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Assessment of clinical factors and new treatment modalities influencing outcomes in cystic fibrosis

PhD thesis

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List of Abbreviations

8-IP	8-isoprostane
8-OHdG	8-hydroxy-2'deoxyguanosine
ABPA	allergic bronchopulmonary aspergillosis
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ASL	airway surface liquid
AST	aspartate aminotransferase
ATS	American Thoracic Society
BMI	body mass index
CAT	catalase
CF	cystic fibrosis
CFLD	cystic fibrosis-related liver disease
CFRD	cystic fibrosis-related diabetes
CFTR	cystic fibrosis transmembrane conductance regulator
COVID-19	coronavirus disease-2019
CRP	C-reactive protein
DTT	dithiothreitol
ELISA	enzyme-linked immunosorbent assay
ENaC	epithelial sodium channel
ETI	elexacaftor/tezacaftor/ivacaftor
FEV_1	forced expiratory volume in 1 second
FVC	forced vital capacity
GGT	gamma-glutamyl transferase
HbA1c	haemoglobin A1c
HEMT	highly effective CFTR modulator therapy
HNCFR	Hungarian National Cystic Fibrosis Registry
ICS	inhaled corticosteroid
IDSA	Infectious Diseases Society of America
IL	interleukin
MABSC	*Mycobacterium abscessus complex*

MAC	*Mycobacterium avium complex*
MF	minimal function mutation
MMP	matrix metalloproteinases
MRSA	methicillin-resistant *Staphylococcus aureus*
N/A	not applicable
NF-κB	nuclear factor-kappa B
NKIP	National Korányi Institute for Pulmonology
NSP	neutrophil serine protease
NTM	non-tuberculous *Mycobacteria*
OR	odds ratio
PD	pulmonary disease
PPI	proton pump inhibitor
pwCF	people with cystic fibrosis
RF	residual function mutation
RNS	reactive nitrogen species
ROS	reactive oxygen species
SARS-CoV2	severe acute respiratory syndrome-related coronavirus
SD	standard deviation
SEM	standard error of the mean
SMX-TMP	sulfamethoxazole-trimethoprim
SOD	superoxide dismutase
WBC	white blood cells

1 Introduction

1.1 Epidemiology of cystic fibrosis

Cystic fibrosis (CF) is a progressive, multisystem genetic disorder that primarily affects the respiratory, digestive and reproductive systems. Despite dramatic improvements in therapeutic approaches over recent decades (**Figure 1**), CF remains associated with reduced life expectancy, with end-stage lung disease continuing to represent the leading cause of mortality. Although numerous factors contributing to the pathological changes in pulmonary tissue have been identified, the underlying mechanisms driving the progression of lung disease in CF remain incompletely understood and are the subject of ongoing investigation.

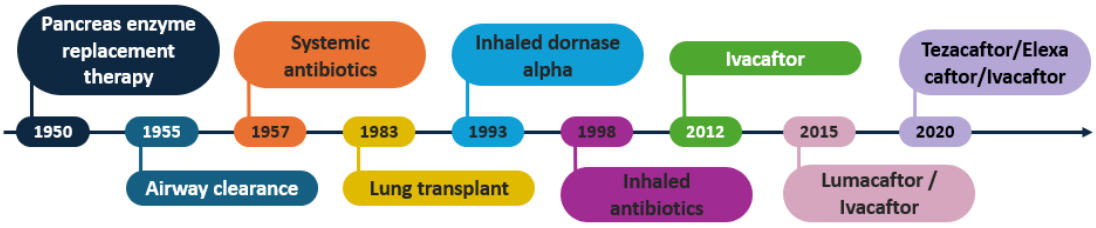

Figure 1. Timetable of evolution in the treatment of CF. Original work of the author.

CF is one of the most common autosomal recessive disorders in the Caucasian population. The incidence of CF is estimated to be approximately 1 in 2,500 to 1 in 3,000 live births in Europe and North America. In contrast, CF is significantly less prevalent in populations of Asian and African ancestry. In Hungary, recent estimates place the incidence at approximately 1 in 5,200 live births, reflecting intermediate prevalence within the European context (1, 2).

1.2 Etiopathogenesis of CF

The cystic fibrosis transmembrane conductance regulator (CFTR) gene is located on the long arm of chromosome 7 at position q31.2. (3). To date, over 2,000 variants of the CFTR gene have been identified in people with cystic fibrosis (pwCF). CF-causing mutations are broadly categorized into six functional classes (I–VI) based on their impact on CFTR protein expression, processing, function and stability (4).

In the lungs, the CFTR protein is expressed on the apical membrane of airway epithelial cells. Activation of the CFTR anion channel requires cAMP-dependent phosphorylation

and the binding and hydrolysis of ATP. The signal transduction induces a conformational change that opens the channel. Once open, chloride ions are transported from the intracellular compartment into the airway lumen, contributing to the hydration of the airway surface (5, 6).

Under normal conditions, CFTR also plays a key regulatory role in inhibiting the epithelial sodium channel (ENaC). This inhibition limits sodium absorption and helps maintain a balance in airway surface liquid (ASL) volume. In CF, dysfunctional or absent CFTR leads to uninhibited ENaC activity, which increases sodium reabsorption across the airway epithelium (7). The combined effect of impaired chloride secretion and excessive sodium absorption disrupts the ionic balance and causes dehydration of the ASL. As a consequence, the mucus layer becomes abnormally viscous and adherent to the epithelial surface, impairing mucociliary clearance. This promotes mucus plugging, persistent microbial colonization, and chronic neutrophil-dominated inflammation, all of which contribute to progressive airway damage and bronchiectasis - hallmarks of CF lung disease (8).

Beyond the airways, CFTR is also expressed in various epithelial tissues, including the sweat glands, gastrointestinal tract, pancreas, biliary system, and vas deferens. Consequently, CF is a multisystemic disorder, with clinical manifestations extending beyond the respiratory system. Common extrapulmonary complications include pancreatic insufficiency, cystic fibrosis-related diabetes (CFRD), meconium ileus, cystic fibrosis-related liver disease (CFLD), and congenital bilateral absence of the vas deferens, which results in male infertility in most cases (9).

1.3 Progression of CF

CF is a hereditary disease that progresses over time and shortens life expectancy primarily due to lung tissue injury. It is suggested that the dysfunctional or absent anion (chloride and bicarbonate) channel in the respiratory epithelium plays an important role in the natural course of CF. The pathological function of the CFTR protein alters the transepithelial ion flux, as well as the pH and hydration levels of the airway surface liquid, resulting in impaired mucociliary clearance (10, 11). The production of thick mucus leads to airway obstruction, promoting local microbial (viral, bacterial and fungal) colonization, infection, and inflammation. The vicious cycle of these pathological

disorders may play a pivotal role in the progression of lung tissue destruction (**Figure 2**) (12).

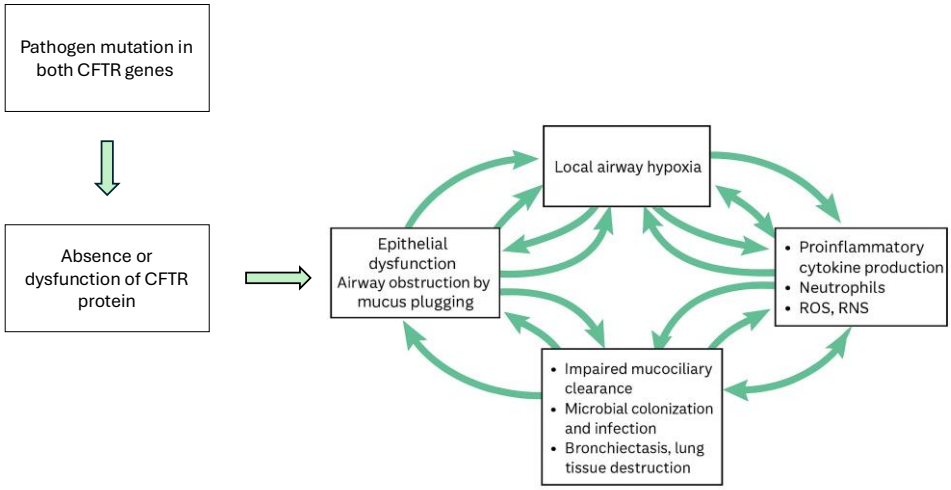

Figure 2. Pathomechanism of CF lung disease.

CFTR: cystic fibrosis transmembrane conductance regulator, **RNS**: reactive nitrogen species, **ROS**: reactive oxygen species. Original work of the author.

Several factors may influence the clinical outcomes in CF. In general, these factors can be categorized as offensive and defensive factors depending on their role (i.e. promote or delay) in the progression of the disease. The most important offensive and defensive factors in CF are summarized in **Table 1** (13, 14). These factors frequently influence the inflammatory cascade in cystic fibrosis, which may, in turn, affect disease prognosis. Next, I give a brief overview of those factors and/or pathologic processes that are closely related to the objectives of this thesis.

Table 1. Summary of matched offensive and defensive factors influencing the clinical course of CF. Original work of the author.

Offensive factors	Defensive factors
Severe CFTR mutations (Class I–III, e.g., F508del homozygosity)	Use of CFTR modulators (e.g., ETI)
Female gender	Male gender
Delayed CF diagnosis	Neonatal screening and early CF diagnosis
CF-related comorbidities (CFRD, GERD, ABPA)	Regular screening for CF-related comorbidities
Decreased FEV_1; chronic respiratory failure	Oxygen supplementation; non-invasive ventilation
Frequent pulmonary exacerbation	Effective prevention (e.g., vaccination) and management of pulmonary exacerbations
Thick, dehydrated mucus due to CFTR dysfunction	Effective airway clearance therapies (e.g., physiotherapy, inhaled saline)
Chronic neutrophilic inflammation	Novel therapeutics (e.g., defensin inhibitors)
Chronic infection with *P. aeruginosa*, MRSA, NTM, or *B. cepacia*	Infection prevention (e.g., hygiene, segregation) and early, effective antibiotic therapy
Oxidative stress; impaired antioxidant defense	Vitamin and micronutrient supplementation
Malnutrition (reduced BMI)	Hypercalorisation
Pancreatic insufficiency	Pancreas enzyme supplementation
Poor adherence to care and treatment	Regular microbial surveillance
Active or passive tobacco smoke exposure	Clean air; low pollution
Depressed mental status; poor socioeconomic status	Increased physical activity; strong social support

ABPA: allergic bronchopulmonary aspergillosis, **BMI**: body mass index, **CFLD**: cystic fibrosis-related liver disease, **CFRD**: cystic fibrosis-related diabetes, **CFTR**: cystic fibrosis transmembrane conductance regulator, **ETI**: elexacaftor/tezacaftor/ivacaftor, **FEV_1**: forced expiratory volume in 1 second, **MRSA**: methicillin-resistant *Staphylococcus aureus*, **NTM**: non-tuberculous *Mycobacteria*.

1.4 Factors and medications modifying the inflammatory responses in CF

1.4.1 Cytokines

Hypoxia caused by airway obstruction results in sterile inflammation already at a very early age. Several studies confirm that respiratory inflammation in pwCF is associated with the excessive production of pro-inflammatory cytokines. Elevated levels of interleukin (IL) -1, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) have been

detected in sputum and bronchoalveolar lavage fluid from pwCF (15, 16). The synthesis of these cytokines is primarily driven by the activation of the transcription factor nuclear factor-kappa B (NF-κB), which regulates multiple intracellular signaling pathways involved in pro-inflammatory responses (17). In contrast, the expression of anti-inflammatory mediators, such as IL-10, interleukin-1 receptor antagonist (IL-1Ra), and soluble TNF-α receptors, is downregulated in CF bronchial epithelial cells. This dysregulation contributes to the persistent and unresolved airway inflammation characteristic of CF (18).

Activation of the NF-κB signaling pathway and reduced surface expression of Toll-like receptor 4 (TLR4) on airway epithelial cells lead to dysregulated type I interferon responses and impaired pulmonary dendritic cell function. In addition, defective T lymphocyte activity and weakened immune surveillance further increase the susceptibility of pwCF to chronic lower respiratory tract inflammation and acute or persistent microbial infections (19-21).

1.4.2 Neutrophils

Airway inflammation in pwCF is predominantly characterised by intense neutrophilic infiltration. Activated neutrophils release reactive oxygen and nitrogen species (ROS, RNS), as well as neutrophil serine proteases (NSPs), including neutrophil elastase (NE), cathepsin G, and proteinase 3, all of which contribute significantly to pulmonary tissue injury (22). An imbalance between these proteases and their endogenous inhibitors, such as alpha-1 antitrypsin (AAT), has been consistently observed in pwCF (23). NSPs exert direct cytotoxic effects on bronchial epithelium through the degradation of extracellular matrix components such as elastin, leading to structural damage, excessive mucus production, impaired mucociliary clearance, and enhanced microbial colonization of the airway surface. This proteolytic activity not only perpetuates tissue destruction but also stimulates the release of additional pro-inflammatory chemoattractants, fuelling a self-sustaining cycle of inflammation that exacerbates progressive lung damage (24, 25).

Neutrophils in pwCF exhibit intrinsic functional defects that compromise their antimicrobial efficacy. One of them is the impaired transport of chloride ions into the phagolysosome. This process is essential for the generation of hypochlorous acid and other free radicals involved in bacterial killing. Additionally, the dysregulated intracellular pH of CF neutrophils contributes to excessive degranulation and

uncontrolled release of granule contents, including serine proteases and peroxidases such as myeloperoxidase (MPO). This aberrant release exacerbates tissue damage and perpetuates inflammation, further amplifying the destructive cycle within the CF airway milieu (26, 27).

Neutrophil extracellular trap (NET) formation, or "NETosis," is a specialized form of neutrophil cell death characterized by the enzyme-mediated decondensation of nuclear chromatin and its release—along with granule-derived proteins such as histones—into the extracellular space. These expelled components form web-like structures that serve as a host defense mechanism by trapping and neutralizing invading pathogens. However, in CF, excessive or dysregulated NET formation contributes to airway obstruction, promotes chronic inflammation, and reflects the dysfunction of the innate immune response (28, 29).

In CF, tissue remodelling of the airways is dysregulated by the overexpression of matrix metalloproteinases (MMPs), particularly MMP-8 and MMP-9, which degrade structural components of the extracellular matrix and disrupt normal tissue architecture. In addition, cytokines such as IL-13 and transforming growth factor-alpha (TGF-α) play a key role in promoting epithelial–mesenchymal transition, mucus hypersecretion, and fibrotic responses. The cycle of persistent epithelial injury and aberrant repair leads to structural remodelling, bronchiectasis, and a progressive decline in pulmonary function in pwCF (30, 31)

1.4.3 Oxidative stress

Recently, it has become widely recognized that CFTR protein deficiency or dysfunction is directly related to increased oxidative stress in CF by multiple molecular mechanisms, including impaired extracellular glutathione transport, alterations in lipid metabolism, and unbalanced autophagy (32). Furthermore, chronic polymicrobial infections result in inflammation and an increased production of reactive oxygen species, which disrupt the oxidant/antioxidant balance and cause oxidative damage to macromolecules. Malabsorption of micronutrients with antioxidant properties also contributes to redox imbalance (33). Over the past decade, several oxidative stress markers have been tested to monitor redox imbalance in CF, with the aim of improving diagnosis and treatment of the condition. Among the products of lipid peroxidation, 8-isoprostane (8-IP) was the most frequently investigated, while protein carbonyls and 8-hydroxy-2’deoxyguanosine

(8-OHdG) were the most analysed markers of oxidative damage to proteins and nucleic acids, respectively. In general, studies have found higher levels of these products in pwCF compared to healthy subjects (34).

Enzymes such as superoxide dismutases (SODs) and catalase (CAT) have been implicated as the main endogenous antioxidant enzymes in the lung. Measuring their activity could therefore be another way of assessing redox status in patients with lung disease (35, 36). However, published data on the role of antioxidants in CF are inconsistent. Some studies have found reduced antioxidant capacity in the disease (37, 38), while others have not (39, 40).

1.4.4 CFTR modulators

Thanks to significant advances in pharmaceutical and clinical research over the past decade, the development of CFTR modulators has revolutionized the treatment of CF, leading to marked improvements in life expectancy and quality of life for pwCF (41-43). These modulators target the underlying protein defect in CF. Two main classes of modulators have been introduced: potentiators and correctors. Potentiators, such as ivacaftor, enhance the gating activity of the CFTR channel. They keep the channel open at the cell surface, thereby allowing effective chloride and bicarbonate ion transport. In contrast, correctors, including lumacaftor, tezacaftor, and elexacaftor, act as pharmacological chaperones. These agents assist in the proper folding of the CFTR protein, promoting its maturation and trafficking to the apical membrane, and preventing intracellular degradation (44).

In particular, triple combination CFTR modulator therapy with elexacaftor/tezacaftor/ivacaftor (ETI) has shown unprecedented improvements in lung function and other clinical outcomes in pwCF with at least one copy of the *F508del* allele (45-47). There is evidence that ETI improves CFTR function in the airways of pwCF to ~40-50% of the normal CFTR activity (48). A recent study also found evidence of a reduction in airway protease burden following ETI treatment, accompanied by a reduction in the concentrations of various pro-inflammatory cytokines and chemoattractants (49). Additionally, ETI therapy may improve the viscoelastic properties of sputum, a shift towards a healthy airway microbiome and a reduction in airway inflammation (50-53). Despite these promising outcomes, it remains unclear whether ETI modulates oxidative stress, a key contributor to CF pathophysiology.

1.4.5 Infections

The presence of thick mucus, airway hypoxia, and impaired mucociliary clearance in CF creates a favourable environment for microbial colonization of the airway epithelium. In addition, CFTR mutations negatively impact innate immunity, as previously discussed, further contributing to susceptibility to persistent infection and chronic inflammation. During childhood, the most common airway pathogens in CF include *Staphylococcus aureus* and *Haemophilus influenzae*. In adolescence and early adulthood, there is a shift toward opportunistic Gram-negative organisms, such as *Pseudomonas aeruginosa, Burkholderia cepacia complex, Stenotrophomonas maltophilia, Achromobacter xylosoxidans*, and *non-tuberculous mycobacteria (NTM)*. The precise role of bacterial colonization and infection in CF disease progression remains incompletely understood. Different microbial strains live in a dynamic and interactive lung network system. Some organisms, such as *Streptococcus mitis*, act as commensals and may exert anti-inflammatory effects. In contrast, others—including *P. aeruginosa*—function as pathogens, capable of inducing chronic inflammation, pulmonary exacerbations, and progressive lung function decline (54, 55).

NTM are opportunistic environmental pathogens that exist as saprophytic bacteria in soil and water, including both natural and municipal sources (56). Human NTM infections are typically not contagious and are most frequently acquired from the environment via inhalation, alimentation, and dermal contact (57, 58). PwCF are particularly vulnerable to respiratory mycobacteriosis due to bronchiectasis, impaired mucociliary clearance, frequent antimicrobial treatment, and the use of corticosteroids (59). A recent multi-center meta-analysis (60) reported an overall NTM prevalence of 7.9% (95% CI, 5.1-12.0%) in CF. However, it shows high geographical and age-related variability. In Europe, the most frequently detected NTM species in pwCF are *Mycobacterium abscessus complex* (MABSC) and *Mycobacterium avium complex* (MAC), with a slight predominance of MABSC (59, 61-63). The management of non-tuberculous mycobacterial pulmonary disease (NTM-PD) is challenging and suboptimal due to prolonged antimicrobial treatment, emerging antibiotic resistance, drug toxicity and frequent relapses (59, 64-66). The PREDICT study aims to standardise the diagnosis of NTM-PD in pwCF by applying evidence-based guidelines to identify individuals who may benefit from targeted treatment (67).

2 Objectives

2.1 Effect of ETI treatment on oxidative stress in CF (Oxidative stress study)

Redox imbalance is considered a central component in the pathomechanism of CF and may significantly contribute to the progressive deterioration observed in affected individuals. Several studies have demonstrated that the triple combination of CFTR modulators (ETI) leads to improved lung function, reduced airway inflammation, and a decreased microbial burden. Based on these considerations, this study aimed to answer the following questions:

a) How do lung function parameters, systemic inflammatory marker levels, BMI, and the cellular composition of sputum change in pwCF undergoing ETI therapy?
b) Is ETI therapy capable of reducing oxidative stress biomarkers in the airways (sputum) and/or systemically (blood) in pwCF, and if so, are these changes associated with clinical parameters?
c) Does ETI therapy influence the activity of antioxidant enzymes in the airways and/or blood?

2.2 Predictors of NTM infections in CF (NTM study)

The airways of pwCF are particularly vulnerable to colonization or infection by environmental NTM species, due to impaired mucociliary clearance, viscous mucus production, dysregulated innate immunity, and frequent use of antibiotics and corticosteroids. However, most of the existing data are derived from paediatric CF populations, while information regarding adult pwCF remains limited. Therefore, this study aimed to investigate the impact of NTM species on the Hungarian adult CF population and to compare these local findings with international observations. An additional objective was to evaluate the effect of ETI therapy on NTM airway colonization and infection. Accordingly, the study addressed the following questions:

a) What is the prevalence of sputum NTM positivity in the Hungarian adult CF population?
b) How are different NTM species distributed, and what are the antibiotic resistance patterns of the MABSC?

c) What are the risk factors associated with airway NTM colonization or infection?
d) Does ETI therapy influence the clearance of NTM species from the respiratory tract?

3 Methods

All patients included in the studies were diagnosed with CF on the basis of family medical history, typical symptoms of the disease, abnormal sweat test results (i.e., sweat chloride concentration ≥60 mmol/L) and genotype analysis, in accordance with Cystic Fibrosis Foundation Consensus criteria (2017)(68). Lung-transplanted pwCF were excluded from the studies.

The research protocol was approved by the Scientific and Research Committee of the Medical Research Council, Budapest, Hungary (BMEÜ/3691-1/2022/EKU). All subjects gave written informed consent to participate in the study. All procedures performed in the study involving human participants complied with the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards.

3.1 Oxidative stress study

3.1.1 Study subjects

Clinically stable pwCF approved for ETI therapy were recruited between August 2022 and November 2023. Of those subjects who were regularly followed up, 30 fulfilled the inclusion criteria and agreed to participate. During follow-up, 4 patients were withdrawn due to unforeseen complications (**Figure 3**). The control group consisted of healthy, age-matched individuals.

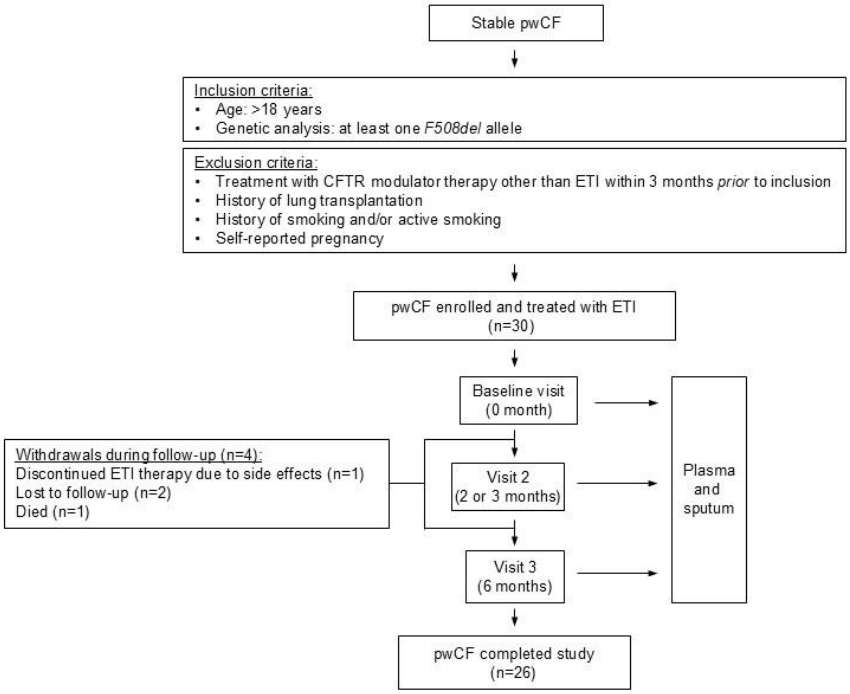

Figure 3. Flow chart of study design

CFTR: cystic fibrosis transmembrane conductance regulator, **ETI**: elexacaftor/tezacaftor/ivacaftor, **pwCF**: people with cystic fibrosis. Adopted from (69) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by-nc-nd/4.0/).

Demographic and clinical characteristics of individuals in the study and control groups are presented in **Table 2**. In addition to inhaled medications, all individuals with CF were receiving vitamin supplementation and pancreatic enzyme replacement therapy. Healthy controls had no history or clinical signs of acute or chronic pulmonary disease and demonstrated normal lung function values. All participants were non-smokers.

Table 2. Demographic and clinical characteristics of study subjects.

Data are presented as mean ± SEM unless stated otherwise. Adopted from (69) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by-nc-nd/4.0/).

	Healthy controls	**pwCF**
Subjects (n)	22	26
Males/Females – n (ratio)	12/10 (0.54/0.45)	12/14 (0.46/0.54)
Age – years	58.9±1.5	33.6±1.7
Genotype (n)		
F508del/F508del	N/A	7
F508del/MF	N/A	14
F508del/RF	N/A	5
CFLD (n)	N/A	10
CFRD (n)	N/A	8
Pancreatic insufficiency (n)	N/A	25
Airway bacterial colonization		
***P. aeruginosa* (n)**	N/A	16
***S. aureus* (n)**	N/A	19
***A. xylosoxidans* (n)**	N/A	8
***B. cepacia complex* (n)**	N/A	4
Other (n)	N/A	3
Inhaled medication		
Antibiotics (n)	N/A	18
Dornase alfa (n)	N/A	26
Hypertonic saline (n)	N/A	26

MF: minimal function mutation, **RF**: residual function mutation, **CFLD**: cystic fibrosis liver disease, **CFRD**: cystic fibrosis-related diabetes, **FVC**: forced vital capacity, **FEV_1**: forced expiratory volume in one second, **CRP**: C-reactive protein, **WBC**: white blood cells, **BMI**: body mass index, **ETI**: elexacaftor/tezacaftor/ivacaftor

3.1.2 Study design

The study was a prospective, observational study of patients starting ETI therapy at the Cystic Fibrosis Unit of the National Korányi Institute for Pulmonology (NKPI). During the baseline visit, spontaneously expectorated sputum and blood samples were collected, and clinical parameters of pwCF were assessed. Patients were then prescribed oral elexacaftor 200 mg/tezacaftor 100 mg/ivacaftor 150 mg to be taken in the morning, and ivacaftor 150 mg in the evening. Patients underwent periodic clinical follow-up, and all clinical examinations, sputum and blood collections were repeated after 2 or 3 months

(visit 2) and 6 months (visit 3) of treatment. Induced sputum samples were collected from healthy controls. Sputum induction, lung function measurement and all other laboratory tests were performed as previously described (70, 71). All samples were stored at -80°C before analysis. Based on previous comparative analysis of biomarker concentrations in induced versus spontaneously expectorated sputum samples, no significant differences were observed between the two sampling methods (71).

3.1.3 Sputum processing

Sputum samples were processed in phosphate-buffered saline (PBS) containing dithiothreitol (DTT), as previously described (70, 72). Cytospins were stained with May-Grunwald-Giemsa for differential cell counting. At least 400 inflammatory cells were counted for each cytospin slide. The number of inflammatory cells in sputum was recorded as a percentage of total non-squamous cells.

3.1.4 Measurement of oxidative stress markers and antioxidants

The levels of 8-IP and 8-OHdG in the sputum supernatant and plasma were determined by enzyme-linked immunosorbent assay (ELISA; Cayman Chemical, Ann Arbor, MI, USA), while protein carbonyl levels and the activity of SOD and CAT enzymes were assessed by colorimetric assays (Cayman). For plasma samples, measurements were performed according to the manufacturer's protocol, while for respiratory samples, DTT was added to the standards at a concentration corresponding to that in the sputum supernatant (0.04%), consistent with other studies (73).

The recommended usable ranges of the 8-IP and the 8-OHdG assays were 2-500 pg/mL and 52-3000 pg/mL, respectively. The dynamic ranges of the SOD and CAT assays were 0.025-0.25 U/mL and 2-35 nmol/min/mL, respectively.

The repeatability of the ELISA and colorimetric assays was determined in pilot studies reported previously (70, 71). The coefficients of variation for repeated measurements were comparable for sputum and plasma and ranged between 12.0 and 18.0%.

3.1.5 Statistical analysis

Data are presented as mean ± SEM or median with interquartile range, as appropriate. Data distribution was analysed by the Kolmogorov-Smirnov test. Clinical variables, oxidative stress marker levels and antioxidant activities in pwCF were compared using the repeated measures ANOVA (parametric data) or the Friedman test (non-parametric data), followed by the Newman-Keuls or the Dunn's test for multiple comparisons, respectively. Sputum cell profiles at visits 2 and 3 were compared to baseline using the Wilcoxon signed-rank test. Correlation coefficients were calculated by Pearson's or Spearman's method. All calculations were performed by GraphPad Prism 4.0 (GraphPad Software Inc., San Diego, CA, USA). A p-value < 0.05 was considered significant.

3.2 NTM study

3.2.1 Study subjects

This study was a retrospective, multicentre cohort study of 232 adult pwCF registered in the Hungarian National Cystic Fibrosis Registry (HNCFR) using data extracted from the participating centres' local electronic medical record systems. NTM-positive (NTM+) patients (cases) were defined as having at least one positive NTM sputum culture within the 1st study period (January 1, 2020 - December 31, 2022). PwCF with only one NTM+ sputum sample were classified as transiently colonized, while those with at least two NTM isolates without clinical or radiological findings were classified as persistently colonized. Subjects who fulfilled the microbiological, radiological, and clinical criteria for NTM caused pulmonary disease (PD) were classified as patients with NTM-PD. All patients enrolled in the 1st study period were CFTR modulator–naïve. Diagnosis of NTM-PD was based on the American Thoracic Society (ATS) (2006) and the Infectious Diseases Society of America (IDSA) (2007) criteria (74).

The 2nd study period extended from July 2023 to July 2025. All patients receiving ETI were included after at least six months of CFTR modulator therapy **(Figure 4)**. Successful NTM clearance was defined as sustained sputum NTM negativity for at least one year after conversion, irrespective of whether anti-mycobacterial treatment was administered.

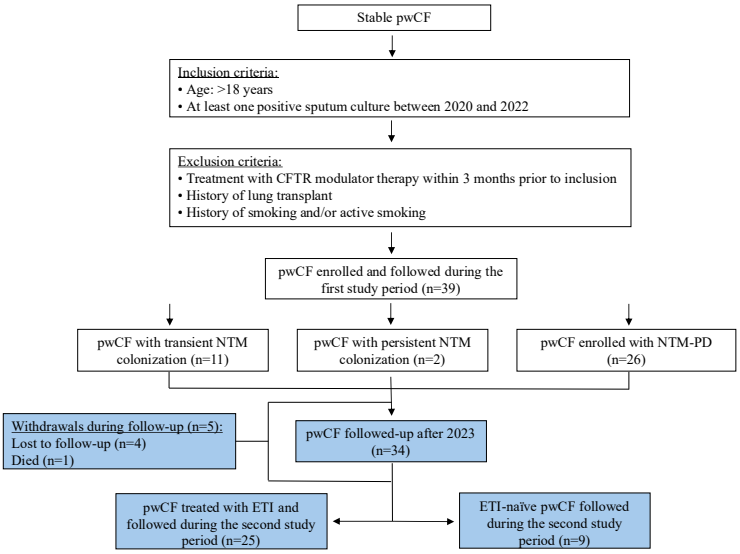

Figure 4. Flow chart of study design.

CFTR: cystic fibrosis transmembrane conductance regulator, **HNCFR**: Hungarian National Cystic Fibrosis Registry, **NTM**: non-tuberculous *Mycobacteria*, **PD**: pulmonary disease, **pwCF**: people with cystic fibrosis. White boxes: 1st study period (January 2020 - December 2022); blue boxes: 2nd study period (July 2023 - July 2025). Original work of the author.

As a part of the study, a case-control comparison was conducted. This included a case group of NTM+ pwCF (n=39) and a control group of NTM-negative (NTM-) pwCF (n=73), based on age and gender as matching criteria (**Table 3**). Those receiving CFTR modulators (which have an impact on clinical parameters) were excluded from this analysis (n=2).

Table 3. Comparison of NTM+ pwCF and controls (NTM-). Demographic data, BMI, FEV_1, CF-related diseases, and co-infecting microbes were recorded at the time of the first NTM isolation. Data of continuous variables are expressed as mean ± SD. $^{\#\#}$: $p < 0.01$ - Fisher exact test; **: $p < 0.01$ - independent samples t-test. Adopted from (75) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by/4.0/).

	NTM-	**NTM +**
Subjects – n	73	39
Age – years	25.8±6.9	26.7±7.5
Males/Females – n (ratio)	33/40 (0.45/0.55)	17/22 (0.44/0.56)
BMI – kg/m^2	20.5±2.5	19.8±3.0
FEV_1 – %	64.9±26.5	**48.0±20.3****
CFRD – n (ratio)	18 (0.25)	11 (0.28)
CFLD – n (ratio)	38 (0.52)	16 (0.41)
Medication in patient history		
ICS – n (ratio)	3 (0.04)	4 (0.10)
Macrolides – n (ratio)	6 (0.08)	7 (0.18)
PPI – n (ratio)	17 (0.23)	8 (0.21)
Previous NTM positivity in sputum – n (ratio)	3 (0.04)	**9 (0.23)**$^{\#\#}$
Number of co-existing pathogens – n	1.51±0.80	1.64±0.83
***P. aeruginosa* co-existing – n (ratio)**	37 (0.51)	25 (0.64)
***S. aureus* co-existing – n (ratio)**	47 (0.64)	20 (0.51)
***A. xylosoxidans* co-existing – n (ratio)**	8 (0.11)	7 (0.18)
***Aspergillus* co-existing – n (ratio)**	8 (0.11)	8 (0.21)
NTM pulmonary disease – n (ratio)	N.A.	26 (0.67)
Persistent NTM infection	N.A.	2 (0.05)
Transient NTM infection	N.A.	11 (0.28)
Genotype		
***F508del* homozygous – n (ratio)**	32 (0.44)	17 (0.44)
***F508del* heterozygous – n (ratio)**	30 (0.41)	16 (0.41)
Other mutations – n (ratio)	11 (0.15)	6 (0.15)

BMI: body mass index, **CF**: cystic fibrosis, **CFLD**: cystic fibrosis related liver disease, **CFRD**: cystic fibrosis related diabetes, **FEV_1**: forced expiratory volume in 1 sec, **ICS**: inhaled corticosteroid, **NTM**: non-tuberculous *Mycobacteria,* **PPI**: proton pump inhibitor

3.2.2 Microbiological identification of NTM species

Identification of NTM species was performed in the Microbiology Laboratory of NKIP guided by protocols of the Clinical and Laboratory Standard Institute. All sputum samples were collected via spontaneous expectoration.

The Ziehl-Neelsen staining method was used to detect acid-fast bacilli in the sputum smear. A direct nucleic acid amplification test (GeneXpert, Cepheid, Sunnyvale, CA, USA) was performed in the presence of Ziehl-Neelsen-positive bacilli to confirm or exclude the presence of the *Mycobacterium tuberculosis complex*. In addition, laboratory confirmation of NTM was based on culturing the bacteria in both solid (Löwenstein-Jensen) and liquid (MGIT) media. Negative NTM results were inferred after the inoculated medium had been incubated for eight weeks with no signs of bacterial growth. A positive NTM result required 7 days or 6 weeks, depending on the medium (liquid or solid) and the NTM species (rapidly or slowly growing). The biomass from the cultures enabled genotypic and phenotypic identification (GenoType Mycobacterium CM, Hain Lifescience GmbH, Nehren, Germany) and drug susceptibility tests using polymerase chain reaction and reverse hybridisation (GenoType NTM-DR, Hain Lifescience GmbH, Nehren, Germany). Microbroth dilution method was used for phenotypic antimicrobial susceptibility testing of rapidly growing *mycobacteria* (RGM). Macrolide resistance was evaluated using genotypic (rrl and erm(41) genes) and phenotypic methods. The minimum inhibitory concentrations were read after 3, 7 and 14 days of incubation. The epidemiological cut-off values were determined in accordance with the recommendations of the European Committee for Antimicrobial Susceptibility Testing (EUCAST), using Sensititre RAPMYCOI plates (Thermo Fisher Scientific Inc., US) (76).

3.2.3 Data collection and statistical analysis

Data were obtained from the records of the HNCFR and the hospital information system of NKIP.

Values of continuous variables were presented as mean ± standard deviation (SD) and comparisons between independent groups were performed using independent samples *t*-test. Fisher's exact test was applied to compare data expressed as proportions. To compare time series data between two groups, two-way repeated-measures ANOVA and Dunnett's post hoc test for multiple comparisons were used. Predictors of NTM acquisition were

identified using logistic regression. Multivariate logistic regression included parameters for which $p < 0.2$ was obtained in the univariate analysis. All statistical analyses were performed using GraphPad Prism 7.0 (San Diego, CA, USA) and Stata 15.1 (College Station, TX, USA), with $p \leq 0.05$ considered statistically significant.

4 Results

4.1 Oxidative stress study

4.1.1 Clinical variables and sputum cell profile during treatment with ETI

As expected, triple-combination CFTR modulator therapy resulted in dramatic improvements in lung function, systemic inflammatory marker levels and body mass index (BMI) in pwCF (**Table 4**). After two to three months of ETI treatment, beneficial changes were already apparent.

During the course of treatment, the number of pwCF able to spontaneously expectorate gradually decreased. Accordingly, four patients (15.3%) no longer had sputum at visit 2, and further nine patients (34.8%) at visit 3. The remaining patients also showed reduced total sputum cell, sputum neutrophil and lymphocyte counts at visit 2 compared to baseline ($p < 0.05$). Total sputum cell count and the cell profile of patients still expectorating at visit 3 were similar to the overall cohort average at baseline (**Table 4**).

Table 4. Demographic and clinical characteristics of study subjects.

Data are presented as mean ± SEM or median (interquartile ranges), unless stated otherwise. $^{***}p < 0.001$, $^{**}p < 0.01$ and $^{*}p < 0.05$ vs. baseline visit (0 month). Adopted from (69) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by-nc-nd/4.0/).

	Healthy controls	CF		
Subjects (n)	22	26		
		Treatment with ETI		
		Baseline visit (0 month)	**Visit 2 (2 or 3 months)**	**Visit 3 (6 months)**
Pulmonary function				
FVC (L)	3.93±0.2	2.7±0.22	3.14±0.22***	3.19±0.21***
FVC (%)	99.8±3.3	69.2±3.82	82.6±3.42***	83.4±3.55***
FEV_1 (L)	3.08±0.14	1.59±0.18	2.00±0.21***	1.99±0.21***
FEV_1 (%)	107.1±3.5	45.4±3.94	55.5±3.91***	56.2±4.1***
Laboratory data				
WBC (G/L)	7.31±0.35	9.44±0.66	7.45±0.51***	7.64±0.55***
CRP (mg/L)	6.07±1.21	14.0±2.34	5.36±1.88***	3.98±1.24***
BMI (kg/m^2)	24.2±1.4	20.4±0.52	21.1±0.53**	21.6±0.59***
Sputum (n)	22	26	22	13
Total cell count ($\times 10^6$/g)	0.32 (0.24-0.69)	11.5 (2.7-30.0)	4.0 (3.0-18.3)*	10.0 (2.1-22.2)
Neutrophils (%)	78.6 (67.6-88.5)	86.0 (68.0-92.0)	72.0 (60.0-86.0)	76.0 (70.0-84.0)
Neutrophils ($\times 10^6$/g)	0.28 (0.2-0.51)	8.6 (1.9-27.6)	2.9 (2.2-13.7)*	8.4 (1.5-20.4)
Macrophages (%)	16.2 (7.9-30.1)	4.0 (0.0-16.0)	8.0 (4.0-15.0)	10.0 (4.0-12.0)
Macrophages ($\times 10^6$/g)	0.08 (0.02-0.11)	0.6 (0.0-2.0)	0.5 (0.2-0.9)	0.8 (0.2-1.2)
Lymphocytes (%)	3.05 (0.84-4.72)	10.0 (6.0-18.0)	8.0 (5.0-15.5)	8.0 (4.0-14.0)
Lymphocytes ($\times 10^6$/g)	0.008 (0.0-0.02)	1.4 (0.6-1.8)	0.4 (0.1-1.6)*	0.4 (0.2-3.0)
Eosinophils (%)	0.0 (0.0-0.5)	0.0 (0.0-2.0)	0.0 (0.0-3.0)	0.0 (0.0-2.0)

Eosinophils ($\times 10^6$/g)	0.0 (0.0-0.002)	0.0 (0.0-0.06)	0.0 (0.0-0.15)	0.0 (0.0-0.27)

BMI: body mass index, **CF***:* cystic fibrosis*,* **CRP**: C-reactive protein, **ETI**: elexacaftor/tezacaftor/ivacaftor, **FEV_1**: forced expiratory volume in one second, **FVC**: forced vital capacity, **WBC**: white blood cells

4.1.2 Oxidative stress markers and antioxidants in sputum

Sputum measurements were only conducted in pwCF who were able to spontaneously expectorate at each visit. While 8-OHdG and 8-IP were detectable in all sputum supernatants tested, protein carbonyl levels fell below the detection limit in sputum samples of six (27.3%) subjects in the control group and one (3.8%) subject in the pwCF group.

At baseline, the concentration of all oxidative markers in sputum was significantly higher in pwCF than in healthy controls ($p < 0.01$, **Figure 5**, Panels A-C). Treatment with ETI led to a decrease in the levels of all these markers in pwCF ($p < 0.05$). Significant reductions in 8-OHdG and protein carbonyl levels were observed as early as visit 2, whereas for 8-IP, this was seen only at visit 3, after six months of treatment. The most pronounced decrease (~3.8-fold) was in protein carbonyl levels. Importantly, both 8-OHdG and protein carbonyl levels remained significantly lower even after six months of treatment.

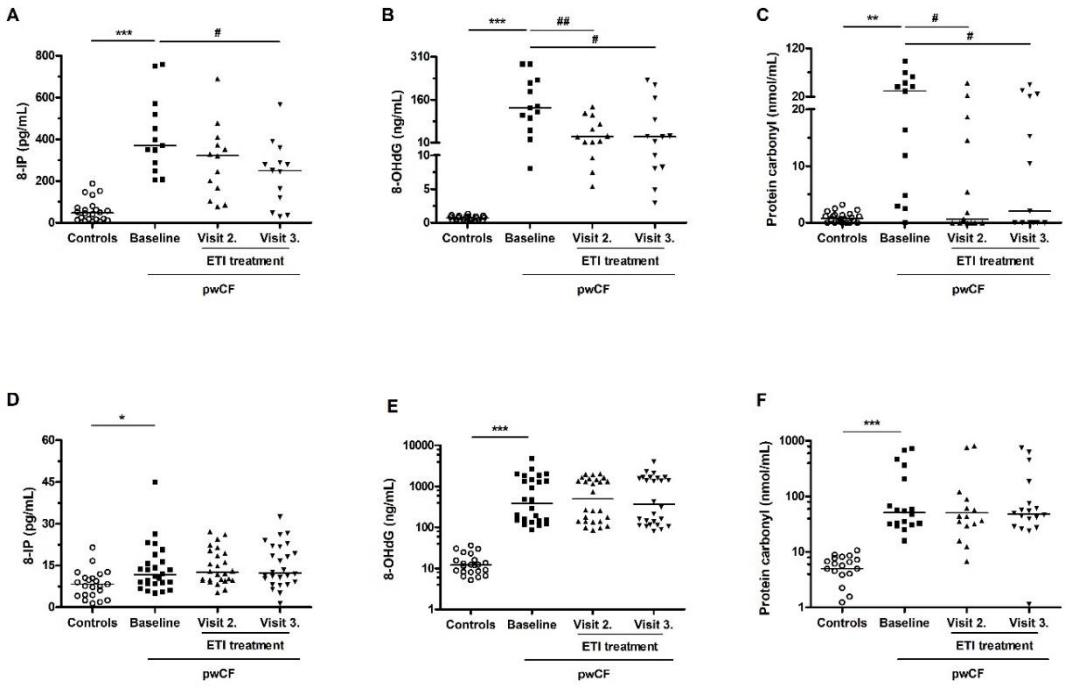

Figure 5. Sputum (Panels A-C) and plasma (Panels D-F) levels of oxidative stress markers in healthy controls and pwCF during follow-up.

In pwCF, 8-isoprostane (8-IP), 8-hydroxy-2-deoxyguanosine (8-OHdG) and protein carbonyl levels were assessed, first, at the time of initiating elexacaftor/tezacaftor/ivacaftor (ETI), a triple cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapy (baseline) and after initiation of modulator treatment at visit 2 and visit 3. Horizontal bars represent median values. **pwCF**: people with cystic fibrosis. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. healthy controls; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ vs. initiating ETI therapy (0 mo). Adopted from (69) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by-nc-nd/4.0/).

Regarding antioxidants, SOD and CAT activity were only detectable in the sputum of 13 (59.1%) and 18 (81.8%) subjects in the control group, respectively. In the pwCF group, SOD activity was below the limit of detection in only one patient, while CAT was detectable in all patients. Compared to healthy controls, pwCF had higher sputum SOD and CAT activity at baseline ($p < 0.01$, **Figure 6**, Panels A_B). After six months, triple

CFTR modulator therapy resulted in decreased SOD activity ($p < 0.05$). Conversely, CAT activity was not affected by modulator treatment.

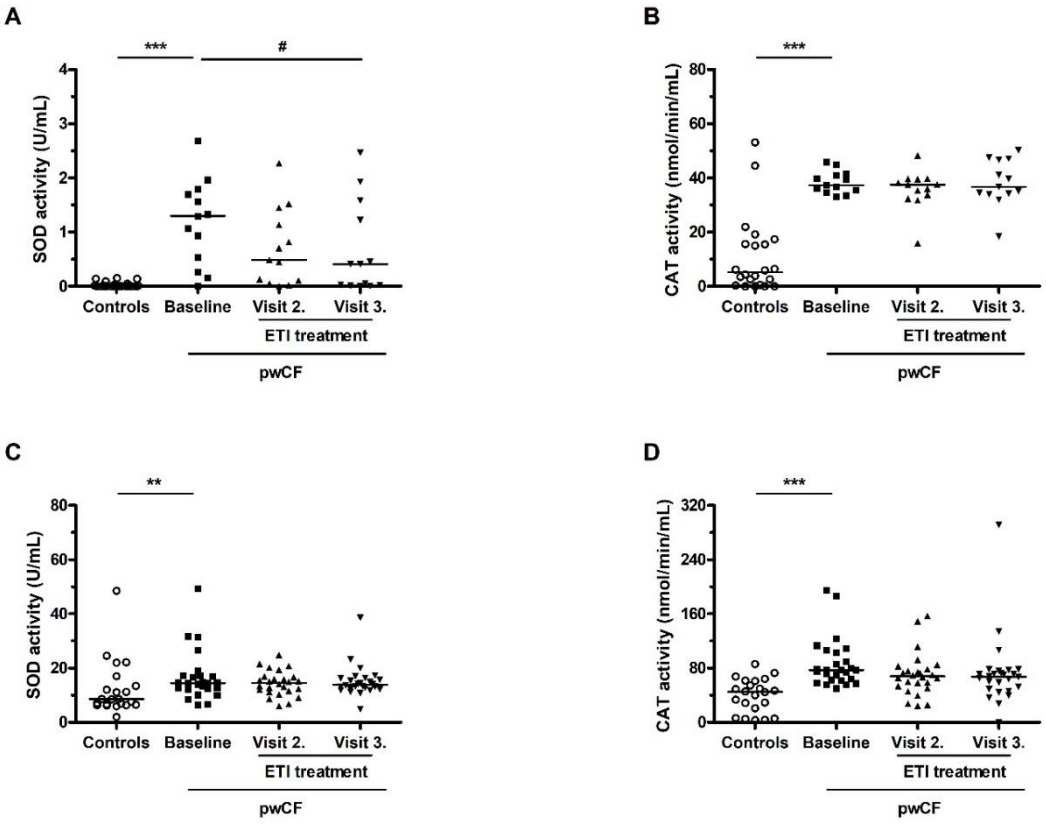

Figure 6. Endogenous antioxidant activities in sputum (Panels A and B) and plasma (Panels C and D) in pwCF during follow-up and in healthy controls.

In pwCF, superoxide-dismutase (SOD) and catalase (CAT) activities were assessed, first, at the time of initiating elexacaftor/tezacaftor/ivacaftor (ETI), a triple cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapy (baseline) and after modulator treatment at visit 2 and visit 3. Horizontal bars represent median values. **pwCF**: people with cystic fibrosis. $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. healthy controls; $^{\#}p < 0.05$ vs. initiating ETI therapy (0 mo). Adopted from (69) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by-nc-nd/4.0/).

4.1.3 Oxidative stress markers and antioxidants in plasma

Oxidative stress markers 8-IP and 8-OHdG as well as the antioxidants SOD and CAT, were detectable in all plasma samples. Plasma protein carbonyl levels were measurable

in all pwCF subjects and in all but four healthy control subjects. Similar to sputum, plasma levels of all oxidative stress markers and the activity of both antioxidants were significantly elevated in pwCF at baseline compared to healthy controls ($p < 0.05$, **Figure 5**, Panels D-F and **Figure 6** C and D, respectively). However, in contrast to what was observed in the airways, ETI treatment did not induce changes in the levels of any of the plasma markers, nor did it affect the plasma activity of any of the enzymes.

4.1.4 Extrapulmonary effects of ETI treatment

To assess the impact of CFTR modulators on CF-associated comorbidities, I first evaluated changes in hepatic and cholestatic enzyme activities as well as haemoglobin A1c (HbA1c) levels in response to ETI therapy. As shown in **Table 5**, data revealed that CFTR modulator treatment did not alter aspartate aminotransferase (AST), alkaline phosphatase (ALP) or gamma-glutamyl transferase (GGT) activity, nor HbA1c levels during the study period. However, significant increases in both alanine aminotransferase (ALT) and total bilirubin were observed with ETI treatment by the end of the study ($p < 0.05$).

Table 5. Aminotransferase and cholestatic enzyme activities, and glycated hemoglobin level in pwCF at baseline and after initiation of elexacaftor/tezacaftor/ivacaftor (ETI) therapy at visit 2 and visit 3.

Data are presented as median (interquartile ranges). Adopted from (69) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by-nc-nd/4.0/).

	pwCF on ETI		
	Baseline visit (0 month)	**Visit 2 (2 or 3 months)**	**Visit 3 (6 months)**
AST (U/L)	22.0 (18.5- 30.0)	26.0 (22.0-30.0)	27.0 (23.5-31.5)
ALT (U/L)	22.0 (15.5- 28.8)	28.0 (20.5-35.0)*	26.5 (20.0-34.0)*
ALP (U/L)	106.0 (72.0- 134.0)	97.0 (80.0-110.0)	96.0 (75.0-113.0)
GGT (U/L)	18.0 (14.5- 28.5)	20.0 (16.0-25.5)	17.0 (15.5-24.5)
Bilirubin (µmol/L)	8.7 (6.75- 9.45)	11.9 (8.7-16.5)**	13.1 (9.25-17.7)***
HbA1c (mmol/mmol)	35.58 (32.48- 41.53)	34.7 (31.49-41.7)	38.17 (32.41-42.85)

ALP: alkaline phosphatase, **ALT**: alanine aminotransferase, **AST**: aspartate aminotransferase, **ETI**: elexacaftor/tezacaftor/ivacaftor, **GGT**: gamma-glutamyl transferase, **HbA1c**: hemoglobin A1c, **pwCF**: people with cystic fibrosis. ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ vs. baseline visit (0 month)

4.1.5 Correlations

At baseline, there was no significant correlation between spirometric and other clinical variables of pwCF and the levels of oxidative stress markers and antioxidant activities in either plasma or sputum (data not shown). Regarding sputum cell profile, both total inflammatory cell counts, and neutrophil cell counts presented significant correlation with sputum protein carbonyl levels ($r=0.65$, $p < 0.05$ and $r=0.83$, $p < 0.05$, respectively). Additionally, SOD activity was found to correlate with the percentage of sputum neutrophils in pwCF at baseline ($r=0.83$, $p < 0.05$). No other clinically relevant correlations were identified.

4.2 NTM study

4.2.1 Prevalence of pwCF with positive NTM sputum

Over the study period, the proportion of pwCF who tested positive for NTM in sputum samples increased steadily. In 2020, 5.7% (11 out of 192) of pwCF were NTM+, rising to 10.2% (20 out of 197) in 2021, and reaching 12.9% (30 out of 232) in 2022. **Figure 7** illustrates the annual increase in the prevalence of respiratory NTM colonization or infection among pwCF.

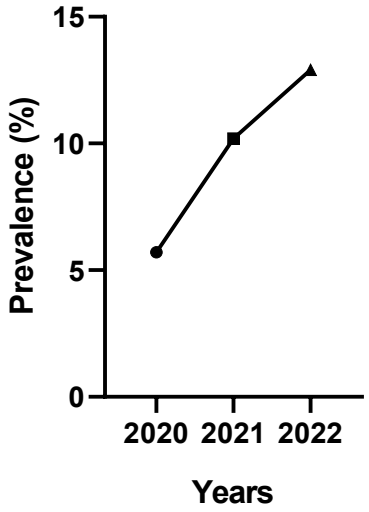

Figure 7. Annual prevalence rate of pwCF with NTM sputum positivity between 2020 and 2022

NTM: non-tuberculous *Mycobacteria*, **pwCF**: people with cystic fibrosis. Original work of the author.

4.2.2 Distribution of NTM species

The following species and subspecies of *Mycobacterium* were identified: MAC (*M. intracellulare and M. avium*), MABSC (*M. abscessus spp. abscessus, spp. massiliense* and *spp. bolletii*), *M. xenopi, M. chelonae, M. gordonae*, and *M. kansasii*. Most patients with positive NTM sputum culture had MAC (16/39) and MABSC (15/39) (**Table 6**). Of these patients, four were positive for multiple NTM species (one for MAC and four for MABSC). In the MAC-positive case, the co-existing pathogen was *M. xenopi*. Of the MABSC-positive subjects, two had *M. chelonae*, one had *M. xenopi* and an unidentifiable NTM strain, and one had *M. gordonae, M. chelonae* and *M. xenopi.* Unidentifiable NTM

isolates were found in three subjects. The third most frequently detectable NTM species was *M. xenopi* (6/39), while *M. chelonae*, *M. gordonae,* and *M. kansasii* were found sporadically (4/39, 2/39 and 1/39, respectively). Of the 16 MAC-positive patients, nine had *M. avium*, five had *M. intracellulare*, and two were co-infected with both species (**Table 6**).

Of the 39 NTM+ patients, 26 (66.67%) were classified as NTM-PD, 11 (28.2%) as transient and two (5.1%) as persistent mycobacterial colonization (**Table 3, Figure 4**). All 15 MABSC-positive cases were classified as NTM-PD (100%), while 11 (68.75%) of the 16 MAC cases were diagnosed as NTM-PD (p=0.04). All patients (n=8) with other than MABSC and MAC had transient mycobacterial colonization (**Table 6**).

Table 6. Distribution of *mycobacteria* in sputum of patients (n=39) with NTM colonization and NTM-PD. Data come from spontaneously expectorated sputum specimens (n=898) collected between 1 January 2020 and 31 December 2022 (annual distribution of specimen numbers: n=105 in 2020, n=294 in 2021 and n=499 in 2022). Adopted from (75) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by/4.0/).

NTM species (Number of pwCF with NTM sputum positivity)	Number of pwCF with NTM colonization (n=13)	Number of pwCF with NTM-PD (n=26)
MAC (16)	5	11
***M. avium* (9)**	2	7
***M. intracellulare* (5)**	2	3
***M. avium* + *M.intracellulare* (2)**	1	1
MABSC (15)	0	15
MABS *spp. abscessus* (13)	0	13
MABS *spp. massiliense* (1)	0	1
MABS *spp. bolletii* (1)	0	1
***M. xenopi* (6)**	6	0
single strain (3)	3	0
***M. chelonae* (4)**	4	0
single strain (1)	1	0
***M. gordonae* (2)**	2	0
single strain (1)	1	0
***M. kansasii* (1)**	1	0
single strain (1)	1	0
Unidentified *mycobacteria* (3)	3	0
single strain (2)	2	0

MABSC: *Mycobacterium abscessus complex*, **MAC**: *Mycobacterium avium complex*, **NTM**: non-tuberculous *Mycobacteria*, **PD**: pulmonary disease, **pwCF**: people with cystic fibrosis

4.2.3 Antibiotic susceptibility pattern of MABSC strains

A total of 20 different MABSC isolates were identified in the sputum samples of 15 patients with pulmonary disease. Thirteen of these were infected with *M. abscessus subsp.*

abscessus, one with *M. abscessus subsp. massiliense*, and one with *M. abscessus subsp. bolletii*. The patient with *spp. bolletii* had four strains, and two patients with *spp. abscessus* had two strains with different antibiotic susceptibility patterns from different sputum samples. The remaining 12 patients presented with a single NTM strain. All isolates were resistant to doxycycline and ciprofloxacin. The resistance rates for moxifloxacin, sulfamethoxazole/trimethoprim (SMX-TMP) and imipenem were high (90.0, 85.0 and 85.0%, respectively). Only 50.0% and 40.0% of MABSC strains were susceptible and intermediate to cefoxitin and tobramycin, respectively. The majority of MABSC isolates showed favourable susceptibility to tygecycline, amikacin, linezolid, and macrolide (100, 95, 100 and 60%, respectively) (**Figure 8**).

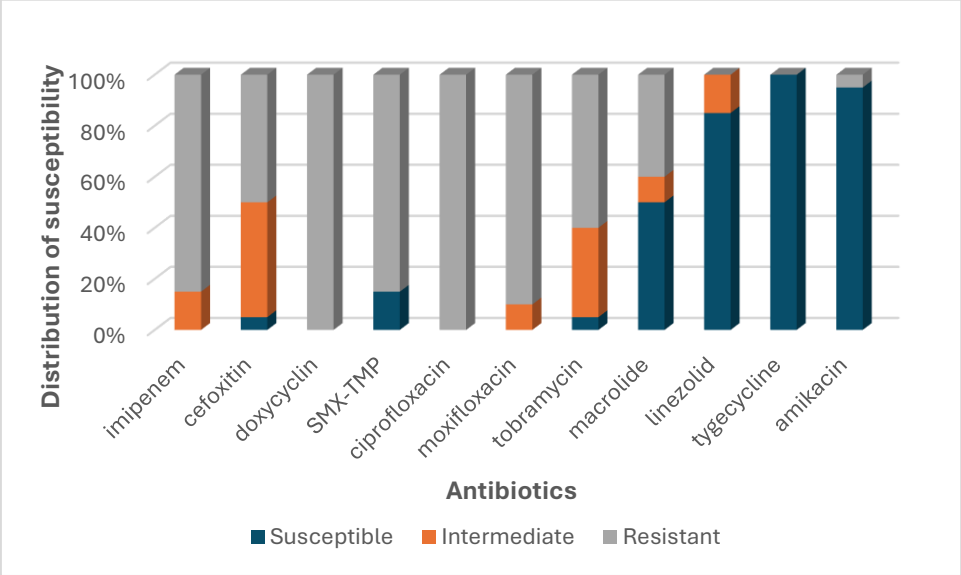

Figure 8. Antimicrobial susceptibility of MABSC isolates (n=20)

MABSC: *Mycobacterium abscessus complex*, **SMX-TMP**: sulfamethoxazole-trimethoprim. Adopted from (75) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by/4.0/).

4.2.4 Microbes other than *mycobacteria* among patients with NTM

The co-existing microbes were identified on either the index date or within a three-month window preceding or succeeding that date. Of the thirty-nine NTM+ patients, only two were free of any co-existing respiratory pathogens at the time of the index. Most patients had *P. aeruginosa* (25/39) and *S. aureus* (20/39) in their sputum. *A. xylosoxidans* (7/39)

and *Aspergillus fumigatus* (8/39) were less common. *B. cepacia*, *S. maltophilia*, *Nocardia* and *Proteus mirabilis* were found sporadically in the subjects (**Table 7**.).

Table 7. Distribution of co-existing microbes in the sputum of pwCF (n=39) with NTM. Data are recorded at the time of the first NTM isolation. Adopted from (75) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by/4.0/).

Bacteria detected in the sputum	Number and percentage of patients with NTM	
	N	%
P. aeruginosa	25	64
S. aureus	20	51
A. xylosoxidans	7	17
Aspergillus fumigatus	8	21
B. cepacia complex	1	3
P. mirabilis	1	3
S. maltophilia	1	3
Nocardia	1	3

NTM: non-tuberculous *Mycobacteria*

4.2.5 Patient characteristics associated with NTM-positivity

For identification of demographic and clinical factors that may predict NTM acquisition in pwCF, a wide spectrum of clinical and laboratory data from 39 NTM+ and 73 NTM- pwCF was assessed (**Table 3**).

No significant differences were observed in demographics, co-existing microbes, CF-related diseases, or the use of proton-pump inhibitors (PPIs), macrolides, or inhaled corticosteroids (ICS) when comparing cases with controls. However, patients with NTM had a significantly lower FEV_1 at the index date than their matched controls (48.0 ± 20.3% vs. 64.9 ± 26.5, $p < 0.01$). Previous sputum NTM positivity was more frequent in cases than in controls (23% vs. 4%, $p < 0.01$). 6 of the 12 cases had the same NTM species at the index date as had been detected previously. The remaining 6 cases had different NTM species at the index date than previously.

4.2.6 Decreased lung function associated with NTM-positivity

As forced expiratory volume in 1 second (FEV_1) was found to be lower in NTM+ pwCF than in NTM- pwCF in the case-control study, I conducted further analysis of their association. The FEV_1 values of pwCF were assessed at the index date and compared with values measured one and two years earlier. These data were available for 36 NTM+ and 57 NTM- pwCF. **Figure 9** shows the differences observed between NTM+ and NTM- subjects regarding the change in FEV_1 over the two-year period. The mean FEV_1 values of NTM+ cases were significantly lower than those of controls at years 0, -1 and -2 relative to the index date (47.97, 50.92 and 55.31% vs. 61.53, 62.58, and 68.81%, $p < 0.05$). No significant difference was found in ΔFEV_1 between the two groups (ΔFEV_1: -7.33±11.91% in the NTM+, and -7.28±10.81% in the NTM- group; p=0.98). Furthermore, multivariate logistic regression models that accounted for previous sputum NTM positivity and FEV_1 values at the index date, as well as in the one- and two-years prior, confirmed that these factors were associated with the risk of NTM acquisition (**Table 8**).

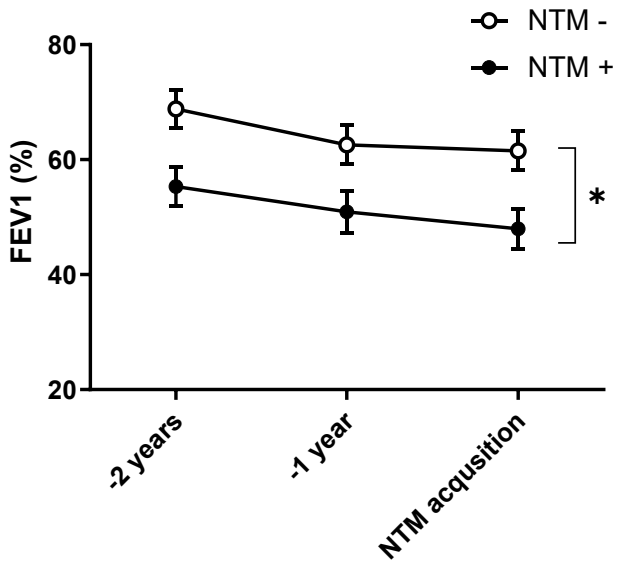

Figure 9. Changes in FEV_1 in the two years preceding NTM acquisition.

FEV_1 values showed declines typical of pwCF in both the NTM- (n=57) and NTM+ (n=36) groups in the 2 years preceding NTM acquisition. However, NTM+ patients had significantly lower values throughout. **NTM***:* non-tuberculous *Mycobacteria,* **FEV_1***:*

forced expiratory volume in 1 second. Statistics: 2-way repeated measurement analysis of variance; *: p≤0.05. Adopted from (75) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by/4.0/).

Table 8. Association of potential risk factors with NTM acquisition.

The results of the logistic regression analysis describe the potential predictors of NTM infection in pwCF. Models 1, 2 and 3 of the multivariate analysis included FEV_1 at the time of NTM detection and FEV_1 one and two years before NTM acquisition, respectively, as well as covariates that showed an association with NTM positivity in the univariate analysis with a p-value of less than 0.15. Adopted from (75) under the terms of the Creative Commons Attribution Non-Commercial Licence (https://creativecommons.org/licenses/by/4.0/).

	Univariate analysis			**Multivariate analysis - Model 1***		
	OR	95 % C. I.	p	OR	95 % C. I.	p
BMI	0.90	0.78;1.05	0.19	-	-	-
FEV_1 - % at the time of NTM detection	0.97	0.95;0.99	**0.002**	0.97	0.96;0.99	**0.004**
FEV_1 - % - 1 year before NTM acquisition	0.98	0.96;0.99	**0.03**	-	-	-
FEV_1 - % - 2 years before NTM acquisition	0.98	0.96;0.99	**0.01**	-	-	-
Macrolide treatment in patient history (n=11)	2.44	0.76;7.86	0.13	2.46	0.63;9.64	0.271
PPI treatment (n=23)	0.85	0.33;2.19	0.74	-	-	-
Previous NTM positivity in sputum (n=11)	7.00	1.77;27.68	**0.01**	5.46	1.25;23.77	**0.024**
Co-existing *P. aeruginosa* (n=56)	1.56	0.66;3.72	0.31	-	-	-
Co-existing *S. aureus* (n=55)	0.65	0.28;1.52	0.32	-	-	-
Co-existing *A. xylosoxidans* (n=12)	1.70	0.50;5.74	0.39	-	-	-
Co-existing *Aspergillus* (n=13)	1.43	0.44;4.65	0.55	-	-	-

	Multivariate analysis - Model 2**			Multivariate analysis – Model 3***		
	OR	95 % C. I.	p	OR	95 % C. I.	p
Body mass index	-	-	-	-	-	-
FEV_1% at the time of NTM detection	-	-	-	-	-	-
FEV_1% - 1 year before NTM acquisition	0.98	0.96; 0.99	**0.049**	-	-	-
FEV_1% - 2 years before NTM acquisition	-	-	-	0.97	0.96; 0.99	**0.027**
Macrolide treatment in patient history	3.05	0.73; 12.7	0.13	2.74	0.52; 6.97	0.16
PPI treatment	-	-	-	-	-	-
Previous NTM positivity in sputum	4.62	1.06;20.11	**0.42**	3.78	0.87; 16.41	0.075

** Hosmer-Lemeshow test: chi^2=4.48, p value=0.11; area under ROC curve: 0.71*

*** Hosmer-Lemeshow chi^2=0.87, p value=0.65, area under ROC curve: 0.66*

**** Hosmer-Lemeshow chi^2=2.17, p value=0.34, area under ROC curve: 0.69*

BMI*:* body mass index, **FEV_1***:* forced expiratory volume in 1 second*,* **NTM***:* non-tuberculous *Mycobacteria,* **PPI***:* proton pump inhibitor

4.2.7 Effect of ETI treatment on sputum NTM status

The findings of the oxidative stress study prompted an expansion of the NTM investigation to evaluate whether ETI therapy influences the sputum NTM status in pwCF. In the 2nd study period, the triple combination CFTR modulator therapy (ETI) became available to most pwCF, providing an opportunity to assess its impact on NTM sputum status in the study population (n = 34; 5 patients were withdrawn from the original cohort) (**Figure 4**). **Table 9** shows the distribution of individuals who achieved sputum NTM clearance (n=23) and those who did not (n=11), categorized according to whether they received anti-mycobacterial therapy during the 1st study period and/or ETI treatment in the 2nd study period.

Table 9. Sputum NTM status in pwCF stratified by ETI treatment and NTM therapy. Original work of the author.

2nd study period / 1st study period	PwCF on ETI (n=25)	ETI-naïve pwCF (n=9)
PwCF treated for NTM (n=24) – n	17	7
Remained NTM+ during the 2nd study period – n	6	3
Converted to NTM- during the 2nd study period – n	11	4
PwCF not treated for NTM (n=10) – n	8	2
Remained NTM+ during the 2nd study period - n	1	1
Converted to NTM- during the 2nd study period – n	7	1

ETI: elexacaftor/tezacaftor/ivacaftor, **NTM**: non-tuberculous *Mycobacteria*, **pwCF**: people with cystic fibrosis

Among the 11 patients who remained NTM+, 7 had the same NTM species as in the 1st study period, while 4 had a different species (**Table 10**).

Table 10. Distribution of NTM species during the 1st and 2nd study period. Original work of the author.

Number of pwCF	Isolated NTM species in the 1st study period	Isolated NTM species in the 2nd study period
Successful NTM clearance		
9	MABSC	N/A
9	MAC	N/A
2	Not-identified *mycobacterium*	N/A
1	*M. chelonae*	N/A
1	*M. xenopi*	N/A
1	*M. gordonae*	N/A
Unsuccessful NTM clearance		
4	MABSC	MABSC
2	MAC	MAC
1	*M. xenopi*	*M. xenopi*
1	MAC	*M. xenopi*
1	MAC	*M. gordonae*
1	MABSC	*M. xenopi*
1	*M. kansasii*	*M. gordonae*

MABSC: *Mycobacterium abscessus complex*, **MAC**: *Mycobacterium avium complex*, **NTM**: non-tuberculous *Mycobacteria*, **pwCF**: people with cystic fibrosis

Statistical analysis using Fisher's exact test revealed that ETI therapy slightly increased the odds of sputum NTM clearance in the total study population (OR: 2.057; 95% CI: 0.501–9.614), although the association was not statistically significant (p=0.4254). Further subgroup analysis indicated an improved ratio for NTM clearance among patients not receiving specific anti-mycobacterial treatment when treated with ETI (OR: 7.00; 95% CI: 0.190–147.6; p=0.378), compared to those receiving both therapies (OR: 1.375; 95% CI: 0.270–7.512; p=0.9999). Nevertheless, none of these associations achieved statistical significance.

5 Discussion

5.1 Oxidative stress study

The study findings showed that pwCF had higher levels of airway and systemic oxidative stress markers and increased antioxidant activity compared to healthy controls. Treatment with ETI, a triple combination CFTR modulator, led to a significant decrease in the levels of airway markers 8-IP, 8-OHdG, and protein carbonyl. However, the treatment did not have any impact on the systemic concentration of these markers. ETI therapy also resulted in reduced SOD activity in the airways with no corresponding changes observed in plasma. Notably, certain pwCF had baseline plasma oxidative stress marker concentrations within the normal range, overlapping with those of controls; this was particularly evident for 8-IP, SOD and CAT. Consequently, further reduction in these markers following ETI treatment may not necessarily be expected. Most of these changes were already visible as early as two or three months after the initiation of modulator treatment. This finding is important as oxidative stress is known to play a substantial role in CF progression and is associated with inflammation and tissue damage in the lungs. Overall, the data suggest that ETI therapy may modulate the redox status in the airway and may help to mitigate these harmful effects and improve overall lung function in pwCF.

Oxidative stress refers to the imbalance between the production of ROS and the ability of antioxidant defenses to neutralize them. In pwCF, the malfunctioning CFTR protein impairs the transport of extracellular glutathione, an important antioxidant that helps protect cells from oxidative damage. Furthermore, CFTR deficiency disrupts lipid metabolism, leading to an altered lipid composition and making cells more susceptible to oxidative damage and inflammation. Additionally, impaired CFTR function can disrupt the autophagy process responsible for removing damaged organelles and proteins. This dysregulation in autophagy can further contribute to oxidative stress in CF (32). Chronic polymicrobial infections exacerbate airway inflammation and enhance the production of ROS, primarily due to abnormal viscoelastic properties of mucus, impaired mucociliary clearance, and mucus plugging. These pathological features, combined with malabsorption of micronutrients with antioxidant properties (33), disrupt the oxidant–antioxidant balance and promote oxidative damage to cellular macromolecules.

This study is the first to demonstrate that triple combination CFTR modulator therapy significantly reduces oxidative injury in the airways, potentially representing a key mechanism underlying the clinical benefits of ETI. The reduction in oxidative stress was evidenced by decreased levels of the lipid peroxidation product 8-IP as well as lower concentrations of oxidative damage markers of nucleic acids and proteins, 8-OHdG and protein carbonyls, respectively. Given that increased deoxyribonucleic acid damage has been reported in pwCF, reducing oxidative stress may also have implications for the elevated malignancy risk observed in this population compared to age-matched individuals without CF (77). Oxidative damage to proteins has been implicated in the pathogenesis of various chronic diseases, including Alzheimer's disease, chronic kidney disease, and diabetes, and may also contribute to skeletal muscle dysfunction in pwCF (78).

In contrast to sputum, ETI treatment did not alter the levels of oxidative stress markers in the blood. Generally, the presence of these markers in plasma reflects systemic oxidative stress, which may originate from various tissues and not necessarily the airways, whereas their presence in respiratory samples is more likely to indicate oxidative processes occurring locally within the lungs (70). The discordance between the local (airway) and systemic (blood) redox response following ETI treatment may explain the lack of improvement in CF-related comorbidities, such as CFLD and CFRD. These comorbid conditions may significantly contribute to free radical production and thus prevent the improvement of the oxidant/antioxidant balance in the blood to the extent seen in the airways. In line with this theory, this study showed a significantly increased plasma level of protein carbonyls in pwCF with CFRD and/or CFLD at baseline compared to patients without such comorbidities. Furthermore, ETI does not fully restore normal CFTR activity; thus, some pwCF may continue to exhibit suboptimal chloride transport and persistent epithelial dysfunction. In addition, ETI does not completely normalize inflammatory signaling pathways, such as NF-κB–mediated activation, which promotes the release of ROS and pro-inflammatory cytokines (51). These cumulative factors may contribute to ongoing cellular damage and, ultimately, to further progression of CF and its related comorbidities despite ETI treatment.

The elevated SOD and CAT activity observed in pwCF is suggestive of a protective regulatory response to mitigate oxidative damage caused by excessive ROS production.

Consistent with this hypothesis, the reduction in airway free radical production following CFTR modulator therapy was accompanied by decreased SOD activity in sputum samples. In contrast, antioxidant enzyme activity in the blood remained unchanged, likely reflecting the absence of significant modulation in systemic redox status. Moreover, patients with *P. aeruginosa* sputum positivity had higher plasma SOD activity at baseline than those without, which may also indicate a protective role against free radicals in the body. Notably, our research group recently reported a similar upregulation of antioxidant defenses, including increased SOD activity, in patients experiencing acute exacerbations of chronic obstructive pulmonary disease (COPD), suggesting a common adaptive response to heightened oxidative burden in chronic airway infections (71).

The effect of ETI therapy on CFLD and CFRD remains controversial in the literature. For example, Steinack et al. observed an improvement in glucose levels as measured by the oral glucose tolerance test, and a significant reduction in HbA1c levels after three months of treatment, while the levels of insulin, C-peptide secretion and the insulinogenic index did not change (79). A recent review article evaluating the effect of ETI therapy on blood glucose levels concluded that there is considerable variability in the glycaemic response to this treatment in pwCF (80). Regarding liver function tests, Schnell et al. reported an increase in ALT activity and total bilirubin levels following ETI treatment, similar to this study (81). These changes may represent a mild adverse effect of the treatment that typically does not necessitate discontinuation of therapy.

The findings described here on improvements in lung function, systemic inflammatory markers, and BMI are consistent with the well-documented clinical benefits of ETI therapy reported in multiple clinical trials (41, 45, 46, 82). A reduction in sputum neutrophil counts as early as three months after initiation of ETI therapy has also been reported by other groups (49). However, among the oxidative stress markers evaluated, only protein carbonyls showed a direct association with sputum neutrophil granulocyte counts. This finding suggests that neutrophilic inflammation contributes to, but does not fully account for, the extent of oxidative injury in the airways.

Finally, the observation that approximately half of the patients no longer expectorated sputum after six months of ETI therapy aligns with findings reported by other research groups (49). Importantly, subgroup analysis revealed no significant differences in blood

levels of oxidative stress markers between patients who continued to produce sputum at the six-month follow-up and those who did not.

This study is not without limitations. First, the sample size was relatively small. Nevertheless, the differences and similarities observed between the control and patient groups appear robust and consistent. Larger, multicentre studies are warranted to confirm and extend these observations. Second, the interval for visit 2 varied between two and three months due to individual scheduling constraints. Presumably, this slight temporal variability did not substantially influence the primary outcomes. Third, the lack of strict age and sex matching between the pwCF and control groups represents another limitation. However, the primary objective was to prospectively evaluate changes in redox status following ETI initiation, and the control group served mainly as a general reference for baseline levels rather than for direct statistical comparison.

5.2 NTM study

The increasing prevalence of NTM infection has become an emerging problem in patients with risk factors, such as CF (83). Clinicians managing NTM infections in pwCF are confronted with several challenges, including the need for prolonged antimicrobial therapy, emerging antibiotic resistance, drug-related toxicity, and frequent relapses. In advanced cases of NTM-PD, empirical antibiotic treatment may be initiated; however, the substantial regional variability in NTM species distribution and antibiotic susceptibility often undermines both empirical and targeted therapeutic strategies. Consequently, region-specific epidemiological data on NTM prevalence and resistance patterns in CF populations are critical to inform and adapt clinical guidelines to local settings.

To date, most published studies on NTM infections in CF have focused primarily or exclusively on paediatric populations (60, 61, 84). However, with the advent of highly effective CFTR modulator therapies, life expectancy in CF has significantly improved over the past two decades, highlighting the need to assess NTM infection rates and characteristics in adult CF cohorts. To address this gap, this study provides detailed data on NTM prevalence, species distribution, and antibiotic resistance in a large adult CF population in Hungary. Additionally, FEV_1 was identified as a potential predictor of subsequent NTM acquisition in this patient group.

The annual prevalence of NTM+ sputum cultures increased from 5.7% to 12.9% over the study period**.** Recent European studies have reported NTM prevalence rates ranging from 8% to 12% (61, 63), although substantial geographical variability has been noted. For instance, Adjemian et al. reported prevalence rates between 0% and 28% depending on the region (85). Several factors may contribute to the observed increase in respiratory NTM colonization, including greater environmental exposure to *mycobacteria* (e.g., household water and shower aerosols), the use of more sensitive diagnostic techniques, and heightened clinician awareness (86). It is assumed that the increased NTM prevalence observed in 2022 may, at least in part, be attributed to the COVID-19 pandemic. During 2020 and 2021, many patients avoided regular visits to the CF clinic due to concerns about coronavirus infection. This likely led to a reduction in the number of sputum cultures obtained during this period (**Table 6**), potentially resulting in some NTM+ cases going undiagnosed. As the pandemic subsided in 2022 and patients returned to their normal routines of regularly attending the CF clinic, previously undetected cases may have been identified, contributing to the apparent rise in NTM prevalence. Additionally, the moderately elevated prevalence observed in this study may also be explained by the fact that the cohort consisted exclusively of adult patients. It is well established that the risk of NTM acquisition increases with age (87), whereas most published prevalence data are derived from study populations that include children (61, 84).

Based on the presented findings, the two predominant NTM species isolated from sputum samples were MAC and MABSC, consistent with previous reports (60, 63, 85). A slight predominance of MAC over MABSC was observed - a distribution pattern typically seen in North America (85), but less common in most European countries (61, 63, 88). Notably, similar MAC dominance has been reported in Portugal (89) and France (84), aligning with these results. Interestingly, the third most frequently identified NTM species in this cohort was *Mycobacterium xenopi*. This finding is atypical, as studies from Germany, France, and Portugal report *M. gordonae* and *M. chelonae* as the more commonly detected species after MAC and MABSC (61, 88, 89). The relatively high prevalence of *M. xenopi* in this study may reflect the influence of local climatic and geographical factors—such as extremely hot summers and the abundance of freshwater sources—that could promote the proliferation and spread of certain NTM species within the Hungarian environment.

All patients infected with MABSC were diagnosed with pulmonary disease, whereas only 69% of those with MAC met the criteria for NTM pulmonary disease. Mycobacterial species other than MAC and MABSC were detected only transiently and were not associated with clinical disease. These findings are in line with previous studies, which have shown that RGM are more likely to cause pulmonary pathology than slow-growing species (90). The higher pathogenic potential of MABSC is thought to be related to specific virulence factors, such as mycobacterial membrane large proteins and surface glycopeptidolipids, which contribute to severe tissue damage and disease progression (91, 92).

Besides NTM, *P. aeruginosa* and *S. aureus* were frequently identified in sputum samples (**Table 7**), consistent with previous studies (54, 93). The role of co-existing airway microbes remains unclear. However, an autoregressive integrated moving average model suggests that the airway microbiome functions as a dynamic bacterial community, with interactions that may influence inflammation levels and infection outcomes (94). Therefore, therapeutic decisions should account for the presence of other respiratory bacteria. Notably, *A. xylosoxidans* has emerged as a significant respiratory pathogen in pwCF. This is particularly concerning, as colonization by *Achromobacter* species has been associated with reduced lung function, frequent exacerbations, and progressive lung damage (95, 96).

PwCF with a history of NTM+ sputum cultures were found to face a higher risk for NTM reacquisition. This finding aligns with a study by Martiniano et al., which also reported a high rate of recurrent NTM+ cultures (97). Analysis of additional factors—including genotypes, CF-related comorbidities, medications, and demographic characteristics—revealed no significant differences between NTM+ and NTM- patients. Furthermore, no association was observed between NTM-positivity and the presence of other respiratory microbes in sputum samples (**Table 8**).

In NTM+ cases, a significant reduction in FEV_1 at the index date was observed compared to controls, consistent with previous reports (97, 98). Notably, this difference was also present one and two years prior to the index date, and lower FEV_1 values at all three time points were associated with subsequent NTM acquisition. These findings suggest that a persistent decline in lung function may serve as a predictor for NTM acquisition; however, confirmation in larger cohorts is needed. This is the first study to document a

two-year trend in lung function preceding NTM detection in sputum. Based on this observation, more frequent NTM screening may be warranted in patients with FEV_1 < 50%.

Despite the lower baseline lung function in NTM+ patients, the rate of FEV_1 decline over two years (ΔFEV_1) was comparable between the NTM and control groups and was not predictive of NTM acquisition. This indicates that the rate of decline in lung function has minimal influence on susceptibility to NTM. Furthermore, contrary to earlier studies, no accelerated FEV_1 decline was observed following NTM acquisition. This may reflect the benefits of routine sputum surveillance and timely initiation of treatment.

Treatment of MABSC remains suboptimal, with only a limited number of randomized controlled trials available (86, 99). Several professional societies—including the ATS, IDSA, European Respiratory Society, and European Cystic Fibrosis Society—have issued consensus-based treatment recommendations (83, 99). According to these guidelines, lung disease caused by MABSC should be treated during the intensive phase with a macrolide-containing regimen (if the strain is macrolide-sensitive), combined with at least three active parenteral agents such as amikacin, imipenem, cefoxitin, tigecycline, or linezolid.

Nevertheless, it has been demonstrated that there are geographical differences in the antibiotic resistance patterns of *Mycobacterium* species. Consequently, knowledge of local data on antimicrobial resistance is essential for the development of effective treatment guidelines. Most MABSC strains detected in the Hungarian CF population have a high rate of resistance to doxycycline, ciprofloxacin, moxifloxacin and SMX-TMP, which may make it difficult to find an effective antimicrobial combination. In contrast, macrolides, amikacin, tigecycline and linezolid have retained antibacterial activity against the majority of MABSC isolates.

Despite the challenges associated with treating pulmonary NTM infections and colonization, as well as the high risk of relapse, more than half of the study population (67.65%) achieved NTM sputum clearance during the 2nd study period. To date, only a few observations have been published regarding the impact of CFTR modulators on airway NTM colonization and infection (100, 101). Consistent with previous reports, our findings indicate a trend toward improved NTM clearance among individuals receiving ETI therapy, with the highest potential benefit observed in patients who had not received

anti-NTM treatment (101-103). Notably, in patients who did not receive anti-mycobacterial treatment, the effect of ETI on NTM clearance appeared more evident. Conversely, in those treated with anti-NTM agents, ETI was associated with only a limited additive or synergistic effect.

It is hypothesised that ETI may contribute to NTM elimination by restoring airway liquid hydration and enhancing mucociliary clearance, which is a key mechanism for eliminating pathogens from the airways (104, 105). The resulting reduction in mucus accumulation may reduce air trapping and atelectasis, which otherwise promote microbial biofilm formation (e.g., by NTM) (106). The absence of biofilm likely increases the susceptibility of pathogens to antibiotics. These favourable changes in the pulmonary milieu may enhance the efficacy of antimicrobial eradication strategies, while also mitigating airway inflammation and oxidative stress. The study has some limitations. First, although data from all adult pwCF in Hungary were available, the number of NTM cases was inherently limited by the size of the national population. Second, the impact of SARS- CoV-2 infections on lung function decline or disease course was not evaluated, as sputum collection, clinical and laboratory tests were limited during the study period due to the COVID-19 pandemic. Despite these limitations, this study provides one of the most comprehensive analyses of NTM prevalence among Hungarian pwCF to date. It is also the first to document a two-year longitudinal trend in lung function decline preceding the detection of NTM in sputum samples, and an assessment of the impact of ETI on sputum NTM positivity

6 Conclusions

1., CFTR modulator-naïve pwCF exhibit elevated levels of oxidative stress biomarkers and increased activity of endogenous oxidoreductase enzymes—both in sputum and plasma—compared to samples from healthy volunteers.

2., ETI treatment significantly reduces the levels of oxidative stress markers and SOD activity in the sputum of pwCF. In contrast, modulator treatment has no effect on the levels of oxidative end-products and antioxidant activities in plasma. The persistence of systemic oxidative stress despite ETI therapy suggests that current CFTR modulators do not fully normalize redox homeostasis at the systemic level.

3., Treatment with the triple combination CFTR modulator therapy led to significant clinical improvements, including enhanced lung function and BMI, decreased systemic inflammatory markers, and a reduction in sputum production. Importantly, these favourable effects were evident as early as two to three months after initiating ETI treatment and remained sustained at the six-month follow-up.

4., The annual prevalence of NTM+ sputum cultures increased from 4.7% to 12.9% among adult pwCF in Hungary between 2020 and 2022. Alarmingly, many MABSC isolates exhibited high resistance rates to multiple antibiotics, a finding with important implications for the ongoing revision of national CF treatment guidelines.

5., Persistently reduced FEV_1 and a history of NTM+ sputum cultures emerged as independent risk factors for subsequent NTM colonization in this cohort. These associations highlight the importance of close monitoring in high-risk patients and advocate for more frequent NTM screening in individuals with these clinical features.

6., Our preliminary observations suggest that CFTR modulator therapy may increase the rate of NTM clearance, particularly in pwCF who do not receive anti-mycobacterial treatment. By enhancing mucociliary function and airway hydration, ETI therapy may contribute to the evolving landscape of pulmonary microbial colonization in pwCF. These findings raise the possibility that CFTR modulation influences not only the oxidative milieu but also host–pathogen dynamics within the lower airways.

7., By analysing key factors influencing CF lung disease progression, this study provides new insights into the clinical and antioxidant benefits of CFTR modulator therapy and its possible role in enhancing NTM clearance, alongside shifts in NTM epidemiology in the

adult CF population. These findings highlight the need for continued therapeutic innovation to address residual pulmonary and systemic disease, and for targeted public health strategies to manage the growing microbial complexity in CF. Future research should validate these observations in larger cohorts, elucidate the mechanisms behind persistent systemic oxidative stress, and clarify the antimicrobial potential of CFTR modulators to optimise personalised treatment and improve long-term outcomes.

7 Summary

Despite recent advancements in therapeutic strategies, CF remains a chronic, progressive, and multisystemic disease. These investigations aimed to explore additional pathogenic factors that may contribute to lung tissue damage, the principal cause of hospitalization and mortality in pwCF.

These findings demonstrate that oxidative stress biomarkers - 8-IP, 8-OHdG, and protein carbonyls - along with the activities of endogenous oxidoreductases such as SOD and CAT, are significantly elevated in both plasma and sputum of CFTR-modulator-naïve pwCF compared to healthy controls. Importantly, treatment with the triple-combination CFTR modulator (ETI) resulted not only in improvements in clinical parameters (BMI, FVC, and FEV_1) but also in a reduction in oxidative stress markers and antioxidant enzyme activities in sputum. However, systemic oxidative damage remained unaltered after 6 months of ETI therapy, suggesting that current CFTR modulators may lack sufficient systemic antioxidant effects. This finding highlights the need for novel therapeutic strategies that target both local and systemic redox imbalances in CF.

Furthermore, lower respiratory tract infections contribute significantly to progressive lung injury. Among these, NTM represent a group of opportunistic environmental pathogens increasingly implicated in adverse clinical outcomes in pwCF. The study identified *MABSC*, *MAC*, and *Mycobacterium xenopi* as the most frequently isolated species in the cohort. Notably, the prevalence of NTM sputum positivity increased from 5.7% to 12.9% between 2020 and 2022, underscoring a growing burden in the Hungarian CF population. A high prevalence of multidrug-resistant MABSC isolates was also observed, and persistently decreased FEV_1 and previous NTM sputum positivity were identified as independent risk factors for subsequent NTM colonization. Of particular note, these findings suggest that ETI therapy may contribute to improved NTM clearance, supporting the broad therapeutic impact of CFTR modulation.

Taken together, these findings emphasize the importance of regular screening and risk-based monitoring for NTM in adult pwCF and support the integration of oxidative stress assessment into future CF management strategies. Further research is warranted to develop comprehensive therapeutic approaches that address both the microbial and redox-driven components of CF pathophysiology.

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9 Bibliography of the candidate's publications

9.1 List of publications related to the dissertation

1., **Örlős, Zoltán** ; Lőrinczi, Lilla Katalin ; Antus, Balázs ; Barta, Imre ; Miklós, Zsuzsanna ; Horváth, Ildikó
Epidemiology, microbiology and clinical impacts of non-tuberculous mycobacteria in adult patients with cystic fibrosis
HELIYON 11 : 1 p. e41324 Paper: e41324 (2025)
IF: 3.6

2., **Örlős, Zoltán** ; Barta, Imre ; Páska, Csilla ; Halász, Adrien ; Antus, Balázs
Impact of long-term elexacaftor/tezacaftor/ivacaftor therapy on oxidative stress in cystic fibrosis: early insights
JOURNAL OF CYSTIC FIBROSIS 24 Paper: S1569-1993(25)01572-3 (2025)
IF: 6.0

9.2 List of other publications

1., Páska, Csilla ; Barta, Imre ; Csoma, Zsuzsanna ; Gajdócsi, Réka ; Szél, Viktória ; Kerpel-Fronius, Anna ; Solymosi, Diána ; **Örlős, Zoltán** ; Antus, Balázs
Alpha1-Antitrypsin in Lung Diseases: A Cross-Sectional Observational Study
INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 26 : 11 p. 5400 (2025)
IF: 4.9

2., **Örlős, Zoltán** ; Miklós, Zsuzsanna ; Horváth, Ildikó
A COVID–19-fertőzéssel és -immunizációval szerzett tapasztalatok felnőtt cystás fibrosisos betegcsoportban
ORVOSI HETILAP 165 : 9 pp. 332-337. , 6 p. (2024)
IF: 0.9

3., **Örlős, Zoltán**
Közösségben szerzett tüdőgyulladás gyermek- és felnőttkorban
ORVOSTOVÁBBKÉPZŐ SZEMLE 31 pp. 19-24. , 6 p. (2024)

4., Kiss, Helga ; **Örlős, Zoltán** ; Gellért, Áron ; Megyesfalvi, Zsolt ; Mikáczó, Angéla ; Sárközi, Anna ; Vaskó, Attila ; Miklós, Zsuzsanna ; Horváth, Ildikó ✉
Exhaled Biomarkers for Point-of-Care Diagnosis: Recent Advances and New Challenges in Breathomics
MICROMACHINES 14 : 2 Paper: 391 , 29 p. (2023)
IF: 3.0

5., **Örlős, Zoltán**
COVID-19 fertőzés cystás fibrosisban
MUCOVISCIDOSIS HUNGARICA 9 pp. 633-640. , 8 p. (2023)

6., **Örlős, Zoltán** ; Halász, Adrien ; Antus, Balázs ; Miklós, Zsuzsanna ; Horváth, Ildikó
Fiatal cisztás fibrózisos betegek tranzíciója a felnőtt ellátásba
MEDICINA THORACALIS (BUDAPEST) 76 : 1 pp. 32-40. , 9 p. (2023)

7., **Örlős, Zoltán** ; Rákóczi, Éva ; Misák, Oléna ; Lénárt, Beáta ; Ócsai, Gabriella ; Kovács, Ilona ; Gorzsás, Szabolcs ; Kardos, László ; Lampé, Zsolt ; Szilvássy, Zoltán ; Várkonyi, István
Outbreak of anthrax in adults and adolescents: A review of nine cases in a regional teaching hospital in East Hungary
CLINICAL MICROBIOLOGY AND INFECTION 23 : 1 pp. 52-54. , 3 p. (2017)
IF: 5.394

8., **Örlős, Zoltán**
Útmutatás a gyermekkori, fertőző légúti betegségek antibiotikum kezeléséhez
GRANUM: ORVOSI SZAKMAI FOLYÓIRAT 16 : 2 pp. 23-25. , 3 p. (2013)

9., **Örlős, Zoltán** ; Nagy, László ; Lakatos, Lajos
Mycoplasma pneumoniae epidémia Hajdú-Bihar megyében
GYERMEKGYÓGYÁSZAT 56 : 6 pp. 617-621. , 5 p. (2005)

10., **Örlős, Zoltán**

A fertőzések etiopatológiája és terápiája congenitális és ciklikus neutropéniában

CSALÁDORVOSI FÓRUM 6 : 2 pp. 20-24. , 5 p. (2005)

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