Biomarker framework for chronic airway diseases. Positioning ADAM17 across immune cells in COPD and asthma

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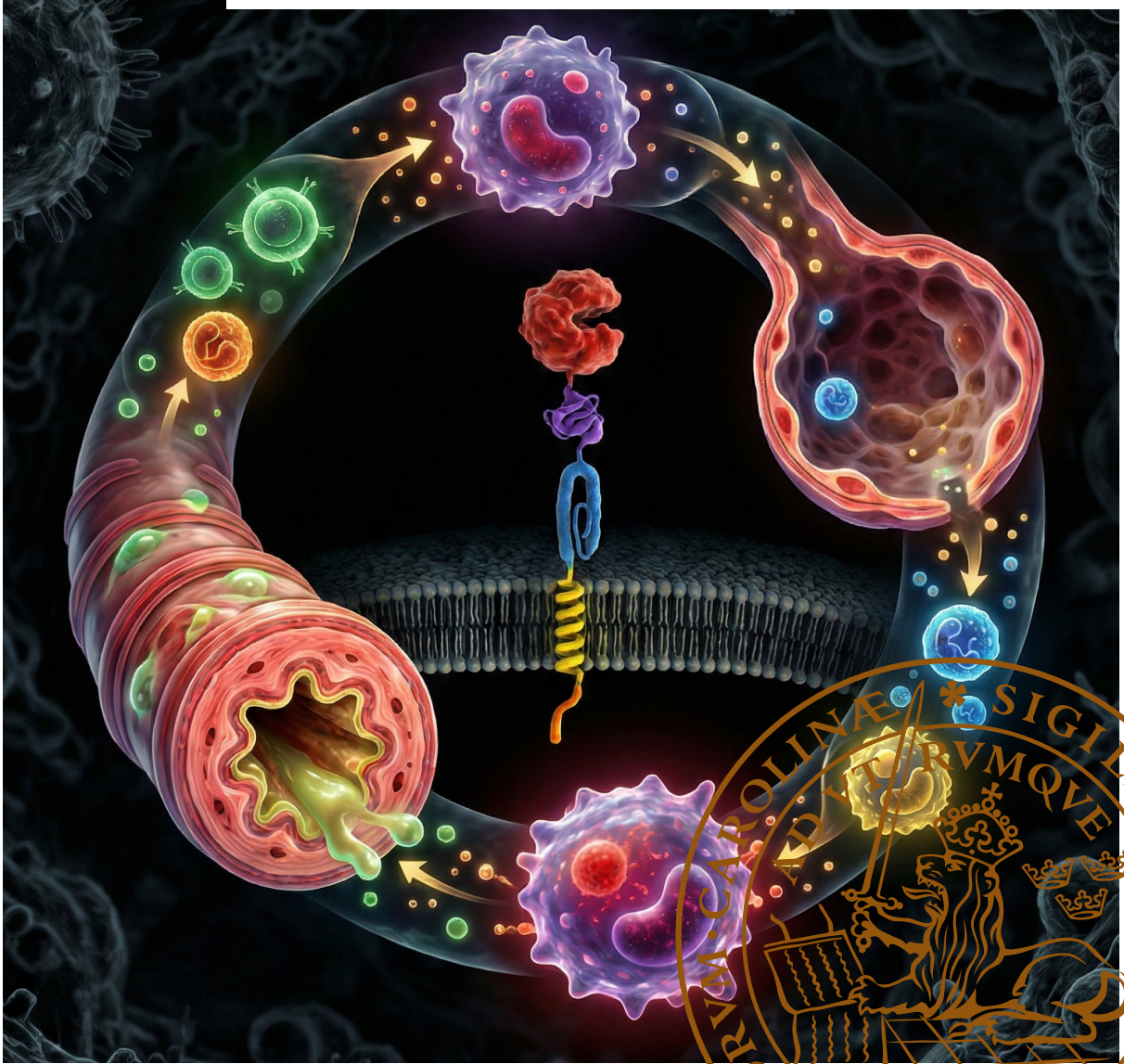
Positioning ADAM17 across immune cells in COPD and asthma

CARIN E. LAU
DEPARTMENT OF CLINICAL SCIENCES LUND | FACULTY OF MEDICINE | LUND UNIVERSITY



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Positioning ADAM17 across immune cells in COPD and asthma

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Carin E. Lau

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UNIVERSITY

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Abstract:

Chronic obstructive pulmonary disease (COPD) and asthma are heterogeneous respiratory diseases with overlapping symptoms but distinct underlying biology. As both diseases involve dysregulated inflammatory signalling, identifying disease- and compartment-specific pathways and biomarkers could improve diagnosis and treatment. A disintegrin and metalloproteinase 17 (ADAM17) is a key sheddase that influences inflammatory signalling and epithelial injury and repair by cleaving over 90 membrane-bound substrates, this process can be enhanced by phosphorylation of ADAM17 (pADAM17).

The aim of this thesis was to broadly characterise protein-based pathways in COPD and asthma by profiling ADAM17-related markers across airway and systemic compartments, and across relevant airway cell subsets. ADAM17 and pADAM17 localisation and expression were assessed in bronchial epithelium, bronchoalveolar lavage (BAL) cytospins, and circulating immune cells. Soluble ADAM17-related substrates were quantified in BAL fluid and blood, as well as in sputum and nasal lavage fluid in asthma, all measures were compared with the respective controls. To test the clinical applicability of assessing ADAM17, changes were also investigated after an exercise intervention.

ADAM17-associated substrate patterns showed more differences relative to controls in COPD BAL fluid than in asthma BAL fluid, and immunofluorescence staining suggested disease-related differences in epithelial ADAM17–pADAM17 localisation (Papers I–II). Given the more frequent substrate differences observed in COPD, exercise-related changes in ADAM17 and pADAM17 cell subsets were evaluated and found to be consistent with a redistribution of immune-cell phenotypes post-exercise, particularly in males (Paper III). Since ADAM17-defined immune-cell subsets differed between COPD and controls, additional cell subsets were profiled using cell-type-resolved GeoMx proteomics, revealing opposing disease–control shifts in cell death-related proteins that were generally decreased in COPD (particularly in neutrophils) but increased in asthma relative to controls (Paper IV).

In conclusion, ADAM17 profiles and immune cell subsets, in general, differ in chronic respiratory diseases compared to both controls, and also between diseases. The ADAM17 profile also tends to be sex-dependent and can shift during therapeutic interventions, such as exercise-associated changes seen in COPD. ADAM17-related cellular phenotypes and soluble substrate patterns can add value as part of a broader biomarker strategy for understanding and phenotyping COPD and asthma.

Key words: ADAM17, ectodomain shedding, COPD, asthma, airway epithelium, BALF, peripheral blood cells, biomarkers, exercise intervention, sex differences

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“Du måste äta elefanten i bitar”

– Gunnar Larsson

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Populärvetenskaplig sammanfattning

Kroniskt obstruktiv lungsjukdom (KOL) och astma är två vanliga lungsjukdomar som ofta ger liknande symtom, till exempel andfåddhet och hosta. Den bakomliggande biologin skiljer sig dock åt och varierar mycket mellan individer, vilket ibland kan göra det svårt att ställa en säker diagnos. För att förbättra diagnostik och behandling behövs därför bättre kunskap om vilka biologiska processer som skiljer sjukdomarna åt och vilka markörer (biomarkörer) som speglar detta.

En biologisk ”nyckelspelare” som kan vara relevant i detta sammanhang är ADAM17. ADAM17 är ett enzym som påverkar inflammation, immunsignalering men även processer som reparerar cellagret (epitelet) som klär insidan av luftvägarna. Enzymet fungerar genom att klippa av delar av proteiner som sitter fast i cellmembranet. De avklippta delarna kan sedan fungera som lösliga signalsubstanser som påverkar andra celler.

För att bidra till bättre förståelse av sjukdomsmekanismer och möjliggöra mer träffsäkra biomarkörer undersökte den här avhandlingen hur ADAM17 och relaterade signalsubstanser skiljde sig mellan KOL och astma. Vi studerade också om mönstren berodde på var i kroppen man mätte, till exempel i nedre luftvägarna, övre luftvägarna eller i blodet. Målet var att få en mer sammanhängande bild av vilka ADAM17-kopplade processer som var aktiva vid respektive sjukdom och om dessa kunde bidra till mer precisa biomarkörer.

För att fånga signaler både i luftvägarna och i hela kroppen analyserades flera typer av prover, bland annat från nedre luftvägarna (lungsköljvätska: BALF), slem från de nedre luftvägarna (sputum), övre luftvägarna (nässköljvätska: NALF) och blod. I en delstudie följde vi även personer med KOL genom ett 12 veckor långt träningsschema med både styrka och kondition. Vi ville se om ADAM17-relaterade mönster i immunceller förändrades när kroppen utsattes för fysisk belastning.

Resultaten visade att ADAM17-relaterade proteiner skilde sig mellan KOL och astma, men på olika sätt. I KOL såg vi fler skillnader i prover från lungorna jämfört med kontrollgrupper (aldrig-rökare och rökare). Vid astma var skillnaderna färre i samma typ av prov men fler i sputum från personer med svårare astma. Det tydde på att mekanismerna i astma är mer beroende av vilken typ av prov man analyserar eller av sjukdomens svårighetsgrad.

Träningsstudien för personerna med KOL antydde att fysisk träning kunde hänga ihop med förändringar i fördelningen av olika immunceller kopplade till ADAM17, snarare än en minskning av det totala antalet. Vi såg också att män och kvinnor kunde reagera olika, vilket tyder på att biologiskt kön är en viktig faktor att ta hänsyn till när man studerar effekter av fysisk aktivitet och rehabilitering vid KOL. Vi utvecklade även en metod för att mäta proteiner i olika celltyper som hade bevarats på ett mikroskopiglas. Metoden kunde ge mer exakt information om vilka proteiner som skilde sig mellan olika immunceller och mellan olika sjukdomar.

Sammantaget tyder våra resultat på att mönstren i ADAM17-relaterade celler och lösliga proteiner kan ingå i ett bredare ”paket” av biomarkörer som komplement till den standardiserade diagnostiken. De kan hjälpa till att visa skillnader mellan KOL och astma samt göra det lättare att identifiera undergrupper av patienter som kan svara på olika typer av läkemedel.

För att detta ska kunna användas mer i vården behövs större studier med välbeskrivna patientgrupper och mer standardiserad provtagning från flera delar av kroppen. Våra resultat tyder också på att det är viktigt att ta hänsyn till biologiskt kön när man tolkar ADAM17-relaterade mönster.

Popular science summary

Chronic obstructive pulmonary disease (COPD) and asthma are two common lung diseases that often cause similar symptoms, such as shortness of breath and coughing. However, the underlying biology is different and can vary a lot between individuals. This similarity of symptoms can sometimes make it hard to be certain of a correct diagnosis. To improve diagnostics and treatment, we need a better understanding of what biological processes separate the diseases, and which markers (biomarkers) can reflect these differences.

One important "key player" that may matter for correct identification is ADAM17. It is an enzyme that affects inflammation and communication between immune cells, and it may also affect how the lining of the airways (the epithelium) is repaired. ADAM17 works by cutting off parts of proteins that sit on the surface of the cells. These cut-off parts then become signals that influence other cells.

To improve the understanding of disease mechanisms and help develop more accurate biomarkers, this thesis examined how ADAM17 and related signals differ between COPD and asthma. We also looked at whether the patterns depended on where in the body the measurements were taken, for example, in the lower airways, upper airways, or in the blood. The goal was to build a clearer picture of which ADAM17-related processes are active in each disease, and whether these could support more precise biomarkers. To capture signals both in the airways and in the whole body, several different sample types were analysed, including lung wash fluid from the lower airways (bronchoalveolar lavage fluid: BALF), mucus from the lower airways (sputum), nasal wash fluid from the upper airways (nasal lavage fluid: NALF), and blood. In one sub-study, individuals with COPD also followed a 12-week training programme which included both strength and endurance exercise. We wanted to see whether ADAM17-related patterns in immune cells changed when the body was exposed to repeated physical exercise.

The results showed that ADAM17-related proteins differed between COPD and asthma, but in different ways. In COPD, we saw more differences in BALF compared with control groups (never-smokers and smokers). In asthma, there were fewer differences in the same type of lung sample, but more differences in sputum from individuals with more severe asthma. This suggests that in asthma, the findings may depend more on the type of sample or how severe the disease is.

The exercise study on individuals with COPD suggested that training may be linked to changes in the balance of different immune cell types connected to ADAM17, rather than simply reducing the total number of cells. We also saw signs that men and women may respond differently, which suggests that biological sex is an important factor to consider when studying exercise and rehabilitation in COPD. We also developed a method to measure proteins in different cell types preserved on a microscope slide. This method can give more detailed information about which proteins differ between immune cell types and between diseases.

Overall, our results suggest that patterns in ADAM17-related cells and soluble proteins could be useful as part of a broader "package" of biomarkers, alongside standard diagnostic methods. They may help show differences between COPD and asthma and make it easier to identify patient subgroups that may respond differently to certain medicines.

Before this can be used in healthcare, larger studies are needed with well-described patient groups and more standardised sampling from several parts of the body. Our results also suggest that biological sex should be considered when interpreting ADAM17-related patterns.

List of Included Papers

Carin E. Lau., Nicole M. D. van der Burg, Jaro Ankerst, Leif Bjermer, and Ellen Tufvesson. 2026. '*ADAM17 and Related Epithelial Injury Markers in Bronchoalveolar Lavage and Blood Distinguish COPD from Controls*'. Respiratory Medicine 251 (January): 108596.

Carin E. Lau, Nicole MD van der Burg, David Aronsson, Jaro Ankerst, Leif Bjermer, Jonas Erjefält, Ellen Tufvesson, 2026. '*Distinct Patterns of ADAM17 Substrate Distribution in Airways of Subjects with Asthma: Differences in Lower but Not Upper Airways'* (Manuscript)

Carin E. Lau, Nicole Van Der Burg, Rana Mansour, Linda Elowsson, Caroline Larsson, Anna Cederberg, Bryan Falcones, Jaro Ankerst, Hamid Akbarshahi, Margareta Emtner, Leif Bjermer, Christer Janson, Gunilla Westergren-Thorsson, Ellen Tufvesson, 2026 '*Immune Modulation of ADAM17 Expression on Circulating Leukocytes Following an Exercise Intervention in Individuals with COPD'* (Manuscript).

van der Burg NMD, **Lau C**, Selander L, Frøssing, L, Ankerst J, Bjermer L, Tufvesson E. 2026 '*High-plex protein profiling on cytospin slides with bronchoalveolar lavage cells from asthma and COPD'*. 2026.01.15.695934 Preprint at https://doi.org/10.64898/2026.01.15.695934 (2026).

Abbreviations

ADAM17	A disintegrin and metalloprotease 17
APP	Amyloid-beta precursor protein
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BMI	Body mass index
COPD	Chronic obstructive pulmonary disease
CD	Cluster of differentiation
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EpCAM	Epithelial adhesion molecule
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GINA	Global initiative for asthma
GOLD	Global initiative for chronic obstructive lung disease
HB-EGF	Heparin-binding EGF-like growth factor
ICS	Inhaled corticosteroids
IL	Interleukin
LABA	Long-acting β2-agonist
LAMA	Long-acting muscarinic antagonist
MIC	MHC class I polypeptide–related sequence
MUC-1	Mucin 1
NALF	Nasal lavage fluid
NK	Natural killer
NKT	Natural killer T

pADAM17	Threonine phosphorylation of ADAM17
PBS	Phosphate-buffered saline
PRISm	Preserved ratio impaired spirometry
ROI	Region of interest
SABA	Short-acting β2-agonist
TACE	TNF-α converting enzyme
TBS	Tris-buffered saline
TGF-α	Transforming growth factor α
TNF-α	Tumour necrosis factor α
TRANCE	TNF-related activation-induced cytokine
TSLP	Thymic stromal lymphopoietin
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells

Preface

In 2019, my grandfather passed away from COPD. He was never the type to complain about anything, so no one understood how severe his disease had become. During his last year, he required an oxygen tank at home, and it was difficult to witness the progression of his illness. He had never been religious, and in the weeks before he passed away, he was admitted to hospital. When the nurses asked if he wanted a priest, he was more offended than comforted. He answered, “What am I going to do with a priest? Give me a doctor that knows something.” His words stayed with me because they pointed to a gap I did not want to see: the gap between what patients hope medicine can do and what it can currently offer in COPD. When I was offered the opportunity to pursue a PhD in respiratory diseases, I wanted to contribute to research that could make a difference for patients and families facing similar circumstances, and this thesis became my way of doing so.

COPD and asthma are often discussed together because they share key respiratory symptoms. Asthma has been recognised since ancient times, while COPD became a distinct concept much later, particularly with increasing tobacco use and air pollution in the 19th century. Over time, improved understanding of inflammation, bronchospasm, and irreversible airflow limitation shaped how these diseases were defined and treated, and therapies progressed from herbs and cigarettes to more targeted approaches.

ADAM17 was discovered in 1997 as the TNF α converting enzyme, responsible for cleaving TNF-α from the cell surface, and it was soon established as a key sheddase for cytokines and growth factors. Early knockout studies revealed severe developmental phenotypes, including mice that were born with their eyes open and died perinatally, and additional phenotypes were linked to impaired epidermal growth factor receptor (EGFR) signalling. From 2009 onwards, studies in asthma linked ADAM17 overexpression to airway inflammation, mucus hypersecretion, and fibrosis. Mechanistic work showed that ADAM17 promoted airway remodelling by shedding EGFR ligands, including heparin binding EGF like growth factor (HB-EGF) and amphiregulin, which drove airway thickening and goblet cell expansion. Although much of the mechanistic evidence was generated in animal and ex vivo models, data from human tissues and cell systems steadily accumulates.

Around 2018, studies reported elevated ADAM17 activity in COPD airways and associated it with epithelial injury markers and EGFR pathway activation, with

increased mucus production, cytokine release, and remodelling. Cigarette smoke exposure activated ADAM17 and linked it to chronic inflammation. A key breakthrough came in 2021 when Saad et al. demonstrated ADAM17 hyperphosphorylation at threonine 735 as a hallmark of emphysema in patients and in mouse models. Follow up studies confirmed and extended these findings, linking dysregulated ADAM17 activity to COPD and lung cancer. ADAM17 inhibitors have been proposed as treatment for different diseases, while ongoing work examine ADAM17 in immune responses and more general lung inflammation.

The paper by Saad et al. sparked my interest in ADAM17 in COPD and became part of the central focus of this thesis.

Introduction

Chronic respiratory disease is an umbrella term for conditions that affect the lungs and airways, such as asthma and chronic obstructive pulmonary disease (COPD). These diseases place a substantial burden on society, including costs related to treatment and hospitalisations following exacerbations[1]. Both asthma and COPD are heterogeneous diseases characterised by airflow obstruction, chronic inflammation, and remodelling[2,3]. Despite extensive research into biomarkers, it remains a challenge diagnosing and treating both asthma and COPD[4–6]. Importantly, several potential biomarkers in asthma and COPD arise from pathways regulated by ADAM17-mediated shedding, a mechanistic link that is frequently under-recognised[7].

Airway Biology

The airway consists of conducting passages in the respiratory tract that transport air from outside the body to the alveoli. It is commonly divided into two connected regions: the upper and lower airways, each with distinct anatomical structures and biological functions. In addition to conducting air, it acts as a filter, humidifies and warms inspired air, and provides defence against pathogens through mucosal and lymphoid tissues[8].

Upper and Lower Respiratory Tract

The upper respiratory tract encompasses the nasal and oral cavities and extends to the larynx, where it is lined by a mucous membrane. In the nasal cavity, airflow becomes turbulent, which improves filtration of inhaled particles and helps humidify inspired air. The paranasal sinuses further condition the air and support voice resonance[8].

The lower respiratory tract starts at the trachea, a tubular conducting airway passage lined with ciliated epithelium and structurally supported by C-shaped connective tissue. It continues into the lungs and divides into the bronchi, which branch repeatedly to form smaller airways. These branches ultimately give rise to the bronchioles, which are approximately one millimetre in diameter and primarily

conduct air to the respiratory zone, where alveolar gas exchange occurs[8]. The different airway compartments are depicted in Figure 1.

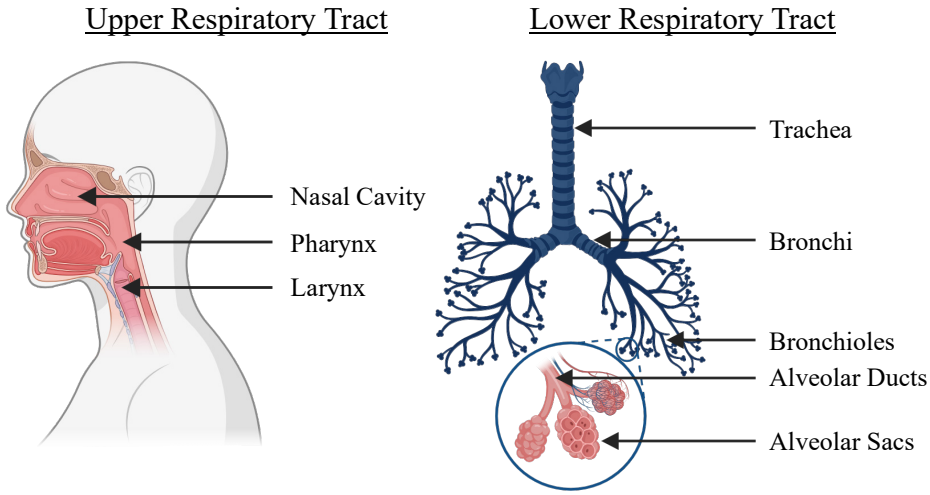

Figure 1: Schematic overview of the respiratory tract
Illustration of the parts that constitute the upper and lower respiratory tracts, including the alveoli, where gas exchange takes place. Image created in BioRender by the author.

The Airway Epithelium

The bronchial epithelium consists of four common cell types: ciliated cells, club cells, goblet cells and basal cells[9].

Ciliated cells are found along the airways and help clear material trapped in mucus from the epithelial surface[10]. These cells originate from basal cells, and their differentiation is tightly regulated by the Notch signalling pathway; suppression of this pathway leads to increased numbers of ciliated cells [11].

Club cells (previously called Clara cells) are secretory cells present in both small and large airways and play an important role in immune defence, the metabolism of foreign compounds (xenobiotics), and repair after injury. Increased Notch signalling has been shown to increase the number of club cells [11–13].

Goblet cells are secretory cells that secrete mucins onto the internal surface of the airways, forming a mucus layer to protect the epithelium [14].

Basal cells are stem cell-like progenitors found in the upper and lower airways and can give rise to either ciliated cells or secretory cells such as goblet and club cells. In chronic respiratory diseases such as COPD and asthma, basal cells can give rise to goblet cell hyperplasia, and this abnormal differentiation can exacerbate airway remodelling[15].

The barrier function of the epithelium depends strongly on the integrity of epithelial cells and their adhesions, particularly tight and adherens junctions. The epithelium also serves as an immune barrier, producing type I interferons to activate the cellular immune response. Antiviral immune responses also include the release of cytokines that can directly affect epithelial barrier function. This is especially true for interleukin 1β (IL-1β), interferon gamma (IFN-γ), and tumour necrosis factor (TNF), which have been linked to increased epithelial permeability[16]. The airway epithelium and the mucus layer protect deeper tissues against inhaled pollutants, allergens, and pathogens. Studies have shown that exposure to particulate matter ≤2.5 μm can disrupt the epithelial barrier by downregulating tight junction proteins, reducing E-cadherin expression, lowering transepithelial electrical resistance, and increasing paracellular permeability[17]. When epithelial integrity is compromised, pattern recognition receptors (PRRs) are activated, triggering the release of epithelial cytokines (alarmins) as well as other cytokines and chemokines. This promotes the recruitment and activation of both innate and adaptive immune cells, supporting antiviral defence and subsequent epithelial repair[18,19].

Epithelial Pathways

Many PRR signalling pathways, mediated via Toll-like receptors (TLRs) and nucleotide-binding oligomerisation domain-containing receptors (NOD receptors), activate the nuclear factor κB (NF-κB) pathway, which controls the expression of a range of inflammatory cytokine genes[20,21]. The NF-κB pathway is a key pathway in chronic respiratory diseases and has been linked to inflammatory processes in both asthma and COPD[22,23].

The epidermal growth factor (EGF) receptor can be activated by multiple stimuli and is involved in mucus production in the airways. It is a main driver of mucin production during goblet cell metaplasia, and the EGFR pathway is involved in a range of responses, including proliferation, differentiation, and motility[24]. The EGFR pathway has also been shown to contribute to neutrophil recruitment via IL-8 production, as well as to the repair of the airway epithelium[25].

Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) refers to a group of diseases that cause a decline in lung function and is the fourth leading cause of death worldwide, responsible for 3.5 million deaths in 2021[26]. COPD is characterised by progressive airflow limitation, chronic bronchitis, and destruction of alveolar tissue, resulting from a persistent inflammatory response in the lungs (Figure 2)[27].

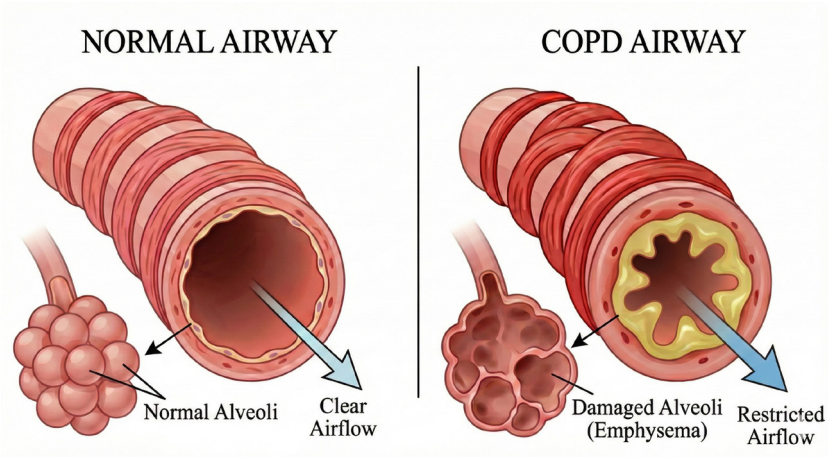

Figure 2: COPD airway pathology
The left panel illustrates a normal airway with a clear lumen and intact alveolar sacs. The right panel depicts pathological changes associated with COPD, including bronchial narrowing, mucus hypersecretion, and the destruction of alveolar walls characteristic of emphysema. These structural changes collectively lead to restricted airflow and impaired gas exchange. Image created using Google Gemini 3 Pro.

Risk Factors

There are many risk factors for the development and progression of COPD. Globally, the largest contributors to COPD burden, based on disability-adjusted life years (DALYs) in the overall adult population, are smoking (34.8% of COPD DALYs), ambient particulate matter pollution (22.2%), household air pollution from solid fuels (19.5%), and occupational exposure to particulate matter, gases, and fumes (15.8%)[28].

COPD in younger individuals (aged 15–49) is also being increasingly recognised. In this age group, the major risk factors broadly mirror those in the overall adult population, although particulate matter pollution contributes proportionally more at younger ages, whereas smoking becomes more influential towards the upper end of this age range[29].

Electronic cigarettes (e-cigarettes, or vaping) have often been presented as a harm-reduction alternative to combustible cigarettes and have been evaluated as aids for smoking cessation. Randomised trials and other evidence indicate that nicotine-containing e-cigarettes can support quitting in some settings[30,31]. However, the long-term respiratory outcomes of e-cigarettes are not yet well defined, but recent observational studies have reported an association between e-cigarette use and higher odds of COPD[32]. A meta-analysis showed a 1.5-fold higher likelihood of developing COPD among individuals who use e-cigarettes without traditional cigarette smoking[33].

While these traditional risk factors remain the primary contributors to COPD development, recent evidence has also highlighted more systemic factors, including metabolic alterations. Many studies now show links between metabolic disorders and increased risk of cardiovascular disease[34], cancer[35], Alzheimer's disease[36], non-alcoholic fatty liver disease[37], diabetes[38], and more. Metabolic syndrome is a cluster of cardiometabolic risk factors that includes obesity, elevated blood pressure, high triglyceride concentrations in the blood, and impaired glucose regulation (insulin resistance)[39]. A recent study found that metabolic syndrome is also associated with COPD development in younger individuals, and that risk increases with a higher number of metabolic syndrome components[40]. Components of metabolic syndrome include reduced insulin sensitivity, high fasting plasma glucose, abdominal obesity, high fasting plasma triglycerides, low HDL cholesterol, and high blood pressure[41].

Risk factors for COPD development differ between males and females, with females generally experiencing more pronounced small airways disease than males for similar tobacco smoke exposure[42]. Leading risk factors also differ globally between sexes: for females, a major driver is household air pollution from solid fuels, largely driven by countries where people still cook over an indoor open fire. For males, the leading risk factor is smoking, which is also the leading risk factor for females in higher-income countries[28].

Diagnosis

The diagnosis and management of COPD remain important challenges, and there is still a lack of measurable biomarkers for diagnosing and treating COPD[43]. Current diagnostic criteria for COPD are defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD)[3] and involve evaluation of relevant symptoms and risk factors. Typical symptoms include dyspnoea, chronic cough, mucus production, and wheeze. The diagnosis is then confirmed using spirometry to measure forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) after bronchodilator use, where the ratio FEV_1/FVC ratio should be <0.70[3].

Prevalence and Misdiagnosis

There is a substantial global variation in the prevalence of COPD globally with estimates suggesting 10-95% of cases are underdiagnosed and 5-60% over-diagnosed. This is mainly due to variations in diagnostic definitions between countries and the limited access to spirometry in rural areas of low- and middle-income countries, where COPD prevalence is often high[44]. In a UK study, 14% of participants had been misdiagnosed with COPD, of these, 40% were found to have asthma rather than COPD[5]. In a Spanish multi-centre study, only 13.9% of those with a COPD diagnosis fulfilled all GOLD criteria[45]. Misdiagnosis is not explained solely by symptom overlap with asthma, although diagnostic confusion between asthma and COPD is common[46].

Pre-COPD, Young COPD, PRISm

Some COPD misdiagnosis may relate to relatively new concepts such as pre-COPD, young COPD, and PRISm. Pre-COPD was first described in 2021 and has been discussed in relation to GOLD stage 0, which referred to the presence of risk factors and symptoms in the absence of spirometric abnormalities. Pre-COPD describes individuals without spirometric obstruction who have symptoms and/or structural or physiological abnormalities that place them at risk of developing COPD in the future[47]. Young COPD was also introduced in 2021 and proposed trials in younger individuals with COPD alongside individuals with pre-COPD, "young" was defined as 20–50 years of age[48]. PRISm (preserved ratio impaired spirometry) was first described in 2014 and refers to individuals with reduced FEV_1 but a preserved FEV_1/FVC ratio (e.g., >0.7)[49]. Together, these terms describe conditions that may or may not precede COPD, meaning that early intervention in these groups could have a substantial impact on the number of individuals diagnosed with COPD each year[50].

Treatment

There is no cure for COPD, treatment aims to relieve symptoms. Smoking cessation is the most effective and important intervention to slow disease progression and reduce exacerbations[3]. Vaccinations are recommended for all individuals with COPD, including annual influenza vaccination and COVID-19 vaccination[51]. Pulmonary rehabilitation improves dyspnoea, exercise capacity, and quality of life. These benefits can be maintained if the individual follows a structured maintenance exercise routine[52,53]. Education, nutrition, and management of comorbidities are also important aspects of COPD treatment[3].

Pharmacological treatment primarily relies on bronchodilators. Combining long-acting beta agonist (LABA) and long-acting muscarinic antagonist (LAMA)

improves lung function compared to placebo[54], and the improvement is consistently greater than either agent alone[55]. Similar additive effects have been reported with short-acting muscarinic antagonists (SAMA) and short-acting beta$_2$ agonists (SABA)[56]. Studies indicate that COPD-associated inflammation has limited responsiveness to inhaled corticosteroids (ICS)[57], and ICS does not appear to modify the long-term decline in FEV_1, instead regular ICS use increases the risk of pneumonia, especially in those with severe disease[58]. In individuals with moderate to very severe disease and exacerbations, ICS combined with a LAMA is more effective than either component alone, improving lung function and health status, and reducing exacerbations[59]. Triple therapy, meaning the combination of LABA, LAMA, and ICS, can be beneficial in individuals with severe airflow obstruction and a history of exacerbations. It has been shown to have a non-significant trend towards lower mortality compared with non-ICS-based therapy[60]. In very severe COPD with resting PaO2 ≤55 mmHg, long-term oxygen therapy has shown survival benefits, but no significant benefit was seen in individuals with moderate desaturation[61,62].

Pulmonary rehabilitation and exercise are emerging as some of the most important treatment options for individuals with COPD, helping to manage breathlessness and improve quality of life[63].

Phenotypes and Endotypes

Although multiple COPD phenotypes have been described, only some are clinically meaningful. In practice, these are defined by their association with clinical outcomes, their ability to consistently classify patients, and their capacity for predicting treatment responses, with validation across cohorts[64]. Historically two major phenotypes associated with COPD have been described: chronic bronchitis, characterised by airway inflammation and chronic cough, and emphysema, associated with alveolar destruction and dyspnoea[65].

Endotypes have also been described in COPD, although they are less established than in asthma. Alpha-1 antitrypsin (AAT) deficiency results from a lack of a protease inhibitor that protects the lungs from neutrophil elastase and other serine proteases. This deficiency can lead to early-onset emphysema, especially in smokers[66,67].

Non-type 2 (neutrophilic) inflammation is often associated with increased sputum neutrophils and mucus hypersecretion, and it is typically unresponsive to corticosteroids, including inhaled corticosteroids (ICS), even at high doses[67–69]. Blocking the chemotactic effects of CXCL8 and related chemokines reduces sputum neutrophils but provides limited or no benefit for exacerbation rate or lung function[70]. Other therapies, such as antibodies targeting TNF-α, IL-1β, and leukotriene B_4, among others, have largely been clinically ineffective[57].

Type 2 inflammation is more debated in COPD. Prevalence estimates vary (13.2–35.7%), and it is commonly characterised by increased sputum and blood eosinophils[71]. However, other studies argue that type 2 inflammation in COPD may reflect misdiagnosed asthma[72,73] although the underlying biological mechanisms do not seem to be identical[74]. Several studies of anti-IL-5 receptor-α antibodies in individuals with severe COPD, recurrent exacerbations, and evidence of peripheral blood eosinophilic inflammation have not shown improvements in FEV_1 or quality of life[75,76]. This suggests that the frequency of this phenotype is likely lower than previously reported[72].

Sex Differences

Biological differences between males and females contribute to variation in airway development and respiratory physiology, which in turn influences susceptibility to inhaled substances such as tobacco smoke[77]. Females generally have smaller airways relative to lung volume, and inflammatory responses within the lungs differ from those observed in males[77,78]. As a result, deposition of inhaled particles from noxious agents, including cigarette smoke, may be greater in the proximal airways of females[42]. Clinically, female smokers often experience a faster annual decline in FEV_1[79] and tend to develop airflow limitation at a younger age and with a lower cumulative smoking exposure than males[80]. This suggests that females may be more vulnerable to the harmful effects of smoking. Furthermore, the observation that females often exhibit more small airways disease, whereas males tend to develop more severe emphysema, may be influenced by sex hormones[81]. Higher testosterone levels have been associated with greater FEV_1 in males, but lower FEV_1 and FVC have been reported in females, suggesting a possible protective effect in males only[82].

Immune cells

Different immune cell types play important roles in COPD development. They contribute to chronic inflammation and airway remodelling in COPD, with an imbalance between pro- and anti-inflammatory responses. A schematic overview of immune cells in COPD is shown in Figure 3.

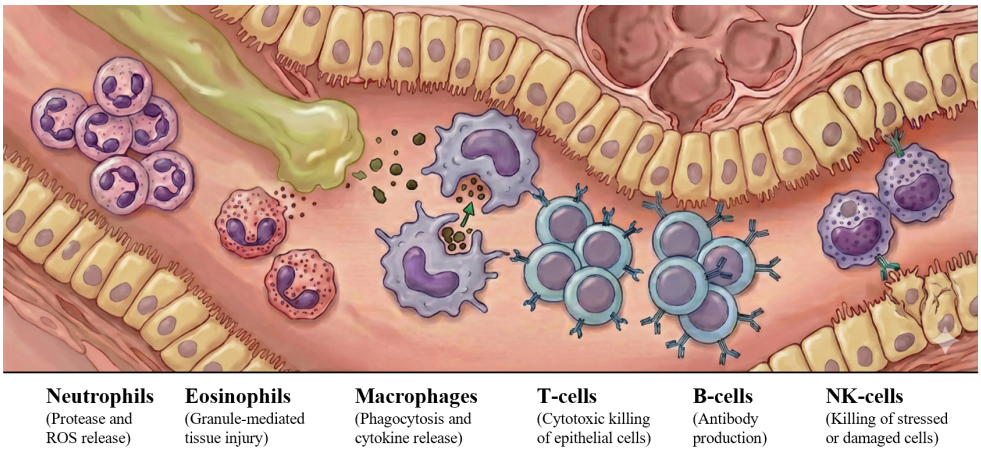

Figure 3: Inflammatory cell profile in COPD
Schematic representation of the chronic inflammatory response in the peripheral airways, involving neutrophils, eosinophils, macrophages, T cells, B cells and NK cells. The interaction between these cells and the epithelium drives the progressive airflow limitation characteristic of COPD. Figure generated using Gemini 3 Pro.

Neutrophils

Neutrophils have long been considered a key inflammatory cell in COPD, with increased sputum neutrophils being the characteristic feature of many individuals. Neutrophilic inflammation is commonly driven by cigarette smoke, infections, and oxidative stress[67]. Neutrophil recruitment to the airways involves initial adhesion to endothelial cells through E-selectin (which is upregulated in individuals with COPD) and migration guided by chemotactic mediators such as LTB_4 and CXCL8. Once recruited into the airways, neutrophils are activated and release serine proteases, including neutrophil elastase (NE), which may contribute to alveolar destruction[83]. Neutrophils from individuals with COPD show increased migratory speed but reduced accuracy compared to controls[84].

Neutrophils typically have a segmented nucleus with distinct lobes; this morphology reflects maturation and enables flexibility during migration. Mature neutrophils typically exhibit 3–5 lobes[85]. In inflammatory conditions, cells with more than four lobes (hypersegmented) have been identified. These subsets show enhanced oxidative burst and functional activation compared with neutrophils with fewer lobes[86].

Although neutrophils play an active role in COPD, targeting neutrophil-driven inflammation (anti-IL-8 and anti-CXCR2)[87,88] and pro-inflammatory cytokines (e.g., anti-TNFα and IL-1) has not been clinically effective[89,90]. This highlights COPD heterogeneity and indicates that more evidence is needed to improve mechanistic understanding and treatment strategies.

Eosinophils

In recent years, eosinophils have received increasing attention in the context of COPD. A subset of individuals with COPD displays an inflammatory profile characterised by elevated numbers of eosinophils and type 2 innate lymphoid cells (ILC2s), similar to what is observed in asthma[74]. Several studies have shown that blood eosinophil count can help predict the magnitude of the response to inhaled corticosteroids (ICS) in preventing future exacerbations[91–93].

Beyond their role as biomarkers, accumulating evidence indicates that eosinophils infiltrate lung tissue in COPD and that their numbers increase with disease severity. Blood eosinophil counts have been shown to correlate with structural lung damage, such as emphysema, and with reduced diffusing capacity for carbon monoxide (DLCO) in individuals with thin airway walls[94]. However, it remains debated whether eosinophils actively contribute to COPD pathogenesis or whether patients with higher eosinophil counts represent a subgroup with asthma-like features rather than "pure" COPD.

Biologic therapies targeting eosinophilic inflammation have been evaluated in COPD with varying results. Some trials have demonstrated a modest reduction in exacerbation rates among individuals with higher blood eosinophil counts, but the overall clinical benefit has been limited and inconsistent between studies[76,95].

Macrophages

Macrophages are effector cells in COPD, and their numbers are markedly increased in the lungs of individuals with COPD. They often accumulate at sites of alveolar wall destruction, which has been correlated with disease severity and the degree of emphysema[96]. These cells are characterised by the production of pro-inflammatory mediators in response to inflammatory stimuli, including cigarette smoke, and may contribute to the elevated concentrations of TNF-α seen in COPD and smokers. Alveolar macrophages also express transforming growth factor beta (TGF-β), which contributes to fibrosis in small airways[97]. Despite the abundance of macrophages in COPD, they show impaired phagocytic ability, leading to defective clearance of bacteria and apoptotic cells. This, in turn, may promote recurrent infections and exacerbations[98]. Macrophages also comprise distinct inflammatory and reparative subsets and exhibit metabolic and epigenetic reprogramming. This includes mitochondrial dysfunction and altered DNA methylation, which may sustain their pro-inflammatory state[98,99].

Attempts have been made to target CCR2, a receptor that recruits monocytes into inflamed tissue. The drug tested appeared to cause a slight decrease in bronchoalveolar lavage (BAL) monocytes after lipopolysaccharide (LPS) challenge, but no formal results have been published[100].

T cells

T cells are involved in chronic inflammation in COPD and can have both pro- and anti-inflammatory roles. $CD8^+$ T cells (cytotoxic T cells) are overrepresented in the lungs of individuals with COPD and contribute to alveolar damage and inflammation. $CD4^+$ T cells, including regulatory T cells (Tregs), play a complex role in COPD: some subsets drive inflammation, whereas others, such as T regs, are vital for immune tolerance. Treg function is impaired in COPD, which could contribute to disease progression[101,102].

B cells

B cells play a complex and often detrimental role in COPD and may contribute to inflammation and alveolar damage through the accumulation of abnormal B cells and autoantibody production. In severe COPD, the number and size of B cell-rich lymphoid follicles in the lung increase. B cells produce pro-inflammatory molecules that promote expansion of these lymphoid follicles by increasing B cell survival. Some B cell functions are protective against infections during exacerbations, but persistent pathogenic B cell responses have been linked to disease progression and emphysema[103].

NK cells

Natural killer (NK) cells play a complex role in COPD, with different functions in the blood and lungs. In the airways, NK cells show increased cytotoxicity, which may contribute to lung tissue destruction and emphysema. In contrast, blood NK cells have been associated with greater susceptibility to COPD exacerbations. This dysregulation of NK cells contributes to inflammation in COPD and may aggravate disease progression. NK cells are commonly divided into two main subsets, $CD56^{dim}$ and $CD56^{bright}$, where $CD56^{bright}$ represents the subset with a greater capacity to produce pro-inflammatory cytokines. $CD56^{dim}$ cells comprise about 90% of peripheral blood NK cells and can induce antibody-dependent cellular cytotoxicity[104].

Asthma

Asthma is among the most common chronic respiratory diseases in low- and middle-income countries and often starts in childhood. Around 260 million people worldwide have asthma, which contributes to a substantial economic burden on society[105,106]. Childhood asthma often begins with wheezing during the preschool years and is more common in boys[107], although symptoms may resolve over time[108]. Adult-onset asthma is often more severe, with a higher symptom burden and greater medication requirements[107]. This form of asthma is more common in females, and

poor asthma control is an independent risk factor for developing fixed airflow obstruction (Figure 4)[109,110].

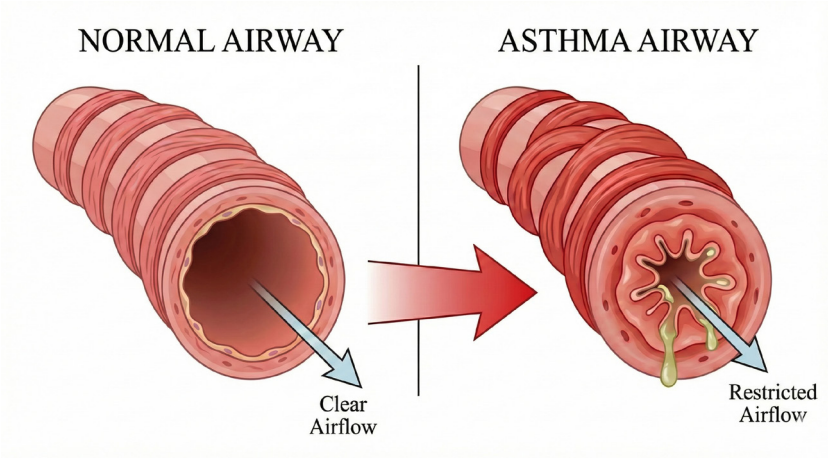

Figure 4: Asthma airway pathology
The left panel depicts a normal bronchiole with a widely patent lumen and relaxed smooth muscle, allowing for clear airflow. The right panel illustrates the pathophysiological changes characteristic of asthma, specifically smooth muscle constriction (bronchospasm) and mucus hypersecretion. These mechanisms collectively narrow the airway lumen, resulting in the restricted airflow shown. Figure generated by Gemini 3 Pro.

Risk Factors

Asthma can develop across the lifespan through interactions between genetic susceptibility and modifiable environmental and lifestyle exposures. Across all ages, exposure to tobacco smoke (active smoking and second-hand smoke) and to vaping or e-cigarette use has been associated with an increased risk of new-onset asthma. Outdoor air pollution, particularly $PM_{2.5}$ and NO_2, is consistently linked to asthma incidence in both children and adults. Risk appears higher in urban environments, where dense development and limited greenery are important contributing factors. Overweight and obesity are also associated with increased asthma risk across age groups, with evidence that higher BMI trajectories through childhood are linked to later airway impairment. In addition, psychosocial stress and adverse experiences have been linked to asthma risk, with associations reported from childhood into adulthood.[105]

Against this background, several early-life exposures are particularly important for childhood wheeze or asthma. Prenatal and perinatal risk factors include maternal smoking, maternal obesity, stress, and medication use (for example, antibiotics or paracetamol) during pregnancy, as well as birth by caesarean section. During the

early postnatal and childhood period, higher risk has been associated with respiratory viral infections and exposure to indoor mould or fungi.[105]

In adulthood, occupational exposures are a major contributor to adult-onset asthma and can account for a substantial proportion of cases. Finally, emerging exposures linked to climate change, including changes in allergenic plant distribution, longer pollen seasons, and smoke exposure from wildfires or weather-related events, might contribute to asthma onset.[105]

Diagnosis

Despite asthma being a common respiratory disease, diagnosing asthma in adults remains challenging, resulting in both over- and under-diagnosis. Typical asthma features include breathlessness, wheeze, cough, and chest tightness, together with objective demonstration of variable airflow limitation. The 2022 European Respiratory Society (ERS) task force proposed an operational definition of asthma based on the presence of typical symptoms together with at least one objective demonstration of variable airflow limitation, applicable in both primary and secondary care[4]. The objectives included:

1) **Excessive variability in lung function**, demonstrated by peak expiratory flow variability ≥20% or a spontaneous increase in forced expiratory volume in 1 second (FEV_1) ≥12% and ≥200 mL
2) **Bronchodilator responsiveness**, defined as an improvement in FEV_1 of ≥12% and ≥200 mL after inhaled bronchodilator
3) **Airway hyperresponsiveness**, shown by a
    a. Methacholine or histamine test with a 20% fall in FEV_1.
    b. Mannitol challenge with a 15% fall in FEV_1.
    c. Exercise challenge with a with a ≥10% fall in FEV_1.
4) **Response to anti-inflammatory treatment**, with an improvement in FEV_1 ≥12% and ≥200 mL following a 2-week course of oral corticosteroids or a 4–6-week course of inhaled corticosteroids.

Prevalence

Asthma remains a substantial health burden among adults worldwide. One analysis estimated that around 260 million people were living with asthma in 2021. Importantly, age-specific patterns show that although asthma peaks in childhood, prevalence continues to rise in later adulthood, and between 25 and 69 years the prevalence rate increases with age. The sex pattern also shifts, from higher

prevalence in boys to higher prevalence in adult females[106]. Although asthma is a relatively uncommon direct cause of death overall, it still contributes to substantial preventable mortality, with approximately 461,000 deaths globally in 2019. Most certified asthma deaths occur in older adults, with death rates rising steeply with age[111].

In Sweden, asthma is also common in adulthood. Swedish clinical and public health summaries commonly report that asthma affects around 10% of the population, reflecting a high prevalence in a high-income setting[112].

Treatment

Asthma treatment is organised into two tracks, each with five steps, as recommended by the Global Initiative for Asthma (GINA, Figure 5). Treatment is guided by disease severity and level of control, with each step involving escalation of inhaled corticosteroids (ICS) and/or add-on therapy. Add-on options include short-acting beta agonists (SABA), long-acting beta agonists (LABA), and long-acting muscarinic antagonists (LAMA)[113].

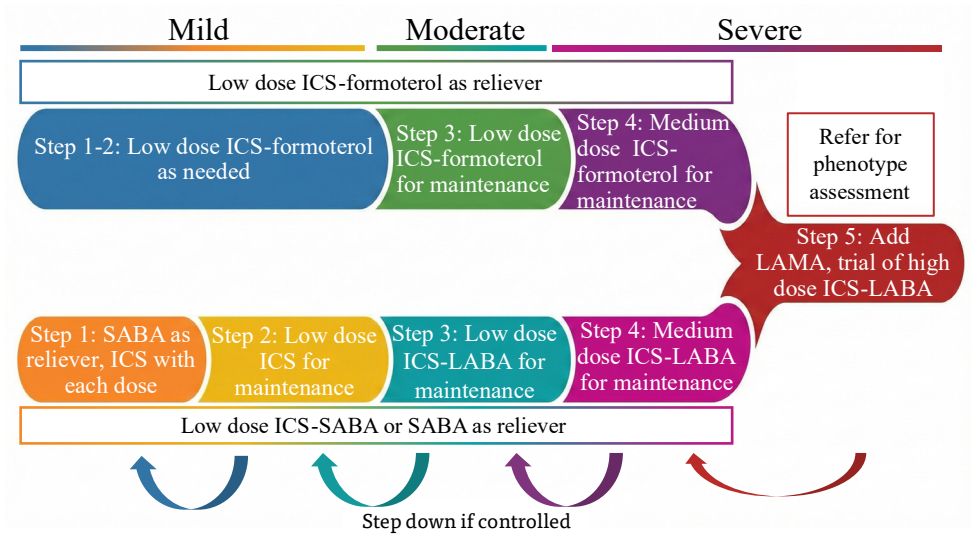

Figure 5: GINA asthma treatment steps
Track 1 (top), the preferred track with five different steps of asthma treatment with increasing severity. Track 2 (bottom), an alternative track with five steps where patients need to adhere to daily controller treatment. ICS: Inhaled corticosteroids, SABA: short-acting β_2-agonist, LABA: long-acting β_2-agonist, LAMA: long-acting muscarinic antagonist. Figure created by the author.

Severe asthma is defined as asthma that is uncontrolled (poor symptom control and frequent exacerbations) despite good adherence to maximally optimised high-dose

ICS–LABA treatment and management of contributory factors, or that worsens when high-dose treatment is reduced[113,114]. Individuals with severe asthma may be eligible for biological treatment after phenotypic assessment, targeting IgE, IL-5, IL-4, IL-13, TSLP, and IL-33[115,116].

Phenotypes and Endotypes

Clinically, asthma is a heterogeneous disease with several phenotypes (clinical patterns) including:

Allergic asthma, the most common form of asthma, often with onset in childhood. It is characterised by allergic sensitisation and high concentrations of eosinophils in the airways[117].

Late-onset eosinophilic asthma, which develops in adulthood and is often severe. It is also often steroid-resistant, and symptoms may not improve adequately despite high doses of inhaled corticosteroids (ICS)[118].

Obesity-related asthma, which has onset in adulthood and is often associated with a high symptom burden and airway hyperresponsiveness, however, this can be reversed with weight loss[119].

Neutrophilic asthma is characterised by low FEV_1, increased air trapping, and sputum neutrophilia. This phenotype is often severe and responds poorly to inhaled corticosteroids (ICS). However, experts have debated whether higher neutrophil counts define a distinct phenotype, as some studies claim that neutrophilia is not more common in asthma than in healthy controls[120]. Other studies suggest that neutrophils may be more pathogenic in this subgroup, which may also show higher bacterial burden and poorer response to standard therapies[121].

Alongside phenotypes, asthma can also be described in terms of endotypes, which are defined by distinct functional or pathobiological mechanisms rather than observable symptoms alone. These include:

Type 2 driven inflammation, the most common asthma endotype, characterised by increased activity of the type 2 immune system, leading to high numbers of eosinophils and inflammatory markers such as IL-4, IL-5, and IL-13[122]. Active eosinophilic inflammation is associated with recurrent asthma exacerbations and irreversible lung function decline. Biomarkers for this endotype include high blood eosinophil counts and fractional exhaled nitric oxide (FeNO)[123].

Non type 2 inflammation, a broader term indicating that inflammation is not type 2-driven but instead reflects other mechanisms, such as neutrophilic or Th17-driven pathways. Th17 cells can release IL-17, which promotes neutrophil infiltration[124]. Non-type 2 inflammation can also be linked to obesity or IL-6, and acts as a mechanistic umbrella for asthma not classified as type 2[125].

Small Airways Disease

Asthma is sometimes referred to as a small airways disease, where the small airways have an internal diameter of <2 mm[126]. The small airways are sometimes known as the "silent zone" because damage is often undetected by standard pulmonary function tests, such as spirometry, until it has become significant[126]. Small airway dysfunction is a major contributor to airflow limitation in asthma and is associated with poor asthma control, more severe bronchial hyperresponsiveness, and higher risk of exacerbations[127]. Structural changes include increased mucus production, increased numbers of goblet cells, thickening of the reticular basement membrane and airway smooth muscle, and changes in the extracellular matrix[128].

Exacerbations

Individuals with severe asthma are more likely to experience an exacerbation, a sudden worsening of symptoms such as shortness of breath, wheeze, cough, and chest tightness. This is often triggered by viral respiratory infections, smoking, allergen exposure, food allergy, air pollution, seasonal changes, or poor adherence to ICS[2,129]. A severe exacerbation can be life-threatening and may necessitate hospitalisation and, in some cases, mechanical ventilation[2].

Immune Cells

Immune cells are central to asthma pathobiology, releasing inflammatory mediators and extracellular traps that damage airway epithelial cells and further activate other immune cells[130]. The relative contribution of different immune cell types varies across asthma phenotypes and endotypes, influencing exacerbation risk and treatment responses. A schematic overview of immune cells in asthma is shown in Figure 6.

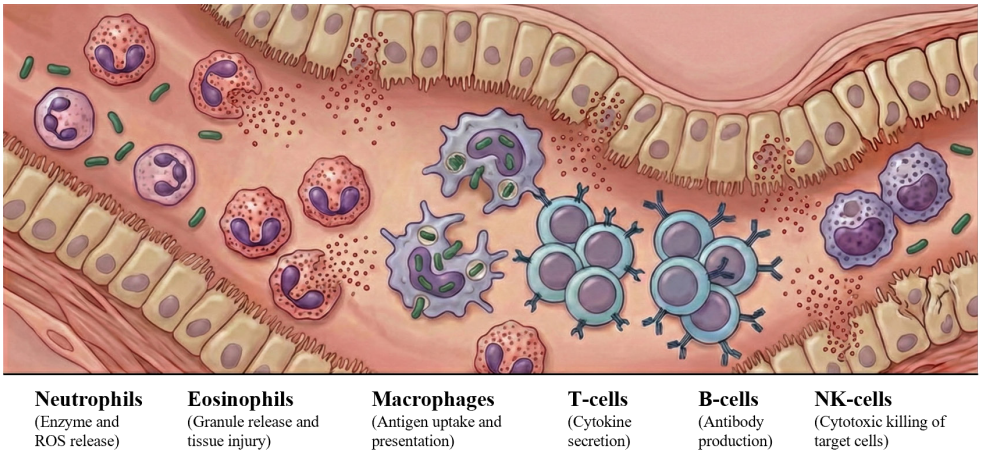

Figure 6: Inflammatory cell profile in asthma
Schematic representation of the inflammatory cascade in an asthmatic bronchiole. Inflammatory cells such as neutrophils, eosinophils, macrophages, T cells, B cells, and NK cells all contribute to ongoing inflammation and structural changes within the airway wall. Figure generated using Gemini 3 Pro.

Neutrophils

Neutrophils have been a debated topic in asthma but are increasingly recognised as important contributors to a subset of the disease characterised by airway neutrophilia, frequent exacerbations, and reduced responsiveness to corticosteroids[121]. Neutrophils are typically the primary defence against infection, but in asthma their function becomes dysregulated, leading to airway pathology that may worsen with age. Neutrophils migrate rapidly to the lungs but exhibit impaired phagocytosis, which can result in bacterial accumulation in the airway. Instead of resolving infection, neutrophils in asthma release cytotoxic substances, including reactive oxygen species, neutrophil extracellular traps (NETs), and elastase, which can cause "bystander" damage to host tissue. Neutrophil elastase release has been linked to mucus hypersecretion, airway smooth muscle proliferation, and structural remodelling, all of which contribute to airflow obstruction in severe asthma.[121]

Eosinophils

Eosinophils are key contributors to type 2 inflammation in asthma, where IL-5-dependent pathways promote eosinophil differentiation, survival, and mobilisation to the airways[131]. In the lungs of individuals with asthma, eosinophils release cytotoxic granules, cytokines, and lipid mediators that contribute to epithelial damage, airway hyperresponsiveness, mucus plugging, and structural remodelling[131,132]. Higher blood or sputum eosinophil counts are associated with increased exacerbation risk and better response to type 2-targeted biologics[132]. Recent work also highlights functional heterogeneity among eosinophil populations, suggesting that eosinophils may contribute differently across asthma phenotypes.

Human eosinophil subsets remain poorly defined, but some evidence suggests that eosinophils comprise heterogeneous populations with distinct developmental programmes and activation states[133].

Macrophages

Macrophages play a complex role in asthma by contributing to both inflammation and its resolution. They are often broadly classified into two phenotypes: one associated with production of pro-inflammatory cytokines, and another associated with antiparasitic functions, immune resolution, and tissue remodelling[134]. Macrophages can promote inflammation through secretion of pro-inflammatory cytokines, which can also recruit other immune cells[135]. In asthma, airway macrophages exhibit impaired phagocytic capacity, reduced clearance of apoptotic cells and pathogens, and an altered inflammatory profile marked by increased production of IL-1β, IL-6, IL-8, and TNF-α[135]. In severe asthma, circulating monocytes show altered differentiation patterns, developing into more profibrotic macrophage-like cells and producing increased amounts of monocyte-derived TGF-β1, linking blood monocytes to airway structural changes and disease severity[136].

T cells

T cells are implicated in asthma through allergen-specific $CD4^+$ T helper cells, which drive chronic airway inflammation, IgE production, and structural remodelling through release of Th2 cytokines[137]. T helper 2 (Th2) cells are key regulators of the type 2 immune response seen in type 2 asthma[138]. They produce cytokines such as IL-4, IL-5, and IL-13, promoting eosinophil recruitment, mucus hypersecretion, airway hyperresponsiveness, and tissue remodelling[138–140]. In contrast, Th1 and Th17 responses are more prominent in non-type 2 (neutrophilic) asthma, where IL-17 is linked to steroid-resistant inflammation. Regulatory T cells (Tregs) normally exert inhibitory effects by producing anti-inflammatory cytokines[140].

B cells

B cells play a pivotal role in asthma and are traditionally recognised for their capacity to produce IgE antibodies upon allergen exposure, which is required to initiate the allergic cascade[141,142]. However, research has revealed significant alterations in B cell populations beyond IgE production in individuals with asthma. IgA+ memory B cells are increased in individuals with asthma, particularly in those with impaired lung function and small airway dysfunction (SAD)[141]. This association suggests that IgA^+ memory B cells may contribute to inflammation in the peripheral lung. In contrast, regulatory B cells (Bregs), which produce anti-inflammatory cytokines such as IL-10 to regulate immune responses, are often decreased in both adults and children with allergic asthma, suggesting impaired suppression of type 2 inflammation[142].

NK cells

Natural killer (NK) cells are innate immune cells that are abundant in the lung, but their involvement in asthma is complex, and they have been suggested to act as both disease promoters and suppressors[143]. NK cells exhibit phenotypic and functional changes in asthma, notably showing a reduced ability to induce eosinophil apoptosis in severe asthma[144,145]. Some findings point towards a pro-inflammatory role, where NK cells are activated by IgE and produce cytokines and chemokines, alongside a reported shift towards IL-4-expressing subsets in allergic asthma[146].

ADAM17

A disintegrin and metalloproteinase 17 (ADAM17), also known as tumour necrosis factor α-converting enzyme (TACE), is a transmembrane protease that plays a vital role in shedding cell surface proteins and regulating signalling pathways. It plays a central role in the pathophysiology of several diseases, and dysregulated ADAM17 expression has been linked to a wide range of autoimmune and inflammatory diseases, cardiovascular disease, and cancer[7].

Structure

ADAM17 consists of 824 amino acids and comprises several domains, shown in Figure 7: the pro-domain (green), metalloprotease domain (red), disintegrin domain (purple), membrane-proximal domain and CANDIS (blue), transmembrane domain (yellow), and cytoplasmic domain (orange)[147].

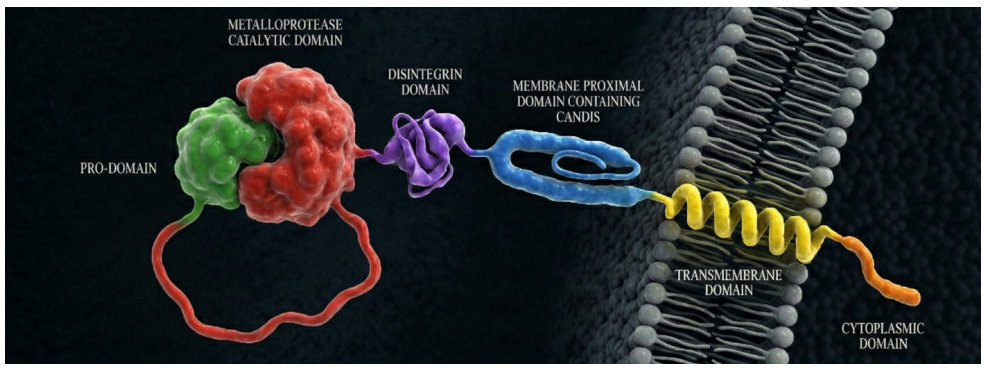

Figure 7: Molecular domains of ADAM17
Schematic representation of the domains of a disintegrin and metalloproteinase 17 (ADAM17), from left to right: prodomain (green), metalloprotease catalytic domain (red), disintegrin domain (purple), membrane-proximal domain containing CANDIS (blue), transmembrane domain (yellow), and the cytoplasmic domain (orange). Figure generated using Gemini 3 Pro.

The pro-domain acts as an inhibitor and is essential for proper folding and maturation of the protein, it is removed during ADAM17 maturation[148].

The metalloprotease (catalytic) domain is the main catalytic region of ADAM17 and requires zinc for its proteolytic activity and cleavage of peptide bonds. Processing of many membrane-bound proteins depends on this domain, including conversion of proTNF-α to its soluble form[149,150].

The disintegrin domain can bind integrins, allowing ADAM17 to mediate cell adhesion with neighbouring cells. It can also activate various receptors, initiating several cell signalling pathways[150].

The membrane-proximal domain contains a highly conserved sequence called CANDIS, which interacts with cell membranes and plays a significant role in ADAM17 function, particularly in substrate recognition and regulation of enzyme activity[151].

The transmembrane domain anchors ADAM17 in the cell membrane and interacts with inactive rhomboid proteins 1 and 2 (iRhom1 and iRhom2), which are crucial for ADAM17 maturation and function. This domain is also necessary for TNF-α shedding[150].

The cytoplasmic domain serves as a key interface for protein–protein interactions, allowing ADAM17 to translate extracellular signals into intracellular responses. It also contains several phosphorylation sites, such as threonine 735 (Thr^{735}), and signalling motifs suggested to play important roles in ADAM17 function, regulation, transport to the cell surface, and recycling. Truncation of the cytoplasmic tail has been shown to reduce ADAM17 substrate shedding in mouse embryonic fibroblasts.[152]

Maturation

For the production of mature ADAM17, removal of the pro-domain by proprotein convertases such as furin is necessary[153], although pro-domain removal does not appear to be required for ADAM17 catalytic activity[154,155]. ADAM17 activity is tightly regulated through various mechanisms, including post-translational modifications such as phosphorylation, conformational changes, and interactions with numerous cellular signalling molecules[7]. Inactive rhomboid proteins (iRhoms) have recently been identified as key regulators of ADAM17 maturation, activation, stability, membrane trafficking, and substrate regulators[156–158]. Mechanistically, mitogen-activated protein kinase (MAPK)-dependent phosphorylation of the iRhom2 cytoplasmic tail weakens its association with ADAM17, thereby enhancing ADAM17 shedding activity[158].

Activity

Phosphorylation and protein–protein interactions within the cytoplasmic tail of ADAM17 have been implicated in regulation of its activity. Removal of this domain in mice caused a partial loss of ADAM17 function due to reduced EGFR signalling, indicating that the cytoplasmic tail contributes to optimal ADAM17 activity[152]. Although phosphorylation of ADAM17's cytoplasmic tail has long been considered an activation step, the cytoplasmic tail has been shown to be dispensable for post-translational activation, suggesting that phosphorylation is not required for ADAM17-induced activity[154,159]. The most studied phosphorylation site on ADAM17 is threonine 735 (Thr^{735}). This phosphorylation is mediated by mitogen-activated protein kinases (MAPKs) and has been shown to enhance ADAM17 shedding of several substrates[160,161].

ADAM17 Substrates

At least 90 substrates have been attributed to ADAM17, and the enzyme is also involved in immune regulation and substrate-mediated signal transduction[162].

The ADAM17 substrates transforming growth factor alpha (TGF-α) and heparin-binding epidermal growth factor (HB-EGF) have been shown to activate the EGFR pathway[163]. Many inflammatory cells produce these ligands, including eosinophils[164,165], macrophages[166,167], neutrophils[168], and T cells[169]. Activation of this pathway by HB-EGF induces phosphorylation of extracellular signal-regulated kinase (ERK) and p38 in lung fibroblasts, in a dose-dependent manner. HB-EGF also promotes proliferation, migration, and collagen secretion in lung fibroblasts, which may enhance airway remodelling in chronic respiratory diseases[170].

TNF-α is one of the best-known substrates of ADAM17 and a crucial inflammatory cytokine that is elevated in chronic respiratory diseases such as COPD and asthma, where it drives inflammation, recruits immune cells, causes oxidative stress, and promotes airway wall thickening[171,172]. TNF-α signals through two distinct receptors: TNF receptor 1 (TNFRI), which is widely expressed and can trigger apoptosis and inflammation[173], and TNF receptor 2 (TNFRII), which has more restricted expression and primarily mediates immune homeostasis, tissue healing, and cell survival[174,175]. ADAM17 shedding of TNF-α generates soluble TNF, which can engage TNFRI and TNFRII. ADAM17 can also shed the extracellular domains of the receptors themselves, modulating receptor availability and downstream signalling[176].

ADAM17 also generates soluble IL-6 receptor (sIL-6R), enabling IL-6 signalling even in cells that lack membrane-bound IL-6R[177]. This pathway is strongly associated with chronic inflammation and airway remodelling in diseases such as asthma and COPD[178,179].

ADAM17 also sheds several adhesion molecules, including L-selectin (CD62L) and intercellular adhesion molecule 1 (ICAM-1). CD62L is expressed on leukocytes and is essential for initiating leukocyte rolling and trafficking to lymph nodes and sites of inflammation[180]. ICAM-1 stabilises leukocyte–endothelial interactions to promote firm adhesion and transmigration of leukocytes to inflamed tissue[181]. Soluble ICAM-1 (sICAM-1), generated by proteolytic shedding (including by ADAM17), is elevated in inflammatory diseases such as asthma and COPD and can exert both pro- and anti-inflammatory effects[182]. At low concentrations, sICAM-1 can stimulate cytokine production via NF-κB and ERK, whereas at higher concentrations it can inhibit leukocyte–endothelial interactions and promote pro-repair immune activity[182].

ADAM17 in inflammation

Inflammatory mediators drive a wide range of physiological and pathophysiological processes, including tissue damage and remodelling, metabolism, cancer, and infections[183]. ADAM17 has been implicated in many inflammatory disorders, mainly through shedding cytokines, chemokines, and adhesion molecules, as well as modulating the functions of various immune cells[184]. The expression and/or activation of ADAM17 is elevated in biopsies from individuals with inflammatory conditions such as COPD or emphysema[185]. Mouse studies inhibiting ADAM17 using different approaches have shown that targeting ADAM17 could be therapeutically beneficial in immune-based disorders[185–187].

Role of ADAM17 in COPD

ADAM17 is an important regulator of airway inflammation and tissue injury in COPD, particularly in the context of cigarette smoke exposure[185]. In primary bronchial epithelial cells from individuals with COPD, cigarette smoke increases ADAM17 activation and shedding of substrates such as interleukin-6 receptor (IL-6R) and amphiregulin, amplifying IL-6 trans-signalling and EGFR-driven epithelial responses[188]. This pathway is associated with chronic inflammation, mucus hypersecretion, and epithelial remodelling in COPD airways[189,190].

Beyond the conducting airways, ADAM17 also appears to contribute to emphysema. Lung tissue from individuals with COPD, as well as emphysema-prone mouse models, shows increased ADAM17 expression or activation. Genetic reduction of ADAM17 in mice protects against emphysema development, supporting ADAM17's role in structural lung damage[185]. While ADAM17 can regulate inflammatory cell trafficking through shedding adhesion molecules such as L-selectin, its contribution to COPD pathogenesis appears to be dominated by epithelial-derived cytokine and growth factor signalling[180,190].

Role of ADAM17 in Asthma

ADAM17 has increasingly been recognised as a regulator of airway inflammation and structural remodelling in asthma, with deletion or pharmacological inhibition reducing type 2 airway inflammation and airway hyperresponsiveness in mouse models[191]. Most studies of ADAM17 in asthma have used animal models or cell lines, and many studies have focused on ADAM17-derived substrates, such as TNF, without necessarily linking them directly to ADAM17[192]. Overexpression of ADAM17 or phosphorylated ADAM17 has been demonstrated in human fibroblasts from individuals with chronic obstructive asthma, suggesting that ADAM17 contributes to subepithelial fibrosis in asthma[193]. In these cells, profibrotic mediators such as hypoxia, TGF-β, and thrombin induce ADAM17-dependent shedding of EGFR ligands and activation of downstream ERK signalling, leading to increased connective tissue growth factor (CTGF) expression and collagen production[194–196].

In addition to its role in fibroblasts, ADAM17 has also been shown to influence immune cell function in asthma mouse models[191,197]. Airway epithelial cells shed syndecan-1 and syndecan-4 through ADAM17-dependent mechanisms, linking the enzyme to epithelial barrier dysfunction and leukocyte recruitment during inflammation[198,199]. Alveolar macrophages from asthma mouse models exhibit TNF-α-induced upregulation of ADAM17, resulting in reduced CD36 expression and impaired phagocytic efficiency, effects that are reversed by ADAM17 knockdown[200].

Aims

The overall aim of this thesis was to explore key aspects of immunity and inflammation in chronic respiratory diseases such as COPD and asthma, with a focus on ADAM17 and its substrates across airway and systemic compartments. It also aimed to characterise how ADAM17-related cellular phenotypes and soluble substrate patterns vary with disease context, airway region, and exercise. The specific aims of the different studies included in the thesis were as follows:

I. To map ADAM17 expression and associated soluble substrate patterns in COPD in bronchoalveolar lavage (BAL) cells, BAL fluid (BALF), and blood, and to distinguish COPD-related changes from those related to smoking.

II. To profile ADAM17-associated soluble substrates across the upper (nasal lavage) and lower (sputum and bronchoalveolar lavage) airways, as well as in blood, in individuals with mild to severe asthma and controls, and to assess how these patterns vary by compartment and disease severity.

III. To determine how a 12-week supervised exercise programme alters circulating immune cell subsets positive for ADAM17 and phosphorylated ADAM17 (pADAM17) in individuals with COPD, and to explore whether these changes differ between males and females.

IV. To spatially characterise cell type-enriched protein signatures in BAL cytospins using NanoString GeoMx, and to identify where within the BAL sample the strongest disease-related differences between COPD and asthma are concentrated, with a focus on macrophages, neutrophils, lymphocytes, and Siglec-8^+ cells.

Methodology

Study populations

BREATHE

In a larger cross-sectional study (n=1492), participants with COPD, asthma and controls were recruited[201]. Participants with other respiratory diseases were excluded. A subset was used in these studies. Controls were classified as Never Smokers and Ever Smokers (≥10 pack-years; current smokers or ex-smokers who quit ≥6 months prior). All available COPD and asthma participants from the Lund cohort, together with matched controls, were included (matching described in the Supplementary Material for Papers I and II). Blood was collected from all participants, while a subgroup (~⅓) underwent bronchoscopy for bronchoalveolar lavage (BAL) and biopsy collection. Baseline characteristics are presented in the individual papers.

- Paper I: BAL: COPD, Ever Smokers, Never Smokers: n=14, 12, 11, blood: n=30, 28, 10
- Paper II: BAL: asthma, controls n=11, 7, blood: n=28, 25
- Paper IV: BAL: COPD, smokers n=9, 7 and asthma, healthy: n=14, 7

SEVA

This study included participants with severe asthma (Paper II), defined according to the 2025 GINA guidelines[113], and controls with no history of respiratory disease. Blood samples were collected from all participants except one (asthma, controls n=12, 9). A subset also provided induced sputum (asthma, controls n=13, 6). Baseline characteristics are presented in Table 2 in Paper II.

Se-NAL

This was a sub-study of SEVA and included participants with predominantly mild asthma[113] and controls with no prior history of respiratory disease. Blood samples (asthma, controls n=11, 11) and nasal lavage (NAL: asthma, controls: n=12, 12) were collected. Baseline characteristics are presented in Table 3 in paper II.

T-REX

Participants with COPD were recruited and screened by a physician to confirm diagnosis according to GOLD criteria[3]. Inclusion criteria included ex-smoker status (≥10 pack-years; ≥1 year since cessation), diagnosis >1 year, a sedentary lifestyle (<2 exercise sessions/week), and clinical stability (≤1 exacerbation/year), as defined in the study protocol[202]. Participants completed a supervised 12-week exercise intervention (combined resistance and endurance training; three sessions/week). Of 33 participants enrolled, 24 completed the intervention; two were excluded due to differences in the cryopreservation protocol, leaving 22 paired pre- and post-intervention samples for analysis. Non-COPD controls (n=8; four ex-smokers and four current smokers) from another study, with no history of respiratory disease, provided baseline comparisons for ADAM17 and pADAM17 expression. Baseline characteristics are presented in Table 1 in Paper III.

Ethics

All the studies were approved by the Regional Ethical Review Board in Lund, Sweden / the Swedish Ethical Review Authority and followed the Declaration of Helsinki. All participants included in the studies gave written informed consent.

BREATHE: 2016/1069

SEVA (Se-NAL): 2020-07261

TREX: 2022-00831-01

Lung physiology

Spirometry was performed to measure FEV_1 and FVC using either a Jaeger MasterScope spirometer (Erich Jaeger GmbH, Würzburg, Germany) or a Medisoft spirometer (Sorinnes, Belgium). Percent predicted values for FEV1 and FVC were calculated using Global Lung Initiative reference equations[203].

Sample collection

Many different samples were used across the papers. An overview is provided in Table 1.

Table 1: Samples used in different papers
Overview of the different sample types used in the papers.

	Paper I	Paper II	Paper III	Paper IV
BAL	✓	✓	X	✓
Biopsies	X	✓	X	X
Sputum	X	✓	X	X
NAL	X	✓	X	X
Plasma/Serum	✓	✓	X	X
PBMC	X	X	✓	X

✓: used in paper, X: not used in paper. BAL: Bronchoalveolar lavage, NAL: Nasal lavage, PBMC: Peripheral blood mononuclear cells separated by Ficoll

Bronchoscopy

Biopsies were taken from the lung subcarina, fixed in 4% formaldehyde, and processed as previously described[204].

BAL was collected from the right middle lobe (2 × 50 mL PBS), filtered, and centrifuged (400 × g, 10 min). The supernatant was stored at −80°C, and the cell pellet was resuspended and cytospun onto SuperFrost Plus slides.

Sputum processing

Sputum was induced as previously described[205]. Mucus plugs were isolated and incubated with Sputolysin (diluted 1:10 in PBS) at a 1:4 ratio for 60 min at 4°C. The suspension was diluted with PBS, filtered (60 µm), and centrifuged (200 × g, 5 min). Supernatants were stored at −80°C, while the cell pellet was resuspended and cytospun onto slides.

Blood sampling

Differential immune-cell counts were analysed in EDTA-treated whole blood samples from all asthma, COPD, and control groups at the accredited Clinical Chemistry routine laboratory at Skåne University Hospital, Lund. These data were used only in Papers I and II. The lower limit of detection was 0.1×10^9 cells/L, values below this were set to half the detection limit (0.05×10^9 cells/L) for plotting and eosinophil analyses.

In addition, plasma was isolated from EDTA-treated whole blood, and serum was prepared from whole blood allowed to clot for 30 min at room temperature. Both were centrifuged at 2000 × g for 10 min, aliquoted, and stored at −80°C.

Ficoll processing

Leukocytes were isolated from EDTA-treated whole blood (6 mL), diluted to 20 mL with PBS, and layered over 15 mL Cytiva Ficoll-Paque PLUS (Uppsala, Sweden). After centrifugation (450 × g, 30 min, light brake), plasma was discarded. The mononuclear cell layer and the Ficoll layer were collected, washed twice with PBS (400 × g, 10 min), and counted using a CellDrop automated cell counter (DeNovix). Cells were resuspended in freezing medium, frozen slowly in a foam box, and stored at −80°C.

Cell count and staining

Airway cell differential count

Live leukocytes were quantified manually using trypan blue exclusion in a Bürker chamber. Slides were stained with Kwik-Diff (Richard-Allan Scientific, USA), scanned at 40×, and analysed using Aperio ImageScope (Leica Biosystems). Differential counts were performed on 400 cells per participant, classified as macrophages, neutrophils, eosinophils, or lymphocytes (COPD; Paper I), or as neutrophils, eosinophils, or “other” (asthma; Paper II).

Immunofluorescence

Immunofluorescence staining for ADAM17 and pADAM17 was performed on bronchial biopsy sections (5 μm) from asthma participants in the BREATHE cohort (including controls) and on BAL cytospins from COPD participants in the BREATHE cohort (including never-smokers and ever-smokers). Due to sample availability in the BREATHE COPD cohort, additional participants were included as detailed in Supplementary Table S1 for Paper I.

Reagents: Primary antibodies against the ADAM17 ectodomain and phosphorylated ADAM17 (pADAM17) were used, followed by fluorescent secondary antibodies.

Staining protocols

- Biopsies: Freshly sectioned slides were baked, underwent heat-induced epitope retrieval, and were blocked for 10 min. Slides were incubated with primary antibodies for 1 h at room temperature. After washing, secondary antibodies were applied for 1 h at room temperature.
- BAL cytospins: Slides were permeabilised, rinsed, and blocked for 60 min at room temperature. Primary antibodies were incubated overnight at 4°C, followed by secondary antibodies for 3 h at room temperature.

Imaging and analysis: All slides were mounted and stored at −20°C until imaging. Biopsy and BAL slides were analysed for positive staining in bronchial cells or cilia, and results were reported as the percentage of positive cells among all cells.

Flow cytometry and cell markers

Frozen peripheral blood mononuclear cells (PBMCs) from each participant and visit were rapidly thawed in room-temperature PBS, washed once more with PBS, and resuspended in flow cytometry staining (FACS) buffer (PBS supplemented with foetal bovine serum, prepared in-house). Cells were transferred to a 96-well plate, where an anti-ADAM17 ectodomain antibody and a secondary antibody were added and incubated for 15 min at room temperature. Following washing, cells were incubated for 30 min with the following surface antibodies: CD3, CD56, CD19, CD14, and a fixable viability stain, in 50 µL FACS buffer.

Cells were washed, fixed, and permeabilised according to the manufacturer's instructions. Intracellular staining for pADAM17 and a secondary antibody was carried out in 50 µL FACS buffer. After washing, cells were resuspended in 100 µL FACS buffer and acquired on a flow cytometer.

Single-colour compensation controls were prepared by staining one tube per fluorochrome. Immune cell populations were defined based on marker expression profiles, as summarised in Table 2.

Table 2: Cell markers used to identify immune cells by flow cytometry
Markers used to identify immune-cell populations in PBMCs by flow cytometry.

Marker	T cells	NK cells	NKT cells	B cells	Monocytes	Undefined
CD3	+	-	+	-	-	-
CD56	-	+	+	-	-	-
CD19	-	-	-	+	-	-
CD14	-	-	-	-	+	-

+ Positive for the marker, - Negative for the marker

Soluble protein analysis

Enzyme-Linked ImmunoSorbent Assay (ELISA)

Soluble ADAM17 concentrations were measured using a human TACE/ADAM17 DuoSet ELISA kit (DY930, R&D Systems, Minneapolis, MN, USA) together with the DuoSet ELISA Ancillary Reagent Kit 2 (DY008B, R&D Systems), according to the manufacturer's instructions, except that the detection antibody was used at a ten-fold higher concentration to increase sensitivity. Serum samples were diluted 1:2 in reagent diluent prior to analysis, whereas airway samples were analysed undiluted.

Luminex Multiplex Assay

The Luminex Discovery Assay (LXSAHM, R&D Systems, Minneapolis, MN, USA) was used to quantify 29 ADAM17 substrates in all BALF samples and matched blood. Based on these results, a reduced 14-substrate panel was analysed in remaining airway samples (sputum and NALF) with matched blood, as well as in never-smokers compared with the COPD participants. Proteins referred to in the main text are listed in Table 3, the full protein list is provided in the Supplementary Tables in Papers I and II. Assays were run according to the manufacturer's instructions with overnight incubation at 4°C. Plasma was diluted 1:1 (sample:diluent) and airway samples 2:1 in reagent diluent.

Table 3: Proteins referred to in the main text
A total of 29 proteins were analysed using Luminex. To keep figures and interpretation concise, only the proteins referred to in the main text of this thesis are shown in this table.

Analyte	Full or Alternative name	Analyte	Full or Alternative name
APP	Amyloid beta precursor protein	MICB	MHC class I polypeptide–related sequence B
CD62L	L-selectin	MUC1	Mucin-1
CD44		Syndecan-1	
CD163		Syndecan-4	
EpCAM	Epithelial cell adhesion molecule	TGF-α	Transforming growth factor alpha
HB-EGF	Heparin-binding EGF-like growth factor	TIM1	T-cell immunoglobulin and mucin protein-1
IL-1RII	Interleukin 1 receptor, type II	TNF-α	Tumour Necrosis Factor-α
IL-6Rα	Interleukin 6 receptor alpha	TNFRI	TNF receptor 1
Lymphotoxin-α	Tumour necrosis factor-beta	TNFRII	TNF receptor 2
MICA	MHC class I polypeptide–related sequence A	TRANCE	TNF-related activation-induced cytokine

GeoMx analysis

Spatial protein profiling was performed using the NanoString GeoMx platform at the KIGene core facility (Karolinska Institutet, Stockholm, Sweden) on bronchoalveolar lavage cells preserved on cytospin slides (Figure 8). Cell types were segmented using a combination of antibody staining and morphology. Protein targets were quantified per cell type using antibody-based chemistry conjugated to NanoString nCounter barcodes via a UV-cleavable linker. For cell collection, the maximum GeoMx region of interest (ROI) was used. Up to four segments were collected within each ROI into separate wells, corresponding to $CD68^+$ macrophages, NE^+ neutrophils, Siglec-8^+ type 2 granulocytes, and the remaining nuclei-positive unstained cells, which were hypothesised to mainly represent lymphocytes. As most collected cell types covered less than the recommended surface collection area per cell type, a pooling plan across several ROIs was created.

Protein panel quantification of pooled samples was performed using the NanoString nCounter platform. Following acquisition, collected cell populations underwent quality control based on immunofluorescence staining, and only samples with >90% target cell purity were included in the final analysis.

Background calculations and normalisation of raw protein counts

For each pooled sample (containing 2–3 ROI collections), background counts were calculated using three background markers; protein counts less than or equal to the geometric mean plus one standard deviation were set to zero. Duplicate or triplicate measurements were summed per cell type to provide a single total count per protein, cell type, and participant. Total protein counts per cell type were normalised to the total collected area for comparisons between cell types and expressed as counts per 1000 μm^2.

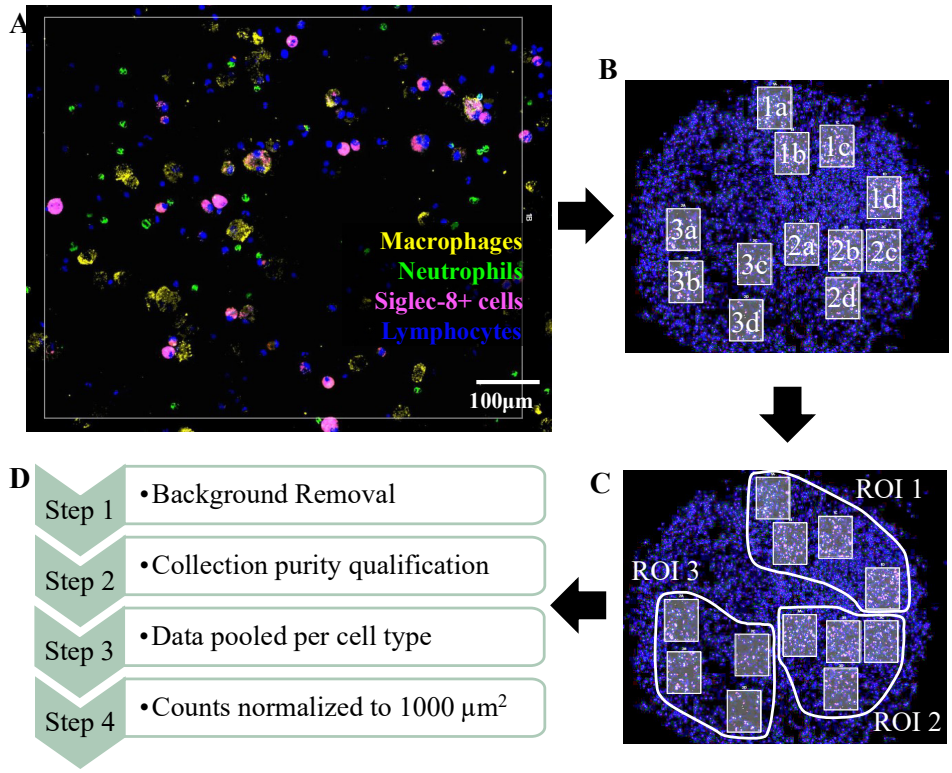

Figure 8: Setup and analysis of cytospins for the NanoString's GeoMx protocol
(**A**) Immunofluorescence staining using four colours. (**B**) Twelve regions of interest (ROIs) positioned on the cytospin. (**C**) Pooling schematic for each cell type. (**D**) Summary of the analysis.

Statistics

All statistical analysis were performed in Python 3 using packages such as Pingouin and Statsmodels[206,207]. When two groups were compared, a Mann Whitney U test was used, and when multiple groups were compared, a Kruskal Wallis test was used, followed by a Dunn's posthoc test with Holm correction for multiple comparisons. For paired samples, a Wilcoxon signed-rank test was used. Ranks for the heat maps were calculated using pandas, where ties were resolved using minimum ranks rather than average ranks.

Results

The following section presents the combined results from all four papers included in this thesis. Additional results can be found in Papers I–IV at the end.

ADAM17 profiles in COPD and asthma in the BREATHE cohort

ADAM17 is a key sheddase that regulates inflammatory signalling and has been implicated in COPD-related emphysema. Its phosphorylated form (pADAM17) has been linked to increased shedding activity. Therefore, ADAM17 (ectodomain) and pADAM17 were assessed in cells on BAL cytospins (COPD and controls) and bronchial biopsies (asthma and controls), and soluble ADAM17 substrates were subsequently analysed.

Epithelial dysregulation of ADAM17 in COPD and asthma

In COPD cohort, morphologically identified bronchial epithelial (BE) cells in BAL cytospins showed group-specific cellular localisation of ADAM17 and pADAM17 (Figure 9A). In Never Smokers, ADAM17 staining was enriched basally around the nucleus, while pADAM17 was distributed throughout the cytoplasm, resulting in overlap mainly in the perinuclear region. In Ever Smokers and COPD, ADAM17 was more widely distributed throughout the cytoplasm, and pADAM17 was predominantly localised to the apical region, indicating a smoking-associated shift in overlap between ADAM17 and pADAM17 from the basal towards the apical region.

In asthma, bronchial biopsy sections showed a looser epithelial layer than controls, and ADAM17 expression was more broadly distributed across the epithelium (Figure 9B). pADAM17 was more concentrated around the nucleus in asthma, while in controls it was more evenly distributed towards the ciliated apical region, indicating a disease-associated shift in pADAM17 localisation.

Together, BE cells in COPD (including Ever Smokers) and asthma appeared to show higher ADAM17 expression at the cell surface. Quantification of staining on

COPD BAL slides confirmed that participants with COPD had the highest proportion of ADAM17^{+} cells compared with both Never Smokers and Ever Smokers (Figure 9C). Quantification of asthma biopsy staining also confirmed more ADAM17^{+} staining per BE cell than in controls (Figure 9F). However, only from participants with COPD showed increased pADAM17^{+} cells compared to controls (Figure 9D), whereas BE cells from participants with asthma showed a downwards trend ($p = 0.071$, Figure 9G).

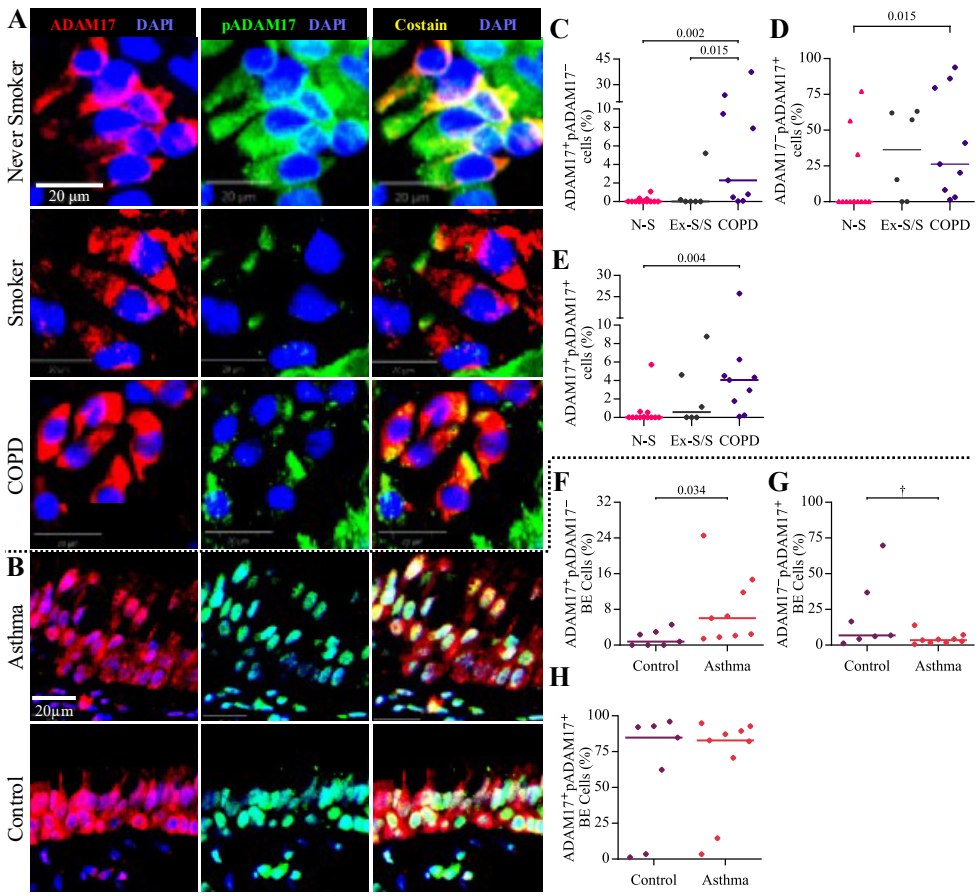

Figure 9: Immunofluorescence staining and quantification of ADAM17 and pADAM17 in BAL cells from participants with COPD and bronchial biopsies from participants with asthma (Papers I and II), and the respective controls

(**A**) BAL-cell staining in never smokers, ever smokers, and COPD. (**B**) Bronchial biopsy staining in asthma and controls. Quantification of (**C**) ADAM17^{+} cells, (**D**) pADAM17^{+} cells, and (**E**) ADAM17^{+}pADAM17^{+} cells in Never Smokers (N-S), Ever Smokers (Ex- and current smokers, Ex-S/S) and COPD. Quantification of (**F**) ADAM17^{+} cells, (**G**) pADAM17^{+} cells, and (**H**) ADAM17^{+}pADAM17^{+} cells in controls and asthma Statistical differences were evaluated using Kruskal–Wallis tests with Dunn's post-hoc test and Holm's correction for multiple comparisons (COPD vs controls), and Mann–Whitney U tests (asthma vs controls), †, $0.05 < p < 0.1$.

Pronounced airway and systemic shedding in COPD, but only systemic shedding in asthma

As ADAM17 BE-cell staining differed between groups, soluble ADAM17 substrates were next investigated in BALF. Several significant differences in soluble ADAM17 substrates were observed in BALF from COPD, but none were detected in BALF from asthma (Figure 10). Both participants with COPD and Ever Smokers had higher concentrations of IL-1RII, TRANCE, APP, MICA, HB-EGF, TGF-α and CD44 than Never Smokers, whereas syndecan-1 was lower (Figure 10A). In asthma, no statistical differences were observed in BALF (Figure 10B). Syndecan-4 showed a non-statistical trend towards higher concentrations in asthma compared with controls ($p = 0.084$).

In blood, TRANCE, HB-EGF and Syndecan-4 were lower, while CD163, TNF-α, EpCAM and IL-6R were all higher in participants with COPD and Ever Smokers than in Never Smokers (Figure 10B). When comparing COPD with Ever Smokers, TNF-α, IL-6R, CD44, and mucin-1 (MUC-1) were lower in COPD. Asthma participants showed higher TRANCE, lymphotoxin-α, IL-6R, IL-1RII, MICA, and MICB, and lower CD44 and syndecan-4 than controls.

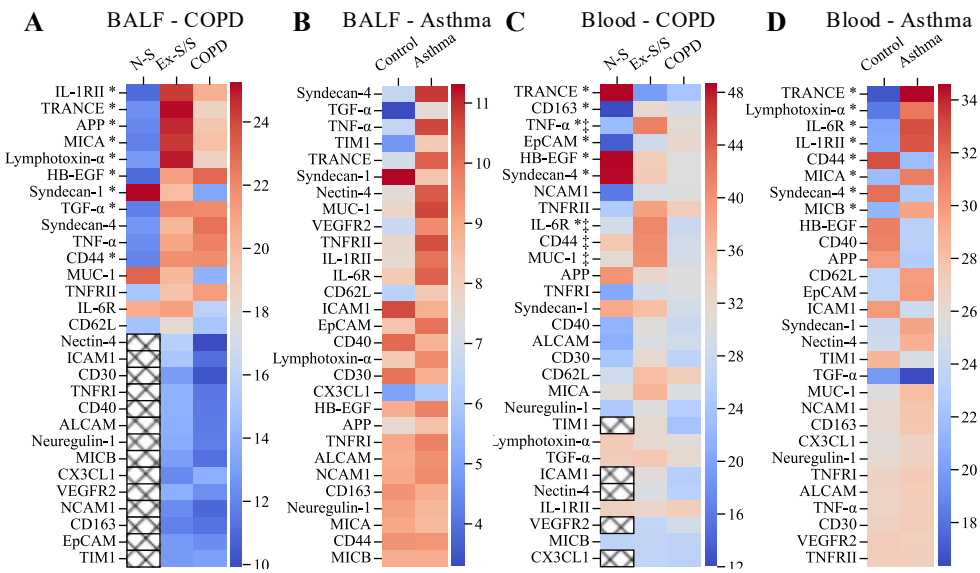

Figure 10: Heatmaps of mean rank differences for ADAM17 substrates in BALF and blood in COPD and asthma (Papers I and II)

ADAM17 substrates were measured in (**A**) COPD BALF, (**B**) asthma BALF, (**C**) COPD blood, (**D**) Asthma blood, all with respective controls: Never Smokers (N–S) and Ever Smokers (ex- and current smokers; Ex-S/S). Rank differences shown within each cohort and ordered by total rank difference; ties were assigned the minimum rank. Statistical differences were evaluated using Kruskal–Wallis tests with Dunn's post-hoc test and Holm's correction (COPD vs controls), and Mann–Whitney U tests (asthma vs controls).*, $p < 0.05$ for all comparisons; ‡, $p < 0.05$ for COPD compared with Ever Smokers.

Cross-compartment comparison of soluble ADAM17 substrates in asthma: sputum, NALF, and BALF

As asthma showed few differences compared with controls in BALF, soluble ADAM17 substrates were examined across airway sample types and between asthma cohorts to assess how levels varied by sampling medium. Across airway sample types, asthma-associated changes in soluble ADAM17 substrates were generally more pronounced in sputum and nasal lavage than in BALF (Figure 11). Several markers increased in asthma sputum relative to controls, including APP, HB-EGF, IL-1RII, TGF-α, and TNFRII. In nasal lavage fluid (NALF), the $\log_2$ ratio suggested higher concentrations of APP, CD44, L-selectin (CD62L), TNF-α, and TNFRII in asthma than in controls. In sputum, the $\log_2$ ratio also suggested higher concentrations of MICA and TNF-α. However, none of these differences were statistically different, indicating greater uncertainty. In contrast, BALF estimates clustered closer to zero across substrates, consistent with smaller, less consistent differences between asthma and controls.

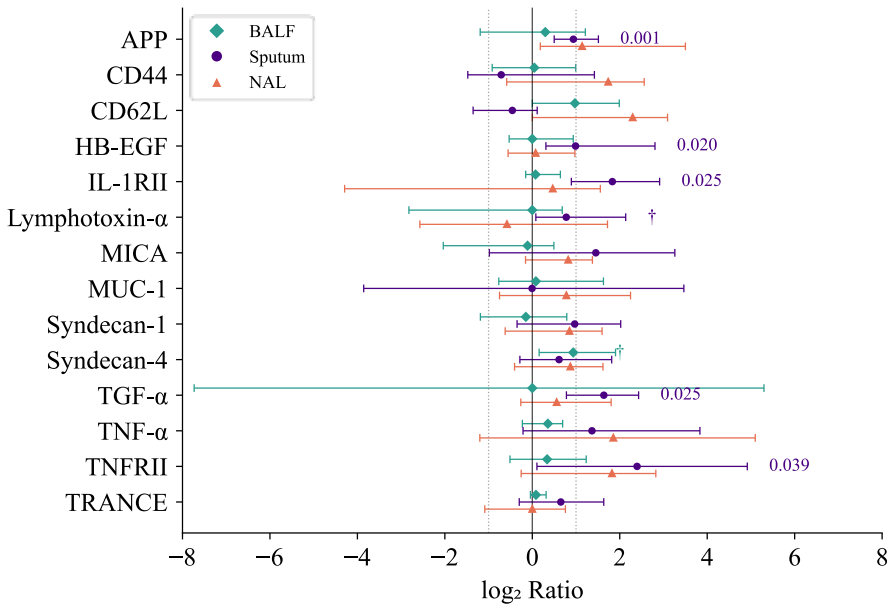

Figure 11: $\log_2$ ratio forest plot of median protein concentrations in asthma and controls in BALF, sputum and NALF (Paper II)

Forest plot of the $\log_2$ ratio of median concentrations (asthma/control) for 14 ADAM17 substrate proteins measured in bronchoalveolar lavage fluid (BALF ◆), induced sputum (●), and nasal lavage (NALF ▲). Error bars represent 95% bootstrap confidence intervals (10 000 iterations). The solid vertical line indicates no difference (ratio = 1, $\log_2 = 0$); dotted lines indicate 2-fold difference thresholds ($\log_2 = \pm 1$). Statistical differences were assessed using Mann–Whitney U tests,p-values are shown in the figure, †, $0.05<p< 0.1$.

Trends in circulating immune cells and their ADAM17 expression

Because soluble ADAM17 substrate patterns in blood differed in COPD, subsequent analyses examined ADAM17 expression on circulating immune-cell subsets to identify potential cellular contributors. ADAM17^{+} clusters were compared between COPD and controls at baseline, and within COPD before and after an exercise intervention, to assess whether immune-cell ADAM17 expression changed with physical exercise.

More circulating monocytes and fewer undefined cells in COPD participants

Flow cytometry showed that participants with COPD had more monocytes and fewer unidentified cells than controls (Figure 12). Participants with COPD also showed a trend towards fewer CD56^{dim} NK cells than controls ($p = 0.078$).

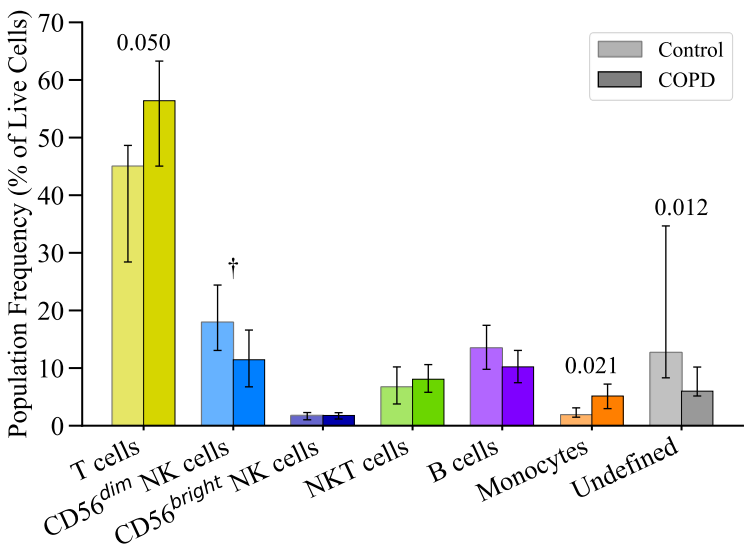

Figure 12: Baseline median differences in immune cells between controls and participants with COPD

Bars show the median frequency of each immune-cell subset measured using flow cytometry (regardless of ADAM17 status), compared between controls and participants with COPD at baseline. Error bars show the interquartile range. Statistical differences were assessed using Mann–Whitney U tests, p-values are shown in the graph, †, 0.05 < p < 0.1.

Exercise intervention effects on circulating immune cells in COPD participants

To determine whether circulating immune cells were affected by physical exercise, cell-type proportions were compared before and after the exercise intervention (Figure 13). Three of the seven measured cell types showed changes in median population frequency after the intervention. Monocytes increased by 2.09%, and natural killer T (NKT) cells decreased by 1.69%. B cells showed a downward trend of 0.44% ($p = 0.085$)

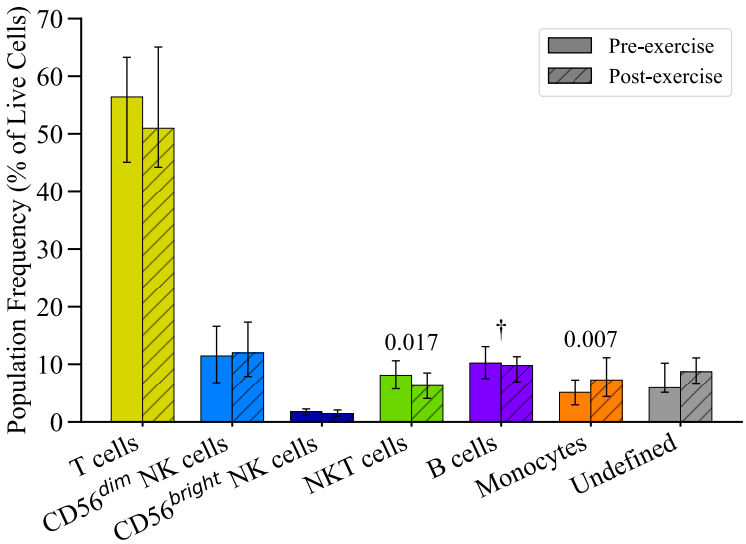

Figure 13: Median differences in immune-cell subsets before and after the exercise intervention in participants with COPD (Paper III)

Bars show the median frequency of each immune-cell subset measured using flow cytometry (regardless of ADAM17 status), compared within participants with COPD before and after the exercise intervention. Error bars show the interquartile range. Statistical differences were assessed using Wilcoxon signed-rank tests, p-values are shown in the graph, †, $0.05 < p < 0.1$.

Exercise increased monocytes and reduced NKT and CD56^{bright} NK cells in COPD

Since COPD was associated with altered ADAM17 expression on circulating immune cells and the exercise intervention shifted immune-cell populations, a more detailed analysis was performed to quantify ADAM17 and pADAM17 expression in immune-cell subsets before and after exercise (Figure 14). Within-participant changes across Hyperfinder-derived cluster gates were assessed using paired analyses of samples collected before and after the exercise intervention. A small subset of cells was negative for all morphology markers in the panel and was classified as unidentified.

After the exercise intervention, ADAM17^{+}pADAM17^{+} monocytes increased by 1.84%, pADAM17^{+} unidentified cells by 0.43% and ADAM17^{-}pADAM17^{-} unidentified cells by 0.21%. ADAM17^{+} NKT cells by 1.69% and ADAM17^{+} CD56^{bright} NK cells by 0.4%. ADAM17^{+} T cells decreased by 0.55% and CD56^{+}ADAM17^{+}pADAM17^{+} monocytes increased by 0.24%, but this did not meet the threshold for statistical difference. The proportion of ADAM17^{+} cells (regardless of cell type) decreased from 42.41% to 36.94% (p = 0.036), corresponding to a median decrease of 4.94%.

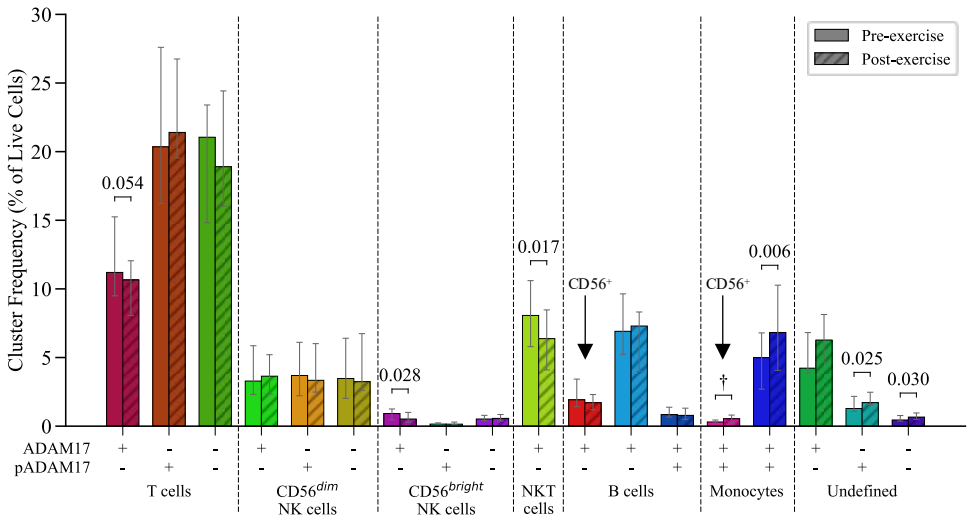

Figure 14: Median changes in cluster frequencies before and after the exercise intervention in participants with COPD (Paper III)

Bars show the median frequency of each cluster, compared within participants with COPD before and after the exercise intervention. ADAM17 status is indicated below the plot: + and − denote positive or negative staining for the ADAM17 ectodomain (ADAM17) and phosphorylated ADAM17 (pADAM17). Error bars show the interquartile range. Statistical differences were assessed using Wilcoxon signed-rank tests, p-values are shown in the graph.

Males exhibit more pronounced post-exercise shifts in immune-cell clusters than females

To assess whether responses to the exercise intervention differed by sex, changes in ADAM17 and pADAM17 immune-cell subsets were compared between males and females (Figure 15). Overall, the heatmap suggested that post-exercise shifts were more pronounced in males. Males showed a larger decrease in ADAM17^{+} CD56^{bright} NK cells (males: −0.47%, females: −0.02%) and a larger increase in ADAM17^{+}pADAM17^{+} monocytes than females (males: +7.56%, females: +1.41%), corresponding to an approximately 5.4-fold larger change in males. ADAM17^{+}pADAM17^{+} B cells changed in opposite directions by sex, decreasing in males (−0.27%) and increasing in females (+0.16%), but showed no statistical difference between sexes.

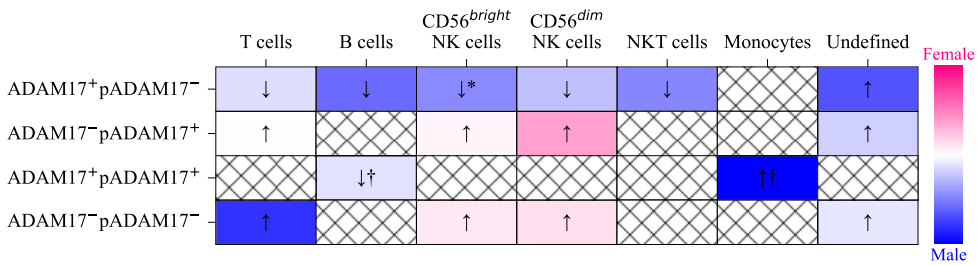

Figure 15: Heatmap of before–after exercise changes in immune-cell cluster frequencies in COPD, stratified by sex (males vs females) (Paper III)

Heatmap showing before–after exercise changes in cluster proportion for each immune-cell cluster. Rows indicate ADAM17 and pADAM17 expression status, and columns represent cell types. Colour denotes which sex showed the larger absolute change in cluster proportion: pink indicates a larger absolute change in females, and blue indicates a larger absolute change in males. Arrows summarise the overall direction of change after the exercise intervention (↑ increase, ↓ decrease), calculated as the average of the male and female median changes. Statistical differences between sexes were assessed using Mann–Whitney U tests and are indicated by symbols: *, $p < 0.05$, †, $0.05 < p < 0.1$. ⊠ indicate cluster combinations that do not exist in the dataset.

Cell-type-specific protein profiling across monocytes, neutrophils, Siglec-8$^+$ cells, and lymphocytes

Since ADAM17-specific subsets differed between COPD and controls, the GeoMx Spatial Profiler was applied to profile additional cell subsets in BAL cytospins from participants with asthma and COPD. Within each cohort, average rank differences were calculated relative to the respective control group (COPD – control, asthma − control). These disease–control differences were then compared between COPD and asthma after normalisation to the number of participants within each segment: CD68$^+$ macrophages, neutrophil elastase$^+$ neutrophils, Siglec-8$^+$ cells, and unstained lymphocytes. Results were visualised as a heatmap, with proteins divided into functional groups (Figure 16).

In the macrophage segment, both diseases showed more protein decreases than increases relative to their respective controls. However, six proteins increased in COPD but decreased in asthma, and three proteins decreased in COPD but increased in asthma. BIM showed the largest divergence, decreasing in COPD and increasing in asthma. In contrast, CD45RO and pan-cytokeratin (PanCK) increased slightly in COPD but decreased in asthma.

In the neutrophil segment, both diseases again showed more decreases than increases relative to controls. Five proteins increased in COPD but decreased in asthma, and eight proteins decreased in COPD but increased in asthma. The most pronounced differences were observed for CD163 and PARP, which decreased in COPD but increased in asthma, while Ki-67 increased in COPD and decreased in asthma.

Because the Siglec-8$^+$ segment was defined by marker positivity rather than eosinophil-specific selection, cellular composition was assessed using morphology in the GeoMx images. This indicated that, in some participants, the Siglec-8$^+$ segment also included mast cells and basophils in addition to eosinophils. The Siglec-8$^+$ segment was therefore interpreted as a broader type 2 granulocyte compartment rather than a pure eosinophil population. This segment showed the strongest divergence between diseases: three proteins increased in COPD but decreased in asthma, whereas 15 proteins decreased in COPD but increased in asthma, relative to their respective controls.

Across segments, the strongest COPD–control and asthma–control contrasts were observed for T-cell markers and cell-death-related proteins. Cell-death-related proteins were generally decreased in COPD (particularly in neutrophils) but increased in asthma relative to controls.

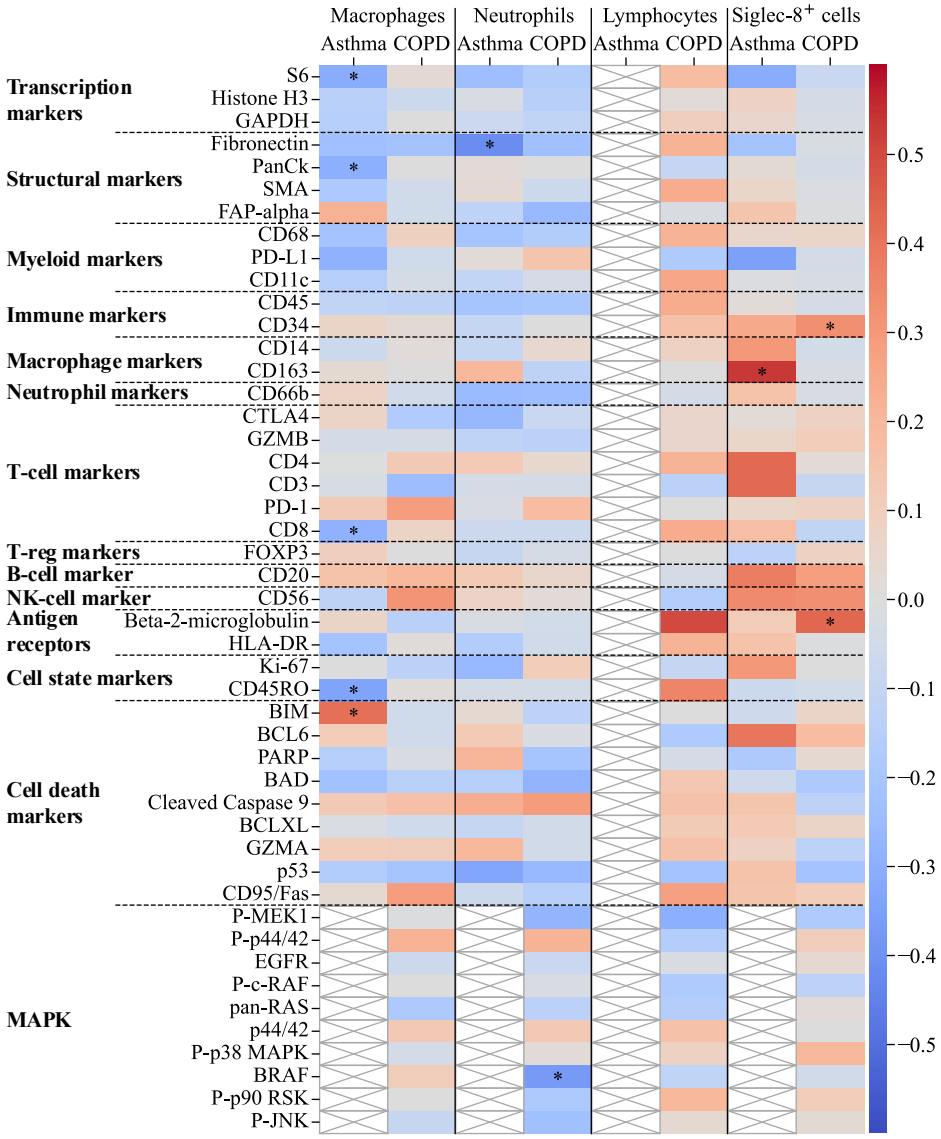

Figure 16: Heatmap of COPD–control and asthma–control protein rank differences across BAL cell segments measured using GeoMx (Paper IV)

Heatmap showing normalised mean rank differences between each disease group and the respective controls for GeoMx-measured proteins across macrophages, neutrophils, lymphocytes, and Siglec-8$^+$ cells in BAL cytospins from participants with asthma and COPD. Values (blue to red) represent normalised mean rank differences between participants with disease and controls, calculated within each disease cohort to account for differences in cohort size and sample-processing time points. Positive values (red) indicate higher average ranks in disease groups relative to controls, whereas negative values (blue) indicate lower average ranks. Columns represent combinations of cell segment and diagnosis, and proteins are grouped by functional categories. Statistical differences were assessed using two-sided Mann–Whitney U tests on minimum-ranked data, *, $p < 0.05$.

Discussion

This thesis highlights how COPD and asthma differ in ADAM17 expression and soluble substrate concentrations across sample types. The included studies identify patterns that refine our understanding of ADAM17 biology in chronic respiratory disease. Key findings were that ADAM17-related patterns differed in chronic respiratory diseases compared to controls and, that a therapeutic intervention, in the form of exercise, can shift these patterns in a way consistent with redistribution or recalibration rather than uniform downregulation. Exploratory analyses suggested a more pronounced post-exercise response in males compared with females, and this aligned with a subsequent analysis that examined possible sex differences in baseline ADAM17 substrate concentrations in another COPD cohort. Although individual substrates were not entirely novel, when considered together they indicated clear alterations in ADAM17 substrates in both COPD and severe asthma, with compartment-specific patterns across asthma airway samples. Finally, looking beyond ADAM17 to broader cell-type-resolved protein patterns, we adapted NanoString's GeoMx protein profiling to begin identifying differences in immune cells preserved in BAL cytospins.

ADAM17-related patterns can be shifted by therapeutic intervention

In the COPD exercise intervention, we did not observe a uniform reduction in $ADAM17^{+}$–$pADAM17^{+}$ immune-cell clusters, instead, the pattern was more consistent with immune-cell redistribution. $ADAM17^{+}pADAM17^{+}$ monocytes increased after the intervention, while $ADAM17^{+}$ NKT cells and $ADAM17^{+}$ $CD56^{bright}$ NK cells decreased. This increase in monocytes is consistent with previous studies reporting an increase in classical monocytes and a decrease in more pro-inflammatory monocyte subsets after a period of exercise in individuals without respiratory disease[208]. Although the exact balance of classical and pro-inflammatory phenotypes could not be determined with the current marker set, the shift of $ADAM17^{+}pADAM17^{+}$ monocytes is thought to reflect classical monocytes. Characteristically, NKT cells can rapidly secrete a wide array of different cytokines[209], whereas $CD56^{bright}$ NK cells also produce high amounts of cytokines

but typically at a later time point[210]. Among these cytokines, monocytes (particularly pro-inflammatory subsets), NKT cells and CD56^{bright} NK cells can all release TNF-α[209–211], a well-established substrate of ADAM17 and a widely studied pro-inflammatory cytokine in COPD[212,213]. Taken together, the reductions in these cell types suggest that the post-exercise immune profile in COPD may be consistent with a reduction in ADAM17-associated, TNF-α–mediated inflammatory potential. These findings suggest that the post-exercise changes in immune markers mainly reflect a redistribution of immune cells and regulatory adaptation. With regular exercise, these repeated responses are associated with improved immune regulation and reduced chronic low-grade inflammation[214]. This helps frame exercise as a driver of immune recalibration rather than a simple "inflammation-dampening switch". Such recalibration may contribute to symptom relief and quality-of-life improvements observed after training, though causal links cannot be inferred from these data alone.

Another cell population that differed from controls and shifted with exercise was an undefined cell type. At baseline, participants with COPD had lower numbers of three clusters of unidentified cells which all increased after the exercise intervention. These undefined clusters may represent granulocyte populations (e.g. neutrophils) that were not characterised because they were expected to survive poorly during the freezing and thawing process[215]. By contrast, in Paper I there were no differences in circulating neutrophil or eosinophil counts between Ever Smokers and COPD. Therefore, the lower numbers of the undefined cell types (presumed granulocytes) may indicate differences in granulocyte survival after cryopreservation rather than true baseline differences. One possible explanation is that neutrophils in COPD may be skewed towards an "aged" phenotype reducing cell survival at baseline[216,217]. If so, exercise might shift neutrophils to a less "aged" phenotype, contributing to the higher post-intervention counts of unidentified cells observed in COPD.

ADAM17-related exercise responses appear more pronounced in males

When exercise responses were analysed separately in males and females, different patterns emerged in ADAM17- and pADAM17-positive immune-cell clusters. In males, ADAM17^{+} CD56^{bright} NK cells decreased and ADAM17^{+}pADAM17^{+} monocytes increased, whereas changes in females were more modest. We did not identify any studies that investigated the sex effects of long-term exercise on the immune system. However, existing evidence suggests that a physically active lifestyle has many immune benefits, including enhanced vaccine responses, reduced baseline inflammatory markers, improved macrophage and neutrophil function, and fewer senescent T cells[208,218,219]. One possible explanation for the larger shifts

observed in males is that they often perform greater absolute work during training (e.g. greater loads or force output), which can increase muscle microdamage and amplify subsequent systemic "repair" signalling, reflected by blood markers such as creatine kinase[220]. In contrast, females may exhibit a more moderate acute immune response, potentially because oestrogen has membrane-stabilising and antioxidant effects that can buffer muscle damage and oxidative stress and support faster recovery and reduce severity of delayed onset muscle soreness[220]. Females also exhibit greater survival benefits at lower activity volumes, which could be advantageous for those with limited respiratory capacity, such as those with COPD[221]. Overall, these data are consistent with sex-dependent modulation of ADAM17-related immune responses in COPD, however, the small study sample highlights the need for larger, sex-specific studies that consider hormonal status, training dose, and baseline disease characteristics.

Because exercise responses differed by sex, we also in a subsequent (post-publication) examined whether baseline soluble ADAM17 substrates differed between males and females with COPD in the BREATHE cohort (Figure 17). In alignment with the exercise findings, most substrates showed a slightly higher $\log_2$ ratio, suggesting higher concentrations in males than in females, although none showed a statistical difference (Figure 17). TNFRI also had a positive ratio, with a trend towards higher concentrations in males ($p = 0.068$). We did not identify any prior COPD studies reporting sex differences in soluble TNFRI. Interestingly, TNFRI has been reported to be higher in healthy females[222]. This might imply that COPD could be associated with a shift in baseline TNFRI concentrations relative to typical sex differences. A potential contributor to these findings is the influence of comorbid sleep apnoea. While only confirmed in one male COPD participant, its known sex-specific effect on elevating TNFRI concentrations[222] suggests that variations in respiratory sleep health across the cohort could impact the observed biomarkers. T-cell immunoglobulin and mucin protein-1 (TIM1), in contrast, tended to be higher in females ($p = 0.0534$), however, we did not identify studies assessing sex differences in soluble TIM1. Overall, our studies found ADAM17-related immune patterns may be linked and shifted in a sex-dependent manner, which should be considered in future studies.

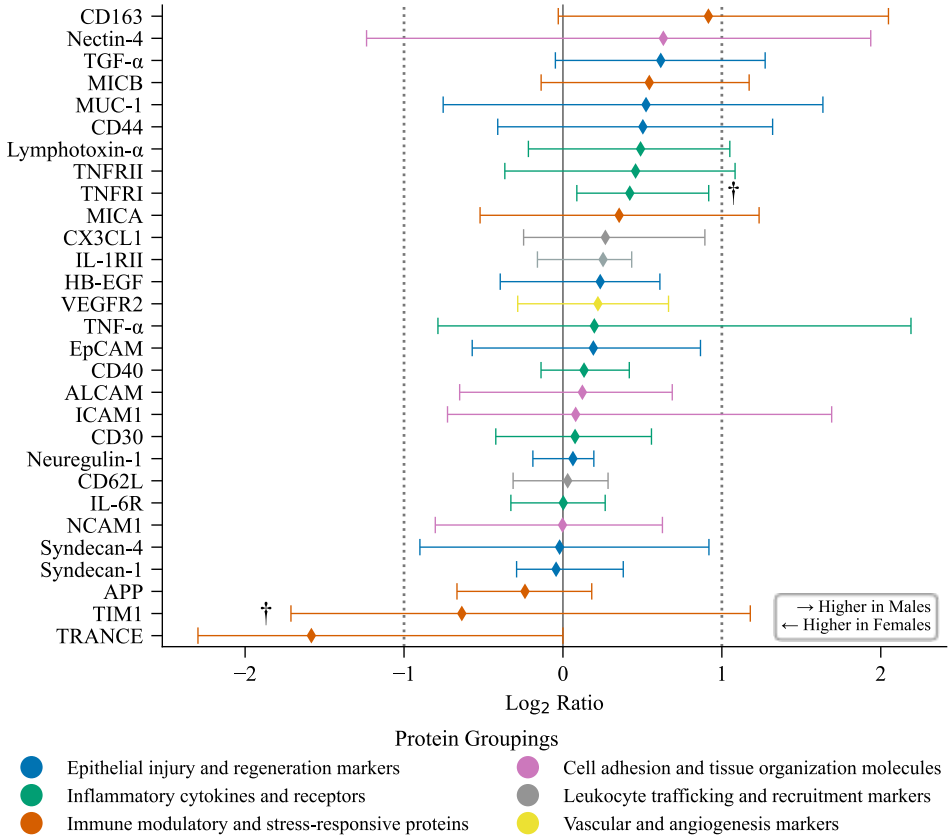

Figure 17: Log_2 ratio forest plot comparing males and females in soluble ADAM17 substrate concentrations in blood from participants with COPD (Paper I)

Forest plot of median soluble ADAM17 substrate concentrations in blood from male and female participants with COPD. Points represent the log_2 ratio of male/female medians, where positive values indicate higher concentrations in males and negative values indicate higher concentrations in females. Error bars show 95% bootstrap confidence intervals (10 000 iterations). Dotted vertical lines at ±1 indicate a 2-fold difference. Colours indicate protein functional groups. Statistical differences were assessed using Mann–Whitney U tests, †, $0.05 < p < 0.1$.

Combined ADAM17-substrate profiling extends biomarker discovery in chronic respiratory diseases to systemic comorbidities

In our studies and others, several ADAM17-linked substrates have differed between diseases and controls not only in the airways but also in the blood. A potential interpretation is that some individuals may have systemic inflammatory or comorbidity-driven profiles that contribute to the overlap in ADAM17 substrates observed in subsets of COPD and asthma. One such comorbidity is metabolic syndrome, which is common in COPD and has also been discussed in asthma phenotyping. Studies have reported that, on average, approximately 33% of males[223–225] and 54% of females[223,224] with COPD have metabolic syndrome which appears to be more common in the early stage of the disease (GOLD I–II)[226,227]. In asthma, a metabolic asthma phenotype has also been proposed, involving mechanisms that are not associated with type 2 immunity[228]. Although metabolic traits were not directly assessed in our studies, insulin resistance has been linked to lower lung function, independent of BMI[229]. We observed higher concentrations of APP in the airways of both participants with COPD and severe asthma. Given links between metabolic dysfunction and APP-related pathways, this overlap may reflect comorbidity-related biology[230–232]. Cardiovascular disease is also a recognised consequence of metabolic syndrome and is a common comorbidity in COPD[233,234]. These observations reinforce the importance of considering systemic comorbidities when interpreting airway and blood biomarker patterns.

ADAM17 substrate profiles are disease-specific

Many substrates of ADAM17 have been reported to differ between respiratory diseases and controls. However, no studies were found that investigated these substrates under the ADAM17 umbrella. As in previous studies, most COPD-associated differences in ADAM17 substrates in our study were detected compared with Never Smokers, although a subset of blood markers differed compared with Ever Smokers, a better control group for COPD. Specifically, TNF-α, IL-6R, CD44 and MUC-1 were all lower in COPD than in Ever Smokers. This highlights that, regardless of smoking status, ADAM17 substrate profiles still differ in chronic respiratory diseases. In our comparative analyses, COPD exhibited more differences in ADAM17-related biology in the lower airways than asthma, relative to the respective controls. However, among substrates that differed between COPD and Never Smokers, several showed opposite directional patterns across compartments, with lower concentrations in blood but higher concentrations in BALF. This

divergence contrasted with asthma, where participants tended to show higher concentrations across both airway and blood, consistent with a more consistent airway–systemic pattern in our cohort. In COPD, the lower substrate concentrations could be due to increased receptor binding driven by upregulated EGFR signalling[190]. In asthma, the higher substrate concentrations might be due to elevated type 2 inflammation during allergy that could enhance ADAM17-mediated shedding and thereby increase circulating substrate levels[191]. Therefore, differences in inflammatory context could explain the different patterns of the ADAM17 substrates seen in COPD compared to asthma.

We observed more differences between asthma and controls in sputum than in BALF and NALF, indicating that ADAM17-related patterns were not uniform across airway compartments. Notably, the sputum samples were obtained from participants with severe asthma which may also contribute to the stronger disease–control signal in this compartment. Interestingly, even in mild asthma, one study reported markedly higher concentrations of soluble biomarkers in sputum than in BALF[235]. Together, these findings suggest that biomarker signals are strongly shaped by sampling compartment and may also vary with disease severity.

Most of the observed differences in COPD were among the epithelial injury and regeneration markers, highlighting airway epithelial dysfunction as a dominant biological signal. Specifically, increased HB-EGF in BALF, and altered epithelial-associated proteins in blood (EpCAM, HB-EGF, syndecan-4, CD44, MUC-1), support persistent epithelial stress and active repair or remodelling in COPD[236]. Such epithelial disturbance is a recognised trigger for alarmin release, including thymic stromal lymphopoietin (TSLP) that can amplify downstream immune activation and has been reported to be altered in COPD airways[237]. In this context, blocking TSLP could be hypothesized to interrupt a feed-forward injury–inflammation loop, particularly in COPD subgroups where epithelial alarmin signalling contributes to exacerbations. However, a clinical trial with anti-TSLP (Tezepelumab) did not reduce exacerbations in the overall COPD population, while a greater reduction was observed in participants with higher blood eosinophil counts, suggesting phenotype-dependent effects[238]. ADAM17-linked epithelial injury and repair markers may, therefore, be useful for identifying subgroups with prominent epithelial–alarmin activity who may be more likely to benefit from such targeted interventions.

Quantifying ADAM17 expression in cells also provided additional supporting context for the differences observed between asthma and COPD. In our substrate studies, COPD showed a higher proportion of $ADAM17^{+}$$pADAM17^{+}$ cells in BAL than in controls, whereas in asthma no difference in the same activation pattern was seen in the bronchial epithelium staining. Together with the knowledge that phosphorylation enhances shedding activity of ADAM17[7], the staining supports the greater changes in the ADAM17 substrate axis in COPD in the lower airways.

Cell-type subsets differ between COPD and asthma

The ADAM17 cell-subset analysis indicated that protein-defined cell subsets differ between asthma and COPD. To obtain higher-dimensional immune-cell profiling than was possible in the PBMC exercise dataset, we applied NanoString GeoMx on preserved cytospins of BAL cells. By applying this technique in an unconventional manner, we enabled cell-type protein profiling of macrophages, neutrophils, Siglec-8^+ cells and lymphocytes. We confirmed that protein profiles in COPD and asthma differed from the respective controls in a cell-type-specific manner, with the largest differences observed in the Siglec-8^+ compartment, particularly for cell-death regulators and T-cell markers. This may partly reflect that asthma participants, especially those with severe disease, had relatively more mast cells that can produce higher levels of inflammatory proteins than COPD participants[239]. Given the array of biological therapeutics targeting the immune pathways involving these cell types, and which have often been tested in both asthma and COPD, better accounting for the cell subsets present in each disease could reduce unnecessary therapeutic testing and associated costs. Overall, cell state, morphology marker selection, and segment purity are important considerations when interpreting disease–control differences in immune cells.

Clinically, our results suggest that ADAM17-related cellular phenotypes and soluble substrate patterns can add value as part of a wider biomarker framework. They may help capture disease- and compartment-specific mechanisms, support phenotyping and subgroup identification, and guide future work on refining clinical subgroups. Moving this toward clinical utility will require larger, well-characterised cohorts and more standardised sampling across compartments, and our findings also suggest that it is important to account for sex when interpreting ADAM17-related patterns at baseline and in response to interventions. These findings reinforce that, alongside efforts to refine phenotyping and targeted treatments, prevention strategies that reduce harmful exposures and support long-term health behaviours may have the greatest population-level impact in chronic respiratory diseases.

Conclusion

Overall, this thesis demonstrates that ADAM17-linked signals in chronic respiratory disease do not follow a single uniform pattern: what is detected depends strongly on the disease, disease severity, the compartment sampled, and the sex of the participant. Importantly for clinical investigations, the profiling of ADAM17 in the blood samples did not always complement airway-derived samples.

A central conclusion is that ADAM17 profiles and immune-cell subsets, in general, differ between chronic respiratory diseases and controls, and also between diseases. The ADAM17 profile tends to be sex-dependent within disease states and can shift with therapeutic intervention, such as the exercise-associated changes observed in COPD. These changes are most consistent with redistribution or recalibration of ADAM17-related immune phenotypes rather than broad immune-cell suppression. This framing supports the idea that interventions such as exercise may reshape immune-cell states in ways that are detectable through ADAM17–pADAM17 patterns.

Among the many pathways in which ADAM17 is involved, the COPD signal was dominated by markers linked to epithelial injury and repair. This finding supports epithelial dysfunction as a major driver of ADAM17-linked substrate patterns in COPD and may help define biologically meaningful subgroups relevant to treatment responses.

ADAM17-related cellular phenotypes and soluble substrate patterns can add value as part of a broader biomarker framework for understanding and phenotyping COPD and asthma. We also suggest that translational potential is likely to be greatest when molecular markers are interpreted alongside systemic comorbidity and patient characteristics, rather than in isolation.

Future Perspectives

As there is still no cure or straightforward way to diagnose chronic respiratory diseases such as COPD and asthma, there remains a need to understand the underlying mechanisms behind these diseases and what happens during treatment and intervention. Based on the findings of this thesis, several potential directions for future research have emerged.

Instead of studying ADAM17 indirectly, a more direct approach, assessing ADAM17 enzymatic activity could provide vital information on which substrates ADAM17 preferentially sheds. This could strengthen the claim about ADAM17 dysfunction in COPD and asthma. A related priority would be to investigate which other enzymes are present in these participants, as not all measured substrates are exclusive to ADAM17. To understand how much ADAM17 itself contributes to the substrate profiles observed in this thesis this will be important to quantify. In addition to measuring the activity level of ADAM17, it could be vital to understand the iRhoms and their role in COPD and asthma as they, particularly iRhom2, are key regulators of ADAM17 maturation and activation. Most work in this field has been conducted in mice, with lung-related data often derived from knockout or mutant models of sepsis-induced acute lung injury. Understanding the role of iRhoms in COPD and asthma may provide information about why ADAM17 is dysregulated in these diseases.

The analysis from Paper II showed more differences in sputum than in BALF and NALF with respect to ADAM17 biology. However, the fact that these came from different individuals made it difficult to interpret whether it is the sample type or the severity of the disease. Repeating this experiment in the same individuals could clarify whether these substrate patterns are primarily driven by disease severity or sampling compartment.

This thesis showed that exercise led to a recalibration rather than suppression of the immune cells, the granulocytes in the samples were assumed not to have survived the freeze–thaw cycle the cells were exposed to. These cells are known to be heavily involved in both COPD and asthma so to understand what happens to these cells during an exercise intervention is important. To address this, staining cytospins from the same samples for neutrophils, eosinophils, ADAM17 ectodomain and pADAM17 could help clarify what happens to these types of cells thereby

broadening our understanding of the biological exercise response in participants with COPD.

Given that NKT cells decreased by 1.69% after the exercise intervention, and no difference was observed between males and females, a logical next step would be to repeat the flow cytometry experiment but to focus on the different subtypes of NKT cells including invariant NKT (iNKT) cells which were not measured in the previous experiment. The NKT cells are a small T-cell subset that can very rapidly secrete a wide range of Th1, Th2 and Th17-type cytokines meaning that they can shape the downstream responses of macrophages, T cells, B cells, NK cells and dendritic cells. As a 'first responder' population, they can push the airway environment in different directions and by better phenotyping these cells could help explain why some participants with COPD exhibits mixed inflammatory patterns or steroid resistance in asthma. Combining this with ADAM17 detection would provide a broader picture of the potential cytokine release as it is the enzyme responsible for cleaving many pro-inflammatory cytokines.

Taken together, these directions suggest that the ADAM17 axis may help explain disease heterogeneity in COPD and asthma and point to new opportunities for intervention.

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I would also like to thank everyone in the BREATHE consortium, especially *Leif Bjermer* and *Jaro Ankerst*, for making the samples available to me. Thank you to Elsa Sjöblad for collecting all the SEVA-NAL samples and thank you to all the participants in these studies—without whom I would not have had a PhD project.

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Use of AI tools

This thesis made use of AI-assisted tools, including ChatGPT, Claude, NotebookLM, Perplexity, and Google Gemini, to support the research process, including image generation. These tools were used for tasks including exploring alternative explanations, checking the clarity of descriptions, reviewing and generating code snippets for plotting graphs, and identifying potentially relevant literature, which was verified manually. All analyses, interpretations, and the final written content were produced by the author. AI outputs were treated as suggestions, independently evaluated, and verified against scholarly sources to ensure accuracy and academic integrity.

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