



LUND UNIVERSITY

Metabolic, Inflammatory, and Microbial Factors Shaping Organ Health in CF

Diemer, Stefanie

2026

Document Version:

Publisher's PDF, also known as Version of record

[Link to publication](#)

Citation for published version (APA):

Diemer, S. (2026). *Metabolic, Inflammatory, and Microbial Factors Shaping Organ Health in CF*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Lund]. Lund University, Faculty of Medicine.

Total number of authors:

1

Creative Commons License:

CC BY

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Metabolic, Inflammatory, and Microbial Factors Shaping Organ Health in CF

STEFANIE DIEMER

DEPARTMENT OF CLINICAL SCIENCES LUND | FACULTY OF MEDICINE | LUND UNIVERSITY



Metabolic, Inflammatory, and Microbial Factors Shaping Organ Health in CF

Metabolic, Inflammatory, and Microbial Factors Shaping Organ Health in CF

Stefanie Diemer



LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD)
at the Faculty of Medicine at Lund University to be publicly defended on the
16th of October 2026 at 09.00 am in Belfragesalen, BMC D15, Lund University.

Faculty opponent

Egil Bakkeheim, Oslo University Hospital, Norway

Organization: LUND UNIVERSITY

Document name: Doctoral dissertation

Date of issue: 16 October 2026

Author(s): Stefanie Diemer

Title and subtitle: Metabolic, Inflammatory, and Microbial Factors Shaping Organ Health in CF

Abstract:

Background: Cystic fibrosis (CF) is a multisystem genetic disease characterized by chronic airway infection and progressive lung function decline. With increasing life expectancy, extrapulmonary comorbidities such as cystic fibrosis–related diabetes (CFRD) and hepatobiliary involvement have become important contributors to disease burden in people with CF (pwCF).

Aim: The overall aim of this thesis was to investigate the role of CFRD and dysglycaemia in CF lung disease, focusing on airway inflammation, proteomic and microbiome signatures, and longitudinal lung function, and to evaluate the impact of CFTR modulator therapy on hepatobiliary involvement in children with CF.

Methods: **Paper I** and **II** were observational studies focusing on airway inflammation (Meso Scale Discovery assays and chromogenic assays), sputum proteomics (tandem mass spectrometry) and airway microbiome analysis (16S rRNA amplification and sequencing). **Paper III** was a retrospective study using data from the Swedish CF Registry, investigating lung function trajectories at baseline and over a three-year follow-up in people with CF across different glycaemic statuses. **Paper IV** retrospectively evaluated the effect of CFTR modulator therapy, elexacaftor/tezacaftor/ivacaftor (ETI), on liver stiffness in children with CF, assessed by two-dimensional shear wave liver elastography.

Results: In **Paper I** pwCF with CFRD had lower lung function and higher levels of airway inflammation, including increased neutrophil elastase, IL-8, and IL-1 β , compared with pwCF without CFRD. Proteomic analyses identified CFRD-specific protein profiles related to neutrophil degranulation and carbohydrate metabolism. **Paper II** showed that pwCF with CFRD had increased airway inflammation and reduced microbial diversity. **Paper III**, demonstrated that CFRD was associated with lower baseline lung function but not with accelerated lung function decline over three years, while impaired glucose tolerance was not associated with inferior pulmonary outcomes.

Paper IV showed that treatment with ETI was associated with reduced liver stiffness and improved pulmonary outcomes, including in children with already established hepatobiliary involvement.

Conclusion: This thesis demonstrates that CFRD represents a distinct metabolic phenotype in CF, associated with specific inflammatory, proteomic, and microbial airway signatures and reduced lung function. The findings highlight the importance of contextualizing metabolic and microbial biomarkers within lung disease severity and support a precision medicine approach integrating metabolic profiling. Furthermore, CFTR modulation can improve hepatobiliary involvement in pwCF.

Key words: cystic fibrosis, cystic fibrosis-related diabetes, airway inflammation, airway microbiome, proteomics, cystic fibrosis-related hepatobiliary involvement, ETI, liver stiffness, elastography

Language: English

Number of pages: 117

ISSN and key title: 1652-8220

ISBN: 978-91-8021-923-5

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature

Date 2026-09-07

Metabolic, Inflammatory, and Microbial Factors Shaping Organ Health in CF

Stefanie Diemer



LUND
UNIVERSITY

Copyright

Pages 1-117 © 2026 Stefanie Diemer, ORCID 0009-0006-3319-611X, (licensed under [CC BY 4.0](#))

Paper 1 © 2025 The authors. Published by ERS publications (licensed under [CC BY 4.0](#)).

Paper 2 © 2026 The authors. Published by Springer (licensed under [CC BY-NC 4.0](#)).

Paper 3 © 2026 The authors. Manuscript unpublished.

Paper 4 © 2025 The authors. Published by Wiley (licensed under [CC BY-NC 4.0](#)).

Cover image by ChatGPT

Published by: Stefanie Diemer

Department of Clinical Sciences Lund

Faculty of Medicine

Lund University

Lund 2026

ISBN 978-91-8021-923-5

ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University,
Lund, 2026



Media-Tryck is a Nordic Swan Ecolabel
certified provider of printed material.
Read more about our environmental
work at www.mediatryck.lu.se

MADE IN SWEDEN 

*To the patients, clinicians, and researchers who have shaped our
understanding of cystic fibrosis. Your commitment and
compassion form the foundation upon which this work stands.*

Table of Contents

List of papers included in the thesis	10
Additional papers not included in the thesis:	11
Abbreviations	12
Abstract	14
Populärvetenskaplig sammanfattning	16
Populärwissenschaftliche Zusammenfassung	17
Thesis at a glance	19
Preface	20
Introduction	21
CF - a genetic disease.....	23
The history and milestones in Cystic Fibrosis.....	24
CF as a multiorgan disease.....	27
Treatment of CF in the era of CFTR modulators	28
The vicious cycle in CF lung disease	31
Airway surface liquid depletion	31
The initiating steps of CF airway disease.....	32
Ongoing chronic inflammation and infection.....	34
Airway microbiome	37
CF-related diabetes.....	40
CFRD – clinical signs.....	40
CFRD and lung disease	42
Screening for CFRD	44
Pathophysiology of CFRD	45
Treatment of CFRD	46
CFRD in times of ETI	46
Cystic fibrosis hepatobiliary involvement.....	47
Definition and clinical signs	47
Pathophysiology of CFHBI	49
Screening and management of CFHBI.....	50

Aims	52
Methods	53
Population	53
Clinical investigations	54
Lung function	54
Oral glucose tolerance test.....	54
Screening for CFHBI.....	55
Laboratory methods	56
Sputum preparation	56
Quantification of cytokines and inflammatory mediators	56
Proteome analysis.....	57
Quantitative Polymerase chain reaction (PCR).....	59
Microbiome	60
Statistics	63
Ethical considerations	64
Results.....	66
Paper I	66
Paper II	69
Paper III.....	72
Paper IV	75
Discussion	78
Driving factors in CFRD airway inflammation.....	78
The lung function dilemma in CFRD.....	81
ETI treatment and its impact in CFHBI	84
Conclusion and further perspectives	87
Use of Generative AI	90
Acknowledgement.....	91
References	94

List of papers included in the thesis

- I. **Diemer S**, Chowdhury S, Sahl C, Happonen L, Pålman LI. Characterisation of airway inflammation and proteomes associated with cystic fibrosis-related diabetes. *ERJ Open Res.* 2025;11(6):00290-2025. doi:10.1183/23120541.00290-2025
- II. **Diemer S**, Kozjek, K, Pålman LI. Cystic fibrosis-related diabetes is associated with reduced airway microbial diversity. *Respir Res.* 2026;27(1):106. doi: 10.1186/s12931-026-03598-2.2026
- III. **Diemer, S**, Olsson O, Krantz C, Pålman LI. Glucose tolerance and its impact on lung function in people with cystic fibrosis in Sweden. Manuscript
- IV. **Diemer S**, Elidottir H, Eklund EA, Pålman LI, Hansen C. The effect of elexacaftor/tezacaftor/ivacaftor on liver stiffness in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr.* 2025;81(1):74-81. doi:10.1002/jpn3.70050

Additional papers not included in the thesis:

Arman, F, **Diemer S**, Happonen LJ, Pålman LI. CFTR modulator therapy reshapes airway inflammation in cystic fibrosis: Insights from proteomics and machine learning. *J Cyst Fibros* 2026; <https://doi.org/10.1016/j.jcf.2026.05.002>

Elidottir H, Hansen CR, **Diemer S**, Eklund E. 2D Shear Wave Elastography, a promising screening tool for Cystic Fibrosis liver disease, shows a correlation between vitamin D and liver stiffness. *J Cyst Fibros* 2022; 21(5):873-877

Elidottir H, **Diemer S**, Eklund E, Hansen CR. Abnormal glucose tolerance and lung function in children with cystic fibrosis. Comparing oral glucose tolerance test and continuous glucose monitoring. *J Cyst Fibros* 2021; 20 (5):779-784

Trojanek JB, Cobos-Correa A, **Diemer S**, Kormann M, Schubert SC, Zhou-Suckow Z, Agrawal R, Duerr J, Wagner CJ, Schatterny J, Hirtz S, Sommerburg O, Hartl D, Schultz C, Mall MA. Airway mucus obstruction triggers macrophage activation and matrix metalloproteinase 12-dependent emphysema. *Am J Respir Cell Mol Biol*. 2014; 51:709-20.

Cobos-Correa A, Trojanek JB, **Diemer S**, Mall MA, Schultz C. Membrane-bound FRET probe visualizes MMP12 activity in pulmonary inflammation. *Nat Chem Biol*. 2009; 5:628-30.

Abbreviations

aCFLD	Advanced cystic fibrosis–related liver disease
AGT	Abnormal glucose tolerance
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ASL	Airway surface liquid
AST	Aspartate aminotransferase
ASVs	Amplicon sequence variants
BAL	Bronchoalveolar lavage
BMI	Body mass index
CF	Cystic fibrosis
CFLD	Cystic fibrosis-related liver disease
CFHBI	Cystic fibrosis-related hepatobiliary involvement
CFRD	Cystic fibrosis–related diabetes
CFTR	Cystic fibrosis transmembrane conductance regulator
CGM	Continuous glucose monitoring
CI	Confidence interval
DIA	Data-independent acquisition
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
ETI	Elexacaftor/tezacaftor/ivacaftor
FEV1pp	Forced expiratory volume in 1 s, expressed as percent of predicted
FH	Fasting hyperglycaemia
GGT	Gamma-glutamyl transferase
GIP	Glucose-dependent insulintropic polypeptide
GLP-1	Glucagon-like peptide-1
HbA1c	Hemoglobin A1c
IGT	Impaired glucose tolerance
INDET	Indeterminate glycaemia

IQR	Interquartile range
kPa	Kilopascal
LCI	Lung clearance index
MBW	Multiple breath washout
MCL	Mucus layer
MS/MS	Tandem mass spectrometry
NE	Neutrophil elastase
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
PCL	Periciliary layer
PCR	Polymerase chain reaction
PHT	Portal hypertension
pwCF	People with cystic fibrosis
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
mRNA	Messenger RNA
SD	Standard deviation
2D SWE	Two-dimensional shear-wave elastography
UDCA	Ursodeoxycholic acid

Abstract

Background

Cystic fibrosis (CF) is a multisystem genetic disease characterized by chronic airway infection, inflammation, and progressive loss of lung function. As life expectancy has increased in people with CF, extrapulmonary comorbidities have become increasingly important determinants of morbidity and mortality. Among these, cystic fibrosis-related diabetes (CFRD) and cystic fibrosis hepatobiliary involvement (CFHBI) are common and clinically significant complications. However, the mechanisms linking CFRD to impaired lung function, the impact of impaired glycaemic control, and the effects of highly effective CFTR modulator therapy on extrapulmonary disease remain incompletely understood.

Aims

The overall aim of this thesis was to investigate the role of CFRD and dysglycaemia in CF lung disease, with a particular focus on airway inflammation, proteomic and microbiome signatures, and longitudinal lung function trajectories, as well as to evaluate the impact of CFTR modulator therapy on hepatobiliary involvement in children with CF.

Methods and results

In Paper I, airway inflammation, bacterial load, and the sputum proteome were compared in people with CF (pwCF) with and without CFRD. Individuals with CFRD exhibited significantly lower lung function and higher sputum levels of neutrophil elastase, IL-8, and IL-1 β . Proteomic analyses identified CFRD-specific protein profiles involved in neutrophil degranulation and carbohydrate metabolism, suggesting distinct inflammatory and molecular signatures associated with CFRD beyond reduced lung function alone.

Paper II examined whether CFRD was associated with alterations in the airway microbiome. PwCF with CFRD had lower lung function, elevated airway inflammation, and reduced bacterial diversity compared to those with CF alone. However, the association between CFRD and reduced microbial diversity was attenuated after adjustment for lung function, indicating that lung disease severity rather than diabetes per se was the primary driver of microbiome dysbiosis.

In Paper III, nationwide Swedish CF registry data were used to explore the relationship between glucose tolerance status and longitudinal lung function decline. PwCF with CFRD had significantly lower baseline lung function compared to those with normal glucose tolerance (NGT), while individuals with impaired glucose tolerance (IGT) did not differ from the NGT group. Importantly, lung function decline over a three-year follow-up period did not differ between glucose

tolerance groups, suggesting that CFRD is associated with established lung function impairment rather than accelerated decline.

Paper IV investigated the impact of the highly effective CFTR modulator therapy elxacaftor/tezacaftor/ivacaftor (ETI) on hepatobiliary involvement in children with CF. Treatment with ETI was associated with a significant reduction in liver stiffness, including in children with CFHBI, alongside improvements in lung function and lung clearance index, indicating a potential beneficial effect of ETI on extrapulmonary disease manifestations.

Conclusion

In conclusion, this thesis demonstrates how metabolic status, airway inflammation, and microbial composition jointly shape pulmonary health in cystic fibrosis, highlighting their relevance for precision medicine-oriented care. CFRD represents a distinct metabolic phenotype associated with reduced lung function and specific inflammatory, proteomic, and microbial airway signatures, whereas impaired glucose tolerance does not appear to confer comparable pulmonary impairment. The observed microbial dysbiosis in CFRD is largely driven by lung disease severity rather than glycaemic status alone, underscoring the need to contextualize biomarkers within individual disease characteristics. Furthermore, the beneficial effects of highly effective CFTR modulation on hepatobiliary involvement illustrate the potential for genotype- and organ-specific therapeutic responses. Collectively, these findings support a precision medicine framework in CF, where metabolic, inflammatory, and microbial profiling can inform risk stratification, guide individualized treatment strategies, including the care and surveillance of extrapulmonary complications, and optimize organ-specific outcomes across the lifespan.

Populärvetenskaplig sammanfattning

Cystisk fibros (CF) är en ärftlig sjukdom som framför allt påverkar lungorna, men som även kan ge upphov till flera andra sjukdomstillstånd i kroppen. Tack vare förbättrad behandling lever personer med CF idag betydligt längre än tidigare, vilket har lett till att komplikationer utanför lungorna har fått allt större betydelse för hälsa och livskvalitet. Två vanliga sådana komplikationer är CF-relaterad diabetes (CFRD) och påverkan på lever och gallvägar.

Denna avhandling undersöker hur ämnesomsättning, inflammation och mikroorganismer samverkar och påverkar organhälsan hos personer med CF. Särskilt fokus ligger på hur CFRD hänger samman med lungfunktion, inflammation i luftvägarna och bakterieflora i lungorna, samt hur modern CFTR-modulerande behandling, en behandling som korrigerar CFTR proteinet cellulärt, kan påverka leversjukdom hos barn med CF.

I de första två studierna analyserades slem från luftvägarna hos personer med CF, med och utan CFRD. Resultaten visar att personer med CFRD har sämre lungfunktion och högre nivåer av inflammationsmarkörer, vilket tyder på en mer aktiv och skadlig inflammation i luftvägarna. Dessutom identifierades särskilda proteinmönster och förändringar i bakterieflora i luftvägarna hos personer med CFRD.

Den tredje studien omfattar hela den svenska CF-populationen och följer lungfunktionen över tid hos personer med normalt blodsocker, nedsatt glukostolerans och CFRD. Studien visar att personer med CFRD har sämre lungfunktion, medan personer med nedsatt glukostolerans inte skiljer sig från dem med normalt blodsocker. Man kan inte se någon skillnad i hur snabbt lungfunktionen försämrades över tid mellan alla tre grupperna under en treårig observationstid.

I den fjärde studien undersöktes effekten av den nya, mycket effektiva CFTR-modulerande behandlingen elexakaftor/tezakaftor/ivakaftor (ETI) på leverpåverkan hos barn med CF. Resultaten visar att leverstyvheten, som är ett mått för leverfibrosutvecklingen, minskade efter insatt behandling, även hos barn som redan hade tecken på leverengagemang. Samtidigt förbättras både lungfunktion och lungornas ventilationsförmåga, vilket tyder på att behandlingen kan ha positiva effekter på flera organ i kroppen.

Sammanfattningsvis visar denna avhandling att samspelet mellan ämnesomsättning, inflammation och bakterieflora har stor betydelse för hur olika organ påverkas vid cystisk fibros. Resultaten av avhandlingen understryker vikten av att anpassa vården efter varje person med CF individuellt, där metabol status, inflammationsnivåer och mikrobiella faktorer vägs samman för att kunna ge bästa möjliga behandling och uppföljning av personer med CF – både vad gäller lungor och andra organ.

Populärwissenschaftliche Zusammenfassung

Zystische Fibrose (CF) ist eine erblich bedingte Erkrankung, die vor allem die Lunge betrifft, aber auch mehrere andere Organe im Körper beeinträchtigen kann. Dank immer wirksamerer Behandlung hat sich die Lebenserwartung von Menschen mit CF in den letzten Jahrzehnten deutlich erhöht. Dadurch gewinnen Begleiterkrankungen außerhalb der Lunge zunehmend an Bedeutung für die Gesundheit und Lebensqualität von Menschen mit CF. Zwei häufige Komplikationen sind die CF-assoziierte Diabeteserkrankung (CFRD) sowie die Beeinträchtigungen von Leber und Gallengängen.

Diese Dissertation untersucht, wie stoffwechselbedingte Veränderungen, Entzündungsprozesse und die bakterielle Besiedelung der Atemwege zusammenwirken und dadurch die Organesundheit bei Menschen mit CF beeinflussen. Der besondere Fokus liegt darauf, wie CFRD mit der Lungenfunktion, mit Entzündung in den Atemwegen und mit der Bakterienflora in der Lunge zusammenspielt. Weiterhin wird untersucht, wie eine moderne Therapie, die am Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) ansetzt und die Funktion des defekten CFTR-Kanals direkt auf zellulärer Ebene verbessert, die Lebererkrankung bei Kindern mit CF beeinflussen kann.

In den ersten beiden Studien wurde das Atemwegssekret von Erwachsenen mit CF analysiert. Die Kohorte wurde in zwei Gruppen eingeteilt: Erwachsene mit CF und CFRD, Erwachsene mit CF ohne CFRD. Die Ergebnisse zeigen, dass Personen mit CFRD eine niedrigere Lungenfunktion und höhere Werte an Entzündungsmarkern aufweisen, was auf eine aktivere Entzündung in den Atemwegen hindeutet. Darüber hinaus wurden bei Personen mit CFRD spezifische Proteinmuster und Veränderungen in der Atemwegs-Bakterienflora identifiziert.

Die dritte Studie umfasst die gesamte schwedische CF-Population ab einem Alter von 10 Jahren und verfolgt ihre Lungenfunktion im Zeitverlauf von 3 Jahren. Die Kohorte wurde in drei Gruppen eingeteilt: Personen mit normalem Blutzucker, Personen mit gestörter Glukosetoleranz und Personen mit CFRD. Die Studie zeigt, dass Personen mit CFRD eine schlechtere Lungenfunktion haben, während sich Personen mit gestörter Glukosetoleranz nicht von denen mit normalen Blutzuckerwerten unterscheiden. Die Veränderung der Lungenfunktion über einen Beobachtungszeitraum von drei Jahren ist in allen drei Gruppen vergleichbar.

In der vierten Studie wurde die Wirkung der neuen, hochwirksamen CFTR-Modulator-Therapie Elexacaftor/Tezacaftor/Ivacaftor (ETI) auf die Lebererkrankung bei Kindern mit CF untersucht. Die Ergebnisse zeigen, dass die Lebersteifigkeit, die ein Maß für die Entwicklung einer Leberfibrose ist, nach Beginn der Behandlung mit ETI abnahm – selbst bei Kindern, die bereits Anzeichen einer Leberbeteiligung aufwiesen. Gleichzeitig verbesserten sich sowohl die Lungenfunktion als auch die Belüftungsfähigkeit der Lunge, was darauf hindeutet,

dass die Behandlung positive Auswirkungen auf mehrere Organe im Körper haben kann.

Zusammenfassend zeigt diese Dissertation, dass das Zusammenspiel von Stoffwechsel, Entzündungsprozesse und Bakterienflora eine große Bedeutung dafür hat, wie verschiedene Organsysteme bei zystischer Fibrose beeinträchtigt werden. Die Ergebnisse der Arbeit unterstreichen, wie wichtig es ist, die Behandlung individuell auf jede Person mit CF abzustimmen, indem Stoffwechselstatus sowie entzündliche und mikrobielle Faktoren zusammen betrachtet werden, um die bestmögliche Behandlung für Menschen mit CF zu gewährleisten. Dies gilt sowohl im Hinblick auf die Lungenerkrankung als auch bezüglich anderer Organsysteme.

Thesis at a glance

Paper	Aim	Method	Results
I	To investigate airway inflammation and sputum proteomics in pwCF with and without CFRD.	Observational study of 33 pwCF. Airway inflammation was assessed by measuring cytokines and inflammatory mediators using Mesoscale and chromogenic assays, while the airway proteome was analysed by tandem mass spectrometry.	PwCF with CFRD had lower lung function and elevated IL-1 β , IL-8, and neutrophil elastase. A distinct airway proteome was observed in pwCF with and without CFRD, with differential expression of proteins involved in neutrophil degranulation and carbohydrate metabolism.
II	To investigate the airway microbiome in pwCF with and without CFRD.	Observational study of 44 pwCF. Airway inflammation was assessed by measuring cytokines and inflammatory mediators using Mesoscale and chromogenic assays, while the airway microbiome was analysed by 16S rRNA gene amplification and sequencing.	PwCF with CFRD had lower lung function and increased airway inflammation, along with reduced microbial diversity and evidence of dysbiosis. This was characterized by enrichment of <i>Abiotrophia</i> , <i>Anaeroglobus</i> , and <i>Escherichia-Shigella</i> .
III	To evaluate lung function trajectories across different glycaemic statuses in pwCF.	Retrospective Swedish CF registry study. Baseline and longitudinal lung function were assessed over a three-year period in pwCF.	PwCF with CFRD exhibited lower baseline lung function; however, the rate of lung function decline over time did not differ between glycaemic status groups.
IV	To evaluate the effect of ETI on liver stiffness in pwCF.	Retrospective study of 21 pwCF. Liver stiffness was evaluated using 2D shear wave liver elastography.	ETI treatment for at least three months was associated with a significant reduction in liver stiffness in the overall cohort, as well as in pwCF with established hepatobiliary involvement.

Preface

When I began my research journey in cystic fibrosis (CF) in 2007, the disease had a completely different face and impact on individuals than it does today. The daily treatment burden, the constant effort required to maintain quality of life, and the struggle to preserve lung function have shaped both the lives of people with CF and our medical approach. Much of this has changed in recent years with the introduction of CFTR modulator therapy, which fundamentally transformed the clinical course and long-term outlook for many people with CF.

Scientifically, I was fortunate to experience this transformation from multiple angles. My journey began in Heidelberg in the laboratory of Prof. Marcus Mall, where I worked with the β ENaC-overexpressing mouse model, a model for CF-like lung disease. There, I studied mechanisms of lung emphysema and the protease/antiprotease imbalance in vivo, gaining deep insight into early pathophysiological processes. Later, at Lund University, my work shifted toward the clinical side, investigating the impact of CFTR modulator therapy on CF-related liver disease and studying the impact of CF-related diabetes on lung disease, reflecting the evolving needs and challenges of an aging CF population.

CF is a disease with many faces - and these faces have changed dramatically over the past decade. As CF clinicians and researchers, we have been compelled to rethink old paradigms, explore new clinical questions, and accompany patients into a fundamentally different “new world” shaped by transformative therapies.

Today, CF research increasingly focuses on CF-related complications. As the disease has shifted from primarily paediatric to predominantly adult, with near-normal life expectancy for many, new challenges have emerged. Understanding metabolic, hepatic, and systemic consequences has become crucial.

The focus of this thesis is therefore twofold:

1. To investigate how CF-related diabetes influences CF lung disease, particularly through inflammatory pathways, and
2. To study how CFTR modulator therapy affects CF related hepatobiliary involvement, an area of growing importance in modern CF care.

Introduction

Cystic fibrosis (CF) is a chronic, inherited multi-organ disease caused by mutations in the *CFTR* gene (1). The *CFTR* gene is located on the long arm of chromosome 7 (7q31.2) encoding the CFTR protein consisting of 1480 amino acids, 27 exons, and 26 introns (2). CF is inherited in an autosomal recessive manner and is the most common inherited disease in the Caucasian population. The cystic fibrosis transmembrane conductance regulator protein is a membrane protein expressed predominantly at the apical surface of epithelial cells in exocrine tissues, including the airways, pancreas, intestine, hepatobiliary system, reproductive tract, and sweat glands.

The CFTR protein (Figure 1) is composed of five major domains: two membrane-spanning domains (MSD1 and MSD2), two cytoplasmic nucleotide-binding domains (NBD1 and NBD2), and a regulatory (R) domain that is unique among ABC transporters (3). Each MSD contains six transmembrane helices that form the pore through which anions traverse. The NBDs bind and hydrolyse ATP, generating conformational changes that open and close the channel pore. Regulation of the CFTR protein begins with phosphorylation of the R domain by protein kinase A (PKA), a process triggered by intracellular cAMP signalling (4). Once phosphorylated, ATP binding at the NBDs induces channel opening, and subsequent ATP hydrolysis results in channel closure. This tightly controlled gating mechanism allows CFTR to respond dynamically to hormonal and intracellular signals, enabling precise regulation of epithelial ion transport.

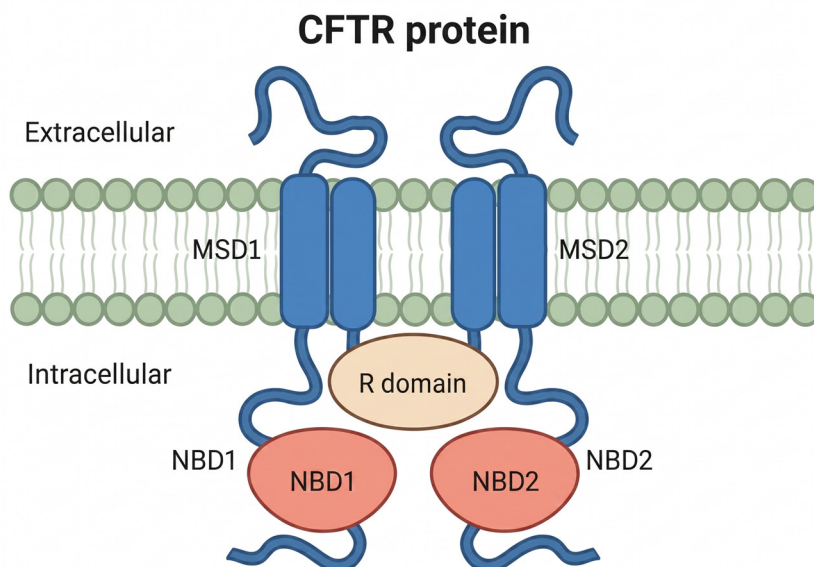


Figure 1.
Structure of the CFTR protein and its domains. AI generated ChatGPT.

CFTR primarily functions as a cAMP-regulated anion channel that transports chloride (Cl^-) and bicarbonate (HCO_3^-) ions across epithelial surfaces (5, 6).

Its main role is to mediate the secretion of chloride ions from the cytoplasm into the lumen. Chloride secretion is a key determinant of epithelial fluid movement, as it generates osmotic gradients that drive passive water transport and, in coordination with the epithelial sodium channel (ENaC), regulates apical Na^+ absorption (7).

CFTR also conducts bicarbonate (HCO_3^-), an anion essential for regulating luminal pH and fluid transport across the epithelium, and for correct mucin expansion (8-10).

Beyond its role as an anion channel, CFTR also regulates other transport systems, including ENaC, other Cl^- channels such as calcium-activated chloride channels, and chloride-bicarbonate exchangers such as SLC26A9 (5, 11, 12).

CFTR is mainly expressed in epithelial cells but has also been described to be expressed in other cell types. CFTR has been found in immune cells, platelets, islet cells and intracellular organelles such as the endoplasmic reticulum, Golgi network, and endosomes (13-16).

In summary, CFTR has multiple functions across different organ systems and intracellular processes. This complicates the understanding and treatment of patients with *CFTR* mutations.

CF - a genetic disease

CF is an autosomal recessive disease affecting around 162,428 people globally (17). It has traditionally been regarded as the most common inherited disease in the Caucasian population with a 1:25 carrier frequency in the Northern European Caucasian population. Recent research shows that the disease is also affecting individuals with genetic backgrounds from Asia and Latin America (18, 19).

In Sweden, around 20 individuals with CF are born each year and currently around 820 individuals with CF are treated at the four CF centres in Sweden - Lund, Gothenburg, Uppsala, and Stockholm (20).

Variants in *CFTR* are classified into six different classes (Figure 2) according to the underlying *CFTR* defect (21, 22). The different classes affect disease severity and current treatment options (23). In the era of highly potent *CFTR* modulators, variant classes are an important tool for individualising CF treatment (24).

The most common variant, F Δ 508, affects 70% of all individuals with CF and is a class II defect. In Sweden, around 80% of all individuals with CF have at least one F Δ 508 variant (20).

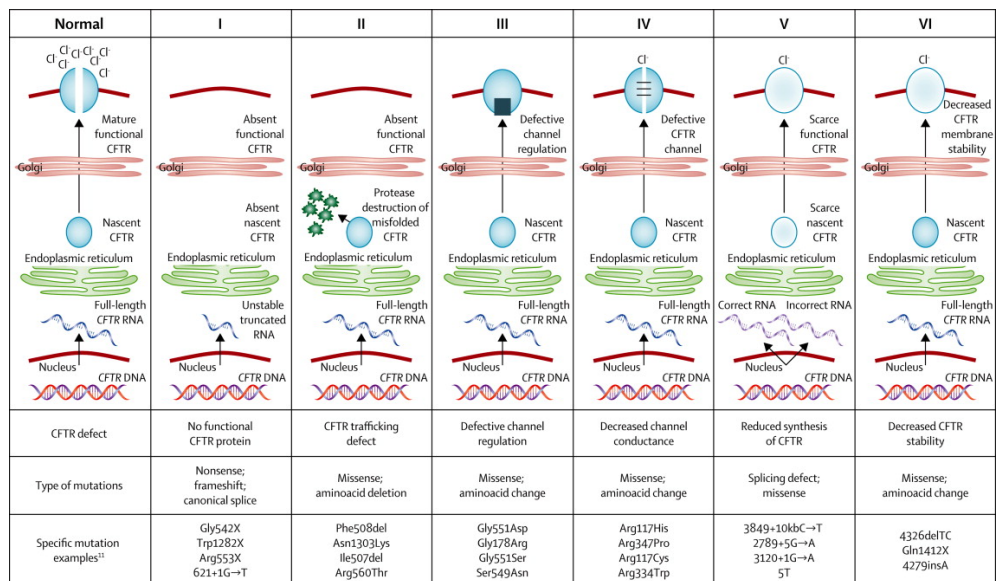


Figure 2.

CFTR variant classes. Reprinted from: Boyle MP, DeBoeck K. A new era in the treatment of cystic fibrosis: correction of the underlying CFTR defect. *Lancet Respir Med.* 2013;1(2):158-163.

Class I variants, caused by CFTR transcription and translation problems, result in either class IA absence of mRNA or class IB absence of CFTR protein due to a premature stop codon variant (21, 25). The “Nordic” mutation 394delTT, which affects about 13% of individuals with cystic fibrosis in Sweden and belongs to Class I, is comparable to 3659delC, which affects roughly 10% of Swedish individuals with CF (20).

Class II variants like FΔ508 are the most prevalent and are caused by misfolding of the CFTR protein, thereby inhibiting trafficking of the protein to the cell membrane (25). Class III variants, mainly known as “gating mutations”, produce a CFTR protein with an insufficient channel opening function at the cell membrane. Class IV variants affect the conductance of the channel, leading to an impaired channel function. Class V variants have a functional protein at the cell membrane but in a reduced amount due to decreased CFTR synthesis. Class VI variants lead to a lower amount of functional protein at the cell surface due to instability of the protein (25).

The disease has a more severe phenotype in classes I-III variants, with predominantly classical CF lung disease with early onset, pancreatic insufficiency, and development of CF-related complications (26). Classes IV-VI cause milder disease, individuals with these classes can be pancreatic sufficient and present with a later onset and a milder disease. Male individuals with class IV-VI variants are sometimes diagnosed during fertility assessments.

The history and milestones in Cystic Fibrosis

Already in medieval times, an Irish folklore described CF: “Woe to the child who tastes salty from a kiss on the brow, for he is cursed and soon will die”(27). In 1595, Pieter Pauw at Leiden University, the Netherlands, performed an autopsy on a 11-year-old child believed to be haunted and described his findings, including an enlarged and shiny pancreas. In the following decades, pancreatic disease was reported recurrently in children, and Carl von Rokiansky described meconium ileus in a fetal autopsy in the 19th century (28). In the same century, Frédéric Chopin suffered from a chronic respiratory disease, malnutrition, and possibly infertility. He died at the age of 39. His sister Emilia also experienced recurrent respiratory infections and died at the age of 14. It has been suggested that Frédéric Chopin may have had cystic fibrosis and could have been the first well-known case of the disease, but this diagnosis has never been confirmed (29).

In the 20th century, Dr. Dorothy Andersen provided the first comprehensive medical description of cystic fibrosis after performing multiple autopsies in children thought to suffer from celiac disease. In 1938, she published a paper defining the disease and detailing its characteristic clinical manifestations involving the pancreas, lungs and intestines (30).

Building on this foundational work, Andersen later contributed to clarifying the inheritance pattern of the disease. In 1946, she co-authored a publication identifying cystic fibrosis as a genetic disorder, marking the first time its hereditary nature was formally described in the scientific literature (31).

During the New York heatwave of 1948, Dr. Paul di Sant'Agnese observed that children with “cystic fibrosis of the pancreas” became severely dehydrated due to excessive salt loss in their sweat. This pivotal clinical observation revealed that individuals with CF have abnormally high sweat electrolyte concentrations and laid the foundation for the development of the sweat test, which later became the gold standard for CF diagnosis (32).

It took more than 50 years before the CFTR gene was discovered in 1989 (1). In the meantime, the sweat test was developed in 1959, and CF care was organized in specialised CF centres, improving survival among patients (33). In the United States, the Cystic Fibrosis Foundation was established in 1955, and treatments such as pancreatic enzyme replacement, airway clearance, and antibiotics were introduced. Improved understanding of bacterial transmission, better nutrition, and the advent of lung transplantation transformed CF between approximately 1970 and 1990 from a fatal childhood disease into a condition increasingly compatible with adult survival.

The 1989 discovery of the CFTR gene marked a turning point in the history of cystic fibrosis, shifting the field from mainly treating symptoms to developing molecularly targeted therapies that directly address disease mechanisms. This breakthrough fundamentally reshaped research strategies and clinical thinking and continues to drive unprecedented improvements in survival and quality of life.

The 21st century has been marked by landmark breakthroughs in CF, driven by advances in genetic sequencing and molecular pathophysiology. These developments enabled a new generation of CFTR-targeted therapies, beginning with the approval of the first potentiator, ivacaftor, in 2012 in people with CF with a class III mutation, which shifted treatment from symptomatic care to correction of the underlying protein defect (34). In 2015, the combination therapy lumacaftor–ivacaftor was approved for individuals with CF homozygous for the F Δ 508 mutation and became the first widely used CFTR modulator therapy in Sweden. Its primary benefits included improvements in FEV₁pp and reductions in pulmonary exacerbations (35).

Building on this foundation, the introduction of the triple-combination therapy ETI (elexacaftor/tezacaftor/ivacaftor) in 2020 in Sweden further transformed and improved outcomes; recent regulatory expansions in Europe now allow its use for people aged 2 years and older with at least one non-class I CFTR mutation (36–38).

At the same time, gene therapy and gene-editing approaches have emerged to address the needs of those who cannot benefit from modulators, particularly individuals with class I mutations, which produce little or no functional CFTR protein. Current research includes airway-directed gene delivery, prime editing, and a first-in-human trial of an inhaled lentiviral CFTR gene therapy designed to provide a mutation-agnostic treatment option (39). These innovations offer realistic potential for disease-modifying therapy across all CF genotypes.

Crucially, this era of therapeutic progress has been paralleled by the implementation of newborn screening, which revolutionized early diagnosis and improves early CF lung disease outcomes, nutritional status, and pancreatic disease (40, 41). Pilot CF newborn screening programs began in the late 1970s and early 1980s through measurement of immunoreactive trypsinogen (IRT) in dried blood spots, and the 1989 discovery of the CFTR gene enabled DNA analysis to be incorporated, improving accuracy (42). By the early 21st century, newborn screening programs had spread widely across the United States and Europe, with most regions adopting IRT-DNA-based algorithms that allow infants to receive nutritional and respiratory interventions before symptoms emerge (43). Unfortunately, in Sweden there is still no newborn screening program available.

Taken together, early detection through newborn screening, the advent of CFTR modulator therapies, and the development of emerging gene-based treatments have transformed the landscape of cystic fibrosis. These advances have markedly improved quality of life, and many individuals with CF now may potentially experience a near-normal life expectancy.

As survival steadily improves, the focus of research has evolved as well. The scientific journey is far from over; instead, it has entered a new phase that concentrates on managing CF-related complications, including CF-related diabetes and CF-related liver disease, both of which are central topics addressed in this thesis.

CF as a multiorgan disease

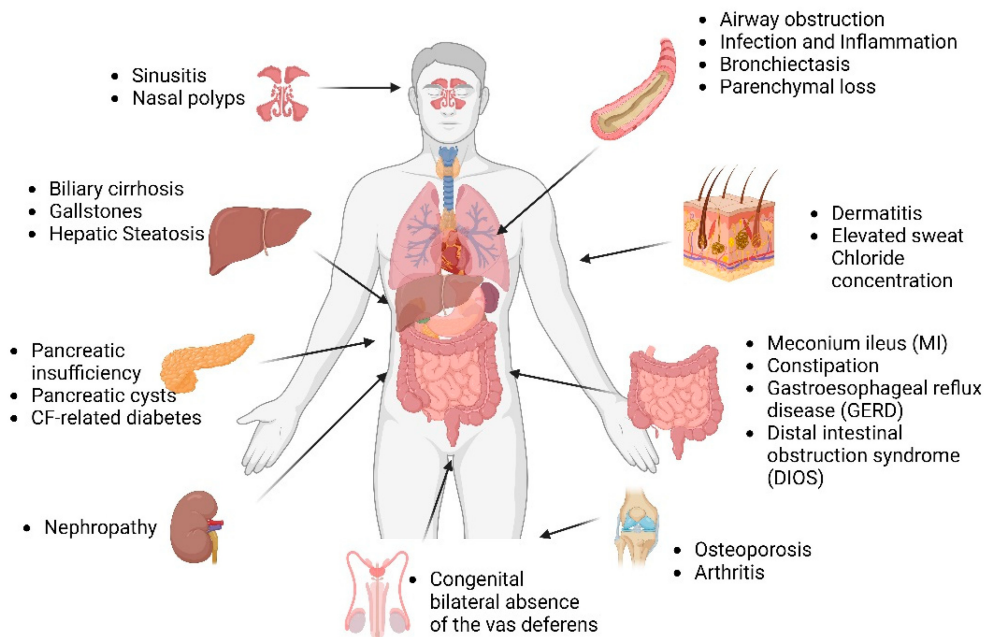


Figure 3.

CF affects multiple organ systems. Reprinted from: Ramananda, Y.; Naren, A.P.; Arora, K. Functional Consequences of CFTR Interactions in Cystic Fibrosis. *Int. J. Mol. Sci.* 2024, 25, 3384.

CF is classically recognized as a multisystem genetic disorder (Figure 3), but its clinical course has historically been dominated by its pulmonary manifestations, which remain the primary cause of morbidity and mortality (26). Defective CFTR-mediated chloride and bicarbonate transport leads to the production of highly viscous airway mucus, impairing mucociliary clearance and creating an environment that promotes chronic bacterial colonization and persistent airway inflammation. Over time, these processes drive the development of bronchiectasis and a progressive decline in lung function, which for decades defined the fatal natural history of the disease in early life (44).

Beyond the lungs, CF exerts profound effects on the pancreas, the gastrointestinal and hepatobiliary systems. Obstruction of the pancreatic ducts by thickened secretions leads to exocrine pancreatic insufficiency early in life, resulting in impaired digestion, malabsorption of fats and proteins, failure to thrive, and fat-soluble vitamin deficiencies (45). Progressive pancreatic injury can extend to the endocrine pancreas, contributing to the development of CFRD, now one of the most common extrapulmonary comorbidities in individuals with CF (46).

Similarly, intestinal involvement results from dehydrated luminal secretions and impaired electrolyte transport, leading to viscous intestinal contents, delayed small intestinal transit, and an altered gut microbiome. This contributes to constipation, distal intestinal obstruction syndrome, and, in around 10% of infants, meconium ileus (47).

Crucially, the same pathophysiological mechanism - abnormal chloride and bicarbonate transport - also affects the hepatobiliary system, giving rise to CFLD. Thickened bile and impaired bile duct secretion lead to cholestasis, periportal inflammation, and focal biliary cirrhosis, which may progress to multilobular cirrhosis and portal hypertension (48).

In addition to gastrointestinal and hepatic complications, CF affects the kidneys, often indirectly. Recurrent dehydration, chronic inflammation, cumulative exposure to nephrotoxic medications, and CFRD can contribute to renal tubular dysfunction, an increased risk of nephrolithiasis, and chronic kidney disease (49, 50).

CF also has a well-recognized impact on bone health. A constellation of factors, including nutritional deficiencies, malabsorption, low-grade chronic inflammation, and glucocorticoid exposure, predisposes individuals to low bone mineral density, osteopenia, and osteoporosis (50, 51).

A further important systemic manifestation concerns the reproductive system in males. Approximately 95% of men with CF present with congenital bilateral absence of the vas deferens, leading to obstructive azoospermia despite preserved spermatogenesis (52, 53).

Collectively, these pulmonary, gastrointestinal, hepatic, renal, skeletal, and reproductive manifestations highlight the deeply systemic and complex nature of cystic fibrosis. As survival improves due to advances in early diagnosis and targeted therapies, the clinical focus increasingly shifts toward understanding, preventing, and managing these long-term multisystem complications.

Treatment of CF in the era of CFTR modulators

Treatment of CF has traditionally focused on symptomatic management across the various organ systems affected by the disease and treatment was planned and revised at CF centres according to the ECFS standards of care (54). A CF centre provides multidisciplinary care through a team consisting of physicians, CF nurses, physiotherapists, dietitians, and psychologists who support and guide individuals living with cystic fibrosis. Regular visits for monitoring and screening for CF-related complications are part of CF management (55).

For decades, the primary therapeutic emphasis was on the respiratory system, as progressive lung damage has been the leading cause of morbidity and mortality in CF. Airway-directed therapies aim to mobilize mucus, rehydrate airways, enhance airway clearance, and control chronic inflammation. Daily physiotherapy, combined with inhaled therapies such as hypertonic saline and dornase alfa or mannitol forms the cornerstone of routine care (56-58). These interventions reduce mucus viscosity and support mucociliary clearance, thereby helping to preserve lung function and reduce pulmonary exacerbations.

A second major pillar of CF management is nutritional support. Due to high incidence of pancreatic exocrine insufficiency in individuals with CF, most individuals require a high-caloric, high-fat diet to counteract malabsorption and increased energy expenditure. Pancreatic enzyme replacement therapy is essential at every meal to ensure proper digestion of fats, proteins, and carbohydrates (59). Supplementation with fat-soluble vitamins (A, D, E, and K) is routinely needed, along with careful monitoring and treatment of constipation and prevention of distal intestinal obstruction syndrome (60, 61).

A third essential component of CF care is antibiotic therapy, which plays a central role in maintaining respiratory health (62). Most individuals with CF develop chronic airway colonization, commonly with *Staphylococcus aureus* in the first decades, shifted later to *Pseudomonas aeruginosa*, requiring recurrent treatment with combinations of inhaled, oral, and intravenous antibiotics (56, 63). Inhaled antibiotics, such as tobramycin and colistin, are used long-term to suppress *Pseudomonas aeruginosa* bacterial load in the airways, while oral antibiotics such as ciprofloxacin or intravenous combination therapy are used for eradication (64, 65). Azithromycin is used for its immunomodulatory properties and anti-inflammatory effects (66). Oral antibiotics are often prescribed for milder infections or anti-staphylococcal prophylaxis, such as flucloxacillin in infants up to school age at the CF Centre in Lund (67). Antibiotic strategies in infections with non-tuberculous mycobacteria, *Burkholderia cepacia* complex, *Achromobacter xylosoxidans* or fungi like *Aspergillus fumigatus* are more individualized and based on microbial colonization patterns and clinical response (50, 63, 68).

In the era of CFTR modulator therapy, the traditional three-pillar approach continues to form the backbone of cystic fibrosis management (69). Ongoing studies are currently evaluating how symptomatic treatments should be adapted within this new therapeutic landscape (70).

Today, the triple CFTR modulator therapy ETI (elexacaftor/tezacaftor/ivacaftor), from the age of two years, is the modulator therapy mainly used in Sweden. Individuals with at least one non-class I variant are eligible for this treatment from the age of 2 years in Europe since 2025. According to the Swedish CF registry, 539 patients in Sweden were receiving ETI in autumn 2025 (71).

ETI is an oral drug consisting of three different substances, elexacaftor/tezacaftor/ivacaftor (Figure 4), that bind to the CFTR protein, acting both as a corrector and a potentiator (72). The corrector (tezacaftor) improves the folding of the CFTR protein intracellularly and enhances trafficking to the cell surface (73). The potentiator (ivacaftor) acts at the CFTR channel level at the cell surface and can facilitate channel opening (74). Elexacaftor acts as both a corrector and a potentiator (75).

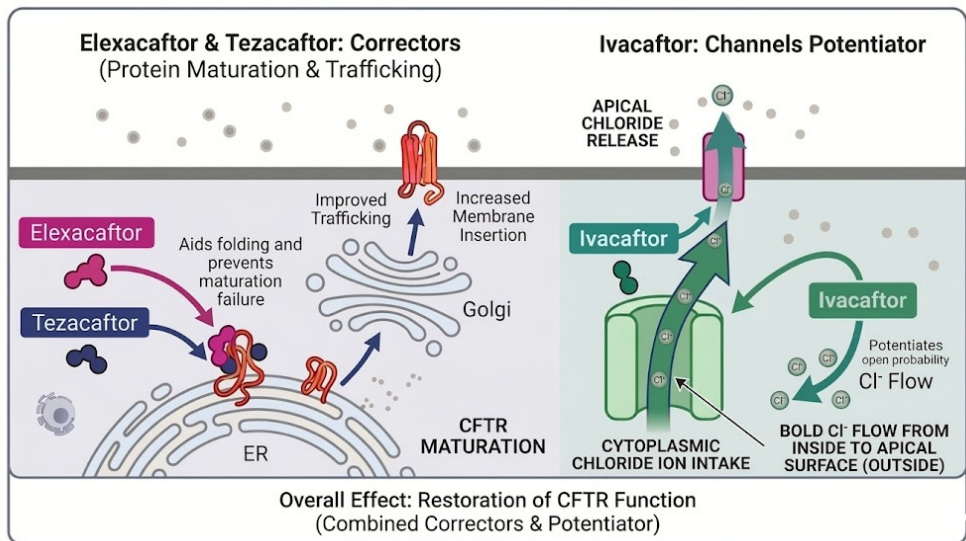


Figure 4.

Elexacaftor and Tezacaftor act as correctors and Ivacaftor as a channel potentiator, AI generated

The safety and positive effects of ETI on the clinical course of cystic fibrosis are well studied in randomized trials (38, 76). ETI therapy has been associated with reduced morbidity, reduced mortality, and a significant improvement in quality of life for most eligible patients. Real-world data reveal a marked reduction in pulmonary exacerbations, a slowing of lung-function decline, and improved nutritional status, reflecting better overall disease stability (77-79). Graeber et al showed that ETI can restore CFTR function in the airways and in the intestine to approximately 50% in patients with at least one FΔ508 variant (80).

Despite these substantial advancements, the long-term impacts of ETI remain an area of active investigation (81). In particular, the influence of ETI on CF-related comorbidities such as CF-related diabetes and CF liver disease is not yet fully understood (82, 83). As patients live longer and with milder pulmonary disease, understanding how CFTR modulation reshapes the broader multisystem manifestations of CF will be essential (84). Another important aspect is the use of

ETI during pregnancy and in utero, an area where evidence is still emerging and requires further investigation in well-designed, multicentre studies. Only small retrospective observations have shown no adverse effects of ETI treatment during pregnancy in mothers and infants (85). Further longitudinal studies are needed to clarify these outcomes and determine whether early and sustained ETI therapy can prevent, delay, or modify the natural history of CF and CF-related complications.

The vicious cycle in CF lung disease

Cystic fibrosis lung disease is often described as a self-perpetuating vicious cycle, where one pathological process amplifies the next one. This cycle begins with the underlying defect in the *CFTR*, which affects the CFTR channel, and progresses through a cascade of airway abnormalities, such as airway surface liquid depletion, impaired ciliary function, and mucus-plugging, to immunological changes with activation of the innate immune response and infection with bacteria or fungi, which reinforce each other over time (86, 87). This vicious cycle leads to damage in the lung parenchyma with development of bronchiectasis and emphysema, causing lung function decline.

Airway surface liquid depletion

The defect in the CFTR channel, leading to impaired chloride and bicarbonate secretion and enhanced sodium absorption through βENaC channels, is probably the primary cause to the airway surface liquid depletion in CF (9, 88, 89). This disturbed ion balance leads to changes in the volume and hydration of the airway surface liquid (ASL) that lines the epithelial cells in the airways (90). Although CFTR is central to ASL regulation, emerging evidence underscores that ASL homeostasis represents the output of a multichannel, multicellular regulatory network rather than a single defective component. Recent research has revealed that other ion channels, including TMEM16A, and transporters such as members of the SLC26 family, in addition to sodium channels like βENaC , contribute significantly to chloride, bicarbonate, and sodium flux across the airway epithelium (91, 92). These pathways may become particularly relevant when CFTR function is pharmacologically modulated, and in the search for new treatment targets, suggesting that CFTR operates within a broader ion transport system.

The ASL forms a thin, protective fluid layer composed of two distinct components: the periciliary layer (PCL), which maintains ciliary mobility, and the mucous layer (MCL), which traps inhaled particles and pathogens. Together, these layers create the foundation for effective mucociliary clearance. The ASL, composed of a balanced mixture of mucus and fluid, is essential for the first line of antimicrobial

defence. By trapping microorganisms and enabling their removal through coordinated ciliary movement, it prevents early pathogen adherence and colonization. The depth and composition of ASL are regulated by osmotic pressure so that the PCL remains sufficiently hydrated to allow ciliary beating, while the mucus layer retains the viscoelastic properties required for transport (93).

In CF, defective regulation of the ASL disrupts this osmotic balance, resulting in ASL depletion. This shift in hydration state compresses the PCL and slows mucus transport through impaired ciliary activity.

As mucus clearance declines, mucus plugging and localized hypoxia develop, creating a microenvironment that is highly conducive to persistent colonization and growth of pathogenic microorganisms (94-96).

Alongside volume regulation, the pH of the ASL plays a fundamental role in host defence. CFTR-mediated bicarbonate secretion maintains a near-neutral pH that is essential for the optimal activity of airway antimicrobial peptides (97). In CF, impaired bicarbonate transport leads to ASL acidification, markedly reducing bacterial killing efficiency (98). This contributes to the formation of excessively viscous mucus and undermines local innate immune defences (99, 100). Studies in CF pigs demonstrated that increasing ASL pH can restore antimicrobial function, highlighting pH dysregulation as an early and primary defect in CF airway defence (101). Moreover, bicarbonate is required for mucin unfolding and proper gel formation; insufficient bicarbonate results in denser, more adhesive mucus that is poorly cleared (9, 102).

The initiating steps of CF airway disease

A long-standing debate in CF research has been the initiating steps in CF airway disease. What triggers early airway inflammation: direct infection, or a cascade of impaired ciliary clearance, mucus plugging, and hypoxia? The correct answer is probably found in animal models – including CF ferret models and murine models of CF-like lung disease. These models show an inflammatory response in sterile conditions, triggered by neutrophilic inflammation (103, 104). Chest imaging in infants and preschool children diagnosed by CF newborn screening confirms, in observational studies, that airway abnormalities such as mucus plugging, bronchial wall thickening, and bronchial dilatation are visible already early in life (105, 106). These findings support impaired ciliary clearance as the initiating step, leading to ongoing inflammation and later infection, as shown in Figure 5.

Mucus plugging and hypoxia in the airways can activate a sterile neutrophilic inflammation driven by IL-1-mediated pathways through hypoxic epithelial cell necrosis (107). In fact, IL-1 β and IL-1 α have been detected in BAL of children with CF in the absence of pathogens, and IL-1 α was associated with structural lung damage (108).

Rosenfeld et al. demonstrated that infants with CF have elevated neutrophilic inflammation and IL-8 in BAL regardless of the presence of detectable pathogens. With increasing age, the prevalence and density of airway pathogens increase together with neutrophils and IL-8 (109). The link between epithelial necrosis, followed by increased neutrophilic inflammation and elevated IL-1 α levels, was also shown in the airways of β ENaC-overexpressing mice (104).

Neutrophilic inflammation as a key player in CF lung disease can also be driven by a cascade of signals mediated by IL-1 α and IL-1RI leading to NF- κ B activation (110, 111). NF- κ B is a potent transcription factor that can induce further inflammatory processes, including the release of IL-8, which causes neutrophil influx (112). IL-10, which normally suppresses NF- κ B, is downregulated in CF - another factor that may perpetuate inflammation.

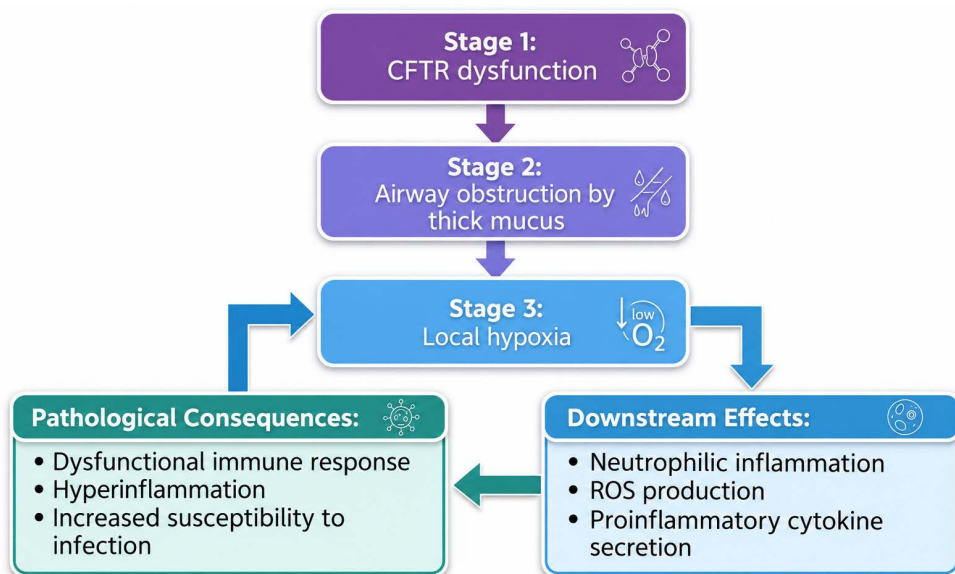


Figure 5.

CFTR dysfunction as the initiating step in CF lung disease. Reproduced by AI from: Petrocheilou A, Moudaki A, Kaditis AG. Inflammation and Infection in Cystic Fibrosis: Update for the Clinician. Children (Basel). 2022 Dec 2;9(12):1898.

Ongoing chronic inflammation and infection

Inflammation in CF airways is a multifactorial and highly dysregulated process, driven by the interplay of persistent bacterial infection, altered innate immunity, and intrinsic epithelial defects. Both extracellular and intracellular mechanisms are involved in the hyperinflammation seen in CF airways, which is measurable as increased neutrophils and pro-inflammatory cytokines in BAL already early in life. Besides the increased inflammatory activity, the inability to terminate and resolve inflammatory processes also plays an important role (113).

Neutrophils

Cystic fibrosis lung disease is marked by a predominantly neutrophil-driven inflammatory response that becomes excessive and self-perpetuating over time. Neutrophils are central components of the innate immune system and are recruited into the airways through interactions with pathogens and inflammatory cytokines. Under normal conditions, activated neutrophils phagocytose and kill microbes, undergo apoptosis, and are subsequently cleared by macrophages. In CF, however, this tightly regulated process is disrupted. Neutrophils exhibit impaired phagocytic capacity, likely related to CFTR dysfunction within the cells, and instead accumulate in large numbers within the airway lumen (114).

Neutrophils also contribute to host defence by releasing neutrophil extracellular traps (NETs), composed of decondensed DNA and antimicrobial proteins, which entrap pathogens. However, in CF, the overproduction of NETs significantly increases mucus viscosity and airway obstruction (115).

In addition, neutrophils contain several granule subsets loaded with pro-inflammatory mediators, including neutrophil elastase (NE) and other serine proteases. The release of these proteases plays a critical role in disturbing the protease–antiprotease balance. NE becomes pathologically abundant in CF due to both excessive neutrophil influx and inadequate antiprotease activity, such as α 1-antitrypsin. Elevated NE activity accelerates disease progression by degrading structural components of the airways and inducing further mucus hypersecretion (116). Notably, increased NE activity in bronchoalveolar lavage fluid has been associated with early bronchiectasis in infants with CF (117). NE itself can further attract neutrophils through IL-8 upregulation in epithelial cells (118).

In parallel, neutrophils generate reactive oxygen species (ROS) as part of the respiratory burst. In CF airways, excessive ROS production represents an additional source of inflammation and tissue damage. This oxidative burden contributes to epithelial injury, further mucus production, and impaired mucociliary clearance, thereby exacerbating airway obstruction and increasing susceptibility to persistent infection (119).

Macrophages

Macrophages are abundant in the lumen of CF airways, where they contribute substantially to the chronic inflammatory environment. Multiple studies have shown that CF macrophages exhibit defective lysosomal or phagolysosomal acidification, impairing their ability to degrade and kill internalized bacteria (120). Additionally, more recent work confirms these defects in CF macrophages, which can be partly rescued by CFTR modulators (121).

Alongside these defects, CF macrophages also display exaggerated innate immune activation with increased production of inflammatory cytokines like IL-1 β , IL-6, and TNF- α indicating that CF macrophages adopt an intrinsically pro-inflammatory phenotype (122). Bruscia et al. demonstrated further that CF macrophages exhibit dysregulated TLR-mediated responses, contributing to airway inflammation via NF- κ B activation and increased cytokine secretion (123).

Lymphocytes

T and B lymphocytes are increasingly recognized as important contributors to the dysregulated immune landscape in CF. Although CF has long been characterized primarily as a neutrophil-driven disease, more recent evidence demonstrates that adaptive immunity is also significantly disrupted (124). Lymphocytes express functional CFTR, and defects in CFTR may disturb intracellular ion homeostasis, leading to altered crosstalk between airway structural cells and lymphocytes, impaired immunoregulation, and weakened host defence mechanisms.

In CF, a characteristic shift from Th1- to Th2-dominated responses has been described, potentially contributing to ineffective pathogen clearance and heightened inflammatory signalling. In addition, Th17 cells, which are key drivers of neutrophilic inflammation, appear to be enriched and hyperactive in the CF airway environment, further amplifying chronic inflammation (125). B-cell function is likewise altered, with evidence of dysregulated activation and antibody responses that may contribute to persistent infection and immune imbalance (126).

Platelets

Platelets have increasingly been recognized as important contributors to the exaggerated inflammatory response characteristic of CF. Beyond their classical role in haemostasis, platelets in CF appear to promote lung injury and impair host defence mechanisms. Observations in CF patients have shown that platelet counts are elevated during acute pulmonary exacerbations, suggesting that platelet activation accompanies episodes of heightened airway inflammation and exacerbations (127).

In addition, levels of soluble CD40 ligand (sCD40L), a pro-inflammatory mediator predominantly released by activated platelets, have been found to be increased in CF. Notably, circulating sCD40L concentrations correlate inversely with FEV1%

predicted, indicating that platelet-derived inflammatory signals may be associated with declining lung function (128).

Platelets express functional CFTR channels in their plasma membrane, and CFTR dysfunction in CF patients leads to altered intracellular ion homeostasis. Specifically, defective CFTR has been associated with increased calcium influx leading to platelet hyperreactivity (129). Platelet hyperreactivity could lead to increased platelet-neutrophil interactions with subsequent NET formation, thereby aggravating the inflammatory response (130).

Moreover, CF platelets produce reduced levels of the anti-inflammatory lipid mediator lipoxin A4 compared with healthy controls (16). Given that lipoxin A4 helps to resolve inflammation, its deficiency may further exacerbate chronic airway inflammation in CF. Altogether, the combined effects of platelet hyperreactivity, elevated platelet-neutrophil complexes, and diminished anti-inflammatory signalling underscore the role of platelets as active participants in CF-related inflammation rather than passive bystanders.

Cytokines and inflammatory mediators

Cytokines and inflammatory mediators (Table 1) in CF lung disease are released in response to the stimuli described above and are widely used as markers of disease progression and inflammation in CF (131, 132).

Table 1. Cytokines and inflammatory mediators investigated in this thesis

	Primary source	Function	Reference
IL-1 β	Macrophages	Neutrophil recruitment, NET formation, release of IL-6 and IL-8	(133)
TNF- α	Macrophages	Potent pro-inflammatory mediator acting in multiple ways (including oxidative stress, cell proliferation, and leucocyte recruitment)	(134)
IL-6	Macrophages, endothelial cells	Mediates acute-phase protein synthesis, stimulates B and T cells	(135)
IL-17	TH17 cells	Activates neutrophils and macrophages	(136)
IL-8	Macrophages, epithelial cells	Attracts and activates neutrophils	(137)
NE	Neutrophils	Degrades elastin and extracellular matrix, induces mucus production, and acts as a pro-inflammatory mediator	(138-140)

Airway microbiome

In recent years, the study of the airway microbiome in CF has expanded considerably, largely due to advances in high-throughput sequencing techniques and improved analytical tools. These developments have enabled more comprehensive and precise investigations into microbial diversity and its role in disease progression.

The human airway hosts a diverse and dynamic microbial community, in contrast to the earlier belief that the lungs were sterile, which is essential for maintaining respiratory health.

In healthy individuals, this airway microbiota consists of bacteria, viruses, and fungi in a dynamic and diverse composition. The function of the airway microbiome is to support immune tolerance, mucosal immunity, and preventing the overgrowth of pathogenic organisms (141, 142). Different studies reveal complex pathways in the interaction between the host immune system and the airway microbiome. Segal et al. demonstrated that in healthy adults an enriched lower airway microbiome with *Prevotella* and *Veillonella* was associated with an increased T_H17 cell-mediated lung inflammation and a diminished Toll-like receptor 4 (TLR4) response by alveolar macrophages (143).

The airway microbiome is commonly divided into the upper and lower airway microbiomes, which differ in microbial density and genus composition (Figure 6). The upper airway microbiome is found in the upper respiratory tract including the nasal cavity, nasopharynx, and oral cavity. It is the most densely colonized part of the airway system and acts as the primary source of microbes found in the lower airways, serving as a gatekeeper for the lower airways.

Dominant genera in healthy upper respiratory tract communities include *Corynebacterium*, *Dolosigranulum*, *Staphylococcus*, *Streptococcus*, *Prevotella* and *Veillonella*. The upper airway microbiota is strongly shaped by age with a lower diversity but higher density in childhood and vice versa in adulthood. Environmental exposures, lifestyle factors, and antibiotic use also influence the microbiome composition (144).

The lower airways were once believed to be sterile, but current evidence shows that healthy lungs contain a low-biomass but structured microbial community. This community largely originates from the upper respiratory tract via microaspiration and inhalation, and its composition is determined by a balance between microbial immigration and clearance mechanisms such as mucociliary transport and coughing. Common taxa detected in healthy lungs include *Streptococcus*, *Veillonella*, *Prevotella*, and *Neisseria*, typically present in low abundance and maintained in a transient, dynamic equilibrium.

There are also reports that a gut-lung axis can have an impact on the airway microbiome (145).

The airway microbiome plays a key role in maintaining respiratory health, and dysbiosis - defined as an imbalance in microbial composition or function - has been strongly linked to several respiratory diseases and, more recently, to the development of lung malignancies (146).

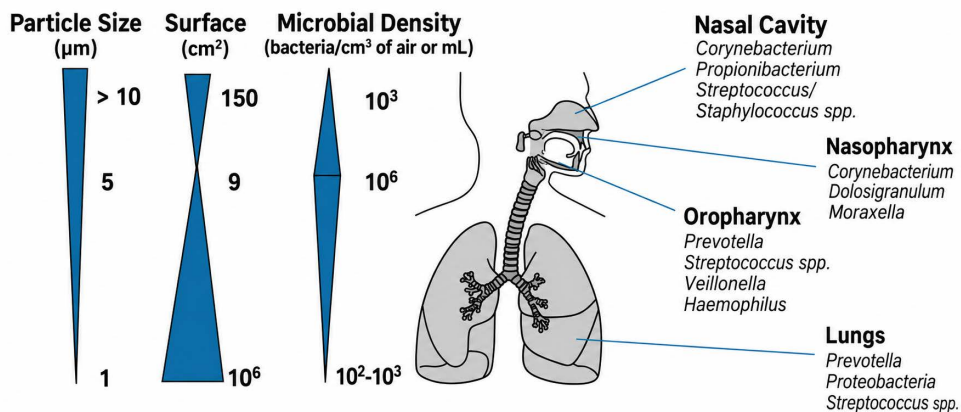


Figure 6.

Airway microbiome. Reprinted from: Glieda, S., Quarta, E., Bottari, B., Lal, V. C., Sonvico, F., & Buttini, F. The role of airways microbiota on local and systemic diseases: a rationale for probiotics delivery to the respiratory tract. *Expert Opinion on Drug Delivery*. 2024; 21(7), 991–1005.

In CF, the airways undergo lifelong cycles of infection, colonization, and inflammation driven by a changing spectrum of microbial pathogens. In early childhood, the airway microbiome in CF is typically more diverse and is often dominated by organisms such as *Haemophilus influenzae* and *Staphylococcus aureus*. As patients age and the disease progresses, this microbial landscape gradually shifts toward a less diverse community structure (Figure 7).

This dysbiosis is characterized by a marked reduction in microbial diversity and increasing dominance of opportunistic pathogens, most notably, *Pseudomonas aeruginosa* and, in many cases, persistent *Staphylococcus aureus*. The transition from a diverse early-life microbiome to a pathogen-dominated ecosystem is closely associated with chronic infection, heightened and sustained airway inflammation, structural lung damage, and an accelerated decline in lung function (147).

This impact has been demonstrated in different studies. Across a multi-centre study, Cuthbertson et al. demonstrated that a decreasing microbiota diversity correlates strongly with worsening lung function and the emergence of pathogen-dominated community structures (148).

Paganin et al. compared the airway microbiome in individuals with stable lung function (annual FEV1pp rate ≤ 1.5) to those with increased annual lung function

decline (annual FEV1pp rate ≥ 5). A correlation between lower diversity and increased dominance by pathogens was observed with reduced FEV₁, whereas patients with stable lung function tend to maintain mixed communities (149). Hampton et al. found another interesting aspect that different microbial dominated communities differ in lung disease severity. In this study, individuals with CF and *Pseudomonas*-dominated communities tended to be older and have reduced FEV1pp compared to subjects with *Streptococcus*-dominated communities (150).

Longitudinal surveillance of the airway microbiome, together with deeper microbial and functional analyses, may provide valuable biomarkers of lung disease progression in CF and help identify novel therapeutic targets.

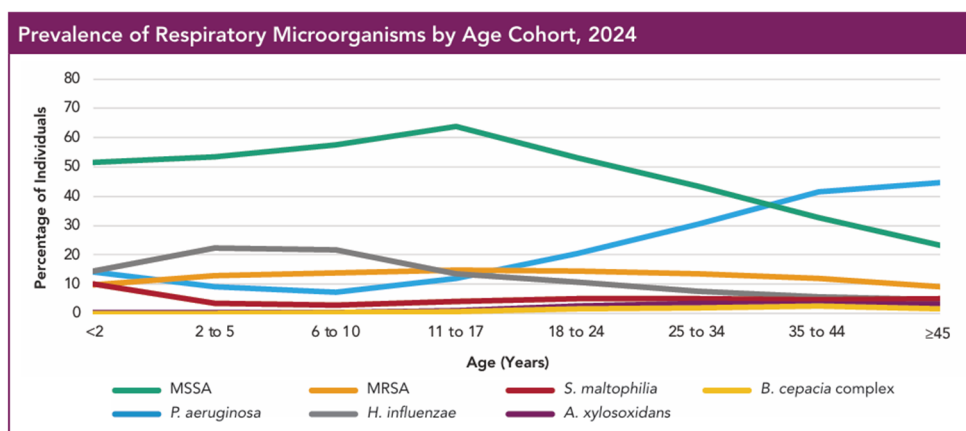


Figure 7.

Prevalence of respiratory microorganisms by age. Reprinted from *Cystic Fibrosis Foundation Patient Registry, 2024 Annual Data Report*; Bethesda, Maryland. © 2025 Cystic Fibrosis Foundation. Reprinted with permission and courtesy of the Cystic Fibrosis Foundation. Data represent individuals with cystic fibrosis receiving care at Cystic Fibrosis Foundation–accredited care centers in the United States who consented to inclusion of their information in the Registry.

CF-related diabetes

CFRD is the most common extrapulmonary complication in CF, affecting around 30% of individuals with CF (151). The incidence of CFRD increase with age, affecting around 1-2% of children, approximately 10-20% of individuals with CF between the ages of 10 and 20 years, and up to 40% in adulthood (152). CFRD is associated with higher morbidity and mortality (153). A clear negative impact of CFRD on lung function and nutritional status is well described (154-156). Airway colonisation with *Pseudomonas aeruginosa* or *Burkholderia cepacia complex* is also increased in CFRD (157). Risk factors for the development of CFRD are pancreatic insufficiency, class I-II mutations, female sex, CF-related liver disease, organ transplantation, gastric tube feeding, vitamin D insufficiency, and a family history of type 2 diabetes (158-160).

CFRD – clinical signs

CFRD represents a distinct clinical entity that shares certain features with both type 1 and type 2 diabetes, yet differs fundamentally in its underlying pathophysiology (161). CFRD typically develops gradually, with incidence increasing with age. In contrast to type 1 diabetes, ketoacidosis and classical symptoms such as polyuria and polydipsia are uncommon. Years before a formal diagnosis, individuals with CF may exhibit intermittent hyperglycaemia, particularly during pulmonary exacerbations. Glucose tolerance often progresses along a continuum - ranging from normal glucose tolerance to indeterminate glucose tolerance (INDET), impaired glucose tolerance (IGT), CFRD without fasting hyperglycaemia (FH), and eventually CFRD with FH.

According to the U.S. Cystic Fibrosis Foundation registry data for 2024 (Figure 8), around 20% of individuals with CF aged 10 years or older have impaired glucose tolerance when screened by an oral glucose tolerance test (OGTT) (162).

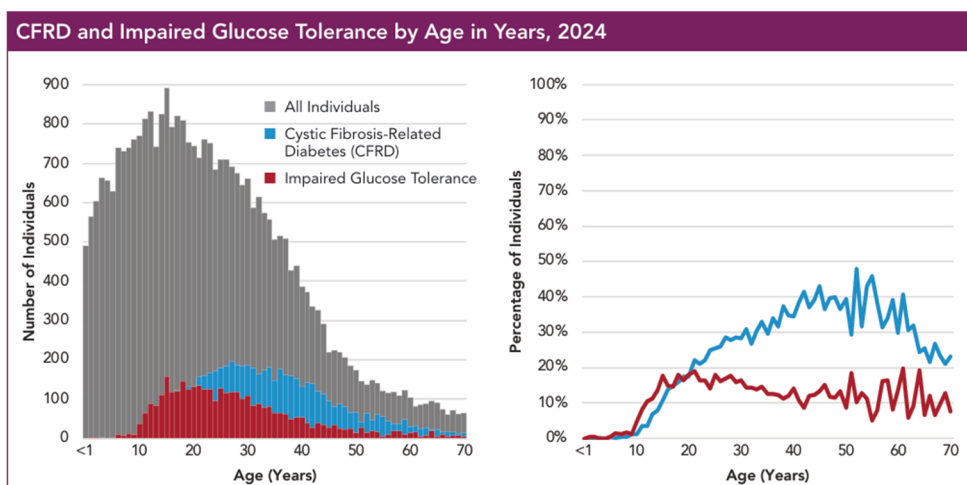


Figure 8.

The prevalence of CFRD and impaired glucose tolerance. Reprinted from *Cystic Fibrosis Foundation Patient Registry, 2024 Annual Data Report*; Bethesda, Maryland. © 2025 Cystic Fibrosis Foundation. Reprinted with permission and courtesy of the Cystic Fibrosis Foundation. Data represent individuals with cystic fibrosis receiving care at Cystic Fibrosis Foundation–accredited care centers in the United States who consented to inclusion of their information in the Registry.

During the early stages of glucose dysregulation, the condition is generally clinically silent. Subtle signs such as unintentional weight loss, decline in lung function, failure to thrive, and pulmonary exacerbations may signal the evolving development of CFRD. The disease is characterized by a combination of insulin deficiency and insulin resistance, but unlike type 1 diabetes, autoimmunity is absent. Patients with CFRD often have normal body weight, and features of metabolic syndrome, which are commonly observed in type 2 diabetes, are typically not present. Fasting hyperglycaemia is not consistently seen; instead, CFRD is frequently dominated by postprandial hyperglycaemia (163).

Microvascular complications, including neuropathy, retinopathy, and nephropathy, have been reported in CFRD (164). However, unlike type 1 and type 2 diabetes, macrovascular complications have rarely been reported. Importantly, the primary determinant of mortality in CFRD is the progressive decline in lung function, rather than cardiovascular disease as seen in type 1 and type 2 diabetes, reflecting the dominant impact of CFRD on pulmonary disease. Milla et al. demonstrated that the degree of impaired glucose tolerance correlates with the annual decline in lung function (165).

CFRD and lung disease

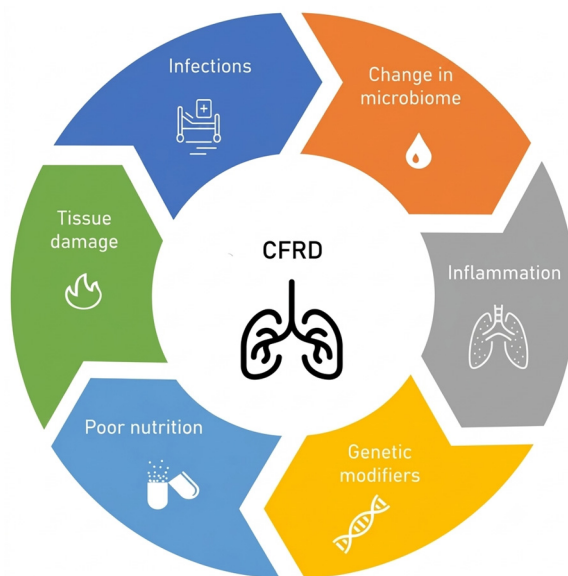


Figure 9.

The multifactorial drivers of CFRD and progressive lung disease. Reprinted from: Prentice BJ, Jaffe A, Hameed S, Verge CF, Waters S, Widger J. Cystic fibrosis-related diabetes and lung disease: an update. *European Respiratory Review*. 2021;30(159):200293.

A strong association between CFRD and lung function was already described 30 years ago (154, 155). In a Swedish cohort study of 377 paediatric CF patients, lung function decline was accelerated in those with CFRD (166). Some studies suggest that the observed decline in pulmonary function starts before the diagnosis of CFRD, but data are inconsistent and form a “the chicken-or-the-egg problem” for researchers studying lung disease in CFRD (167).

The fact that there is an association has been demonstrated in several studies, and there are signs that already impaired glycaemic control has a negative impact on lung function and that the decline rate is dependent on the severity of abnormal glucose tolerance.

Milla et al demonstrated in a prospective cohort study that both FEV₁pp and FVC declined over 4-year period, with the greatest decline rate observed in CFRD without FH and a greater decline rate in the IGT versus the NGT group at baseline. The same study demonstrated that patients with the lowest levels of insulin during OGTT had the greatest rate of lung function decline (165). Colombo et al. demonstrated ventilation inhomogeneity on the multiple breath washout test in paediatric CF patients with a disturbed first-phase insulin response (168). Similarly, Leclercq et al. identified pre-diabetic glucose abnormalities by continuous glucose

monitoring (CGM) in CF patients with a normal OGTT. Participants in this study with elevated glucose levels on CGM had significantly lower lung function than those without glucose abnormalities and had higher rates of *Pseudomonas aeruginosa* colonisation (169). We previously demonstrated, in a study with 32 paediatric CF patients, changes in lung clearance index (LCI) and FEV1pp in patients with AGT (170).

Abnormal glucose tolerance in CF is associated not only with declining lung function but also with structural lung changes detectable on CT scans. Notably, in CFRD, the annual rate of progression of structural lung damage was increased, even in the absence of measurable changes in lung function (171).

The pathophysiology underlying the accelerated lung function decline and structural changes in CFRD remains poorly understood, but is likely multifactorial (Figure 9), and many questions remain unanswered (172, 173). Impaired glucose homeostasis with hyperglycaemic peaks in the airways may affect airway epithelial cells, promote the growth of *S. aureus* and *P. aeruginosa*, and increase oxidative stress (174-176). Colonisation with *P. aeruginosa* is more common in individuals with CFRD, and Hunt et al. demonstrated impaired bacterial clearance of *P. aeruginosa* in CF-diabetic mice compared to CF-non-diabetic mice and controls (174). Garnett et al. reported that elevations in glucose levels promoted the growth of *P. aeruginosa* on primary CF and non-CF human bronchial epithelial cell monolayers (177).

Inflammatory processes driven by oxidative stress, infection, or hyperglycaemia may play an important role in the development of CFRD-related lung disease. Hyperglycaemia can induce NET formation, increase IL-6 and IL-8 levels, and act as a pro-inflammatory stimulus (178, 179). In addition, the higher infection burden in CFRD may contribute to disturbances in the airway microbiome.

Since lung development and function are closely linked to nutrition, poor nutritional status secondary to insulin deficiency may increase the risk of pulmonary exacerbations and accelerate lung function decline (180).

Structural parenchymal changes and alveolar microangiopathy, well described in diabetes-related lung disease, may also occur in individuals with CFRD (181).

Furthermore, the established association between genetic modifiers for type 2 diabetes and CFRD suggests that genetic factors may also influence lung disease in this population (182).

Screening for CFRD

Regular screening for CFRD is recommended according to European and U.S. CF guidelines and is essential to minimise the negative impact of CFRD (183, 184). The diagnosis of CFRD may be delayed by as much as eight years when based solely on the detection of hyperglycaemia rather than on routine screening (185).

Usually, screening starts at the age of 10 with an annual OGTT according to WHO guidelines as gold standard.

Additional screening is recommended during pregnancy or following organ transplantation.

OGTT should be performed in a clinically stable period by oral administration of 1.75g/kg dextrose (maximum 75g) after a fasting period of 8-12 hours. Blood glucose levels are then measured at 0, 30, 60, 90, and 120 minutes after administration. Unlike in type 2 diabetes, mid-OGTT glucose values play a particularly important role in CFRD. Several studies indicate that mid-OGTT elevations are more strongly associated with declines in lung function in children with CF and are highly predictive of future abnormalities in glucose tolerance within the CF population (186, 187). Continuous glucose monitoring (CGM) is increasingly being used in CF care as a screening method for abnormalities in glucose regulation (170, 188). However, clear consensus statements and standardized guidelines regarding CGM thresholds and its role in screening for CFRD are still lacking.

Traditionally, HbA1c has been considered insensitive for detecting CFRD when using thresholds established for type 1 and type 2 diabetes. This reduced sensitivity is largely due to increased red blood cell turnover in CF (189). However, more recent research suggests that revised HbA1c thresholds for CF care, or combined approaches, may enhance its diagnostic performance in CF (190).

The diagnostic criteria for CFRD in a stable patient with CF are listed below in Table 2.

Table 2. Diagnostic criteria for CFRD in clinically stable patients

		Notes
2 hour OGTT value	≥11.1 mmol/L	
Fasting plasma glucose	≥7.0 mmol/L	
HbA1C	≥48 mmol/mol	
Random plasma glucose	≥11.1 mmol/L	Combined with classic symptoms of diabetes (polyuria, polydipsia, or unexplained weight loss)

Unless the patient presents with classic symptoms of diabetes accompanied by a random glucose level ≥11.1 mmol/L, or meets at least two additional diagnostic criteria (2 hour OGTT/ fasting plasma glucose/HbA1C) for CFRD the results should be confirmed with repeated testing.

Pathophysiology of CFRD

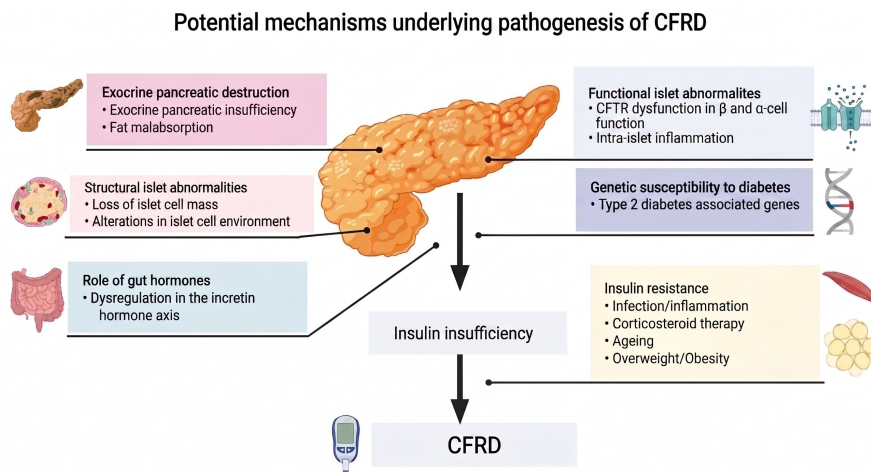


Figure 10.

Reprinted from: Moheet A, Moran A. New Concepts in the Pathogenesis of Cystic Fibrosis–Related Diabetes. *The Journal of Clinical Endocrinology & Metabolism*. 2022;107(6):1503-9.

The pathophysiology of CFRD is multifactorial (Figure 10) and remains incompletely understood, with several mechanistic aspects still under investigation. The earliest changes arise from the impact of CFTR dysfunction on the exocrine pancreas, leading to viscous secretions, intraductal obstruction, inflammation with cytokine activation, and progressive fibrosis, altogether processes that drive the gradual loss of islet cell mass and function (191). In CFRD, there is not only a reduction in β -cell mass, but also β -cells dysfunction, which may lead to the observed insulin deficiency in CF (192).

There have been conflicting reports regarding whether CFTR is expressed in β -cells; however, studies in animal models and in humans have confirmed CFTR expression in β -cells (46). Edlund et al. reported that, under glucose-stimulated conditions, F508del mice exhibited reduced β -cell exocytosis, increased proinsulin release, and an exaggerated secretion of glucagon (193). These findings may indicate a direct impact of CFTR dysfunction in β -cells. Oxidative stress and induction of NF- κ B in β -cells are other potential mechanisms involved in β -cell dysfunction (194). CFTR was even found in α -cells, leading to altered glucagon secretion (13).

Since the earliest insulin defect in CF is the loss of the first-phase insulin response, which leads to postprandial hyperglycaemia, this pattern points toward a disturbance in the incretin axis, as hormones such as GIP and GLP-1 normally enhance insulin release and suppress glucagon after meals. Studies have shown that people with CFRD have reduced GLP-1 levels compared with healthy controls (195).

Genes that increase diabetes risk in the general population may also contribute to susceptibility to CFRD. For example, having a family history of type 2 diabetes raises the risk of developing CFRD threefold. The type 2 diabetes-associated locus TCF7L2 has likewise been linked to a higher likelihood of CFRD and an earlier age at diagnosis (196).

Insulin resistance, arising from factors such as inflammation, corticosteroid treatment, malnutrition, older age, or overweight, may also play an important contributing role (46).

Treatment of CFRD

The current treatment guidelines for CFRD consist of non-pharmacological treatments like regular physical activity and individualised dietary recommendations, together with pharmacological therapy (197). Insulin treatment is the first choice, and rapid-acting insulin administered pre-meal is the standard of care in CFRD without fasting hyperglycaemia (183). In CFRD with fasting hyperglycaemia, long-acting insulin is added to the treatment regime. Insulin pump therapy in CFRD has so far only been evaluated in small studies, but it may represent a promising alternative treatment option in the future (197). Oral diabetes agents have not been implemented in the international guidelines for CFRD. However, randomized controlled trials comparing insulin versus repaglinide have shown no clear advantage of repaglinide (198, 199).

CFRD in times of ETI

The introduction of highly effective CFTR modulators has markedly advanced the treatment of cystic fibrosis, with ETI demonstrating profound improvements in pulmonary outcomes, nutritional status, and overall health. Despite these substantial clinical benefits, the effects of CFTR modulators on glucose metabolism and CFRD remain complex and not yet fully characterized (200). Current evidence indicates considerable variability across studies, reflecting heterogeneity in patient populations, disease severity, and treatment regimes.

Emerging findings suggest that CFTR modulators may provide modest improvements in metabolic parameters (82). These improvements are thought to result from enhanced β -cell function, reduced systemic inflammation, and stabilization of the overall metabolic environment, as well as improved glucagon secretion.

Kelly et al. showed an increase in insulin secretion after four months of treatment with ivacaftor (201). Nielsen et al. reported a decline in HbA1c after one year of ETI (202). Nevertheless, available studies consistently indicate that modulator therapy does not reverse established CFRD, but may reduce insulin requirements

and reduce lung function decline but does not clearly seem to improve β -cell function (203-206).

In individuals with long-standing diabetes, irreversible pancreatic islet damage limits the potential for full restoration of insulin secretion, and consequently, improvements in glycaemic control tend to be modest and inconsistent.

In parallel, the nutritional effects of CFTR modulators introduce additional metabolic considerations. Enhanced appetite, better nutrient absorption, and increased weight and fat mass are commonly observed following treatment initiation (36, 207). Although these changes are generally beneficial for long-term health, they may increase insulin resistance, potentially exacerbating glucose intolerance in susceptible individuals.

Given the current uncertainties, existing recommendations for CFRD screening and treatment remain unchanged. Ongoing research and systematic monitoring will be essential to clarify the effects of ETI on CFRD, and to guide individualized metabolic management in the era of CFTR modulator therapy.

Cystic fibrosis hepatobiliary involvement

Cystic fibrosis-related hepatobiliary involvement (CFHI) is another frequent extrapulmonary complication of CF, although it typically develops much earlier in life than CFRD, often beginning in early childhood with intrahepatic changes, where excessive mucus in biliary ducts, focal biliary cirrhosis and periportal changes can already be observed in the first months of life (208). CFHI affects around 30% of individuals with cystic fibrosis by 25 years of age (209). Advanced CF liver disease (aCFLD) affects up to 10 % of individuals with CF by age 30 (210). In an Irish cohort study, the incidence of CFHI was highest in paediatric CF patients under the age of 10 (211). In the same study, CF patients who had normal clinical and radiological liver findings at the age of ten were not affected by CFHI during a 10-year follow-up, pointing to an early onset of CFHI in the lifetime of individuals with CF.

Definition and clinical signs

CFHI is a heterogeneous condition with a wide spectrum of clinical manifestations, which has historically made it difficult to establish a precise and universally accepted definition. Previously, the definition proposed by Debray et al., Koh et al. or Colombo et al. were broadly used (210, 212, 213). To address this challenge, a new consensus paper and updated clinical guidelines were published in 2024,

providing clearer diagnostic criteria and recommendations for the management of CFHI (214).

Since 2024, the new classification defines CF liver disease into two categories:

Advanced CF Liver Disease (aCFLD) refers to having one (or more) of the following: nodular liver, advanced fibrosis (F4), multilobular cirrhosis with or without portal hypertension, or non-cirrhotic portal hypertension.

CF Hepatobiliary Involvement (CFHBI) refers to having one (or more) of the following without features of aCFLD: hepatomegaly, liver fibrosis (<F4), increased liver stiffness by elastography (<F4), hepatic steatosis, focal biliary cirrhosis, cholestasis, persistent elevation of serum liver enzyme levels, abnormal liver imaging, cholelithiasis, sclerosing cholangitis, or hepatolithiasis.

CFHI is associated with increased morbidity and mortality. According to the U.S. CF Foundation, CFHI and aCFLD accounted for 4.5% of deaths in individuals with CF in the United States in 2023 (215). In individuals with CFHI, the morbidity is increased, with higher hospitalisation rates and an increased risk of CF-related diabetes, CF bone disease, and malnutrition (216). Issa et al. showed that individuals with aCFLD present with lower lung function (217).

Portal hypertension (PHT) appears to play an important role in morbidity and mortality, as it is the most clinically significant and severe manifestation of aCFLD (218). PHT can lead to splenomegaly, thrombocytopenia, ascites, gastrointestinal varices, and variceal bleeding. In recent years, there have been increasing reports that PHT can occur through different pathways in aCFLD, including cirrhotic PHT in early life and a non-cirrhotic PHT (also called CF-related hepatic venopathy) in adulthood (219).

In the early stages, CFHI may be clinically silent. When present, signs can include persistently elevated liver enzymes, hepatosplenomegaly, steatosis, fibrosis, and cirrhosis. In neonates with CF, the condition may manifest as cholestasis. Histological studies of neonates and infants with CF have already shown abnormal liver parenchyma, including broadened portal tracts, portal fibrosis, ductular proliferation, and inspissated eosinophilic material within the bile ducts (220).

Risk factors for the development of CFHI are male sex, minimal function variants of class I-III, pancreatic insufficiency, meconium ileus, and genetic modifiers such as *SERPINA1* resulting in a heterozygosity for α_1 -antitrypsin z allele (209, 221, 222).

Pathophysiology of CFHBI

The wide range of disease manifestations suggests that the pathophysiology of CFHBI is multifactorial, and many aspects remain unresolved.

A central mechanism involves the CFTR defect in cholangiocytes. CFTR is expressed on the apical membrane of these epithelial cells lining the bile ducts and gallbladder. When CFTR function is impaired, bile composition is altered, affecting bile acid concentration, viscosity, and pH, which can lead to biliary obstruction with inspissated bile, accumulation of toxic bile acids, and depletion of hepatic antioxidants. This cascade contributes to hepatocellular injury and inflammation, initially manifesting as focal periportal inflammation and fibrosis, progressing to focal biliary cirrhosis, and, in some cases to multifocal biliary cirrhosis with portal hypertension (223, 224).

Another contributing factor may be dysregulation of the innate immune response in CFTR-defective cholangiocytes. In a murine model, CFTR deficiency in cholangiocytes was shown to activate an epithelial protein complex involving Src tyrosine kinase-mediated stimulation of Toll-like receptor 4. This pathway activates NF- κ B, leading to pro-inflammatory signalling and cytokine release, causing inflammation, disruption of the apical junctional complex, and impaired epithelial barrier function (225).

A further proposed mechanism involves the gut–liver axis. Alterations in the gut microbiome, together with increased mucosal inflammation and permeability, may permit microbial products and inflammatory mediators to enter the portal circulation, thereby triggering hepatic inflammation and contributing to portal fibrosis (226).

The recently described form of non-cirrhotic portal hypertension, also referred to as CF-related hepatic venopathy and predominantly observed in adults, remains poorly understood. Proposed mechanisms include endothelial dysfunction, platelet activation with subsequent microthrombosis, and translocation of bacterial endotoxins into the portal circulation due to impaired tight-junction integrity (83, 227).

Screening and management of CFHBI

Because CFHBI can be asymptomatic and may already be present in neonates, early childhood screening is essential. The 2024 consensus recommendations propose annual screening beginning at the time of CF diagnosis, including a physical abdominal examination and liver function tests. In addition, abdominal ultrasound should be performed every two years starting at age three (214).

According to the new consensus guidelines, individuals diagnosed with CFHBI should undergo a baseline liver elastography assessment to evaluate liver stiffness and identify aCFLD (214). Thereafter, liver elastography should be performed at least annually to monitor disease progression. Elastography is a non-invasive method that measures liver tissue elasticity; increasing stiffness reflects underlying fibrotic processes in the liver parenchyma. It is a validated tool for staging fibrosis across several chronic liver diseases (228). Liver elastography can be performed using multiple modalities, including vibration-controlled transient elastography (VCTE), ultrasound-based techniques such as acoustic radiation force impulse imaging (ARFI) and two-dimensional shear-wave elastography (SWE), as well as magnetic resonance elastography (MRE). Our previous work demonstrated that SWE is a valuable, non-invasive, and rapid method for assessing CFHBI (229). SWE has proven to be an effective tool for routine follow-up and for detecting early-stage CFHBI in both adults and children (230-232).

Additional assessments in individuals with CFHBI include standardized evaluations for other liver diseases, magnetic resonance cholangiopancreatography, and liver histology when indicated. Ongoing monitoring should be performed regularly and includes liver function tests including INR and platelet counts every six months, abdominal ultrasound, liver elastography, non-invasive fibrosis indices, nutritional and pharmacological guidance, screening for CFRD, and surveillance for hepatobiliary malignancies.

Management of CFHBI and aCFLD remains challenging, and currently no clear evidence-based treatment or proven preventive strategy exists. Medical therapy - most commonly ursodeoxycholic acid (UDCA), continues to be widely used despite conflicting evidence regarding its long-term efficacy in a systematic Cochrane review (233). UDCA, a hydrophilic secondary bile acid, is thought to improve bile secretion, reduce bile viscosity, and decrease the proportion of toxic hydrophobic bile acids (234). Although small studies have reported improvements in liver enzyme levels, there is no convincing evidence that UDCA prevents the development or progression of aCFLD, although it may have beneficial effects in mild CFHBI (235). The current consensus guidelines do not recommend the routine use of UDCA to prevent aCFLD.

For advanced CF-related liver disease, surgical options such as portosystemic shunting may be considered in selected cases, whereas liver transplantation remains

the definitive treatment for end-stage disease or complications such as portal hypertension.

The potential benefit of CFTR modulator therapy for CFHBI and aCFLD remains uncertain. Large randomized controlled trials with liver-specific endpoints are lacking, and the available evidence is limited to small observational studies. An additional challenge is that many individuals with established CFHBI or aCFLD have limited access to CFTR modulators, either due to their CFTR genotype or concerns about potential hepatotoxicity (236, 237).

Treatment with lumacaftor/ivacaftor has been associated with significant reductions in gamma-glutamyl transferase and alkaline phosphatase levels, whereas ETI has shown mixed effects on these markers, along with reported increases in total and direct bilirubin concentrations (238). A case report in a 17-year-old girl with CF further described an improvement in CFLD-related steatosis after two years of ivacaftor treatment (239).

Current guidelines recommend the use of highly effective CFTR modulators in individuals with CFHBI and, with careful monitoring, also in those with compensated aCFLD. Their precise impact on the progression of CF-related liver disease, however, remains to be fully determined.

Several emerging therapeutic strategies are under investigation. These include vitamin D supplementation and probiotics aimed at modulating the gut–liver axis, norursodeoxycholic acid (norUDCA), which may increase biliary flow through enhanced bicarbonate secretion, and anti-inflammatory approaches targeting TLR4 signalling (83, 240). These interventions may offer future treatment possibilities, but further research and clinical evidence are needed.

Aims

This thesis aims to elucidate the complex interplay between cystic fibrosis-related metabolic dysregulation and multi-organ health, including its impact on pulmonary disease progression and hepatobiliary involvement in the context of CFTR modulator therapy.

Paper I–II focus specifically on the impact of metabolic disturbances in CF on pulmonary health. In these studies, we sought to elucidate inflammatory pathways involved in CFRD-associated lung disease, examine the expression of key regulatory proteins, and explore the role of the airway microbiome.

Given the close association observed between lung function decline, heightened inflammation, and microbiome alterations, we extended our investigation in **Paper III** to assess how FEV1pp is influenced by abnormal glucose tolerance (AGT) in the Swedish CF population, using a retrospective observational study design.

In **Paper IV**, we examined the effects of ETI - a therapy approved in Sweden during my doctoral studies - on CF-related liver disease, with a particular focus on changes in liver elastography measurements.

Methods

Population

CF care in Sweden is organised as a centralised healthcare system, with approximately 800 individuals currently living with cystic fibrosis. CF care is provided at four specialised CF centres located in Stockholm, Uppsala, Gothenburg, and Lund. All patients attend at least one annual visit at their CF centre, following a standardised review programme conducted by a multidisciplinary team. Due to the geographical distances within Sweden, routine clinical care between annual visits is delivered through a shared-care model in collaboration with local healthcare providers.

In Papers I–II, the cohort of this observational study consisted of individuals with CF receiving treatment at the adult unit of the CF centre in Lund, Skåne University Hospital. At the adult unit, 140 individuals with CF are treated. All sputum samples and clinical data were collected during scheduled visits at the CF centre in Lund. The clinical data were collected from the Swedish CF registry.

In Paper III, individuals with CF from the age of 10 years who were included in the Swedish national CF registry were enrolled in this retrospective observational study. All four CF centres in Sweden report to the national CF registry, and all participants have provided informed consent for data collection and use. The CF registry was established in 2012 and contains data from all four CF centres. According to the 2024 annual report, the registry comprises 811 patients.

Data from each patient's annual review at their CF centre are entered into the CF registry, with the annual review scheduled in the same month each year for each patient. The Swedish CF registry is part of the European CF Registry, and Swedish data are reported annually to the European platform. The Swedish CF registry is certified within the national system for knowledge-driven management in Swedish healthcare and is considered a robust and high-quality registry, with a coverage rate exceeding 99% in 2024.

In the retrospective cohort study of Paper IV, individuals with CF who underwent regular assessments for CFHBI at the paediatric unit of the CF centre in Lund were included. The paediatric unit provides care for approximately 70 patients.

Annual evaluation for CFHBI is initiated at an early age and includes liver function tests, abdominal ultrasound, and liver elastography measurements. Data were collected retrospectively from patients' medical records after informed consent, covering the period from 2018 to 2022.

Clinical investigations

Lung function

Lung function in individuals with CF is regularly assessed using spirometry. For this thesis, lung function data were obtained from the annual review, which is performed during a clinically stable period. FEV1_{pp} values were calculated using the Global Lung Function Initiative equations (241). FEV1_{pp} measures airflow limitation and is a parameter for the limitation in the central airways. It is a well-established measure of CF lung disease severity and is widely used as a key endpoint in CF research.

In Paper IV, lung clearance index (LCI) was used as a lung function parameter. LCI was calculated using multiple breath washout (MBW) with Exhalyzer®D (Ecomedics, version 3.3.1), using three consecutive measurements in nitrogen washout mode, representing inhomogeneous ventilation (242). The LCI value is an indicator of ventilation in the peripheral airways and is considered a more sensitive method than FEV1_{pp} in paediatric CF patients (243).

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) is performed annually from the age of 10 years in individuals with CF, in accordance with CF care guidelines. The procedure follows WHO standards, using an oral glucose load of 1.75 g/kg (maximum 75 g), with blood glucose measured in mmol/L at baseline (0 minutes) and at 30, 60, 90, and 120 minutes (170). HbA1c (mmol/mol) is also obtained at baseline.

The classification of OGTT is shown in Table 3 below (244):

Table 3. Oral glucose tolerance test results

	Fasting plasma glucose (mmol/L)	2-h glucose (mmol/L)	Notes
NGT	<7.0	<7.8	All glucose levels <11.1
INDET	<7.0	<7.8	Mid-OGTT glucose $\geq$ 11.1
IGT	<7.0	7.8-11.1	
CFRD FH-	<7.0	$\geq$ 11.1	
CFRD FH	$\geq$ 7.0		

NGT: Normal glucose tolerance, INDET: Indeterminate glycemia, IGT: Impaired glucose tolerance, CFRD FH-: Cystic fibrosis-related diabetes without fasting hyperglycaemia, CFRD FH: Cystic fibrosis-related diabetes with fasting hyperglycaemia

Screening for CFHBI

At the paediatric unit of the CF centre in Lund, all individuals with CF undergo regular screening for CFHBI as part of the annual review. The screening includes liver function tests alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and direct and indirect bilirubin. A clinical abdominal examination and an abdominal ultrasound with liver elastography are also performed. Liver elastography has been included as part of the CFHBI screening for all paediatric patients in Lund since 2017. Before liver elastography is performed, the patient must fast for three hours. Liver elastography measures the elasticity of the liver parenchyma, which is reported in kilopascals (kPa). In Lund, liver elastography is performed by paediatric radiologists using 2D SWE on the Canon/Toshiba Aplio i70 system. The 2D SWE technique generates shear waves within the tissue and calculates their propagation speed, which correlates with liver stiffness and is expressed in kPa. 2D SWE is a non-invasive and safe method that can be used repeatedly for monitoring and screening. It has an increasing role in fibrosis screening in paediatric patients and individuals with CF (229, 231, 245).

Laboratory methods

Sputum preparation

Sputum samples from individuals with CF were used for the analyses in Paper I and Paper II. The samples were obtained during routine visits at the CF centre in Lund. As part of standard clinical care, sputum samples are collected at every CF centre visit and sent for microbiological analysis. Samples are obtained during the physiotherapy session as spontaneously expectorated sputum, primarily after inhalation with hypertonic saline.

For this research project, one additional sputum sample was donated after informed consent. The sample was immediately transported to the laboratory at BMC, Lund University, and liquefied using 0.1% dithiothreitol (DTT) in PBS. Following incubation at 37°C with continuous mixing for 20 minutes, the sample was divided into two portions: one was used for DNA extraction, and the other was centrifuged at $1000 \times g$ for 10 minutes, after which the cell-free supernatant was collected for cytokine analysis (246).

Quantification of cytokines and inflammatory mediators

In Papers I and II, we quantified the cytokines IL-1 β , IL-8, IL-6, IL-17, TNF- α , as well as the inflammatory mediator NE.

For the analysis of NE activity, a chromogenic assay from Sigma-Aldrich was used. The assay quantifies NE activity based on its ability to proteolytically cleave a synthetic substrate and release the fluorophore AFC, which enables measurement of enzymatic activity by fluorescence (246).

For analysis of IL-1 β , IL-8, IL-6, IL-17, and TNF- α , a multiplex immunoassay on the Meso Scale Discovery (MSD) platform was used.

The MSD platform (Mesoscale Diagnostics LLC., Maryland, USA) is based on the same immunological principles as a traditional sandwich ELISA but uses electrochemiluminescence for detection. In a traditional sandwich ELISA, the target analyte is captured between two antibodies: a plate-bound capture antibody that binds the analyte from the sample, and a detection antibody that recognizes a different epitope on the same molecule. This antibody–antigen–antibody “sandwich” is then visualized through an enzyme-linked detection system that produces a measurable signal. This dual-antibody binding provides high specificity and makes sandwich ELISA well suited for quantifying low-abundance proteins such as cytokines.

Similar to this format, the MSD system (Figure 11) also uses a capture antibody immobilized within each well and a detection antibody labelled with an electrochemiluminescent tag. However, MSD incorporates two major advancements. First, it uses electrochemiluminescence detection with a SULFO-TAG, which produces light only when an electrical current is applied, resulting in very low background noise and high sensitivity. Second, MSD plates contain multiple spatially separated antibody spots within a single well, allowing the simultaneous measurement of several cytokines from the same small sample volume. This multiplex design reduces pipetting steps, minimizes handling errors, prevents sample loss, and ensures that all analytes are measured under identical conditions.

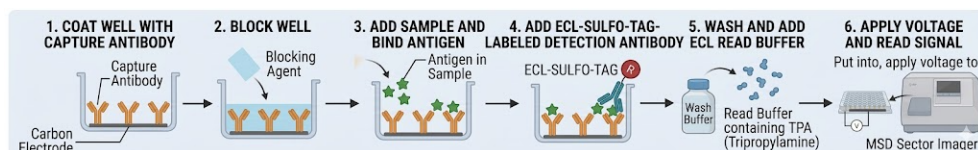


Figure 11.
Meso Scale Discovery assay, AI generated

Proteome analysis

Proteome analysis of sputum provides a comprehensive overview of all proteins present in the sample and enables a detailed investigation of airway inflammation. With major advances in analytical instrumentation and computational bioinformatics, proteomics has become an increasingly powerful tool in biomedical research, offering high sensitivity, a broad dynamic range, and the ability to identify hundreds to thousands of proteins within a single sample.

In Paper I, we applied tandem mass spectrometry-based proteomics to characterize the sputum proteome (Figure 12). The modern proteomic workflow begins with sample preparation, during which sputum is homogenized, and proteins are extracted. We used a bottom-up mass spectrometry approach, in which proteins are enzymatically digested into peptide fragments. For this purpose, proteins were digested with trypsin and incubated at 37 °C for 18 hours (247).

The resulting peptide mixture was then separated using liquid chromatography, which resolves peptides based on their hydrophobicity. We used an Orbitrap Eclipse mass spectrometer coupled to an ultra-high-performance liquid chromatography Dionex Ultra 3000 system (Thermo Scientific). In tandem mass spectrometry (LC-MS/MS), peptides first enter the mass spectrometer, where they are ionized in the first MS stage and subsequently fragmented. Fragmentation generates characteristic fragment-ion spectra, which function as “molecular fingerprints.” These fragments then enter the second MS stage, where they are detected according

to their mass-to-charge ratio (m/z). By using a data-independent acquisition method, all peptides present in the sample are repeatedly fragmented and analysed in the second MS, enabling the detection of even low-abundance peptides.

The MS/MS data were analysed using Spectronaut (version 18.4.231017.55695) against the UniProt reviewed human database containing 20,418 entries. Quantification of identified proteins was performed using the Perseus software platform (version 2.0.9.0), and gene-enrichment analyses were conducted using Metascape (248).

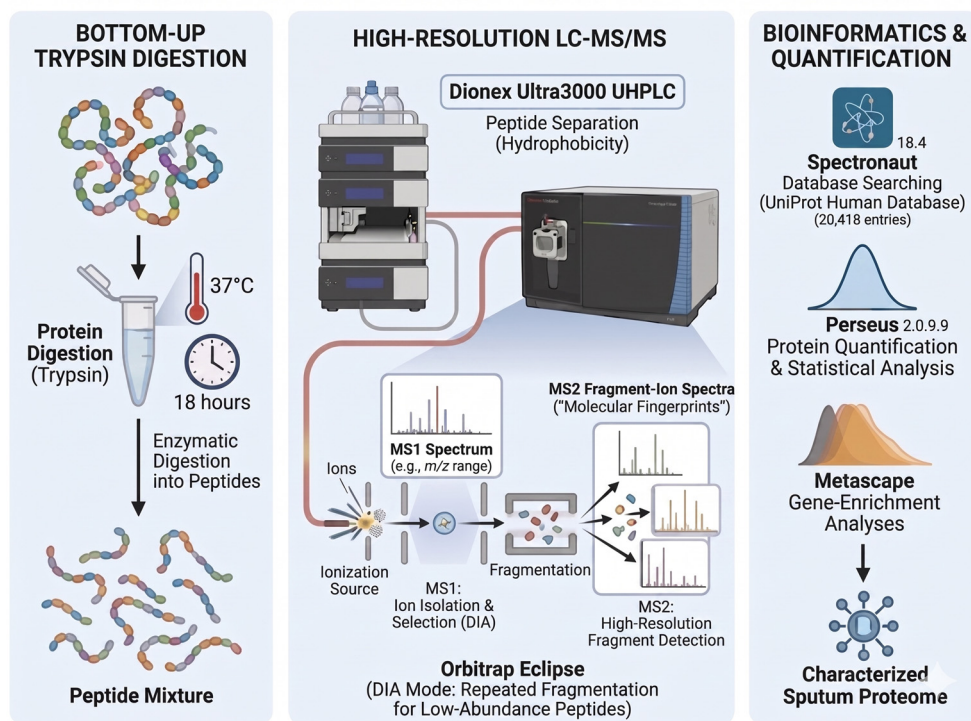


Figure 12.
Sputum proteome analysis, AI generated

Quantitative Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a molecular technique used to amplify small amounts of DNA (Figure 13), enabling subsequent quantification and identification of specific genetic targets.

In Paper I and Paper II, quantitative PCR was used to measure the bacterial load in sputum samples. Total bacterial load was assessed using quantitative PCR (qPCR) targeting the bacterial 16S rRNA gene, a genetic marker present in all bacteria. The 16S rRNA gene contains two types of regions: conserved regions and hypervariable regions. The conserved regions are largely unchanged throughout evolutionary history and are therefore used as binding sites for universal primers during PCR amplification. In contrast, the hypervariable regions (V1–V9) exhibit sequence diversity between bacterial taxa and function as a molecular fingerprint, allowing species-level or genus-level identification in microbiome analyses. Amplification was performed using previously published primer sequences binding in the conserved region in the 16S rRNA gene at a 59.4°C annealing temperature, allowing sensitive and accurate measurement of the overall bacterial burden in each sample (249).

As first step, total DNA was extracted from sputum using Pathogen Lysis Tubes S, followed by the QIAamp UCP Pathogen Mini Kit to efficiently lyse bacterial cells. The extraction workflow involved mechanical bead-beating, followed by purification of total DNA.

Amplification proceeds through repeated temperature cycles consisting of denaturation (94–98°C), annealing (50–65°C), and extension (68–72°C). During denaturation, double-stranded DNA separates into single strands. In the annealing step, primers bind to their complementary sequences on the template DNA. During extension, DNA polymerase synthesizes new complementary strands by adding nucleotides to the primer–template complex. These cycles are repeated 30–45 times, resulting in exponential amplification of the target DNA sequence.

Quantification of 16S rRNA gene copies during PCR amplification was performed using iTaq Universal SYBR Green Supermix (Bio-Rad). Serial 10-fold dilutions of *P. aeruginosa* DNA at known concentrations were analyzed in parallel and used to generate a standard curve.

THE POLYMERASE CHAIN REACTION (PCR): AMPLIFICATION CYCLE

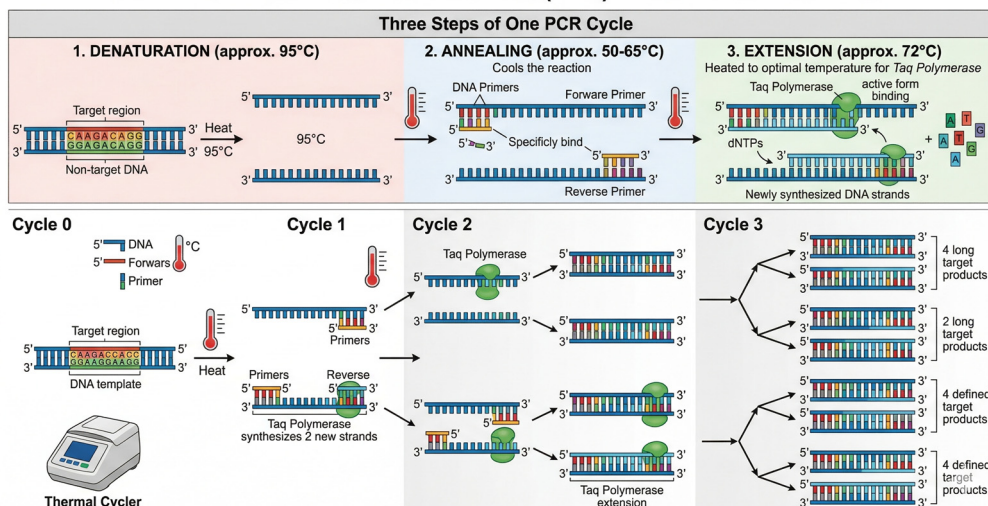


Figure 13.
PCR steps, AI generated

Microbiome

The complex analysis of the bacterial airway microbiome in Paper II was conducted with support from the Center for Translational Genomics (CTG), Lund University, Lund, Sweden, and from the National Bioinformatics Infrastructure Sweden (NBIS), SciLifeLab, Department of Laboratory Medicine, Lund University, Lund, Sweden.

Microbiome profiling begins with the collection of biological material containing microbial DNA. In this study, sputum samples were collected during routine clinical visits. To ensure reliable downstream analysis, samples were handled under standardized conditions to minimize contamination and preserve microbial integrity.

Total DNA was extracted as described above. The resulting DNA contained the mixed genetic material from the sputum and served as the template for subsequent amplification.

To characterize the bacterial composition of the sputum microbiome, we performed targeted amplification of the 16S rRNA gene. Amplicon libraries were prepared using primers targeting the hypervariable V3–V4 region of the bacterial 16S rRNA gene for identification of different bacterial taxa. Specifically, we used the 515F/806R primer pair, a widely adopted primer set suitable for broad bacterial detection.

PCR amplification enriched the bacterial 16S rRNA fragments from the extracted DNA, enabling sensitive detection of microbial community members. The amplification protocol followed standard cycling conditions, including denaturation, annealing, and extension steps optimized for efficient and specific amplification of the V3–V4 amplicon as described above.

After amplification, the amplicons were analysed by sequencing on the Illumina NextSeq 2000 platform. Sequencing data were processed using the nf-core/ampliseq pipeline (version 2.9.0), part of the nf-core community-curated workflow collection in fully reproducible computational environments using Bioconda and Biocontainers. Bioconda and Biocontainers provide reproducible software environments by offering pre-built bioinformatics tools (Bioconda) and standardized containerized versions of these tools (Biocontainers), ensuring consistent and reliable execution of the nf-core/ampliseq workflow.

Initial sequence quality was assessed with FastQC, and summary reports across all samples were compiled using MultiQC. Primer trimming was performed using Cutadapt, and sequences lacking the required primer sequences after trimming were discarded to ensure high-quality amplicon reconstruction.

Amplicon sequence variants (ASVs), representing single-nucleotide-resolved bacterial sequence features, were inferred using the DADA2 pipeline and were taxonomically classified against the SILVA 138.1 prokaryotic SSU database. This combined workflow provides precise, high-resolution characterization of the sputum microbiome and minimizes amplification bias and sequencing artifacts.

For further analysis regarding the diversity in the airway microbiome (Figure 14), including analyses of the richness (number of taxa) and evenness (their distribution), ASVs were aggregated at the genus level.

For alpha diversity, we used the Shannon index (reflecting both richness and evenness) and observed genera (richness only). For beta diversity, we used Bray-Curtis dissimilarity, which accounts for both the presence and abundance of taxa, and this was visualized by principal coordinate analysis. A principal coordinate analysis plot shows how similar or different microbial communities are across samples: closer points mean more similar composition, axis percentages show how much variation each coordinate explains, and clustering or separation indicates biological patterns.

R statistical software (version 4.1.1) was used to perform the statistical analysis.

Alpha diversity

Alpha diversity describes the diversity within a single sample. It can be assessed using metrics such as the Shannon index, which reflects both the number of taxa and their distribution, and observed genera, which measure the total number of genera present.

Beta diversity

Beta diversity describes the diversity between samples. It provides insight into how distinct or similar microbial communities are across samples. Bray-Curtis dissimilarity was used as a metric, which quantifies differences based on the abundance of shared taxa. Higher Bray-Curtis values indicate greater dissimilarity between samples.

Analysis of Microbiome Data

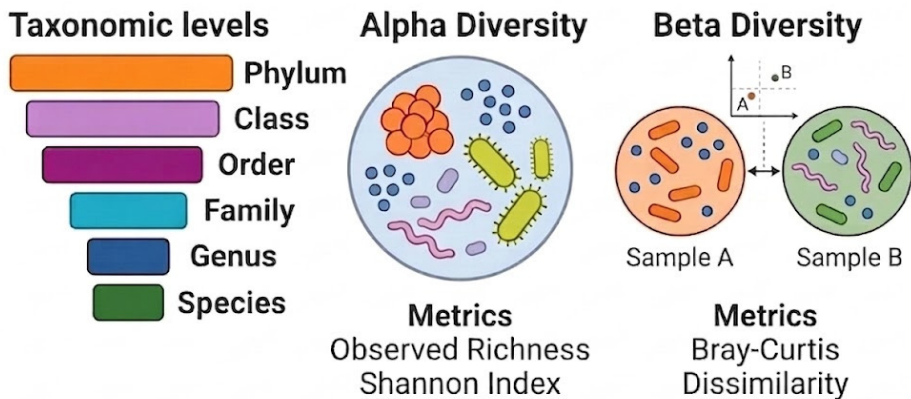


Figure 14.
Analysis of microbiome data in the thesis, AI generated.

Statistics

Different statistical software and tests were used in this thesis.

Paper I-II

Statistical analyses of clinical data, cytokines, and bacterial load were performed using GraphPad Prism version 10.0.2 (GraphPad Software, San Diego, CA). Comparisons between groups were conducted using the Mann–Whitney U test for continuous non-parametric data. Fisher’s exact test was applied for comparisons of categorical variables. A p-value ≤ 0.05 was considered statistically significant.

Statistical analyses of the proteomic data were performed using the Perseus platform (version 2.0.9.0) by Lotta Happonen and Sounak Chowdhury, Lund University, Department of Clinical Sciences, Lund, Sweden.

Microbiome analyses were conducted in R (version 4.1.1) by Katja Kozjek, National Bioinformatics Infrastructure Sweden (NBIS), SciLifeLab, Department of Laboratory Medicine, Lund University, Lund, Sweden.

Multiple regression analyses examining the association between the Shannon index, CFRD, and lung function were performed using SPSS version 30.0 (IBM SPSS Inc., Chicago, IL, USA).

Paper III

All statistical analyses were performed using SPSS (IBM SPSS Statistics, version 30.0) and were conducted by Olivia Olsson, Lund University, Department of Clinical Sciences, Lund, Sweden, with support from Clinical Studies Sweden, Forum South, Lund. For baseline characteristics, bivariate analyses (chi-square tests and Kruskal-Wallis tests) were used to compare the three glucose tolerance status groups. When the Kruskal-Wallis test indicated significant group differences, Bonferroni-adjusted Dunn’s post hoc tests were performed. A p-value ≤ 0.05 was considered statistically significant. Multiple linear regression analysis was used to assess differences in lung function between the three glucose tolerance groups after adjusting for relevant confounders. Changes in lung function over time were analysed using a linear mixed-effects model.

Paper IV

Statistical analyses were performed using GraphPad Prism version 10.0.2 (GraphPad Software, San Diego, CA, USA). The Wilcoxon matched-pairs signed-rank test was used to compare pre-ETI and post-ETI values. Descriptive statistics were applied for clinical data and are presented as non-parametric measures, including the median and interquartile range (IQR). A p-value ≤ 0.05 was considered statistically significant.

Ethical considerations

All studies included in this thesis were conducted in accordance with ethical principles for medical research according to the Declaration of Helsinki and were approved by the Swedish ethics review boards. Written informed consent was obtained from all participants, or from legal guardians for participants under the age of 15 in for Papers I, II and IV.

Sputum samples were collected during routine, planned clinical visits at the CF centre, ensuring that no additional procedures beyond standard care were required for sample acquisition. Clinical data used in this thesis were obtained from patient records or from the Swedish CF Registry, which contains data collected annually during the standardised review at each CF centre. All participants consented to the inclusion of their clinical data in these projects.

Liver elastography measurements analysed in this thesis were performed as part of the routine annual CF review and did not involve any additional examinations beyond normal clinical practice. Thus, no extra burden was imposed on participants.

All data were processed in accordance with the General Data Protection Regulation (GDPR), ensuring lawfulness, confidentiality, and integrity of personal data, and minimizing the risk of unauthorized access or data leakage.

The results from these studies are not intended to provide a direct benefit to the individual participants, but rather to generate knowledge that may contribute to the improvement of care for the broader cystic fibrosis community.

Paper I

The study was approved by the local Medical Ethics Committee in Lund (DNR 2018/54 with amendments 2021-05475-02 and 2023-06075-02).

Paper II

The study was approved by the Swedish Ethical Review Authority, Uppsala, Sweden (DNR 2018/54 with amendments 2021-05475-02 and 2023-06075-02 and DNR 2021-01191).

Paper III

The study was approved by the Swedish Ethical Review Authority, Uppsala, Sweden (DNR 2023-06024-01) and the Swedish CF Registry Steering Committee.

Paper IV

This study was approved by the local Medical Ethics Committee in Lund (DNR 2018/54).

Results

Paper I

The main aim of Paper I was to investigate the inflammatory profile in sputum in individuals with CF without diabetes compared to those with CF and diabetes.

A total of 40 individuals with CF were enrolled at the adult unit, CF Centre Lund, of whom seven were excluded (one due to organ transplantation and six due to uncertain glucose tolerance status), resulting in a final cohort of 33 participants (Table 4). Eighteen participants (55%) had CFRD, and 13 of them (72%) were treated with insulin. HbA1c levels were significantly higher and lung function significantly lower in individuals with CFRD.

Table 4. Patient characteristics, Paper I

	All patients (n=33)	PwCF without CFRD (n=15)	PwCF with CFRD (n=18)	p-value
Age years, median (IQR)	38 (27–39)	32 (25–39)	39 (29–40)	0.27
female n (%)	10 (30)	5 (33)	5 (28)	>0.99
Homozygous $\Delta F508$ n (%)	18 (55)	9 (60)	9 (50)	0.73
BMI (kg/m ²) median (IQR)	23 (21–25)	24 (21–24)	23 (20–27)	0.88
FEV ₁ pp median (IQR)	71 (54–86)	86 (70–96)	60 (45–74)	<0.01
Chronic <i>P. aeruginosa</i> n (%)	22 (67)	8 (53)	14 (78)	0.16
Pancreatic insufficiency n (%)	32 (97)	14 (93)	18 (100)	0.45
Treatment with CFTR modulator n (%)	20 (61)	10 (67)	10 (56)	0.72
HbA1c (mmol/mol) median (IQR)	41 (36–45)	37 (34–41)	43 (41–50)	<0.001

CFRD: cystic fibrosis-related diabetes; BMI: body mass index; FEV₁pp: forced expiratory volume in 1 second, expressed as percent of predicted.

To compare airway inflammation between individuals with and without CFRD, NE activity and inflammatory cytokines were measured in sputum. NE activity, IL-8, and IL-1 β concentrations were significantly higher in the CFRD group, whereas IL-6 levels were lower (Figure 15). No group differences were observed for IL-17, TNF- α , or total bacterial DNA.

Because airway inflammation is closely linked to lung function and individuals with CFRD in our cohort presented with lower FEV₁pp, participants were matched by

FEV1pp (within ± 5 percentage points) to control for this potential confounder, resulting in two comparable groups (n=10 per group) with similar lung function (median FEV1pp 72 (IQR 61-82), vs 73 (62-86), $p=0.75$), age, and *P. aeruginosa* colonisation. In these matched groups, sputum levels of NE, cytokines, and bacterial load did not differ significantly, although IL-1 β showed a non-significant trend toward higher levels in individuals with CFRD (Figure 15).

In summary, CFRD appears to be associated with increased airway inflammation, but this relationship is largely explained by lower lung function, making it difficult to determine whether CFRD directly drives airway inflammation.

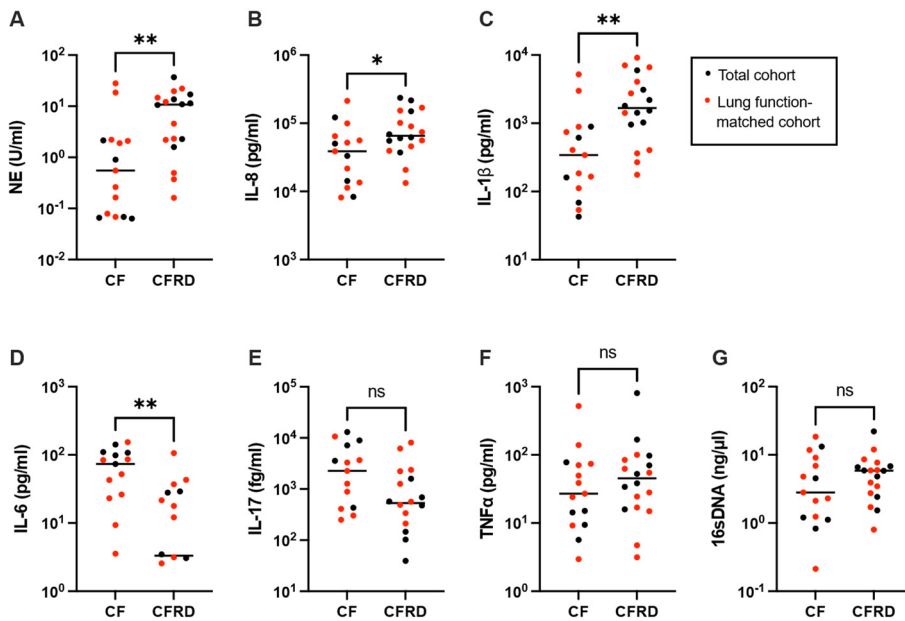


Figure 15. Neutrophil elastase activity and cytokine expression in sputum samples.

Sputum samples from pwCF with (n=18) or without CFRD (n=15) were analysed for neutrophil elastase (NE) activity using a chromogenic assay (A), the inflammatory cytokines IL-8 (B), IL-1 β (C), IL-6 (D), IL-17 (E) and TNF α (F) using Mesoscale U-PLEX assays, and bacterial DNA using qPCR of the 16S rRNA gene (G). Red dots represent patients matched on lung function. Bars represent median values. *= $p < 0.05$, **= $p < 0.01$, ns = not significant.

To more deeply examine airway inflammation in relation to CFRD, we analysed the sputum proteome using DIA LC-MS/MS. Across all samples, 2313 human proteins were identified. In the total cohort, 27 proteins were significantly associated with CFRD and 83 with non-CFRD. Gene-enrichment analysis showed that CFRD-associated proteins were enriched in neutrophil degranulation and carbohydrate metabolism, while proteins associated with non-CFRD were linked to humoral immunity and platelet activation (Figure 16).

We then compared proteomes in pwCF with and without CFRD matched for lung function. No proteins reached significance in the enrichment analysis. However, among the 27 CFRD-associated proteins identified in the total cohort, ten remained significantly more abundant in the CFRD group after matching, including Prothymosin α (P06454), previously implicated in diabetes and insulin regulation. Conversely, 27 of the 83 proteins associated with non-CFRD were significantly more abundant in the matched non-CFRD group, including ezrin (P15311), TGFB1 (Q15582), both linked to protective roles in diabetes, and α_1 -antitrypsin (P01009), essential for protease–antiprotease balance in the airways.

Taken together, we demonstrated that there is an increased airway inflammation in individuals with CFRD and distinct airway protein expression patterns.

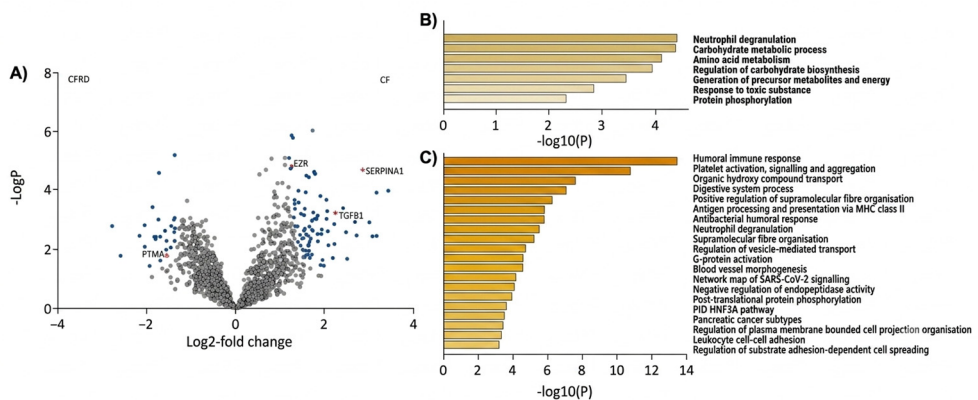


Figure 16. Sputum proteins significantly associated with CFRD.

(A) Volcano plot showing proteins significantly associated (blue dots) with CFRD and non-CFRD. (B-C) Gene enrichment analyses of the 27 proteins significantly associated with CFRD (B) and the 83 proteins associated with non-CFRD (C) using Metascape.

Paper II

The main aim of paper II was to compare the airway microbiome in individuals with CF without diabetes to that in individuals with CFRD.

A total of 44 patients were included, of whom 26 (59%) had a confirmed diagnosis of CFRD at the time of sputum collection (Table 5). Individuals with CFRD exhibited significantly lower lung function and higher HbA1c levels compared to those without CFRD. Among those with CFRD, 18 patients (69%) were treated with insulin.

Table 5. Patient characteristics, paper II

	All patients (n=44)	PwCF without CFRD (n=18)	PwCF with CFRD (n=26)	p-value
Age in years median (IQR)	36 (27 – 40)	31 (25-39)	39 (28 - 42)	0.28
Female n (%)	12 (27)	5 (28)	7 (27)	0.95
Homozygous F508del n (%)	28 (64)	12 (67)	16 (62)	0.73
BMI (kg/m ²) median (IQR)	23.1 (20.7-24.6)	23.2 (21-24.1)	22.7 (20.6-25.4)	0.98
FEV1pp median (IQR)	68.9 (56.6-86.3)	86.2 (67.1- 97)	61.9 (47.7-74.3)	0.006
Chronic <i>P. aeruginosa</i> n (%)	27 (61)	11 (61)	16 (62)	0.98
Pancreatic insufficiency n (%)	42 (95)	16 (89)	26 (100)	0.08
HbA1c mmol/mol median (IQR)	41 (36.3-45.8)	37 (33.8-40)	44.5 (40.8-54.8)	<0.001
Treatment with ETI n (%)	5 (11)	3 (16)	2 (7)	0.36

CFRD: cystic fibrosis-related diabetes; BMI: body mass index; FEV1pp: forced expiratory volume in 1 second, expressed as percent of predicted. ETI: elexacaftor/tezacaftor/ivacaftor

Cytokine analysis showed significantly higher sputum levels of IL-1 β ($p = 0.039$) and significantly lower IL-6 ($p = 0.0015$) in individuals with CFRD. No differences in sputum bacterial load were observed between the groups.

Sequencing of the 16S rRNA gene was used to analyse the lung microbiome in individuals with and without CFRD. *Pseudomonas* and *Staphylococcus* were frequent in both groups and tended to dominate when present (Figure 17).

Microbiome diversity was assessed using alpha and beta diversity metrics. The number of observed genera did not differ between groups, indicating similar richness.

In contrast, the Shannon index was significantly lower in the CFRD group ($p = 0.027$), reflecting reduced richness and a more uneven microbial community (Figure 17).

This was supported by unadjusted regression analysis, where CFRD was associated with lower Shannon diversity ($B = -0.46$ (-0.90 to -0.23), $p = 0.039$). However, after adjusting for FEV1pp, the association was no longer significant ($B = 0.001$ (-0.43 to 0.44), $p = 1.0$), while lung function remained strongly associated with Shannon diversity ($B = 0.022$ (0.012 to 0.033), $p < 0.001$).

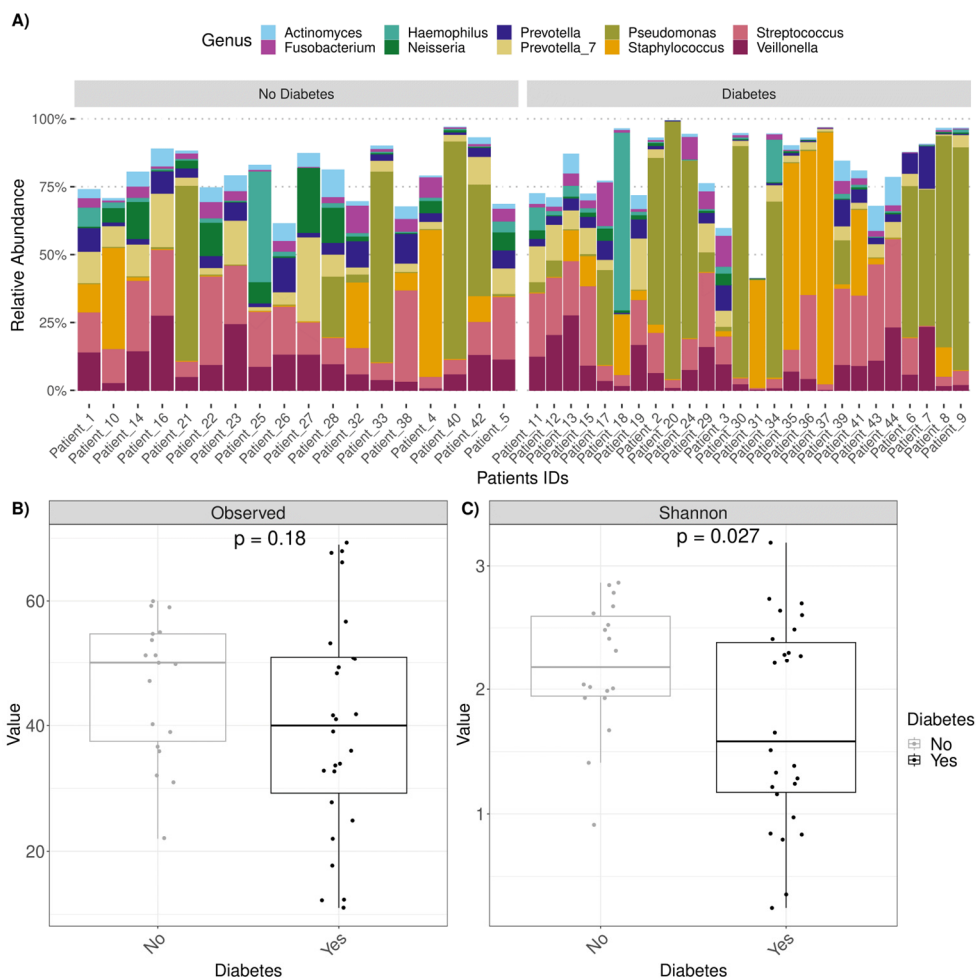


Figure 17. Lung microbial composition of the study samples at the genus level.

The figure shows the microbiome of all included study samples from each patient. The patients' diabetes status (no diabetes and diabetes) is indicated above the bars, and each bar displays the relative abundance of the 10 most common bacterial genera in this study. (B) Alpha diversity of the sputum samples of pwCF without and with CFRD. The microbiome composition of each sample was assessed based on genus richness (number of observed genera) (C) and genus diversity (Shannon index). * $p < 0.05$, ** $p < 0.01$, ns = not significant.

Together, these findings indicate that the reduced Shannon diversity observed in individuals with CFRD is primarily driven by impaired lung function rather than CFRD itself.

Beta diversity was measured using the Bray-Curtis dissimilarity index. Principal coordinates analysis demonstrated no significant clustering between the CFRD and non-CFRD groups, indicating that CFRD alone did not drive major differences in overall microbial community structure. However, beta diversity correlated significantly with lung function, measured as FEV1pp ($p = 0.001$) and sputum IL-1 β levels ($p = 0.002$), suggesting that airway inflammation and disease severity were stronger determinants of microbial community composition than CFRD status.

Differential abundance analysis showed that *Abiotrophia*, *Anaeroglobus*, and *Escherichia-Shigella* were enriched in the CFRD group, whereas *Neisseria*, *Prevotella*, and *Streptococcus* were more abundant in individuals with CF without CFRD.

Overall, the airway microbiome in CFRD tends to show reduced diversity and signs of dysbiosis.

Paper III

In Papers I and II, we observed increased airway inflammation and indications of reduced microbial diversity and dysbiosis in individuals with CFRD. However, the strong influence of lung function decline within the CFRD group makes it difficult to determine the isolated effect of CFRD on airway inflammation.

In Paper III, we therefore aimed to examine baseline lung function and longitudinal decline in lung function over a three-year period in a Swedish cohort of individuals with different glycaemic statuses - NGT, IGT and CFRD - based in OGGT results or CFRD diagnoses.

For Paper III registry data were obtained from the Swedish CF registry. A total of 411 patients were included in the cross-sectional analyses to define baseline lung function (Table 6). For the longitudinal analysis, 146 patients were excluded due to missing visits or fluctuation in glycaemic status, and a final cohort of 265 patients was included in the longitudinal analysis.

Table 6. Patient characteristics, Paper III, cross-sectional analysis

	All pwCF (n=411)	NGT (n=202)	IGT (n=57)	CFRD (n=152)	p- value
Age in years median (IQR)	25 (16-36)	22 (15-30.3)	22 (13-33)	31 (21-41.8)	<0.001
Female n (%)	180 (43.8)	86 (42.6)	25 (43.9)	69 (45.4)	0.869
Homozygous F508del n (%)	210 (51.1)	107 (53)	28 (49.1)	75 (49.3)	0.756
BMI (kg/m ²) median (IQR)	21.4 (19.2-23.6)	21.3 (19.1-23.3)	21.5 (18.2-23.4)	21.7 (19.5-24.1)	0.367
FEV1pp median (IQR)	80.4 (61.6-93.7)	82.3 (67.1-96.3)	85.9 (73-96.7)	70.8 (51.6-86.8)	<0.001
Chronic <i>P. aeruginosa</i> n (%)	178 (43.3)	81 (40.1)	17 (29.8)	80 (52.6)	0.005
Pancreatic insufficiency n (%)	389 (94.6)	189 (93.6)	51 (89.5)	149 (98)	0.032
HbA1c mmol/mol median (IQR)	37 (35-42)	36 (34-38)	37 (35-38)	44 (39-53)	<0.001
Treatment with CFTR modulator n (%)	210 (51)	104 (51.5)	28 (49)	78 (51)	0.94

CFRD: cystic fibrosis-related diabetes; BMI: body mass index; FEV1pp: forced expiratory volume in 1 second, expressed as percent of predicted. ETI: ellexacaftor/tezacaftor/ivacaftor

In the cross-sectional baseline analysis, 49.1% were classified as NGT, 13.9% as IGT and 37% as CFRD. There was a clearly lower FEV1pp in the CFRD group compared to both the NGT ($p<0.001$) and IGT groups ($p<0.001$). No difference in lung function was observed between NGT and IGT ($p=1.000$) (Figure 18). After adjusting for confounding factors including age, pancreatic insufficiency and *P. aeruginosa* colonisation, the differences in FEV1pp between NGT and CFRD remained significant ($B = -5.24$ (-9.48 to -0.99), $p = 0.016$) in the multiple linear regression analysis.

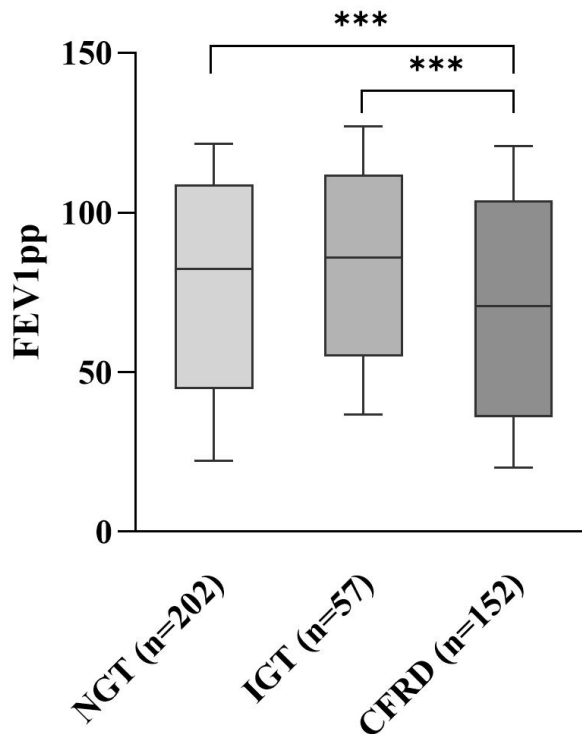


Figure 18. Lung function in the different glucose tolerance groups in the cross-sectional analysis. The figure shows the lung function (FEV1pp) in NGT (n=202), IGT (n=57) and CFRD (n=152) in the cross-sectional analysis. The boxplots display the median, interquartile range, and range. ***= $p<0.001$.

In the longitudinal analysis of 265 patients, the results of lung function at baseline were consistent with the results in the cross-sectional analysis. Lung function over time was then analysed using a linear mixed model, based on 627 observations across up to three years. The interaction between glucose tolerance status and time was not significant ($p = 0.671$), indicating that the rate of lung function decline did

not differ between groups. However, the main effect of glucose status on FEV1pp remained significant ($p = 0.01$). The CFRD group ($B = -6.14$ (-10.39 to -1.89), $p = 0.005$) had significantly lower lung function than the NGT group, while no difference was observed between IGT ($B = 1.32$ (-6.42 to 9.06), $p = 0.738$) and NGT (Figure 19).

Chronic *P. aeruginosa* colonisation, pancreatic insufficiency, and age were also significant predictors (all $p < 0.001$). Time itself was not significant ($B = -0.64$ (-2.51 to 1.24), $p = 0.505$), suggesting no substantial change in FEV1pp over the three-year period after adjustment for covariates.

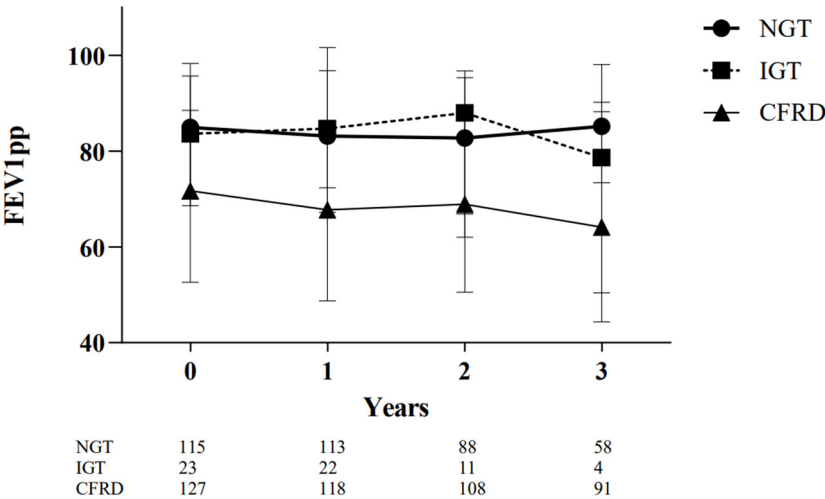


Figure 19. Lung function in the different glucose tolerance groups in the longitudinal analysis. The figure shows the lung function in FEV1pp in NGT, IGT and CFRD in the longitudinal analysis over a three-years time period. Data points represent the median FEV1pp, and error bars indicate the IQR. The table below the x-axis displays the number of patients with available data at each annual time point for each group.

In summary, individuals with CFRD exhibited lower lung function compared with those with NGT or IGT, whereas lung function did not differ between individuals with IGT and those with NGT. However, over the three-year follow-up period, the rate of lung function decline was similar across all glycaemic status groups. These findings confirm a strong association between CFRD and reduced lung function, while lung function in individuals with IGT appears comparable to that in individuals with NGT.

Paper IV

During this thesis, the highly effective elexacaftor/tezacaftor/ivacaftor (ETI) therapy became available in Sweden. In Paper IV, we aimed to assess the impact of ETI on CFHBI, with a specific focus on its effect on liver stiffness measured by liver elastography.

In this retrospective study, we included 21 paediatric patients (Table 7) from the CF centre in Lund, Sweden, who underwent regular 2D SWE screening between 2018 and 2024 and initiated ETI treatment in late 2022 or early 2023. Three measurement points were in the pre-ETI study period (Pre ETI 1-3) and one measurement point was after initiating ETI treatment Post ETI 1 (Figure 20)

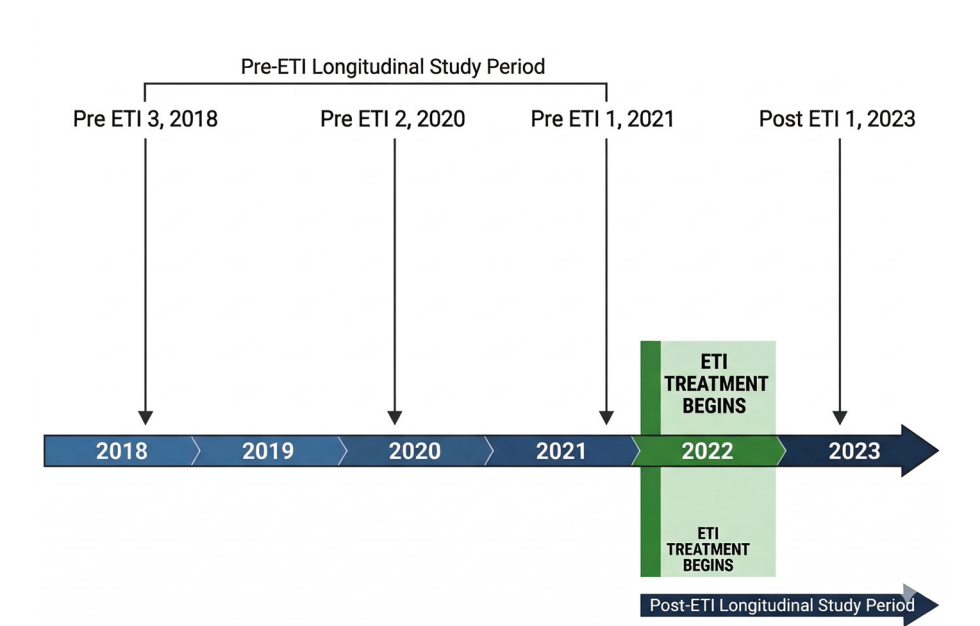


Figure 20.
Timeline for CFHBI assessment

At the last measurement point before initiation of ETI, Pre ETI 1, twelve patients (57%) were classified as having CFHBI according to the recent classification, none was classified having aCFLD.

Table 7. Patient characteristics, Paper IV

	Pre ETI 1 Total cohort (n=21)	Pre ETI 1 CFHBI (n=12)	Post ETI Total cohort (n=21)
Age in years median (IQR)	14 (13-16)	14 (12.3-15.8)	16 (14-18)
Homozygous F508del n (%)	14 (66)	11 (91,6)	14 (66)
Female n (%)	11 (52)	7 (58)	11 (52)
BMI SD (kg/m ²) median (IQR)	0.2 (-0.6 to 0.6)	0.3 (-1.2 to 0.5)	0.3 (-0.8 to 1.1)
FEV1pp median (IQR)	85.6 (73.8-97.9)	85.4 (72.9-98)	94.9 (85.1-105.1)
Lung Clearance index (IQR)	7.4 (6.7-9.2)	7.4 (6.8-9.1)	6.6 (6.4-7.0)
Chronic <i>P. aeruginosa</i> n (%)	3 (14)	1 (8)	0 (0)
Pancreatic insufficiency n (%)	21 (100)	12 (100)	21 (100)
Treatment with ursodeoxycholic acid n (%)	7 (33)	7 (58)	6 (29)
Treatment with lumacaftor-ivacaftor n (%)	12 (57)	7 (58)	0 (0)

BMI: body mass index; CFHBI: Cystic Fibrosis Hepato-Biliary Involvement; FEV1pp: forced expiratory volume in 1 second, expressed as percent of predicted.

Analysis of liver stiffness by 2D SWE (Figure 21) revealed a significant reduction from Pre ETI 1 (median 5.6; IQR 4.6–8.3) to Post ETI (median 5.1; IQR 4.2–5.9) across the total cohort ($W = -125$, $n = 21$, $p = 0.0283$).

Regarding liver enzymes, we observed a significant reduction in AST from Pre ETI 1 to Post ETI in the total cohort. In contrast, GGT levels showed a slight but statistically significant increase following ETI treatment. Nevertheless, both AST and GGT values remained within the normal reference range before and after ETI treatment. There was no change in ALT levels from Pre ETI 1 compared to Post ETI in the total cohort.

In a subsequent sub-analysis, we compared the effect of ETI in individuals with CFHBI to those without CFHBI. Participants with CFHBI had higher liver stiffness at Pre ETI 1 (median 6.8 kPa; IQR 4.9–9.2) compared with those without CFHBI (median 4.9 kPa; IQR 3.9–6.3). Within the CFHBI subgroup, liver stiffness decreased significantly during ETI treatment, reaching a median of 5.7 kPa (IQR 3.9–7.1) ($W = -60$, $n = 12$, $p = 0.0161$). In contrast, individuals without CFHBI showed only a slight reduction in liver stiffness after ETI.

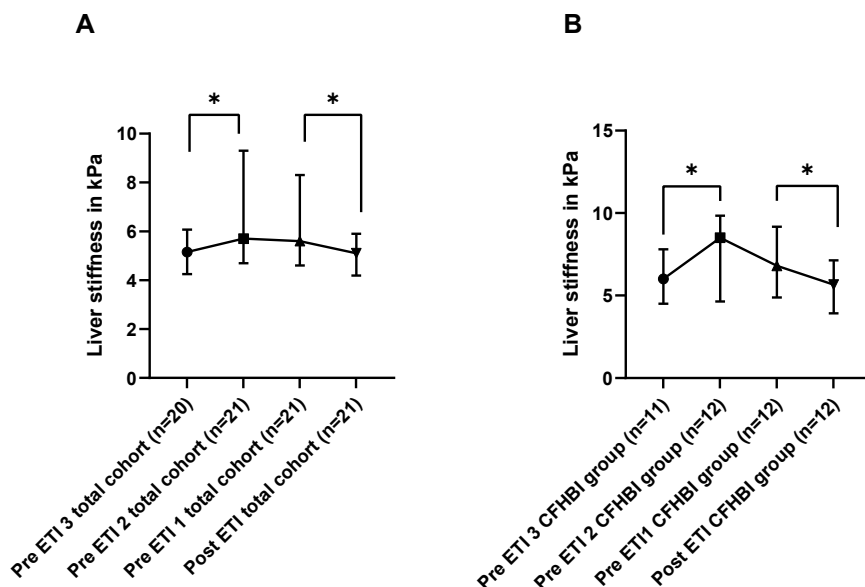


Figure 21. Liver stiffness measured by 2D shear wave elastography

(A) Liver stiffness over time in the total cohort. (B) Liver stiffness over time in patients with CFHBI.

*= $p < 0.05$, **= $p < 0.01$

Regarding liver enzymes, individuals with CFHBI exhibited significantly higher AST levels at Pre ETI 1 compared with those without CFHBI. However, AST levels declined significantly Post ETI in the CFHBI subgroup.

In a second sub-analysis, we examined whether lumacaftor–ivacaftor influenced liver stiffness. Twelve participants (57%) were already receiving this modulator at Pre ETI 1. Liver stiffness measurements taken before initiating lumacaftor–ivacaftor (Pre ETI 3, 2018) and during treatment (Pre ETI 2, 2020) showed an increase from a median of 6 kPa (IQR 4.4–7.8) to 8 kPa (IQR 5.1–9.7) ($W = 70$, $n = 12$, $p = 0.0034$). No other significant changes were observed at the remaining time points in this sub-analysis.

In summary, ETI had a beneficial effect on liver stiffness, particularly in individuals with CFHBI, whereas lumacaftor–ivacaftor did not show a comparable improvement. Liver enzyme elevations - often reported as ETI-related side effects - were mild and remained within normal ranges.

Discussion

Driving factors in CFRD airway inflammation

The starting point of this thesis was an unexpected observation made in an earlier project. In a small cohort of paediatric patients at our CF centre, we detected variations in lung function and lung clearance index that appeared to relate to differences in glycaemic status (170). A search for pathophysiological mechanisms explaining the lung function decline characteristic of CFRD identified a notable gap in the literature, particularly regarding sputum-based analyses of airway inflammation. This knowledge gap ultimately motivated and guided the work presented in this thesis.

In Paper I, we applied modern and comprehensive methods to address this knowledge gap. By integrating cytokine profiling with proteomic analyses, we obtained a more detailed understanding of the underlying inflammatory processes. To our knowledge, this is the first study to characterise airway inflammation and the full airway proteome in sputum from adults with CF, both with and without CFRD. In Paper II, we extended our work by analysing the airway microbiome together with the associated cytokine profile.

In summary, we showed that CFRD is linked to higher airway NE activity, increased sputum levels of IL-8 and IL-1 β , characteristic alterations in the airway proteome, and trends towards more dysbiosis and decreased microbial diversity.

Our findings regarding cytokine activation and increased NE activity are consistent with earlier work showing elevated airway neutrophils and IL-8 expression in CF airways during periods of hyperglycaemia, where NE itself can drive epithelial IL-8 production and perpetuate neutrophilic inflammation (139, 179).

Evidence from outside the CF field further supports the concept that dysglycaemia directly adversely affects the airways. Type 2 diabetes is highly comorbid with COPD and contributes to worsened disease progression and greater respiratory morbidity (250). Hyperglycaemia also increases susceptibility to respiratory infections and promotes higher airway bacterial growth, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, by increasing glucose in ASL and impairing epithelial barrier integrity (177, 251, 252). In asthma, hyperglycaemia has similarly been linked to worsened symptom control and increased exacerbation risk, and the

association between asthma and type 2 diabetes has recently been confirmed in a large Swedish cohort (253, 254).

A plausible explanation for these effects is that elevated airway glucose creates a nutrient-rich milieu favouring microbial proliferation and dysbiosis. In CF specifically, dysglycaemia has been associated with reduced microbial diversity and an increased abundance of dominant taxa such as *Pseudomonas aeruginosa*, consistent with the shifts observed in Paper II (255). However, bacterial load was not influenced by dysglycaemia in Paper I or II and these results are in alignment with another recently published study (255).

Several mechanistic pathways may contribute to the hyperglycaemia-inflammation-microbial colonisation cycle. Oxidative stress and NF- κ B activation are well recognised drivers of IL-8 and TNF- α upregulation in CF airways. IL-1 β may be amplified through ROS-mediated activation, contributing to disturbances in the protease-antiprotease balance. This is compatible with our finding of increased NE activity together with reduced antiprotease availability in the sputum proteome of pwCF with CFRD. Additionally, dysregulation of IL-1 β -TGF- β 1 pathways can influence Th17 biology and may contribute to airway inflammation (256).

IL-1 β may also worsen hyperglycaemia itself by impairing insulin signalling in β -cells and higher glucose levels could lead to secretion of IL-1 β in human islet cells (257). TGF- β 1, which was more highly expressed in individuals with CF without CFRD, has been demonstrated to promote pancreatic islet survival and function, and to attenuate type 1 diabetes development via immunoregulatory mechanisms in mouse models of diabetes (258, 259).

Our proteomic data identified ezrin as more highly expressed in pwCF without diabetes. Ezrin is a key regulator of epithelial barrier function, linking transmembrane proteins to the cytoskeleton, and its downregulation under hyperglycaemic conditions has been shown to weaken tight junctions and increase epithelial permeability (260). Reduced ezrin may therefore facilitate increased bacterial ingress and augment airway inflammation and could also be a factor involved in microbial changes.

Interestingly, IL-6 was decreased in CFRD in our cohort, aligning with its described roles in epithelial repair, regeneration, and interactions with TGF- β 1 signalling (261).

The lower expression of α_1 -antitrypsin in the sputum proteome of pwCF with CFRD is a sign of more impaired protease/antiprotease balance and reduced protective effects of α_1 -antitrypsin (262). Interestingly, low levels of α_1 -antitrypsin have also been associated with both type 1 and type 2 diabetes (263-265).

Finally, prothymosin α , which we observed to be associated with CFRD, has been identified as a genetic modifier of CFRD and is involved in oxidative stress, inflammation, and regulation of insulin sensitivity via TLR4 (182, 266). Its

established role in pulmonary emphysema pathogenesis, including its ability to potentiate NF- κ B activation and protease expression, supports the biological relevance of its increased abundance in the sputum of pwCF with CFRD (267, 268).

The additional finding in Paper II of reduced microbial diversity, together with a trend toward a more dysbiotic airway microbiome characterised by an increased relative abundance of *Escherichia/Shigella* in the CFRD group, further reinforces our cytokine and proteomic results demonstrating heightened airway inflammation and a greater susceptibility to pathogen invasion. Notably, *Escherichia/Shigella* has also been reported to be more abundant in the gut microbiome of individuals with type 2 diabetes compared with healthy controls, suggesting a possible direct association between shifts in microbial communities and dysglycaemia, potentially mediated through a gut–pancreas axis (269, 270).

Emerging evidence indicates that gut bacteria may translocate to the pancreas, where they can trigger both local and systemic inflammatory responses. Such microbial translocation has been implicated in pancreatic injury, altered islet immune homeostasis, and impaired glucose metabolism (271, 272). In this context, the enrichment of *Escherichia/Shigella* in the airways of pwCF with CFRD may reflect broader diabetes-associated microbial shifts that extend beyond the lungs and could contribute to inflammatory signalling pathways relevant to both airway disease and metabolic dysfunction.

In our microbiome analysis, colonisation with *P. aeruginosa* and overall bacterial load were comparable between pwCF with and without CFRD. Similarly, the number of observed taxa did not differ, and we did not detect clustering or shifts in Bray-Curtis dissimilarity. These findings contrast with the results of two previously published studies on pwCF with CFRD, which reported increased alpha diversity in one study and distinct microbial community structures in two studies (255, 273). A key methodological difference is the lung function profile of the study populations: in our cohort, both the NGT and CFRD groups exhibited higher overall lung function than those described in prior studies.

Given that alterations in the airway microbiome are well documented in pwCF with declining lung function, this discrepancy likely reflects differences in disease severity rather than the presence of CFRD alone. Supporting this interpretation, we identified significant correlations between beta diversity and both FEV1pp and IL-1 β , indicating that lung function and airway inflammation exert stronger effects on microbial community composition than CFRD status per se.

A major strength of Paper I and II is the use of modern, highly detailed analytical techniques. In both studies, we employed methods capable of capturing the full depth of the sputum proteome and airway microbiome, enabled by a bottom-up proteomics workflow combined with data-independent acquisition (DIA). For the microbiome analyses, we applied robust data-cleaning pipelines and machine-learning-based approaches, allowing for sensitive detection of bacterial

taxa even at low abundance. Cytokine quantification was performed using the Meso Scale Discovery platform, which reduces hands-on pipetting steps and dilution errors, thereby improving precision. Furthermore, sputum handling procedures in our laboratory follow well-established and validated protocols, ensuring high sample integrity.

Paper I contribute entirely new data to the field, while Paper II builds upon two previously published datasets, which have reported conflicting results. Our integrated approach therefore provides important clarification and adds methodological strength to existing findings.

The main limitations of Paper I and II include the single-centre design and the relatively small sample sizes, which may reduce generalisability. In addition, the CFRD cohort exhibited substantially lower lung function, which complicates interpretation of disease-specific effects independent of lung function decline. While lung function decline is part of the clinical presentation of CFRD, it highlights the difficulty in separating inflammatory and microbiome alterations driven specifically by CFRD from those arising secondary to impaired lung function. Further studies with larger well-phenotyped cohorts and longitudinal design would be required to clarify the specific contributions of CFRD to airway inflammation. Paper II also included an unintentional overrepresentation of male participants, which may have introduced sex-related bias.

As the effects of ETI on airway physiology still are not fully understood, longitudinal assessments following ETI initiation would provide important insights into how CFRD modifies both the airway microbiome and local inflammatory responses.

The lung function dilemma in CFRD

CFRD and lung function are closely intertwined, and reduced lung function in individuals with CFRD was already reported by Lanng et al. in 1992 (155). Although several studies suggest that lung function decline may begin early in the dysglycaemic state, the evidence remains inconsistent. In both Paper I and Paper II, we faced the challenge that our CFRD cohort exhibited markedly lower lung function compared with the non-diabetic group. To minimise the potential confounding effect of lung function in paper I, we applied lung function-matched pair analysis, whereas in Paper II we used multiple linear regression adjusting for FEV1pp. However, because CFRD and declining lung function are so tightly linked, adjusting for lung function risks controlling for a clinically meaningful component of the disease process itself. This raises the classic “chicken-or-egg” question: does dysglycaemia drive lung function decline, or does worsening lung disease precipitate metabolic deterioration?

To further address this question, we conducted a retrospective longitudinal study using data from the Swedish CF Registry. In the cross-sectional analyses, we confirmed that individuals with CFRD consistently exhibited lower lung function compared with those without diabetes. However, we did not observe a difference in lung function between NGT and IGT based on OGTT categories. As in our cross-sectional findings, the systematic review by Iwanicki et al. detected no lung function differences between IGT and NGT groups, but reaffirmed that CFRD is associated with lower lung function in individuals with CF (274). Conversely, Lavie et al. and Patel et al. reported that individuals with CF and IGT displayed markedly reduced lung function relative to those with NGT (275, 276).

In the longitudinal analysis, the results from our cross-sectional observations were confirmed with decreased lung function in CFRD but no difference between NGT and IGT. Importantly, across the three-year follow-up, the trajectory of lung function decline was comparable among individuals with NGT, IGT, and CFRD.

Our observations in the longitudinal study are in line with those of Rakotoarisoa et al. who demonstrated no association between 2-hour OGTT glucose levels and lung function decline in a 5-year prospective study (277). Similarly, Boudreau et al. observed that elevated 1-hour OGTT glucose values were not linked to either lower baseline lung function or the subsequent rate of decline during a four-year follow-up (278). Further support comes from Reynaud et al., who found no association between glucose patterns derived from fasting and 2-hour OGTT values and FEV1pp in children, and no association between 1-hour glucose patterns and FEV1pp in adults. However, they did identify a significant decline in FEV1pp over time among adults with high and increasing 2-hour glucose patterns compared with those displaying low and stable 2-hour glucose patterns (279).

In summary, our findings align well with the existing literature. However, in our earlier pilot study, we observed differences between NGT, IGT, and CFRD groups similar to the patterns reported by Lavie et al. and Patel et al. (170, 275, 276). One contributing challenge is the uneven group sizes: both our cohort in Paper III and that of Patel et al. included markedly unbalanced numbers across glucose-tolerance categories. In the study by Lavie et al., an additional limitation was the unequal genetic background among participants in the different groups, which may have influenced the observed outcomes.

Another limitation is that glycaemic status defined by OGTT may be an imprecise method. Studies using CGM as a screening tool for CFRD may be more sensitive for detecting early dysglycaemia. In our previous work, we observed that CGM values exceeding 8 mmol/L correlated with glucose levels at 30, 60, and 90 minutes during the OGTT, but not with the 120-minute measurement, in paediatric patients with CF (170). Moreover, we found a significant decline in FEV1pp among paediatric patients with CF undergoing CGM, with those experiencing ≥ 0.5 daily

peaks of interstitial glucose above 11 mmol/L showing worse lung function than those with fewer peaks.

Similarly, although Rakotoarisoa et al. reported no association using standard OGTT metrics, they did identify a significant decline in FVC and FEV1pp associated with increases in CGM-derived AUC > 7.8 mmol/L, further supporting the notion that CGM may capture early dysglycaemia more effectively than OGTT (277).

A further limitation of our study is the lack of mid-OGTT glucose values in the Swedish CF Registry, which prevented us from identifying individuals with indeterminate glycaemia (INDET). INDET is defined as fasting glucose < 7 mmol/L and a 2-hour OGTT glucose < 7.8 mmol/L, but with at least one intermediate OGTT value (30, 60, or 90 minutes) ≥ 11.1 mmol/L (244). This category represents a pre-diabetic metabolic state positioned between IGT and CFRD and may capture individuals with early, intermittent post-prandial dysglycaemia that is not reflected in the standard OGTT endpoints.

Emerging evidence suggests that this intermediate phenotype may hold clinical relevance. Coriati et al. reported that although broader IGT groups may not show differences in pulmonary outcomes, specific subgroups - including those classified as INDET - can exhibit reduced lung function comparable to individuals with newly diagnosed CFRD (280). Their findings raise the possibility that INDET may represent an early stage of CFRD-related metabolic decline with potential respiratory consequences. The absence of mid-OGTT data in our dataset therefore limits our ability to detect this subgroup and may lead to an underestimation of the true impact of early dysglycaemia on lung function in CF.

A key strength of this study is its population-based approach. The initial cohort comprised all individuals listed in the Swedish CF Registry, which captures approximately 95% of the national CF population (6). The final cohort of 411 participants therefore represents more than half of all Swedish pwCF (around 800), supporting the robustness and external validity of our findings.

Overall, we were unable to conclusively answer the longstanding “chicken-or-egg” question regarding whether dysglycaemia or lung function decline occurs first. This highlights the need for larger, well-designed longitudinal studies and reinforces the importance of early detection with more sensitive tools like CGM and LCI, as well as continuous monitoring of dysglycaemia in CF.

ETI treatment and its impact in CFHBI

During my PhD studies, ETI became available in Sweden in December 2022. By that time, ETI therapy had already been accessible in many European countries for more than eighteen months. Because of this delayed national introduction, Swedish studies evaluating the clinical effects of ETI on the course of cystic fibrosis developed later than those in other countries. This created a need to focus on research questions that had not yet been thoroughly explored elsewhere.

Within this context, we decided to concentrate our research on cystic fibrosis hepatobiliary involvement (CFHBI) and on liver elastography, a diagnostic method that our group had previously studied (229). This focus allowed us to address clinically relevant knowledge gaps and to build on our earlier methodological experience.

In Paper IV, we evaluated the effect of ETI therapy on 2D shear wave liver elastography. Our findings demonstrated an overall reduction in liver stiffness measurements following the initiation of ETI. Notably, this decline was significant among the twelve patients with pre-existing CFHBI, indicating that ETI may have a particularly beneficial impact on those already exhibiting hepato-biliary involvement.

Liver elastography represents a key non-invasive modality for monitoring CFHBI and is included in the latest updated guidelines as part of recommended follow-up (281). Moreover, it has been proposed as a useful monitoring tool for assessing treatment response to ETI (83).

At our CF centre, we have performed annual liver elastography using 2D shear wave elastography (2D SWE) in paediatric patients since 2018. The observed reduction in liver stiffness following ETI initiation aligns with the recently published findings by Terlizzi et al. (282). In their cohort of 27 children with CF aged 6–12 years, they similarly reported an overall decline in liver stiffness, with a significant reduction specifically in patients with established CFHBI.

Comparable results have also been described in older populations. Calvo et al. examined 55 individuals with CF and demonstrated a significant overall decrease in mean liver stiffness after six months of ETI therapy. Notably, among patients with baseline liver stiffness ≥ 5 kPa, a measurable decline was already evident after just one month of treatment (283). Another study in adults reported a significant reduction in liver stiffness after a median of 21 months ETI treatment among 14 participants with elevated stiffness (>6.8 kPa) prior to therapy, although no corresponding changes were observed in liver enzymes or non-invasive fibrosis indices (284). Recently, a study from Greece was published with an even longer ETI treatment period, with a median of 39 months. This study confirmed our findings

over a longer observation period, showing that liver stiffness declined in the overall cohort and also in CFHBI patients (285).

In contrast, Schnell et al. reported an increase in liver stiffness after six months of ETI therapy in a prospective cohort of 20 individuals with CF, comprising 10 participants younger than 20 years and 10 older than 20 years (286). These studies were based on relatively small cohorts, underscoring the need for further research in larger populations to better clarify the impact of ETI on liver stiffness.

In the early phase of ETI introduction, transient elevations in liver enzymes were reported in paediatric CF patients (76). In our cohort, however, liver enzyme values remained within the normal range both before and after ETI initiation, although we noted a slight increase in GGT and a decrease in AST among individuals with CFHBI. A systematic review by Moiceanu et al. similarly described heterogeneous findings regarding liver function tests following ETI initiation, highlighting that biochemical responses vary across studies (238).

The effects of ETI on CF-related liver physiology are not yet fully understood, and several potential mechanisms may be involved. One possibility is that ETI improves biliary flow by enhancing CFTR function in the biliary epithelium, thereby reducing cholestasis. Additionally, ETI may attenuate systemic and hepatic inflammation, which in turn could positively influence liver stiffness and biochemical markers. Further mechanisms, such as improved nutritional status, reduced hepatocellular stress, or indirect metabolic effects, may also contribute, underscoring the need for more detailed mechanistic studies.

The strengths of our study include the focus on a paediatric cohort within the age range in which CFHBI typically begins to manifest, allowing us to capture early hepatic changes relevant to disease development. All participants were of school age, exhibited pancreatic insufficiency, and the cohort demonstrated a balanced sex distribution, enhancing the internal comparability of the group. Another notable strength is the long-standing implementation of 2D SWE at our centre. This technique has been used routinely since 2018, meaning that the investigators are highly experienced, and serial measurements spanning more than five years were available for analysis. This consistency in methodology enhances the reliability of our elastography measurements and minimizes variability related to operator dependency. 2D SWE is integrated into the ultrasound system and provides real-time, two-dimensional elasticity mapping with direct visual control, which improves the validity of liver stiffness assessments. In addition, 2D SWE is less operator-dependent and has a higher applicability rate than conventional transient elastography (287).

However, the study also has important limitations. The retrospective design limits control over both the timing and the completeness of data collection. The cohort was relatively small, largely due to missing assessments and the transition of adolescents to the adult CF unit, where 2D SWE surveillance was not continued, resulting in

gaps in longitudinal follow-up. These factors may reduce the generalisability of the findings and lower the statistical power to detect more subtle or gradual changes in liver stiffness. Furthermore, liver enzyme measurements in CF are under debate and have relatively low sensitivity for detecting early hepatic involvement. More advanced biomarkers like aspartate aminotransferase to platelet ratio index (APRI) or Fibrosis-4 index, could provide a more nuanced assessment of liver health and strengthen the interpretation of our elastography results (288).

To strengthen the evidence base, larger prospective long-term longitudinal studies are required. Such studies are essential to establish robust reference values for liver stiffness in pwCF, define the expected developmental trajectory of liver stiffness, and clarify how ETI influences the natural history of hepatic involvement across the lifespan.

Conclusion and further perspectives

Paper I focuses on airway inflammation in CFRD. In this study, we were able to demonstrate, for the first time, a distinct airway proteomic profile in individuals with CFRD, characterised by an overexpression of prothymosin α and elevated concentrations of inflammatory mediators such as IL-1 β , IL-8, and NE. These findings highlight potential biomarkers and therapeutic targets relevant to the management of CFRD-associated lung disease. Notably, IL-8, IL-1, and NE are already recognised treatment targets, and several therapeutic agents directed against these inflammatory pathways are currently in development and progressing toward clinical application.

Looking ahead, a more personalised precision-medicine approach for individuals with CFRD may become feasible, with the potential to positively affect not only airway inflammation but also broader systemic inflammatory and metabolic processes. Prothymosin α may function as a genetic modifier for CFRD, offering promise as an early biomarker for identifying individuals at increased risk of developing CFRD and CFRD-associated lung disease. Earlier detection, combined with proactive monitoring and optimised glycaemic control, could slow disease progression and ultimately improve long-term outcomes and quality of life.

Paper II focuses on the airway microbiome in individuals with CFRD. We observed a tendency towards greater dysbiosis and reduced microbial diversity in the airways of those with CFRD.

The enrichment of *Escherichia/Shigella* in the CFRD airway microbiome - together with previous reports linking *Escherichia/Shigella* to diabetes through a gut-pancreas axis - supports the idea that inflammation, microbiome composition, and metabolic changes are closely interconnected. By examining both the gut and airway microbiomes and applying targeted probiotic treatments, it may be possible to restore a more balanced microbial community, achieve improved glycaemic control, and potentially slow disease progression. Recent clinical trials and meta-analyses in type 1 and type 2 diabetes further show that probiotic supplementation can modestly improve glycaemic parameters such as HbA1c and fasting plasma glucose, although the magnitude of effect varies (289, 290). Strains of *Lactobacillus* and *Bifidobacterium* were often used in these studies. Probiotics could act via diverse mechanisms, such as reducing overall inflammation and oxidative stress, enhancing β -cell function, and GLP-1 secretion (291). The role of probiotics in CF has been

discussed in several studies, and there is evidence that they could improve pulmonary function, decrease pulmonary exacerbation, and have a positive impact on gastrointestinal health, but to date there is no published study investigating the effect of probiotics in CFRD (292, 293). A multicentre, randomized controlled trial would be valuable for clarifying the potentially beneficial effects of probiotics in CFRD.

Paper III examined the progression of lung function in individuals with CFRD. We observed an overall lower lung function in those with CFRD compared with individuals who had CF and impaired glucose tolerance or normal glucose regulation. However, over the three-year retrospective observation period, we did not detect a steeper rate of lung function decline in individuals with CFRD compared with those with impaired glucose tolerance or normal glucose regulation.

Lung function decline and CFRD are closely interconnected. Because loss of lung function is the most critical complication in CF and ultimately leads to respiratory failure, understanding the timing and pattern of lung function decline in CFRD is essential for prevention. More sensitive monitoring tools, such as the lung clearance index, may improve the detection and characterization of early lung function changes in CFRD. Another methodological challenge is the early identification of dysregulated glycaemia. The current “gold standard,” the OGTT, may not adequately capture early postprandial glucose peaks. Integrating lung clearance index measurements with continuous glucose monitoring (CGM) could represent a promising strategy for earlier and more precise detection of disease progression.

Paper IV investigated the impact of ETI on liver stiffness. In our paediatric cohort, we observed a decrease in liver stiffness following the initiation of ETI therapy. Notably, individuals with CFHBI also showed a reduction in liver stiffness after starting ETI, and we did not observe any negative effects of ETI on liver enzyme levels.

The role of ETI in managing extra-pulmonary complications of CF remains under discussion but is gaining increasing relevance as the CF population ages.

In this study, we investigated the course of liver stiffness exclusively in paediatric patients, an age group in which CFHBI often first becomes apparent. Extending these analyses to adults with more established CFHBI after initiating ETI over a longer observation period would be valuable in determining whether ETI can still modify more advanced hepatic involvement.

Our study provides conclusions only regarding liver stiffness, which represents just one parameter used to monitor CFHBI. Including additional outcome measures to assess the effects of ETI on CFHBI would allow for more comprehensive interpretations. Combining liver elastography with biomarkers of liver fibrosis, such as the aspartate aminotransferase-to-platelet ratio index (APRI) or the fibrosis-4

score (FIB-4), could offer a more robust assessment of hepatic disease progression and treatment response.

This thesis provides new insights into several understudied areas of CF, including CFRD-related lung disease and CFHBI in the era of modulator therapy. By 2036, CF care will likely be characterized by highly personalized treatment, with modulator therapies available to all individuals - including those living in Eastern Europe - and mRNA-based treatments accessible for people with class I variants. Improved management of the CFTR defect, together with broader implementation of newborn screening programs in CF - hopefully also in Sweden - has the potential to reduce CF-related complications and overall disease burden for future generations.

However, individuals with CF who are currently in their twenties and thirties will age into a period where severe CF-related complications may still arise. This thesis may serve as a step toward more preventive care strategies addressing metabolic, inflammatory, and microbial health in the CF population. Such future approaches may involve modern biomarkers, probiotic therapies, and advanced screening for CF-related complications.

Use of Generative AI

I declare that generative AI tools (including ChatGPT, Microsoft Copilot, and Gemini AI) were used in the preparation of this thesis. These tools were used to generate figures, as indicated in the corresponding figure captions, and to create the cover image. The AI-generated figures were reviewed, revised where necessary, and approved by the author. Generative AI was also used for language editing and to improve the clarity and readability of the text throughout the thesis.

For the research projects presented in Papers I–IV, all analyses, scientific content, interpretations, and conclusions were conceived, performed, evaluated, and written by the authors. Generative AI tools were not used to generate, analyse, interpret, or draw scientific conclusions from the research data.

Acknowledgement

Many people have been part of my clinical and research journey in cystic fibrosis across Europe over the past 15 years. Without your support, encouragement, and guidance, this thesis would not have been possible.

First and foremost, I would like to thank all people with cystic fibrosis in Davos, Switzerland, and in Lund, Sweden. You have taught me so much about living with CF and its impact on daily life, providing invaluable insights and inspiring my continued interest in this field. Thank you for participating in the clinical studies at Lund University.

When I came to Sweden, it took some time before I met my main supervisor, Lisa Pålman. Lisa, I'm so glad to have met you. I still clearly remember our first "garden" meeting with little Anna in my arms. Thank you for believing in my ideas, for your patience, and for your invaluable guidance throughout this journey. You have been a cornerstone in my busy and sometimes turbulent research and professional life. Your precision and dedication to our projects were essential to completing this thesis. Our meetings always ended on an encouraging note, with the feeling that everything is possible. I will miss these meetings and sincerely hope that our research journey will continue.

My co-supervisor Erik Eklund encouraged me to pursue a Swedish PhD and connected me to clinical research at Lund University. Thank you for sharing your experience and for your valuable comments - also now as a colleague in my role as section-head.

My co-supervisor Christine Hansen - you are an outstanding clinician and have taught me so much. Thank you for your contributions to the clinical projects and for being my friend for many years. Opera visits, churros nights in Madrid, and trips to Iceland are wonderful memories.

My co-supervisor Jonas Erjefält - thank you for introducing me to the field of immunohistochemistry and for your valuable scientific input.

A big thank you to the lab team at BMC - Cecilia, Malgorzata, Jenny and Anita - your excellent technical and project support has been invaluable.

I would also like to thank my co-authors: Lotta Happonen for her help with the proteomics project, Katja Kozjek for her support in the microbiome project, and

Olivia Olsson for her excellent statistical work on the registry project. Your contributions have been essential to this thesis.

My sincere thanks also go to my mentor Robin Kahn for your guidance and for generously sharing your experience.

A warm thank you to all members of the CF team at Skåne University Hospital, both in the paediatric and adult units, including nurses, physiotherapists, dietitians, psychologists, and administrative staff. Your daily dedication to patient care is truly inspiring, and together we form a wonderful team - reflected in CF centre meetings, summer and Christmas celebrations, and conference trips.

I am also grateful to all colleagues at VO Barnmedicin, including Ulrika, Anna, Erik, Ioannis, Kees-Jan, together with the management team, and especially all ST doctors, for your support, collaboration, and for creating such a stimulating working environment. Special thanks to Emmie Walentynowicz and Louise Kolben for your support when I am immersed in the research world. Johan Lundgren, thank you for supporting and trusting in me during my first years at VO Barnmedicin.

A special thank you to Frida, Anna P, Tina, Petter, Helena, Nadine, Kristjan, Hanna, Jacob, Huda, Karolina, Sara, Maria M, Kristel, Moa and Sandra for all your support, lunch breaks, and short catchups during working days - these moments are worth so much for my working environment and well-being.

I am deeply grateful to my clinical mentors throughout the years. In Davos, I would like to thank Christian Geidel, Andreas Jung, and Charly Stirner. In Lund, I thank Peter Meyer and Lena Mared - without you, I might never have come to Lund. I would also like to thank Inger Ervander for sharing her knowledge and supporting me when I started in Lund as an ST doctor. Furthermore, I wish to thank Christine Hansen, Helga Elidottir, and, from the adult unit, Ulrika Lindberg and Lennart Hansson. You have always been available for discussions, and I treasure the many memories from our ECFS and ERS conferences over the years. Thank you very much for reading this thesis and for your valuable comments. Helga, I truly miss you in Lund but visiting you in Iceland is a great consolation - and a glass of bubbles is never amiss.

During my medical studies in Heidelberg, Germany, I had the opportunity to join the cystic fibrosis research group in Professor Marcus Mall's laboratory. Without this formative experience, I would probably never have started a Swedish PhD. I am very grateful for those years, which introduced me to research, animal models, and preclinical projects. Marcus, thank you for your trust and support. I also thank the former lab members - Stephanie, Jolanthe, Zhe, Diana, Julia, and Penelope - for creating such an inspiring and collaborative environment. Penelope, it is always a pleasure to see you at least once a year and catch up over a pizza or dinner.

Outside of work, I have been incredibly fortunate to be part of such supportive communities. Thank you to everyone who made Lund feel like home - for all the evenings and weekends shared with “Pingvinmammorna”, the friends from the German book club and Ladies Circle Lund.

A special thank you to my German friends Verena and Katrin in Lund, and to my friends back home - Chrissi, Tina, Beni, Philro, and Ines - for your enduring friendship and support across distances.

To my extended Swedish family and specially to my parents-in-law Anneli, Björn, and Marie, thank you for your incredible support, particularly for helping with the children during busy workdays and conference trips.

To my German family - my parents Irmgard and Norbert and my brother Simon - thank you for your unwavering love, belief in me, and constant encouragement throughout all these years. Your visits to Lund during research school, my half-time seminar, and conferences meant so much and were invaluable to us as a family.

Finally, to my husband Aron and my children Ida, Nils, and Anna - thank you for your endless love, patience, and support, even during periods of heavy workload and many activities. You are my greatest source of strength and motivation. This thesis is as much yours as it is mine.

References

1. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science*. 1989;245(4922):1066-73. Doi:10.1126/science.2475911.
2. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science*. 1989;245(4922):1059-65. Doi:10.1126/science.2772657.
3. Infield DT, Strickland KM, Gaggar A, McCarty NA. The molecular evolution of function in the CFTR chloride channel. *Journal of General Physiology*. 2021;153(12) Doi:10.1085/jgp.202012625.
4. Gadsby DC, Vergani P, Csanády L. The ABC protein turned chloride channel whose failure causes cystic fibrosis. *Nature*. 2006;440(7083):477-83. Doi:10.1038/nature04712.
5. Saint-Criq V, Gray MA. Role of CFTR in epithelial physiology. *Cell Mol Life Sci*. 2017;74(1):93-115. Doi:10.1007/s00018-016-2391-y.
6. Anderson MP, Gregory RJ, Thompson S, Souza DW, Paul S, Mulligan RC, et al. Demonstration that CFTR is a chloride channel by alteration of its anion selectivity. *Science*. 1991;253(5016):202-5. Doi:10.1126/science.1712984.
7. Reddy MM, Quinton PM. Functional interaction of CFTR and ENaC in sweat glands. *Pflügers Arch*. 2003;445(4):499-503. Doi:10.1007/s00424-002-0959-x.
8. Poulsen JH, Fischer H, Illek B, Machen TE. Bicarbonate conductance and pH regulatory capability of cystic fibrosis transmembrane conductance regulator. *Proc Natl Acad Sci U S A*. 1994;91(12):5340-4. Doi:10.1073/pnas.91.12.5340.
9. Quinton PM. Cystic fibrosis: impaired bicarbonate secretion and mucoviscidosis. *Lancet*. 2008;372(9636):415-7. Doi:10.1016/s0140-6736(08)61162-9.
10. Novak I, Wang J, Henriksen KL, Haanes KA, Krabbe S, Nitschke R, Hede SE. Pancreatic bicarbonate secretion involves two proton pumps. *J Biol Chem*. 2011;286(1):280-9. Doi:10.1074/jbc.M110.136382.
11. Schwiebert EM, Benos DJ, Egan ME, Stutts MJ, Guggino WB. CFTR Is a Conductance Regulator as well as a Chloride Channel. *Physiological Reviews*. 1999;79(1):S145-S66. Doi:10.1152/physrev.1999.79.1.S145.
12. Fong P. CFTR–SLC26 transporter interactions in epithelia. *Biophysical Reviews*. 2012;4(2):107-16. Doi:10.1007/s12551-012-0068-9.

13. Edlund A, Pedersen MG, Lindqvist A, Wierup N, Flodström-Tullberg M, Eliasson L. CFTR is involved in the regulation of glucagon secretion in human and rodent alpha cells. *Scientific Reports*. 2017;7(1) Doi:10.1038/s41598-017-00098-8.
14. Lukasiak A, Zajac M. The Distribution and Role of the CFTR Protein in the Intracellular Compartments. *Membranes (Basel)*. 2021;11(11) Doi:10.3390/membranes11110804.
15. Hartl D, Gaggar A, Bruscia E, Hector A, Marcos V, Jung A, et al. Innate immunity in cystic fibrosis lung disease. *Journal of Cystic Fibrosis*. 2012;11(5):363-82. Doi:10.1016/j.jcf.2012.07.003.
16. Mattoscio D, Evangelista V, De Cristofaro R, Recchiuti A, Pandolfi A, Di Silvestre S, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) expression in human platelets: impact on mediators and mechanisms of the inflammatory response. *The FASEB Journal*. 2010;24(10):3970-80. Doi:https://doi.org/10.1096/fj.10-159921.
17. Guo J, Garratt A, Hill A. Worldwide rates of diagnosis and effective treatment for cystic fibrosis. *J Cyst Fibros*. 2022;21(3):456-62. Doi:10.1016/j.jcf.2022.01.009.
18. Scotet V, Gutierrez H, Farrell PM. Newborn Screening for CF across the Globe—Where Is It Worthwhile? *International Journal of Neonatal Screening*. 2020;6(1):18.
19. Ioannou L, McClaren BJ, Massie J, Lewis S, Metcalfe SA, Forrest L, Delatycki MB. Population-based carrier screening for cystic fibrosis: a systematic review of 23 years of research. *Genet Med*. 2014;16(3):207-16. Doi:10.1038/gim.2013.125.
20. Krantz CL, Anders. Årsrapport 2024. <https://www.cf-registret.se/publikationer>: Swedish CF registry; 2025.
21. De Boeck K, Amaral MD. Progress in therapies for cystic fibrosis. *The Lancet Respiratory Medicine*. 2016;4(8):662-74. Doi:10.1016/S2213-2600(16)00023-0.
22. Marson FAL, Bertuzzo CS, Ribeiro JD. Classification of CFTR mutation classes. *The Lancet Respiratory Medicine*. 2016;4(8):e37-e8. Doi:10.1016/S2213-2600(16)30188-6.
23. Anwar S, Peng J-L, Zahid KR, Zhou Y-M, Ali Q, Qiu C-R. Cystic Fibrosis: Understanding Cystic Fibrosis Transmembrane Regulator Mutation Classification and Modulator Therapies. *Advances in Respiratory Medicine*. 2024;92(4):263-77.
24. Martiniano SL, Sagel SD, Zemanick ET. Cystic fibrosis: a model system for precision medicine. *Curr Opin Pediatr*. 2016;28(3):312-7. Doi:10.1097/mop.0000000000000351.
25. Boyle MP, De Boeck K. A new era in the treatment of cystic fibrosis: correction of the underlying CFTR defect. *Lancet Respir Med*. 2013;1(2):158-63. Doi:10.1016/s2213-2600(12)70057-7.
26. Elborn JS. Cystic fibrosis. *The Lancet*. 2016;388(10059):2519-31. Doi:10.1016/S0140-6736(16)00576-6.
27. Sankari A, Sharma S. Cystic Fibrosis. *StatPearls*. Treasure Island (FL): StatPearls Publishing Copyright © 2025, StatPearls Publishing LLC.; 2025.
28. Trouvé P, Saint Pierre A, Férec C. Cystic Fibrosis: A Journey through Time and Hope. *Int J Mol Sci*. 2024;25(17) Doi:10.3390/ijms25179599.

29. O'Shea JG. Was Frédéric Chopin's illness actually cystic fibrosis? *Med J Aust*. 1987;147(11-12):586-9. Doi:10.5694/j.1326-5377.1987.tb133693.x.
30. Andersen DH. Cystic fibrosis of the pancreas and its relation to celiac disease: A clinical and pathologic study. *American Journal of Diseases of Children*. 1938;56(2):344-99. Doi:10.1001/archpedi.1938.01980140114013.
31. Andersen DH, Hodges RG. Celiac syndrome; genetics of cystic fibrosis of the pancreas, with a consideration of etiology. *Am J Dis Child* (1911). 1946;72:62-80. Doi:10.1001/archpedi.1946.02020300069004.
32. Di Sant'Agnese PA, Darling RC, Perera GA, Shea E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas; clinical significance and relationship to the disease. *Pediatrics*. 1953;12(5):549-63.
33. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. *Pediatrics*. 1959;23(3):545-9.
34. Ramsey Bonnie W, Davies J, McElvaney NG, Tullis E, Bell Scott C, Dřevínek P, et al. A CFTR Potentiator in Patients with Cystic Fibrosis and the G551D Mutation. *New England Journal of Medicine*. 365(18):1663-72. Doi:10.1056/NEJMoa1105185.
35. Wainwright CE, Elborn JS, Ramsey BW, Marigowda G, Huang X, Cipolli M, et al. Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. *N Engl J Med*. 2015;373(3):220-31. Doi:10.1056/NEJMoa1409547.
36. Middleton PG, Mall MA, Dřevínek P, Lands LC, McKone EF, Polineni D, et al. Elexacaftor–Tezacaftor–Ivacaftor for Cystic Fibrosis with a Single Phe508del Allele. *New England Journal of Medicine*. 2019;381(19):1809-19. Doi:10.1056/NEJMoa1908639.
37. Heijerman HGM, McKone EF, Downey DG, Van Braeckel E, Rowe SM, Tullis E, et al. Efficacy and safety of the elexacaftor plus tezacaftor plus ivacaftor combination regimen in people with cystic fibrosis homozygous for the F508del mutation: a double-blind, randomised, phase 3 trial. *The Lancet*. 2019;394(10212):1940-8. Doi:https://doi.org/10.1016/S0140-6736(19)32597-8.
38. Kapouni N, Moustaki M, Douros K, Loukou I. Efficacy and Safety of Elexacaftor-Tezacaftor-Ivacaftor in the Treatment of Cystic Fibrosis: A Systematic Review. *Children*. 2023;10(3):554. Doi:10.3390/children10030554.
39. Davies JC, Polineni D, Boyd AC, Donaldson S, Gill DR, Griesenbach U, et al. Lentiviral Gene Therapy for Cystic Fibrosis: A Promising Approach and First-in-Human Trial. *Am J Respir Crit Care Med*. 2024;210(12):1398-408. Doi:10.1164/rccm.202402-0389CI.
40. Travert G, Heeley M, Heeley A. History of Newborn Screening for Cystic Fibrosis-The Early Years. *Int J Neonatal Screen*. 2020;6(1):8. Doi:10.3390/ijns6010008.
41. Castellani C, Southern KW, Brownlee K, Dankert Roelse J, Duff A, Farrell M, et al. European best practice guidelines for cystic fibrosis neonatal screening. *J Cyst Fibros*. 2009;8(3):153-73. Doi:10.1016/j.jcf.2009.01.004.
42. Crossley JR, Elliott RB, Smith PA. Dried-blood spot screening for cystic fibrosis in the newborn. *Lancet*. 1979;1(8114):472-4. Doi:10.1016/s0140-6736(79)90825-0.

43. Gonska T, Ratjen F. Newborn screening for cystic fibrosis. *Expert Rev Respir Med*. 2015;9(5):619-31. Doi:10.1586/17476348.2015.1085804.
44. Fajac I, Burgel P-R. Cystic fibrosis. *La Presse Médicale*. 2023;52(3):104169. Doi:<https://doi.org/10.1016/j.lpm.2023.104169>.
45. Singh VK, Schwarzenberg SJ. Pancreatic insufficiency in Cystic Fibrosis. *Journal of Cystic Fibrosis*. 2017;16:S70-S8. Doi:<https://doi.org/10.1016/j.jcf.2017.06.011>.
46. Moheet A, Moran A. New Concepts in the Pathogenesis of Cystic Fibrosis–Related Diabetes. *The Journal of Clinical Endocrinology & Metabolism*. 2022;107(6):1503-9. Doi:10.1210/clinem/dgac020.
47. van der Doef HPJ, Houwen RHJ. Constipation and cystic fibrosis. Slow movement. *Journal of Cystic Fibrosis*. 2022;21(3):385-6. Doi:10.1016/j.jcf.2022.04.003.
48. Colombo C, Russo MC, Zazzeron L, Romano G. Liver Disease in Cystic Fibrosis. *Journal of Pediatric Gastroenterology and Nutrition*. 2006;43(S1):S49-S55. Doi:10.1097/01.mpg.0000226390.02355.52.
49. Quon BS, Mayer-Hamblett N, Aitken ML, Smyth AR, Goss CH. Risk Factors for Chronic Kidney Disease in Adults with Cystic Fibrosis. *American Journal of Respiratory and Critical Care Medicine*. 2011;184(10):1147-52. Doi:10.1164/rccm.201105-0932OC.
50. Burgel P-R, Southern KW, Addy C, Battezzati A, Berry C, Bouchara J-P, et al. Standards for the care of people with cystic fibrosis (CF); recognising and addressing CF health issues. *Journal of Cystic Fibrosis*. 2024;23(2):187-202. Doi:10.1016/j.jcf.2024.01.005.
51. Aris RM, Merkel PA, Bachrach LK, Borowitz DS, Boyle MP, Elkin SL, et al. Guide to bone health and disease in cystic fibrosis. *J Clin Endocrinol Metab*. 2005;90(3):1888-96. Doi:10.1210/jc.2004-1629.
52. Wong R, Gu K, Ko Y, Patel P. Congenital absence of the vas deferens: Cystic fibrosis transmembrane regulatory gene mutations. *Best Practice & Research Clinical Endocrinology & Metabolism*. 2020;34(6):101476. Doi:<https://doi.org/10.1016/j.beem.2020.101476>.
53. de Souza DAS, Faucz FR, Pereira-Ferrari L, Sotomaior VS, Raskin S. Congenital bilateral absence of the vas deferens as an atypical form of cystic fibrosis: reproductive implications and genetic counseling. *Andrology*. 2018;6(1):127-35. Doi:<https://doi.org/10.1111/andr.12450>.
54. Conway S, Balfour-Lynn IM, De Rijcke K, Drevinek P, Foweraker J, Havermans T, et al. European Cystic Fibrosis Society Standards of Care: Framework for the Cystic Fibrosis Centre. *Journal of Cystic Fibrosis*. 2014;13:S3-S22. Doi:10.1016/j.jcf.2014.03.009.
55. Southern KW, Castellani C, Lammertyn E, Smyth A, VanDevanter D, van Koningsbruggen-Rietschel S, et al. Standards of care for CFTR variant-specific therapy (including modulators) for people with cystic fibrosis. *J Cyst Fibros*. 2023;22(1):17-30. Doi:10.1016/j.jcf.2022.10.002.
56. Flume PA, Robinson KA, O'Sullivan BP, Finder JD, Vender RL, Willey-Courand DB, et al. Cystic fibrosis pulmonary guidelines: airway clearance therapies. *Respir Care*. 2009;54(4):522-37.

57. Yang C, Montgomery M. Dornase alfa for cystic fibrosis. *Cochrane Database Syst Rev.* 2021;3(3):Cd001127. Doi:10.1002/14651858.CD001127.pub5.
58. Wark P, McDonald VM. Nebulised hypertonic saline for cystic fibrosis. *Cochrane Database Syst Rev.* 2018;9(9):Cd001506. Doi:10.1002/14651858.CD001506.pub4.
59. Somaraju U, Solis-Moya A. Pancreatic enzyme replacement therapy for people with cystic fibrosis. *Paediatric respiratory reviews.* 2015;16(2):108-9.
60. Wilschanski M, Munck A, Carrion E, Cipolli M, Collins S, Colombo C, et al. ESPEN-ESPGHAN-ECFS guideline on nutrition care for cystic fibrosis. *Clinical Nutrition.* 2024;43(2):413-45. Doi:10.1016/j.clnu.2023.12.017.
61. Colombo C, Ellemunter H, Houwen R, Munck A, Taylor C, Wilschanski M. Guidelines for the diagnosis and management of distal intestinal obstruction syndrome in cystic fibrosis patients. *J Cyst Fibros.* 2011;10 Suppl 2:S24-8. Doi:10.1016/s1569-1993(11)60005-2.
62. Döring G, Flume P, Heijerman H, Elborn JS. Treatment of lung infection in patients with cystic fibrosis: Current and future strategies. *Journal of Cystic Fibrosis.* 2012;11(6):461-79. Doi:10.1016/j.jcf.2012.10.004.
63. Taccetti G, Terlizzi V, Campana S, Dolce D, Ravenni N, Fevola C, et al. Antibiotic treatment of bacterial lung infections in cystic fibrosis. *Eur J Pediatr.* 2024;184(1):82. Doi:10.1007/s00431-024-05905-9.
64. Mogayzel PJ, Jr., Naureckas ET, Robinson KA, Brady C, Guill M, Lahiri T, et al. Cystic Fibrosis Foundation pulmonary guideline. pharmacologic approaches to prevention and eradication of initial *Pseudomonas aeruginosa* infection. *Ann Am Thorac Soc.* 2014;11(10):1640-50. Doi:10.1513/AnnalsATS.201404-166OC.
65. Hewer SCL, Smyth AR, Brown M, Jones AP, Hickey H, Kenna D, et al. Intravenous versus oral antibiotics for eradication of *Pseudomonas aeruginosa* in cystic fibrosis (TORPEDO-CF): a randomised controlled trial. *Lancet Respir Med.* 2020;8(10):975-86. Doi:10.1016/s2213-2600(20)30331-3.
66. Principi N, Blasi F, Esposito S. Azithromycin use in patients with cystic fibrosis. *European Journal of Clinical Microbiology & Infectious Diseases.* 2015;34(6):1071-9. Doi:10.1007/s10096-015-2347-4.
67. Smyth AR, Rosenfeld M. Prophylactic anti-staphylococcal antibiotics for cystic fibrosis. *Cochrane Database Syst Rev.* 2017;4(4):Cd001912. Doi:10.1002/14651858.CD001912.pub4.
68. Zemanick E, Burgel PR, Taccetti G, Holmes A, Ratjen F, Byrnes CA, et al. Antimicrobial resistance in cystic fibrosis: A Delphi approach to defining best practices. *J Cyst Fibros.* 2020;19(3):370-5. Doi:10.1016/j.jcf.2019.10.006.
69. Fajac I, Burgel P-R, Martin C. New drugs, new challenges in cystic fibrosis care. *European Respiratory Review.* 2024;33(173):240045. Doi:10.1183/16000617.0045-2024.
70. Donaldson SH, Corcoran TE, Pilewski JM, Laube BL, Mogayzel P, Ceppe A, et al. The effect of discontinuing hypertonic saline or dornase alfa on mucociliary clearance in elxacaftor/tezacaftor/ivacaftor treated people with cystic fibrosis: The SIMPLIFY-MCC Study. *Journal of Cystic Fibrosis.* 2024;23(3):457-60. Doi:10.1016/j.jcf.2024.02.003.

71. Krantz CL, Anders. Fifth semiannual report - Kafrtio in Sweden <https://www.cf-registret.se/publikationer>: Swedish CF registry; 2025.
72. Fiedorczuk K, Chen J. Molecular structures reveal synergistic rescue of $\Delta 508$ CFTR by Trikafta modulators. *Science*. 2022;378(6617):284-90. Doi:10.1126/science.ade2216.
73. Van Goor F, Hadida S, Grootenhuis PD, Burton B, Stack JH, Straley KS, et al. Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proc Natl Acad Sci U S A*. 2011;108(46):18843-8. Doi:10.1073/pnas.1105787108.
74. Eckford PD, Li C, Ramjeesingh M, Bear CE. Cystic fibrosis transmembrane conductance regulator (CFTR) potentiator VX-770 (ivacaftor) opens the defective channel gate of mutant CFTR in a phosphorylation-dependent but ATP-independent manner. *J Biol Chem*. 2012;287(44):36639-49. Doi:10.1074/jbc.M112.393637.
75. Laselva O, Bartlett C, Gunawardena TNA, Ouyang H, Eckford PDW, Moraes TJ, et al. Rescue of multiple class II CFTR mutations by elexacaftor+tezacaftor+ivacaftor mediated in part by the dual activities of elexacaftor as both corrector and potentiator. *Eur Respir J*. 2021;57(6) Doi:10.1183/13993003.02774-2020.
76. Mall MA, Brugha R, Gartner S, Legg J, Moeller A, Mondejar-Lopez P, et al. Efficacy and Safety of Elexacaftor/Tezacaftor/Ivacaftor in Children 6 Through 11 Years of Age with Cystic Fibrosis Heterozygous for F508del and a Minimal Function Mutation: A Phase 3b, Randomized, Placebo-controlled Study. *Am J Respir Crit Care Med*. 2022;206(11):1361-9. Doi:10.1164/rccm.202202-0392OC.
77. Sutharsan S, Dillenhoefer S, Welsner M, Stehling F, Brinkmann F, Burkhart M, et al. Impact of elexacaftor/tezacaftor/ivacaftor on lung function, nutritional status, pulmonary exacerbation frequency and sweat chloride in people with cystic fibrosis: real-world evidence from the German CF Registry. *Lancet Reg Health Eur*. 2023;32:100690. Doi:10.1016/j.lanepe.2023.100690.
78. Bower JK, Volkova N, Ahluwalia N, Sahota G, Xuan F, Chin A, et al. Real-world safety and effectiveness of elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis: Interim results of a long-term registry-based study. *Journal of Cystic Fibrosis*. 2023;22(4):730-7. Doi:<https://doi.org/10.1016/j.jcf.2023.03.002>.
79. Daines CL, Tullis E, Costa S, Linnemann RW, Mall MA, McKone EF, et al. Long-term safety and efficacy of elexacaftor/tezacaftor/ivacaftor in people with cystic fibrosis and at least one F508del allele: 144-week interim results from a 192-week open-label extension study. *Eur Respir J*. 2023;62(6) Doi:10.1183/13993003.02029-2022.
80. Graeber SY, Vitzthum C, Pallenberg ST, Naehrlich L, Stahl M, Rohrbach A, et al. Effects of Elexacaftor/Tezacaftor/Ivacaftor Therapy on CFTR Function in Patients with Cystic Fibrosis and One or Two F508del Alleles. *Am J Respir Crit Care Med*. 2022;205(5):540-9. Doi:10.1164/rccm.202110-2249OC.
81. Taylor-Cousar JL, Robinson PD, Shteinberg M, Downey DG. CFTR modulator therapy: transforming the landscape of clinical care in cystic fibrosis. *The Lancet*. 2023;402(10408):1171-84. Doi:10.1016/S0140-6736(23)01609-4.

82. Scully KJ, Marchetti P, Sawicki GS, Uluer A, Cernadas M, Cagnina RE, et al. The effect of elexacaftor/tezacaftor/ivacaftor (ETI) on glycemia in adults with cystic fibrosis. *Journal of Cystic Fibrosis*. 2022;21(2):258-63. Doi:<https://doi.org/10.1016/j.jcf.2021.09.001>.
83. Dana J, Debray D, Beaufrère A, Hillaire S, Fabre M, Reinhold C, et al. Cystic fibrosis-related liver disease: Clinical presentations, diagnostic and monitoring approaches in the era of CFTR modulator therapies. *Journal of Hepatology*. 2022;76(2):420-34. Doi:10.1016/j.jhep.2021.09.042.
84. Middleton PG, Taylor-Cousar JL. Reflections 5 years on from the approval of ETI therapy. *The Lancet Respiratory Medicine*. 2024;12(10):752-4. Doi:10.1016/S2213-2600(24)00259-5.
85. Taylor-Cousar JL, Jain R. Maternal and fetal outcomes following elexacaftor-tezacaftor-ivacaftor use during pregnancy and lactation. *J Cyst Fibros*. 2021;20(3):402-6. Doi:10.1016/j.jcf.2021.03.006.
86. Nichols D, Chmiel J, Berger M. Chronic inflammation in the cystic fibrosis lung: alterations in inter- and intracellular signaling. *Clin Rev Allergy Immunol*. 2008;34(2):146-62. Doi:10.1007/s12016-007-8039-9.
87. Berger M. Inflammation in the lung in cystic fibrosis. A vicious cycle that does more harm than good? *Clin Rev Allergy*. 1991;9(1-2):119-42. Doi:10.1007/978-1-4612-0475-6_8.
88. Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. *N Engl J Med*. 2015;372(4):351-62. Doi:10.1056/NEJMra1300109.
89. Mall M, Grubb BR, Harkema JR, O'Neal WK, Boucher RC. Increased airway epithelial Na⁺ absorption produces cystic fibrosis-like lung disease in mice. *Nat Med*. 2004;10(5):487-93. Doi:10.1038/nm1028.
90. Haq IJ, Gray MA, Garnett JP, Ward C, Brodrie M. Airway surface liquid homeostasis in cystic fibrosis: pathophysiology and therapeutic targets. *Thorax*. 2016;71(3):284-7. Doi:10.1136/thoraxjnl-2015-207588.
91. Mall MA, Burgel P-R, Castellani C, Davies JC, Salathe M, Taylor-Cousar JL. Cystic fibrosis. *Nature Reviews Disease Primers*. 2024;10(1):53. Doi:10.1038/s41572-024-00538-6.
92. Balázs A, Mall MA. Role of the SLC26A9 Chloride Channel as Disease Modifier and Potential Therapeutic Target in Cystic Fibrosis. *Front Pharmacol*. 2018;9:1112. Doi:10.3389/fphar.2018.01112.
93. Matsui H, Randell SH, Peretti SW, Davis CW, Boucher RC. Coordinated clearance of periciliary liquid and mucus from airway surfaces. *The Journal of Clinical Investigation*. 1998;102(6):1125-31. Doi:10.1172/JCI2687.
94. Matsui H, Grubb BR, Tarran R, Randell SH, Gatzky JT, Davis CW, Boucher RC. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. *Cell*. 1998;95(7):1005-15. Doi:10.1016/S0092-8674(00)81724-9.
95. Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, et al. Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest*. 2002;109(3):317-25. Doi:10.1172/jci13870.

96. Boucher RC. New concepts of the pathogenesis of cystic fibrosis lung disease. *European Respiratory Journal*.23(1):146-58. Doi:10.1183/09031936.03.00057003.
97. Kim D, Liao J, Hanrahan JW. The buffer capacity of airway epithelial secretions. *Front Physiol*. 2014;5:188. Doi:10.3389/fphys.2014.00188.
98. Borowitz D. CFTR, bicarbonate, and the pathophysiology of cystic fibrosis. *Pediatr Pulmonol*. 2015;50 Suppl 40:S24-s30. Doi:10.1002/ppul.23247.
99. Shah VS, Meyerholz DK, Tang XX, Reznikov L, Abou Alaiwa M, Ernst SE, et al. Airway acidification initiates host defense abnormalities in cystic fibrosis mice. *Science (New York, NY)*. 2016;351(6272):503-7. Doi:10.1126/science.aad5589.
100. Simonin J, Bille E, Crambert G, Noel S, Dreano E, Edwards A, et al. Airway surface liquid acidification initiates host defense abnormalities in Cystic Fibrosis. *Scientific Reports*. 2019;9(1):6516. Doi:10.1038/s41598-019-42751-4.
101. Pezzulo AA, Tang XX, Hoegger MJ, Abou Alaiwa MH, Ramachandran S, Moninger TO, et al. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. *Nature [Internet]*. 2012 2012/07//; 487(7405):[109-13 pp.]. Available from: <http://europepmc.org/abstract/MED/22763554>
102. Quinton PM. Role of epithelial HCO₃⁻ transport in mucin secretion: lessons from cystic fibrosis. *Am J Physiol Cell Physiol*. 2010;299(6):C1222-33. Doi:10.1152/ajpcell.00362.2010.
103. Rosen BH, Evans TIA, Moll SR, Gray JS, Liang B, Sun X, et al. Infection Is Not Required for Mucoinflammatory Lung Disease in CFTR-Knockout Ferrets. *American Journal of Respiratory and Critical Care Medicine*. 2018;197(10):1308-18. Doi:10.1164/rccm.201708-1616OC.
104. Fritzscheing B, Zhou-Suckow Z, Trojanek JB, Schubert SC, Schatterny J, Hirtz S, et al. Hypoxic Epithelial Necrosis Triggers Neutrophilic Inflammation via IL-1 Receptor Signaling in Cystic Fibrosis Lung Disease. *American Journal of Respiratory and Critical Care Medicine*. 2015;191(8):902-13. Doi:10.1164/rccm.201409-1610OC.
105. Sly PD, Brennan S, Gangell C, de Klerk N, Murray C, Mott L, et al. Lung disease at diagnosis in infants with cystic fibrosis detected by newborn screening. *Am J Respir Crit Care Med*. 2009;180(2):146-52. Doi:10.1164/rccm.200901-0069OC.
106. Stahl M, Steinke E, Graeber SY, Joachim C, Seitz C, Kauczor H-U, et al. Magnetic Resonance Imaging Detects Progression of Lung Disease and Impact of Newborn Screening in Preschool Children with Cystic Fibrosis. *American Journal of Respiratory and Critical Care Medicine*. 2021;204(8):943-53. Doi:10.1164/rccm.202102-0278OC.
107. Balázs A, Mall MA. Mucus obstruction and inflammation in early cystic fibrosis lung disease: Emerging role of the IL-1 signaling pathway. *Pediatric Pulmonology*. 2019;54(S3):S5-S12. Doi:<https://doi.org/10.1002/ppul.24462>.
108. Montgomery ST, Dittrich AS, Garratt LW, Turkovic L, Frey DL, Stick SM, et al. Interleukin-1 is associated with inflammation and structural lung disease in young children with cystic fibrosis. *Journal of Cystic Fibrosis*. 2018;17(6):715-22. Doi:10.1016/j.jcf.2018.05.006.

109. Rosenfeld M, Gibson RL, McNamara S, Emerson J, Burns JL, Castile R, et al. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. *Pediatr Pulmonol*. 2001;32(5):356-66. Doi:10.1002/ppul.1144.
110. Sadikot RT, Han W, Everhart MB, Zoia O, Peebles RS, Jansen ED, et al. Selective I kappa B kinase expression in airway epithelium generates neutrophilic lung inflammation. *J Immunol*. 2003;170(2):1091-8. Doi:10.4049/jimmunol.170.2.1091.
111. Montgomery ST, Mall MA, Kicic A, Stick SM. Hypoxia and sterile inflammation in cystic fibrosis airways: mechanisms and potential therapies. *European Respiratory Journal*. 2017;49(1):1600903. Doi:10.1183/13993003.00903-2016.
112. Bodas M, Vij N. The NF-kappaB signaling in cystic fibrosis lung disease: pathophysiology and therapeutic potential. *Discov Med*. 2010;9(47):346-56.
113. Nichols DP, Chmiel JF. Inflammation and its genesis in cystic fibrosis. *Pediatric Pulmonology*. 2015;50(S40):S39-S56. Doi:https://doi.org/10.1002/ppul.23242.
114. Painter RG, Marrero L, Lombard GA, Valentine VG, Nauseef WM, Wang G. CFTR-mediated halide transport in phagosomes of human neutrophils. *J Leukoc Biol*. 2010;87(5):933-42. Doi:10.1189/jlb.1009655.
115. Law SM, Hardisty G, Gillan JL, Robinson NJ, Davidson DJ, Whyte MKB, et al. Neutrophil extracellular traps are associated with airways inflammation and increased severity of lung disease in cystic fibrosis. *ERJ Open Res*. 2024;10(6) Doi:10.1183/23120541.00312-2024.
116. Gehrig S, Duerr J, Weitnauer M, Wagner CJ, Graeber SY, Schatterny J, et al. Lack of neutrophil elastase reduces inflammation, mucus hypersecretion, and emphysema, but not mucus obstruction, in mice with cystic fibrosis-like lung disease. *Am J Respir Crit Care Med*. 2014;189(9):1082-92. Doi:10.1164/rccm.201311-1932OC.
117. Sly PD, Gangell CL, Chen L, Ware RS, Ranganathan S, Mott LS, et al. Risk Factors for Bronchiectasis in Children with Cystic Fibrosis. *New England Journal of Medicine*. 2013;368(21):1963-70. Doi:doi:10.1056/NEJMoa1301725.
118. Walsh DE, Greene CM, Carroll TP, Taggart CC, Gallagher PM, O'Neill SJ, McElvaney NG. Interleukin-8 Up-regulation by Neutrophil Elastase Is Mediated by MyD88/IRAK/TRAF-6 in Human Bronchial Epithelium. *Journal of Biological Chemistry*. 2001;276(38):35494-9. Doi:https://doi.org/10.1074/jbc.M103543200.
119. Van den Bossche S, De Broe E, Coenye T, Van Braeckel E, Crabbé A. The cystic fibrosis lung microenvironment alters antibiotic activity: causes and effects. *European Respiratory Review*. 2021;30(161):210055. Doi:10.1183/16000617.0055-2021.
120. Di A, Brown ME, Deriy LV, Li C, Szeto FL, Chen Y, et al. CFTR regulates phagosome acidification in macrophages and alters bactericidal activity. *Nat Cell Biol*. 2006;8(9):933-44. Doi:10.1038/ncb1456.
121. Badr A, Eltobgy M, Krause K, Hamilton K, Estfanous S, Daily KP, et al. CFTR Modulators Restore Acidification of Autophago-Lysosomes and Bacterial Clearance in Cystic Fibrosis Macrophages. *Frontiers in Cellular and Infection Microbiology*. 2022;Volume 12 - 2022 Doi:10.3389/fcimb.2022.819554.

122. Lévêque M, Le Trionnaire S, Del Porto P, Martin-Chouly C. The impact of impaired macrophage functions in cystic fibrosis disease progression. *Journal of Cystic Fibrosis*. 2017;16(4):443-53. Doi:<https://doi.org/10.1016/j.jcf.2016.10.011>.
123. Bruscia EM, Zhang P-X, Satoh A, Caputo C, Medzhitov R, Shenoy A, et al. Abnormal Trafficking and Degradation of TLR4 Underlie the Elevated Inflammatory Response in Cystic Fibrosis. *The Journal of Immunology*. 2011;186(12):6990-8. Doi:10.4049/jimmunol.1100396.
124. Berg M, Krabbendam L, van der Ploeg EK, van Nimwegen M, van der Veer T, Banchero M, et al. Evidence for altered immune-structural cell crosstalk in cystic fibrosis revealed by single cell transcriptomics. *Journal of Cystic Fibrosis*. 2025;24(5):849-60. Doi:10.1016/j.jcf.2025.01.016.
125. Hector A, Schäfer H, Pöschel S, Fischer A, Fritzsche B, Ralhan A, et al. Regulatory T-cell impairment in cystic fibrosis patients with chronic pseudomonas infection. *Am J Respir Crit Care Med*. 2015;191(8):914-23. Doi:10.1164/rccm.201407-1381OC.
126. Polverino F, Lu B, Quintero JR, Vargas SO, Patel AS, Owen CA, et al. CFTR regulates B cell activation and lymphoid follicle development. *Respiratory Research*. 2019;20(1):133. Doi:10.1186/s12931-019-1103-1.
127. Özdemir A, Ersoy M. Platelets: A Neglected Cell in Cystic Fibrosis Lung Inflammation. *The Journal of Pediatric Research*. 2024
128. Falco A, Romano M, Iapichino L, Collura M, Davì G. Increased soluble CD40 ligand levels in cystic fibrosis. *Journal of Thrombosis and Haemostasis*. 2004;2(4):557-60. Doi:10.1111/j.1538-7836.2004.00683.x.
129. Ortiz-Muñoz G, Yu MA, Lefrançois E, Mallavia B, Valet C, Tian JJ, et al. Cystic fibrosis transmembrane conductance regulator dysfunction in platelets drives lung hyperinflammation. *The Journal of Clinical Investigation*. 2020;130(4):2041-53. Doi:10.1172/JCI129635.
130. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest*. 2012;122(7):2661-71. Doi:10.1172/jci61303.
131. Osika E, Cavaillon JM, Chadelat K, Boule M, Fitting C, Tournier G, Clement A. Distinct sputum cytokine profiles in cystic fibrosis and other chronic inflammatory airway disease. *European Respiratory Journal*. 1999;14(2):339-46. Doi:10.1034/j.1399-3003.1999.14b17.x.
132. Courtney JM, Ennis M, Elborn JS. Cytokines and inflammatory mediators in cystic fibrosis. *Journal of Cystic Fibrosis*. 2004;3(4):223-31. Doi:10.1016/j.jcf.2004.06.006.
133. Fantone KM, Gokanapudi N, Rada B. Neutrophil extracellular traps and interleukin-1 β in cystic fibrosis lung disease. *Frontiers in Immunology*. 2025;Volume 16 - 2025 Doi:10.3389/fimmu.2025.1595994.
134. Malaviya R, Laskin JD, Laskin DL. Anti-TNF α therapy in inflammatory lung diseases. *Pharmacology & Therapeutics*. 2017;180:90-8. Doi:<https://doi.org/10.1016/j.pharmthera.2017.06.008>.

135. Tanaka T, Narazaki M, Kishimoto T. IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harbor Perspectives in Biology. 2014;6(10):a016295-a. Doi:10.1101/cshperspect.a016295.
136. Hagner M, Albrecht M, Guerra M, Braubach P, Halle O, Zhou-Suckow Z, et al. IL-17A from innate and adaptive lymphocytes contributes to inflammation and damage in cystic fibrosis lung disease. *Eur Respir J*. 2021;57(6) Doi:10.1183/13993003.00716-2019.
137. Jundi K, Greene CM. Transcription of Interleukin-8: How Altered Regulation Can Affect Cystic Fibrosis Lung Disease. *Biomolecules*. 2015;5(3):1386-98.
138. Zeng W, Song Y, Wang R, He R, Wang T. Neutrophil elastase: From mechanisms to therapeutic potential. *Journal of Pharmaceutical Analysis*. 2023;13(4):355-66. Doi:<https://doi.org/10.1016/j.jpha.2022.12.003>.
139. Nakamura H, Yoshimura K, McElvaney NG, Crystal RG. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *The Journal of Clinical Investigation*. 1992;89(5):1478-84. Doi:10.1172/JCI115738.
140. Shao MXG, Nadel JA. Neutrophil Elastase Induces MUC5AC Mucin Production in Human Airway Epithelial Cells via a Cascade Involving Protein Kinase C, Reactive Oxygen Species, and TNF- α -Converting Enzyme1. *The Journal of Immunology*. 2005;175(6):4009-16. Doi:10.4049/jimmunol.175.6.4009.
141. Wu BG, Segal LN. Lung Microbiota and Its Impact on the Mucosal Immune Phenotype. *Microbiol Spectr*. 2017;5(3) Doi:10.1128/microbiolspec.BAD-0005-2016.
142. Man WH, de Steenhuijsen Piters WA, Bogaert D. The microbiota of the respiratory tract: gatekeeper to respiratory health. *Nat Rev Microbiol*. 2017;15(5):259-70. Doi:10.1038/nrmicro.2017.14.
143. Segal LN, Clemente JC, Tsay JC, Koralov SB, Keller BC, Wu BG, et al. Enrichment of the lung microbiome with oral taxa is associated with lung inflammation of a Th17 phenotype. *Nat Microbiol*. 2016;1:16031. Doi:10.1038/nmicrobiol.2016.31.
144. Odendaal M-L, de Steenhuijsen Piters WAA, Franz E, Chu MLJN, Groot JA, van Logchem EM, et al. Host and environmental factors shape upper airway microbiota and respiratory health across the human lifespan. *Cell*. 2024;187(17):4571-85.e15. Doi:10.1016/j.cell.2024.07.008.
145. Li R, Li J, Zhou X. Lung microbiome: new insights into the pathogenesis of respiratory diseases. *Signal Transduction and Targeted Therapy*. 2024;9(1):19. Doi:10.1038/s41392-023-01722-y.
146. Zhao L, Luo J-L, Ali MK, Spiekerkoetter E, Nicolls MR. The Human Respiratory Microbiome: Current Understandings and Future Directions. *American Journal of Respiratory Cell and Molecular Biology*. 2023;68(3):245-55. Doi:10.1165/remb.2022-0208TR.
147. Zemanick ET, Wagner BD, Robertson CE, Ahrens RC, Chmiel JF, Clancy JP, et al. Airway microbiota across age and disease spectrum in cystic fibrosis. *Eur Respir J*. 2017;50(5) Doi:10.1183/13993003.00832-2017.

148. Cuthbertson L, Walker AW, Oliver AE, Rogers GB, Rivett DW, Hampton TH, et al. Lung function and microbiota diversity in cystic fibrosis. *Microbiome*. 2020;8(1):45. Doi:10.1186/s40168-020-00810-3.
149. Paganin P, Fiscarelli EV, Tuccio V, Chianciani M, Bacci G, Morelli P, et al. Changes in cystic fibrosis airway microbial community associated with a severe decline in lung function. *PLoS One*. 2015;10(4):e0124348. Doi:10.1371/journal.pone.0124348.
150. Hampton TH, Thomas D, Gast Cvd, O'Toole GA, Stanton BA. Mild Cystic Fibrosis Lung Disease Is Associated with Bacterial Community Stability. *Microbiology Spectrum*. 2021;9(1):10.1128/spectrum.00029-21. Doi:doi:10.1128/spectrum.00029-21.
151. Orenti A ZA, Jung A, van Rens J et al. ECFSPR Annual Report 2021. <https://www.ecfs.eu/projects/ecfs-patient-registry/annual-reports; 2021>.
152. Moran A, Dunitz J, Nathan B, Saeed A, Holme B, Thomas W. Cystic Fibrosis–Related Diabetes: Current Trends in Prevalence, Incidence, and Mortality. *Diabetes Care*. 2009;32(9):1626-31. Doi:10.2337/dc09-0586.
153. Chamnan P, Shine BS, Haworth CS, Bilton D, Adler AI. Diabetes as a determinant of mortality in cystic fibrosis. *Diabetes Care*. 2010;33(2):311-6. Doi:10.2337/dc09-1215.
154. Koch C, Rainisio M, Madessani U, Harms HK, Hodson ME, Mastella G, et al. Presence of cystic fibrosis-related diabetes mellitus is tightly linked to poor lung function in patients with cystic fibrosis: Data from the European Epidemiologic Registry of Cystic Fibrosis. *Pediatric Pulmonology*. 2001;32(5):343-50. Doi:10.1002/ppul.1142.
155. Lanng S, Thorsteinsson B, Nerup J, Koch C. Influence of the development of diabetes mellitus on clinical status in patients with cystic fibrosis. *European Journal of Pediatrics*. 1992;151(9):684-7. Doi:10.1007/BF01957574.
156. Hameed S, Morton JR, Jaffé A, Field PI, Belessis Y, Yoong T, et al. Early glucose abnormalities in cystic fibrosis are preceded by poor weight gain. *Diabetes Care*. 2010;33(2):221-6. Doi:10.2337/dc09-1492.
157. Marshall BC, Butler SM, Stoddard M, Moran AM, Liou TG, Morgan WJ. Epidemiology of cystic fibrosis-related diabetes. *J Pediatr*. 2005;146(5):681-7. Doi:10.1016/j.jpeds.2004.12.039.
158. Pincikova T, Nilsson K, Moen IE, Fluge G, Hollsing A, Knudsen PK, et al. Vitamin D deficiency as a risk factor for cystic fibrosis-related diabetes in the Scandinavian Cystic Fibrosis Nutritional Study. *Diabetologia*. 2011;54(12):3007-15. Doi:10.1007/s00125-011-2287-1.
159. Peng Y, Wu M, Alvarez JA, Tangpricha V. Vitamin D Status and Risk of Cystic Fibrosis-Related Diabetes: A Retrospective Single Center Cohort Study. *Nutrients*. 2021;13(11):4048.
160. Perrem L, Stanojevic S, Solomon M, Carpenter S, Ratjen F. Incidence and risk factors of paediatric cystic fibrosis-related diabetes. *Journal of Cystic Fibrosis*. 2019;18(6):874-8. Doi:10.1016/j.jcf.2019.04.015.

161. Konrad K, Scheuing N, Badenhoop K, Borkenstein MH, Gohlke B, Schöfl C, et al. Cystic fibrosis-related diabetes compared with type 1 and type 2 diabetes in adults. *Diabetes Metab Res Rev*. 2013;29(7):568-75. Doi:10.1002/dmrr.2429.
162. Cystic Fibrosis Foundation Patient Registry 2024 Annual Data Report Bethesda, Maryland; 2024.
163. Moran A, Becker D, Casella SJ, Gottlieb PA, Kirkman MS, Marshall BC, Slovis B. Epidemiology, pathophysiology, and prognostic implications of cystic fibrosis-related diabetes: a technical review. *Diabetes Care*. 2010;33(12):2677-83. Doi:10.2337/dc10-1279.
164. Schwarzenberg SJ, Thomas W, Olsen TW, Grover T, Walk D, Milla C, Moran A. Microvascular complications in cystic fibrosis-related diabetes. *Diabetes Care*. 2007;30(5):1056-61. Doi:10.2337/dc06-1576.
165. Milla CE, Warwick WJ, Moran A. Trends in Pulmonary Function in Patients with Cystic Fibrosis Correlate with the Degree of Glucose Intolerance at Baseline. *American Journal of Respiratory and Critical Care Medicine*. 2000;162(3):891-5. Doi:10.1164/ajrcm.162.3.9904075.
166. Schaedel C, de Monestrol I, Hjelte L, Johannesson M, Kornfält R, Lindblad A, et al. Predictors of deterioration of lung function in cystic fibrosis. *Pediatric Pulmonology*. 2002;33(6):483-91. Doi:https://doi.org/10.1002/ppul.10100.
167. Granados A, Chan CL, Ode KL, Moheet A, Moran A, Holl R. Cystic fibrosis related diabetes: Pathophysiology, screening and diagnosis. *Journal of Cystic Fibrosis*. 2019;18:S3-S9. Doi:https://doi.org/10.1016/j.jcf.2019.08.016.
168. Colombo C, Alicandro G, Gambazza S, Mileto P, Mari A, Grespan E, et al. Ventilation inhomogeneity is associated with OGTT-derived insulin secretory defects in cystic fibrosis. *Pediatric Pulmonology*. 2019;54(2):141-9. Doi:https://doi.org/10.1002/ppul.24212.
169. Leclercq A, Gauthier B, Rosner V, Weiss L, Moreau F, Constantinescu AA, et al. Early assessment of glucose abnormalities during continuous glucose monitoring associated with lung function impairment in cystic fibrosis patients. *J Cyst Fibros*. 2014;13(4):478-84. Doi:10.1016/j.jcf.2013.11.005.
170. Elidottir H, Diemer S, Eklund E, Hansen CR. Abnormal glucose tolerance and lung function in children with cystic fibrosis. Comparing oral glucose tolerance test and continuous glucose monitoring. *J Cyst Fibros*. 2021;20(5):779-84. Doi:10.1016/j.jcf.2021.01.002.
171. Widger J, Ranganathan S, Robinson PJ. Progression of structural lung disease on CT scans in children with cystic fibrosis related diabetes. *J Cyst Fibros*. 2013;12(3):216-21. Doi:10.1016/j.jcf.2012.09.005.
172. Coderre L, Debieche L, Plourde J, Rabasa-Lhoret R, Lesage S. The Potential Causes of Cystic Fibrosis-Related Diabetes. *Front Endocrinol (Lausanne)*. 2021;12:702823. Doi:10.3389/fendo.2021.702823.
173. Prentice BJ, Jaffe A, Hameed S, Verge CF, Waters S, Widger J. Cystic fibrosis-related diabetes and lung disease: an update. *European Respiratory Review*. 2021;30(159):200293. Doi:10.1183/16000617.0293-2020.

174. Hunt WR, Zughaier SM, Guentert DE, Shenep MA, Koval M, McCarty NA, Hansen JM. Hyperglycemia impedes lung bacterial clearance in a murine model of cystic fibrosis-related diabetes. *Am J Physiol Lung Cell Mol Physiol*. 2014;306(1):L43-9. Doi:10.1152/ajplung.00224.2013.
175. Baker EH, Clark N, Brennan AL, Fisher DA, Gyi KM, Hodson ME, et al. Hyperglycemia and cystic fibrosis alter respiratory fluid glucose concentrations estimated by breath condensate analysis. *Journal of Applied Physiology*. 2007;102(5):1969-75. Doi:10.1152/japplphysiol.01425.2006.
176. Vazquez Cegla AJ, Jones KT, Cui G, Cottrill KA, Koval M, McCarty NA. Effects of hyperglycemia on airway epithelial barrier function in WT and CF 16HBE cells. *Sci Rep*. 2024;14(1):25095. Doi:10.1038/s41598-024-76526-3.
177. Garnett JP, Gray MA, Tarran R, Brodrie M, Ward C, Baker EH, Baines DL. Elevated Paracellular Glucose Flux across Cystic Fibrosis Airway Epithelial Monolayers Is an Important Factor for *Pseudomonas aeruginosa* Growth. *PLoS ONE*. 2013;8(10):e76283. Doi:10.1371/journal.pone.0076283.
178. Joshi MB, Lad A, Bharath Prasad AS, Balakrishnan A, Ramachandra L, Satyamoorthy K. High glucose modulates IL-6 mediated immune homeostasis through impeding neutrophil extracellular trap formation. *FEBS Lett*. 2013;587(14):2241-6. Doi:10.1016/j.febslet.2013.05.053.
179. Prentice BJ, Ooi CY, Strachan RE, Hameed S, Ebrahimkhani S, Waters SA, et al. Early glucose abnormalities are associated with pulmonary inflammation in young children with cystic fibrosis. *J Cyst Fibros*. 2019;18(6):869-73. Doi:10.1016/j.jcf.2019.03.010.
180. Terliesner N, Vogel M, Steighardt A, Gausche R, Henn C, Hentschel J, et al. Cystic-fibrosis related-diabetes (CFRD) is preceded by and associated with growth failure and deteriorating lung function. *J Pediatr Endocrinol Metab*. 2017;30(8):815-21. Doi:10.1515/jpem-2017-0005.
181. Hsia CC, Raskin P. The diabetic lung: relevance of alveolar microangiopathy for the use of inhaled insulin. *Am J Med*. 2005;118(3):205-11. Doi:10.1016/j.amjmed.2004.09.019.
182. Aksit MA, Pace RG, Vecchio-Pagán B, Ling H, Rommens JM, Boelle P-Y, et al. Genetic Modifiers of Cystic Fibrosis-Related Diabetes Have Extensive Overlap With Type 2 Diabetes and Related Traits. *The Journal of Clinical Endocrinology & Metabolism*. 2020;105(5):1401-15. Doi:10.1210/clinem/dgz102.
183. Moran A, Brunzell C, Cohen RC, Katz M, Marshall BC, Onady G, et al. Clinical care guidelines for cystic fibrosis-related diabetes: a position statement of the American Diabetes Association and a clinical practice guideline of the Cystic Fibrosis Foundation, endorsed by the Pediatric Endocrine Society. *Diabetes Care*. 2010;33(12):2697-708. Doi:10.2337/dc10-1768.
184. Castellani C, Duff AJA, Bell SC, Heijerman HGM, Munck A, Ratjen F, et al. ECFS best practice guidelines: the 2018 revision. *J Cyst Fibros*. 2018;17(2):153-78. Doi:10.1016/j.jcf.2018.02.006.

185. Noronha RM, Damaceno N, Muramatu LH, Monte O, Calliari LEP. Importance of screening with oral glucose tolerance test for early diagnosis of cystic fibrosis-related diabetes mellitus. *Pediatric Diabetes*. 2014;15(4):309-12. Doi:<https://doi.org/10.1111/pedi.12094>.
186. Brodsky J, Dougherty S, Makani R, Rubenstein RC, Kelly A. Elevation of 1-hour plasma glucose during oral glucose tolerance testing is associated with worse pulmonary function in cystic fibrosis. *Diabetes Care*. 2011;34(2):292-5. Doi:10.2337/dc10-1604.
187. Prentice BJ, Chelliah A, Ooi CY, Hameed S, Verge CF, Plush L, Widger J. Peak OGTT glucose is associated with lower lung function in young children with cystic fibrosis. *J Cyst Fibros*. 2020;19(2):305-9. Doi:10.1016/j.jcf.2019.05.005.
188. Prentice B, Hameed S, Verge CF, Ooi CY, Jaffe A, Widger J. Diagnosing cystic fibrosis-related diabetes: current methods and challenges. *Expert Rev Respir Med*. 2016;10(7):799-811. Doi:10.1080/17476348.2016.1190646.
189. Hardin DS, Grilley K, Baron B, Hale KA. Accelerated Red Blood Cell Turnover Can Invalidate the Use of Hemoglobin A1c as a Diagnostic Test for Cystic Fibrosis Related Diabetes. *Pediatric Research*. 1999;45(7):90-. Doi:10.1203/00006450-199904020-00537.
190. Scully KJ, Brenner L, Martin K, Ruazol M, Sawicki GS, Uluer A, et al. Continuous glucose monitoring and advanced glycation endproducts for prediction of clinical outcomes and development of cystic fibrosis-related diabetes in adults with CF. *Front Endocrinol (Lausanne)*. 2024;15:1293709. Doi:10.3389/fendo.2024.1293709.
191. Couce M, O'Brien TD, Moran A, Roche PC, Butler PC. Diabetes mellitus in cystic fibrosis is characterized by islet amyloidosis. *J Clin Endocrinol Metab*. 1996;81(3):1267-72. Doi:10.1210/jcem.81.3.8772610.
192. Moheet A, Moran A. New Concepts in the Pathogenesis of Cystic Fibrosis–Related Diabetes. *The Journal of Clinical Endocrinology & Metabolism*. 2022;107(6):1503-9. Doi:10.1210/clinem/dgac020.
193. Edlund A, Barghouth M, Hühn M, Abels M, Esguerra JSE, Mollet IG, et al. Defective exocytosis and processing of insulin in a cystic fibrosis mouse model. *J Endocrinol*. 2019;241(1):45-57. Doi:10.1530/joe-18-0570.
194. Ntimbane T, Mailhot G, Spahis S, Rabasa-Lhoret R, Kleme M-L, Melloul D, et al. CFTR silencing in pancreatic β -cells reveals a functional impact on glucose-stimulated insulin secretion and oxidative stress response. *American Journal of Physiology-Endocrinology and Metabolism*. 2016;310(3):E200-E12. Doi:10.1152/ajpendo.00333.2015.
195. Hillman M, Eriksson L, Mared L, Helgesson K, Landin-Olsson M. Reduced levels of active GLP-1 in patients with cystic fibrosis with and without diabetes mellitus. *J Cyst Fibros*. 2012;11(2):144-9. Doi:10.1016/j.jcf.2011.11.001.
196. Blackman SM, Hsu S, Ritter SE, Naughton KM, Wright FA, Drumm ML, et al. A susceptibility gene for type 2 diabetes confers substantial risk for diabetes complicating cystic fibrosis. *Diabetologia*. 2009;52(9):1858-65. Doi:10.1007/s00125-009-1436-2.

197. Ode KL, Ballman M, Battezzati A, Brennan A, Chan CL, Hameed S, et al. ISPAD Clinical Practice Consensus Guidelines 2022: Management of cystic fibrosis-related diabetes in children and adolescents. *Pediatric Diabetes*. 2022;23(8):1212-28. Doi:<https://doi.org/10.1111/pedi.13453>.
198. Moran A, Pekow P, Grover P, Zorn M, Slovis B, Pilewski J, et al. Insulin Therapy to Improve BMI in Cystic Fibrosis–Related Diabetes Without Fasting Hyperglycemia: Results of the Cystic Fibrosis Related Diabetes Therapy Trial. *Diabetes Care*. 2009;32(10):1783-8. Doi:10.2337/dc09-0585.
199. Ballmann M, Hubert D, Assael BM, Staab D, Hebestreit A, Naehrlich L, et al. Repaglinide versus insulin for newly diagnosed diabetes in patients with cystic fibrosis: a multicentre, open-label, randomised trial. *The Lancet Diabetes & Endocrinology*. 2018;6(2):114-21. Doi:10.1016/S2213-8587(17)30400-X.
200. Vuralli D. From Pathophysiology to Treatment: Contemporary Approaches to CFRD in the Pediatric and Adolescent Population. *Pediatr Diabetes*. 2026;2026:5539725. Doi:10.1155/pedi/5539725.
201. Kelly A, De Leon DD, Sheikh S, Camburn D, Kubrak C, Peleckis AJ, et al. Islet Hormone and Incretin Secretion in Cystic Fibrosis after Four Months of Ivacaftor Therapy. *American Journal of Respiratory and Critical Care Medicine*. 2019;199(3):342-51. Doi:10.1164/rccm.201806-1018OC.
202. Nielsen BU, Olsen MF, Mabuza Mathiesen IH, Pressler T, Ritz C, Katzenstein TL, et al. Decline in HbA1c during the first year of elexacaftor/tezacaftor/ivacaftor treatment in the Danish cystic fibrosis cohort: Short title: Decline in HbA1c after elexacaftor/tezacaftor/ivacaftor treatment. *Journal of Cystic Fibrosis*. 2024;23(1):103-8. Doi:10.1016/j.jcf.2023.11.005.
203. Norris AW. Is Cystic Fibrosis–related Diabetes Reversible? New Data on CFTR Potentiation and Insulin Secretion. *American Journal of Respiratory and Critical Care Medicine*. 2019;199(3):261-3. Doi:10.1164/rccm.201808-1501ED.
204. Amini M, Yu K, Liebich J, Ahir V, Wood E, Albert S, Dhindsa S. The Changing Landscape of Treatment for Cystic Fibrosis Related Diabetes. *J Clin Transl Endocrinol*. 2024;35:100332. Doi:10.1016/j.jcte.2024.100332.
205. Lurquin F, Gohy S, Hermans MP, Preumont V. Combined CFTR modulator therapies are linked with anabolic benefits and insulin-sparing in cystic fibrosis-related diabetes. *J Clin Transl Endocrinol*. 2023;33:100320. Doi:10.1016/j.jcte.2023.100320.
206. Piona C, Mozzillo E, Tosco A, Volpi S, Rosanio FM, Cimbalo C, et al. Impact of CFTR Modulators on Beta-Cell Function in Children and Young Adults with Cystic Fibrosis. *J Clin Med*. 2022;11(14) Doi:10.3390/jcm11144149.
207. Granados A, Chan CL, Moheet A, Vigers T, Arbeláez AM, Larson Ode K. The impact of elexacaftor/tezacaftor/ivacaftor on body composition in a small cohort of youth with cystic fibrosis. *Pediatric Pulmonology*. 2023;58(6):1805-11. Doi:<https://doi.org/10.1002/ppul.26388>.
208. Oppenheimer EH, Esterly JR. Hepatic changes in young infants with cystic fibrosis: possible relation to focal biliary cirrhosis. *J Pediatr*. 1975;86(5):683-9. Doi:10.1016/s0022-3476(75)80351-9.

209. Boëlle PY, Debray D, Guillot L, Clement A, Corvol H. Cystic Fibrosis Liver Disease: Outcomes and Risk Factors in a Large Cohort of French Patients. *Hepatology*. 2019;69(4):1648-56. Doi:10.1002/hep.30148.
210. Colombo C, Battezzati PM, Crosignani A, Morabito A, Costantini D, Padoan R, Giunta A. Liver disease in cystic fibrosis: A prospective study on incidence, risk factors, and outcome. *Hepatology*. 2002;36(6):1374-82. Doi:<https://doi.org/10.1053/jhep.2002.37136>.
211. Rowland M, Drummond J, Connolly L, Daly E, McCormick PA, Bourke B. The natural history of cystic fibrosis liver disease a prospective cohort study. *J Cyst Fibros*. 2023;22(6):1054-61. Doi:10.1016/j.jcf.2023.07.002.
212. Debray D, Kelly D, Houwen R, Strandvik B, Colombo C. Best practice guidance for the diagnosis and management of cystic fibrosis-associated liver disease. *Journal of Cystic Fibrosis*. 2011;10:S29-S36. Doi:[https://doi.org/10.1016/S1569-1993\(11\)60006-4](https://doi.org/10.1016/S1569-1993(11)60006-4).
213. Koh C, Sakiani S, Surana P, Zhao X, Eccleston J, Kleiner DE, et al. Adult-onset cystic fibrosis liver disease: Diagnosis and characterization of an underappreciated entity. *Hepatology*. 2017;66(2):591-601. Doi:<https://doi.org/10.1002/hep.29217>.
214. Sellers ZM, Assis DN, Paranjape SM, Sathe M, Bodewes F, Bowen M, et al. Cystic fibrosis screening, evaluation and management of hepatobiliary disease consensus recommendations. *Hepatology*. 2023;10.1097/HEP.0000000000000646. Doi:10.1097/hep.0000000000000646.
215. Faro A. Cystic Fibrosis Foundation Patient Registry 2023 Annual Data Report Bethesda, Maryland: Cystic Fibrosis Foundation; 2024.
216. Singh H, Coffey MJ, Ooi CY. Cystic Fibrosis-related Liver Disease is Associated With Increased Disease Burden and Endocrine Comorbidities. *Journal of Pediatric Gastroenterology and Nutrition*. 2020;70(6):796-800. Doi:<https://doi.org/10.1097/MPG.0000000000002694>.
217. Issa Z, Gohy S, Zech F, Baldin P, Delire B, Dahlqvist G. Prevalence and characteristics of cystic fibrosis liver disease: a study highlighting the lack of histological diagnosis. *Clinics and Research in Hepatology and Gastroenterology*. 2022;46(9):101977. Doi:<https://doi.org/10.1016/j.clinre.2022.101977>.
218. Stonebraker JR, Ooi CY, Pace RG, Corvol H, Knowles MR, Durie PR, Ling SC. Features of Severe Liver Disease With Portal Hypertension in Patients With Cystic Fibrosis. *Clinical Gastroenterology and Hepatology*. 2016;14(8):1207-15.e3. Doi:<https://doi.org/10.1016/j.cgh.2016.03.041>.
219. Witters P, Libbrecht L, Roskams T, De Boeck K, Dupont L, Proesmans M, et al. Liver disease in cystic fibrosis presents as non-cirrhotic portal hypertension. *Journal of Cystic Fibrosis*. 2017;16(5):e11-e3. Doi:<https://doi.org/10.1016/j.jcf.2017.03.006>.
220. Lykavieris P, Bernard O, Hadchouel M. Neonatal cholestasis as the presenting feature in cystic fibrosis. *Arch Dis Child*. 1996;75(1):67-70. Doi:10.1136/adc.75.1.67.
221. Bartlett JR, Friedman KJ, Ling SC, Pace RG, Bell SC, Bourke B, et al. Genetic Modifiers of Liver Disease in Cystic Fibrosis. *JAMA*. 2009;302(10):1076-83. Doi:10.1001/jama.2009.1295.

222. Schaefer B, Mandorfer M, Viveiros A, Finkenstedt A, Ferenci P, Schneeberger S, et al. Heterozygosity for the alpha-1-antitrypsin Z allele in cirrhosis is associated with more advanced disease. *Liver Transplantation*. 2018;24(6):744-51. Doi:10.1002/lt.25057.
223. Wilschanski M, Durie PR. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. *Gut*. 2007;56(8):1153-63. Doi:10.1136/gut.2004.062786.
224. Fiorotto R, Strazzabosco M. Pathophysiology of Cystic Fibrosis Liver Disease: A Channelopathy Leading to Alterations in Innate Immunity and in Microbiota. *Cellular and Molecular Gastroenterology and Hepatology*. 2019;8(2):197-207. Doi:https://doi.org/10.1016/j.jcmgh.2019.04.013.
225. Fiorotto R, Villani A, Kourtidis A, Scirpo R, Amenduni M, Geibel PJ, et al. The cystic fibrosis transmembrane conductance regulator controls biliary epithelial inflammation and permeability by regulating Src tyrosine kinase activity. *Hepatology*. 2016;64(6):2118-34. Doi:10.1002/hep.28817.
226. Flass T, Tong S, Frank DN, Wagner BD, Robertson CE, Kotter CV, et al. Intestinal Lesions Are Associated with Altered Intestinal Microbiome and Are More Frequent in Children and Young Adults with Cystic Fibrosis and Cirrhosis. *PLOS ONE*. 2015;10(2):e0116967. Doi:10.1371/journal.pone.0116967.
227. De Gottardi A, Rautou PE, Schouten J, Rubbia-Brandt L, Leebeek F, Trebicka J, et al. Porto-sinusoidal vascular disease: proposal and description of a novel entity. *Lancet Gastroenterol Hepatol*. 2019;4(5):399-411. Doi:10.1016/s2468-1253(19)30047-0.
228. De Lédinghen V, Le Bail B, Rebouissoux L, Fournier C, Foucher J, Miette V, et al. Liver Stiffness Measurement in Children Using FibroScan: Feasibility Study and Comparison With Fibrotest, Aspartate Transaminase to Platelets Ratio Index, and Liver Biopsy. *Journal of Pediatric Gastroenterology and Nutrition*. 2007;45(4):443-50. Doi:10.1097/mpg.0b013e31812e56ff.
229. Elidottir H, Hansen CR, Diemer S, Eklund E. 2D Shear Wave Elastography, a promising screening tool for Cystic Fibrosis liver disease, shows a correlation between vitamin D and liver stiffness. *J Cyst Fibros*. 2022;21(5):873-7. Doi:10.1016/j.jcf.2022.06.009.
230. Højte C, Jørgensen MH, Jensen F, Katzenstein TL, Skov M. Extended Screening for Cystic Fibrosis-related Liver Disease Including Elastography in Children and Adolescents. *Journal of Pediatric Gastroenterology and Nutrition*. 2020;71(5):663-8. Doi:https://doi.org/10.1097/MPG.0000000000002872.
231. Levitte S, Lee LW, Isaacson J, Zucker EJ, Milla C, Barth RA, Sellers ZM. Clinical use of shear-wave elastography for detecting liver fibrosis in children and adolescents with cystic fibrosis. *Pediatric Radiology*. 2021;51(8):1369-77. Doi:10.1007/s00247-021-05015-w.
232. Klotter V, Gunchick C, Siemers E, Rath T, Hudel H, Naehrlich L, et al. Assessment of pathologic increase in liver stiffness enables earlier diagnosis of CFLD: Results from a prospective longitudinal cohort study. *PLOS ONE*. 2017;12(6):e0178784. Doi:10.1371/journal.pone.0178784.

233. Cheng K, Ashby D, Smyth RL. Ursodeoxycholic acid for cystic fibrosis-related liver disease. *Cochrane Database Syst Rev*. 2017;9(9):Cd000222. Doi:10.1002/14651858.CD000222.pub4.
234. Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. *Hepatology*. 2002;36(3):525-31. Doi:10.1053/jhep.2002.36088.
235. Staufer K. Current Treatment Options for Cystic Fibrosis-Related Liver Disease. *International Journal of Molecular Sciences*. 2020;21(22):8586. Doi:10.3390/ijms21228586.
236. Keating D, Marigowda G, Burr L, Daines C, Mall MA, McKone EF, et al. VX-445-Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis and One or Two Phe508del Alleles. *N Engl J Med*. 2018;379(17):1612-20. Doi:10.1056/NEJMoa1807120.
237. Colombo C, Ramm GA, Lindblad A, Corti F, Porcaro L, Alghisi F, et al. Characterization of CFTR mutations in people with cystic fibrosis and severe liver disease who are not eligible for CFTR modulators. *Journal of Cystic Fibrosis*. 2023;22(2):263-5. Doi:https://doi.org/10.1016/j.jcf.2023.01.012.
238. Moiceanu ES, Leucuța DC, Nițescu VG, Lescaie A, Iacobescu M, Stan IV, et al. Impact of CFTR Modulator Therapies on Liver Function in Cystic Fibrosis Patients: A Systematic Review of Hepatic Biomarkers. *J Gastrointestin Liver Dis*. 2024 Doi:10.15403/jgld-5879.
239. Hayes D, Jr., Warren PS, McCoy KS, Sheikh SI. Improvement of hepatic steatosis in cystic fibrosis with ivacaftor therapy. *J Pediatr Gastroenterol Nutr*. 2015;60(5):578-9. Doi:10.1097/mpg.0000000000000765.
240. Martinekova P, Obeidat M, Topala M, Vánca S, Veres DS, Zolcsák Á, et al. Role of Vitamin D Supplementation in Chronic Liver Disease: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrition Reviews*. 2025;83(11):2043-54. Doi:10.1093/nutrit/nuaf117.
241. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *European Respiratory Journal*. 2012;40(6):1324-43. Doi:10.1183/09031936.00080312.
242. Robinson PD, Goldman MD, Gustafsson PM. Inert Gas Washout: Theoretical Background and Clinical Utility in Respiratory Disease. *Respiration*. 2009;78(3):339-55. Doi:10.1159/000225373.
243. Gustafsson PM, De Jong PA, Tiddens HAWM, Lindblad A. Multiple-breath inert gas washout and spirometry versus structural lung disease in cystic fibrosis. *Thorax*. 2008;63(2):129-34. Doi:10.1136/thx.2007.077784.
244. Moran A, Pillay K, Becker D, Granados A, Hameed S, Acerini CL. ISPAD Clinical Practice Consensus Guidelines 2018: Management of cystic fibrosis-related diabetes in children and adolescents. *Pediatr Diabetes*. 2018;19 Suppl 27:64-74. Doi:10.1111/pedi.12732.

245. Akyuz M, Gurcan Kaya N, Esendagli G, Dalgic B, Ozhan Oktar S. The evaluation of the use of 2D shear-wave ultrasound elastography in differentiation of clinically insignificant and significant liver fibrosis in pediatric age group. *Abdom Radiol (NY)*. 2021;46(5):1941-6. Doi:10.1007/s00261-020-02844-5.
246. Hovold G, Palmcrantz V, Kahn F, Egesten A, Pålman LI. Heparin-binding protein in sputum as a marker of pulmonary inflammation, lung function, and bacterial load in children with cystic fibrosis. *BMC Pulm Med*. 2018;18(1):104. Doi:10.1186/s12890-018-0668-7.
247. Happonen L, Hauri S, Svensson Birkedal G, Karlsson C, De Neergaard T, Khakzad H, et al. A quantitative *Streptococcus pyogenes*–human protein–protein interaction map reveals localization of opsonizing antibodies. *Nature Communications*. 2019;10(1) Doi:10.1038/s41467-019-10583-5.
248. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun*. 2019;10(1):1523. Doi:10.1038/s41467-019-09234-6.
249. Nadkarni MA, Martin FE, Jacques NA, Hunter N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology*. 2002;148(1):257-66. Doi:https://doi.org/10.1099/00221287-148-1-257.
250. Anghel L, Ciubară A, Patraş D, Ciubară AB. Chronic Obstructive Pulmonary Disease and Type 2 Diabetes Mellitus: Complex Interactions and Clinical Implications. *Journal of Clinical Medicine*. 2025;14(6):1809.
251. Hughes EM, Kiedrowski MR. Effects of Elevated Glucose on Bacterial Respiratory Infections in Cystic Fibrosis and Chronic Airway Diseases. *International Journal of Molecular Sciences*. 2025;26(12):5597.
252. Garnett JP, Baker EH, Naik S, Lindsay JA, Knight GM, Gill S, et al. Metformin reduces airway glucose permeability and hyperglycaemia-induced *Staphylococcus aureus* load independently of effects on blood glucose. *Thorax*. 2013;68(9):835-45. Doi:10.1136/thoraxjnl-2012-203178.
253. Narendra DK, Khurana S. Asthma and Hyperglycemia: Exploring the Interconnected Pathways. *Diagnostics*. 2024;14(17):1869.
254. Mubanga M, Gong T, Smew AI, Wikström A, Caffrey Osvald E, Eeg-Olofsson K, et al. Association between asthma and type 2 diabetes in a Swedish adult population: a register-based cross-sectional study. *Thorax*. 2025;80(6):385-91. Doi:10.1136/thorax-2024-222819.
255. Brenner LN, Huang C-Y, Kim M, Bringham L, Richards CJ, Sicilian L, et al. Dysglycemia and the airway microbiome in cystic fibrosis. *PLOS ONE*. 2025;20(10):e0331847. Doi:10.1371/journal.pone.0331847.
256. Wijdan SA, Bokhari SMNA, Alvares J, Latif V. The role of interleukin-1 beta in inflammation and the potential of immune-targeted therapies. *Pharmacological Research - Reports*. 2025;3:100027. Doi:https://doi.org/10.1016/j.prerep.2025.100027.

257. Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, et al. Glucose-induced β cell production of IL-1 β contributes to glucotoxicity in human pancreatic islets. *The Journal of Clinical Investigation*. 2002;110(6):851-60. Doi:10.1172/JCI15318.
258. Han B, Luo H, Raelson J, Huang J, Li Y, Tremblay J, et al. TGFBI (β IG-H3) is a diabetes-risk gene based on mouse and human genetic studies. *Hum Mol Genet*. 2014;23(17):4597-611. Doi:10.1093/hmg/ddu173.
259. Wu C, Liu W, Liu Y, Xu T, Li M, Li X, et al. Human umbilical cord mesenchymal stem cell-derived TGFBI attenuates streptozotocin-induced type 1 diabetes mellitus by inhibiting T-cell proliferation. *Hum Cell*. 2023;36(3):997-1010. Doi:10.1007/s13577-023-00868-9.
260. Yu H, Luo C, Linghu R, Yang J, Wu H. Ezrin Contributes to the Damage of Airway Epithelial Barrier Related to Diabetes Mellitus. *J Inflamm Res*. 2024;17:2609-21. Doi:10.2147/jir.S449487.
261. Zhang XL, Topley N, Ito T, Phillips A. Interleukin-6 regulation of transforming growth factor (TGF)- β receptor compartmentalization and turnover enhances TGF- β 1 signaling. *J Biol Chem*. 2005;280(13):12239-45. Doi:10.1074/jbc.M413284200.
262. Oriano M, Amati F, Gramegna A, De Soyza A, Mantero M, Sibila O, et al. Protease–Antiprotease Imbalance in Bronchiectasis. *International Journal of Molecular Sciences*. 2021;22(11):5996.
263. Sandström CS, Ohlsson B, Melander O, Westin U, Mahadeva R, Janciauskiene S. An association between Type 2 diabetes and alpha-antitrypsin deficiency. *Diabet Med*. 2008;25(11):1370-3. Doi:10.1111/j.1464-5491.2008.02584.x.
264. Belongie KJ, Ferrannini E, Johnson K, Andrade-Gordon P, Hansen MK, Petrie JR. Identification of novel biomarkers to monitor β -cell function and enable early detection of type 2 diabetes risk. *PLoS One*. 2017;12(8):e0182932. Doi:10.1371/journal.pone.0182932.
265. Park SS, Mai M, Ploszaj M, Cai H, McGarvey L, Mueller C, et al. Type 1 diabetes contributes to combined pulmonary fibrosis and emphysema in male alpha 1 antitrypsin deficient mice. *PLoS One*. 2023;18(10):e0291948. Doi:10.1371/journal.pone.0291948.
266. Mosoian A, Teixeira A, Burns CS, Sander LE, Gusella GL, He C, et al. Prothymosin- α inhibits HIV-1 via Toll-like receptor 4-mediated type I interferon induction. *Proceedings of the National Academy of Sciences*. 2010;107(22):10178-83. Doi:10.1073/pnas.0914870107.
267. Su B-H, Tseng Y-L, Shieh G-S, Chen Y-C, Shiang Y-C, Wu P, et al. Prothymosin α overexpression contributes to the development of pulmonary emphysema. *Nature Communications*. 2013;4(1):1906. Doi:10.1038/ncomms2906.
268. Su BH, Tseng YL, Shieh GS, Chen YC, Wu P, Shiau AL, Wu CL. Over-expression of prothymosin- α antagonizes TGF β signalling to promote the development of emphysema. *The Journal of Pathology*. 2016;238(3):412-22. Doi:10.1002/path.4664.

269. Yarmohammadi H, Soltanipur M, Rezaei M, Ejtahed HS, Raei M, Razavi A, et al. The Comparison of the Gut Microbiome Composition, Serum Inflammatory Markers and Faecal Short-Chain Fatty Acids Among Individuals With Type 1 and 2 Diabetes Mellitus With Healthy Controls: A Case-Control Study. *Endocrinol Diabetes Metab.* 2025;8(4):e70071. Doi:10.1002/edm2.70071.
270. Yi H-M, Won S, Pak J, Park S-E, Kim M-R, Kim J-H, et al. Fecal Microbiome and Urine Metabolome Profiling of Type 2 Diabetes. *J Microbiol Biotechnol.* 2025;35(3):1-9. Doi:10.4014/jmb.2411.11071.
271. Chen Q, Gao Y, Li F, Yuan L. The role of gut–islet axis in pancreatic islet function and glucose homeostasis. *Diabetes, Obesity and Metabolism.* 2025;27(4):1676-92. Doi:https://doi.org/10.1111/dom.16225.
272. Jankowski WM, Fichna J, Tarasiuk-Zawadzka A. A systematic review of the relationship between gut microbiota and prevalence of pancreatic diseases. *Microbial Pathogenesis.* 2025;199:107214. Doi:https://doi.org/10.1016/j.micpath.2024.107214.
273. Vasiljevs S, Witney AA, Baines DL. The presence of cystic fibrosis-related diabetes modifies the sputum microbiome in cystic fibrosis disease. *American Journal of Physiology-Lung Cellular and Molecular Physiology.* 2024;326(2):L125-L34. Doi:10.1152/ajplung.00219.2023.
274. Iwanicki C, Logomarsino JV. Impaired glucose tolerance, body mass index and respiratory function in patients with cystic fibrosis: A systematic review. *Clin Respir J.* 2019;13(6):341-54. Doi:10.1111/crj.13019.
275. Lavie M, Fisher D, Vilozni D, Forschmidt R, Sarouk I, Kanety H, et al. Glucose intolerance in cystic fibrosis as a determinant of pulmonary function and clinical status. *Diabetes Research and Clinical Practice.* 2015;110(3):276-84. Doi:10.1016/j.diabres.2015.10.007.
276. Patel M, McCracken C, Daley T, Stecenko A, Linnemann R. Trajectories of oral glucose tolerance testing in cystic fibrosis. *Pediatric Pulmonology.* 2021;56(5):901-9. Doi:https://doi.org/10.1002/ppul.25207.
277. Rakotoarisoa L, Weiss L, Lefebvre F, Porzio M, Renaud-Picard B, Ravoninjatovo B, et al. Early glucose abnormalities revealed by continuous glucose monitoring associate with lung function decline in cystic fibrosis: A five-year prospective study. *J Diabetes Complications.* 2024;38(4):108703. Doi:10.1016/j.jdiacomp.2024.108703.
278. Boudreau V, Reynaud Q, Denis A, Colomba J, Touzet S, Desjardins K, et al. Impact of 1h oral glucose tolerance test on the clinical status of adult cystic fibrosis patients over a 4-year period. *PLOS ONE.* 2021;16(3):e0246897. Doi:10.1371/journal.pone.0246897.
279. Reynaud Q, Rabilloud M, Roche S, Poupon-Bourdy S, Iwaz J, Nove-Josserand R, et al. Glucose trajectories in cystic fibrosis and their association with pulmonary function. *J Cyst Fibros.* 2018;17(3):400-6. Doi:10.1016/j.jcf.2017.09.010.
280. Coriati A, Ziai S, Azar M, Berthiaume Y, Rabasa-Lhoret R. Characterization of patients with cystic fibrosis presenting an indeterminate glucose tolerance (INDET). *Journal of cystic fibrosis : official journal of the European Cystic Fibrosis Society.* 2016;15 1:127-32.

281. Bodewes FAJA, Freeman AJ, Weymann A, Debray D, Scheers I, Verkade HJ, Narkewicz MR. Towards a Standardized Classification of the Hepatobiliary Manifestations in Cystic Fibrosis (CFHBI): A Joint ESPGHAN/NASPGHAN Position Paper. *Journal of Pediatric Gastroenterology and Nutrition*. 2024;78(1):153-65. Doi:10.1097/mpg.0000000000003944.
282. Terlizzi V, Fevola C, Cecchetti M, Terminiello A, Curci F, Bartolini E, et al. Effect of elexacaftor-tezacaftor-ivacaftor on liver transient elastography, fibrosis indices and blood tests in children with cystic fibrosis. *J Cyst Fibros*. 2025 Doi:10.1016/j.jcf.2024.12.010.
283. Calvo PL, Giugliano L, Evangelista A, Bignamini E, Pinon M. Effects of CFTR modulator therapies on liver stiffness and bile flow: A single-centre experience. *Journal of Hepatology*. 2023;79(2):e76-e8. Doi:10.1016/j.jhep.2023.02.006.
284. Tewkesbury DH, Scott J, Barry PJ, Bright-Thomas RJ, Hanley KP, Athwal V, Jones AM. Effects of elexacaftor/tezacaftor/ivacaftor on liver fibrosis markers in adults with cystic fibrosis. *Journal of Cystic Fibrosis*. 2023 Doi:https://doi.org/10.1016/j.jcf.2023.09.006.
285. Manioudaki S, Vasilieva L, Geladari E, Mani I, Athanassa Z, Elefsiniotis I, et al. Improvement of Cystic Fibrosis-Associated Liver Disease in Adults on Long-Term Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Modulators. *Life*. 2025;15(12):1794.
286. Schnell A, Jüngert J, Klett D, Hober H, Kaiser N, Ruppel R, et al. Increase of liver stiffness and altered bile acid metabolism after triple CFTR modulator initiation in children and young adults with cystic fibrosis. *Liver International*. 2023;43(4):878-87. Doi:https://doi.org/10.1111/liv.15544.
287. Poynard T, Pham T, Perazzo H, Munteanu M, Luckina E, Elaribi D, et al. Real-Time Shear Wave versus Transient Elastography for Predicting Fibrosis: Applicability, and Impact of Inflammation and Steatosis. A Non-Invasive Comparison. *PLOS ONE*. 2016;11(10):e0163276. Doi:10.1371/journal.pone.0163276.
288. Leung DH, Khan M, Minard CG, Guffey D, Ramm LE, Clouston AD, et al. Aspartate aminotransferase to platelet ratio and fibrosis-4 as biomarkers in biopsy-validated pediatric cystic fibrosis liver disease. *Hepatology*. 2015;62(5):1576-83. Doi:10.1002/hep.28016.
289. Hejazi N, Ghalandari H, Rahmanian R, Haghpanah F, Makhtoomi M, Asadi A, Askarpour M. Effects of probiotics supplementation on glycemic profile in adults with type 2 diabetes mellitus: A grade-assessed systematic review and dose–response meta-analysis of randomized controlled trials. *Clinical Nutrition ESPEN*. 2024;64:133-43. Doi:10.1016/j.clnesp.2024.09.014.
290. Baroni I, Fabrizi D, Luciani M, Magon A, Conte G, De Angeli G, et al. Probiotics and synbiotics for glycemic control in diabetes: A systematic review and meta-analysis of randomized controlled trials. *Clinical Nutrition*. 2024;43(4):1041-61. Doi:10.1016/j.clnu.2024.03.006.
291. Ağagündüz D, Çelik E, Cemali Ö, Yesildemir O, Demirci KŞ, Akkus G, et al. Mechanism of actions of probiotics on type 2 diabetes: Development and complications. *Biomedicine & Pharmacotherapy*. 2025;191:118421. Doi:https://doi.org/10.1016/j.biopha.2025.118421.

292. Van Dorst JM, Tam RY, Ooi CY. What Do We Know about the Microbiome in Cystic Fibrosis? Is There a Role for Probiotics and Prebiotics? *Nutrients*. 2022;14(3):480. Doi:10.3390/nu14030480.
293. Cruz Mosquera FE, Perlaza CL, Naranjo Rojas A, Murillo Rios S, Carrero Gallego A, Fischersworring SI, et al. Effectiveness of Probiotics, Prebiotics, and Symbiotic Supplementation in Cystic Fibrosis Patients: A Systematic Review and Meta-Analysis of Clinical Trials. *Medicina*. 2025;61(3):489.

About the author

Stefanie Diemer is a pediatrician at the Department of Pediatrics, Skåne University Hospital, and at the Department of Clinical Science, Lund University, Sweden. Her doctoral research investigates the interplay of metabolic, inflammatory, and microbial factors in cystic fibrosis (CF), alongside the effects of highly effective CFTR modulator therapies on CF-associated hepatobiliary disease.

