredning av lungsjukdomar. Metoderna har potential att upptäcka tidiga förändringar i små luftvägar och ventilationens fördelning, långt innan de syns med konventionella tester som spirometri. Genom att använda dessa metoder parallellt med etablerade tekniker kan tidig diagnos, mer individanpassad behandling och bättre uppföljning möjliggöras – vilket på sikt kan bromsa sjukdomsutvecklingen, minska symtom och förbättra livskvaliteten.

Abstract

Chronic obstructive pulmonary disease (COPD) and asthma both involve inflammation and structural changes in the peripheral airways that are not detectable with current standard tools such as spirometry. Accurate and sensitive assessment of lung function is crucial for early diagnosis, monitoring disease progression, and guiding appropriate treatment.

The overall aim of this thesis was to investigate alternative and less explored methods for detecting early or subtle dysfunction in small airway physiology in individuals with COPD, asthma, and healthy smokers, and to compare their diagnostic value with established pulmonary function tests (PFTs).

In Papers I and II, more sophisticated measures of respiratory oscillometry were investigated in different settings in COPD and asthma. Paper III evaluatedcomprehensive PFTs, including respiratory oscillometry, in never-smokers with COPD. Paper IV explored the severity of airway obstruction and preserved lung function assessed by ventilation/perfusion (V/P) SPECT in young and older healthy smokers and subjects with COPD.

In Paper I, within-breath measures of respiratory oscillometry, reflecting expiratory flow limitation, were most prominent in parameters measuring peripheral resistance and reactance. Paper II demonstrated that higher baseline inspiratory resistance, primarily reflecting peripheral airways, was an indicator of a positive mannitol challenge test in asthma. Paper III provided further evidence that COPD in never-smokers presents with mild pathophysiological changes and constitutes a distinct phenotype affecting the small airways. Paper IV identified increased ventilation impairment in a subgroup of young healthy smokers despite normal spirometry. In the older cohort, most variables indicated impaired lung function, with higher obstruction grades observed in the COPD group.

In conclusion, respiratory oscillometry is a promising technique for assessing peripheral airway function, and its clinical utility should be further explored alongside conventional methods. V/P SPECT is a sensitive imaging modality that can detect ventilation heterogeneities earlier in the disease process than standard lung function tests.

Abbreviations

ACT	Asthma Control Test
ACQ	Asthma Control Questionnaire
ATS	American Thorax Society
AQLQ	Asthma Quality of Life Questionnaire
AX	Reactance area
BEC	Blood Eosinophil Counts
CAT	COPD Assessment Test
CCQ	Clinical COPD Questionnaire
CT	Computed Tomography
DLCO	Diffusion capacity of the lung for carbon monoxide
DTPA	Diethylenetriaminepentaacetate
EFL	Expiratory flow limitation
ERS	European Respiratory Society
FEV_1	Forced Expiratory Volume in 1 second
FOT	Forced Oscillation Technique
FRC	Functional Residual Capacity
Fres	Resonans frequency
FVC	Forced Vital Capacity
GINA	Global Initiative for Asthma
GLI	Global Lung Function Initiative
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HRCT	High Resolution Computed Tomography
ICS	Inhaled Corticosteroids
IOS	Impulse Oscillometry System
LABA	Long-acting β2-agonist
LAMA	Long-acting muscarinic antagonist
LLN	Lower limit of normal
MAA	Macro-aggregated human albumin

MRC	Medical Research Council questionnaire
OG	Obstruction Grade
RAWs	Specific airway resistance
SABA	Short-acting β2-agonist
SAD	Small airway disease
SAMA	Short-acting muscarinic antagonist
PFT	Pulmonary function tests
PLF	Preserved Lung Function
PRISm	Preserved ration impaired spirometry
RV	Residual Volume
R5	Resistance at 5 Hz
R19	Resistance at 19 Hz
Rexp	Expiratory flow resistance of the airways
Rinsp	Inspiratory flow resistance of the airways
TLC	Total Lung Capacity
VA	Alveolar volume
V/P SPECT	Ventilation/Perfusion Single-Photon Emission Computed Tomography
X5	Reactance at 5 Hz
Xexp	Expiratory flow reactance of the airways
Xinsp	Inspiratory flow reactance of the airways

Introduction

Before beginning my doctoral studies, my clinical experience with patients with chronic obstructive pulmonary disease (COPD) came primarily through performing spirometry and ventilation/perfusion (V/P) SPECT in my role as a technologist. I frequently encountered patients with emphysema and advanced airway obstruction, which often led me to reflect on why these individuals had not sought medical attention at an earlier stage. This recurring question sparked a growing interest in the disease.

My interest in COPD deepened during a course on contemporary illness, where I became increasingly engaged in understanding the complexity of the disease. This engagement led me to focus my master's thesis on investigating whether the severity of airway obstruction seen in ventilation studies influenced the detection of pulmonary embolism using V/P SPECT imaging. Upon beginning my doctoral studies, I was encouraged to find that the research projects included in my thesis aimed to explore novel methods for identifying COPD at an early stage.

Background

The Respiratory System

The respiratory system begins with the nasal and oral cavities. During inspiration, air passes through the oropharynx and larynx before entering the trachea. At the carina, the trachea divides into the right and left main bronchi. The right main bronchus branches into three lobar bronchi, while the left divides into two lobar bronchi. These further subdivide into segmental and subsequently subsegmental bronchi. After multiple divisions, the airways transition into bronchioles, then alveolar ducts, and ultimately terminate in more than 300 million alveoli. The alveoli represent the primary sites of gas exchange, where oxygen diffuses into the surrounding capillary network and enters the arterial circulation, while carbon dioxide is simultaneously removed from the blood.

The bronchial tree is generally divided into large and small airways, which differ in anatomical location, structure, and function. Large airways encompass generations 1–8 of the bronchial tree and have diameters greater than 2 mm. They include the trachea, primary, secondary, and tertiary bronchi, and their main function is to conduct air to the deeper regions of the lungs. In contrast, small airways include generations 9–23 and have diameters less than 2 mm. This group comprises bronchioles, terminal bronchioles, respiratory bronchioles, alveolar ducts, and alveoli (Figure 1).

During normal breathing, lung expansion and contraction are primarily driven by the diaphragm, which moves downward during inspiration and upward during expiration. In situations requiring increased respiratory effort, such as physical activity or respiratory disease, ventilation becomes more dependent on the expansion of the chest cavity, assisted by elevation of the rib cage and activation of intercostal and accessory muscles.

Peripheral airways

The peripheral regions of the lungs represent about 95% of the total lung volume. However, due to the dramatic increase in total airway cross-sectional area for each generation of branching, only 10-20% of the total airway resistance to flow in healthy adult originates from the peripheral airways (1). Spirometry, the standard

lung function test, measures flow reflecting intermediate and large airways. Therefore, pronounced changes in the peripheral airway physiology can occur before they are detected as a reduction in flow by spirometry. Numerous studies have been conducted to increase the understanding of COPD and asthma, and recent reports have revealed that these disorders affect the small airways and that patients with more involvement of the peripheral airways have more symptoms and a more severe disease (2-4).

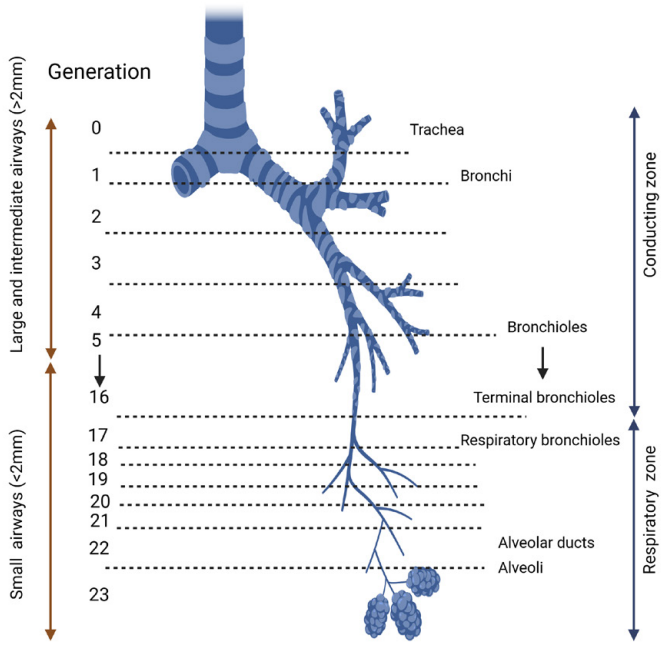

Figure1. Schematics of the bronchial tree by Weibel's model. A schematic figure of the bronchial tree, beginninig with the trachea and leading down to the alveolar sacs. The conducting airways and respiratory zone airways. Created in Biorender. https://BioRender.com

The anatomy of the conducting zone

The trachea is lined, adjacent to the lumen, by respiratory epithelium composed of pseudostratified ciliated columnar cells. This epithelial layer includes ciliated cells, goblet cells, columnar brush cells (with afferent nerve endings functioning as sensory receptors), basal cells, and small granule cells.

Goblet cells are responsible for producing mucus, which traps inhaled particles and pathogens. The cilia function to transport mucus upward toward the pharynx, particularly during coughing. Basal cells act as progenitor cells, playing a key role in regenerating and maintaining the epithelial lining. The granule cells are part of

the diffuse neuroendocrine system. The epithelia rests on a basement membrane which consists of extracellular matrix of connective tissue. Beneath this layer is lamina propria and serous glands. This layer has a layer of smooth muscle and is connected to the C-shaped hyaline cartilage rings that keep the lumen open by resisting the intrathoracic pressure (5, 6).

The respiratory epithelium lines bronchi also and lays on basal membrane. The number goblet cells decreases as bronchi become smaller and are substituted by an increased number of club cells. The function of the club cells is to regulate the immune system and have a role as progenitor cells. There is a layer of smooth muscle cells between the lamina propria and the cartilage. The main bronchi are surrounded by cartilage which becomes more irregular as the bronchi become smaller. This allows the airways to become more elastic (5).

Bronchioles are also lined with respiratory epithelium that is shorter as the epithelia become cuboidal when divided into smaller bronchioles. Club cells are most frequent in bronchioles laying on a smooth muscle layer without serous glands or cartilage (5).

The anatomy of the respiratory zone

The terminal bronchioles divide into respiratory bronchioles having a similar shape as the bronchioles but the difference that their walls are disrupted with sacks like alveoli. The gas exchange begins here, and we can find some types I pneumocytes. Clara cells are the predominant secretory cell type of the respiratory bronchioles and are also found in the smaller conducting bronchioles. The number of mucus-secreting cells diminish in size and frequency distally along the airways. (5, 6).

The wall of the alveolar ducts consists only of alveolar openings without cuboidal epithelium in between and is lined with attenuated squamous alveolar cells. In the proximal parts of the alveolar ducts, the lamina propria contains a smooth muscle cell layer which lacks in the distal parts. There is only a connective tissue consisting of elastic fibres and collagens that support the alveolar ducts (5).

The alveolar wall consists of an epithelial layer composed of type I pneumocytes (the most abundant) and surfactant-producing type II pneumocytes. The lumen of the alveoli is lined with surfactant, which prevents alveolar collapse and plays a role in the immune defence of the lungs. Beneath the epithelium lies a thin layer of connective tissue that supports a dense network of capillaries.

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is used as an umbrella term including two conditions, chronic bronchitis and emphysema. It is the fourth leading cause to death globally (7). In 2021, it caused 3.5 million deaths, approximately 5% of all global deaths. That vast majority of COPD-related deaths in individuals under 70 years occur in low- and middle-income countries. In high-income countries, tobacco smoking is the leading cause of COPD. In low- and middle-income countries, household air pollution is the predominant cause, while tobacco smoking accounts for 30-40% of COPD cases. However, recent research has shown that COPD is the end result of a series of dynamic and cumulative gene–environment interactions over the lifetime that go beyond smoking, can begin early in life and result in varying lung function trajectories (8, 9), of which many of them may lead to COPD as adult. (10). COPD results in a substantial economic and social burden for countries, leading to increased healthcare costs, loss of productivity and reduced quality of life for affected individuals (11).

According to The Global Initiative for COPD (GOLD), the diagnosis of COPD is based on the ratio $FEV_1/FVC < 0.7$ obtained from spirometry. GOLD classification of airflow limitation depends on FEV_1%pred (Table 1), the frequency of exacerbations and the evaluation of breathlessness and symptoms using either the modified Medical Research Council Questionnaire (mMRC) or COPD Assessment Test (CAT) (12, 13).

In the papers included in this thesis, staging and classification of COPD were done according to the GOLD criteria which states that a post-bronchodilator FEV_1/FVC value <0.7 is diagnostic for COPD, as shown in table1, and the severity/staging of COPD was only based on the value of FEV_1%pred.

The Global Initiative for Chronic Obstructive Lung Disease (GOLD)

A non-profit organization established in **1998** in collaboration with National Institutes of Health, USA, National Heart, Lung and Blood Institute and World Health Organization.

First report *Global Strategy for the Diagnosis, Management and prevention of COPD* was launched in **2001.**

GOLD Report was revised in **2006**, **2011** and **2017**, and updated annually thereafter.

GOLD collaborates with health care professionals and public health officials worldwide to raise awareness of COPD and to improve prevention and treatment of this lung disease

The GOLD science Committee (established in 2002) reviews published research on COPD management and prevention to post annual updates on the GOLD website.

GOLD is working to improve the lives of patients with COPD around the world through the development of evidence-based guidelines for COPD management and events such as the annual celebration of World COPD Day (14).

Using a fixed FEV_1/FVC ratio for COPD diagnosis may lead to overestimation of disease prevalence in elderly individuals, as normal aging is associated with a decline in lung function, particularly in FEV_1, leading to a reduced FEV_1/FVC ratio.

An alternative approach is to use the lower limit of normal (LLN), defined as the lower fifth percentile of the FEV_1/FVC ratio, which accounts for age-related changes and reduces overdiagnosis in older populations. However, the application of LLN may increase the risk of underdiagnosis in younger individuals (15-17).

Numerous studies have been conducted to increase the understanding of COPD, and recent reports have revealed that these disorders affect the small airways and that patients with more involvement of the peripheral airways have more symptoms and a more severe disease. Spirometry, the most common type of pulmonary function tests, mostly measures intermediate and large airways. Therefore, there is a need to find new methods that measure the small airways (18).

Table 1. GOLD grades and severity of airflow obstruction in patients with COPD with FEV_1/FVC <0.7 (based on post-bronchodilator FEV_1%predicted) (19)

GOLD stage	Stage	FEV_1 (%pred postbronchdilator)
GOLD 1	Mild	≥80
GOLD 2	Moderate	50-79
GOLD 3	Severe	30-49
GOLD 4	Very Severe	<30

In the 2023 report, GOLD revised the ABCD combined assessment tool to better reflect the clinical impact of exacerbations, independently of the level of symptoms. The update introduced Group E, which merges the former Groups C and D and is defined by a high frequency of exacerbations. This change aims to improve risk stratification and guide treatment more effectively by recognizing exacerbation history as an independent factor. The current assessment framework combines the degree of airflow limitation (GOLD grades 1–4; Table 1) with both symptom burden and exacerbation frequency (GOLD groups A, B, and E) (18).

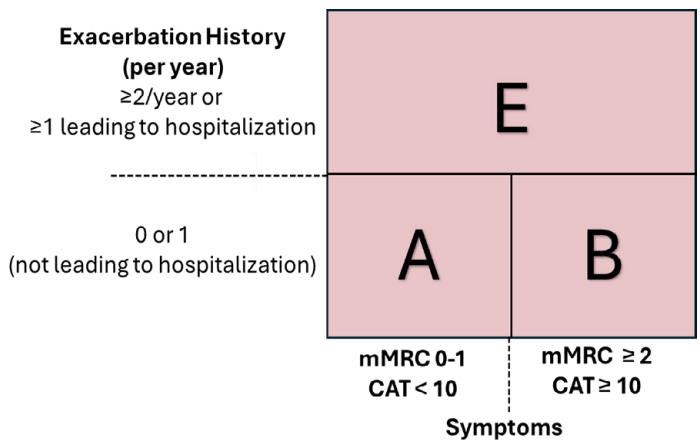

Figure 2 GOLD ABE Assessment Tool (18)

Symptoms in COPD

Symptoms of COPD often develop gradually over an extended period and may go unnoticed until significant lung damage has occurred. One of the most common and often earliest symptoms is coughing, which may be intermittent initially but typically becomes persistent over time. Sputum production is another common symptom, usually accompanying the cough and often more pronounced in the morning. Shortness of breath (dyspnea) is also an early symptom, typically first noticed during physical exertion (e.g., climbing stairs) and gradually worsening to

occur even at rest as the disease progresses. These symptoms are frequently dismissed by patients as signs of poor physical fitness or a prolonged cold.

Many individuals with COPD adapt to their declining respiratory status without realizing it, which contributes to delays in seeking medical attention. Additionally, feelings of shame or denial may lead patients to underreport or ignore their symptoms, further postponing diagnosis and treatment.

As airflow limitation advances, patients often experience reduced exercise tolerance. Moreover, individuals with COPD are more prone to frequent respiratory infections and report increased fatigue, largely due to the elevated work of breathing and impaired gas exchange.

Diagnosis of COPD

COPD is a heterogenous disease characterized by chronic, progressive and partially irreversible airflow obstruction which is often difficult to detect in its early stages. The most common symptoms include dyspnea, cough, sputum production, and recurrent exacerbations. These manifestations result from structural and functional abnormalities in the airways, such as chronic bronchitis and bronchiolitis and/or in the alveoli, as seen as in emphysema, ultimately leading to persistent, and frequently progressive airflow limitation. The clinical characteristics for COPD are summarized in figure 3.

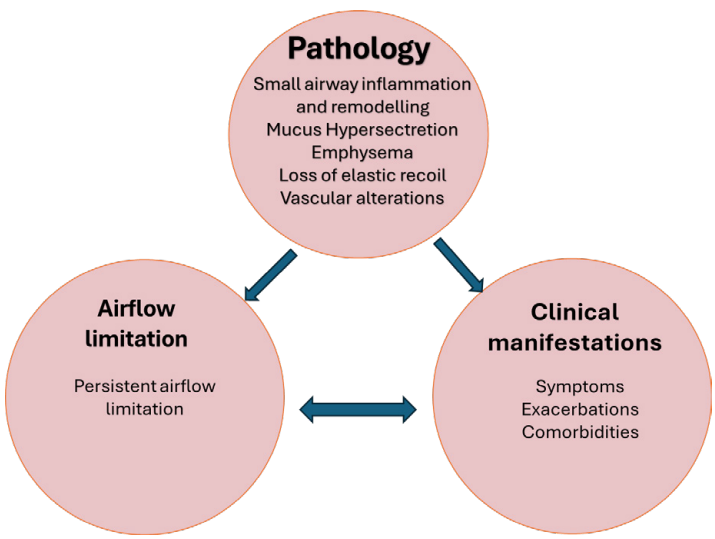

Figure 3. Clinical characteristics of COPD. Key pathological features of COPD drive airflow limitation and clinical outcomes, with worsening lung function increasing symptoms and exacerbation risk, while frequent exacerbations accelerate functional decline. Adapted from GOLD-2021-Chapter-1.pdf

In COPD, chronic airflow limitation arises from a combination of small airway remodelling and parenchymal destruction (emphysema), with the relative contribution of each varying between individuals. Notably, significant structural abnormalities, including emphysema, small airway disease, airway wall thickening, and gas trapping, may be present even in individuals without spirometric evidence of airflow limitation. These pathological changes progress at different rates and may not occur simultaneously (20, 21).

Chronic inflammation plays a central role in COPD pathogenesis by contributing to the narrowing of small airways, loss of alveolar attachments, and reduced lung elastic recoil, all of which promote airway collapse during expiration. Additionally, mucociliary dysfunction impairs the clearance of mucus and inhaled particles, resulting in mucus accumulation and further airway narrowing. The loss of small airways, also referred to as small airway obliteration or the "silent zone", is a hallmark of early COPD and significantly contributes to airflow limitation, even before emphysematous destruction becomes apparent (22). In most patients, COPD is associated with significant concomitant chronic diseases, which increases its morbidity and mortality (23).

Importantly, chronic respiratory symptoms may precede measurable airflow limitation and are associated with increased risk of acute respiratory events. Many smokers with normal spirometry still exhibit structural impairments in the lungs, highlighting the limitations of relying solely on lung function tests for early detection (18, 24).

Pre-COPD and PRISm

Pre-COPD refers to individuals who exhibit respiratory symptoms, with or without structural and/or functional abnormalities, despite having no airflow obstruction, defined as a post-bronchodilator FEV_1/FVC ratio ≥ 0.7, and who may develop persistent airflow limitation over time (10, 25, 26). The term *Preserved Ratio Impaired Spirometry* (PRISm) has been introduced to describe those with a normal FEV_1/FVC ratio but reduced spirometric values. Individuals with Pre-COPD or PRISm are at increased risk of progressing to detectable airflow obstruction, although this is not inevitable in all cases (27, 28).

Airway remodelling in COPD

COPD is also associated with chronic inflammation and progressive structural remodelling of the lungs, but with distinct features and a later onset in life. Key remodelling characteristics include (29, 30):

- Epithelial mucous metaplasia and airway wall fibrosis, especially in small airways.
- Loss of alveolar attachments around the bronchioles, contributing to airflow limitation.
- Increased bronchiolar smooth muscle mass, although less prominent than in asthma (31).
- Emphysema, particularly in more severe cases, characterized by destruction of alveolar walls. Interestingly, focal fibrosis and thickening of alveolar walls may also be observed, despite the underlying tissue destruction (32).
- Hypertrophy of mucus glands, similar in extent to that seen in asthma.
- Pulmonary vascular remodelling, which may include increased wall thickness and vessel number, particularly in advanced disease.

Structural abnormalities of the airways, alveoli, and pulmonary circulation in patients with COPD disrupt the normal ventilation–perfusion ($\dot{V}A/\dot{Q}$) relationships. In COPD, airway narrowing, inflammation, and mucus plugging can cause regions of low ventilation relative to perfusion, while destruction of alveolar walls and capillary beds in emphysema creates areas with high ventilation but poor perfusion. These mismatches represent the primary mechanism underlying impaired pulmonary gas exchange, leading to varying degrees of arterial hypoxemia, with or without hypercapnia (33).

Parenchymal destruction due to emphysema reduces the total alveolar surface area available for gas exchange and decreases the density of the pulmonary capillary network. As the disease progresses, the combined effects of worsening $\dot{V}A/\dot{Q}$ mismatch and reduced diffusing capacity result in progressive deterioration of pulmonary gas exchange (18, 34). Unlike asthma, the inflammation in COPD is dominated by neutrophils, macrophages, and CD8+ T cells, with a remodeling process that primarily affects the small airways and alveolar regions (35). An increase in blood eosinophil counts can be seen in some patients with COPD, especially if there is clinical overlap with asthma (36, 37).

Treatment of COPD

The treatment of COPD is not straightforward as it is a complex disease with varying causes, severity levels and individual patient responses to treatment.

There are two main goals for the treatment of stable COPD, first to reduce symptoms by using medications, improving exercise tolerance and improving health status. The other goal is to reduce risks to prevent disease progression, exacerbations and

mortality. Non-pharmacological and pharmacological treatments are required to achieve an appropriate management of COPD (38).

Non-pharmacological treatments

There is strong evidence that a range of non-pharmacological treatments—including smoking cessation, educational interventions that promote self-management, regular physical activity, and good nutrition—provide clinically meaningful benefits for patients with COPD. In addition to these widely applicable strategies, advanced non-pharmacological interventions such as surgical (e.g., lung volume reduction surgery, lung transplantation) and bronchoscopic procedures (e.g., endobronchial valves or coils) may be considered for selected patients with severe disease. These approaches are complementary to pharmacological treatments and together form the basis of comprehensive COPD management (39).

Among non-pharmacological options, smoking cessation remains the most effective and cost-effective intervention to slow the progression of COPD. Identifying and addressing tobacco dependence is therefore an essential part of disease management. Effective strategies include psychological support, evidence-based pharmacological aids, and therapeutic education, all of which increase the likelihood of sustained abstinence (40, 41).

Pharmacological treatment

Pharmacological therapy for COPD is used to reduce symptoms, the frequency and severity of exacerbations and improve exercise tolerance and health status (18). Although, clinical trials have shown diverse results of the effect of pharmacotherapy on the rate of decline in FEV_1 (42-44).

The most common medications in COPD are short- (SABA)and long-acting beta2-agonists (LABA), short- (SAMA) and long-acting antimuscarinic (anticholinergic) (LAMA) and inhaled corticosteroids (ICS). Other, less commonly used drugs in both general treatment and exacerbation management include phosphodiesterase-4 inhibitors, antibiotics, mucolytics, oral glucocorticoids, and biologics. Vaccinations are also recommended to decrease the risk of exacerbations (45).

Beta$_2$-agonists work by stimulating beta$_2$-adrenergic receptors in airway smooth muscle, leading to relaxation of the muscle through an increase in cyclic AMP. This counters bronchoconstriction. Short-acting beta$_2$-agonists (SABA) begin working within 10–15 minutes, last about 4–6 hours and can still provide additional relief even when used alongside long-acting beta$_2$-agonists (LABA). LABAs have a duration of action of 12 hours or more (18, 46, 47).

Antimuscarinic drugs prevent bronchoconstriction by primarily blocking the action of acetylcholine on M3 muscarinic receptors in airway smooth muscle. Short-acting antimuscarinics (SAMA) also block M2 receptors on nerves, which may

unintentionally promote bronchoconstriction through vagal reflexes. Long-acting muscarinic antagonists (LAMA) bind to M3 receptors for a prolonged period and detach more quickly from M2 receptors, extending their bronchodilator effect while minimizing unwanted M2 blockade (18, 48, 49).

Inhaled corticosteroids (ICS) are used to reduce inflammation in the airways, but the use of them must be in selected patients with COPD, namely those with increased exacerbation risk plus higher level of blood eosinophil count (50), earlier or current history of asthma can benefit from ICS treatment (51). In addition, a combination of LABA and LAMA can give more bronchodilation and less side effects than increasing dosage of just one of them (52, 53).

According to the GOLD proposal (18), the pharmacological management of COPD should be performed according to an individualized assessment of symptoms and exacerbations risk following the group classifications ABE (figure 2) as follows:

Group A: Short-acting bronchodilator (SABA)

Group B: LABA or LAMA

Group E: LABA+LAMA, if blood eosinophil ≥300 consider adding ICS.

Exacerbations

Inclusion criteria for patients with COPD and asthma in this thesis required that they had no airway infection at a specified time prior to the trial. However, it is important to note that both patients with COPD and asthma may experience exacerbations, which are acute worsening or flare-ups of symptoms triggered by factors such as viral or bacterial infections, environmental pollutants, or unknown causes. These episodes often require additional treatment or hospitalization and may lead to an accelerated decline in lung function (18, 45, 54).

Tobacco Smoking

Smoking, Lung Function, and COPD Risk

Tobacco smoking is the leading environmental risk factor for COPD, associated with greater respiratory symptom burden, accelerated decline in FEV_1, increased exacerbation frequency, and higher mortality compared to non-smokers (55). However, only about 20% of smokers develop COPD, indicating that additional mechanisms and modifying factors influence susceptibility. Globally, particularly in low- and middle-income countries, up to half of all COPD cases are attributable to causes other than tobacco, including indoor and outdoor air pollution, childhood respiratory infections, poor nutrition, chronic asthma, impaired lung development, low socioeconomic status, and genetic predisposition (56, 57).

Pathophysiology of Smoking-Induced Lung Damage

Cigarette smoke induces persistent inflammation in the airways and lung parenchyma, characterised by neutrophil, macrophage, and $CD8^+$ T lymphocyte infiltration. These cells release proteolytic enzymes, causing alveolar wall destruction and emphysema. Smoking also promotes mucus hypersecretion, impairs mucociliary clearance, and increases oxidative stress, contributing to chronic bronchitis, small airway disease (SAD), and airflow limitation. SAD, common even in smokers with preserved spirometry, is linked to airway wall thickening, loss of elastic recoil, impaired quality of life, and increased cardiovascular risk (58).

Healthy Smokers and PRISm

Healthy smokers are individuals who smoke, with or without respiratory symptoms, but who maintain normal spirometric lung function. However, advanced imaging, i.e. HRCT and V/P SPECT, and sensitive physiological tests such as respiratory oscillometry have revealed early airway and parenchymal changes in a subset. Similarly, in PRISm, smokers with a preserved FEV_1/FVC ratio but reduced FEV_1, are often accompanied by respiratory symptoms and structural abnormalities (59).

Additional Risk Factors

Beyond active smoking, COPD risk is influenced by genetic susceptibility (e.g., alpha-1 antitrypsin deficiency (60)), sex differences, early-life factors such as low birthweight, and socioeconomic deprivation. Other inhalational exposures (pipes, cigars, waterpipes, marijuana) and passive environmental tobacco smoke also contribute. Prenatal tobacco exposure may induce epigenetic changes that impair lung growth and immune function, increasing COPD susceptibility later in life through gene–environment interactions (61, 62).

Asthma

Asthma is a major chronic pulmonary disease, affecting people of all ages. According to the World Health Organization (WHO), it is estimated that approximately 300 million people around the world are affected by asthma and causing 1000 deaths per day which makes asthma a serious global health problem. (54). Asthma is a heterogenous, chronic inflammatory airway disease characterized by variable airflow obstruction, airway inflammation, airway hyperresponsiveness (AHR), and structural remodeling. These pathological features are interrelated and contribute collectively to the clinical presentation (Figure 4).

Typical symptoms include wheeze, shortness of breath, chest tightness and cough which may vary in frequency and intensity, and can be triggered by viral infections, allergens, pollution and irritants or exercise. Exacerbations may occur, sometimes

be life-threatening including variable expiratory flow limitation and leads sometimes to life-threatening, particularly due to variable expiratory flow limitation (54).

The most well-known and common asthma endotype is T2-high asthma, defined by increased levels of blood and airway eosinophils. Upon activation, eosinophils release broncho active and pro-inflammatory mediators, including T2 cytokines, which sustain airway inflammation and promote the development of airway hyperresponsiveness (63). However, asthma also includes non-Type-2 phenotypes, which are often associated with more severe disease manifestations and reduced responsiveness to standard treatments (54).

The Global Initiative for Asthma (GINA)

Established in **1993** through a collaboration between the National Heart, Lung, and Blood Institute, the National Institutes of Health, USA, and the World Health Organization.

Guided by committees of leading asthma experts and patient representatives worldwide.

Main objectives:

-Increase awareness of asthma and its public health impact

-Reduce morbidity and mortality

-Identify factors contributing to the rising prevalence

-Explore links between asthma and environmental factors.

-Improve asthma management

-Ensure access to effective therapies (54).

Diagnosis of asthma

According to the Global Initiative for Asthma (GINA) guidelines, the diagnosis of asthma is made by the presence of variable respiratory symptoms and a confirmed variable expiratory flow limitation with objective pulmonary function tests (PFTs) (64).

Commonly used PFTs include spirometry, peak expiratory flow (PEF), and bronchoprovocation testing. A reversibility test, assessing the response to a bronchodilator, is often performed to support the diagnosis by demonstrating reversible air flow limitation.

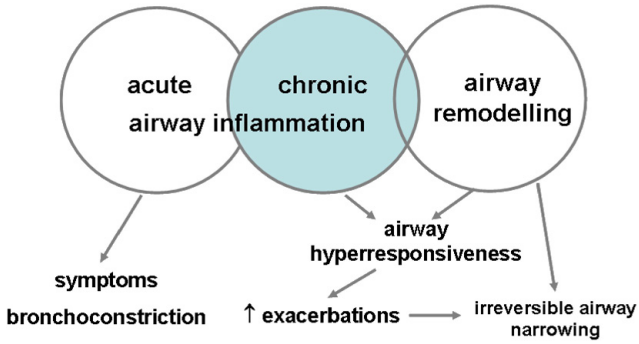

Figure 4. Schematic relationship among asthma characteristics (63)

Airway remodelling in Asthma

Asthma is a chronic inflammatory disease of the airways characterized by early-onset structural remodelling. Several of these alterations begin in childhood, even before significant loss of lung function. Key remodelling features in asthma include:

- Epithelial fragility, with the airway epithelium being more easily damaged compared to COPD.
- Thickening of the reticular basement membrane (RBM), which is a hallmark of asthmatic remodelling.
- Marked hypertrophy and hyperplasia of bronchial smooth muscle, contributing to airway narrowing.
- Absence of emphysema in non-smoking asthmatics.
- Hypertrophy of submucosal mucus-secreting glands, which is present and comparable to that in COPD.
- Increased vascularity within the bronchial wall, particularly in patients with severe, corticosteroid-dependent asthma (35).

Small airways in asthma

Asthma has been considered as a disease that predominantly involves the large airways previously. This concept has been challenged lately with increasing evidence showing that abnormalities in the small airways also contribute to the clinical expression of asthma. The small airways can be affected by inflammation, remodeling, and changes in the surrounding tissue, all contributing to small-airways dysfunction. This dysfunction is associated with worsened asthma control with increases in symptoms, exacerbations, severity of exercise induced bronchoconstriction and late phase allergic responses (65-71).

Lung function tests

Respiratory Oscillometry

Conventional lung function measures are effort dependent and may be insensitive to changes, particularly in the peripheral airways where small airway disease may originate or manifest. Respiratory oscillometry, also known as the Forced oscillation technique (FOT), is a non-invasive, objective and effort-independent method for assessing the mechanical properties of the respiratory system. It measures impedance in the upper and intrathoracic airways, lung tissue, and chest wall by applying external pressure waves generated by a loudspeaker at the mouth during tidal breathing (72). First described by Dubois et al. in 1956, oscillometry has gained widespread use in clinical and research settings, especially over the past 10-15 years (73). Several oscillometry devices are commercially available, with slight variations in data sampling and analysis. While their fundamental principles remain consistent, measurement techniques may still differ (72, 74).

Impedance is a key parameter in oscillometry, representing the total opposition to airflow in the respiratory system. It consists of two components: resistance and reactance.

1. **Resistance**: represents the overall resistance to airflow in the respiratory system, including the airways, lung tissue, and chest wall. It is determined by the difference in amplitude between pressure and flow oscillations.
2. **Reactance** describes the dynamic behaviour of the respiratory system, influenced by inertance (which resists changes in airflow) and elastance (which represents lung recoil). It is quantified as the phase shift between pressure and flow oscillations.

Inertance represents the force that resists the initiation of airflow, causing pressure oscillations to precede the flow. Elastance, on the other hand, reflects the restoring force of the lung and is generated after some airflow has already occurred, acting as a form of "back pressure" and therefore lagging the flow.

As a result, resistance is measured as the component of the pressure-flow relationship that is in-phase with the flow, while reactance corresponds to the component that is out-of-phase with the flow. According to established norms, inertial forces are assigned a positive sign, while elastic forces are assigned a negative sign.

In oscillometry, external pressure waves of different frequencies are superimposed on normal tidal breathing to assess airflow resistance and compliance. Low-frequency waves penetrate deeper and reflecting total respiratory system resistance. High-frequency waves, however, are absorbed earlier in the bronchial tree,

providing insights into central airway properties. Thus, the frequency dependence of resistance helps differentiate between central and peripheral airway obstruction (figure 5).

A key advantage of respiratory oscillometry is that it requires minimal patient effort, making it particularly useful in children, elderly patients, and individuals with limited ability to perform forced breathing manoeuvres, such as those required in spirometry. Oscillometry outperforms spirometry in detecting regional heterogeneities, enabling differentiation between large and small airway obstruction. This facilitates early detection of subtle small airway dysfunction and enhances long-term disease monitoring (72, 75).

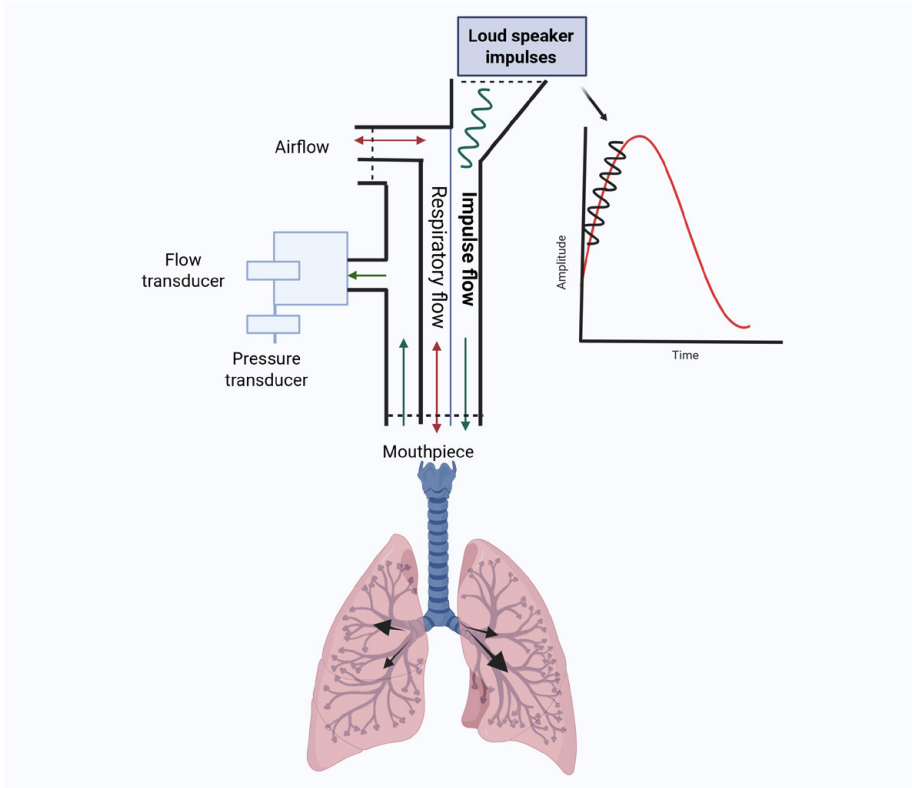

Figure 5. A schematic illustration of the measure of respiratory oscillometry. A loudspeaker generates pressure impulses that are superimposed on the patient's tidal breathing through a mouthpiece. The resulting changes in airflow and pressure are measured by a flow and pressure transducer system. These measurements allow for the calculation of respiratory impedance, including airway resistance (R) and reactance (X), across various frequencies. Respiratory oscillometry enables non-invasive assessment of both central and peripheral airway function during tidal breathing. Image created partly in Biorender https://BioRender.com

Key-parameters of respiratory oscillometry

There are six key parameters obtained from measurements of resistance and reactance using respiratory oscillometry (Figure 6):

Resistance at low frequency (R5): Reflects the resistance of the entire respiratory system.

Resistance at high frequency (R19 or R20): Primarily reflects the resistance of the central (large) airways.

Frequency dependence of resistance (R5–R19/20): Represents the difference between low- and high-frequency resistance and reflects resistance in the peripheral airways.

Reactance at low frequency *(X5):* Reflects the elastic properties of the lungs and chest wall. Negative X5 values indicate reduced elastic recoil. In emphysema, increased compliance leads to more negative X5 values.

Resonant Frequency (Fres): The frequency at which the elastic and inertial forces of the respiratory system are equal. Shifts in Fres can indicate changes in lung mechanics.

Reactance Area (AX): The integrated area of reactance between X5 and Fres. It reflects the extent of airway obstruction and ventilation heterogeneity.

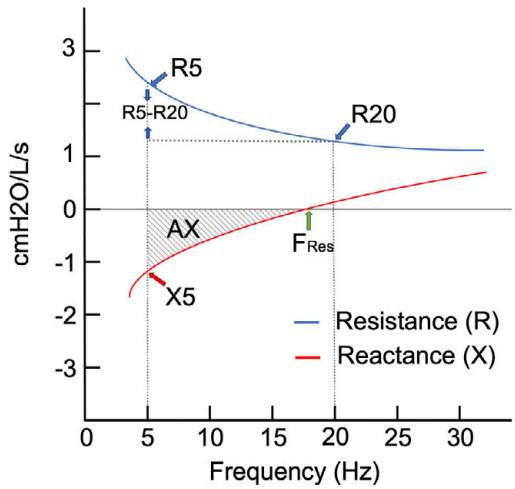

Figure 6. Illustration of oscillometry technique indices locations in resistance and reactance curves. The red line is the reactance line, and oscillation at 5 Hz is (X5). When reactance pressure reaches 0, this is the point of resonant frequency (Fres). The area under the curve between X5 and Fres is the area of reactance (AX). The blue line is the resistance line, and resistance at 5 Hz is the total lung resistance (R5). Resistance at 20 Hz is the large airways resistance (R20). The difference in resistance between R5 and R20 is considered as the small airways resistance (R5-R20) (71). In some devices odd numbers of frequencies are used, i.e. 5,11,19 Hz. Therefore, R19 and R5-R19 are used instead of R20 and R5-R20.

Expiratory Flow Limitation

Expiratory flow limitation (EFL) occurs when flow cannot increase regardless of increasing expiratory effort. It is a common finding among patients with COPD and indicates increased disease severity. EFL is commonly detected using body plethysmography shown as prominent expiratory loops during tidal breathing. This results in incomplete emptying of the lungs causing air trapping and hyperinflation. EFL has been identified as a key determinant of disease severity, prognosis, and response to therapy in obstructive lung diseases (76-79).

Mannitol challenge test

Airway hyperresponsiveness (AHR) is often present in patients with asthma and occurs also in COPD (80, 81). AHR can be assessed using direct airway challenge tests, such as methacholine or histamine, or indirect methods, such as mannitol inhalation or exercise (82-84) (Figure 7).

Inhalation of mannitol causes dehydration and an increased osmolarity of the airway surface in a way that mimics the effect of exercise. Indirect challenge tests trigger airway narrowing through the release of endogenous mediators or cytokines that provoke contraction of the airway smooth muscles.

Mannitol responsiveness has been linked to eosinophil- and mast cell–mediated airway inflammation. A positive mannitol test is highly predictive of a favorable response to inhaled corticosteroids (81) and is more associated with increased involvement of the peripheral airways (85). In asthma, individuals who tested positive to mannitol have shown more peripheral airway involvement at baseline, supporting the idea peripheral airway pathology is an important predictor of AHR (69).

In COPD, certain inflammatory phenotypes are associated with airway eosinophilia. Identifying sputum eosinophils has clinical value in predicting corticosteroid responsiveness (86). Accordingly, hyperresponsiveness to mannitol in COPD is associated with biomarkers of airway inflammation (87, 88). These findings underscore the importance of assessing small airway function when evaluating AHR to mannitol.

FeNO

Nitric oxide (NO), produced in the airways by nitric oxide synthase enzymes, is detectable in the exhaled breath of all individuals (89). Fractional exhaled nitric oxide (FeNO) serves as a non-invasive biomarker of eosinophilic airway inflammation and in predicting the response to inhaled mannitol (90, 91).

According to ATS guidelines, FeNO can predict corticosteroid responsiveness and monitor airway inflammation, correlating with other inflammatory markers and aiding treatment decisions in severe asthma (54). The ERS defines FeNO < 25 ppb as indicating a low likelihood of eosinophilic inflammation, and > 50 ppb as suggesting a high probability of corticosteroid responsiveness (92).

While FeNO is commonly elevated in asthma, findings in COPD are inconsistent, likely due to the NO-lowering effect of smoking. Nonetheless, higher FeNO levels have been reported in severe, unstable COPD compared with stable disease, suggesting potential clinical relevance in selected COPD phenotypes (88, 93).

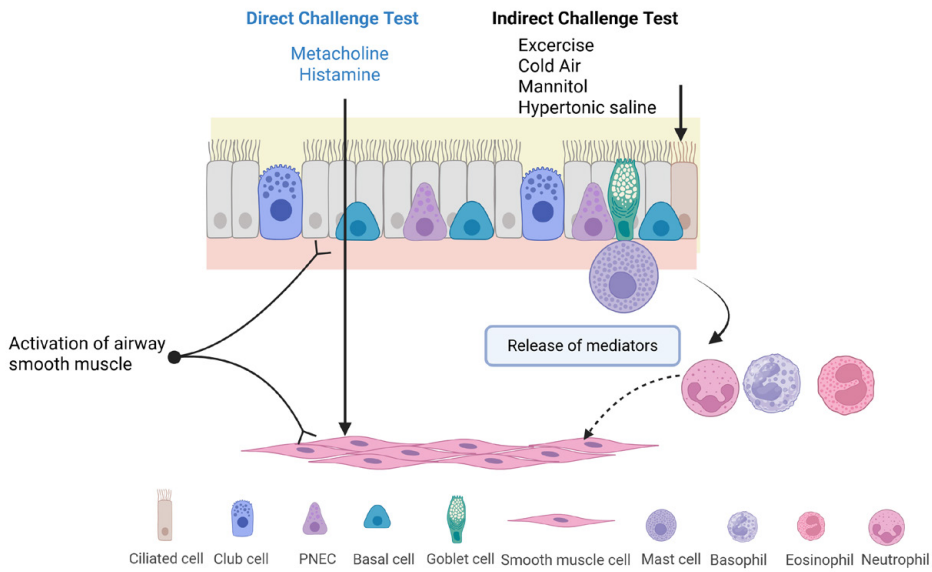

Figure 7. Direct and indirect challenge tests. Direct challenge tests (e.g., methacholine or histamine) act directly on airway smooth muscle receptors to induce bronchoconstriction, independent of inflammatory cell activation. In contrast, indirect challenge tests (e.g., mannitol, exercise, cold air, hypertonic saline) trigger bronchoconstriction by stimulating inflammatory cells such as mast cells and eosinophils to release mediators (e.g., histamine, leukotrienes), which subsequently lead to airway smooth muscle contraction. The indirect response reflects the presence of airway inflammation and is more specific to asthma. Image created partly in Biorender https://BioRender.com

Ventilation/Perfusion Single Photon Emission Computed Tomography (V/P SPECT)

V/P SPECT was first introduced in the late 1980s and 1990s, providing 3D images that were superior to the earlier planar V/P scintigraphy in detecting ventilation and perfusion abnormalities for the diagnosis of pulmonary embolism (PE). However, image acquisition initially took a long time, approximately 40 minutes. In 2001, the introduction of dual-head gamma cameras improved the feasibility of V/P SPECT by reducing acquisition time and enhancing the resolution and reconstruction of the acquired images. V/P SPECT has applications beyond the diagnostic work-up of PE, as it provides evidence for other pathologies such as COPD, pneumonia and left heart failure (94-96).

Over the past decade, hybrid imaging systems have become increasingly common. Combining morphological imaging with computed tomography (CT) and functional imaging with SPECT improves the specificity for diagnosing pulmonary embolism (PE) (97, 98). This approach also provides additional benefits in patients with COPD, who are at increased risk of lung cancer and other comorbidities (99). Low-dose CT enables the visualization of pulmonary abnormalities such as emphysema, other parenchymal changes, or extrinsic vascular compression, thereby adding diagnostic value when interpreting perfusion defects (96, 99, 100).

V/P SPECT is an imaging technique that maps and quantifies the distribution of air and blood in the lungs using radioactive tracers. Ventilation imaging is performed using technetium-99m (99mTc)-labelled aerosols, such as Technegas. Technegas consists of ultra-fine carbon particles with a diameter of approximately 0.005-0.2 μm. These particles are generated by heating at extremely high temperatures and then suspending them in a gas. Due to their small size, Technegas particles can penetrate deep in the airways, reaching the peripheral regions where they are deposited in the bronchioles and alveoli primarily by diffusion. Technegas should be used within 10min of generation as the particles tend to aggregate over time (96).

During the procedure, the patient-lying in supine position-, inhales 99mTc-Technegas through a hose connected to a mouthpiece while wearing a nose clip. The scan is conducted with the patient in the same supine position to ensure even aerosol distribution and minimize the effects of gravity. Ventilation imaging takes approximately 10 minutes, after which ^{99m}Tc-macroaggregated albumin (MAA) is administered intravenously for lung perfusion imaging, which takes about 5 minutes. The ^{99m}Tc-MAA particles will be transported with the blood and reach the pulmonary arteries and capillaries through the right atrium and ventricle and further to systemic circulation. It is crucial that the subject maintains the same position during both ventilation and perfusion imaging because the resulting images are merged to detect mismatches between ventilation and perfusion (ref).

The administered activities followed the recommended by the European Association of Nuclear Medicine (EANM) in our study. An approximate dose of 30 MBq of Technegas was used for the ventilation scan, and 120–140 MBq of ^{99m}Tc-MAA for the perfusion scan. The effective radiation dose from V/P SPECT is approximately 2 mSv. Technetium-99m (^{99m}Tc) has a half-life of six hours and emits gamma radiation with a photopeak energy of 140 keV.

What is an aerosol?

The word aerosol is derived from the Greek aer (air) and Latin solutio (solution). An aerosol is a suspension of fine solid or liquid particles in a gas. When a radioactive tracer is attached to an aerosol, it is called a radioaerosol. Radioaerosols can consist of either solid or liquid particles. Particle size is a key factor influencing how aerosols deposit within the lungs.

Different radiotracers for ventilation SPECT

Both inert gases such as ^{133}Xe, ^{81m}Kr and radiolabelled aerosols ^{99m}Tc-DTPA and ^{99m}Tc-Technegas can be used for mapping of the regional ventilation.

Historically, ^{133}Xe was used for ventilation imaging. However, its low photon energy (81 keV) results in significant signal attenuation, particularly affecting the anterior lung regions when posterior images are acquired. Additionally, its use in the diagnosis of pulmonary embolism (PE) is limited due to restricted availability in Europe.

^{81m}Kr is an inert noble gas used in ventilation imaging that does not produce artefacts related to central airway deposition. One of its key advantages is the ability to perform simultaneous ventilation and perfusion imaging, as its gamma energy (193 keV) is higher than that of ^{99m}Tc (140 keV), which is commonly used for perfusion scans. ^{81m}Kr has an extremely short half-life of 13 seconds, meaning that its elimination from the alveoli occurs primarily through radioactive decay, rather than exhalation. When delivered continuously, the regional activity at steady state accurately reflects regional ventilation. However, in patients with COPD, the time required to reach steady state may be prolonged, potentially limiting its effectiveness in such cases. Additionally, clinical use of ^{81m}Kr is restricted by logistical and economic factors. The parent isotope, ^{81}Rb, has a short half-life of only 4.6 hours, necessitating daily delivery of a costly, cyclotron-produced generator, which limits routine availability.

^{99m}Tc-DTPA is a commonly used radiolabeled aerosol with a particle size of 1.2-2µm. ^{99m}Tc-DTPA is cleared from the alveolar region by transepithelial diffusion and allows the study of alveolo-capillary permeability. Resorbed ^{99m}Tc-DTPA is

excreted by glomerular filtration in the kidneys. The size of particles causes central deposition of the particles often encountered in COPD.

Technegas® is an aerosol consisting of ^{99m}Tc-labelled solid graphite hydrophobic particles, with diameters ranging from approximately 0.005 to 0.2 μm. Developed in Australia, it has been widely used in clinical practice since 1986. Due to their extremely small size, the particles behave almost like a gas, enabling the aerosol to reach the peripheral regions of the lungs. Deposition occurs primarily in the bronchioles and alveoli via diffusion. Compared to ^{99m}Tc-DTPA, Technegas® significantly reduces the issue of central airway deposition, thereby improving image quality and facilitating interpretation, particularly in patients with COPD (101).

Technegas production

The commercially available Technegas generator operates using ^{99m}Tc-sodium pertechnetate eluted from a ^{99}Mo/^{99m}Tc generator. A graphite crucible, with a maximum capacity of 0.17 mL, is used for preparation. After adding the ^{99m}Tc solution, the simmering phase begins, during which the liquid is evaporated at 70°C for 6 minutes in an ultrapure argon environment. This is followed by the combustion phase, where an alternating current arc ablates the graphite and ^{99m}Tc, reaching temperatures of approximately 2,750°C for 15 seconds. This process produces ultrafine carbon nanoparticles. Operation of the Technegas generator is straightforward when the stepwise protocol is carefully followed (102). The particles tend to grow by aggregation and should, therefore, be used within 10 min after generation.

Obstruction grade

The penetration index for Technegas may be used to evaluate the grade of COPD severity by assessing its distribution pattern in the airways. The degree of obstruction is then visually evaluated according on a 4-point scale as follows (Figure 8) (103, 104):

Grade 0 Normal: Even distribution of Technegas with good penetration into the peripheral airways and no gas accumulation (hotspots).

Grade 1 Mild airway obstruction: Slightly uneven distribution with some deposition in small and intermediate airways. A few areas in the peripheral airways show reduced penetration.

Grade 2 Moderate airway obstruction: Deposition of Technegas in the intermediate and large airways with diminished peripheral penetration and maximum accumulation in the central part of the lungs.

Grade 3 Severe airway obstruction: Central deposition in the large airways and severely diminished penetration of Technegas, resulting in extensive areas with reduced or abolished function.

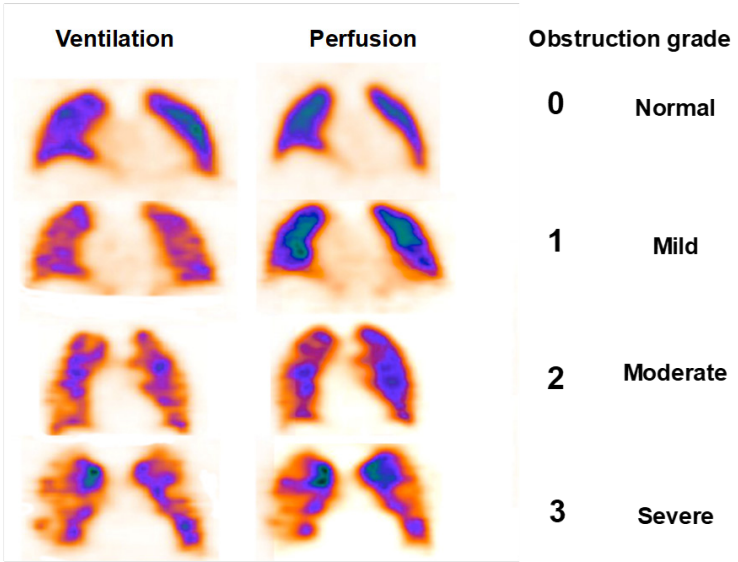

Figure 8. Obstruction grade of COPD. Grade 0 Normal: Even distribution of Technegas with good penetration into the peripheral airways and no gas accumulation (hotspots). Grade 1 Mild airway obstruction: Slightly uneven distribution with some deposition in small and intermediate airways. A few areas in the peripheral airways show reduced penetration. Grade 2 Moderate airway obstruction: Deposition of Technegas in the intermediate and large airways with diminished peripheral penetration and maximum accumulation in the central part of the lungs. Grade 3 Severe airway obstruction: Central deposition in the large airways and severely diminished penetration of Technegas, resulting in extensive areas with reduced or abolished function (103).

Assessment of preserved lung function

Total preserved lung function (PLF) can be quantified semi-quantitatively and described in % of the total estimated lung volume. The extent of matched, mismatched, and reverse mismatched defects is expressed as a percentage of the total lung volume. The sum of these is used to estimate the extent of the total reduction in lung function. An area is considered to have fully preserved lung function if both ventilation and perfusion were normal and aligned (96, 104).

The assessment of preserved lung function and grading airway obstruction, using V/P SPECT, have been explored in previous studies and identified as a new tool to diagnose COPD and grade its severity. These measures have shown good correlations with both spirometric lung function, and the extent of emphysema as shown on HRCT (103, 105, 106).

Aims

The overall aim of the thesis was to enhance our understanding of the use of novel methods which are less explored such as respiratory oscillometry and V/P SPECT in comparison with comprehensive pulmonary function tests.

Paper I

The primary aim of the study was to investigate the difference between inspiratory and expiratory resistance and reactance as a measure of expiratory flow limitation (EFL) in patients with asthma, COPD, healthy smokers and healthy never-smokers using respiratory oscillometry. We also wanted to investigate the associations between EFL and symptoms and quality of life using standardized questionnaires

In a subgroup, we aimed to investigate EFL in association with air trapping measured as the ratio of residual volume/ Total lung capacity (RV/TLC) by body plethysmography. Moreover, we wanted to compare the with-in-breathe measurements from two respiratory oscillometry devices.

Paper II

The primary aim of this study was to explore airway hyperresponsiveness to mannitol in asthma and COPD in respect to inspiratory versus expiratory resistance and reactance. A secondary aim was to investigate inflammation markers, such as fractional exhaled nitric oxide (FeNO), blood eosinophils, allergen sensitization and symptom scores in relationship to AHR to mannitol

Paper III

The main purpose of the study was to thoroughly examine the appearance of airflow obstruction using different advanced lung function measurements in never-smokers with COPD compared to a normal population of healthy never-smokers.

Paper IV

The aim of this study was to evaluate whether V/P SPECT can quantify the percentage of preserved lung function (%PLF) and determine obstruction grade (OG) in healthy young and older smokers whose lung impairments are not detectable by conventional methods. The focus was on assessing the ability of V/P SPECT to identify early functional changes in smokers with normal spirometry.

Methods

Study population and study design

Paper I and II

Data for study I and II were collected from the BREATHE cohort (107), a cross-sectional study of real-life patients with asthma and/or COPD in Denmark and Sweden. The participants were either newly referred patients, patients at regular controls at the primary care clinic, or subjects recruited through web-based advertising. However, only individuals from the two sites in Skane, Sweden, where included in PaperI and II as it was a different type of respiratory oscillometry device used at Danish sites. The exclusion criteria were malignant (lung) disease or any other lung disease of clinical significance, pregnancy, lower airway infection or exacerbation requiring prednisolone or antibiotics within six weeks. All subjects were asked to refrain from short-acting β2agonists (SABA) for at least 6hours, inhaled corticosteroids and long-acting β2agonists for at least 12 hours prior the examination. Healthy current/former smokers were defined as individuals with a smoking history of >5 packyears without self-reported respiratory symptoms and preserved spirometry.

In paper I, 471 subjects were included (311 with asthma, 96 with COPD, 30 healthy smokers and 34 healthy never-smokers). All subjects underwent comprehensive respiratory oscillometry and spirometry in the given order. A subgroup performed body plethysmography and additional measures of respiratory oscillometry using another device (impulse oscillometry, IOS) for comparison. All subjects (exclusive healthy never-smokers) answered questionnaires regarding symptoms and quality of life.

We conducted the mannitol challenge test on 292 subjects (238 with asthma, 25 with COPD, 14 healthy smokers and 15 healthy never-smokers). All subjects had a FEV_1 >70% of predicted normal. Fractional exhaled nitric oxide (FeNO) was measured prior to the test. Spirometry was measured before the test and repeated after each dose of mannitol. Respiratory oscillometry was measured prior to the test and after the last dose of mannitol. All subjects (except healthy never-smokers) answered questionnaires regarding symptoms and quality of life.

Paper III

We investigated never-smokers with airway obstruction, defined as an FEV_1/FVC ratio below 0.70, identified from the BRONCHO-SCAPIS study. These individuals were selected for detailed lung function assessments. Totally 155 subjects were included (35 never-smokers with COPD, 30 never-smokers with lower lung function, 26 COPD never-smokers with large lungs, 41 healthy never-smokers and 23 healthy smokers). The classification of the groups of never-smokers with low FEV_1/FVC ratio followed strict criteria (Table3). All participants underwent comprehensive lung function tests, including spirometry, body plethysmography, diffusion capacity, and respiratory oscillometry (IOS). All subjects answered the CAT questionnaire to evaluate their symptoms.

Paper IV

We had totally 125 subjects consisting of five different groups, young smokers and never-smokers, older smokers and never-smokers and a group of subjects with COPD. All subjects performed measurements of spirometry, diffusion capacity, body plethysmography and IOS followed by V/P SPECT. All subjects answered the CCQ questionnaire regarding symptoms.

Pulmonary function tests

Spirometry

Spirometry manoeuvres were performed according to the recommended standards by the American Thoracis Society/ European Thoracis Society (108, 109). Reference values were established using GLI (110).

Spirometry is a fundamental method of assessing lung function by measuring the volume of air that the patient can expel from the lungs after a maximal inspiration. The indices derived from this forced exhaled maneuver have become the most accurate and reliable way of supporting a diagnosis of COPD. When these values are compared with predicted normal values determined based on age, height, sex, and ethnicity, a measure of the severity of airway obstruction can be determined. It is on these values that COPD guidelines around the world base the assessment of mild, moderate, and severe disease levels.

Body Plethysmography

Static lung volumes were measured using body plethysmography (Paper I, III-IV) (Masterscreen Body, Erich Jaeger GmbH) according to manufacturer's instructions and ERS/ATS recommendations (111). Reference values were established using GLI (110).

The maneuver is performed with the patient seated upright in a closed box with an air-tight seal. The patient breathes through a mouthpiece while wearing a nose clip and supports their cheeks with their hands. This cheek support minimizes the movement of soft tissues around the mouth during respiratory efforts, particularly when the shutter is closed, ensuring accurate measurement of thoracic volume changes (Figure 10).

This procedure provides three primary measurements:

1. **Specific airway resistance (sRAW):** an assessment of total airway obstruction and indicates volume-and resistance-dependent work of breathing needed to generate a reference flow rate of L/s. Though sRAW does not differentiate between central and peripheral airway components. (Figure 9)
2. **Thoracic gas volume (TGV):** representing the volume of gas within the thoracic cavity.
3. **Lung volume measurements:** including both static and dynamic lung volumes.

Measurement of specific airway resistance (sRAW) is performed at the beginning of the maneuver, with the patient breathing at a frequency of approximately 20 breaths per minute, until 5 to 10 technically acceptable breaths are recorded. Each breath is plotted with the shift volume on the X-axis and the flow on the Y-axis.

The measurement of thoracic gas volume (TGV) is based on **Boyle's law**, which states that pressure and volume are inversely related under isothermal conditions. Following the specific airway resistance (sRAW) measurement, the patient performs gentle panting against a temporary occlusion of the mouthpiece.

During this maneuver, the relationship between pressure changes within the plethysmograph chamber and those at the mouthpiece reflects changes in TGV. Since the amount of gas in the thoracic cavity remains constant, volume changes will result in corresponding pressure changes, allowing TGV to be calculated.

Functional residual capacity (FRC) can then be derived from TGV by subtracting the volume of the apparatus dead space and any inspired air above the resting end-expiratory lung volume at the moment of occlusion.

Airway resistance (Raw) is calculated as the ratio of sRAW and FRC. It is a measure of flow resistance, i.e. ratio of alveolar driving pressure minus mouth pressure to flow rate. Raw indicates the alveolar pressure needed to generate a reference flow rate of 1 L/s.

After the TGV maneuver, the patient is asked to exhale slowly all air in their lungs followed by a spirometry maneuver. Residual volume (RV) is calculated as the difference between FRC and expiratory reserve volume (ERV). Total lung capacity (TLC) is calculated as the sum of maximal vital capacity (VC) and RV (figure6) (112).

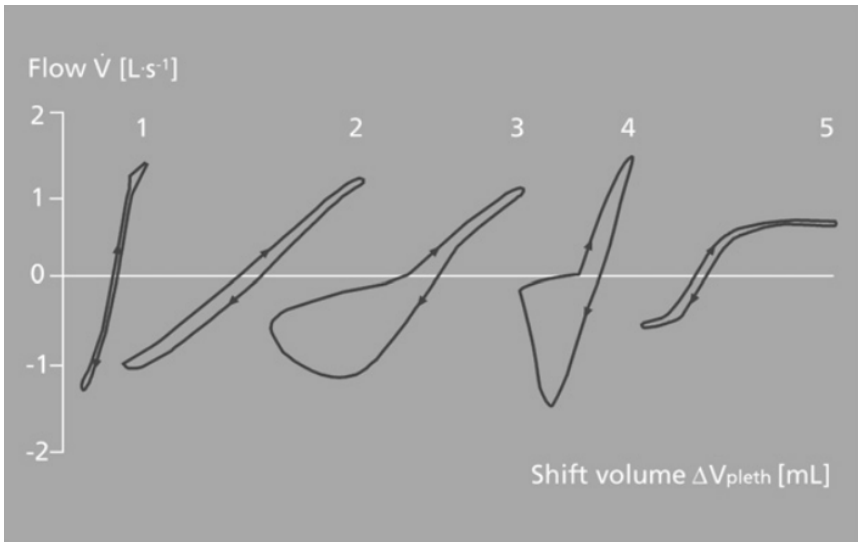

Figure 9. Schematic representation of specific resistance loops (sRaw) in a normal subject (1), a subject with chronic airflow resistance (2), a subject with chronic airflow obstruction (3), a subject with obesity or diaphragmatic paralyis (4), and a subject with upper airway obstruction (5) (112).

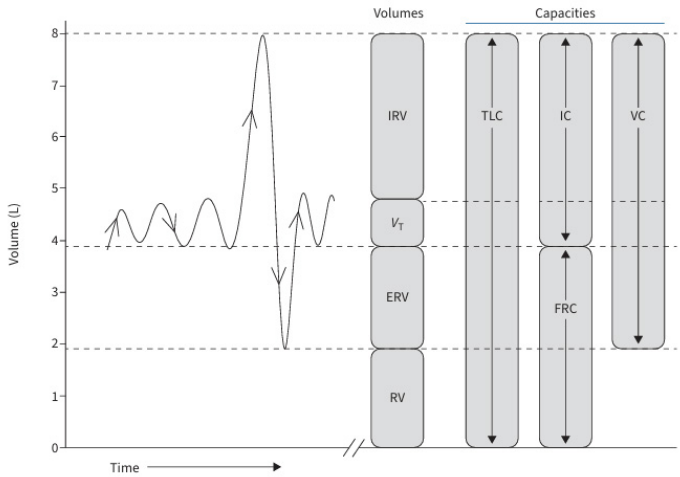

Figure10. Static lung volumes measured in body plethysmography. Static lung volumes and capacities thhat can be measured/calculated using body plethysmography based on a volume-time spirogram. IRV: inspiratory reseve volume; V_T: tidal volume; ERV: expiratory reserve volume; RV: residual volume; TLC: total lung capacity; IC: inspiratory capacity; FRC: functional residual capacity; VC: vital capacity (111).

Diffusion Capacity

The diffusion capacity test for carbon monoxide (DLCO) is a well-established PFT and has been used in clinical settings for more than 60 years (113). DLCO provides essential information beyond spirometry by assessing the lung´s ability to transfer gas from inhaled air to the pulmonary capillaries. In COPD, DLCO is particularly valuable as reduced values have been associated with a higher symptom burden, impaired exercise capacity, increased risk of exacerbations and higher mortality.

The reference values established by Quanjer were previously used for the interpretation of DLCO, but in 2017 new reference values were published by The Global Lung Function Initiative (GLI) using lower limit of normal (LLN) the 5th percentile to define impaired DLCO (114, 115).

The diffusion capacity of carbon monoxide (DLCO) test is performed using a two-valve system connected to a mouthpiece, with the patient wearing a nose clip. The patient first exhales fully to residual volume (RV) and then rapidly inhales a test gas mixture containing 0.3% carbon monoxide (CO) and 0.3% methane (CH_4) (previously, helium was used). The patient then holds their breath for approximately 10 seconds at total lung capacity (TLC) before exhaling.

During exhalation, the first portion of expired gas is discarded (to remove dead space air), while the end-expiratory portion is analyzed. The fraction of methane in the inspired and expired gas is measured using the methane dilution technique,

allowing for the calculation of alveolar volume (VA). The diffusion capacity of the lungs is then determined based on the uptake of CO, as CO readily binds to hemoglobin in red blood cells.

Respiratory Oscillometry

Resmon Pro Full and Impulse Oscillometry System (IOS) are two different variants of forced oscillation technique (FOT) used to assess the mechanical properties of the respiratory system. It applies external pressure waves, generated by a loudspeaker, at the mouth opening during tidal breathing. The relationship between flow oscillations and pressure oscillations is used to calculate the impedance of the respiratory system, which includes the upper and intrathoracic airways, lung tissue, and chest wall. Reference values were established by Oostveen et al (116).

Impedance consists of two components:

1. Resistance (R): Represents airway resistance.
2. Reactance (X): Represents the combined effects of inertance (the resistance to changes in airflow) and elastance (the elastic recoil of the lungs) (72).

Low-frequency impulses (5 Hz) penetrate deeply into the lungs and are therefore used to assess the total resistance of the respiratory system. In contrast, high-frequency impulses (19–20 Hz) are attenuated earlier in the bronchial tree and primarily reflect the properties of the central airways. Peripheral airway resistance is estimated as the difference between R5 and R19 (or R20) (Figure 6).

Resmon Pro Full

In paper I and II, a commercial respiratory oscillometry device called Resmon Pro Full (Restech srl, Milan, Italy) was used. It applies a composite of sinusoidal pressure waves with frequencies of 5, 11 and 19 Hz superimposed over tidal breathing and analyses reflected signals. Resmon Pro uses prime signals to avoid harmonic interference between the individual sine waves that make the composite signal.

Impulse Oscillometry System (IOS)

IOS (MasterScreen, Erich Jaeger GmbH, Wurzburg, Germany) was performed in a subgroup in paper I and in papers III-IV. The measurement is performed with the patient sitting upright, breathing through a mouthpiece, wearing a nose clip, and holding their cheeks to minimize upper airway shunting which can impact the values of the test. In adults a measurement takes 30 seconds and is repeated three times, with the mean value calculated. In IOS, square-shaped impulses at 5 Hz are introduced, and additional frequencies are extracted using Fourier analysis.

Mannitol challenge test

A mannitol powder kit (Aridol®, Pharmaxis, Frenchs Forest, Australia), consisting of a handheld dry powder inhaler and pre-filled mannitol capsules, was used. Gradually increasing doses were administered in eight steps according to the manufacturer's instructions, with a maximum cumulative dose of 635 mg. FEV_1 was measured before the test as a baseline value and repeated one minute after each dose step. The challenge was considered positive if FEV_1 decreased by ≥15% at any point compared to baseline or if FEV_1 decreased more than 10% between consecutive doses.

FeNO

Fractional exhaled nitric oxide (FeNO) was measured prior to the mannitol challenge. For the FeNO measurement, subjects inhaled nitric oxide–depleted ambient air and exhaled into a mouthpiece at a constant flow rate of 50 mL/s using a nitric oxide analyzer (Medisoft FENO+, Sorinnes, Belgium). The test was performed three times, and the average of the three measurements was used (117).

Blood analyses

Blood Eosinophil Counts

Blood eosinophil counts (BEC) and sputum are biologic inflammatory markers that link asthma to a specific pathophysiologic phenotype (118), and responsiveness to specific medications. The GOLD 2019 report recommended using BEC as part of a precision strategy to identify the most suitable patients for ICS treatment. Retrospective studies have reported that elevated blood eosinophil count was associated with increased asthma exacerbations in concurrent and future years in general cohorts of patients with persistent asthma (119).

Atopy

The majority of participants recruited in Papers I and II were part of the BREATHE study and consisted of patients with asthma. Atopy is a known risk factor for adult-onset asthma. To assess atopic status, screening for allergen sensitization was performed using Phadiatop and total immunoglobulin E (IgE) levels measured from venous blood samples (120). The specific IgE test was considered positive if at least

one of the IgE levels >0.35kU/L and a positive Phadiatop test. Atopy was defined as positive specific IgE or positive Phadiatop (107).

Questionnaires

CCQ

The Clinical COPD questionnaire (CCQ) was used to measure symptoms and functional state. It has been validated for studies of clinical control in COPD patients. The 10-item CCQ is self-administered, and patients are instructed to recall symptoms experienced during the last 7 days (121, 122). The scores range from 0 to 6, where 0 denotes the lowest score and 6 represents the highest.

CCQ	
1.	Short of breath at rest
2.	Short of breath doing physical activities
3.	Concerned about getting a cold or your breathing getting worse
4.	Depressed because of your breathing problems
5.	How much didi you cough
6.	Did you produce phlegm
7.	Strenuous physical activities
8.	Moderate physical activities
9.	Daily activities at home
10.	Social activities

CAT

The COPD Assessment Test (CAT) is designed to assess the impact of COPD on a patient´s health status and to help clinicians evaluate symptom severity, monitor disease progression, and response to treatment. The test consists of eight questions, each question addressing a different aspect of COPD symptoms and their effect on daily life. Every question has six possible answers, scored from 0 to 5 where 0 indicates no impairment and 5 indicates maximum impairment. The total score ranges from 0 to 40 categorizing the impact of the disease as low (0-10), medium (11-20), high (21-30) and very high (31-40) (123). A change of 2 or more units over 2-3 months suggests a clinically significant change in health status (124).

CAT	
1.	I never cough…… I cough all the time
2.	I have no plegh (mucus) in my chest at all… My chest is completely full of plegh (mucus)
3.	My chest does not feel tight at all… My chest feels very tight
4.	When I walk up a hill or a flight of stairs I am not breathless…When I walk up a hill or a flight of stairs I am very breathless
5.	I am not limited doing any activities at home… I am very limited doing activities at home
6.	I am confident leaving my home despite my lung condition…I am not confident at all leaving my home despite my lung condition
7.	I sleep soundly…I don´t sleep soundly because of my lung condition
8.	I have lots of energy…Ihave no energy at all

ACT

The Asthma Control Test (ACT) is evaluating the control of the disease during last 4 weeks. It consists of 5 questions, each scoring from 1 to 5. A total of 25 indicates a well-controlled disease, a score between 20-24 indicates partly controlled, <20 uncontrolled and < 16 poorly controlled disease (125, 126).

ACT	
1.	During the last 4 weeks, how much of the time has your asthma kept you from getting as much done t work, school or home?
2.	During the last 4 weeks, how often have you had shortness of breath?
3.	During the last 4 weeks, how often have your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) woken you up at night or earlier than usual in the morning?
4.	During the last 4 weeks, how often have you used your rescue inhaler or nebuliser medication (such aas Salbutamol)?
5.	How would you rate your asthma control during the last 4 weeks?

ACQ

The Asthma Control Questionnaire (ACQ) is a clinical tool used to measure how well a patient´s asthma has been controlled over the past week. It helps both clinicians and patients evaluate symptoms, medication use and lung function. There are several versions of the ACQ and some of the questions resemble those in the ACT, but with different answer options, and the addition of a question that includes a measure of FEV_1% predicted. Each question is scored from 0 to 6, where 0 indicates well-controlled and 6 poorly controlled disease. The total score is calculated as the average of all completed items, resulting in a final score ranging from 0 to 6. An ACQ score of ≤0.75 indicates well-controlled asthma, 0.76-1.49 indicates not well-controlled, and ≥ 1.5 indicates poorly controlled asthma. A

change of ≥0.5 in the ACQ-score is considered clinically significant meaning the patient´s asthma has either improved or worsened meaningfully (127).

ACQ-5
Circle the number of the response that best describes how you have been during the last week.
1. On average, how often were you woken by your asthma during the night?
2. On average, how bad were your asthma symptoms when you woke up in the morning?
3. In general, how much shortness of breath did you experience because of your asthma?
4. In general, how limited were you in your activities because of your asthma?
5. In general, , how much of the time did you wheeze?
6. On average, how many puffs of a short-acting bronchodilator have you used each day
To be completed by a member of the clinic staff:
7. FEV_1%predicted prebronchodilator

MiniAQLQ

Mini AQLQ is a shorter version of the Asthma Quality of Life Questionnaire, AQLQ, and was published in 1999. It is a self-administered tool that consists of 15 items to measure the functional impairments that are most troublesome to adult patients with asthma. The miniAQLQ evaluates four domains:1) Symptoms 2) Activity Limitation 3) Emotional Function and 4) Environmental Exposure. Each item is scored on a 7-point scale, with higher scores indicating better quality of life (127).

Statistics

Most variables were not normally distributed; therefore, non-parametric tests were used. Data are presented as medians (interquartile ranges). Categorical variables are expressed as numbers with percentages.

Statistical analyses were performed using Excel, Graphpad Prism and IBM SPSS Statistics 29.0 (SPSS Inc. Chicago, Illinois 60606) for Windows. Mann-Whitney test was used when comparing only two unpaired groups. Kruskal-Wallis test with Dunn´s Multiple Comparison post-test was used on groups containing three groups and above. Correlations were analysed using the Spearman´s nonparametric correlation test. Linear regression was used in paper I to analyse the relationship between FOT-variables while adjusting for potential cofounders (age and sex).

Categorical variables were compared using Chi-squared analysis.

A p-value of <0.05 was considered statistically significant.

Results

Paper I:

In this study we explored the difference between expiratory and inspiratory, as delta-values, in resistance and reactance and its correlation with air trapping, symptoms and quality of life in patients with obstructive lung disease. Delta-values were used as a measure of expiratory flow limitation during tidal breathing. We included 311 patients with asthma, 96 patients with COPD, 34 healthy never-smokers and 30 healthy smokers. Healthy never-smokers matched the asthma-patients and healthy smokers matched patients with COPD.

We found higher delta value between expiratory and inspiratory parameters of total resistance (ΔR5) in patients with COPD and healthy smokers than in healthy subjects and asthmatics. The delta value of peripheral resistance (ΔR5-R19) was also higher in patients with COPD than in patients with asthma and healthy subjects. Patients with COPD had a significantly lower (more negative) delta value in reactance (ΔX5) than patients with asthma and healthy controls (Figure 11).

There were correlations between the RV/TLC (as a measure of air trapping) and ΔR5, ΔR5-R19 and ΔX5 (but not ΔR19) among all subjects. When investigating the separate groups, these correlations were only found in patients with COPD (Figure 12).

In patients with asthma, we observed significant associations between changes in total airway resistance (ΔR5), peripheral resistance (ΔR5–R19), and reactance (ΔX5) with scores from symptom questionnaires (ACT and ACQ) and the asthma-specific quality of life questionnaire (AQLQ). In contrast, among patients with COPD and healthy smokers, associations were identified between changes in reactance (ΔX5) and symptoms assessed by the CCQ and CAT questionnaires, whereas no such associations were observed for resistance parameters. Notably, no correlations were found between changes in central airway resistance (ΔR19) and symptom scores in either the asthma or COPD groups (Table 2).

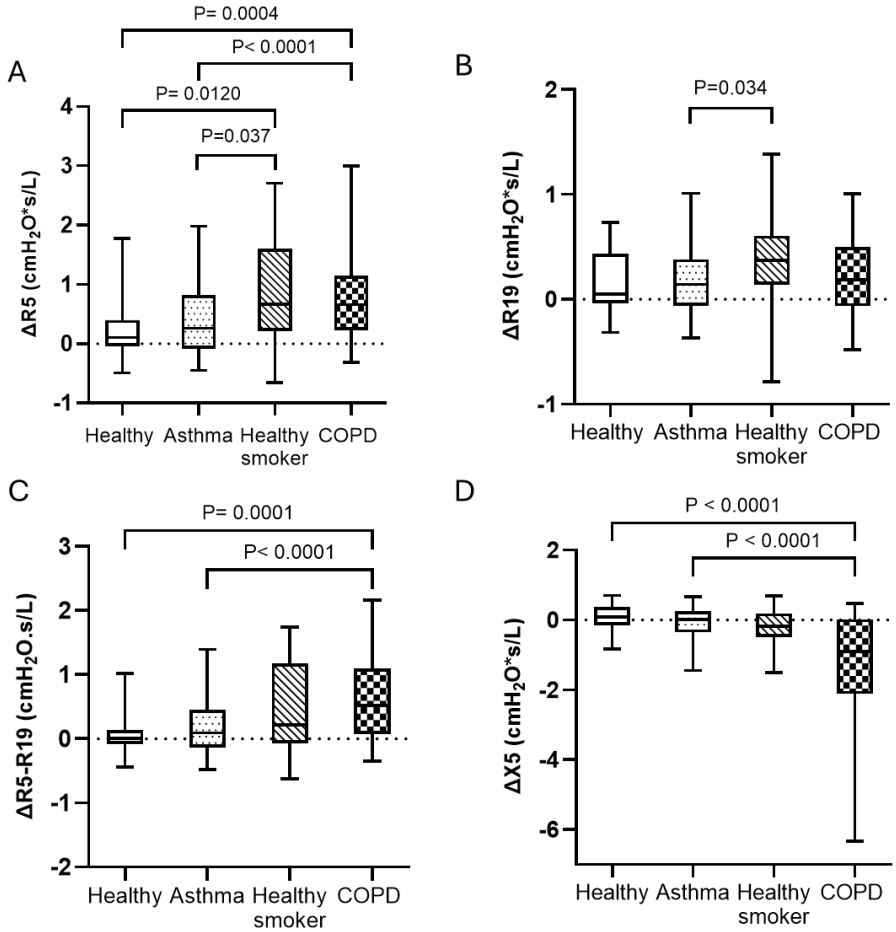

Figure 11. Boxplots showing the delta-value between expiratory and inspiratory R5 (A), R19 (B), R5-R19 (C) and X5 (D).

When comparing the two FOT devices, Resmon Pro Full and IOS, our data demonstrated good agreement between the delta-values from Resmon Pro Full and corresponding measurements using IOS. However, the linear regression analysis of the Bland-Altman plots showed a significant positive slope in ΔR5, ΔR19/20 and ΔX5. Resmon Pro Full showed some lower values in ΔX5 compared with IOS.

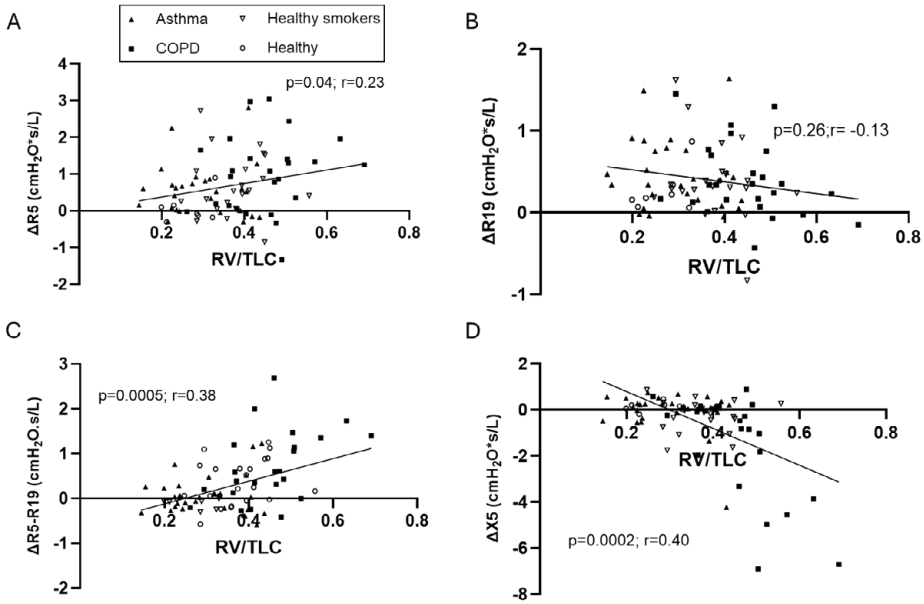

Figure 12. Spearman correlation plots between RV/TLC and the delta-value between expiratory and inspiratory R5 (A), R19 (B), R5-R19 (C) and X5 (D)

Table 2. Correlations between delta-FOT-variables and symptoms, after adjustment for age and sex. FOT-variables are presented as pre-bronchodilator delta-values between expiratory and inspiratory measurements.

	Questionnaire		ΔR5	ΔR19	ΔR5-R19	ΔX5
Asthma	ACT	p	0.021	0.21	0.007	<0.001
		β1	-0.73	-0.39	-0.847	1.15
	ACQ	p	0.001	0.062	0.002	<0.001
		β1	1.24	0.68	1.145	-1.37
	AQLQ	p	0.003	0.63	0.23	0.007
		β1	-3.185	0.42	-1.31	2.51
COPD	CAT	p	0.94	0.15	0.53	<0.001
		β1	-0.056	-0.97	0.61	-1.30
	CCQ	p	0.97	0.20	0.28	<0.001
		β1	-0.030	-1.05	1.29	-1.89
Healthy Smokers	CAT	p	0.33	0.035	0.80	0.038
		β1	0.96	1.36	0.35	-3.39
	CCQ	p	0.16	0.10	0.48	0.020
		β1	1.44	1.13	1.03	-3.92

Paper II.

In this study we explored airway hyperresponsiveness (AHR) to mannitol in asthma and COPD in respect to inspiratory versus expiratory resistance and reactance. We included individuals from the cohort of Paper I with a FEV_1>70%pred. The Mannitol challenge test was conducted on 292 subjects: 238 with asthma, 25 with COPD, 14 healthy smokers, and 15 healthy never-smokers. The response was assessed using both spirometry and respiratory oscillometry, with inspiratory and expiratory resistance and reactance measured separately.

A positive mannitol test was confirmed in 35% of patients with asthma, 52% patients with COPD, and 50% of healthy smokers. Subjects with asthma who had a positive mannitol test had a higher inspiratory total (R5) and central resistance (R19) at baseline. Additionally, the change from baseline to last dose measurements were larger in both inspiratory and expiratory R5, R5-R19, and X5 (but not R19), compared to asthma subjects with a negative test, figure 13. A similar tendency was seen among subjects with COPD and healthy smokers.

In asthma, there were negative correlations between baseline value of FEV_1 and respiratory oscillometry variable, both with and without a positive mannitol test. These correlations were observed in both inspiratory and expiratory values of resistance (R5 and R19) and reactance (X5). There was a similar pattern in COPD in R5 and R5-R19 and X5, but more pronounced with subjects with a positive response to mannitol and in expiratory compared to inspiratory values.

To minimize the impact of the differences in lung volumes, the relative change in % from baseline to last dose in FEV_1 ($\Delta\%$ FEV_1) and relative changes in respiratory oscillometry were also analyzed. In the asthma groups there were no correlations between $\Delta\%FEV_1$ and relative changes in respiratory oscillometry variables. However, in subjects with COPD with a negative mannitol test, there was a correlation between $\Delta\%FEV_1$ and the relative change in inspiratory X5 (Δ%X5insp). These correlations were weak in healthy smokers.

Subjects with asthma with a positive mannitol test had more symptoms than subjects with a negative test, while this was not shown among subjects with COPD and healthy smokers.

In both asthma and COPD, higher levels of blood eosinophils were found compared to healthy never-smokers and healthy smokers. However, no differences were found between subjects with a positive mannitol test compared to those with a negative mannitol test.

Among subjects with asthma, 54 (64%) individuals with a positive mannitol test and 94 (61%) individuals with a negative mannitol test had positive sensitization to airborne allergens.

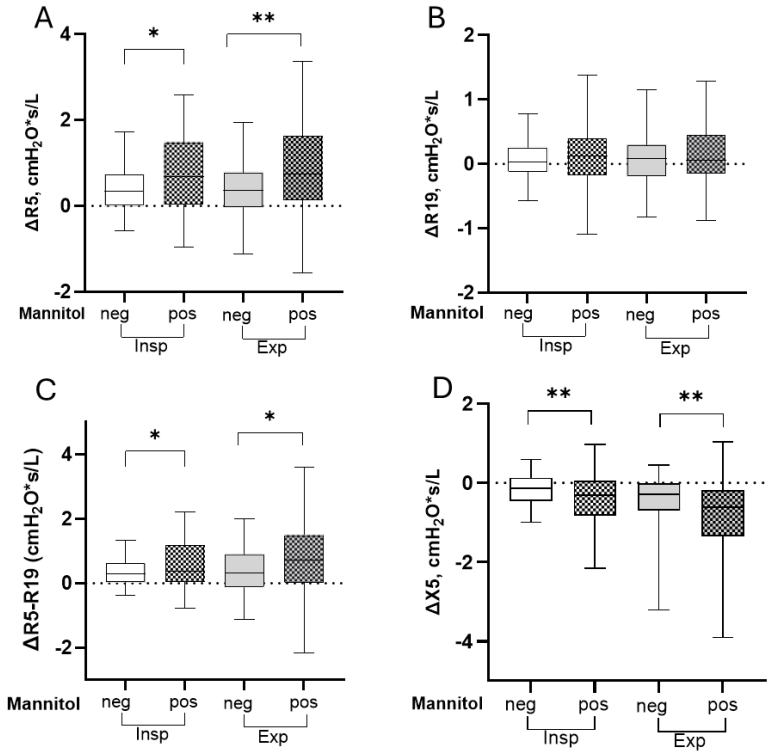

Figure 13. Inspiratory (Insp) and expiratory (Exp) FOT-indices, R5, R19, R5-R19 and X5, presented as the absolute change from baseline to last dose of mannitol in subjects with asthma with a negative (neg) versus positive (pos) response to mannitol challenge test.

Paper III.

A total of 155 subjects, of which 41 were healthy never-smokers (HNS), 35 were never-smokers with COPD (NS-COPD), 30 were never-smokers with lowered lung function (NS-low), 26 were never-smokers with large lungs (NS-large) and 23 were healthy smokers (HS), were included in this study (Table 3).

Never-smokers with COPD had % of predicted values of FVC, VC, TLC, RV and VA that did not differ significantly from healthy never-smokers, suggesting a mild lung function deficiency with preserved lung volumes in these subjects, despite having an FEV_1/FVC ratio below lower limit of normal. (Figure 14).

Table 3. Classification of subjects according to smoking status and post-bronchodilator spirometry results.

Description	Smoking status	FEV_1/FVC ratio	FEV_1/FVC, z-score	FEV_1, %pred
Healthy never-smokers *(HNS)*	Never[a]	≥ 0.70	≥-1.64	>90%
Never-smokers with COPD *(NS-COPD)*	Never[a]	< 0.70	< -1.64	50 – 99 %
Never-smokers with low lung function *(NS-low)*	Never[a]	< 0.70	≥-1.64	50 – 99 %
Never-smokers with large lungs *(NS-large)*	Never[a]	< 0.70	No limit	>100%
Healthy smokers *(HS)*	Current with ≥10 pack-years	≥ 0.70	≥-1.64	>90%

a <100 cigarettes in total and no smoking for the last 2 years.

In contrast, the never-smokers with large lungs (with an FEV_1/FVC ratio <0.7 but FEV_1>100% pred) showed a high FVC % pred, which explains that the low FEV_1/FVC ratios are due to their high values of FVC. Similarly, this group shows higher % of predicted values for VC, TLC and VA compared to all other groups, which further confirms large lung volumes. Additionally, the airway resistance in this group was at the same level as never-smoker controls without airway obstruction but lower resistance level (R5%pred) compared to never-smokers with COPD (Figure 15).

Never-smokers with COPD did not differ in DLCO or DLCO/VA expressed as % of predicted compared to the other groups (Figure 14). The never-smokers with large lungs, though, showed higher DLCO as % of predicted, compared to never-smokers with low lung function as well as to healthy smokers. This was most probably because of their larger alveolar volume, as the difference was not found in DLCO/VA % of predicted.

Both never-smokers with COPD and never-smokers with large lungs reported relatively few symptoms, as indicated by low CAT scores. In contrast, the healthy smoker group exhibited significantly higher symptom burden, as reflected by elevated CAT scores compared with all other groups.

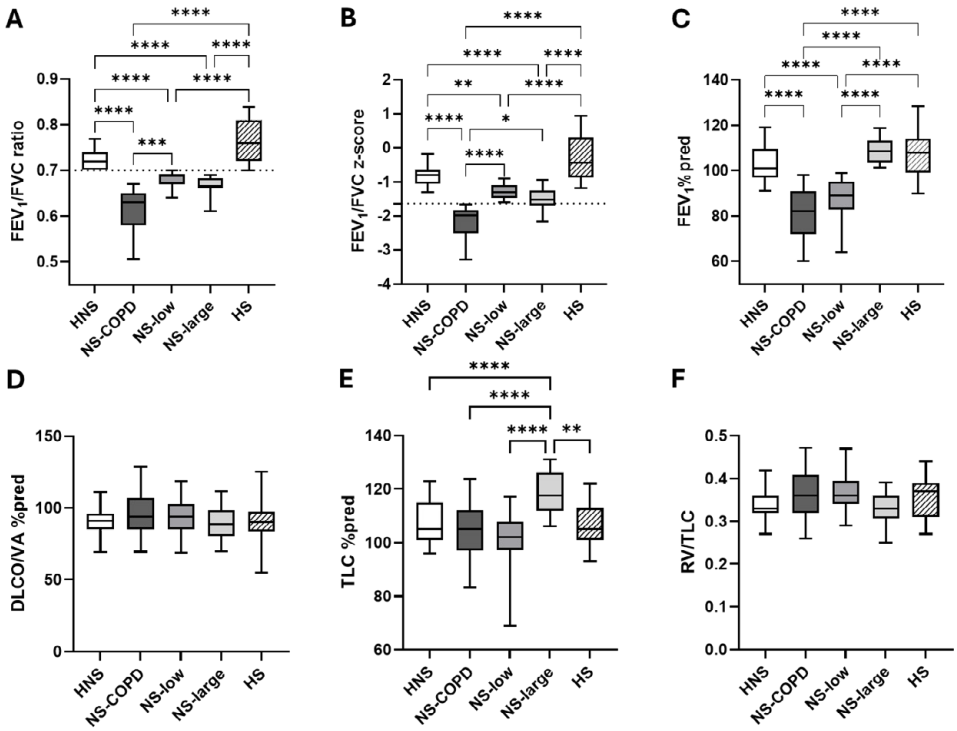

Figure 14. Boxplot of post-bronchodilator spirometri FEV_1/FVC (A) (as ratio and z-score (B)), FEV_1%pred (C), diffusion capacity (DLCO/VA%pred)(D), and body plethysmography (total lung capacity and residual volume) TLC%pred (E) and RV/TLC (F).

The measures of resistance and reactance by respiratory oscillometry showed that never-smokers with COPD had higher total resistance of the airways (R5) compared with the lower levels in never-smokers with large lungs. In addition, the latter group had the lowest level of median peripheral resistance and significantly lower than the healthy smokers (Figure15).

We assessed the total, inspiratory and expiratory resistance measures obtained by body plethysmography. Never-smokers with COPD presented higher both expiratory and inspiratory resistance compared to the never-smokers with large lungs. Expiratory flow limitation was assessed as the difference between the expiratory and inspiratory resistance, showed no difference between the groups.

Similarly, never-smokers with COPD were found to be more bronchodilator responsive in the variables of IOS, significantly more in R5, X5, Fres and AX than in the healthy never-smokers. As expected, never-smokers with large lungs showed lower %pred values of R5-R20, X5, Fres and AX (Figure15).

In the group of never-smokers with COPD, 26% had a bronchodilator response in FEV_1, a proportion that was higher than any other group. All never-smoking subjects showed higher bronchodilator responsiveness in FEV_1 compared with healthy smokers. Interestingly, no significant differences in bronchodilator responsiveness for FVC were observed among the groups.

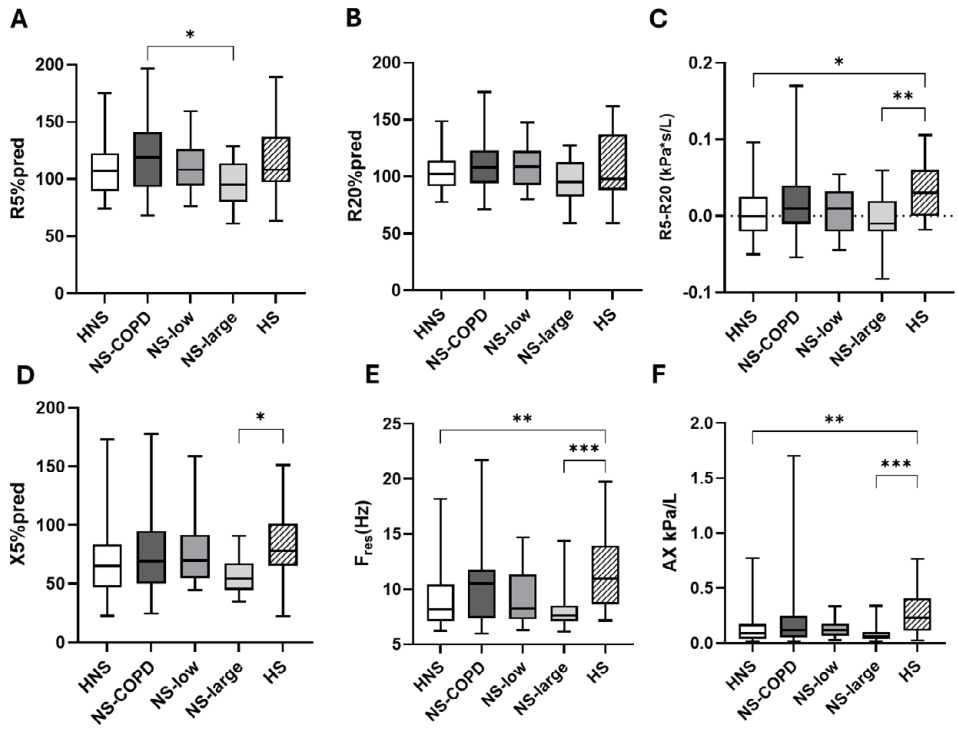

Figure 15. Boxplot of post-bronchodilator respiratory oscillometry measures , R5%pred (A), R20%pred (B), R5-R20 (C), X5%pred (D), Fres (Hz) (E) and AX (F).

Paper IV

We found a higher proportion of young smokers with obstruction grades 1–2 compared to young never-smokers, who all had grades 0–1, despite both groups having spirometric values within the normal range. These ventilation impairments, illustrated as varying obstruction grades, are shown in Figure 16. Young smokers also demonstrated lower diffusion capacity than young never-smokers (Figure 17).

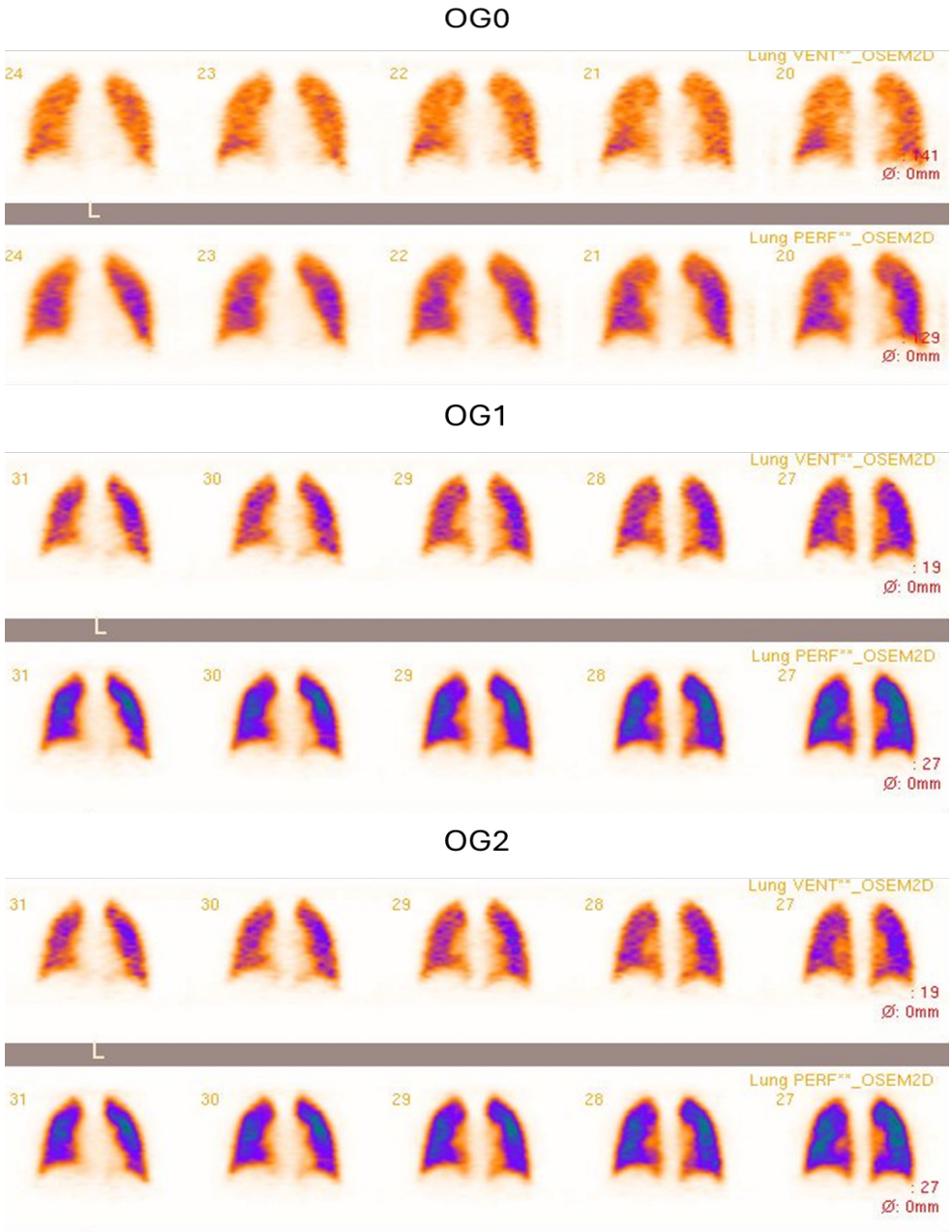

Figure 16. Images from V/P SPECT in young healthy smokers with varying grades of airway obstruction. Ventilation images are shown in the upper row and perfusion images in the lower row. The top two rows represent young smokers with obstruction grade 0, the middle rows obstruction grade 1 and the bottom rows obstruction grade 2.

In the older cohort, most lung function variables were impaired, with the highest obstruction grades observed in the COPD group. Among participants with the same obstruction grade, the COPD group had more pronounced abnormalities in resistance and reactance than other groups. Almost all older never-smokers and healthy smokers had grades 0–2, whereas the COPD group ranged from grades 1–3 (Figure 18).

Young never-smokers with obstruction grade 2 had lower %PLF than obstruction grade 0. Notably, all young smokers with obstruction grade 1 had ≥80% PLF, while those with obstruction grade 2 had ≤ 80% PLF, with two subjects with values even at 60% PLF.

No significant differences were observed in the correlations between PFTs and V/P SPECT findings when comparing older smokers to older never-smokers.

Particularly among subjects with COPD, there were significant correlations between %PLF and FEV_1/FVC, FEV_1% predicted, as well as diffusion capacity. Additional correlations were observed with body plethysmography metrics such as residual volume, air trapping (residual volume/total lung capacity), as well as with symptom scores (CCQ). Furthermore, %PLF correlated with airway resistance and reactance, and most pronounced in the peripheral airways (Figure19).

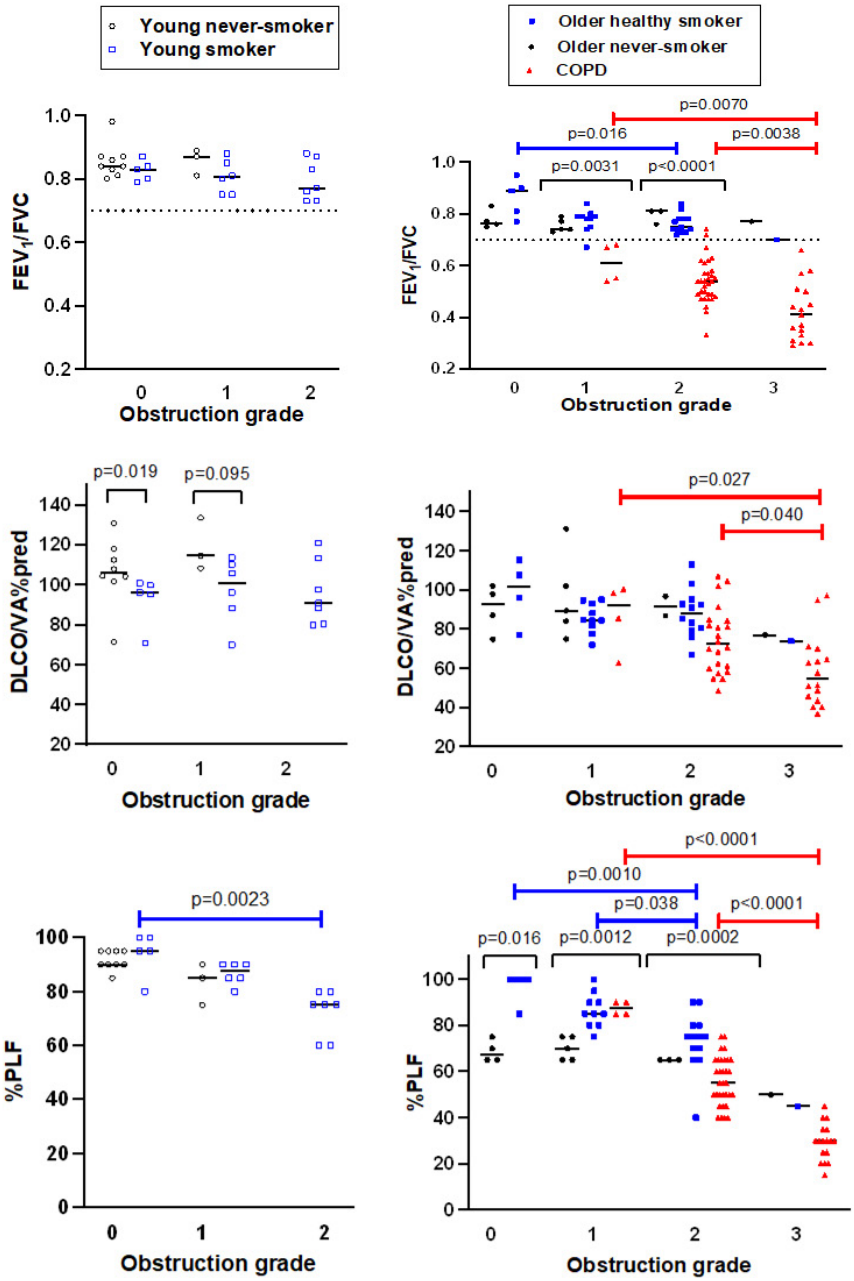

Figure 17. Young never-smokers and young smokers are shown on the left side, while older never-smokers, older smokers, and subjects with COPD are shown on the right side. Black brackets indicate differences between groups. Blue brackets highlight differences between healthy smoker with different obstruction grades (OG). Red brackets indicate differences between subjects with COPD with different OG.

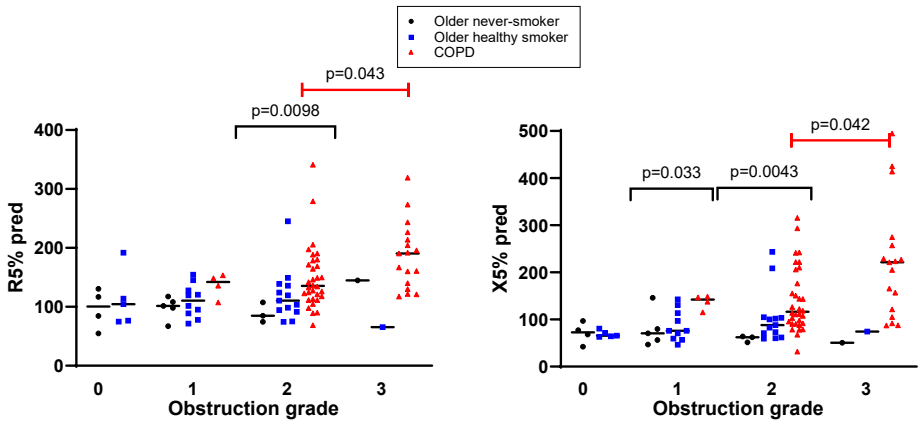

Figure 18. Respiratory oscillometry measures, resistance (R5%pred) and reactance (X5%pred), in the older groups. Black brackets indicate differences between groups, while red brackets show differences between subjects with COPD with different obstruction grades (OG).

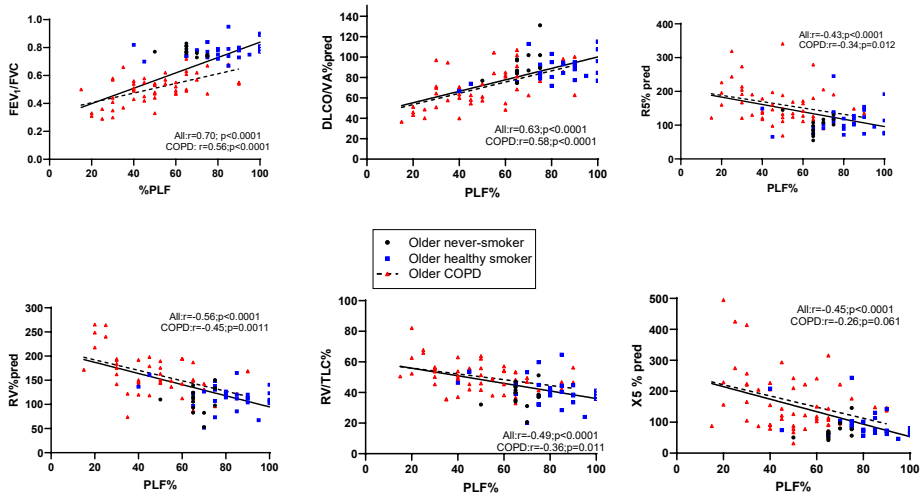

Figure 19. Correlations between Preserved lung function (PLF) in % and pulmonary function tests (PFTs) in the older groups. Strong to moderate correlations were shown between PLF % and FEV_1/FVC, diffusion capacity (DLCO/VA %pred), residual volume (RV%pred), RV/TLC %, and respiratory oscillometry measures; resistance (R5%pred) and reactance (X5%pred).

Discussion and limitations

In this thesis, small airway impairments in the peripheral regions of the lungs, in the context of airway obstruction, were investigated using less commonly applied diagnostic methods, respiratory oscillometry and V/P SPECT. Advanced analytical approaches were employed, including the separate evaluation of inspiratory and expiratory signals, expressed as absolute values, predicted values, and within-breath measures defined as the difference between inspiration and expiration.

One of the notable strengths of the papers included in this thesis is the investigation of two distinct obstructive pulmonary diseases, COPD and asthma. Although both conditions share certain pathophysiological features, such as airway inflammation and remodeling, they differ in their underlying mechanisms, clinical presentation, and disease progression. By including both patient groups, it was possible to explore similarities and differences in small airway involvement and functional impairments. This comparative approach provided a broader and more nuanced perspective for interpreting the results, allowing us to identify disease-specific patterns as well as shared pathophysiological mechanisms.

Our studies demonstrated that respiratory oscillometry is a sensitive method for detecting small airway impairments that may go undetected by spirometry. In standard clinical reporting, oscillometry outcomes are typically expressed as total values over the entire respiratory cycle. While this approach is appropriate in healthy individuals where minimal differences between inspiration and expiration are expected it can mask clinically relevant discrepancies, as total values represent an average of both phases.

To address this limitation, we conducted a more detailed analysis in Paper I by examining the difference between inspiratory and expiratory signals as a measure of expiratory flow limitation (EFL). EFL was most pronounced in delta-values reflecting the peripheral airways. This approach provided additional diagnostic insights, highlighting the potential of respiratory oscillometry for characterizing the mechanical properties of the respiratory system in diverse clinical contexts.

In Paper II, we further explored the separate evaluation of inspiratory and expiratory signals during the mannitol challenge test, where higher inspiratory resistance in patients with asthma was associated with a positive response to mannitol. However, the number of subjects with COPD included was considerably lower than that in asthma, limiting the statistical power and the ability to draw robust conclusions for

the COPD group. To translate these findings into clinical practice, further research is warranted to establish reference values, define relevanta cut-off points, and validate predictive equations for different FOT devices.

Including healthy smokers, never-smokers with impaired lung function, and healthy never-smokers in our studies broadened our understanding of the heterogeneity of COPD. Notably, the group of healthy smokers demonstrated heterogeneous outcomes across the studies. In Paper I, their within-breath variations resembled those of the COPD group. A similar phenomenon was observed in Paper II, where the proportion with a positive mannitol challenge was similar to that of COPD patients. In Paper III, healthy smokers demonstrated increased values in oscillometry measures reflecting the peripheral airways compared to never-smokers. Paper IV further revealed distinct phenotypes: some young smokers displayed early signs of COPD, whereas older smokers had preserved lung function and obstruction grades comparable to older never-smokers, as assessed by V/P SPECT. Collectively, these findings highlight the diversity within the "healthy smoker" category and emphasize the influence of environmental, non-environmental, and genetic factors on disease onset and progression.

In Paper III, we demonstrated that reliance solely on an FEV_1/FVC ratio below 0.7 is insufficient for diagnosing COPD in never-smokers. The subgroup with large lungs exhibited preserved lung function, comparable to that of healthy never-smokers, and their reduced ratio was attributable to an increased vital capacity. This pattern contrasts with PRISm, in which the FEV_1/FVC ratio remains preserved despite impaired spirometric values.

It is remarkable that airway obstruction in the subgroup of young healthy smokers, observed by V/P SPECT, was not detected by respiratory oscillometry but was evident in measures of lung diffusion capacity. This finding suggests that diffusion capacity may be affected early in the smoking-induced lung destruction process and could serve as an early marker of lung function impairment. This aligns with previous reports indicating that the diffusion capacity for carbon monoxide (DLCO) is a sensitive tool for detecting early COPD in otherwise healthy smokers.

Building on this, the V/P SPECT results demonstrate how advanced imaging can uncover subtle functional changes invisible to conventional tests. The method identified a subgroup of healthy young smokers with reduced ventilation, suggesting early alterations that may precede clinically detectable COPD. Combined with the broader phenotypic patterns observed across our studies, these findings support the view that COPD development is multifactorial and highly variable. Selective integration of V/P SPECT into research and clinical practice could facilitate earlier detection, guide targeted interventions, and ultimately improve patient outcomes. Raising clinician awareness of its potential, alongside systematic reporting and grading of ventilation impairments, could further enhance its adoption

Beyond these methodological limitations, there are also practical challenges that affect the broader applicability of V/P SPECT. The technique is less widely available than conventional CT scanners, as it requires both radiotracers and gamma camera, which are not present in all hospitals. Moreover, it is not feasible for clinicians to refer all smokers with respiratory complaints for V/P SPECT in the same way they would for conventional pulmonary function tests, such as spirometry, body plethysmography, diffusion capacity, and respiratory oscillometry. Before performing any examination involving ionizing radiation, including V/P SPECT, a justification assessment is essential to ensure that the expected diagnostic benefit outweighs the potential radiation risk, even though the absorbed dose is relatively low (≈2.2 mSv). When available, however, V/P SPECT should serve as a reference method in clinical trials evaluating simpler diagnostic techniques. Using V/P SPECT as the gold standard would enable these studies to more accurately determine the validity and reliability of alternative methods. Such an approach could help identify techniques that are both accurate and feasible for use in primary health care, thereby supporting earlier detection and improved management of obstructive airway diseases in settings with limited access to advanced imaging. This study contributes to that goal by enhancing the understanding of how V/P SPECT compares with conventional diagnostic methods.

We acknowledge that separate analyses of inspiratory and expiratory FOT-measures, as well as within-breath variations, consistent with the methodology in the other studies of this thesis, were considered during the data analysis in the V/P SPECT study. However, this was not feasible, as the software used for the COPD group and approximately half of the older healthy smokers did not support this functionality at the time the measurements were performed. We believe that including such analyses could have provided a better understanding of our findings.

Overall, the work presented in this thesis demonstrates that advanced physiological and imaging methods uncover small airway impairments not captured by conventional diagnostics. Combining respiratory oscillometry with V/P SPECT provides complementary insights into disease mechanisms, improves early detection, and highlights the heterogeneity of obstructive airway diseases. These findings underscore the need for broader adoption of sensitive diagnostic approaches and continued efforts to refine and validate tools for use in both research and clinical settings.

Conclusions and Future Perspectives

In Paper I, we demonstrated that expiratory flow limitation, expressed as the within-breath difference between expiratory and inspiratory oscillometry measurements, was most pronounced in subjects with COPD and, notably, also in healthy smokers. This observation suggests that healthy smokers may represent a distinct phenotype compared to asthma. The strong association between expiratory flow limitation in the peripheral airways and both respiratory symptoms and quality of life was evident even when using COPD-specific questionnaires for healthy smokers. These findings raise the possibility that respiratory oscillometry could enable the detection of disease at a very early stage.

Establishing robust reference values for respiratory oscillometry would further enhance its clinical utility, enabling the definition and monitoring of a broad spectrum of obstructive lung disease phenotypes.

Inspiratory resistance, as measured by respiratory oscillometry in Paper II, was found to predict hyperresponsiveness to mannitol in patients with asthma. Moreover, respiratory oscillometry detected a significant post-challenge increase in resistance, particularly in the peripheral airways, in these subjects. Combining resistance and reactance measurements with traditional spirometry may therefore provide a more comprehensive assessment of the hyperreactive airway response.

Future studies should investigate baseline inspiratory oscillometry measures in larger cohorts to establish clinically relevant cutoff values for bronchial challenge tests. It would also be valuable to replicate this work using other devices, such as impulse oscillometry (IOS), since the device used in our study is not widely available in routine clinical practice. Additionally, exploring whether inspiratory resistance can serve as a predictor of positive responses to other bronchial hyperreactivity tests, such as the methacholine challenge, could determine whether oscillometry offers complementary or additive diagnostic value in assessing airway responsiveness.

Looking ahead, advances in artificial intelligence may support the automated interpretation of oscillometry results, facilitating its integration into primary care. Such an approach could improve the early detection of airway impairments particularly among smokers at risk of developing COPD.

Paper III provides further evidence that COPD in never-smokers represents a distinct phenotype, characterized by involvement of small airways. Our findings also show that individuals with spirometrically defined airway obstruction due to large lung volumes do not exhibit pathological patterns of lung physiology. Further research is needed to investigate the underlying pathophysiological mechanisms in these groups, as well as to explore potential treatment strategies specifically for the never-smoking COPD phenotype.

In Paper IV, we confirmed that V/P SPECT is a sensitive imaging modality capable of detecting early signs of airway obstruction and grading its severity in COPD. Importantly, it identified ventilation impairment in a subgroup of young healthy smokers despite normal spirometry, suggesting that V/P SPECT can reveal ventilation heterogeneities earlier in the disease process than conventional lung function assessments. A long-term follow-up study—such as re-examining these young smokers after 10 years—would provide valuable evidence to support and strengthen our current findings.

It is also worth considering the use of alternative radionuclides beyond technetium, as V/P SPECT has certain technical limitations, including lower spatial resolution, longer acquisition times, reduced count statistics, and motion blurring caused by respiration. Advances in nuclear medicine and molecular imaging, particularly with hybrid imaging systems and novel PET tracers, offer opportunities to overcome these challenges. Compared to SPECT, PET provides higher sensitivity, improved spatial resolution, better temporal resolution, and more precise quantitative measurements (128-130).

Future studies could therefore explore the use of PET tracers in young smokers to assess ventilation and perfusion abnormalities with greater precision. Incorporating artificial intelligence for the quantification and classification of airway obstruction and preserved lung function could further enhance diagnostic accuracy and support the integration of these imaging techniques into routine clinical practice for early detection of small airway disease (131). In addition, emerging imaging modalities such as oxygen-enhanced MRI (O_2MRI), which enables mapping of ventilated lung regions without ionizing radiation, hold promise as complementary or alternative approaches.

Acknowledgments

I would like to extend my deepest and most heartfelt thanks to everyone who has been part of the journey that led to this thesis. It has been so much more than an academic project. it has been a chapter of my life filled with learning, growth, challenges, and countless moments of joy. Along the way, I have been surrounded by kindness, encouragement, and generosity that I will carry with me forever.

Your support has been my anchor on difficult days and my source of inspiration on the good ones. I truly hope that one day I can give back even a fraction of what I have been given. Words will never be enough to express my gratitude, but I would like to especially acknowledge some of the wonderful people who made this journey unforgettable.

My supervisor, **Ellen Tufvesson**, from the moment you accepted me as your PhD student, you believed in me and gave me the confidence to take on this journey. Your endless patience, encouragement, and genuine care have meant more to me than words can express. You have guided me with both wisdom and kindness, always finding the right balance between support and independence. Your deep knowledge and passion for research have been an inspiration, and I feel truly privileged to have had you by my side through every step of this work

My co-supervisor, **Leif Bjermer**, your creativity, passion, and endless curiosity are a constant source of inspiration for every researcher. You have the rare ability to spark new ideas and to see possibilities where others might see limitations.

My co-supervisor, **Linnea Jarenbäck**, thank you for welcoming me into all the projects, patiently teaching me new methods, and sharing your expertise so generously.

Jaro Ankerst, you have always been there to answer my questions, offer wise advice, and share your knowledge with enthusiasm. Your support and encouragement have been invaluable throughout my doctoral journey.

Georgia Papapostolou, working with you has been a joy. Your enthusiasm and warm personality made our collaborations not only productive but also truly enjoyable.

Marika Bajc, you encouraged me to take one of the most important steps in my life: applying for a PhD. I am deeply grateful for that push, as well as for our valuable discussions and fruitful collaboration on our project.

Ulf Nihlén and Finn Radner, I sincerely appreciate your help, guidance, and support, as well as the productive and insightful cooperation we have had in our work together.

Bodil Andersson, thank you for believing in me, giving me opportunities to grow in my career, and for your continuous support throughout my doctoral studies.

To all current and former colleagues at the Research Clinic**, Anna, Karina, Mette, Leif S., Tina, Susann, Emma, and Tate**, I am deeply grateful. From my very first day, you welcomed me with warmth and kindness, making me feel like part of your big family. It has been a true joy to work alongside you, and I sincerely appreciate all the support and help you gave me as I found my way into your research unit.

To my colleagues at the Department of Clinical Physiology and Nuclear Medicine: your friendship, encouragement, and support have been a source of strength and motivation. I am fortunate to have been surrounded by such a warm and inspiring team.

To my dear friend and sister **Ilona**, you are someone I can always count on, at any time and in any situation. Thank you for patiently listening to my frustrations about my studies and for constantly reminding me to take care of my health through exercise and staying active. Your encouragement has been a gentle reminder that good physical health is the foundation for maintaining mental well-being.

To my mother **Hayjar**, no words can truly express the depth of my gratitude and love for you. Your unwavering support, endless encouragement, and unconditional care have been the foundation upon which I could build this journey. You have always stood by me, believing in me even when I doubted myself, and for that I am forever grateful.

To my siblings **Rasha, Nabil, Rana**, **Bassem** and my sister-in-law **Niven** thank you for your endless support, encouragement throughout my studies and for always believing in me.

To my sunbeam, soulmate and daughter **Sarah**, thank you for always encouraging and reminding me of the light at the end of the tunnel during my ups and downs.

To my treasures, my sons **Iyad**, **Khalil** and **Jonas,** my son-in-law **Moe** and my daughters-in-law **Susann** and **Diana** thank you for your support, encouragement and believing in me.

To my husband **Yehia**, thank you for being by my side all these years and your constant encouragement.

And finally, to the joy of my life and my greatest source of love, my grandchildren, **Karim** and **Yasmine**.

Generative AI tool (ChatGPT) was used solely to refine the language and grammar of the text, and to assist the design of the cover page. All generated material was critically reviewed by me, the author, and I take full responsibility for the final content.

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