Aspects on the Diverse Manifestations of Cystic Fibrosis

Elidottir, Helga

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HELGA ELÍDÓTTIR
PAEDIATRICS | FACULTY OF MEDICINE | LUND UNIVERSITY

The drawing of *65 roses* by Gregory Beylerian was donated to an auction to raise funds and awareness for Cystic Fibrosis. Since 1965, the term 65 Roses has represented Cystic Fibrosis. It comes from a small boy with CF overhearing his mother making phone calls for CF fundraising. He told his mom he now knew she was working for 65 roses.

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Abstract: Background. Cystic Fibrosis (CF) is a multiorgan disease in which the pulmonary consequences are most devastating. Other aspects of the disease include CF-related diabetes (CFRD) and liver disease (CFLD), which are essential to screen for, starting in childhood. The diverse complications interact and influence the disease progression both separately and additively. The prevalence of CF differs globally, and the F508del variant is the most common, accounting for approximately 90% of disease-causing gene variants. This thesis aims to describe the different methods available for screening the co-morbidities of CFRD and CFLD and to understand how the different aspects of the disease influence each other and affect lung function. Furthermore, the thesis explores the impact of lumacaftor-ivacaftor (LUM/IVA) on glucose tolerance. Finally, the prevalence of CF, genetic variants and CF care in Iceland will be described.

Methods. A comparative cohort study was performed at Lund Pediatric CF Centre in 2019, where children aged 7-18 were prospectively recruited to compare oral glucose tolerance tests and continuous glucose monitoring (CGM). The impact of abnormal glucose tolerance (AGT) on lung function was investigated by spirometry and multiple breath washout. A retrospective cohort study from the same CF centre followed where individuals included were evaluated for CFLD by ultrasound and two-dimensional shear wave elastography (2D SWE). Data regarding liver disease, lung function, nutrition and glucose tolerance was collected. The third part presents a cohort study from The Swedish National CF Registry. Individuals included in the study received treatment with LUM/IVA and had done an OGTT before and during treatment, starting in 2018-2019. Finally, a retrospective descriptive analysis was performed of the Icelandic CF cohort between 1955 and 2021 with demographic and clinical data extracted from medical charts.

Results. Thirty-two participants, with a median age of 11.5, delivered 28 pairs of CGMs and OGTTs. The CGM percentage of measurements above 8mmol/L and the number of peaks per day above 11 mmol/L correlated with the intermediate time points on OGTTs but not the 2-hour glucose value. Individuals with AGT had inferior lung function than those with normal glucose tolerance (NGT), as demonstrated by both forced expiratory volume in the first-second percentage of predicted (FEV1pp) and lung clearance index (LCI). Fifty-one children, with a median age of 11, had performed an ultrasound of the liver and a 2D SWE. Four children had significantly increased liver stiffness and confirmed liver cirrhosis on biopsy. Children with AGT had increased liver stiffness compared to children with NGT. A negative correlation was found between liver stiffness, vitamin D levels, and FEV1pp, respectively. Seventy-eight individuals, 32 children and 46 adults received LUM/IVA and had performed an OGTT before and during treatment. Forty-seven of them had AGT, and 31 had NGT. There was a significant decrease in the two-hour glucose value after the onset of treatment for individuals with AGT and NGT. No significant improvement was found in fasting glucose, one-hour values or HbA1c. A trend for increased lung function was seen but was not significant. The prevalence of CF in Iceland was 0.372:10.000 inhabitants. The F508del is the most common CF transmembrane conductance regulator (CFTR) variant (46.4%), closely followed by N1303K (44.6%). Modern CF medications, including the recent CFTR modulators, are available.

Conclusion. CGM results do not seem to correlate to the 2-hour glucose value in OGTTs; instead, they correspond more to the intermediate time points of the OGTTs. LCI demonstrates, as does FEV1pp, inferior lung function in children and adolescents with AGT. Liver stiffness measurement by 2D SWE is a feasible addition to CFLD screening in youth. Increased liver stiffness is associated with lower vitamin D levels, inferior FEV1pp and AGT.LUM/IVA appears to positively impact glucose tolerance in people with CF, as demonstrated by improvements in the two-hour glucose value of OGTTs. This gives rise to the hope that the more potent CFTR modulators could diminish the burden of CFRD in people with CF. Even though Iceland has a relatively low prevalence of CF, it holds the highest known prevalence of the N1303K variant. Access to necessary treatment is satisfactory, but improvements are advisable for some aspects of the routine assessment.

Key words: Continuous glucose monitoring, Cystic Fibrosis, Cystic Fibrosis liver disease, Cystic Fibrosis-related diabetes, glucose tolerance, lumacaftor/ivacaftor, lung function, N1303K variant, oral glucose tolerance test.

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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 Sep;21(5):873-877. doi: 10.1016/j.jcf.2022.06.009. Epub 2022 Jul 3. PMID: 35794060.

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Abbreviations

AGT	Abnormal glucose tolerance
BMI	Body mass index
CF	Cystic Fibrosis
CFF	Cystic Fibrosis Foundation
CFLD	Cystic Fibrosis liver disease
CFLI	Cystic Fibrosis liver involvement
CFRD	Cystic Fibrosis related diabetes
CFTR	Cystic Fibrosis transmembrane conductance regulator
CGM	Continuous glucose monitoring
ECFS	European Cystic Fibrosis Society
ETI	Elexacaftor-tezacaftor-ivacaftor
FEV1pp	Forced expiratory volume in the first-second percentage of predicted
IGT	Impaired glucose tolerance
INDET	Indeterminate glycemia
LCI	Lung clearance index
LUM/IVA	Lumacaftor-ivacaftor
MBW	Multiple breath washout
MR	Magnetic resonance
NGT	Normal glucose tolerance
OGTT	Oral glucose tolerance test
PH	Portal hypertension
pwCF	People with CF
NBS	Newborn screening
2D SWE	Two-dimensional shear wave elastography

Abstract

Background. Cystic Fibrosis (CF) is a multiorgan disease in which the pulmonary consequences are most devastating. Other aspects of the disease include CF-related diabetes (CFRD) and liver disease (CFLD), which are essential to screen for, starting in childhood. The diverse complications interact and influence the disease progression both separately and additively. The prevalence of CF differs globally, and the F508del variant is the most common, accounting for approximately 90% of disease-causing gene variants. This thesis aims to describe the different methods available for screening the co-morbidities of CFRD and CFLD and to understand how the different aspects of the disease influence each other and affect lung function. Furthermore, the thesis explores the impact of lumacaftor-ivacaftor (LUM/IVA) on glucose tolerance. Finally, the prevalence of CF, genetic variants and CF care in Iceland will be described.

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Conclusion. CGM results do not seem to correlate to the 2-hour glucose value in OGTTs; instead, they correspond more to the intermediate time points of the OGTTs. LCI demonstrates, as does FEV1pp, inferior lung function in children and adolescents with AGT.

Liver stiffness measurement by 2D SWE is a feasible addition to CFLD screening in youth. Increased liver stiffness is associated with lower vitamin D levels, inferior FEV1pp and AGT.

LUM/IVA appears to positively impact glucose tolerance in people with CF, as demonstrated by improvements in the two-hour glucose value of OGTTs. This gives rise to the hope that the more potent CFTR modulators could diminish the burden of CFRD in people with CF.

Even though Iceland has a relatively low prevalence of CF, it holds the highest known prevalence of the N1303K variant. Access to necessary treatment is satisfactory, but improvements are advisable for some aspects of the routine assessment.

Svensk sammanfattning

Cystisk fibros är en sk. recessiv ärftlig sjukdom, där patienten ärver en sjukdomsorsakande variant från var sin förälder i genen *CFTR*. Detta resulterar i att ett protein (äggviteämne) som sitter på cellmembranen (en jonkanal för kloridjoner) inte fungerar som det ska. Detta leder till stora problem i många organ, men lungorna och bukspottkörteln är de organ som drabbas värst. Barnläkaren och patologen Dorothy H. Andersen gav sjukdomen dess namn 1938 när hon såg sambandet mellan lunginfektioner och cystor i bukspottkörteln hos små barn som dog. Två år tidigare hade dock läkaren Guido Fanconi beskrivit ett liknande tillstånd hos två barn. I lungorna leder sjukdomen till att det bildas mycket segt slem i luftvägarna, vilket gör att mikroorganismer kan få fäste där och orsaka infektioner och kronisk inflammation som förvärras med åren. De flesta med CF föds med nedsatt funktion i bukspottkörteln, så att den inte producerar tillräckligt med viktiga matsmältningsenzymer och personer med CF kan därför inte ta upp näringsämnen som vitaminer, fett och protein utan att behandlas med speciella matsmältningsenzymer. Andra sjukdomar som ofta uppkommer vid CF är diabetes, som ofta utvecklas senare i livet, men är varken diabetes typ 1 eller 2. CF-relaterad diabetes utvecklas under många år och får allvarliga konsekvenser för dem som utvecklar den. Leversjukdom kan också följa med CF och de flesta som utvecklar CF-relaterad leversjukdom börjar utveckla mätbara tecken på det redan som barn. Både diabetes och leversjukdom förblir dock utan klara kliniska symptom under lång tid, varför man måste screena för dessa komplikationer. Blodsockerbelastning används vid CF-diabetes screening, men på vissa ställen används också kontinuerliga blodsockermätningar där blodsockret kan övervakas dygnet runt med en sensor som fästs på huden. CF-relaterad leversjukdom upptäcks genom blodprov och ultraljud eller biopsi av levern, men andra tekniker har utvecklats för att bedöma leverns stelhet.

Mycket har hänt i utvecklingen av behandling för CF. Matsmältningsenzymer, inhalationsmediciner, andningsövningar och antibiotika av olika slag är exempel på behandlingar som under åren successivt förbättrat prognosen så att det numera finns fler vuxna än barn med CF i de flesta länder där förekomsten är känd. År 2012 introducerades en ny typ av medicinering som drastiskt förändrat prognosen för personer med denna svåra sjukdom, mediciner som kallas *CFTR-modulatorer*. Många genvarianter är kända i CF-genen, där den som kallas F508del är den absolut vanligaste. Några CFTR-modulatorer fungerar väldigt bra hos patienter med denna genvariant men även vid andra varianter finns nu läkemedel som fungerar väl.

Det forskningsprojekt som inkluderas i denna avhandling har fyra delar. I det första utreds om det är möjligt att screena för CF-relaterad diabetes hos barn och ungdomar med kontinuerlig blodsockermätning på ett jämförbart sätt med blodsockerbelastning, samt om nedsatt glukostolerans påverkar lungfunktionen uppmätt med två olika metoder. Den andra delen utvärderar om det är att föredra att

mäta leverns stelhet med en apparat som heter 2DSWE. Sambandet mellan leversjukdom och diabetes, näringsfaktorer och lungfunktion hos barn med CF beskrivs också. Den tredje delen undersöker om behandling med en kombination av de relativt nya CFTR-modulatorerna, lumacaftor och ivacaftor, påverkar glukostoleransen mätt hos barn och vuxna med CF. Slutligen beskrivs CF på Island; prevalensen, vilka genvarianter och komplikationer som är vanligast och hur behandlingen och uppföljningen går till.

De viktigaste resultaten från de olika delstudierna var : att kontinuerlig blodsockermätning inte bedömde samma faktorer som blodsockerbelastning; den fångade inte samma värden och är mer relaterat till faktorer som tyder på nedsatt glukostolerans. Detta är viktigt att veta eftersom man genom att använda kontinuerlig blodsockermätning inte verkar vara tillräckligt precis för att kunna diagnostisera diabetes hos barn med CF, men det kan ge ytterligare information. De barn som hade en onormal blodsockerbelastning hade markant sämre lungfunktion. Att använda mätning av leverstelhet utöver ultraljud och blodprover för att utvärdera CF-relaterad leversjukdom visade sig vara ett användbart komplement. Ökad stelhet i levern var associerad med nedsatt glukostolerans, lägre D-vitaminnivåer i gruppen och sämre lungfunktion. Därför kan man tänka sig att barn som utvecklar leversjukdom behöver övervakas särskilt noggrant och till och med screenas tidigare för diabetes. Det är också viktigt att noga övervaka lungfunktionen hos alla med CF, men kanske särskilt hos dem med nedsatt glukostolerans och/eller leversjukdom. Lumacaftor-ivacaftor verkar kunna förbättra glukostoleransen något, men ytterligare forskning behövs, särskilt på nyare CFTR-modulatorer. CF är inte lika vanligt på Island som i de flesta europeiska länder. En genvariant som heter N1303K är mycket vanlig på Island, och faktiskt har Island den högsta kända frekvensen av denna mutation. Moderna behandlingsalternativ är tillgängliga i landet, vissa aspekter av uppföljningen kan förbättras och komplikationer liknar de som ses på andra håll i världen.

Íslensk samantekt

Cystic fibrosis nefnist slímseigjusjúkdómur á íslensku en þó er oftast einfaldlega talað um CF. Þessi sjúkdómur er arfgengur þannig að einstaklingur erfir tvo erfðabreytileika hvorn frá sínu foreldri sem leiða til þess að ákveðið prótein sem situr á frumuhimnum virkar ekki sem skyldi. Þetta leiðir til mikilla vandræða í mörgum líffærum en lungun og brisið eru þau líffæri sem verða verst út. Í lungum leiðir sjúkdómurinn til þess að það myndast mikið og seigt slím í loftvegunum sem verður til þess að örverur geta sest þar að, valdið sýkingum og alvarlegum bólgum í lungum sem síðan versnar eftir því sem árin líða. Flestir með CF fæðast með vanstarfsemi í brisinu þannig að það framleiðir ekki lífsnauðsynleg meltingarensím og fólk með CF getur ekki tekið upp næringu, vítamin, fitu og prótein án þess að taka inn sérstök meltingarensím. Það var reyndar barnalæknirinn og meinafræðingurinn Dorothy H Andersen sem gaf sjúkdómnum nafn árið 1938 þegar hún sá tengslin milli þessara tveggja þátta hjá börnum sem dóu mjög ung og tveimur áður áður hafði læknir í Sviss að nafni Guido Fanconi lýst sambærilegu hjá tveimur börnum. Fleira getur fylgt CF, eins og sykursýki sem oft kemur fram seinna á ævinni en CF sykursýki er hvorki sykursýki eitt né tvö. CF sykursýki þróast á mörgum árum og hefur alvarlegar afleiðingar fyrir þá sem fá hana. Lifrarsjúkdómur getur líka fylgt CF en flestir sem fá CF lifrarsjúkdóm fá fyrstu merki hans sem börn. Bæði sykursýkin og lifrarsjúkdómurinn eru lengi einkennalaus og þess vegna þarf að skima fyrir þessum fylgikvillum á ákveðinn hátt. Fyrir sykursýkina eru notuð sykurþolspróf en einnig er sums staðar farið að nota samfelldar blóðsykurmælingar þar sem hægt er að fylgjast með blóðsykrinum allan sólarhringinn með nema sem er festur á húðina. Lifrarsjúkdómurinn finnst hins vegar með blóðprufum og ómun eða sýnatöku en einnig hefur verið þróuð tækni til að meta stífleika lifrarinnar.

Margt hefur gerst í þróun á meðferð við CF. Meltingarensím, innúðalyf, öndunaræfingar, sýklalyf af ýmsu tagi ásamt fleiru hafa komið fram á liðnum árum og bætt horfurnar smám saman þannig að nú á dögum eru fleiri fullorðnir en börn með sjúkdóminn í flestum löndum þar sem algengi er þekkt. Árið 2012 kom síðan fram ný tegund lyfja sem gerbreytir horfum fólks með þennan erfiða sjúkdóm en þessi lyf kallast *CFTR modulators*. CF er að finna um allan heim þó algengi sé nokkuð misjafnt eftir löndum. Mjög margar genabreytur eru þekktar í CF geninu en ein þeirra sem kallast F508del er lang algengust. Nýjustu lyfin virka einmitt mjög vel á þessa genabreytu og svo bætast við fleiri tegundir lyfja sem gagnast fleiri genabreytum.

Þær rannsóknir sem fjallað er um í þessu doktorsverkefni eru í fjórum hlutum. Í þeim fyrsta er verið að kanna hvort hægt sé að skima fyrir CF tengdri sykursýki hjá börnum og unglingum með samfelldri blóðsykurmælingu á sambærilegan hátt eða til viðbótar við sykurþolspróf og hvort skert sykurþol hafi áhrif á lungnastarfsemi sem mæld er með tveimur mismunandi aðferðum. Í öðrum hluta er verið að meta hvort það sé ákjósanlegt að mæla stífleika lifrarinnar með tæki sem kallast 2DSWE.

Einnig er gerð grein fyrir tengslum lifrarsjúkdóms við sykursýki, næringarþætti og lungnastarfsemi hjá börnum með CF. Í þriðja hlutanum er kannað hvort meðferð með hinu nýja CFTR modulator lyfi, lumacaftor-ivacaftor, hafi áhrif á sykurþol sem mælt er með sykurþolsprófum hjá börnum og fullorðnum einstaklingum með CF. Að lokum er skýrt frá CF á Íslandi; hvert algengið er hér, hvaða genabreytur og fylgikvillar eru algengust og hvernig meðferð og eftirliti er háttað.

Niðurstöðurnar eru þær að samfelld blóðykurmæling mat ekki sömu þætti og sykurþolsprófið; það fangaði ekki sömu gildi og tengist frekar þáttum sem benda til skerts sykurþols. Þetta er mikilvægt atriði því ekki virðist vera nægjanlegt að nota samfellda blóðsykurmælingu til að greina sykursýki hjá börnum með CF en það getur þó gefið viðbótarupplýsingar. Þau börn sem voru með óeðlilegt sykurþolspróf voru með marktækt lakari lungnastarfsemi. Það reyndist fýsileg viðbót að nota mælingu á stífleika lifrarinnar til viðbótar við hefðbundna ómun og blóðprufur til að meta CF tengdan lifrarsjúkdóm. Aukinn stífleiki í lifrinni tengdist skertu sykurþoli, lægra D vítamíngildi í hópnum og lakari lungnastarfsemi. Þannig er hægt að hugsa sér að fylgjast þurfi sérstaklega vel með þeim börnum sem fá lifrarsjúkdóm og jafnvel að skima fyrr fyrir sykursýki hjá þeim. Einnig er mikilvægt að fylgjast vel með lungnastarfsemi allra með CF en ef til vill sérstaklega hjá þeim sem eru með skert sykurþol og/eða lifrarsjúkdóm. Lumacaftor-ivacaftor virðist geta bætt sykurþol að einhverju leyti en frekari rannsókna er þörf og þá sérstaklega á enn nýrri CFTR modulator lyfjum. CF er ekki eins algengt á Íslandi og í flestum öðrum löndum Evrópu. Genabreyta sem kallast N1303K er mjög algeng á Íslandi og hæsta þekkta tíðni þessarar stökkbreytingar er reyndar á Íslandi. CF meðferðarmöguleikar eru góðir í landinu, viss atriði eftirlits mætti bæta og fylgikvillar eru svipaðir og annars staðar.

Preface

The story of Cystic Fibrosis (CF) is a story of great victories and deep sorrows. The achievements are those of the children and adults with CF rising to the challenges of the disease every day of their lives. The successes are also those of research, continuously moving forward and with every year bringing us closer to finding the cure for CF. Nevertheless, along the way, there are many defeats and disappointments, lost battles, and the loss of many young lives.

I started working with children with CF in 2012 and soon became the chief physician for the pediatric division of Lund CF Centre. The fantastic group of children who came to the clinic was why I chose to stay with CF. There is something extraordinary about them and their families, and I was amazed by the children's bravery, honesty, and resilience.

I realised that research is important for the families of children with CF, and our CF centre had aspirations to launch more research. In the daily practice, I noticed that of all the tests our children with CF had to undergo at the clinic, one of the most dreaded ones was to have the oral glucose tolerance test (OGTT) done every year. I wanted to know if there was any way to do the diabetes screening differently. Therefore, we started using continuous glucose monitoring combined with the traditional OGTT with the help of the diabetes team. From then on, I decided that a research project would be the most beneficial way to explore this in more detail. In addition, we had just gotten the Exhalyzer-D to do multiple breath washouts for lung function monitoring, and I also wanted to include that in the study. And that was the start of my first project. I did not have much experience in research, so with the help of my fantastic colleagues, the CF team and my supervisors, I enlisted as a PhD student, which I anticipated would increase the quality of the study. And then I had to get some more ideas…

The second project, regarding CF liver disease (CFLD), emerged when the radiology department acquired the equipment for liver elastography. It would have been favourable to have done a prospective study on it. Unfortunately, it was somewhat complicated in praxis, so instead, I decided to explore it retrospectively and try to ascertain if there were any hidden issues regarding the risk factors for CFLD that this method could help us identify, thereby evaluating whether the elastography could aid in the screening process which is not always straight forward.

Towards the end of my time at Lund CF Centre, we gained access to a new CF medication, the CFTR modulator lumacaftor-ivacaftor. Those were exciting times. It was the beginning of a new life for many children and adults with CF. The third project developed during those years when we gathered clinical information for the CF registry regarding this new treatment.

The last project was obviously not on the table initially but was initiated after I moved back home to Iceland. I was curious to know how CF had been diagnosed and treated in Iceland through the years and if there were any aspects we should approach differently.

When writing this thesis, I better understood what a unique disease CF is and how research on its pathophysiology and genetics has advanced medical research, even in other fields of medicine. With the miraculous medication of CFTR modulators, we are now experiencing more optimistic times than ever. However, we should remember that this treatment is not available to all. In some parts of the world, even the essential medication, much less expensive, is difficult or even impossible for people to access.

On Christmas Day, some years ago, I was doing the rounds at the Children´s Hospital in Lund. On that day, I got a glimpse back to the past when fundamental treatment for CF was scarce. I met this small boy with CF who had travelled a long way with his family in search of better health care for his CF to save his life. He instantly caught my heart with the warmest smile and hope in his eyes. To him and all the wonderful kids with CF I have met through the years, I humbly dedicate my drop in the ocean: this thesis.

Introduction

The milestones of Cystic Fibrosis

The discoveries

At a time of extraordinary medical advances, when we finally have a highly effective treatment for most people with CF (pwCF), it is intriguing to begin this thesis by reviewing some of the crucial milestones of CF. All these discoveries have paved the way to where we now find ourselves.

CF was first described in two children in 1936 by the Swiss physician Guido Fanconi, but it was Dr Dorothy H. Andersen, pediatrician and pathologist, doing autopsies on children previously thought to have celiac disease who effectively put it on the map in 1938. (1-3). Dr Andersen subsequently came up with the name Cystic fibrosis of the pancreas (3, 4). When reading the children 's medical charts, she found that they had all suffered from failure to thrive, steatorrhea from malabsorption, and lung infections (3, 5). Initially, the hypothesis was that the pancreatic damage leading to the loss of pancreatic enzymes accounted for such severe malnutrition that it would lead to serious pneumonia and bronchiectasis with bacterial infections, which often ended these children´s lives. Dr. Andersen also discovered a deficiency of pancreatic enzymes in these children and suggested that a replacement therapy would be beneficial (5, 6). The thick and sticky mucus filling the glandular ducts and the pancreatic enzyme deficiency were the focus of research in these early years, further trying to clinically distinguish these children from the ones who suffered from celiac disease. It became more apparent that children with similar gastrointestinal symptoms could be differentiated by normal versus abnormal pancreatic function, the latter being a fatal condition early in life where an autopsy would reveal the cystic fibrosis of the pancreas (6, 7). Several papers followed, which presented the role of trypsin in duodenal secretions and stool analysis as an indication of pancreatic dysfunction, proposing that the absence of tryptic activity suggested cystic fibrosis and much focus was on the mucus-filled ducts as the primary cause of the disease (6, 7). Dr Andersen and colleagues soon discovered that the absorption of fat-soluble vitamins was inadequate in these children; there were signs of biliary cirrhosis, enlarged liver, and abnormal glucose

curves (8). The clinical picture of CF was there, and pieces of the puzzle were starting to align.

In 1949, a young pediatrician, Paul di Sant'Agnese, made another revolutionary discovery that furthered the understanding of the disease, revealing that the problem was not only the mucus-filled ducts. During a heat wave in New York, he discovered that small children with 'cystic fibrosis of the pancreas' had a highly elevated sweat sodium and chloride, which made these children more prone to heat exhaustion (9). The subsequent pilocarpine iontophoresis technique of Gibson and Cooke offered a novel diagnostic test model that set the ground for diagnosing CF by sweat test (10). The method evolved through the years, and today, the macroduct method is considered the most reliable, where a sweat chloride value greater than 59 mmol/L is consistent with a diagnosis of CF (11). During these early years of discovering CF, researchers and clinicians soon began to suspect that the disease was inherited, and for some time, having a close relative with the disease was included in the diagnostic criteria (6).

Preclinical research progressed significantly in the 1980s when Dr. Welsh and colleagues discovered that the CF protein was a chloride and bicarbonate ion channel at the cell membranes on the surface of the epithelial mucosa (12). Further experiments revealed a more detailed function of the Cystic Fibrosis transmembrane conductance regulator (CFTR) and the fact that mutant CFTR trafficking can be corrected. These discoveries and many others led to advancements in partially restoring CFTR function (12).

The discovery of the CFTR gene in 1989 was not only an important milestone for CF but also a breakthrough in human genetics since it was the first disease-causing gene to be identified by positional cloning (13, 14). The work was led by Dr Lap-Chee Tsui and Dr Jack Riordan at the Hospital for Sick Children in Toronto in collaboration with Dr Francis Collins at the University of Michigan, who was later the long-term director of the National Institutes of Health. The sequencing of the CFTR gene allowed for a more refined diagnosis of CF and a better understanding of its protein product. Investigating the CFTR protein, its composition, and its mechanism has led to a better understanding of the disease pathophysiology. This has, in turn, driven the medical advances in CF treatment, bringing forth the CFTR modulators (15-17). A new era in CF has begun with these revolutionary medications, and the future has become much brighter for pwCF (18).

Cystic Fibrosis care

The formation of specific CF centres started in the 1950s when standardised surveillance of people with CF (pwCF) was initiated. The CF centres established the three pillars of CF care: nutrition, airway clearance, and aggressive treatment of respiratory infections, which have further developed through the years (19-21).

Nutritional challenges are common in CF, which has been known since the disease's discovery. A significant advancement occurred when enteric-coated microspheres containing pancreatic enzymes partially substituting pancreatic dysfunction became available in the 1980s (22). Adding fat-soluble vitamins and nutritional supplements has further improved growth, weight gain, and nutritional status (21).

An increased understanding of pulmonary exacerbations in CF and better ways to identify and treat them has improved the prognosis, and the treatment of lung infections soon became central in the clinical management of CF. In addition, the diverse airway clearance techniques and mucolytic agents came early in the history of the disease and coincided with the treatment of infections. Recombinant DNA technology made way for the development of human DNase, which proved effective in reducing pulmonary exacerbations (23). Various antibiotics have surfaced, including inhaled antibiotics and anti-inflammatory agents, further strengthening the force against lung deterioration (24-27).

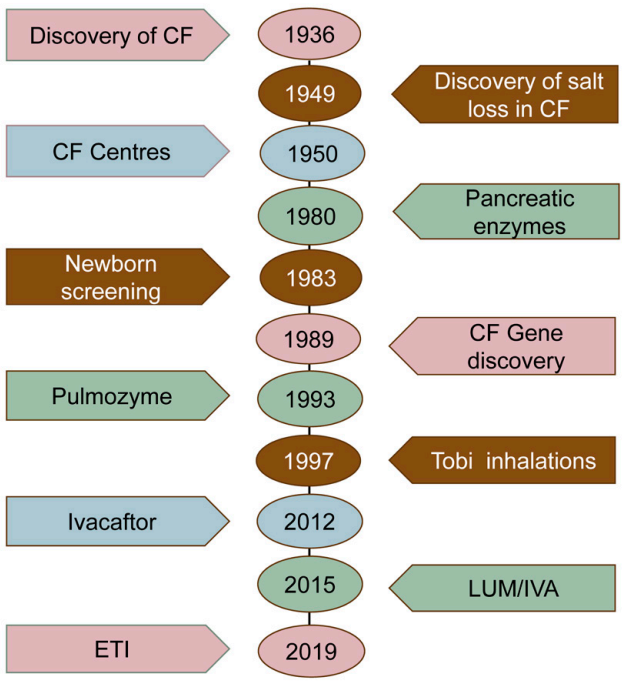


Figure 1. The Milestones of Cystic Fibrosis. Tobi=tobramycin; ETI= elexacaftor-tezacaftor-ivacaftor; LUM/IVA= lumacaftor-ívacaftor

The first pilot project of newborn screening (NBS) for CF started in 1983 in Wisconsin and Colorado, USA. The initial projects demonstrated that NBS for CF was both feasible and effective in identifying infants with CF before they became symptomatic (28-30). NBS has gradually become more widespread throughout the globe, and further research has been added to the beneficial outcome of NBS (31-36).

When Dr Andersen and Dr Fanconi first described CF in the 1930s, the median life expectancy of these children was less than a year. Today, the majority of pwCF are adults, and with recent treatment advances, a large proportion of the CF population may have a near-normal life expectancy (37). However, there is still work to do so that every individual with CF has the same treatment opportunities (38).

From genotype to phenotype

Finding the CFTR gene and protein were truly breakthrough discoveries. However, many questions still need to be answered. One of them, which clinicians often puzzle over, is why the presentation of the disease can vary so much between individuals with the same genetic variants (39).

Even though over 2000 CFTR gene variants are known, not all result in the CF. In the CFTR2 database www.CFTR2.org, there are 719 disease-causing variants listed and 49 of varying clinical consequences. The CFTR gene resides on chromosome 7; it is 250 kB long and encodes a protein of 1,480 amino acids (40). The CFTR is an anion channel within the cell membranes that controls the flow of anions such as chloride and bicarbonate through the cell membrane and regulates the epithelial sodium channel ENaC (12, 41).

The type of genetic variant determines the defect of the protein (6, 40). The conventional classification of gene variants in CF ranging from class 1 to 6 is presented in Figure 2 (42). Appreciating the effects of genetic variability has proved crucial in designing molecular treatments for CF. Class 1 variants are mostly nonsense or stop variants resulting in a non-functional protein. Class 2 variants are the most common ones. They cause protein misfolding, which prevents adequate trafficking, resulting in a decreased number of CFTR proteins with minimal function to reach the cell membrane. Class 2, F508del, is the most common CFTR variant, and 90% of pwCF bear at least one allele of this variant. Another example of a class 2 variant is the N1303K, which is much less common in most countries. Class 3 variants are often called gating mutations or variants since they cause a substantial reduction in CFTR channel opening time (42). The G551D variant is an example of a class 3 variant, and the first CFTR modulator in clinical practice, ivacaftor, targets the gating process (16). Class 4 variants are similar to gating mutations but affect channel conductivity instead of regulation. In class 5 variants,

there is decreased CFTR protein synthesis, resulting in a lower number but functional ion channels at the cell membrane. Class 6 variants affect the CFTR protein´s stability, leading to a functional protein being downregulated, resulting in fewer of them at the surface. A specific variant can hold elements that belong to different classes. The F508delta variant, for example, not only has the misfolding and failed trafficking (class 2) but also has a defective gating mechanism (class 3), and it is downregulated too quickly from the cell membrane (class 6) (22). Class 1-3 variants are considered more severe than class 4-6 variants. The minimal function variants of class 1-3 involve pancreas insufficiency and are more frequently associated with a heavier burden of the disease, faster decline in lung function, CF-related diabetes (CFRD), and CF-liver disease (CFLD) (22). However, on an individual level, the prognosis is multifactorial. The phenotype of CF cannot solely be explained by the genotype since individual variation regarding the clinical course varies substantially (43, 44). The degree of organ system involvement differs considerably among affected individuals holding the same genotype. Genetic modifiers and environmental and social factors probably contribute to some of the disease development and progression (40,45-51). Furthermore, the pathophysiology of some aspects of the disease, such as the evolution of CFRD, still needs to be better understood. Therefore, despite the tremendous success of new medications, there is still the need to examine every corner to increase knowledge, hopefully promoting new treatment options.

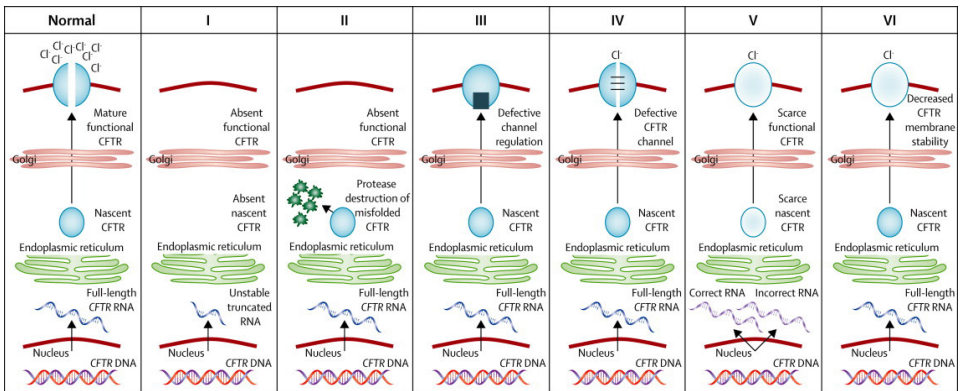


Figure 2. Classification of CFTR defects. From *Boyle and De Boeck* (42).

Cystic Fibrosis across the globe

It is estimated that at least 100,000 people have CF worldwide, and the incidence of CF is quite different across the globe (Figure 3) (52-54). CF is most common in populations with Northern European ancestry where the F508del variant predominates (55). Even within Northern Europe, the prevalence differs substantially between different countries. The median European prevalence is 0.737:10.000, with Ireland having the highest CF prevalence at 2.98:10.000 (55). In Sweden, the prevalence is at 0.7:10.000, but in the neighbouring country Finland, the prevalence is only approximately one-tenth of that (38, 56). In the USA, the overall prevalence is estimated at 1:8000, but it differs substantially between ethnicities (52, 57). CF is much less common in Africa and Asia, and less is known about the CF population in these continents (58, 59).

CFTR gene variant variability is found worldwide, and the F508del variant is less dominant in non-white populations (60-63). The prevalence of the F508del variant decreases from Northwest to Southeast Europe. All other variants are much less frequent, with fewer than 20 gene variants occurring at more than 0.1% worldwide frequency. In specific populations, it is possible to find a higher frequency for rare variants, and the diversity of genetic variants is increased in more southern countries (43, 64).

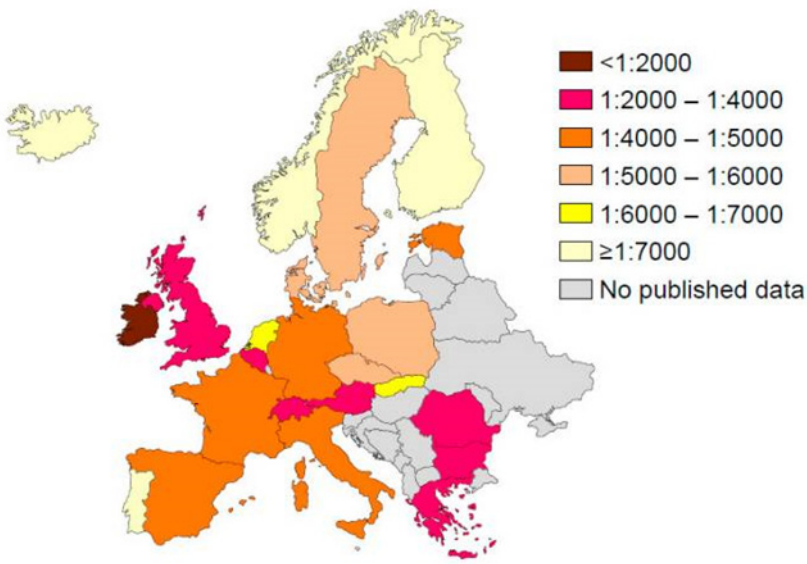


Figure 3. The incidence of CF in Europe. From *Scotet et al.* (65).

The diverse manifestations of Cystic Fibrosis

Cystic Fibrosis is a multiorgan disease, as shown in Figure 4. The most significant morbidity and mortality are caused by pulmonary decline, characterised by recurrent and chronic infections, bronchiectasis, and progressive respiratory impairment. Significant comorbidities occur in the pancreas, gastrointestinal tract, liver, upper airways, sweat glands, and reproductive organs. How these different comorbidities affect each individual can vary substantially. Some get diabetes and/or liver disease, while others do not. People with more severe variants are more prone to suffer from diverse complications. Still, at diagnosis, it is impossible to accurately predict exactly how things will turn out individually. The greatest focus always lies on the lungs, the most important determinant of life expectancy. The addition of CFRD and CFLD undoubtedly is of high clinical relevance both for quality of life and overall prognosis (66, 67). With the increased life expectancy of pwCF, particularly in light of the future effects of CFTR modulators, these comorbidities are increasingly important (38).

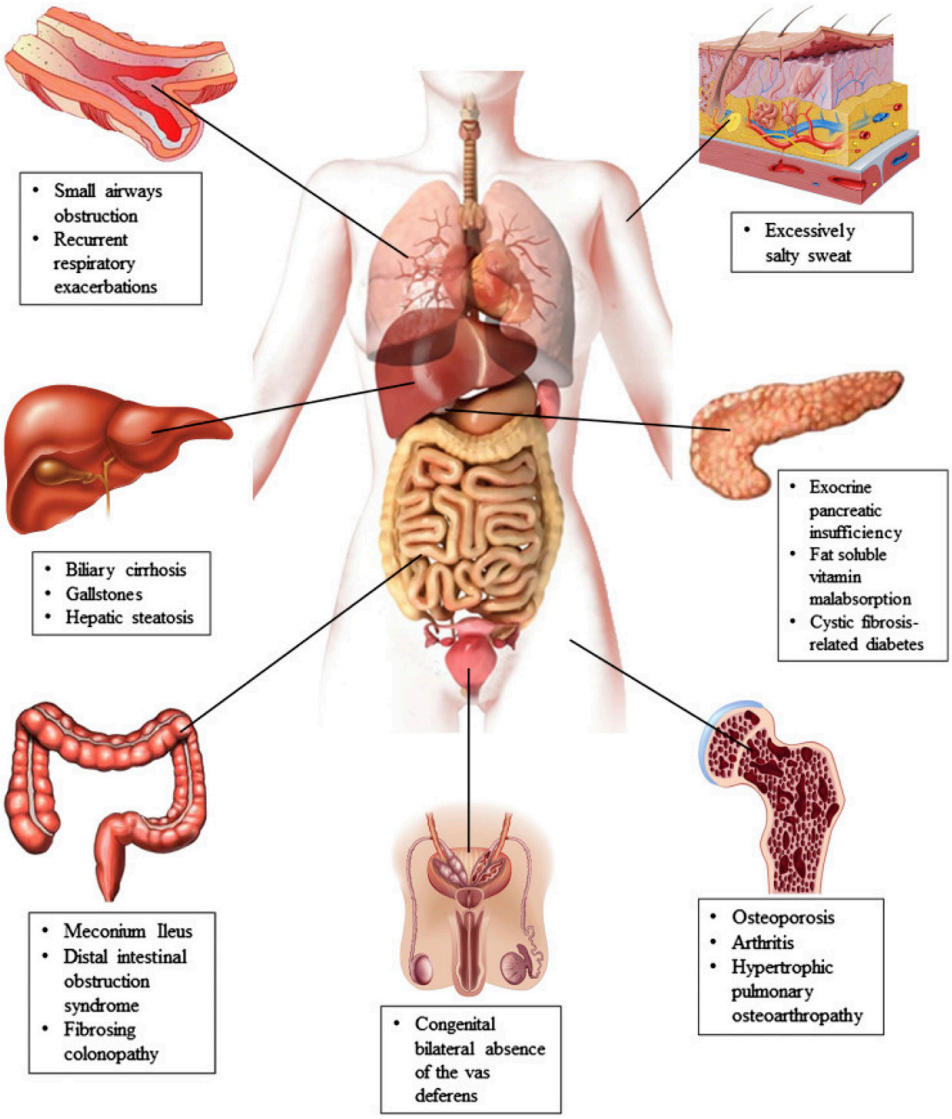


Figure 4. Clinical manifestations of Cystic Fibrosis. From *Molina and Hunt* (68).

The lungs

The lungs are a major focus in CF care, and lung health is the strongest parameter regarding morbidity and mortality. Lung involvement is present in CF from infancy, where structural changes in the airways have been demonstrated from early childhood, even before symptoms are unveiled (69). Recurrent and chronic infections, neutrophil inflammation, and thick, abundant mucus are characteristic of CF lung disease. Bronchiectasis develops over time, and recent research has revealed that this process even starts in young children (70-72).

Surveillance and aggressive treatment of airway infections are central in clinical management, together with interventional methods for airway clearance and lung function monitoring. *Staphylococcus aureus* is the most common airway pathogen in childhood; later in life, it is replaced in prevalence by *Pseudomonas aeruginosa*. Other important pathogens include *Burkholderia cepacia complex*, Achromobacter species, and nontuberculous mycobacteria (NTM). All these pathogens are known to accelerate the decline in lung function, as presented in Figure 5 (73). Some fungal infections, particularly the hypersensitivity reaction, allergic bronchopulmonary aspergillosis (ABPA), can also influence lung function and lead to structural damage (74).

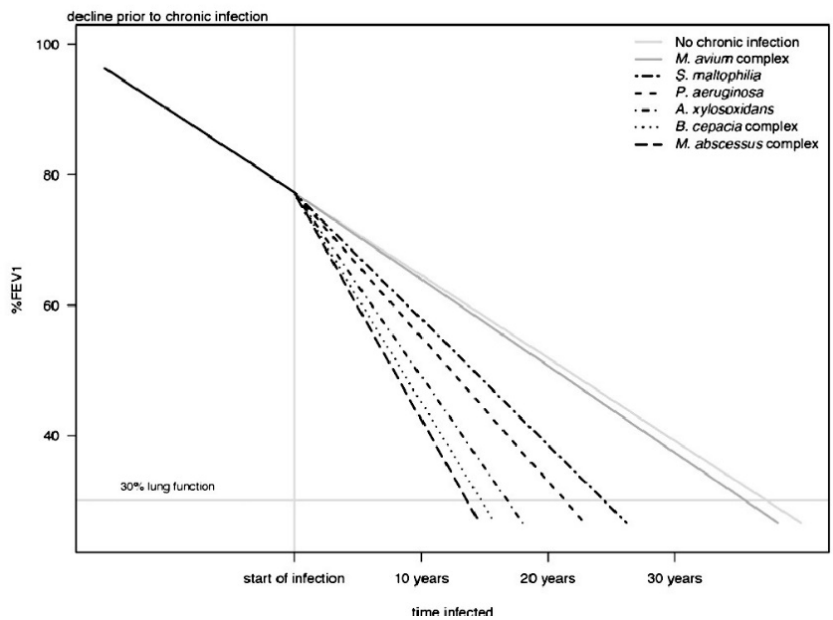


Figure 5. Different bacteria cause lung function decline at different rates. *Mycobacterium abscessus complex* has the most significant effect, followed by *B. Cepacia complex*, *A. xylosoxidans,* and *P. aeruginosa.* From *Qvist et al.* (73).

Lung function measurements are performed regularly, where spirometry is the most conventional method (20). In recent decades, multiple breath washout (MBW) has proved valuable for lung function monitoring, especially in children (75, 76).

Lung function decline in CF is influenced by other factors besides infections. Pancreas insufficiency and compromised nutritional status are associated with worse lung function. CFRD and even abnormal glucose tolerance are also well-known risk factors in this respect, and women tend to have a faster decline in lung function than men (67, 77-80). The association between lung function and CFLD is a matter of discordance. Some research indicate a relationship between these two components of CF, while others do not (66, 81, 82).

Cystic Fibrosis related diabetes

After years of fighting infections, managing all kinds of treatments for the airways, performing the daily routine of inhalations and airway clearance, coping with digestive problems, and still living life with friends and family, the blow of getting a diagnosis of diabetes on top of everything else can indeed be extremely hard. Having CFRD adds a whole new load to the daily treatment with the addition of glucose controls and subcutaneous insulin administration.

CFRD is a common co-morbidity associated with CF. CFRD affects approximately 1-2% of children <10, 10-20% of the age group between 10 and 20, and 40-50% of adults with CF (67, 83). CFRD is an important prognostic factor of morbidity and mortality in CF (84). It is associated with inferior lung function, lower body mass index (BMI), female gender, vitamin D insufficiency, previous ABPA, and liver disease (79, 85, 86).

CFRD is different from both type 1 and type 2 diabetes. The pathophysiology of CFRD is not entirely understood, and a multifactorial aetiology is probable. A diagram of the proposed pathogenesis of CFRD is presented in Figure 6. The cardinal sign of CFRD is reduced insulin secretion. The inborn and continuous fibrotic destruction of the exocrine and endocrine pancreas is thought to lead to a slower and diminished insulin release over time. The islets can be smaller from the beginning, and the islet mass further diminishes over time, affecting insulin secretion (87). Research over the last decades has indicated that islet-intrinsic defects are also present in CF (88). The CFTR is expressed to some extent in the β and α cells of the pancreas, which can affect insulin exocytosis and the mechanism of the controlling hormones glucagon and somatostatin (88-91). However, the relevance of these findings is still debated (92). In addition, chronic inflammation in CF and inflammatory cytokines could be involved in the deterioration and function of the β cells (93). As with other comorbidities in CF, some genetic modifiers are known to influence the development of CFRD, some of which are linked to type 2 diabetes (47). A family history of type 2 diabetes increases the risk

of CFRD (94). The complex pathogenesis of CFRD likely involves, to some extent, all of these aspects mentioned above: fibrosis, cell loss, and dysfunction with the addition of genetic and inflammatory co-factors.

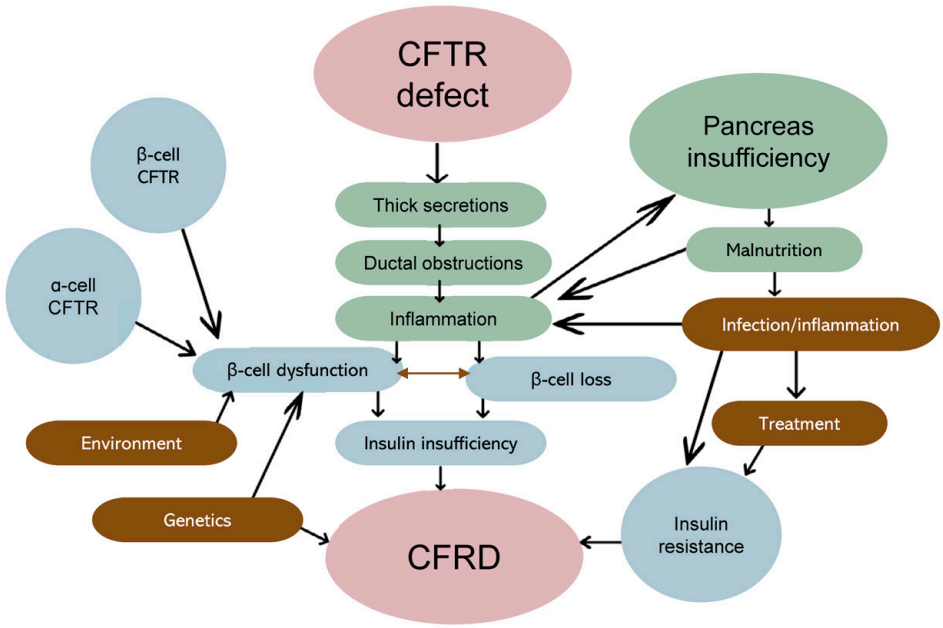


Figure 6. A schematic diagram demonstrating the complex pathogenesis currently associated with CFRD. Adapted from *Granados et al.* (94).

The insidious onset of CFRD is a central element of its development. If screening should not be practised, then the diagnosis of CFRD would be delayed by approximately eight years (95). Guidelines of the European Cystic Fibrosis Society (ECFS) and the CF Foundation (CFF) state that all CF patients, from the age of 10, not having been diagnosed with diabetes, should be screened for CFRD using the standard WHO protocol for oral glucose tolerance test (OGTT) (20, 96). Although CFRD is rare before ten years of age, abnormal glucose metabolism is present from infancy, and CFRD can actually occur at any age. Therefore, some CF centres begin screening before age 10, and recent guidelines from Italy recommend screening for glucose alterations from at least six years of age (97-100).

The OGTT is the gold standard for CFRD screening. The OGTT is performed during clinical stability by oral administration of 1,75g/kg dextrose (maximum dose 75g) after overnight fasting, and blood glucose levels are measured at 0, 30, 60, 90, and 120 minutes.

CFRD is defined as fasting blood glucose ≥ 7 mmol/L (126 mg/dl) and/or a 120-minute blood glucose value ≥ 11.1 mmol/L (200 mg/dl). An impaired glucose tolerance (IGT) is defined as fasting blood glucose < 7 mmol/L (126 mg/dl) and a 2-hour blood glucose value ≥7.8 mmol/L (140 mg/dl), but <11.1 mmol/L (200 mg/dl). Indeterminate glycemia (INDET) is a term used in CF for fasting blood glucose < 7 mmol/L (126 mg/dl) and a 120-minute blood glucose value of < 7.8 mmol/L (140 mg/dl), but at least one intermediate blood glucose value ≥ 11.1 mmol/L (200 mg/dl). Abnormal glucose tolerance (AGT) is the collective term of CFRD, IGT, and INDET (94).

A CFRD diagnosis is made if the diagnostic criteria are met on two separate OGTTs or one OGTT if both fasting and 2-h values are pathological. CFRD diagnosis can also be made by one diabetic result from an OGTT and an HbA1c > 48mmol/mol (6.5%). Other criteria apply during enteral feeding and acute illness, including systemic steroid treatment when an OGTT is not applicable.

Although the mid-OGTT values may not be measured in all centres, they are generally considered important to include in CFRD screening. There is some evidence that the mid-OGTT glucose levels (INDET) may be even more predictive of clinical decline than the 2-h level regarding lung function, and the combination of IGT and elevated intermediate glucose values is a strong predictive indicator of evolving CFRD (78, 101).

An impaired glucose tolerance precedes the CFRD diagnosis, and pwCF can have IGT and diabetic glucose levels periodically, long before the definite CFRD diagnosis. They can move from one category to another for many years before the CFRD diagnosis becomes established. Periods of hyperglycemia are often associated with pulmonary exacerbations and system steroid treatment (102). Nutritional treatment via gastrointestinal tube, puberty, and pregnancy are also circumstances when special attention is needed (95).

Several studies have demonstrated a clinical decline in the years before the diagnosis of CFRD, both in terms of growth and lung function (79, 100, 103). Even in infants, there is evidence of an association between abnormal glucose metabolism and pulmonary inflammation (104). However, it is unclear if intensified glucose control can prevent these worsening clinical parameters. Some have tried initiating insulin therapy earlier, but data on the benefits of this strategy are limited (100, 105).

Although OGTT is the standard CFRD screening method, other alternatives for screening have been sought. The OGTT is a challenging process for the individual and is limited by sensitivity, specificity, and reproducibility (106). Up to one-third of pwCF do not adhere to the annual OGTT screening protocol. Therefore, some CF centres have used continuous glucose monitoring (CGM) more frequently for diagnostic workup and surveillance (107). CGM has been validated for children and adolescents with CFRD, but there are no official clinical guidelines concerning CGM use for the screening of CFRD. CGM has the advantage of examining

glycemic control in real-life settings and offers data on interstitial glucose levels with a sensor placed on the subject's arm (108). However, its concordance with OGTT is not straightforward, and the findings of CGM cannot substitute the OGTT in diagnosing diabetes (109, 110).

Glycemic variability and hyperglycemia measured by CGM correlate with worse nutritional status and lung function in people with CFRD (111). Furthermore, glucose abnormalities demonstrated by CGM are associated with impaired lung function in pwCF without CFRD (112, 113).

The HbA1c has, in general, been considered unreliable in screening for CFRD, and previous research is somewhat conflicting. Technical variations may explain the discrepancy to some extent (114). There may be some situations where the HbA1c can be of value in the diagnosis or as a complementary method in the diagnostic process of CFRD and AGT, especially when people are reluctant or unable to participate in the conventional screening (115-117). Recent studies have proposed that an HbA1c below 37-40 mmol/mol (5.5-5.8.%) is consistent with a very low risk of CFRD. This approach might sometimes allow a stepwise screening protocol (95, 114).

With the ageing population of pwCF, not only the pulmonary and nutritional consequences of CFRD are of issue, but also the more traditional diabetes complications. Understandably, few papers have addressed this issue, but microvascular complications of diabetes will probably soon become a more imminent problem for people with CFRD (118).

Cystic Fibrosis liver disease

The liver is one of the vital organs affected by CF. The spectrum of liver involvement is wide, with the most severe form having dire consequences. Interestingly, CFLD is primarily a childhood diagnosis with a continuing path to adulthood. Therefore, screening for CFLD starts early in life, and it is an important aspect of CF care (119). A lack of specific and sensitive screening tools and different classifications of CFLD complicates its diagnosis.

Liver disease is an early co-morbidity of CF, with a cumulative incidence of 27-35%. It is most often, but not exclusively, diagnosed in childhood, with the median age of diagnosis of ten years old (66, 120). Many individuals develop signs of liver abnormalities, including hepatomegaly, steatosis, and elevated liver enzymes, and 5-10% of children with CF develop clinically significant liver disease with multilobular cirrhosis and portal hypertension (PH) (120, 121). Because of the diverse liver involvement in pwCF, there have been difficulties in clearly defining CFLD (122, 123). The term CFLD has been applied for a broad spectrum of symptoms and findings ranging from neonatal cholestasis, elevated liver enzymes, gallstones, and gallbladder abnormalities to the most severe multilobular cirrhosis

that can lead to PH. The North American CFF distinguishes between CFLD and CF liver involvement (CFLI). The former includes cirrhosis with or without PH, and the latter is without cirrhosis and PH (124, 125). *Debray et al*. (119) and *Koh et al*. (126) have presented other diagnostic criteria. The three different criteria are shown in Table 1.

Table 1. The three classifications of Cystic Fibrosis liver disease. Based on *Kamal et al*. (125)

	CF Foundation		**Debray criteria**	**Koh criteria**
	CFLD + cirrhosis +/-PH[3]	CFLI[2] - cirrhosis - PH	CFLD	CFLD
Physical exam	X		X	
Biochemistry		X	X	X
Histology	X	X	X	X
Imaging	X	X	X	X
Elastography				X
Noninvasive Biomarkers [1]				X
Laparoscopy	X			

1. Biomarkers are the APRI = the AST-Platelet-Ratio Index , FIB-4= Fibrosis 4 Index, and AAR =AST to ALT Ratio. **2.** CFLI = CF liver involvement; **3.** PH=portal hypertension. **CFF criteria:** CFLD is present if there is evidence of cirrhosis +/- PH based on clinical examination, imaging, histology, or laparoscopy. CFLI is when at least one of the other categories is abnormal: persistently or intermittently elevated AST, ALT, GGT >2x upper limit, steatosis or fibrosis on biopsy, cholangiopathy by imaging, or ultrasound abnormalities inconsistent with cirrhosis (124). **Debray criteria:** CFLD should be considered if at least two of the four categories are abnormal (119). **Koh criteria:** CFLD is present when there is radiological or histological evidence of cirrhosis or diffuse liver disease or when two of the other three categories are abnormal, i.e., biochemistry, elastography, and the noninvasive biomarkers APRI, FIB-4, and AAR (126).

The pathognomonic histopathological liver lesion of CF is periportal biliary cirrhosis. It is often subclinical and without alterations in biochemistry and ultrasound. The CFTR is localised in the apical surface of the bile duct epithelium and not in the hepatocytes (127). The pathophysiology of CFLD is not entirely understood. One proposed pathway is that the defective CFTR promotes alkalinisation and dehydration of bile acids, leading to inspissated bile in the small bile ducts with plugging and subsequent inflammation and periportal fibrosis (128). However, focal biliary cirrhosis does not necessarily lead to multilobular cirrhosis (124). When multilobular cirrhosis affects the liver, it becomes hard and nodular, and it can become enlarged, often leading to PH. Once PH is present, splenomegaly and esophageal varices can jeopardise the affected individual's life. Controversially, there are reports on pwCF presenting with PH without previous cirrhosis presumed to be due to obliterative venopathy, which seems to be a different presentation of the disease (129, 130).

CFLD has been associated with severe genotypes, a history of meconium ileus, malnutrition, CFRD, and male sex (66, 120). Environmental factors and genetic

modifiers may influence the evolvement of CFLD, and those who are heterozygous for the α-1 antitrypsin (SERPINA1) Z allele have an increased risk for developing CF-related cirrhosis (131).

CFLD is of high clinical relevance, especially with the increasing survival of the CF population. CFLD seems to increase both morbidity and mortality, which is becoming more evident with decreasing mortality from extrahepatic causes (132, 133).

There is no effective treatment for CFLD. The ursodeoxycholic acid is most widely used and recommended in guidelines (119), but its clinical benefit is not entirely convincing (133, 134). A liver transplant is the end-stage treatment, and it is considered when PH and esophageal varices become present (135).

CFLD is often challenging to diagnose initially, and pwCF rarely present with symptoms or signs of hepatobiliary disease such as jaundice (128). Annual screening is recommended, which usually consists of abdominal examination, biochemical evaluations, and ultrasound of the liver and the spleen, with the addition of further investigations when indicated (119). Routine liver biochemistries alone are unreliable indicators of cirrhosis or the risk of developing cirrhosis. There is evidence that ultrasound investigations are more sensitive for detecting CFLD than biochemical measurements, and combining ultrasound and a biochemical work-up is often warranted (136). Although liver biopsy has represented the gold standard for diagnosing liver fibrosis, it can be less reliable in CFLD because of the uneven distribution of lesions. Furthermore, a liver biopsy is an invasive procedure and not without complications (119). Therefore, additional methods for the evaluation of CFLD have been explored. One of these methods is elastography, which measures the tissue stiffness or elasticity (137). Different types of elastography are on the market, for example, magnetic resonance elastography and acoustic transient elastography (TE or FibroScan) (137-140). The 2D Shear Wave Elastography (2D SWE) is another method for evaluating tissue stiffness. It is provided as an application on various ultrasound systems, and with this technique, visual localisation of the organ is present, in contrast to methods such as TE (137). Several other noninvasive measures have been applied to the diagnostic workup and for research purposes. These include the AST to ALT Ratio (AAR) (141), the AST-Platelet-Ratio Index (APRI) (142) and the Fibrosis-4 Index (FIB-4) (143). These are sometimes used in combination with other screening methods. Often, the screening of CFLD involves multiple examinations and re-evaluations. Individual and site-specific differences can account for the wide variation in the diagnostic workup.

Other liver involvements in CF without cirrhosis and PH include steatosis, cholestasis, cholangiopathy, and gallbladder disease. Hepatic steatosis is quite common in CF, with a prevalence of 23-75% of the CF population. However, the more severe form is becoming less frequent because of better nutrition and earlier diagnosis. Hepatic steatosis is considered a benign complication of CF and generally

does not lead to cirrhosis (124). Neonatal cholestasis is the earliest manifestation of liver involvement in CF and presents with prolonged jaundice in the newborn. However, this presentation is rare, affecting less than 2% of infants with CF. Meconium ileus is a known risk factor for cholestasis and cirrhosis development later in life (120, 144). Gallbladder abnormalities are found in 24-50% of pwCF, including microgallbladder, gallbladder dysfunction or distention, and gallstones (124, 145). It is clear that liver involvement in CF is quite common and can become present in all stages of life, although the most crucial time for CFLD screening is in youth. The optimal way of CFLD screening is yet to be discovered.

The importance of teamwork

When a child gets diagnosed early in life, there is initially much focus on nutrition, with enzyme supplements and close follow-up. The airway clearance and surveillance of infections is a parallel process that, over time, becomes increasingly imminent. As the child grows, various infections of different calibres occur, requiring additional treatment strategies. Thereafter the screening for other complications, such as CFLD and CFRD, begins; in most cases, things start piling up. Periodically, the road can be uneventful from CF perspective, but there are always many hills ahead, and some of them are quite steep. The CF care must meet all these factors, and it is not only about the disease. CF care is multi-factorial; it also involves psychosocial and everyday aspects and changes through the different phases of life.

Due to these complexities in CF, there is often a firm liaison between caregivers, researchers, pwCF, and their families. The CFF in the USA advocates and financially supports research and acts as a network for pwCF in the USA and their families. The CFF was active in establishing CF centres that are now present in most Western countries. The CFF, the ECFS, and numerous national CF organisations are essential in introducing clinical guidelines and promoting standardised CF care (11, 146). The multi-disciplinary team of the CF centres is recommended to consist of doctors and nurses, microbiologists, physiotherapists, dietitians, pharmacists, psychologists, social workers, clinical geneticists and allied healthcare professionals, all experienced in CF care (147). Outcomes for patients cared for in specialist CF centres are better than for those who are not; therefore, specialised teamwork in CF care is crucial (148). National patient registries serve as quality control of CF care and constitute a backbone in clinical research. The European CF Registry (ECFR) was founded and run by the ECFS. The first pilot report was published in 2003 with data from the seven pioneering countries (149). Since then, the work has continued and grown, with new members joining every year, and the CF community is still growing. CF care has many aspects and follows the individual throughout life. It is a unique chain of cooperation with the individual at the centre.

Aims

The overall objective is to describe the different methods available for screening the co-morbidities CFRD and CFLD and to understand how the various aspects of CF influence each other.

Specific aims are the following:

- To investigate the relationship between OGTT and CGM in children with CF
- To explore the association of lung function, examined by both spirometry and multiple breath washout, and blood glucose abnormalities.
- To evaluate the effects of lumacaftor-ivacaftor on glucose tolerance
- To examine the correlation of liver stiffness to other indications of CFLD.
- To investigate the relationship between liver stiffness and nutritional status, lung function, and glucose tolerance.
- To determine the prevalence of CF in Iceland.
- To describe the CFTR variants present in pwCF in Iceland.
- To explore lung function, respiratory infections, complications, treatment, and the structure of CF care in Iceland.
- To describe the strengths and weaknesses of CF care in Iceland.

Population and methods

Population

Paper I and II: The CF centre in Lund is one of four CF centres in Sweden. The pediatric part of the centre cares for approximately 65 children and adolescents. Paper I presents a comparative cohort study performed at Lund Pediatric CF Centre in 2019, where children aged 7-18 were prospectively recruited to compare OGTT and CGM. Paper II presents a retrospective cohort study from the same CF centre. The individuals included had been evaluated for CFLD by ultrasound and 2D SWE between 2018 and 2020.

Paper III: The Swedish National CF Registry holds information from all four CF centres in Sweden: Gothenburg, Lund, Stockholm and Uppsala. The paper presents a cohort study from the registry. Individuals included in the study were receiving treatment with LUM/IVA and had done an OGTT before and during treatment.

Paper IV: This paper presents a retrospective description of the Icelandic cohort of pwCF, which includes all Icelandic individuals with a confirmed CF diagnosis between 1955 and 2021.

Methods

The Swedish National Cystic Fibrosis Registry was a data source for papers I-III concerning age, specific CFTR variants, height (z score), weight (z score), bacterial colonisation, serum calcifediol (vitamin D), and lung function results, including forced expiratory volume in 1-second percent predicted (FEV1pp) and lung clearance index (LCI). Furthermore, results from the OGTTs and the LUM/IVA initiation date for paper III were extracted.

The oral glucose tolerance test was performed according to WHO guidelines. Blood glucose levels were measured in mmol/L at 0, 30, 60, 90, and 120 minutes, along with baseline HbA1c in mmol/mol.

OGTT classification:

CFRD: fasting blood glucose ≥ 7 mmol/L OR at 120-minutes ≥ 11.1 mmol/L

IGT: fasting blood glucose < 7 mmol/L
+ at 120 minutes 7.8 - 11.0 mmol/L

INDET: fasting blood glucose < 7 mmol/L AND at 120 minutes < 7.8 mmol/L
AND at least one intermediate blood glucose value ≥ 11.1 mmol/L

The continuous glucose monitoring was conducted with the Freestyle libre® equipment. A sensor was applied on the subject´s upper arm, and the participants were asked to scan the sensor with their monitor minimally every 8 hours, when possible, for 14 days, provided the sensor stayed in place. The term intermittent scan CGM (isCGM) is used because the user intermittently scans the sensor. The isCGM data were downloaded to the Diasend® net application. Data collection from the download included the total number of days registered, the total number of measurements, the daily number of measurements, average interstitial glucose values in mmol/L, maximal and minimal values of interstitial glucose in mmol/L, and the standard deviation (SD). The number of measurements ≥ 11.1 mmol/L from each analysis was counted, and the proportion of measurements > 8 mmol/L was calculated.

Lung function was provided by spirometry and MBW measurements**.** FEV1pp values were obtained via spirometry using the Global Lung Function Initiative equations. FEV_1 and the rate of FEV_1 decline are the most significant predictors of mortality in pwCF. The FEV_1 measures airflow limitation, and it is one of the most frequently used endpoints in clinical studies on CF (150).

MBWs were performed on Exhalyzer-D with three consecutive measurements per individual, resulting in an average lung LCI. The LCI represents ventilation inhomogeneity. Evidence shows it is a more sensitive outcome measure than FEV_1 and correlates better with structural lung changes, especially in younger individuals (151).

A questionnaire was provided in study I. After completing both OGTT and isCGM, the children could answer a questionnaire regarding the different methods. The children were asked to grade the OGTT and the isCGM experiences and the ability to remember the sensor‘s scanning, using the scale: (1)very easy, (2) easy, (3) neither easy nor difficult, (4) difficult or (5) very difficult. They also had the opportunity to write their own comments. (Appendix 1)

The impact of lumacaftor-ivacaftor on glucose tolerance was retrospectively evaluated in paper III**.** Data was extracted from the Swedish CF Registry for individuals who started treatment with lumacaftor-ivacaftor in 2018 and 2019 and who had performed an OGTT before and during treatment with the medication.

Exclusion criteria were a previous CFRD diagnosis. The extracted data included age, gender, the start date for lumacaftor-ivacaftor treatment, OGTT results of 0,60 and 120 minutes, OGTTs‘ investigation dates and FEV1pp and HbA1c results before and during LUM/IVA treatment. The results of the OGTTs at baseline, within three months of treatment start, and the subsequent follow-up or annual assessment, including an OGTT, were compared.

Liver disease screening included biochemistry measures of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Glutamyl transferase (GT), ultrasound, and 2D SWE of the liver. An ultrasound is performed yearly from age five at the CF centre as a part of the annual assessment. 2D SWE was added to the CFLD screening at the centre in 2017. Pediatric radiologists performed the ultrasound, and at the same visit, further evaluation of the liver was done using the 2D SWE from Canon/Toshiba Aplio i70. The results from the pathology assessments were registered in those who had undergone a liver biopsy.

Statistical analysis was performed using IBM SPSS Statistics versions 26, 27 and 29 (IBM Svenska AB, Stockholm, Sweden). Correlations between isCGM and OGTT were calculated using Pearson’s correlation test. Continuous data were analysed for normality of distribution. If normally distributed, the independent samples t-tests and paired t-tests were used. Otherwise, a Mann-Whitney U test was performed. The Kruskal-Wallis test was used to compare more than two independent variables. The results of these analyses are presented as mean (SD) or median (range), respectively. The level of significance was set at a p-value ≤ 0.05.

Registered data of paper IV

A medical records search for CF diagnosis codes in Iceland for 1955-2021 was performed. Furthermore, data from an unpublished Icelandic CF study, which identified some of the study's participants, was available.

The categories of variables are demographic data (year of birth, gender, and age of death), symptoms at diagnosis, diagnostic data of sweat tests and genetic analysis, results of spirometries, microbiological culture results and antibody testing, variables related to nutritional status and growth, CF-related complications, treatment information, and follow-up data.

Lung function from spirometries for 2011-2021 was extracted for FEV1pp and FVCpp, age at first spirometry, and the number of yearly tests.

Chronic infections were defined according to Leeds criteria as > 50% of positive cultures for the same pathogen in the preceding 12 months.

Statistical analysis was descriptive with the use of Microsoft Excel and R Studio. Median values (range) are presented for descriptive purposes. Information regarding population numbers in Iceland was obtained from Statistics Iceland Hagstofa Íslands (www.hagstofa.is).

Ethical considerations

CF is a chronic progressive disease, and the burden and complications of the disease increase with increasing age. CFRD can occur at any age, and screening for diabetes usually begins at age 10. Young children are unaware of this complication, but as soon as the screening starts, it most certainly becomes real. We examined lung function in relation to abnormal glucose tolerance and compared two different methods of CFRD screening in children from the age of 8, both of which are used in clinical practice. As a result of this clinical research project, we focused on this challenging complication, CFRD, earlier than before, and our children and families became more aware of CFRD. This could be an ethical consideration in that this might put stress and anxiety on the families earlier, making this complication more real to them. An essential part of pediatrics is letting children be children as long as possible; that is to say, we want to shield them from adult problems and worries as long as possible. That task can be challenging when working with children suffering from severe and chronic diseases. In our study, we sought to investigate the children´s own perspectives on the matter why the first study included a questionnaire for them to answer regarding how they felt about the two different screening methods. In that way, we could get a hint of negative experiences if such occurred and try to find an approach to address them.

Another ethical consideration of the studies is that some of the participants were my patients. The families depend on their caregivers, the CF team, which could influence their study participation. In designing the study, we decided not to put an extra burden on the children, and all the prospective study´s objectives were part of their routine visits. When designing clinical research, I discovered this is an important aspect. From the clinician´s point of view, I know that my children with CF already have a particular disease burden, and adding to that burden with various new investigations was not an option. However, from the researcher´s point of view, a research project can be challenged if your feelings for the participants influence the study design negatively. The ethical aspect regarding research and the doctor-patient relationship has two sides. Not only is the patient dependent on their doctor, but the doctor also cares for the patient differently than a researcher who does not know the patient.

The local ethical review boards approved all studies: Paper I-II (#2018/54), Paper III (#2019-00417), and Paper IV (VSN-21-007 and 53/2020).

Results and discussion

Glucose tolerance in Cystic Fibrosis. Paper I and III

Oral glucose tolerance test versus continuous glucose monitoring

Thirty-two children and adolescents provided data on 35 isCGMs and 36 OGTTs. Twenty-eight participants provided 33 measurements of both isCGM and OGTT; for each participant, one OGTT and one isCGM were used for correlation calculations.

Table 2. Demographic and clinical data of the 32 participants from *Elidottir et al.* (152)

Age years, median (range)	11.5 (7-16.3)
Female (%)	14 (43.75)
Homozygous F508del n (%)	24 (75)
FEV1pp, median (range)	87.35 (57.7-120.9)
LCI, median (range)	8.26(5.78-14)
Height z-score, median (range)	-0.63 (-2.99-1.8)
Weight z-score, median (range)	-0.51 (-2.95-1.19)

A high proportion of the participants investigated with OGTT, or 21 individuals (67.7%), had AGT. Two (6.5%) had diabetogenic results (DG), seven (22.5%) had IGT, and INDET was found in another twelve (38.7%).

Likewise, the isCGM showed a relatively high proportion of measurements with interstitial glucose values above 8 mmol/L and a high number of peak values per day above 11.0 mmol/L. The median percentage of measurements >8 mmol/L was 11.3% (0.6-31.6). All but two individuals had at least one glucose peak above 11 mmol/L, with a median of 0.5 daily peaks (0-3.6).

These results present a relatively high proportion of glucose abnormalities both in OGTTs and CGMs. It could partially be explained by the fact that all participants in our study population have a class 1 or a clas 2 CFTR variant in each allele and are more likely to have a severe phenotype. Glucose abnormalities are prevalent in CF, including the pediatric population. INDET values and CGM, which are included and presented here, can have the advantage of providing more subtle changes in glucose tolerance. These results could also reflect the importance of abnormal

glucose tolerance in CF and not only the diagnosis of CFRD since the vast majority in this sample have AGT. Furthermore, different approaches to CFRD screening seem to capture more glucose abnormalities.

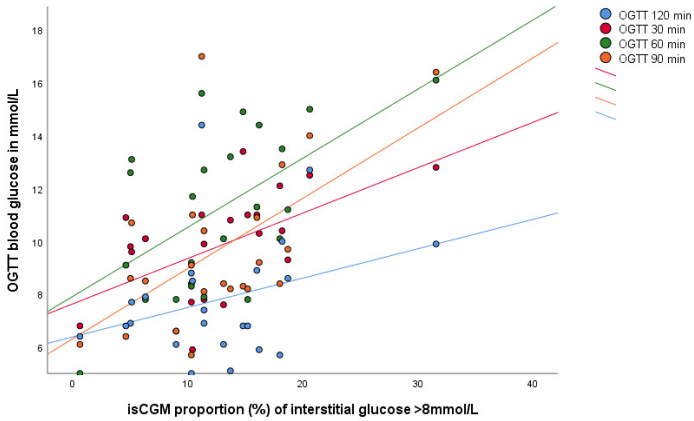
Table 3. Results of isCGMs and OGTTs (number) from *Elidottir et al.* (152).

Measurements	Median	Maximum	Minimum
isCGM total number of measurements (29)	1012	2404	488
isCGM number of measurements per day(29)	69.5	114	41
isCGM glucose peak value mmol/L (29)	13.6	17.2	9.4
isCGM percentage of glucose measurements >8 mmol/L (29)	11.3	31.6	0.6
isCGM daily average number of glucose peak values >11 mmol/L (29)	0.5	3.6	0
isCGM standard deviation (29)	1,4	2,3	1,1
isCGM number of days monitored (29)	14	15	6
OGTT fasting blood glucose in mmol/L (31)	5.4	8.5	4.4
OGTT 30 min blood glucose in mmol/L (26)	9.5	12.5	5.9
OGTT 60 min blood glucose in mmol/L (29)	11.1	16.1	5.0
OGTT 90 min blood glucose in mmol/L (24)	9.5	17.0	5.7
OGTT 120 min blood glucose in mmol/L (31)	7.5	14.4	5.0
HbA1c mmol/mol (28)	36	43	32

There was a statistically significant correlation between the percentage of glucose measurements above 8mmol/L in the isCGMs and elevated blood glucose levels found at 30 minutes (p=0.005), 60 minutes (p=0.007), and 90 minutes (p=0.003) in the OGTTs (Figure 7 a). Furthermore, a statistically significant correlation between the average number of peak glucose values above 11.0 mmol/L in the isCGMs and elevated blood glucose levels found at 60 minutes (p=0.035) and 90 minutes (p=0.005) in the OGTTs was found (Figure 7 b).

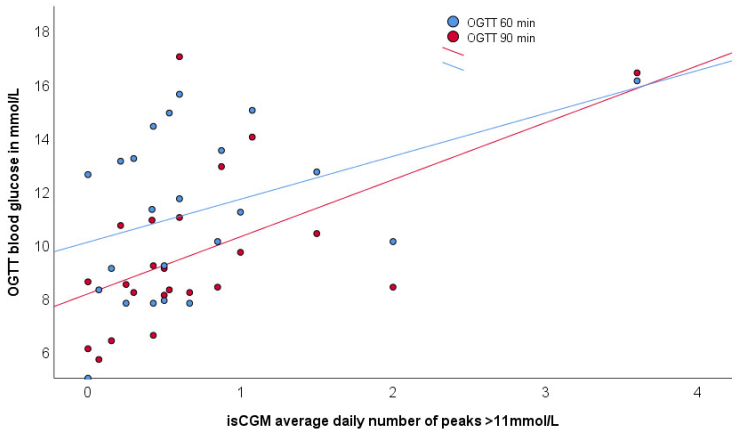
Interestingly, there were no correlations between the isCGM results and 2-h values from the OGTTs. This could indicate that the intermediate glucose values from the OGTT correspond more to isCMG glucose elevations than the 2-h value. Furthermore, the lack of correlation between isCGM and the 2h value from the OGTTs suggests that CFRD diagnosis could be missed if only CGMs were used in CFRD screening.

A



Correlations between the percentage of measurements above eight mmol/L during isCGM and OGTT blood glucose values in mmol/L at 30 minutes r(25)= 0.546, p= 0.005; 60 minutes, r(26)= 0.515, p= 0.007; 90 minutes r(23)= 0.585, p= 0.003; and 120 minutes r(28)=0.311, p=0.1.

B



Correlations between the daily number of peaks of interstitial glucose values above 11 mmol/L during isCGM and the glucose values from the OGTT at 60 minutes r(26)= 0.415, p= 0.035, and at 90 minutes r(23)= 0.561, p= 0.005.

Figure 7. Correlations between the OGTTs and isCGM results from *Elidottir et al.*(152).

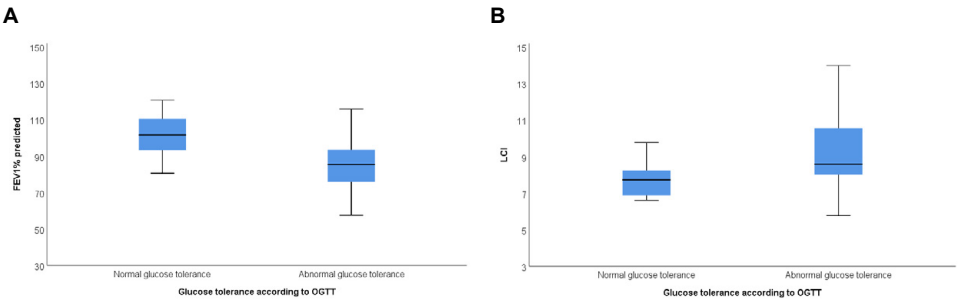
Glucose tolerance and lung function

Data from 32 children at Lund CF Centre were used to explore lung function in relation to glucose tolerance. INDET values on OGTTs and the average peak glucose above 11 mmol/L on isCGM were most significantly associated with lower lung function values.

Spirometry and MBW demonstrated lung function differences between individuals with AGT and NGT. There was a significant difference in lung function between children with NGT and AGT, both by LCI (p= 0.022) and FEV1pp (p=0.008) (Figure 8). Participants having INDET had a significantly lower FEV1pp (p=0.01) and a higher LCI (p=0.043) than children with NGT. This result indicates a relationship between the mid glucose values of the OGTT and lung function. The same difference was not seen in regards to IGT and CFRD, but the smaller number of children in these groups may involve some explanation for that.

The association between the results of the isCGM and lung function was not as strong as the OGTT counterparts. There was a significant difference in lung function, measured as FEV1pp, between those with equal or more than 0.5 peaks > 11mmol/L per day in interstitial glucose and those with fewer than 0.5 peaks per day (p=0.018; Figure 9). The same trend was seen for LCI and daily peaks but did not reach statistical significance (p=0.052). No difference was found in lung function regarding the proportion of measurements with interstitial glucose above 8 mmol/L or the average interstitial glucose values on isCGM. It is essential to establish whether glucose abnormalities in CGM are clinically relevant. These results are particularly important for CF centres relying more on CGM results than OGTT in CFRD screening.

The lung function of 78 individuals registered in the Swedish National CF registry was evaluated in relation to glucose tolerance. The lung function measured by FEV1pp was similar in the groups of AGT (80.9% (23.3)) and NGT (82.6% (19.3)) (t(74) -0.316, p=0.37). The FEV1pp at baseline before LUM/IVA treatment was 82.0% and 85.4% at follow-up (t(74) 1.439, p=0.077). Therefore, these results do not confirm that lung function is influenced by glucose tolerance and the effects of LUM/IVA on lung function seems modest. However, the cohorts of these two studies are not entirely comparable, and the settings also differ.



FEV1pp in NGT: (N=10), mean 100.95, SD 12.52
FEV1pp in AGT: (N=21), mean 85.56, SD 14.79
95%CI 4.30-26.49, p=0.008

LCI in NGT: (N=10), mean 7.88, SD 1.09
LCI in AGT: (N=19), mean 9.46, SD 2.39
95%CI -2.91 - -0.25, p= 0.022

Figure 8. Children with AGT have inferior lung function compared to children with NGT demonstrated by a lower FEV1pp (a) and a higher LCI (b). Adapted from *Elidottir et al.* (152).

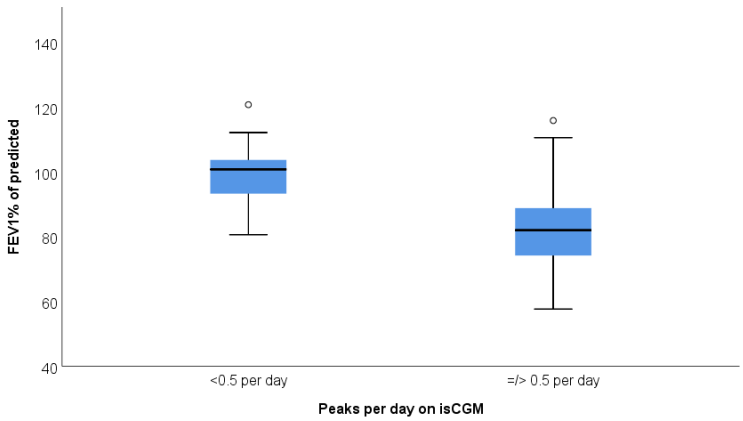


Figure 9. Individuals with ≥0.5 glucose peak values above 11mmol/L per day on isCGM have lower FEV1pp than the ones with <0.5 peaks per day. FEV1pp with ≥ 0.5 peaks per day: (N=15), mean 83.0, SD 16.57, 95%CI 4.89-27.40; FEV1pp with <0.5 peaks per day: (N=13), mean 99.2, SD 11.48. p 0.018; ° outlier.

The Questionnaire

Twenty children answered the questionnaire on OGTT and CGM (appendix 1). The isCGM was well tolerated by all. The majority was more favourable towards isCGM than OGTT, and all but two felt that the isCGM was very easy or easy to carry and remember to scan. In contrast, opinions on OGTT were more evenly spread with positive and negative experiences. Most comments were regarding the OGTT, including comments on the bad taste of the glucose liquid and problems with intravenous access.

The effects of lumacaftor-ivacaftor on glucose tolerance

Data from 78 individuals receiving LUM/IVA treatment were found in the Swedish CF Registry, from whom baseline OGTT and follow-up results were available. Thirty-two (41%) were children and adolescents aged 6.9-18 years. Thirty-one participants (40%) were female. The mean duration of treatment between OGTTs was 399 days, and the baseline OGTTs were all performed within three months of the beginning of treatment. Forty-seven individuals (60%) were identified with AGT according to their baseline OGTT, and 31 had NGT (40%). Nineteen of the 32 children (59%) had AGT, 11 (34%) had IGT, and eight (25%) had INDET. Twenty-eight adults (60%) had AGT, seven (15%) had CFRD, 11 (24%) had IGT, and 10 (22%) had INDET.

LUM/IVA treatment significantly affected the two-hour glucose value of the OGTTs. For the group of AGT, there was a mean of 8.6 mmol/L (SD=2.5) at baseline and 7.5 mmol/L (SD=2.4) at follow-up (t (46) 3.2, p=0.01). For the NGT group, the glucose value at baseline had a mean of 6.9 mmol/L (SD=2.5) and 6.1 mmol/L (SD=1.1) at follow-up (t (30) -1.8, p=0.043) (Table 4).

Since CFRD is much more common in adults, we did separate calculations for adults and children. Only the adult group with AGT sustained a significant change between the two-hour glucose value, with a mean of 9.2 mmol/L (SD=2.9) at baseline and 7.7 mmol/L (SD=2.6) at the follow-up date (t(27) 3, p=0.002). There was the same trend for the children with AGT with a mean glucose value of 7.8 mmol/L (SD=1.4) at baseline and 6.8 mmol/L (SD=2.4) at follow-up (t(18) 1.5, p=0.075).

The cohort had fewer children than adults, which could explain these differences. Children, in general, also have a less advanced disease; therefore, the effects of lumacaftor-ivacaftor in children may be more subtle.

Table 4. Results of the 78 OGTTs for individuals with AGT (47) and NGT (31), presented by mean (SD).

AGT n=47	**Baseline glucose in mmol/L (SD)**	**Follow-up glucose in mmol/L (SD)**	**P value**
Fasting glucose	5.6 (0.7)	5.7 (0.9)	0.2
1-hour glucose	10.8 (0.4)	8.8 (0.6)	**0.03**
2-hours glucose	8.6 (2.5)	7.5 (2.4)	**<0.01**
HbA1c mmol/mol	37.6 (3.8)	37.4 (4.4)	0.5
NGT n=31			
Fasting glucose	5.7 (0.6)	5.6 (5.5)	0.176
1-hour glucose	8.9 (0.3)	9.7 (0.4)	0.185
2-hours glucose	6.4 (1.9)	6.1(2.5)	**0.043**
HbA1c mmol/mol	36.6 (3.5)	36.0 (4.0)	0.6

Cystic Fibrosis liver disease. Paper II

Demographics

Fifty-one children were included in the study. The median age was 11 years (range 5-18 years), and 23 (45%) were female. Twenty-five participants (49%) were homozygous for the F508del variant, and 12 (23.5%) were heterozygous. Almost all the participants had pancreatic insufficiency (98%). Eleven (21.6%) were receiving treatment with ursodeoxycholic acid at the time of the evaluations.

Table 5. Demographic and clinical data of the study population from *Elidottir et al.* (153).

Age in years, median (range)	11 (5-18)
Female n (%)	23 (45)
Homozygous F508del n (%)	25 (49)
FEV1pp, median (range)	90.6 (44.4-115.8)
LCI, median (range)	7.8 (5.8-18.5)
Height z-score, median (range)	-0.27 (-2.99-1.8)
Weight z-score, median (range)	-0.3 (-2.98-2.1)

Liver stiffness and glucose tolerance

Thirty-nine of the 51 individuals were eligible for defining glucose tolerance, either by an OGTT or a previous CFRD diagnosis. The remaining 12 children had not done an OGTT due to their young age. Twenty-nine had AGT, and ten had NGT. Thirteen had INDET, 10 had IGT, and 6 had CFRD. Children with AGT had

significantly increased liver stiffness (M place = 22.47) compared to children with normal glucose tolerance (M place = 12.85; U= 216, p=0.021). Within the different categories of AGT, there was a statistically significant difference in liver stiffness between CFRD, IGT, INDET, and normal glucose tolerance (H (3)=12.4, p=0.006) (Figure 10).

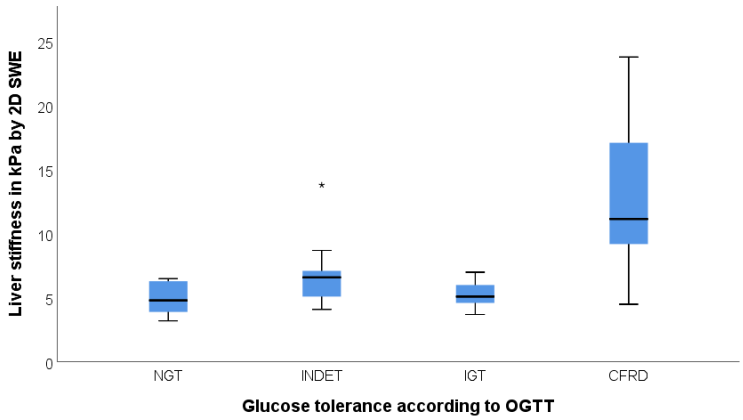


Figure 10. Liver stiffness in relation to glucose tolerance of 39 children as measured by 2D SWE in kPa for NGT (n=10), INDET (n=13), IGT(n=10) and CFRD(n=6) (p=0.006). From *Elidottir et al.* (153).

These results demonstrate that children and adolescents with AGT have increased liver stiffness. This could indicate a partial common pathogenic pathway for CFRD and CFLD, but no causal relationship can be established. During the evolvement of CFRD from AGT, possible associated disease progression in other organs should be evaluated. Likewise, these findings support early screening with OGTT for children with signs of liver involvement.

Liver stiffness and other screening methods

Ultrasound: In six of the 51 individuals (11.7%), the liver was described as nodular or fibrotic on ultrasound. They had increased liver stiffness compared to those who had a normal ultrasound (32 cases, 62.7%), echogenic (9 cases, 17.6%), or hepatomegaly (4 cases, 7.8%), (H (3)=19.3, p=0.001). An advantage of investigating liver stiffness by 2D SWE is its non-invasive nature and the possibility of combining the investigation with standard ultrasound. However, at the same time, there is a risk of bias in the evaluation.

Biochemistry: Regarding liver function tests, the only correlation found was between liver stiffness in kPa and ALT levels (r(49)=0.331, p=0.021). When the individuals treated with ursodeoxycholic acid were omitted, this correlation was no longer statistically significant.

Biopsy: Four children (7.8%) with liver cirrhosis, confirmed by biopsy, had significantly increased liver stiffness compared to all the other children, with a median value of 15.45 kPa (13.0-23.8) (p=0.001). Two individuals had periportal fibrosis on biopsies; their levels were 6.3 kPa. From our small sample of biopsy results of fibrosis and cirrhosis, it is impossible to draw specific conclusions from these results.

Liver stiffness, nutrition, and lung function

Vitamin D deficiency has been linked to inferior lung function and CFRD, but less data is found on similar associations to CFLD. Interestingly, vitamin D deficiency was associated with increased liver stiffness (r (48)= -0.352, p= 0.014). This might indicate suboptimal nutritional status and, as such, associated with CFLD. However, there could also be some elements of the effects of vitamin D in terms of inflammation or other processes that might be important.

The liver produces 25-OH vitamin D, which can lead to vitamin D deficiency in people with severely compromised liver function. Liver disease can also lead to impaired vitamin D absorption (154). However, it is unlikely that this would explain the lower vitamin levels in individuals with increased liver stiffness in this cohort.

There was a negative correlation between 2D SWE levels and height (r (51)= -0.949, P=0.001) and weight (r(51)= -0.707, p=0.054), but the latter did not reach statistical significance.

There was a statistically significant negative correlation between liver stiffness and lung function as measured by FEV1pp (r (48)= -0.299, p= 0.039). However, no correlation was found between liver stiffness and lung function as measured by LCI (r (41)=0.021, p=0.87). This correlation does not consider any specific definition of CFLD, and no causal relationship is claimed. However, it is still an interesting observation regarding the possible relationship between lung function and CFLD.

Cystic Fibrosis in Iceland. Paper IV

Prevalence, genetic variants, and diagnosis

Thirty individuals, 17 males and 13 females, had a CF diagnosis during the study period from 1955 to 2021. The prevalence of CF in Iceland is calculated in 2021 at 0.372:10.000 inhabitants, and the yearly incidence is estimated at 1:10.000 births. The results confirm that CF is uncommon in Iceland, or half the median prevalence of most European countries.

Seventeen of these 30 individuals were alive at the end of the study period: eight children (47.1%) and nine adults (52.9%). The median age of survivors was 22 (2-61 years). Eleven of the deceased individuals were born between 1955 and 1972. Their median age of death was four years (0.5-46 years), and only one became more than 18 years old. Five of the 11 individuals reaching adulthood had become parents.

Complete genetic analysis was found for 27 of the 30 individuals. Two individuals had only a single known CFTR variant; their diagnosis was based on clinical grounds. For one individual, the diagnosis was based only on a sweat test and typical clinical symptoms.

Five CFTR variants were found in the cohort: F508del, N1303K, p.F316LfsTer12, G551D, and W1282X. The F508del variant was seen in 26 alleles of the 56 available (46.4%). The N1303K was found in 25 alleles (44.6%), the p.F316LfsTer12 and the W1282X variants were found in 2 alleles each (3.6%), and the G551D was found in a single allele (1.8%). The heterozygous F508del/N1303K combination was the most common one; it was present in 11 of 27 individuals. Six individuals were homozygous for the F508del variant, and 14 were heterozygous. Six individuals were homozygous for the N1303K variant, and 13 were heterozygous. This indicates Iceland has the highest known prevalence of the N1303K variant, possibly due to a founder effect.

Information regarding the age and symptoms at diagnosis was found for 19 individuals. The median age of diagnosis was 4.5 months (4 days-26 months and one outlier at 31 years). The most common symptoms at diagnosis were respiratory symptoms (n=13) and failure to thrive (n=12). Three children were born with meconium ileus. Iceland does not have NBS, but two children were diagnosed abroad by NBS. From a clinical perspective, it is advisable to initiate NBS in Iceland to secure early diagnosis and treatment, which improves the prognosis, as demonstrated by numerous studies (34, 36, 155).

Lung function

Data on lung function tests were available for 12 individuals, six children and six adults. Figure 11 shows each individual's best FEV1pp values from 2011-2021. During this period, the median FEV1pp for the children was 99.9% (72.8-128.2%) and 90.8% for the adults (57-117%).

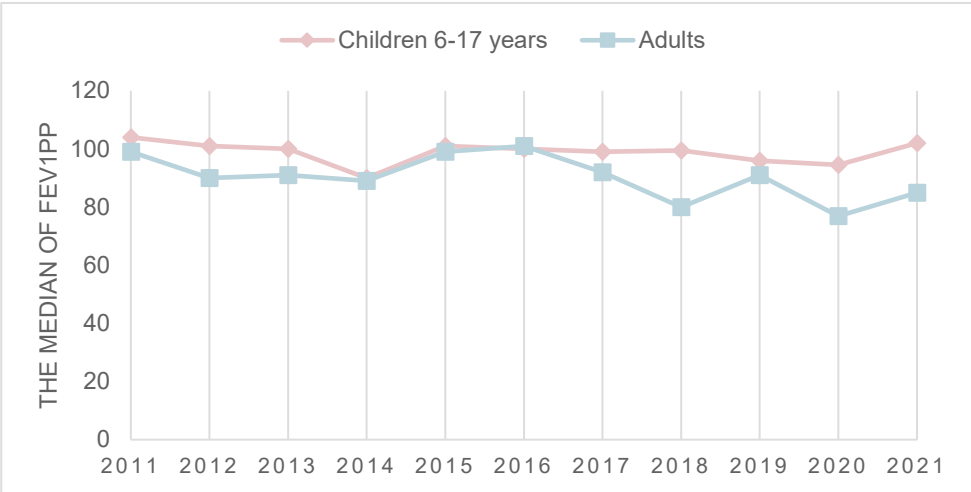


Figure 11. The median values of FEV1pp for the CF population, six children and six adults, in Iceland during the years 2011-2021

Airway infections

Reliable data on airway infections were found for 19 individuals from 2000-2021. The median age of the first respiratory infection was 4.5 months (2-24 months). The most common pathogen was *S. aureus* (n=7). Chronic bacterial colonisation of the airways was found in 14 of the 19 individuals (74%) according to Leeds criteria, five of which were children. *S. aureus* was the most common pathogen in this cohort. All but one individual had at least one positive bacterial culture with this bacterium. *S. aureus* was found in the airways of ten children during the first two years of life. Only one individual had a methicillin-resistant *S. aureus* (MRSA) respiratory infection. *P. aeruginosa* was found in 12 individuals sometime during the study period. Seven children had been infected by this bacterium before the age of 2, two of whom had *P. aeruginosa* as their first known infection shortly after diagnosis at ages 14 and 24 months. Five individuals were diagnosed with chronic *P. aeruginosa* infection, one child and four adults. *Aspergillus fumigatus* was found in seven individuals; three had a chronic infection. Only one child had suffered from ABPA.

Co-morbidities

Information regarding co-morbidities was available for 20 individuals. The most common complication was nasal polyposis (n=9), followed by CFRD (n=8). CFLD was present in four individuals, and no one had undergone a liver transplantation. Two individuals had undergone lung transplantation; both were males in their forties at the time of transplantation.

Treatment and surveillance

Information on CF specific treatment and surveillance was available for 20 individuals, 14 of whom were under the care of the Icelandic CF team at the end of the study period. All individuals were treated with pancreatic enzymes. All children received hypertonic saline and dornase alfa inhalation treatment. Most of the adults had one or both treatment options. Nine individuals were either homozygous or heterozygous for the F508del variant, and all are currently receiving CFTR modulator therapy. The last one started on ETI in 2022 after the approval of the medication for children 6-12 years.

The study suggests that the respiratory status of the CF population, respiratory infections, and most complication rates are similar to those of other European countries. However, CFRD seems relatively common. Access to care is adequate and follows international standards and guidelines. However, the data also indicates that surveillance can be further improved. Modern CF medications, including the most recent CFTR modulators, are readily available.

Table 6. Surveillance data for 20 individuals with CF in Iceland in 2011-2021

Type of surveillance	Median number (range)
Yearly hospital admissions (n=20)	0.5 (0-5)
Yearly outpatient visits (n=20)	8 (1-25)
Yearly cough swabs (children n=9)	7 (1-13)
Yearly sputum culture (children n=9)	8.4 (0·4-16·6)
Yearly sputum culture (adults n=10)	5.6 (1·6-9)
Age of first spirometry (n=20)	6 (5-16)
Yearly number of LFTs (n=19)	3 (1-7)
Age of first CT thorax (n=17)	15 (0-34)
Age of first OGTT (n=9)	12 (10-20)

Strengths and weaknesses

The study presented in Paper I is limited by the small number of participants, even though many eligible patients from the pediatric CF centre in Lund participated. There are certain limitations to using the Freestyle libre equipment, such as the need to scan the sensor repeatedly every eight hours, which is not always possible in real life, and missing data can be substantial. More recent technology can minimise this problem and give more accurate evaluations. The main strength of this study is the prospective design of the comparison between OGTT and isCGM, which adds to current knowledge of this area. In addition, using both spirometry and MBW adds quality to evaluating the relationship between AGT and lung function.

The main weaknesses of the second paper are the study´s retrospective design and the limited number of subjects with CFLD. There is also a risk for low inter-rater reliability since different pediatric radiologists performed the examinations combined with an ultrasound. The fact that various techniques are available to measure liver stiffness makes the comparison between other studies somewhat difficult, and the reference values of one approach do not apply to another. The lack of reference values for children for the 2D SWE method and the lack of validation and standardisation for the different techniques are also troublesome. The study's main strength is that it describes a feasible, non-invasive method for evaluating CFLD and encompasses a fair number of children with CF. Additionally, the possible implications of vitamin D in CFLD are attractive for future research.

The third paper's main weakness is the retrospective approach and the fact that the follow-up dates are not all at the same time point. The strength of the study is that it includes a fair number of participants, children and adults, and it represents real-life data on LUM/IVA treatment.

The fourth study is limited by the small size of the Icelandic population and, thus, the relatively small group of people with CF. This is a descriptive study; no conclusive statistical comparison to other countries can be made. However, the paper presents the first overview of CF in Iceland and demonstrates the country´s unique situation well.

Conclusions

There need to be comprehensive and multiple ways to screen for the co-morbidities of CF. The CGM is a feasible addition to the conventional OGTT in CFRD screening, and it can detect more subtle glucose abnormalities. These two screening methods demonstrate different aspects of glucose variability in CF, and the CGM results correlate better with the intermediate values of the OGTT than the 2-h value.

AGT is associated with inferior lung function in children and adolescents with CF, and this is demonstrated by multiple breath washout as well as spirometry.

Increased liver stiffness is associated with abnormal glucose tolerance, inferior lung function, and lower vitamin D levels. 2D SWE is a feasible method for determining liver stiffness in CF and can be used in addition to the screening process.

LUM/IVA appears to positively affect glucose tolerance in people with CF, demonstrated by improvements in OGTTs.

Iceland has a relatively low prevalence of CF or half the median of most European countries. However, Iceland holds the highest known prevalence of the N1303K variant. Access to CF care, including treatment with CFTR modulators, is adequate and follows international standards. The respiratory status of the CF population, respiratory infections, and most complication rates are similar to those of other European countries. However, CF surveillance and screening of comorbidities can be further improved, especially for CFRD, which is relatively common in the cohort.

Clinical implications and future perspectives

Screening of co-morbidities – why, when, and how?

In CF, we know that it is necessary to screen for various complications because of their insidious onset yet serious impact. The recommendation for CFRD is to start screening at the age of 10, but we know that glucose abnormalities are found much earlier in life (104). We also know there are signs of clinical deterioration before CFRD diagnosis (78). We need to determine whether treating AGT before CFRD diagnosis is feasible and whether starting the screening at a younger age is clinically relevant. Furthermore, we don´t know if it is possible to hinder or halt the evolution of AGT to CFRD. Even if the guidelines recommend a screening start at a specific age, it might be advisable to begin screening at an earlier age, at least where risk factors are present. It is sensible to be very observant of clinical deterioration, decreasing lung function, and liver involvement and, in these circumstances, screen for glucose abnormalities irrespective of age.

Although OGTT does not seem to be replaceable by CGM, the latter method can be useful in tracking glucose abnormalities. It can reveal more subtle glucose abnormalities, which can have clinical relevance, especially in the younger population (112, 113). It is even possible to alternate these two methods and, in that way, follow individuals at increased risk more closely. Using CGM more frequently can also help pwCF adjust to this technique, which might facilitate matters when CFRD becomes a reality.

CFLD screening also starts in childhood. Screening is necessary because of this complication´s silent onset, which can lead to severe disease. The combination of clinical examination, biochemistry, and ultrasound is frequently used for screening, and more than one method is required (119). Even with liver biopsy, considered the gold standard for cirrhosis diagnosis, it is impossible to exclude CFLD because of the initial patchy distribution of lesions. A liver biopsy is not recommended as a systematic investigation because of its invasiveness and shortcomings (119). Other examination methods are needed to come further in the evaluation of CFLD. *Yavuz et al.* revealed in their prospective study that children with CF had increased liver stiffness, measured by 2D SWE, compared to healthy controls. They reason that

liver involvement can occur much earlier than ultrasound detects (156). Other types of liver stiffness measurements have been investigated, such as the FibroScan and Magnetic Resonance (MR) elastography. The FibroScan is an easily applicable instrument, while MR elastography is a more complex procedure requiring anaesthesia or sedation in young children (157). *Levitte et al.* compared 2D SWE to MR elastography and found a significant correlation between these two methods (158). Likewise, there seems to be a good correlation between FibroScan and 2D-SWE (159, 160). It is probably more important for CF clinics to follow each individual regularly with the same application to monitor the disease and inspect the slope of increased liver stiffness than which technique is available (161). Adding ways to evaluate the liver in CF is beneficial; hopefully, we will see more of these in the future.

Is vitamin D important?

An unexpected finding in our study was the relationship between vitamin D and liver stiffness, suggesting that lower vitamin D levels could be related to CFLD. Fat-soluble vitamins are standard supplements in CF nutritional recommendations because of the well-known risk of deficiency. Vitamin D has been the focus of research in a broad context. Most cells in the body have a vitamin D receptor, and $1,25(OH)_2D$ influences the expression levels along with other factors of up to one-third of the human genome. Therefore, it is unsurprising that numerous studies have demonstrated an association of vitamin D deficiency with an increased risk of various diseases such as cancers, diabetes, cardiovascular diseases, and autoimmune and inflammatory diseases. The biological actions of vitamin D are complex and broad (162). One of these is the stimulation of insulin production, which might explain the relationship between vitamin D deficiency and CFRD (86, 163). Some studies have also demonstrated an association between vitamin D and lung function in CF, which might be explained by the anti-inflammatory effects of vitamin D (164-166). Both of these mechanisms of action can influence CFLD evolvement, but further studies are needed in this area. The compiling evidence for vitamin D benefits in CF should at least indicate recommendations for keeping the vitamin D levels in the upper normal reference area and regularly tracking these.

Determinants of prognosis

Since CF is a multiorgan disease, it is not surprising that the individual outcome is multifactorial. Likewise, it is expected that the different manifestations of the disease influence each other since they all arrive from the same faulty CFTR protein.

However, the pathogenesis of different co-morbidities is complicated, and several factors besides the CFTR function are involved. The environment, genetic modifiers, immunological, and other individual aspects can contribute substantially to the morbidity, influence CF prognosis, and to which extent the different co-morbidities evolve. One can imagine that these factors will be a more prominent focus of research in the future when the CFTR function is more or less restored by CFTR modulators.

It will be interesting to see if early treatment with CFTR modulators can alter or decrease the prevalence of CFRD and CFLD. PwCF having AGT appear to follow a more severe clinical trajectory characterised by a faster decline of lung function, increased exacerbations and infections, poorer nutritional status, and earlier mortality (67). There are few studies on the extrapulmonary effects of CFTR modulators. Most studies involve adults, and some reveal improved glycemic control to varying extents (167). Five years of registered data from the UK and USA indicate decreased CFRD prevalence on IVA (168). Other studies are often limited by the number of participants and study design, but overall, they show a positive trend in glucose control with CFTR modulator therapy (167). Data concerning young children and the effects of CFTR modulators on glucose tolerance is scarce. A pediatric pilot study suggests improved glucose tolerance after short-term treatment with ETI, as demonstrated by OGTT but not CGM (169).

A few case studies of the intrauterine effects of CFTR modulators are promising for the preventive possibilities of these medications (170, 171). The most intriguing question is whether early administration of CFTR modulators can prevent glucose abnormalities and progress toward CFRD. Early pancreatic disease in infants may be more likely to be reversible than a longstanding disease in the older population. Thus, future research in this area should aim to discover the optimal window for initiating CFTR modulator therapy. (172).

What about the N1303K variant?

The N1303K variant is a missense variant located in the nucleotide-binding domain 2 of the CFTR protein. It belongs to class 2 folding defects of CFTR variants since it induces little or no CFTR at the cell surface and scarce ion channel activity (173). The N1303K variant is one of the five most common CFTR variants globally, with a previously described prevalence of 2.15 – 2.4% (53, 173, 174). Other countries with a relatively high prevalence of the N1303K variant are Mediterranean countries, Italy (5.5%), Southwest of France (7.8%), Egypt (6%), and Lebanon (9.4-27%) (53, 175, 176). *Farhat et al.* speculate in their paper on the possibility of the N1303K variant originating in Lebanon and spreading with sailors (177). In that case, it has perhaps reached the north at some point. The relatively high prevalence

of otherwise rare variants is a well-known observation in certain populations, called the founder effect hypothesis. This may occur in religious, ethnic, or, as in the case of Iceland, geographic isolates (178).

Although the N1303K variant belongs to the same class as the F508del variant, the initial CFTR modulators did not seem to fit this variant. However, recent evidence indicates that ETI is effective even for individuals homozygous for the N1303K variant, which is also our experience in Iceland, not presented in this thesis (179, 180). Hopefully, we will see further expansion of the indications of ETI by the end of this year.

Global perspectives

The CF community of the Western world rejoices over the success of CFTR modulators. There are great aspirations that treatment will be found for all CF gene variants. However, we have not crossed the finish line, and access to CFTR modulators is not universal. There are gene variants the current CFTR modulators do not fit, such as the class 1 and some class 2 variants. Furthermore, these new medications are extremely expensive, and it is hard to imagine that countries where basic CF medications are hardly available will be able to afford the CFTR modulators at the current price. In countries where CF is less prevalent, there is also the risk that the cost of these medications will be even higher since each country´s authorities individually negotiate with the pharmaceutical companies. Perhaps the CF organisations, The ECFS, and the CFF could influence these steps and give even greater support to economically compromised countries. Of course, this tremendous inequality is not isolated to CF but affects health and well-being in the broadest perspective.

The CF community is a united one. It is built on the belief that a cure will be found for every person with CF. Historically, CF demonstrates cooperation achievements, both on the smaller scale at the CF centres and on the larger scale of international liaisons. It is characterised by the partnership of researchers, clinicians, healthcare providers, parents, adults, and children with CF. This story will continue, and the CF community will hopefully reach the ultimate goal.

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"Which is more important," asked Big Panda,
"the journey or the destination?"
"The company," said Tiny Dragon.
(J.Norbury)

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Appendix

Skånes universitetssjukhus
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Bilaga 15

Frågeformulär efter glukosbelastning

Studie Lungfunktion, diabetes och inflammation vid cystisk fibros

Glukosbelastning

Att göra glukosbelastning tycker jag gick:

Mycket bra ☐
Bra ☐
Varken bra eller dålig ☐
Dåligt ☐
Mycket dåligt ☐

Att göra glukosbelastning tycker jag är:

Mycket lätt ☐
Lätt ☐
Varken svårt eller lätt ☐
Svårt ☐
Mycket svårt ☐

Det jag hade svårt med var:_____

_____

Det jag skulle vilja ha annorlunda med glukosbelastningen är:_____

_____

_____

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Blodsockermätare i hemmet

Att ha blodsockermätaren på tycker jag gick:

Mycket bra ☐
Bra ☐
Varken bra eller dåligt ☐
Dåligt ☐
Mycket dåligt ☐

Att ha blodsockermätaren på mig tycker jag är:

Mycket lätt ☐
Lätt ☐
Varken svårt eller lätt ☐
Svårt ☐
Mycket svårt ☐

Att komma ihåg att mäta blodsocker gick:

Mycket bra ☐
Bra ☐
Varken bra eller dåligt ☐
Dåligt ☐
Mycket dåligt ☐

Det jag hade svårt med var:_____

_____

_____

Det jag skulle vilja ha annorlunda med blodsockermätaren är:_____

_____