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ORIGINAL PAPER

Species distribution and clinical features of infection and colonisation with non-tuberculous mycobacteria in a tertiary care centre, central Germany, 2006–2016

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Abstract

Purpose NTM are ubiquitous bacteria that can cause colonisation and infection in immunocompetent and compromised hosts. The aim of this study was to elucidate the epidemiology of infection or colonisation with NTM for the metropolitan region of Frankfurt, Germany.

Methods All patients from whom NTM were isolated within the period from 2006 to 2016 were included in this retrospective analysis. Patient data were retrieved using the local patient data management system. Different groups were formed according to clinical manifestations, underlying diseases and mycobacterial species. They were compared in regard to mortality, duration of infection/colonisation and their geographical origins.

Results A total of 297 patients with a median of 28 new patients each year were included. Most patients suffered from lung infection or colonisation (72.7%, $n=216$), followed by disseminated mycobacteriosis (12.5%, $n=37$). The majority were HIV-positive, suffering from malignoma or cystic fibrosis (29.3%, $n=87$, 16.2%, $n=48$, and 13.8%, $n=41$, respectively). 17.2% of patients showed no predisposing condition ($n=51$). *Mycobacterium avium* complex (MAC) species were most frequently isolated (40.7%, $n=121$). Infection/colonisation was longest in CF patients (median of 1094 days). The mortality was highest in malignoma patients (52.4%), while CF patients had the lowest overall mortality rate (5.3%). But mortality analysis showed non-significant results within different mycobacterial species and clinical manifestations.

Conclusion NTM remain rare but underestimated pathogens in lung and disseminated disease. MAC were the species most frequently isolated. Depending on species and underlying predispositions, the duration of infection/colonisation can be unexpectedly long.

Keywords NTM · Non-tuberculous mycobacteria · Mycobacterium avium complex · Mycobacterium abscessus complex · Cystic Fibrosis · HIV

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Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous environmental microorganisms. They can be regarded as opportunistic pathogens that affect humans in certain circumstances, for example when immunosuppression is present. Still, infections may occur in otherwise healthy subjects [1].

NTM can be subdivided into slow growing mycobacteria (SGM) and rapid growing mycobacteria (RGM). SGM include—besides others—*Mycobacterium avium* complex (MAC, with *M. avium, M. intracellulare, M. chimaera*), *M. kansasii, M. xenopi, M. simiae*, whereas *M. abscessus* complex (MAbsC, with *M. abscessus* subsp. *abscessus*, subsp. *bolletii* and subsp. *massiliense*), *M. fortuitum* and *M.*

chelonae are important members of the RGM-family [2]. NTM are ubiquitous in nature and may inhabit several ecological niches, such as water reservoirs, shower heads, but also animals, all depending on the mycobacterial species [3, 4]. Recently, a contamination of heater cooler systems for cardiac surgery has caused a considerable outbreak of nosocomial infections with *M. chimaera* all over the world [5]. Although known since the advent of bacteriology and the discovery of *M. tuberculosis*, the clinical importance of NTM is increasingly recognized.

There are two main clinical manifestations caused by NTM in adult patients: pulmonary disease due to NTM (NTM-PD), and disseminated mycobacteriosis [1].

In NTM-PD, possible predisposing factors are lung transplantation, chronic obstructive pulmonary disease (COPD), bronchiectasis, malignoma of the lung and cystic fibrosis (CF) [6]. NTM-PD is mainly caused by the SGM-species MAC*, M. kansasii, M. xenopi* and RGM-species of the MAbsC (especially in CF patients) [7]. Disseminated disease has been described in immunosuppressed hosts, hereby HIV patients with low CD4-cell counts are the most important group. This entity is mainly caused by MAC [8].

In children, generalized or local lymphadenitis is the most important clinical manifestation, which could be regarded as the primary infection caused by NTM [9]. Nevertheless, many more manifestations such as affection of the skin (e.g. aquarium granuloma with *M. marinum*) or the central nervous system exist [10].

In the last years, the prevalence and awareness of diseases caused by NTM, as well as positive microbiological results for NTM, has probably risen [11]. The prevalence in Germany was estimated at 2.3–3.3 cases/100.000 [12]. Mortality depends mainly on NTM species, clinical and radiological findings [13–16].

Differentiation between colonisation and infection can be challenging, especially when NTM are isolated from respiratory specimen [1, 17, 18]. The American Thoracic Society (ATS) and British Thoracic Society (BTS) have, therefore, developed diagnostic criteria that comprise clinical and microbiological aspects alike [1, 19]. Diagnostic and therapeutic recommendations for CF patients were proposed, as well [20].

Objective of this study was to elucidate the role of NTM infections in a tertiary care centre covering the Frankfurt metropolitan area in central Germany. Clinical manifestations were compared and underlying predispositions, if present, were examined. In addition, mortality rates for different mycobacteria, predispositions and clinical groups were investigated.

Methods

Clinical database analysis

This retrospective study was approved by the local ethics committee under the reference number 473/16. Clinical data were extracted from the local hospital database (ORBIS, Agfa Health Care, Bonn, Germany) by two manners: first, all patients coded with ICD-10 diagnosis A31 (Infection due to other Mycobacteria) from 01/2006 to 12/2016 were retrospectively identified by a local hospital information system-database query. Second, a list with all positive microbiological results (PCR and culture) was correlated with the ICD-10 diagnosed patient group for the same period of time. Patients, for whom clinical data were not available, were omitted. Remaining patients were then analysed for mycobacterial species, clinical manifestations, underlying dispositions, date of hospitalization, date of first and last positive culture, in-hospital mortality during the observation period and duration of observation. For geospatial analysis, resident cities of patients were recorded.

Further statistical analysis

Geospatial analysis was performed with EpiInfo 7 (CDC, Centers for Disease Control and Prevention, Atlanta, [21]).

Patients were subgrouped by disposition (CF, HIV, malignoma, other structural lung diseases—SLD—, none and others. Within this groups clinical manifestations, as well as mycobacterial species were compared using libre office calc (The document foundation) and R [22]. Continuous variables, such as age, were compared by conducting ANOVA-analysis, categorial variables using Pearson's Chi-squared test. Dodged barplots were drawn with R's package ggplot2 [23].

Duration of positive microbiological cultures was calculated by taking the timespan between first and last positive culture of the same mycobacterium in one patient. Their median was calculated with R for every mycobacterial species and predisposition, whereas groups with less than ten individuals and *M. gordonae* for being a frequent contamination were subgrouped into others. Boxplots were calculated with R's ggplot 2 package [23]. Lengths of durations were compared between different groups using the Welch two sample *t* test for unequal variances.

For in-hospital-mortality analysis an additional period until 01/2017 was included. Death or survival during the treatment provided by our institution, as well as the observation time, were recorded. Kaplan–Meier curves were drawn with R's survival package for a 5-year period [24, 25]. Numbers at risks were added to the resulting graphs, as well as lethal events during the preceding time period. Differences in mortality were calculated using G-rho family of tests within R's survival package.

Results

Baseline characteristics and temporal trend

Between 2006 and 2016, a total of 1105 positive cultures or PCR results in 360 different patients were generated in our hospital. Of those, 203 patients were coded with ICD-10 A31. 63 patients were omitted due to unavailable clinical data. Thus, 297 patients could be included in this study according to the criteria mentioned above.

Those were patients either showing at least one positive culture for NTM, positive PCR results or both (91.2%, $n = 271$; 14.1%, $n = 42$, and 7.4%, $n = 22$, respectively). For 2.0% of the cases the diagnostic method was not documented, but NTM species or disease was registered within the patient's history during the observation period (for example in previous hospital stays, or other hospitals).

A median of 28 new patients was hospitalized annually. Patients being hospitalized several times were only counted once. 65.0% of the patients were male ($n = 193$), 35.0% female ($n = 104$). The median age was 46.6 years at time of diagnosis for all patients (1.35–90.1, mean = 47.7, SD = 20.2). It was 30.9 years in CF patients (mean = 31.5, SD = 10.8), 43.4 years in all HIV patients (mean = 41.5, SD = 11.3), 63.9 years in cancer patients (mean = 66.1, SD = 12.6), 68.2 in patients with structural lung diseases as, e.g. COPD, bronchiectasis or fibrosis (mean = 67.2, SD = 15.2), 50.3 years in patients with other predispositions (mean = 53.1, SD = 19.7) and 45.5 years in patients without any apparent disposition (51.2, SD = 29.26) (Table 1, Fig. 1).

Geographic distribution

290 patients had their residence in Germany. Seven patients were from the US, UK, Russia, Kazakhstan, Egypt and Saudi Arabia. 252 patients resided in the Frankfurt area, 265 from the federal district of Hessia (Fig. 2).

Clinical manifestations

72.7% of all patients were suffering from lung infection or colonisation by NTM ($n = 216$). 12.5% had disseminated disease ($n = 37$), 5.7% lymphadenitis ($n = 17$), and 4.0% a dermatological manifestation due to NTM ($n = 12$). Other clinical manifestations were, for example, neurological, gastrointestinal and hepatic manifestations ($n = 15$, in total 5.1%) (Table 1, Fig. 3).

Table 1 Table of baseline characteristics. Patients were subdivided into groups by disposition (CF, HIV, malignoma, SLD, others and none)

Parametre		All		Cystic Fibrosis		HIV		Malignoma		SLD		Others		None		
Cases		297		41		87		48		47		23		51		
Age	Median	46.6		30.9		43.4		63.9		67.2		50.3		45.5		ANOVA
	Mean	47.7		31.5		41.5		66.1		68.2		53.1		51.2		p <0.001
	SD	20.1		10.9		11.3		12.6		15.2		19.7		29.3		
		abs [n]	rel [%]	abs [n]	rel [%]	abs [n]	rel [%]	abs [n]	rel [%]	abs [n]	rel [%]	abs [n]	rel [%]	abs [n]	rel [%]	Chi-squared-Test
Gender	male	193	65.0	18	43.9	64	73.6	36	75.0	26	55.3	16	69.6	33	64.7	p=0.01
	female	104	35.0	23	56.1	23	26.4	12	25.0	21	44.7	7	30.4	18	35.3	
CD4 Cells	<50/µl	NA		NA		56	64.4	NA		NA		NA		NA		
	>50/µl					31	35.6									
Myobacterial species	MAC	121	40.7	15	36.6	49	56.3	14	29.2	16	34.0	10	43.5	17	33.3	
	MabsC	24	8.1	16	39.0	1	1.1	2	4.2	4	8.5	0	0.0	1	2.0	
	M. gordonae	54	18.2	5	12.2	2	2.3	17	35.4	16	34.0	5	21.7	9	17.6	
	M. xenopi	34	11.4	0	0.0	16	18.4	8	16.7	4	8.5	3	13.0	3	5.9	
	M. fortuitum	13	4.4	0	0.0	4	4.6	2	4.2	2	4.3	2	8.7	3	5.9	
	M. kansasii	18	6.1	2	4.9	5	5.7	4	8.3	3	6.4	0	0.0	4	7.8	
	M. marinum	9	3.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	9	17.6	
	M. simiae	8	2.7	2	4.9	2	2.3	1	2.1	1	2.1	1	4.3	1	2.0	
	M. celatum	3	1.0	0	0.0	2	2.3	0	0.0	0	0.0	1	4.3	0	0.0	
	M. chelonae	3	1.0	1	2.4	0	0.0	0	0.0	0	0.0	1	4.3	1	2.0	
	M. chubuense	1	0.3	0	0.0	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	
	M. genavense	3	1.0	0	0.0	3	3.4	0	0.0	0	0.0	0	0.0	0	0.0	
	M. gilvum	1	0.3	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	2.0	
	M. haemophilum	1	0.3	0	0.0	1	1.1	0	0.0	0	0.0	0	0.0	0	0.0	
	M. malmoense	3	1.0	0	0.0	1	1.1	0	0.0	0	0.0	0	0.0	2	3.9	
	M. szulgai	1	0.3	0	0.0	0	0.0	0	0.0	1	2.1	0	0.0	0	0.0	
Clinical manifestation	NTM PD or Colonisation	216	72.7	41	100.0	44	50.6	45	93.8	46	97.9	16	69.6	24	47.1	Chi-squared-Test
	Disseminated Mycobacteriosis	37	12.5	0	0.0	33	37.9	0	0.0	0	0.0	2	8.7	2	3.9	p <0.001
	Lymphadenitis	17	5.7	0	0.0	2	2.3	0	0.0	1	2.1	1	4.3	13	25.5	
	Dermatological Manifestation	12	4.0	0	0.0	1	1.1	1	2.1	0	0.0	1	4.3	9	17.6	
	Others	15	5.1	0	0.0	7	8.0	2	4.2	0	0.0	3	13.0	3	5.9	
>1 positive culture		125	42.1	30	73.2	44	50.6	14	29.2	14	29.8	9	39.1	14	27.5	Chi-squared-Test P<0.001

Categorial variables were compared using the Chi-squared test, continuous variables using ANOVA

Differences in age, number of patients with more than one positive culture, gender and clinical manifestations were significant

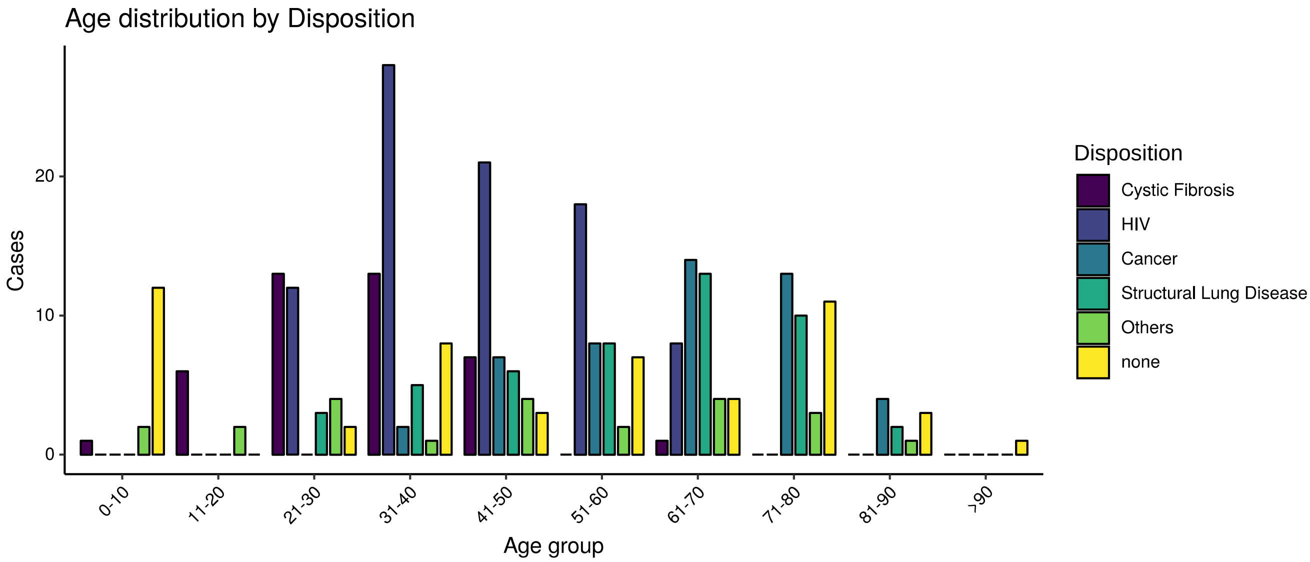

Fig. 1 Age distribution divided by different clinical manifestations. Age range was from 1.35 to 90.1 years, with a median age of 46.6 years. The youngest group was CF patients with a median age of 30.9 years, whereas the oldest group was composed of patients suffering from SLD (median age of 68.2 years). Patients without any apparent disposition showed a peak in the youngest age group mirroring the occurrence of lymphadenitis with NTM in immunocompetent children. Differences in age distribution were significant according to ANOVA

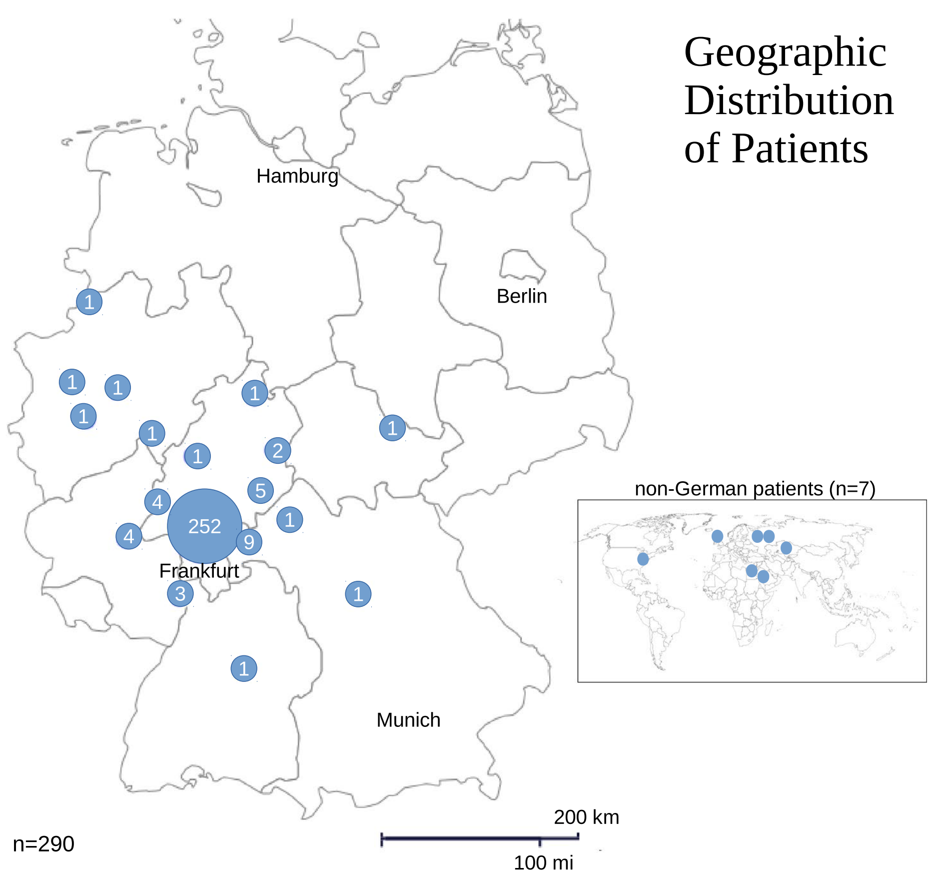

Fig. 2 Map depicting geographical origin of patients in which NTM were found. Most patients were living in and around Frankfurt. Seven patients from the US, UK, Russian Federation, Egypt, Saudia Arabia and Kazakhstan are shown on the world map on the right. Circle size represents number of cases, if more than one clinical case in one area was present, numbers in circles are depicted

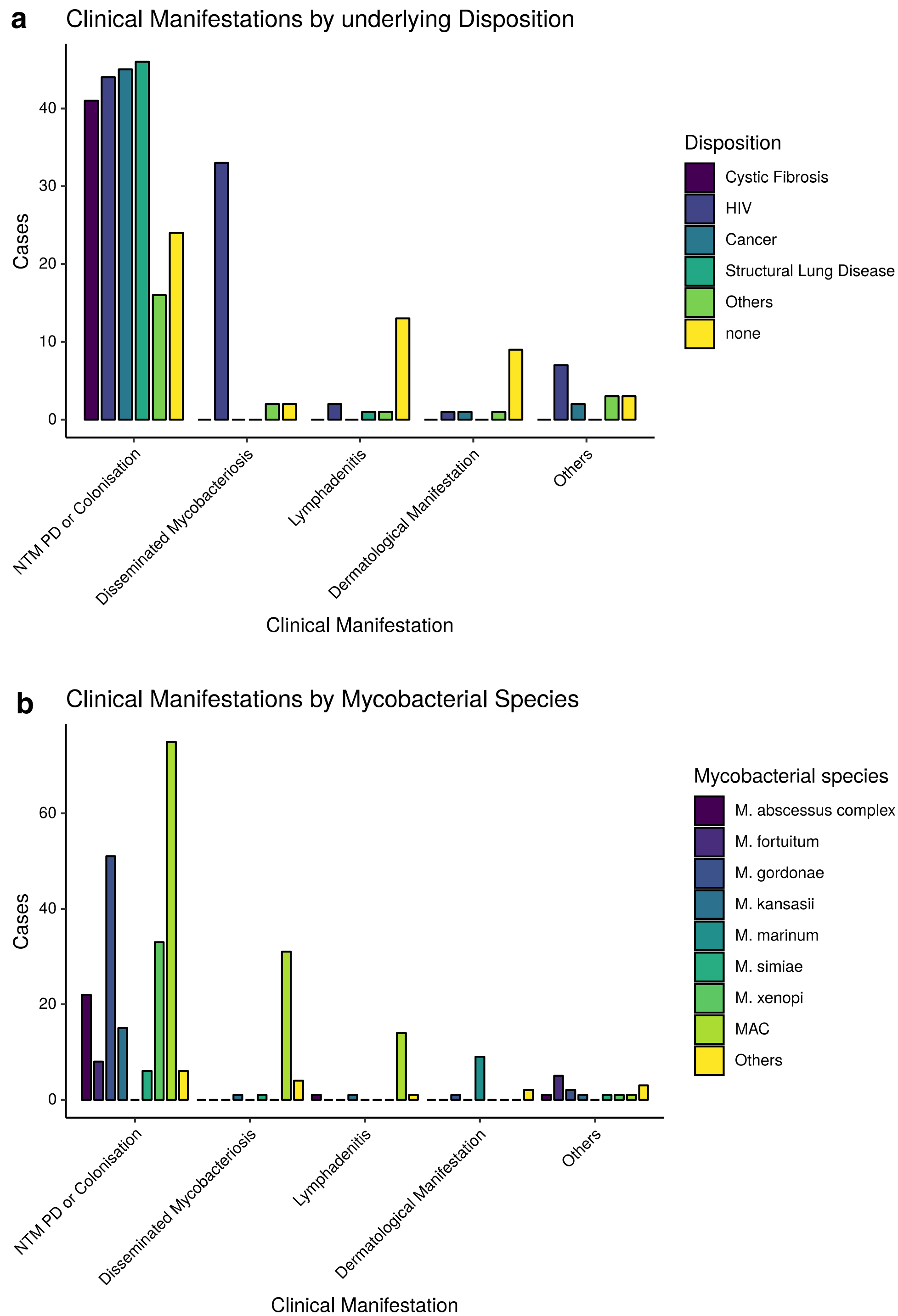

Fig. 3 Bar graph depicting frequency of certain clinical manifestations divided by underlying dispositions (**a**) and different mycobacterial species (**b**) (*n* = 297). Disseminated mycobacteriosis and NTM-PD/colonisation were the most frequent clinical manifestations. Lung manifestations outnumbering the others with 72.7% (*n* = 216) **.** 40.7% of mycobacterial species were belonging to MAC, followed by *M. gordonae*, *M. xenopi*, MAbsC, *M. kansasii*, *M. fortuitum*, *M. marinum* and others

NTM species distribution

Most of the cases were caused by MAC-species (including *M. avium*, *M. intracellulare* and *M. chimaera*). They accounted for 40.7% of all cases (*n* = 121), followed by *M. gordonae* (18.2%, *n* = 54), *M. xenopi* (11.5%, n = 34), MAbsC (8.1%, *n* = 24), *M. kansasii* (6.1%, *n* = 18), *M. fortuitum* (4.4%, *n* = 13) and *M. marinum* (3.0%, *n* = 9). Species that made up less than 3% were *M. simiae*, *M. celatum*, *M. chelonae*, *M. malmoense*, *M. genavense*, *M. gilvum*, *M. haemophilum*, *M. chubuense* and *szulgai* (*n* = 24, in total 8.1%) (Table 1, Fig. 3b).

NTM most frequently isolated in lung infection or colonisation were MAC (33.3%, *n* = 71), followed by *M. gordonae* (23.6%, *n* = 51), *M. xenopi* (15.2%, *n* = 33) and MAbsC (9.3%, *n* = 21). Disseminated disease was most frequently caused by MAC (81.1%, $n=30$), dermatological manifestations by *M. marinum* (75.0%, $n=9$).

Immunosuppression and other predispositions

Most of the patients were suffering from HIV and had lower CD4 cell counts than 50/µl (18.9%, $n=56$). The second most important predisposition was malignoma with 16.2% ($n=48$), followed by CF with 13.8% ($n=41$). 10.4% were HIV patients with CD4 cell counts above 50/µl ($n=31$). HIV patients accounted in total for 29.3% of patients ($n=87$). Other dispositions were structural lung changes, bronchiectasis, COPD, lung transplantation, tuberculosis, rheumatic disease and different immune deficiencies. Interestingly, for 17.2% of the patients no apparent predisposition or immunodeficiency could be found ($n=51$) (Table 1, Fig. 3a).

Altogether, underlying lung diseases (CF and structural lung diseases as COPD, non-CF-bronchiectasis, tuberculosis or a patient having undergone lung transplantation) were the most frequent predisposition (29.6%, $n=88$). Naturally, these were even more important in NTM-PD or colonisation (38.4%).

In disseminated mycobacteriosis the leading cause was an HIV infection with CD4 cell counts below 50/µl (81.0%, $n=30$). No patients with CF, bronchiectasis, COPD or cancer were suffering from disseminated mycobacteriosis during the observation period.

Both, in dermatological manifestations and lymphadenitis, patients without any known disposition were the largest group.

Patients with long-term NTM infection/colonisation

125 patients showed more than 1 positive culture or PCR result for NTM during the 11-year observation period. Within this group, HIV infection with low CD4 cell count, CF and malignoma were once again the most important underlying predispositions (28.8, 24.0, and 11.2%). CF patients showed the longest periods of infection or colonisation with a median length of 1094 days (0–3591 days, SD = 1130 days, Fig. 4a). This was significantly longer than in HIV patients with a median length of 13 days (0–3654, SD = 639.44), or all other underlying predispositions taken together, with 15 days as well (0–3654, SD = 659.91) (CF vs. HIV, $p<0.001$, CF vs. all others, $p=0.001$). The CF-patient with the longest duration of infection or colonisation showed positive MAbsC cultures for 3591 days within the observation period (9.84 years).

In all clinical manifestations and underlying dispositions, MAbsC were the mycobacterial species with the longest median duration of positive cultures (898.5 days, 70–3591 days, SD = 1066). In comparison MAC-species showed a median of 30 days (MAbsC vs. MAC, $p<0.001$, MAbsC vs. all others, $p=0.01$, Fig. 4b).

In 13 patients more than 1 single species of NTM could be cultured (4.4%). The maximum being four mycobacterial species (MAC, *M. gordonae*, *M. chelonae* and *M. tuberculosis*) in an immunocompetent patient suffering from XDR-tuberculosis concurrently.

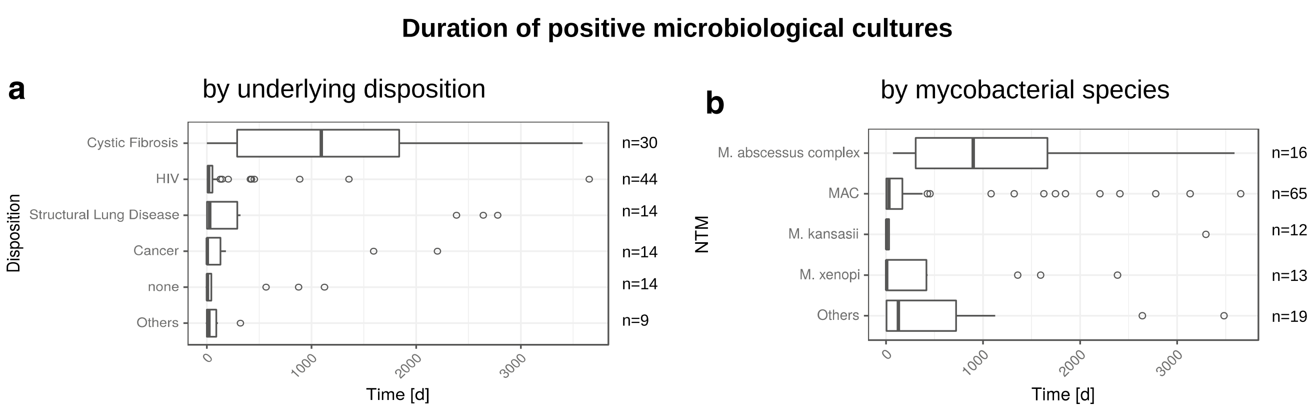

Fig. 4 **a** Boxplot showing the median length of cultivation in different dispositions as CF, HIV, malignoma, SLD, others and none, in patients that had at least two positive cultures/PCRs ($n=125$). CF patients showed the longest time of cultivation with a median of 1094, equaling 3.00 years, as in comparison to 13 days in HIV patients and 15 days in patients with all other underlying predispositions. **b** Median length of cultivation in different mycobacterial species in patients that had at least two positive cultures or PCRs ($n=125$). MAbsC showed a median cultivation period of 895.5 days. MAC-species had short cultivation periods with a median of 30.0 days

In-hospital-mortality in patients with NTM infection or colonisation

5-year all-cause mortality was compared divided by predisposition, mycobacterial species, and clinical manifestation. Median observation time was 643 days (0–4305 days, SD = 1186 days).

As expected, patients suffering from malignoma had highest overall mortality with 52.4% in the first 5 years after detection of NTM, they were followed by patients with SLDs (20.5%) and HIV (all CD4 cell counts included, 19.1%). Interestingly, CF patients had the lowest mortality rates with 5.3%, even outranking patients without any (known) disposition whatsoever (14.7% 5-year mortality rate). According to G-rho family of tests analysis these differences in mortality were significant ($p = 0.02$, Fig. 5).

For all underlying dispositions and clinical manifestations, *M. kansasii* was the mycobacterial species with the highest mortality rate in our observation group (34.8%). Patients colonised with *M. gordonae* died in 21.8% of cases, with MAC-species in 23.1%. However, significance was not reached for mortality rates between different mycobacterial species ($p = 0.7$).

Disseminated mycobacteriosis showed a 5-year in-hospital-mortality of 27.9%, whereas patients with lung infection or colonisation died in 20.4%. Patients with other clinical manifestation had fatal outcomes in only 5.2% of cases. However, this tendency was not significant, either ($p = 0.1$).

Discussion

This study shows that even in one single tertiary care centre there is a significant amount of patients colonised or infected with NTM. Although renowned a rare infection, NTM have to be considered in different clinical settings.

MAC-species were the most frequently isolated species in this cohort, followed by *M. gordonae*, and *M. xenopi*. This was also described earlier by a worldwide study conducted by NTM-NET with 20,182 patients from 60 countries [11]. As the differentiation of MAC-species became just available during the observation period, they were considered as one group. The same is the case for MAbsC. In proportion, with 8.1% this group was more frequent in our study than as it was in the international results. This might be linked to the local focus on treatment of patients with CF. As this study enrolled only 297 patients the statistical power is a certain limitation and makes analyses within the small groups of our cohort more difficult to assess.

Interestingly, our cohort showed a broad range in age distribution, whereas according to predispositions CF patients were the youngest to be colonised or infected with NTM

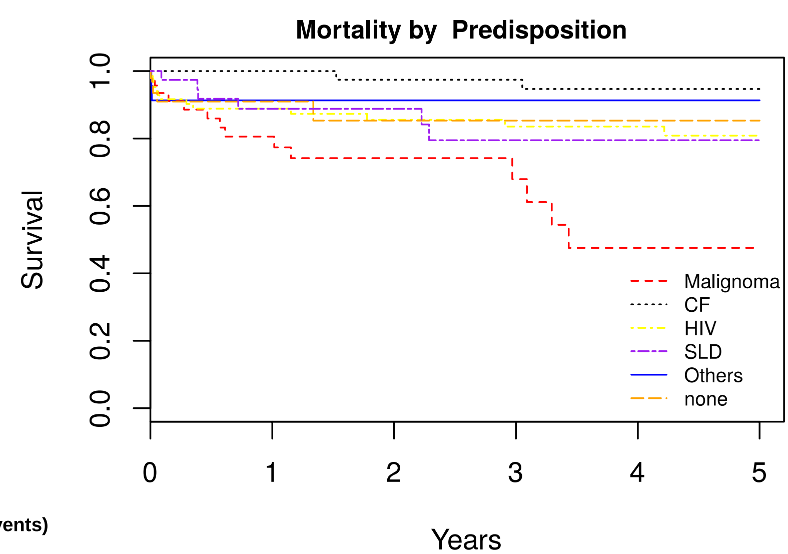

Fig. 5 Kaplan–Meier curves depicting mortality divided by underlying disposition. Tables underneath show numbers at risk and lethal events in the preceding time. Time duration in years. Mortality in different underlying dispositions was significantly distinct ($p = 0.02$), whereas expectedly patients with malignomas had highest 5-year overall mortality. Different clinical manifestations and mycobacterial species did not show significant differences in mortality and are not shown ($p = 0.1$ and $p = 0.7$)

Numbers at risk (Events)

Malignoma	48 (1)	26 (7)	17 (2)	11 (1)	7 (3)	7 (0)
CF	41 (0)	39 (0)	38 (1)	36 (0)	31 (1)	29 (0)
HIV	87 (0)	59 (9)	46 (2)	42 (1)	33 (0)	25 (1)
SLD	47 (0)	28 (4)	19 (0)	13 (2)	12 (0)	11 (0)
others	23 (0)	15 (2)	11 (0)	10 (0)	9 (0)	6 (0)
none	51 (0)	19 (4)	13 (1)	9 (0)	8 (0)	4 (0)

and patients with other structural lung diseases the oldest ones. Our group of patients without any apparent disposition shows two distinct age peaks (age group 0–10 and > 50). This demonstrates the role of MAC in lymphadenitis in immunocompetent children [26].

The most frequent clinical groups were lung infection or colonisation, thus mirroring the importance of underlying lung diseases as CF or other structural lung diseases as non-CF-bronchiectasis, COPD and others. The duration of infection or colonisation was longest in CF patients with a median of 1094 days (0–3591 days, SD = 1130 days). This underlines the necessity for effective treatment options in those patients as well as the need for antimicrobial resistance testing in NTM to adapt antibiotic treatment. HIV patients were another relevant group with shorter duration of microbiological culture positivity. Nevertheless, there were some patients with long periods of positive cultures (max. 10 years). A possible explanation is that NTM in HIV patients are highly susceptible to the degree of T-cell deficiency, and the cure will highly depend on whether there is immune reconstitution which depends on patient compliance to the antiretroviral therapy or the occurrence of malignoma. Astonishingly, the second most frequent group was patients without any known predisposition at all, which is clearly showing the relevance in this group of patients who would be considered as being otherwise healthy. However, contaminations cannot be completely ruled out if cultures originate from respiratory specimen.

Our study could show a high 5-year in-hospital mortality of 52.4% in malignoma patients colonised or infected with NTM species. Of course, this is most likely due to the underlying disease and additional comorbidities in this cohort. Thus, the colonisation with NTM might just be a sign for the compromised immune system. Additionally, many of those patients were suffering from lung cancer and got bronchial washes during diagnostic or therapeutic bronchoscopies performed for other indications, so that the relevance of mycobacterial infection remains debatable, yet interesting to study further. This is underlined by the high amount of positive cultures for *M. gordonae,* which is generally considered a colonisation and not an infection [1]. Unfortunately application of the ATS criteria in patients with positive respiratory samples was not possible due to partial lack of clinical information, which might be seen as a another weakness of our analysis.

The clinical manifestation with the highest mortality of 27.9% was disseminated mycobacteriosis, mostly in HIV patients with low CD4 cell counts. For different predispositions, mortality in HIV patients was 19.1%. Another study found lower mortality rates (10%) in patients with HIV and disseminated infections [27]. Comorbidities due to other opportunistic infections, or the use of MAC prophylaxis, which is not done in Germany, might explain this discrepancy. Nevertheless, this group requires specialized clinical care in experienced centres.

Interestingly, in our group CF patients showed lowest mortality rates with 5.3%. This might be explained by several factors: first, patients with CF are engaged in special treatment programs, a deterioration in lung function leads more often to treatment of NTM infection and might thus explain their low mortality. Second, being a chronic disorder with constant clinical care, patients might more often opt for palliative care, so that a lethal event did not occur in our hospital, but in other institutions. Third, and most importantly, the median age in the CF group was 31.5 years, rendering this group significantly younger than the other cohorts ($p < 0.001$). This group requires long-term follow-up and repeated mycobacterial cultures to detect the advent of an infection or colonisation with NTM. Unfortunately, our overall median observation time was only 643 days, while CF patients are treated for years.

Finally, our study found highest mortality in patients with *M. kansasii* infection or colonisation (non-significant). Because of the small case numbers within this group ($n = 18$) and the fact that most of these patients were HIV-positive with low CD4 cell counts, interpretation of these results seems difficult. Although, *M. kansasii* pulmonary disease is generally regarded milder, different studies have found the mortality within this group to be higher than MAC-PD [14, 16]. Nevertheless, data on this topic remain scarce.

The retrospective design of our study just allowed us to show the overall mortality, which includes all reasons for fatal events. Thus, a causal relationship cannot be deducted. Further studies regarding mortality in NTM infections are needed. Here, patients will have to be analysed further in respect to their comorbidities or other risk factors. Control groups without NTM infection or colonisation will have to be included as well. Therefore, our study delivers preliminary data on mortality rates within different disease groups.

Conclusion

NTM are underestimatedly linked to mortality and morbidity in different clinical settings. Especially, HIV, malignoma and CF patients are the ones at risk. CF patients show longest durations of positive microbiological results. Mortality analysis did not show significant results for different mycobacterial species or clinical manifestations, but as a tendency HIV patients with disseminated mycobacteriosis and patients with SLD are the ones with high mortality rates and require special clinical care. Interestingly, CF patients have low mortality rates in our study, which is most likely due to their young age in our cohort.

While treatment remains difficult, NTM will have an important role in the future, as they affect immunocompromised patients and patients without immunodeficiency alike.

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Compliance with ethical standards

Conflict of interest Nils Wetzstein, Thomas Wichelhaus, Michael Hogardt, Olaf Eickmeier, Gerrit Kann and Gudrun Just-Nübling declare that they have no conflict of interest. Christian Hügel has received congress fees and travel expenses from Gilead and Novartis. Claus-Philippe Küpper-Tetzel reports personal fees from Gilead, Janssen and Abbvie. Christoph Stephan reports personal honorary fees from scientific advisory board activities for pharmaceutical companies (MSD, Gilead, ViiV, Janssen Cilag) and travel grants for participation in medical conferences (BMS, Gilead, Janssen). Timo Wolf reports to have received lecture fees and travel grants by Janssen, Gilead and Merck Sharp Dome. All outside the submitted work.

References

1. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An Official ATS/IDSA Statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med. 2007;175:367–416. https://doi.org/10.1164/rccm.200604-571ST.
2. van Ingen J, Boeree MJ, van Soolingen D, Mouton JW. Resistance mechanisms and drug susceptibility testing of nontuberculous mycobacteria. Drug Resist Updat. 2012;15:149–61. https://doi.org/10.1016/J.DRUP.2012.04.001.
3. Feazel LM, Baumgartner LK, Peterson KL, Frank DN, Harris JK, Pace NR. Opportunistic pathogens enriched in showerhead biofilms. Proc Natl Acad Sci USA. 2009;106:16393–9. https://doi.org/10.1073/pnas.0908446106.
4. Falkinham JO, Iseman MD, de Haas P, van Soolingen D. Mycobacterium avium in a shower linked to pulmonary disease. J Water Health. 2008;6:209–13. https://doi.org/10.2166/wh.2008.032.
5. van Ingen J, Kohl TA, Kranzer K, Hasse B, Keller PM, Katarzyna Szafrańska A, et al. Global outbreak of severe Mycobacterium chimaera disease after cardiac surgery: a molecular epidemiological study. Lancet Infect Dis. 2017. https://doi.org/10.1016/s1473-3099(17)30324-9.
6. Prevots DR, Loddenkemper R, Sotgiu G, Migliori GB. Nontuberculous mycobacterial pulmonary disease: an increasing burden with substantial costs. Eur Respir J. 2017;49:1700374. https://doi.org/10.1183/13993003.00374-2017.
7. Johnson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections. J Thorac Dis. 2014;6:210–20. https://doi.org/10.3978/j.issn.2072-1439.2013.12.24.
8. Henkle E, Winthrop K. Nontuberculous mycobacteria infections in immunosuppressed hosts. Clin Chest Med. 2015;36:91–9. https://doi.org/10.1016/j.ccm.2014.11.002.
9. Wolinsky E. Mycobacterial lymphadenitis in children: a prospective study of 105 nontuberculous cases with long-term follow-up. Clin Infect Dis An Off Publ Infect Dis Soc Am. 1995;20:954–63.
10. Veraldi S, Çuka E, Vaira F, Nazzaro G. Mycobacterium marinum skin infection in a sushi cook. G Ital Di Dermatol E Venereol Organo Uff Soc Ital Di Dermatol E Sifilogr. 2016;151:569–70.
11. Hoefsloot W, van Ingen J, Andrejak C, Angeby K, Bauriaud R, Bemer P, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. Eur Respir J. 2013;42:1604–13. https://doi.org/10.1183/09031936.00149212.
12. Ringshausen FC, Wagner D, de Roux A, Diel R, Hohmann D, Hickstein L, et al. Prevalence of nontuberculous mycobacterial pulmonary disease, Germany, 2009–2014. Emerg Infect Dis. 2016;22:1102–5. https://doi.org/10.3201/eid2206.151642.
13. Vinnard C, Longworth S, Mezochow A, Patrawalla A, Kreiswirth BN, Hamilton K. Deaths related to Nontuberculous mycobacterial infections in the United States, 1999–2014. Ann Am Thorac Soc. 2016;13:1951–5. https://doi.org/10.1513/AnnalsATS.201606-474BC.
14. Kotilainen H, Valtonen V, Tukiainen P, Poussa T, Eskola J, Järvinen A. Clinical findings in relation to mortality in non-tuberculous mycobacterial infections: patients with Mycobacterium avium complex have better survival than patients with other mycobacteria. Eur J Clin Microbiol Infect Dis. 2015;34:1909–18. https://doi.org/10.1007/s10096-015-2432-8.
15. Diel R, Lipman M, Hoefsloot W. High mortality in patients with Mycobacterium avium complex lung disease: a systematic review. BMC Infect Dis. 2018;18:206. https://doi.org/10.1186/s12879-018-3113-x.
16. Gommans EPAT, Even P, Linssen CFM, van Dessel H, van Haren E, de Vries GJ, et al. Risk factors for mortality in patients with pulmonary infections with non-tuberculous mycobacteria: a retrospective cohort study. Respir Med. 2015;109:137–45. https://doi.org/10.1016/j.rmed.2014.10.013.
17. Chien J-Y, Lai C-C, Sheng W-H, Yu C-J, Hsueh P-R. Pulmonary infection and colonization with nontuberculous mycobacteria, Taiwan, 2000–2012. Emerg Infect Dis. 2014;20:1382–5. https://doi.org/10.3201/eid2008.131673.
18. Alvarez-Uria G, Falcó V, Martín-Casabona N, Crespo M, del Saz SV, Curran A, et al. Non-tuberculous mycobacteria in the sputum of HIV-infected patients: infection or colonization? Int J STD AIDS. 2009;20:193–5. https://doi.org/10.1258/ijsa.2008.008300.
19. Haworth CS, Banks J, Capstick T, Fisher AJ, Gorsuch T, Laurenson IF, et al. British Thoracic Society Guideline for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). BMJ Open Respir Res. 2017;4:e000242. https://doi.org/10.1136/bmjresp-2017-000242.
20. Flume PA. US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis. J Cyst Fibros. 2016;15:139–40. https://doi.org/10.1016/S1569-1993(16)00018-7.
21. Dean AG, Arner TG, Sunki GG, Friedman R, Lantinga M, Sangam S, Zubieta JC, Sullivan KM, Brendel KA, Gao Z, Fontaine N, Shu M, Fuller G, Smith DC, Nitschke DA, and Fagan RF. Epi Info™, a database and statistics program for public health professionals. CDC, FR. Epi Info™, a database and statistics program for public health professionals. 2011. https://www.cdc.gov/epiinfo/index.html.
22. R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2018. https://www.R-project.org/
23. Wickham H. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag; 2016. http://ggplot2.org.
24. Therneau TM. A package for survival analysis in S. version 2.38. 2015. https://CRAN.R-project.org/package=survival.
25. Therneau Terry M, Grambsch Patricia M. Modeling survival data: extending the cox model. New York: Springer; 2000.
26. Garcia-Marcos PW, Plaza-Fornieles M, Menasalvas-Ruiz A, Ruiz-Pruneda R, Paredes-Reyes P, Miguelez SA. Risk factors of non-tuberculous mycobacterial lymphadenitis in children: a case–control study. Eur J Pediatr. 2017;176:607–13.
27. Miguez-Burbano MJ, Flores M, Ashkin D, Rodriguez A, Granada AM, Quintero N, et al. Non-tuberculous mycobacteria disease as a cause of hospitalization in HIV-infected subjects. Int J Infect Dis. 2006;10:47–55. https://doi.org/10.1016/j.ijid.2004.11.005.