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Research article

Epidemiology, microbiology and clinical impacts of non-tuberculous mycobacteria in adult patients with cystic fibrosis

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ABSTRACT

Background: Due to its increasing prevalence and suboptimal treatment, non-tuberculous mycobacterial (NTM) infection is an emerging problem in patients with cystic fibrosis (CF). Detailed description of regional NTM prevalence and distribution, and identification of predictors of NTM acquisition in CF are essential to optimise treatment and surveillance guidelines.
Methods: A retrospective, multi-center analysis was conducted between the years 2020 and 2022 on data from 232 adult patients registered in the Hungarian CF Registry in 2022. In a case-control analysis of NTM-positive (n = 39) and NTM-negative (n = 73) CF patients, demographic, clinical, and microbiological data were analysed to identify potential predictors for NTM acquisition. The distribution of NTM species, their antibiotic susceptibility patterns were also evaluated.
Results: The prevalence of NTM-positive sputum increased from 4.7 % to 12.9 % over study period. The most prevalent NTMs were *M. avium complex* (41.0 %), *M. abscessus complex* (MABSC) (38.5 %) and *M. xenopi* (15.4 %). MABSC strains were highly resistant to doxycycline, fluoroquinolones, and sulfonamides, while amikacin, macrolides, tigecycline and linezolid were often effective. Forced expiratory volume in 1 s (FEV_1) was lower in the NTM-positive group at the index date and 1 and 2 years before NTM detection ($p < 0.01$), predicting NTM infection. Previous NTM-positive sputum culture enhanced the risk of NTM reacquisition in the airway (odds ratio: 7).
Conclusion: The results demonstrate a high prevalence of NTM in the Hungarian adult CF population and a high rate of multidrug-resistant MABSC isolates in their sputum. The risk of acquiring airway NTM is higher in CF patients with significantly impaired lung function and previous respiratory mycobacteriosis.

Abbreviations: ATS, American Thoracic Society; BMI, body mass index; CFLD, cystic fibrosis-related liver disease; CFRD, cystic fibrosis-related diabetes; COPD, chronic obstructive lung disease; COVID-19, coronavirus disease-2019; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; DST, drug susceptibility testing; EUCAST, European Committee for Antimicrobial Susceptibility Testing; FEV_1, forced expiratory volume in 1 s; ICS, inhaled corticosteroid; IDSA, Infectious Diseases Society of America; MABSC, Mycobacterium abscessus complex; MAC, *Mycobacterium avium* complex; NKIP, National Korányi Institute for Pulmonology; NTM, non-tuberculous mycobacteria; OR, odds ratio; PD, pulmonary disease; PPI, proton-pump inhibitor; RGM, rapidly growing Mycobacterium; SCC, sputum culture conversion; SD, standard deviation; SMX-TMP, sulfamethoxazole-trimethoprim.

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1. Introduction

Non-tuberculous mycobacteria (NTM) are opportunistic environmental pathogens that exist as saprophytic bacteria in soil and water, including natural and municipal water sources [1]. Their biofilm-forming ability confers numerous survival advantages under a diverse range of conditions [2]. Human NTM infections are typically not contagious and are most frequently acquired from the environment via inhalation, alimentation, and dermal contact [3,4]. However, there is some evidence that the transmission of NTM can occur among people with CF. To date, more than 190 NTM species have been identified [5].

The increasing incidence of NTM infection in developed countries is an emerging problem, largely because the ageing population suffers from several diseases and conditions that enhance the risk of acquiring NTM. In addition, people with a history of chronic lung disease, immunocompromised conditions or hospitalisation are also at a higher risk of developing NTM infection [6–9]. Individuals with cystic fibrosis (CF) are particularly susceptible to respiratory mycobacteriosis, due to bronchiectasis, impaired mucociliary clearance that are typical of the disease; as well as due to frequent antimicrobial treatment, use of systemic and inhaled corticosteroids, and macrolides [10]. For this reason, current CF guidelines recommend regular annual NTM screening [11–13].

A recent multi-center meta-analysis [14] 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. While previous studies on national CF patient registries reported an NTM prevalence of 12 % in the United States [15] and France [16], it was estimated to be 8 % in Germany [17] and lower than 5 % in Australia, Brazil [14] and the United Kingdom [18]. In Europe, *Mycobacterium abscessus complex* (MABSC) and *Mycobacterium avium complex* (MAC) are the most frequent NTM species detected in CF patients, with a slight predominance of MABSC [10,17,19,20].

The management of non-tuberculous mycobacterial pulmonary disease (NTM-PD) is challenging and suboptimal. In certain cases, treatment must be initiated empirically before the results of drug susceptibility testing (DST) results are available, and even DST-based targeted antibiotic regimens fail to provide the expected benefit, especially in lung disease. Furthermore, existing NTM treatment guidelines may not always take into account regional specificities of NTM species occurrence and antimicrobial resistance, increasing the likelihood of treatment failure [10,21,22]. Therefore, studies to assess regional NTM prevalence, NTM species distribution and antimicrobial resistance patterns, especially in vulnerable patient groups such as CF patients, are essential to tailor recommendations for better treatment outcomes.

Data on NTM prevalence and characteristics in patients with CF in Central Europe are scarce in the literature, therefore there is ambiguity concerning the reasons for frequent treatment failure. The main aims of our study were to characterise the prevalence and distribution of NTM species in the Hungarian adult CF cohort, and to reveal the typical antimicrobial resistance patterns in MABSC.

Another focus of our study was to identify epidemiological and clinical characteristics of CF patients that may be associated with NTM acquisition, in order to help clinicians identify individuals who should be given special attention during NTM screening. For this purpose, we carried out a case-control study.

2. Material and methods

2.1. Subjects

The study was a retrospective, multicentre cohort study with embedded case-control analysis of NTM acquisition risk factors and FEV_1 trends. The study population comprised 232 non-transplanted adult Hungarian CF patients registered in the Hungarian National Cystic Fibrosis Registry in 2022. Data were analysed between January 1, 2020 and December 31, 2022. The diagnosis of CF was established according to the Cystic Fibrosis Foundation Consensus criteria (2017) [11]. Data obtained from the entire cohort were used for assessment of NTM prevalence patterns and retrospective evaluation of FEV_1 changes. NTM-positive patients (cases) were identified by at least one positive NTM sputum culture within the study period. CF patients with only one NTM-positive sputum were classified as transiently, those with at least two NTM isolates without clinical and radiological findings as persistently colonized, and those fulfilling microbiological, radiological, and clinical criteria for NTM-PD as patients with NTM-PD. Diagnosis of NTM-PD was based on the American Thoracic Society (ATS) (2006) and the Infectious Diseases Society of America (IDSA) (2007) criteria [12].

For identifying characteristics of CF patients that might be associated with NTM-positivity, a case control study was designed including a subgroup (n = 112) of the CF studied cohort. All involved NTM-positive (NTM+) CF patients constituted the case group (n = 39), and the control group (n = 73) was selected from NTM-negative (NTM-) CF patients based on age and gender as matching criteria. Those, receiving cystic fibrosis transmembrane conductance regulator (CFTR) modulators (which have an impact on clinical parameters) were excluded from this analysis (n = 2).

2.2. Microbiological identification of NTM species

In Hungary, patients without suspected NTM infection are routinely screened once a year. 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. In the presence of Ziehl-Neelsen-positive bacilli, a direct nucleic acid amplification test (GeneXpert/Cepheid) was performed to exclude or confirm the presence of *Mycobacterium tuberculosis complex*. Additionally, laboratory confirmation of NTM was based on culture in both solid (Löwenstein-Jensen) and liquid (MGIT) media. NTM-negative results were inferred after incubation of inoculated media for 8 weeks. A positive NTM result required 7 days or 6 weeks, depending on the medium (liquid or solid) or the NTM species (rapidly or slowly growing). The

biomass generated from the culture enabled genotypic and phenotypic identification (Genotype Mycobacterium CM and AS/Hain Lifescience GmbH, Nehren, Germany) and drug susceptibility test based on polymerase chain reaction and reverse hybridization (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 also evaluated using both genotypic (rrl and erm (41) genes) and phenotypic methods. Minimum inhibitory concentrations were read after 3, 7 and 14 days incubated. The epidemiological cut-off values were determined by the recommendations of European Committee for Antimicrobial Susceptibility Testing (EUCAST), using Sensititre RAPMYCOI plates (Thermo Fisher Scientific Inc., US) [23].

2.3. Data collection

Data were obtained from the records of the Hungarian National Cystic Fibrosis Registry and the hospital information system of the National Korányi Institute for Pulmonology (NKIP), Budapest, Hungary. Demographic, microbiological, and clinical data, including patient gender, age, body mass index, forced expiratory volume in 1 s (FEV_1), CF-related diseases, concomitant medications, and sputum culture results were collected. The date of data collection for NTM-positive patients was the time of first NTM identification (index date). To analyse changes in lung function, FEV_1 values measured one and two years before the index date were also recorded.

2.4. Statistical analysis

Values of continuous variables were presented as mean ± standard deviation (SD) and were compared between independent groups using independent samples *t*-test. For comparing data expressed as proportions, Fisher's exact test was applied. To compare time series data between 2 groups, two-way repeated measurement ANOVA and Dunnett's *post hoc* tests for multiple comparisons were used. Predictors of NTM acquisition were identified by logistic regression. Multivariate logistic regression included parameters for which $p < 0.2$ was obtained in univariate analysis. Statistical analyses were performed using GraphPad Prism 7.0 (San Diego, CA, USA) and Stata 15.1 (College Station, TX, USA) and $p \leq 0.05$ was considered as statistically significant throughout.

3. Results

3.1. Prevalence and distribution of NTM species

Of the 232 CF patients followed up between 2020 and 2022, 39 (16.8 %; 22 females and 17 males, mean age: 26.7 ± 7.5 years) had at least one positive NTM sputum culture (Table 1). Interestingly, 30 of these were identified in 2022. Therefore, we also defined the

Table 1
Comparison of CF patients who acquired NTM (NTM +) and their controls (NTM-). Controls are age- and sex-matched CF patients. Demographics, BMI, FEV_1, CF-related diseases, co-infective microbes are recorded at the time of the first NTM isolation. Data of continuous variables are expressed as mean ± SD. **: $p < 0.01$ vs. NTM-independent samples *t*-test;$^{\#\#}$: $p < 0.01$ vs NTM- Fisher exact test. Abbreviations: BMI, body mass index; FEV_1, Forced expiratory volume in 1 s; CFRD, cystic fibrosis related diabetes; CFLD, cystic fibrosis related liver disease; ICS, inhaled corticosteroid; PPI, proton pump inhibitor.

	NTM-	NTM +
Number of patients – n	73	39
Age – yrs	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/m²	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-infective pathogens – n	1.51 ± 0.80	1.64 ± 0.83
P. aureginosa co-infection – n (ratio)	37 (0.51)	25 (0.64)
S. aureus co-infection – n (ratio)	47 (0.64)	20 (0.51)
A. xylosoxidans co-infection–n(ratio)	8 (0.11)	7 (0.18)
Aspergillus co-infection – 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		
F580del homozygous – n (ratio)	32 (0.44)	17 (0.44)
F580del heterozygous – n (ratio)	30 (0.41)	16 (0.41)
Other mutations – n (ratio)	11 (0.15)	6 (0.15)

annual prevalence of NTM-positivity, which showed an increasing trend over the study period (5.7 % (11/192) in 2020, 10.2 % (20/197) in 2021 and 12.9 % (30/232) in 2022) (Table 2).

The following Mycobacterium species and subspecies 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 3). Among them, four patients were positive for multiple NTM species (one of MAC and four of MABSC). In the MAC-positive case, the co-existing pathogen was *M. xenopi*. Of the MABSC-positive subjects, two had *M. chelonae*, another had *M. xenopi* and an unidentifiable NTM strain, and the third had *M. gordonae, M. chelonae* and *M. xenopi*. Unidentifiable NTM isolates were found in three individuals. 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). Out of the 16 MAC-positive patients, 9 had *M. avium* and 5 had *M. intracellulare* species, while two subjects were co-infected with both MAC species (Table 3).

Of the 39 NTM-positive patients, 26 (66.67 %) were defined as patients with NTM-PD, 11 (28.2 %) as transiently and 2 (5.1 %) as persistently mycobacterial colonized (Table 1). All 15 MABSC-positive cases were classified as NTM-PD (100 %), while only 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 3).

3.2. Antibiotic susceptibility pattern of MABSC strains

A total of 20 different MABSC isolates were identified from the sputum of 15 patients with pulmonary disease. Of these, 13 were infected with *M. abscessus* spp. *abscessus*, one with *spp. massiliense* and one with *spp. bolletii*. The patient with *spp. bolletii* had 4, two patients with *spp. abscessus* had 2 strains of different antibiotic susceptibility patterns from different sputum samples during the study period. The remaining 12 patients presented with a single NTM strain. All isolates were resistant to doxycycline and ciprofloxacin. Resistance rates to 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 tygecyclin, amikacin, linezolid, and macrolide (100, 95, 100 and 60 %, respectively) (Fig. 1).

3.3. Co-existing 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. Only two of the thirty-nine NTM-positive patients were free of any co-existing respiratory pathogens at the time of the index. Most patients had *Pseudomonas aeruginosa* (25/39) and *Staphylococcus aureus* (20/39) in their sputum. The species *Achromobacter xylosoxidans* (7/39) and *Aspergillus fumigatus* (8/39) were less common. *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Nocardia* and *Proteus mirabilis* were found sporadically in the subjects (Table 4).

3.4. Patient characteristics associated with NTM-positivity

For identification of demographic and clinical factors that may predict NTM acquisition in CF patients, a wide spectrum of clinical and laboratory data from 39 NTM-positive and 73 NTM-negative CF patients was assessed (Table 1).

All patients were Caucasian and had a mean age of 26.7 ± 7.5 in the NTM-positive group and 25.8 ± 6.9 in the NTM-negative group at index dates. When comparing cases with controls, there were no significant differences in demographics, co-existing microbes, CF-related diseases, use of proton-pump inhibitors (PPIs), macrolides, and inhaled corticosteroids (ICS). Patients with NTM had a significantly lower FEV_1 at the index date compared with their matched controls (48.0 ± 20.3 % vs. 64.9 ± 26.5, $p = 0.00$). Previous sputum NTM positivity was more frequent in cases than in controls (23 % vs. 4 %, $p = 0.00$). 6 of the 12 cases had the same NTM species at the index date as previously detected. The remaining 6 cases had different NTM species at the index date than previously. The CFTR mutation pattern was similar in the two groups, with a predominance of F508del homozygous and heterozygous genotypes.

3.5. Decreased lung function is associated with NTM-positivity

As FEV_1 proved to be lower in NTM + patients in the case-control study, we further analysed their association. We collected the FEV_1 values of CF patients measured at index date, and measured 1 and 2 years before. These data were available in 36 NTM+ and 57 NTM-patients. Fig. 2 shows the differences observed between NTM-positive and NTM-negative patients in terms of the change in FEV_1

Table 2
Annual prevalence rate of non-transplanted adult CF patients between 2022 and 2022. The total number of CF patients was obtained from the Hungarian National Cystic Fibrosis Registry. Abbreviations: NTM, non-tuberculous mycobacteria.

Years	Total number of CF patients	Number of NTM-positive patients	Annual prevalence rate of NTM-positive patients (%)
2020	192	11	5.7
2021	197	20	10.2
2022	232	30	12.9

Table 3
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). Some patients are positive for multiple NTM species. Abbreviations: MAC: *Mycobacterium avium* complex, MABSC: Mycobacterium abscessus complex, NTM, non-tuberculous mycobacteria, PD, pulmonary disease.

NTM species (Number of NTM-positive patients)	Number of patients with NTM colonization (n = 13)	Number of patients 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)	–	–
single strain (3)	3	0
M. chelonae (4)	–	–
single strain (1)	1	0
M. gordonae (2)	–	–
single strain (1)	1	0
M. kansasii (1)	–	–
single strain (1)	1	0
Unidentified mycobacteria (3)	–	–
single strain (2)	2	0

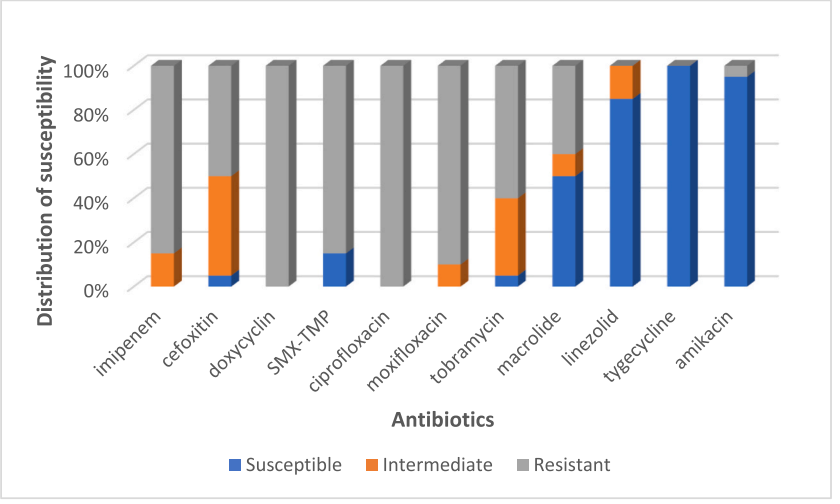

Fig. 1. Antimicrobial susceptibility of MABS isolates (n = 20). MABSC, Mycobacterium abscessus complex; SMX-TMP, sulfamethoxazole-trimethoprim.

Table 4
Distribution of co-existing microbes in sputum of patients (n=39) with NTM. Data are recorded at the time of the first NTM isolation.

Respiratory microbiology	Number and percentage of patients with NTM	
	n	%
Pseudomonas aeruginosa	25	64
Staphylococcus aureus	20	51
Achromobacter xylosoxidans	7	17
Aspergillus fumigatus	8	21
Burkholderia cepacia complex	1	3
Proteus mirabilis	1	3
Stenotrophomonas maltophilia	1	3
Nocardia	1	3

over the two-year interval. The mean FEV_1 values of NTM-positive 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 %, $p \leq 0.05$. Comparing the two groups, no significant difference was found in ΔFEV_1 over the studied 2 years (ΔFEV_1: 7.33 ± 11.91 % in the NTM+, and −7.28 ± 10.81 % in the NTM-group; $p = 0.98$). Moreover, a higher FEV_1 at index date, and 1 year and 2 years before index date was associated with decreased susceptibility to NTM acquisition in univariate regression models (Table 5). Multivariate logistic regression models including previous sputum NTM positivity and macrolide treatment beside FEV_1 values also confirmed this and showed that previous sputum NTM positivity is also associated with the risk of NTM acquisition (Table 5).

4. Discussion

The increasing prevalence of NTM infection has become an emerging problem in patients with risk factors, such as CF [5]. Clinicians managing NTM infection in CF patients face prolonged antimicrobial treatment, emerging antibiotic resistance, drug toxicity and frequent relapses. Furthermore, advanced NTM-PD may require the initiation of empirical antibiotic therapy. However, the high regional variability in NTM species distribution and antibiotic susceptibility often renders empirical and even targeted therapies unsuccessful. Therefore, studies providing data on the regional NTM prevalence and characteristics in CF patients are essential to help adapt guidelines to local circumstances. In addition, most published data on NTM infection characteristics in CF patients come from studies that partly or exclusively include children [14,16,17]. However, owing to novel successful therapies the life expectancy of CF patients has increased in the last 2 decades, which requires an assessment of the NTM infection rate and characteristics in the adult CF population. Our study aimed to fill this gap and provides detailed data on NTM prevalence, species distribution and antibiotic resistance in a large Hungarian adult CF cohort. In addition, we identified FEV_1 as a factor that can be considered as a predictor of subsequent NTM acquisition in CF patients.

The annual prevalence of NTM-positive sputum cultures increased from 4.7 % to 12.9 % over the study period. Recent European studies [16,17,20] have reported NTM prevalence rates between 8 and 12 %, although Adjemian et al. found significant geographical variation in NTM prevalence (0–28 %) [15]. In addition, increased exposure to mycobacteria (e.g., household water, shower aerosols), more sensitive diagnostic methods and increased clinician awareness may also influence the incidence of respiratory NTM colonization [13]. We assume that the increased NTM prevalence in 2022 could –at least partly-be explained by the COVID-19 pandemic. Many patients did not attend our CF clinic regularly in the years 2020 and 2021 due to fear of coronavirus infection. The reduction in the number of sputum cultures obtained during this period (Table 1) may have resulted in some NTM-positive cases being undiagnosed. In 2022, as the COVID-19 pandemic seemed to be subsiding, CF patients returned to their normal routines of attending the CF clinic regularly. We assume that the NTM cases detected in 2022 include some of the undiagnosed cases of the previous two years. The fact that our study group consisted only of adult patients is another explanation for the moderately elevated NTM prevalence. It is well established that the risk of acquiring NTM increases significantly with age [24] and prevalence data for CF patients are usually obtained from study groups that include children [14,16,17].

Based on our results, the two predominant NTM species in sputum were MAC and MABSC, in agreement with other studies [14,15,20]. We confirmed the slight dominance of MAC over MABSC, a distribution that is typical in North America [15] but not in the most European countries [17,20,25]. However, in Portugal [26,27] and France [15] a similar MAC dominance was found as in our results. Interestingly, the third most common NTM species detected was *M. xenopi*. According to German, French, and Portuguese observations [17,25,27], the most common mycobacteria other than MAC and MABSC are *M. gordonae* and *M. chelonae*. The relatively high prevalence of *M. xenopi* is unusual. It is assumed that the local climatic and geographical conditions (e.g., extremely hot summers, many fresh water sources) have an impact on the Hungarian flora and fauna, which may favour the reproduction and spread of certain NTM species.

All patients with MABSC had pulmonary disease, while 69 % of those with MAC received this diagnosis. Mycobacteria other than

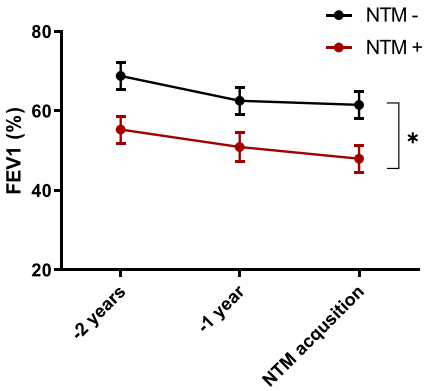

Fig. 2. Changes of FEV1 in the 2 years preceeding NTM acquisition. FEV1 values showed declines typical of CF patients in both the NTM- (n = 57) and NTM+ (n = 36) groups in the 2 years preceeding NTM acquisition. However, NTM + patients had significantly lower values throughout. Statistics: 2-way repeated measurement analysis of variance; *: $p \leq 0.05$.

Table 5
Association of potential predictors with NTM acquisition. Results of logistic regression analysis to describe potential predictors of NTM infection in CF patients (n = 93). Multivariate model 1, 2 and 3 included FEV_1 at the time of NTM detection, and FEV_1 1 year and 2 years before NTM acquisition, respectively; as well as covariates that showed association with NTM positivity in univariate model with a p value < 0.15. Abbreviations: FEV_1, Forced expiratory volume in 1 s; PPI, proton pump inhibitor.

	Univariate analysis			Multivariate analysis - Model 1[a]			Multivariate analysis - Model 2[b]			Multivariate analysis – Model 3[c]		
	OR	95 % C. I.	p	OR	95 % C. I.	p	OR	95 % C. I.	p	OR	95 % C. I.	p
Body mass index	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**	–	–	–	0.98	0.96; 0.99	**0.049**	–	–	–
FEV_1 - % - 2 years before NTM acquisition	0.98	0.96; 0.99	**0.01**	–	–	–	–	–	–	0.97	0.96; 0.99	**0.027**
	2.44	0.76; 7.86	0.13	2.46	0.63; 9.64	0.271	3.05	0.73; 12.7	0.13	2.74	0.52; 6.97	0.16
Macrolide treatment in patient history (n = 11)												
	0.85	0.33; 2.19	0.74	–	–	–	–	–	–	–	–	–
PPI treatment (n = 23)												
Previous NTM positivity in sputum (n = 11)	7.00	1.77; 27.68	**0.01**	5.46	1.25; 23.77	**0.024**	4.62	1.06; 20.11	**0.42**	3.78	0.87; 16.41	0.075
	1.56	0.66; 3.72	0.31	–	–	–	–	–	–	–	–	–
Co-existing P. aureginosa (n = 56)												
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	–	–	–	–	–	–	–	–	–

[a] Hosmer-Lemeshow test: $chi^2 = 4.48$, p value = 0.11; area under ROC curve: 0.71.
[b] Hosmer-Lemeshow $chi^2 = 0.87$, p value = 0.65, area under ROC curve: 0.66.
[c] Hosmer-Lemeshow $chi^2 = 2.17$, p value = 0.34, area under ROC curve: 0.69.

MAC and MABSC were detected transiently, and never led to pathological conditions. Other studies [17,28] have also shown that RGM species are more likely to induce lung disease than slow growing species. Virulence factors of MABSC (i.e., Mycobacterial membrane large proteins, surface glycopeptidolipids) play a key role in the development of severe tissue damage [29,30].

Besides NTM, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were regularly identified in sputum samples (Table 1), consistent with previous research [16,17,31]. The role of microbes co-existing in the airways is not yet clear. According to an autoregressive integrated moving averages model, the airway microbiome functions as a bacterial community network with dynamics and interactions that can influence the level of inflammation and thus the outcome of infections [32]. Therefore, the choice of therapy should also consider other respiratory bacteria present. Remarkably, *Achromobacter xylosoxidans* has risen to prominence as a respiratory pathogen in CF patients, a matter of significant concern given that colonization by *Achromobacter* species has been linked to decreased lung function, frequent acute exacerbations, and damage to lung tissue [33,34].

We found that CF patients with previous NTM-positive sputum cultures had a higher risk of NTM reacquisition. An analysis by Martiniano et al. also showed a high rate of secondary positive cultures for NTM [35]. When other factors such as genotypes, CF-related diseases, medications, or demographics were analysed, no significant differences were found between NTM-positive and NTM-free patients. We did not find association between NTM-positivity and the presence of other respiratory microbes in the sputum, either (Table 5).

In NTM cases, we observed a significant decrease in FEV_1 at the index date compared with controls as previously reported by others [35,36]. More importantly, this significant difference was also observable one and two years preceding the index date, and lower FEV_1 measured at index date and 1 and 2 years before showed association with NTM acquisition. Our results suggest that persistent decline in lung function may represent a predictor for acquisition of NTM, however this finding needs to be confirmed in larger studies. To our knowledge, this is the first study to report a two-year trend in lung function prior to NTM detection in sputum. Based on this observation, more frequent sputum screening for NTM should be considered in patients with $FEV_1 \leq 50$ %. The rate of FEV_1 decline over 2 years was similar in the NTM-positive and control groups and ΔFEV_1 was not predictive of NTM acquisition, demonstrating that the rate of lung function decline has a negligible effect on susceptibility to NTM. Furthermore, in contrast to previous reports we did not observe an increased FEV_1 decline associated with NTM acquisition. This may be the favourable consequence of regular sputum surveillance for NTM and prompt initiation of treatment.

The treatment of MABSC is still not optimal and only a few randomized controlled trials have been performed [13,37]. Several societies including ATS, IDSA, European Respiratory Society, European Cystic Fibrosis Society have developed consensus-based treatment recommendations [5,37]. According to these guidelines, lung disease caused by MABSC should be treated in the intensive phase with a macrolide-containing antimicrobial regimen (if the strain is macrolide-sensitive) that includes at least three active parenteral drugs (amikacin, imipenem, cefoxitin, tigecycline, 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. A national CF guideline is being harmonised and we advocate implementing these observations.

This study has certain limitations: 1) even though we have access to data of all adult CF patients in Hungary, the data could only be collected from a limited number of NTM cases due to the limiting factor of the size of the Hungarian population. 2)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. 3) the availability of CFTR modulator therapy in Hungary during the second half of 2022 introduced a bias in clinical parameters that prevented the long-term impact of NTM persistence on lung function from being evaluated. 4) the potential effects of variations in treatment regimens on the clinical course and risk of NTM acquisition were not investigated, although they may act as confounders. Nevertheless, this study represents one of the most comprehensive analyses of NTM prevalence among Hungarian CF patients to date.

5. Conclusion

Our study sheds light on the high prevalence of multidrug-resistant MABSC isolates among adult CF patients in Hungary. Furthermore, persistently decreased FEV_1 and previous sputum NTM positivity were identified as potential independent risk factors for the development of NTM airway colonization in the CF population. Therefore, patients with these risk factors should be monitored more frequently for sputum NTM. However, studies in larger CF populations and in non-CF patients at risk of NTM infection are still needed to corroborate our findings and to draw further conclusions.

CRediT authorship contribution statement

Zoltán Örlős: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Lilla Katalin Lőrinczi:** Writing – review & editing, Validation. **Balázs Antus:** Writing – review & editing, Supervision. **Imre Barta:** Writing – review & editing, Formal analysis. **Zsuzsanna Miklós:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Data curation. **Ildikó Horváth:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Ethics declaration

The study was reviewed and approved by the Scientific and Research Committee of the Medical Research Council, Budapest, Hungary (approval number: BMEÜ/3691-1/2022/EKU, dated 22nd November 2022) and complies with the ethical standards of the Declaration of Helsinki. As the study is based on retrospective analysis of the medical records of the hospital, the need for informed consent was waived by the Scientific and Research Committee of the Medical Research Council, Budapest, Hungary (approval number: BMEÜ/3691-1/2022/EKU, dated 22nd November 2022).

Data availability statement

Original data used for analysis in this study are not shared in a public data repository. Data will be made available on request.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Balazs Antus reports financial support was provided by Hungarian Research, Development and Innovation Office. Zoltan Orlos reports article publishing charges was provided by Association of Cystic Fibrosis Patients - ACFP. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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