Thesis

Trends and clinical characteristics of non-tuberculous mycobacterial infections over the past 10 years in Graz, Austria

A retrospective cohort study

submitted by

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Graz, 24.04.2025

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Graz, 24.04.2025 Tobias Alexander Weiß m.p.

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Zusammenfassung

Grundlagen: Als Nicht-tuberkulöse Mykobakterien (NTM) werden alle Mykobakterien zusammengefasst, die nicht zum Mycobacterium-tuberculosis-Komplex zählen oder *Mycobacterium leprae* sind. Von den über 200 Arten, die hauptsächlich in Gewässern und im Boden zu finden sind, können einige beim Menschen zu Krankheiten führen, wobei vor allem die Lunge betroffen ist. Weltweit wird über einen Anstieg von NTM-Infektionen berichtet, doch aus Österreich liegen dazu keine Daten vor. Deshalb ist es Ziel dieser Arbeit, NTM-Infektionen in Graz über die letzten 10 Jahre zu analysieren.

Methodik: Es wurde eine retrospektive single-center Kohortenstudie an der Abteilung für Infektiologie des Landeskrankenhauses (LKH)-Universitätsklinikums Graz (Medizinische Universität Graz), durchgeführt. Eingeschlossen wurden Personen, die mindestens eine auf NTM positiv getestete Probe im Zeitraum 01.06.2014-01.06.2024 hatten und an der Abteilung für Infektiologie diagnostiziert und/oder behandelt wurden. Das Hauptinteresse gilt dabei den Infektionslokalisationen, den identifizierten Spezies und der Therapie inklusive Outcome.

Ergebnisse: 43 Personen wurden in die Studie eingeschlossen. 26/43 Personen (60.5%) waren männlich und das Durchschnittsalter betrug 56.1 Jahre (±17.9). 9/43 Infektionen (20.9%) waren pulmonal lokalisiert und 34/43 (79.1%) extrapulmonal. Insgesamt wurden 44 Mykobakterien-Isolate identifiziert, wobei der Mycobacterium-avium-Komplex mit 12/44 (27.3%) am häufigsten nachgewiesen wurde. Danach folgten *Mycobacterium abscessus* (18.2%) und die Mycobacterium-fortuitum-Gruppe (15.9%). Eine medikamentöse Therapie gegen NTM wurde bei 41/43 Personen (95.3%) angewendet. Dabei wurden bei 37/41 Personen (90.2%) Makrolide verwendet, 19/41 Personen (46.3%) erhielten Fluoroquinolone und 18/41 Personen (43.9%) Tuberkulostatika. Von 41 behandelten Personen hatten 27 (65.9%) eine klinische und fünf Personen (12.2%) eine mikrobiologisch gesicherte Heilung. Ein Behandlungsversagen trat nur in einem Fall (2.4%) auf und war auf mangelnde Adhärenz zurückzuführen. Drei der behandelten Personen (7.3%) hatten ein Wiederauftreten des Mykobakteriums, welches auch die erste Infektion verursacht hatte.

Zusammenfassung: In den letzten 10 Jahren wurden an der Klinischen Abteilung für Infektiologie des LKH-Universitätsklinikums Graz 43 NTM-Infektionen diagnostiziert/behandelt, wobei der Großteil extrapulmonal lokalisiert war. Die häufigsten nachgewiesenen Spezies waren der Mycobacterium-avium-Komplex, *Mycobacterium*

abscessus und die Mycobacterium-fortuitum-Gruppe. In 78% der behandelten Fälle konnte eine klinische oder mikrobiologische Heilung erreicht werden.

Abstract

Background: Nontuberculous mycobacteria (NTM) are mycobacteria other than *Mycobacterium leprae* and the Mycobacterium tuberculosis complex. Some of the over 200 known species, commonly found in water and soil, can cause disease in humans. Most infections are located pulmonary. A worldwide increase in NTM infections has been reported, but there is no data available from Austria, which is why the aim of this thesis is to investigate NTM infections in Graz over the last 10 years.

Methods: A single-center retrospective cohort observational study was conducted at the Division of Infectious Diseases at the LKH-University Hospital of Graz. Patients with at least one positive sample for NTM during 01.06.2014 and 01.06.2024 have been included, if the Division of Infectious Diseases at the LKH-University Hospital of Graz (Medical University of Graz) was involved in diagnosis and/or treatment. The main interest of this study are the sites of infection, identified species as well as therapy and outcome.

Results: 43 patients were included in this study. 26/43 patients (60.5%) were male and the mean age was 56.1 years (±17.9). 9/43 infections (20.9%) were pulmonary and 34/43 (79.1%) were extrapulmonary. A total of 44 mycobacteria isolates were identified. Mycobacterium avium complex was most common and isolated 12 times (27.3%). Other frequently found NTM were *Mycobacterium abscessus* (18.2%) and the Mycobacterium fortuitum group (15.9%). Medical treatment was administered to 41/43 patients (95.3%). Macrolides were used in 37/41 patients (90.2%), fluroquinolones in 19/41 pateints (46.3%) and antituberculosis drugs in 18/41 patients (43.9%). Out of the 41 treated patients, 27 (65.9%) had clinical cure and five (12.2%) had microbiological cure. Treatment failure occurred in one case (2.4%) and was attributed to poor adherence. In 3/41 treated patients (7.3%) recurrence occurred.

Conclusion: Over the last 10 years 43 NTM infections were diagnosed and/or treated at the Division of Infectious Diseases at the Medical University of Graz. Most cases were extrapulmonary. Most frequent NTM were the Mycobacterium avium complex, *Mycobacterium abscessus* and the Mycobacterium fortuitum group. In 78% of patients that were treated for NTM infections, clinical or microbiological cure was achieved.

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Abbreviations and their explanations

ALIS	Amikacin liposome inhalation suspension
ALT	Alanine aminotransferase
AP	Alkaline phosphatase
ART	Antiretroviral therapy
AST	Aspartate transaminase
ATS	American Thoracic Society
BAL	Bronchioalveolar lavage
BMI	Body mass index
CD	Cluster of differentiation
COPD	Chronic obstructive pulmonary disease
COVID19	Coronavirus disease 2019
CRP	C-reactive protein
CT	Computed tomography
ERS	European Respiratory Society
ESCMID	European Society of Clinical Microbiology and Infectious Diseases
GFR	Glomerular filtration rate
GGT	Gamma-glutamyltransferase
GM-CSF	Granulocyte macrophage colony stimulating factor
HIV	Human immunodeficiency virus
HRCT	High-resolution computed tomography
IDSA	Infectious Diseases Society of America
i.v.	intravenous(ly)
ICD	International Statistical Classification of Diseases and Related Health Problems
IL	Interleukin
JAK2	Janus kinase 2
LDH	Lactate dehydrogenase
LKH	Landeskrankenhaus
M.	Mycobacterium
MALDI-TOF	Matrix-assisted laser desorption ionization-time of flight

MIC	Minimal inhibitory concentration
MRI	Magnetic resonance imaging
NaOH	Sodium hydroxide
NEMO	Nuclear factor kappa light chain enhancer of activated B-cells essential modulator
NF-κB	Nuclear factor kappa light chain enhancer of activated B-cells
NTM	Nontuberculous mycobacterium (and Nontuberculous mycobacteria)
PCR	Polymerase chain reaction
PD	Peritoneal dialysis
pH	Potential of hydrogen
PY	Pack years
STAT	Signal transducer and activator of transcription
TNF-α	Tumor necrosis factor-α
TYK2	Tyrosine kinase 2

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1 Introduction

Nontuberculous mycobacteria (NTM) are a diverse group of mycobacteria which are neither *Mycobacterium (M.) leprae* nor do they belong to the M. tuberculosis complex. They can cause a variety of diseases, especially in immunocompromised patients and individuals with preexisting chronic pulmonary diseases.(1) While 77% of NTM infections cause pulmonary disease, NTM can also infect soft tissue, skin, the lymphatic system and other sites. Disseminated infections are rare but may occur.(2)

NTM are rather normal inhabitants than contaminants of the environment. They can be found in soils, dusts from agriculture, drainage waters, natural waters and drinking waters. Especially drinking water systems are an ideal habitat for NTM because they are more resistant to frequently used water disinfectants, such as chlorine, than other microorganisms. Hence, NTM have most of the necessary nutrients for themselves.(3)

Currently, more than 200 species and subspecies of NTM are known to exist, with some being strict or opportunistic pathogens.(4) They are divided into slowly growing and rapidly growing mycobacteria. This classification is based on the time it takes them to form colonies on subculture. Whilst rapidly growing mycobacteria grow within seven days, slowly growing mycobacteria require more than seven days to form mature colonies.(5) Among the rapidly growing NTM, *M. abscessus* is most frequently found in patients with pulmonary disease whereas most common types of slowly growing NTM are the Mycobacterium avium complex (MAC), *M. kanasii* and *M. xenopi*.(6)

The MAC originally consisted of *M. avium* and *M. intracellulare*. The purpose of defining this complex was the clinical similarity between the two and the limited possibilities for further differentiation. In 2018, a new classification, which added 10 more mycobacteria to the MAC, was proposed: *M. chimaera, M. colombiense, M. arosiense, M. vulneris, M. bouchedurhonense, M. timonense, M. marseillense, M. yongonense, M. paraintracellulare and M. lepraemurium*.(7)

Most frequently isolated species of NTM are listed in table 1.

Table 1: List of frequently isolated NTM

Slowly growing NTM	Rapidly growing NTM
MAC	*M. abscessus*
M. arupense	*M. alvei*
M. asiaticum	*M. aurum*
M. bohemicum	*M. boenickei*
M. branderi	*M. brumae*
M. celatum	*M. chelonae*
M. europaeum	*M. confluentis*
M. florentinum	*M. elephantis*
M. genavense	*M. fortuitum*
M. gordonae	*M. goodii*
M. haemophilum	*M. holsaticum*
M. heckeshornense	*M. immunogenum*
M. interjectum	*M. iranicum species nova*
M. intermedium	*M. margeritense*
M. kanasii	*M. mucogenicum*
M. kubicae	*M. peregrinum*
M. lentiflavum	*M. phocaicum*
M. malmoense	*M. septicum*
M. marinum	*M. thermoresistibile*
M. nebraskense	
M. palustre	
M. saskatchewanense	
M. scrofulaceum	
M. shimodei	
M. simiae	
M. szulgai	
M. ulcerans	
M. xenopi	

Adapted from (1)

1.1 Epidemiology of NTM

Although there is limited data regarding the worldwide and country specific epidemiology of NTM infections, most studies suggest that there is a general increase in NTM infections. A recently conducted systematic review of 47 studies regarding pulmonary NTM infection and disease found, that 82.1% of the included studies showed an increase in pulmonary NTM infection and 66.7% reported an increase in pulmonary NTM disease (with infection being defined as the absence of symptoms and radiological findings of pulmonary disease).(8) Possible causes for the rising of NTM incidence may be the increasing number of immunocompromised patients, more complex surgical and medical procedures and better diagnostic capabilities.(9)

1.1.1 North America

The annual age adjusted prevalence of patients with a positive specimen for NTM in five US-states (Maryland, Mississippi, Missouri, Ohio, Wisconsin) rose from 8.7/100,000 in 2008 to 13.9/100,000 in 2013.(10) A more recent study from the USA analysed NTM pulmonary disease cases in patients above the age of 65 from 2010 to 2019. They found that the incidence has continued to rise in all regions and among males and females. The highest incidence was noted in the South with 28.6/100,000 in 2019.(11) Another study reported that the prevalence of NTM lung disease in the US rose from 6.78/100,000 in 2008 to 11.70/100,000 in 2015.(12) In Ontario, Canada, the NTM pulmonary disease prevalence increased from 4.65/100,000 in 1998 to 9.08/100,000 in 2010. The prevalence of extrapulmonary NTM disease varied between 0.65/100,000 and 0.79/100,000 in the mentioned time period and did not change considerably.(13)

1.1.2 South America

Generally, epidemiologic data regarding NTM infections in South America is rare. In Uruguay, the incidence of NTM increased from 0.33/100,000 in 2006 to 1.57/100,000 in 2018.(14) A study from Brazil showed that the number of patients from Rio de Janeiro with NTM pulmonary disease has been stable from 1993 to 2005 with five to seven identified cases per year. In 2006, the patient count had tripled with approximately 20 cases per year. Since then, the numbers remained approximately the same till 2010 with 20-40 registered yearly. The authors note, that the true prevalence of NTM infections in Brazil remains

unknown.(15) The numbers from South America might be an underestimate due to poorer detection capabilities compared to those in North America.

1.1.3 Asia and Oceania

In Taiwan, the incidence of NTM associated disease increased from 2.7/100,000 in 2000 to 10.2/100,000 in 2008. During this period, NTM accounted for 39.2% of all positive mycobacterial cultures with the most common NTM being MAC and *M. abscessus*.(16) In South Korea, the incidence of NTM infection increased from 6/100,000 in 2008 to 19/100,000 in 2016.(17) At the Kyoto University hospital, the number of patients newly diagnosed with NTM pulmonary disease almost doubled from 24 in 2000 to 54 in 2013. MAC accounted for 85% of the cases.(18) Previously, a doubling in NTM related deaths was reported in Japan from 2000 to 2010.(19)

In Queensland, Australia, the incidence of NTM cases increased from 11.10/100,000 in 2001 to 25.88/100,000 in 2016. An increase was noted for all common species except *M. kanasii*.(20)

1.1.4 Europe

In England, Wales and Northern Ireland, the rate of NTM reports increased from 0.9/100,000 in 1995 to 2.9/100,000 in 2006. Almost half the cases were due to an infection with MAC.(21) From 2007 to 2012 the NTM incidence increased in the same countries from 5.6/100,000 to 7.6/100,000. With 35.6%, MAC was once again the most frequently cultured NTM.(22) In Germany, the prevalence for NTM pulmonary disease climbed from 2.3/100,000 in 2009 to 3.3/100,000 in 2014.(23) Furthermore, an average annual increase of NTM pulmonary disease associated hospitalisations of 4.9% was noted between 2005 and 2011.(24) In Denmark, the incidence rate for patients with at least one positive NTM culture appears to have remained stable between 1991 and 2015. It was reported to be 2.57/100,000. The most frequently detected NTM species among the Danish people were MAC and *M. gordonae*.(25)

1.2 Pathogenesis of NTM

1.2.1 Environmental sources of NTM

It is known that several factors contribute to the existence and distribution of NTM in the environment. Due to their hydrophobic outer membrane, their ability to grow at a low potential of hydrogen (pH) and their temperature resistance, NTM can not only be found in natural water and drinking water systems, but also in hospital and household plumbing, hot tubs and spas, acidic, brown-water swamps, potting soils, boreal forest soils and metal removal fluid systems.(26)

The main reason for their presence in the environment is the existence of a lipid-rich outer membrane with long chain mycolic acids, linked to arabinogalactan. They contribute to the impermeability and hydrophobicity of the mycobacteria.(27)

1.2.2 Transmission of NTM

Due to their hydrophobicity, NTM living in water can stick to air bubbles rising through it. When the bubbles hit the surface, they burst and eject up to four droplets containing NTM to a height of several centimetres. When these aerosols dry, they shrink to a size small enough to pervade the human lung.(28)

This pathway of transmission is supported by a study that compared the occurrence of NTM in home environmental samples (shower aerosols, bathroom faucets, kitchen faucets and indoor and outdoor soil) between the homes of patients diagnosed with MAC pulmonary disease and a control population. The NTM isolation from shower aerosols had an odds ratio of 4.0 associated with disease, whilst the other samples did not show a significant correlation with disease.(29) In another study, researchers found, that the pulmonary infection with *M. avium* of a patient was most likely caused by the same clone that was found on the patient's showerhead.(30)

A further pathway of transmission is the inhalation of dust from gardening soil colonized with NTM. It has been proven that the mycobacteria found in the patient's garden's soils were the same species that caused their pulmonary diseases.(31)

Especially in children with cervical lymphadenitis, the route of infection seems to be through wounds caused by tooth eruption. This theory is supported by the fact, that most cases of cervical lymphadenitis are reported in children between the ages of six months and two years.(26)

For the fishing tank granuloma, which is a rare skin infection caused by *M. marinum*, fish tank exposure is the main source of infection. The mycobacteria enter the skin through open wounds and small lesions, often seen in patients with psoriasis. Although it is not generally recommended, people with open skin lesions should wear waterproof gloves when cleaning their fish tanks or avoid water contact.(32)

The only known person-to-person transmission of NTM appears to be in cystic fibrosis patients infected with *M. abscessus*. The route of transmission seems to be indirect but further research needs to be done.(33)

1.2.3 Immune response to NTM infection

In immunocompetent individuals, mononuclear phagocytes absorb mycobacteria in phagosomes. This process stimulates the release of Interleukin (IL) 12 which then binds to the IL-12-receptor on natural killer cells and T cells. In further consequence, via tyrosine kinase 2 (TYK2) and Janus kinase 2 (JAK2) a signal is given to the cell nucleus which then leads to activation of signal transducer and activator of transcription (STAT) 4. Interferon-γ is released and activates a signalling cascade in macrophages where STAT1 plays an important role. In the end, macrophages release further IL-12 and tumor necrosis factor-α (TNF-α). The latter stimulates additional macrophages and is important for the formation of granulomas. The granulomas include infected macrophages and other antigen-presenting cells surrounded by lymphocytes.(34) Under these circumstances macrophages are capable of killing intracellular mycobacteria by nutrient deprivation, induction of autophagy and exposure to reactive oxygen species and antimicrobial peptides.(35) Another important factor for immunity against mycobacteria is the nuclear factor kappa light chain enhancer of activated B-cells (NF-κB) essential modulator (NEMO)-mediated pathway.(36)

1.2.4 Risk factors

Studies show that a comorbid respiratory disease is associated with a high odds ratio for NTM pulmonary disease. The strongest association is reported for bronchiectasis, followed by a history of tuberculosis, interstitial lung disease and asthma.(37) Another risk factor is the use of immunosuppressive drugs. Inhaled corticosteroids show the highest odds ratio, followed by oral corticosteroids and other immunosuppressants. Other factors that seem to increase the risk of NTM pulmonary disease are solid tumours, cardiovascular disease, the presence of pneumonia and treatment for rheumatoid arthritis. Whilst being overweight

seems to reduce the risk of NTM pulmonary disease, it remains unclear whether infected patients have lost weight due to disease and/or treatment or have been underweight before.(37) A correlation between scoliosis and pectus excavatum and NTM pulmonary disease has been noted, especially in women.(38)

Another important risk factor for both pulmonary and extrapulmonary NTM infections is an infection with the human immunodeficiency virus (HIV). Especially individuals with cluster of differentiation (CD) 4 counts <50 cells/µl are at high risk of disseminated disease caused by MAC with the risk being inversely proportional to CD4 count.(5) It is important to note, that the rate of MAC infections in HIV-positive individuals decreased after the implementation of antiretroviral therapy (ART).(39)
Furthermore, several rare primary immunodeficiency states are associated with a higher risk of disseminated NTM disease: Anti-interferon γ-antibodies, Anti-granulocyte macrophage colony stimulating factor (GM-CSF)-antibodies, NEMO-mutations, STAT1 deficiency and IL-12 mutations.(40)
Risk factors for extrapulmonary NTM disease are cosmetic surgeries, prosthetic devices and implants, organ transplantation, dental procedures, invasive devices such as pacemakers, trauma and injections into joints, muscle and dermis.(40,41)

1.3 Clinical manifestations of NTM infections

With the main manifestation of NTM disease being pulmonary disease, only 20-30% of NTM infections are reported to originate from extrapulmonary sites such as skin and soft tissue, lymphatic tissue, the musculoskeletal system and disseminated disease. The clinical presentation varies by site of infection.(41)

1.3.1 Pulmonary disease

The symptoms of NTM pulmonary disease may vary and are unspecific. Almost all patients report chronic or recurring cough. Other symptoms are increased sputum production, malaise, fatigue, fever, haemoptysis, chest pain, weight loss and dyspnea. In progressive NTM pulmonary disease, the number of symptoms tends to increase. Nevertheless, it is important to note that the evaluation of symptoms is often complicated by coexisting lung diseases like chronic obstructive pulmonary disease (COPD), cystic fibrosis or

bronchiectasis. When auscultating diseased individuals, findings may be rhonchi, crackles, squeaks and wheezes.(5)

1.3.2 Lymphatic disease

NTM lymphatic disease is primarily seen in children with the main location being the head and neck area.(42,43) Occasionally, other regions, like mediastinal lymph nodes, are affected. Involved lymph nodes are typically indurated and mostly unilateral. The lymph nodes may show fast growth and even rupture, but systemic symptoms are not common. The two major complications are recurrence and infection with a prolonged course happening rarely.(42) NTM associated lymphadenitis is possible in adults, but happens infrequently, unless immunosuppressed. Most common NTM causing lymphadenitis are *M. avium, M. haemophilum, M. intracellulare and M. malmoense*.(41)

1.3.3 Skin infections

Skin infections happen frequently following trauma and surgical procedures.(41) A special type of infection, the fishing tank granuloma caused by *M. marinum*, was already mentioned earlier. Clinically, it presents as a granulomatous pustule or nodule that ulcerates over time. Occasionally multiple lesions can be observed and extension along lymphatic pathways may happen. Disseminated skin lesions due to *M. marinum* are only found in immunosuppressed patients.(44)

Another skin manifestation is the Buruli ulcer caused by *M. ulcerans*. Clinical manifestation shows a subcutaneous nodule or a papule, looking like an insect bite at beginning. The lesions usually show a diameter of 3 cm and more and are typically painless. Some patients present with the oedematous form characterised by non-pitting diffuse swelling. As the disease progresses further, subcutaneous ulcers develop. Wound edges are typically undermined and necrotic tissue can be seen at the wound base. Buruli ulcer usually affects limbs and face and is not only limited to skin but can also cause osteomyelitis. If not treated early, scars and contractures may occur.(45)

Skin infections with *M. chelonae*, often found after cosmetic surgeries, may lead to wound dehiscence, erythema, induration and abscess formation.(41) A study that analysed skin infections caused by three species of rapidly growing NTM came to the following conclusions: Especially infections with *M. chelonae* and *M. abscessus* cause multiple skin lesions, whilst *M. fortuitum* primarily causes singular lesions. Diagnosis of skin infections

is often delayed due to a lack of microbiological testing of surgical wounds and skin biopsies.(46)

1.3.4 Musculoskeletal infections

Symptoms of musculoskeletal infections include pain in the affected body part, restricted joint movement and swelling. Up to 50% of the affected patients complain about fever. Further symptoms are unhealed wounds, bleeding and redness and warmth at the affected site.(47,48)

Tendosynovitis caused by MAC shows a gradual growth of tendon and synovium. Underlying structures like muscles, bones and joint structures are usually not affected.

Another infection caused by NTM is osteomyelitis. Most frequently found species are *M. abscessus, M. fortuitum and M. chelonae*. The infection may occur following trauma and joint replacement and is associated with disseminated disease and immunosuppression. Especially when the spine is involved, symptoms are bone pain, neuropathic pain and neurological symptoms.(41)

1.3.5 Disseminated disease

NTM disseminated disease is a major cause of morbidity in HIV-positive individuals with low CD4 cell counts. Although the most common NTM responsible for disseminated disease is MAC, other species like *M. kanasii* and *M. genavense* can cause it as well. Symptoms include fever, night sweat and weight loss. These three are seen significantly more frequently in HIV-positive patients with disseminated NTM disease than in HIV-positive patients without this infection and therefor are a good predictor for mycobacterial disease in this population.(49) Further symptoms reported in disseminated MAC disease in individuals with HIV are diarrhea, severe anaemia with haematocrit <26%, abdominal pain and an elevated alkaline phosphatase.(50)

Although not reported often, disseminated disease in immunocompetent can be caused by rapidly growing mycobacteria. Symptoms include chronic bilateral cervical lymphadenopathy and reactive skin manifestations. Some patients manifest with a Sweet's syndrome, which is characterised by fever, leucocytosis and erythematous plaques infiltrated by neutrophils.(51)

1.3.6 Other clinical manifestations

Some NTM are known to cause peritoneal dialysis (PD)-related infection. They may lead to exit-site and tunnel infections, but also peritonitis.(52) In case of peritonitis, symptoms include nausea, vomiting, abdominal pain and pseudo bowel obstruction. Loculated ascites and abdominal adherences are rare.(41) A typical sign for tunnel infection is purulent drainage from exit site of the catheter.(53,54)

NTM can also cause infection of prosthetic heart valves. Symptoms include fever, anaemia, pancytopenia, weight loss and valve insufficiency.(41) A study found, that NTM infective endocarditis is significantly more common in patients with bioprosthetic valves compared to mechanical valves and native valves.(55)

1.4 Diagnosis of NTM infections

Diagnosis of NTM infection varies by site of infection and is based on radiological, histopathological and microbiological diagnostics combined with the clinical symptoms. The most important method of diagnosis is the microbiological identification of NTM from sputum or other samples obtained.(1,41,56)

1.4.1 NTM pulmonary disease

The American Thoracic Society (ATS)/European Respiratory Society (ERS)/European Society of Clinical Microbiology and Infectious Diseases (ESCMID)/Infectious Diseases Society of America (IDSA) diagnostic criteria for NTM pulmonary disease from 2020 include the criteria listed in table 2.

Table 2: ATS/ERS/ESCMID/IDSA criteria for diagnosis of NTM pulmonary disease

1.Clinical criteria	Pulmonary or systemic symptoms
and	
2. Radiologic criteria	Nodular or cavitary opacities on chest radiograph, or high-resolution computed tomography (HRCT) scan showing bronchiectasis with multiple small nodes.
and	Exclusion of other diagnoses
3. Microbiologic criteria	1) positive cultures from at least two separate sputum samples or 2) positive culture from ≥1 bronchial wash or lavage or 3) lung biopsy with mycobacterial histologic features and positive culture for NTM or biopsy showing mycobacterial features and ≥1 positive culture from sputum or bronchial washings.

Adapted from (6)

Radiologic features of NTM pulmonary disease include emphysema, ground-glass opacities, consolidation, cavities, bronchiectasis, tree-in-bud pattern, nodules and pleural effusion.(57) The two most common types of NTM pulmonary disease in immunocompetent patients are the cavitary or classic form and the bronchiectatic form. For the first type, typical findings in chest radiograph are upper lobe cavities, cicatricial atelectasis and pleural thickening. Differentiation between the cavitary form and postprimary tuberculosis is difficult, but cavities tend to be smaller and thinner walled in NTM infections. In the bronchiectatic form, nodular opacities can be found randomly distributed, whist cavities are uncommon. Any segment of the lung can be involved, with the right middle lobe and lingula being the main locations. In computed tomography (CT), tree-in-bud patterns and cylindric bronchiectasis are common.(58) While the bronchiectatic form is most common in pulmonary disease caused by MAC, it is infrequently seen in *M. xenopi* infections, which more commonly exhibit a cavitary pattern or cannot be ascribed to any of the mentioned types.(59)

CT may also play in important role in in predicting outcome in patients with MAC pulmonary disease.(60,61) Atelectasis, pleural thickening and cavities detected by HRCT are associated with worse therapeutic response.(60)

For laboratory diagnosis of NTM pulmonary disease, the 2020 ATS/ERS/ESCMID/IDSA guidelines recommend that at least three respiratory samples should be examined over an interval of at least one week. This is essential to differentiate between transient contamination and actual pulmonary infection.(6) Studies regarding the sensitivity of cultures generated from bronchioalveolar lavage (BAL) fluid, bronchial washing and expectorated sputum suggest different results. It remains unclear whether sputum is less sensitive than bronchial washing.(62–64) Although the diagnostic method has no impact on disease severity and progression, performing bronchoscopy is recommended if sputum cannot be obtained.(65)

After sample collection, decontamination by 0.25% N-acetyl-L-cysteine and 1% Sodium hydroxide (NaOH) is crucial to supress accompanying flora. The following culture is performed on both solid and liquid media.(6) The optimal temperature for incubation of NTM is 36±1°C but pulmonary samples from patients with cystic fibrosis should be concurrently incubated at lower temperatures. This is due to frequent infections with *M. abscessus*, a mycobacterium that grows at lower temperatures. Incubation time varies between six weeks for liquid media and eight weeks for solid media with longer periods being necessary occasionally.(1)

For correct identification of NTM, which is vital to determine clinical relevance and ideal treatment, both molecular and mass spectrometry-based methods are disposable. For molecular identification probe-based assays and gene sequencing are available. Whilst probe-based assays are easier to perform, gene sequencing has higher discriminatory power. Another approach to identify NTM is the matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry method.(6) By using a short laser to ionize the samples, particles are accelerated through an electric field. Subsequently, the time of flight, which varies by bacteria, is detected. The obtained data is then compared with a reference database for bacterial identification. MALDI-TOF is a rapid method to determine mycobacteria at a species level.(66)

Regarding susceptibility testing of NTM, it should only be performed for clinically significant isolates using the method of broth microdilution. Using this procedure, the minimal inhibitory concentration (MIC) is determined. The MIC is the smallest concentration of a specific antibiotic that inhibits microbial replication. Especially in slowly growing mycobacteria, there is limited data regarding the correlation of in vitro testing results and clinical outcome.(67)

Acid fast bacteria like NTM can be detected by microscopy. Although this diagnostic method would be cheap and fast, differentiation between NTM and *M. tuberculosis* is not possible. Therefore, positive microscopy can suggest NTM infection but is not meaningful without further testing.(68)

1.4.2 Extrapulmonary NTM disease

For diagnosing extrapulmonary NTM infection, microbiological cultures, molecular investigations and histopathology might be necessary, depending on site of infection.(41)

For NTM lymphadenitis there exists no diagnostic method that can provide both optimal sensitivity and optimal specificity which makes the combination of several diagnostic methods crucial. An NTM antigen skin test containing purified protein derivative provides high specificity and is, if positive, indicative for NTM lymphadenitis. For definite diagnosis, microbiological or polymerase chain reaction (PCR) detection of NTM is necessary. The sample should be obtained by fine needle aspiration.(69,70) If NTM lymphadenitis is suspected, but not yet confirmed by PCR or culture, the lymph node is often surgically removed without definite diagnosis. The tissue is then analysed by PCR or culture to optimize further antibiotic treatment, if necessary.(70)

Ultrasonographic findings in NTM lymphatic disease include hypoechoic lymph nodes that show liquefaction with intranodal necrosis and surrounding soft tissue oedema.(71)

To confirm NTM skin infections, only skin biopsies for culture and histology are needed. Imaging is only necessary if deep tissue infection is suspected.(41)

Diagnosis of musculoskeletal NTM infection includes mycobacterial cultures from synovial biopsy, synovial fluid, abscess puncture and tissue biopsy.(47,48) Magnetic resonance

imaging (MRI) is performed regularly but shows nonspecific findings like soft tissue swelling, joint effusion, sinus tracts and osteopenia.(47)

For diagnosis of disseminated disease, blood cultures, urine cultures and stool cultures combined with biopsy of bone narrow may be necessary. Positron Emission Tomography-CT can be useful to diagnose suspected disseminated NTM infection.(41)

1.5 Treatment of NTM infections

Because NTM isolation does not always imply infection or disease, the decision between initiating treatment or choosing a watch and wait procedure may vary depending on several factors.(72) While the main therapy relies on administration of a combination of multiple antibiotics, surgical interventions also plays a role in treating NTM disease. This is the case for both pulmonary and extrapulmonary infections.(6,41) Regarding pharmaceutical therapy, at least two effective antibiotics should be used to prevent resistance. Depending on site of infection and severity of disease the use of up to four antibiotics may be necessary. For certain species treatment is divided into two phases: In the initial phase, drugs are often administered intravenously (i.v.) for up to three months, while in the maintenance phase, oral administration is sufficient.(73)

1.5.1 Treatment of pulmonary NTM disease

The 2020 ATS/ERS/ESCMID/IDSA guidelines for treatment of pulmonary NTM disease advise initiation of treatment for all patients who meet the criteria listed in table 2 rather than watchful waiting. The treatment should be susceptibility-based and varies by NTM.(6)
For treating MAC pulmonary disease, a 3-drug regimen including a macrolide like azithromycin or clarithromycin is recommended. In patients with severe bronchiectatic or cavitary pulmonary disease the administration of parenteral amikacin or streptomycin is suggested for 2-3 months. Treatment should be continued for at least 12 months after culture conversion. If culture conversion does not happen after six months of treatment, the additional administration of amikacin liposome inhalation suspension (ALIS) is indicated.(6)
For the second most common NTM causing pulmonary disease, *M. abscessus*, treatment is complicated. This is since two of the three subspecies (*M. abscessus subspecies abscessus* and *M. abscessus subspecies bolletii*) have an inducible macrolide resistance gene, resulting

in clinical macrolide resistance. The third subspecies, *M. abscessus subspecies massiliense*, does not have the affected gene and is therefore usually susceptible to macrolide antibiotics. Nevertheless, it is important to note that some *M. abscessus* subspecies have a special mutation of the mentioned gene, which preserves their macrolide susceptibility. Therapy for macrolide susceptible *M. abscessus* isolates should consist of a 3-drug regimen including one macrolide. In the initial phase at least one antibiotic should be administered parenterally. Macrolide-resistant isolates should be treated with at least four antibiotics. Azithromycin should be included for its immunomodulatory properties. Optimal drug therapy in the maintenance phase is unknown, but a combination of inhaled amikacin, clofazimine and omadacycline is recommended. Adding tedizolid or linezolid can be considered. If *M. abscessus* is unresponsive to initial treatment, switching to maintenance therapy is not associated with better outcome.(74)

For treatment of *M. kanasii* and *M. xenopi* pulmonary disease, a 3-drug regimen of rifampicin, ethambutol and a macrolide is recommended. The macrolide can be replaced by isoniazid in *M. kanasii* and fluoroquinolone in *M. xenopi* infections. In severe cases, parenteral amikacin can be added in the initial phase. *M. kanasii* should be treated for a total of 12 months and *M. xenopi* for 12 months after culture conversion.(6)

In selected patients, surgical intervention in addition to pharmacological treatment may improve outcomes and is therefore recommended.(6,75) The most frequent indication for surgery in pulmonary NTM disease is failure of pharmaceutical therapy. Some patients require surgery to control worrying symptoms like haemoptysis and in a limited number of cases surgical debulking is performed to slow down disease progression.(75) Procedures include lobectomies, segmentectomies, pneumonectomies and mixed procedures.(76) They are performed by thoracotomy and thoracoscopy.(77) Regarding the outcome, patients who are both treated surgically and medically seem to have culture conversion more often than those who are only treated with antibiotics. The mortality rate does not differ significantly.(78)

Postoperative complications occur in up to 20% of patients and include bronchopleural fistulae, respiratory failure, bleeding, pneumothorax, pyothorax, prolonged air leak, atrial fibrillation and atelectasis. Operative mortality varies between 0% and 2.6% depending on

the study. In conclusion, adjuvant pulmonary resection combined with medical therapy is beneficial for patients with localized pulmonary NTM disease.(76,77,79)

1.5.2 Treatment of extrapulmonary NTM disease

The treatment regimen for extrapulmonary NTM varies by manifestation of disease and triggering pathogen. There are no guidelines existent for duration of treatment, but it often takes 6-18 months. In contrast to pulmonary disease, pharmacological treatment is not mandatory in all extrapulmonary NTM infections.(41)

For NTM lymphadenitis, typically found in children, surgical removal of involved lymph nodes has proven to be superior to medical treatment. In case of recurrence after excision, which is seen in a limited number of patients, therapy with a combination of clarithromycin and rifabutin for a duration of three months was reported to be successful.(80)

Therapy of skin and soft tissue infections depends on the causative NTM species. Antibiotics should be chosen based on the results of drug sensitivity testing. Especially in uncomplicated cases antibiotic monotherapy might be sufficient, while a multidrug regimen can be necessary in more severe cases. In case of medical therapy failure, abscess formation or widespread lesion, surgical debridement should be performed. Two to four months of treatment should be adequate in most patients. Typical endpoints for treatment are substantial disappearance of skin lesions, no new skin lesions or unchanged skin lesions after a certain time.(81) In skin infections caused by *M. ulcerans, M. chelonae* and especially *M. marinum*, adding thermotherapy to treatment regimen has been reported to be beneficial.(82) Furthermore photodynamic therapy in addition to antibiotic treatment has been reported to shorten the course of treatment in NTM skin infections.(83,84)

Treatment for NTM musculoskeletal infections usually requires both surgical and medical management. Multidrug therapy should be based on antibiotic sensitivities and happen over a period of at least six months. In severe cases, a treatment duration of 12 months or longer might be necessary.(85) In case of persistent signs of infection after surgery and despite effective antimicrobial medication, more aggressive resection of affected tissue is required. If prosthetic joints are infected, they need to be removed.(86)

For disseminated MAC disease, frequently seen in HIV-infected patients, the most effective therapy is a 3-drug regimen based on clarithromycin, ethambutol and rifabutin.(87) It is important to both treat NTM and HIV infection in disseminated MAC disease. If immune restauration cannot be achieved by ART, patients with MAC disseminated disease should receive lifelong maintenance therapy.(88) In case of successful immune restauration, antibiotics should be administered for 6-12 months.(5)

1.6 Aim of this study

The aim of this diploma thesis is to investigate NTM infections diagnosed and/or treated at the Division of Infectious Diseases at the Landeskrankenhaus (LKH)-University Hospital of Graz (Medical University of Graz) over the past decade, with a focus on detected species, sociodemographic parameters, sites of infection and treatment outcomes. Additionally, the work seeks to identify the development of NTM infections in Graz over time.

2 Material and methods

A single-center retrospective cohort observational study was conducted at the Division of Infectious Diseases at the LKH-University Hospital of Graz (Medical University of Graz). The necessary data were collected from the hospital information system.

The patient cohort included all patients with at least one positive molecular or microbiological specimen (BAL, biopsy material, blood, cerebrospinal fluid, PD-outflow, sputum, urine, wound swap) for NTM during 01.06.2014 and 01.06.2024 who were diagnosed and/or treated in cooperation with the Division of Infectious Diseases at the LKH-University Hospital of Graz (Medical University of Graz).

2.1 Inclusion and Exclusion criteria

Inclusion criteria

- Male and female patients
- All age groups
- At least one positive molecular/microbiological sample taken from biopsy material, blood, cerebrospinal fluid, PD-outflow, respiratory tract (BAL/sputum), urine or wound swap between 01.06.2014 and 01.06.2024
- Diagnosis and/or treatment in cooperation with the Division of Infectious Diseases at the LKH-University Hospital of Graz (Medical University of Graz)

Exclusion criteria

- No positive result for NTM
- No involvement of the Division of Infectious Diseases in diagnosis and/or treatment of NTM infection
- No contact to patient after detection of NTM

2.2 Parameters

The relevant data included demographic characteristics, physical measurements, medical history, social history, microbiological findings, laboratory findings, treatment and outcome. All the collected parameters are listed in table 3.

Table 3: Collected parameters sorted by subgroups

Variable	Unit/Scale
Demographic characteristics	
Date of Birth	Date
Sex	Male/female
Country of birth	Categorial
Citizenship	Categorial
Postcode of registered address	Categorial
Physical measurements	
Height	cm
Weight	kg
Body mass index (BMI)	kg/m^2
Medical history	
Comorbidities (at time of NTM detection)	Categorial
Concomitant medication (at time of NTM detection)	Categorial
Social history	
Highest level of education	Categorial
Profession	Categorial
Smoking	Pack years (PY)
Alcohol abuse	Yes/no
Illicit drug use	Yes/no
Travel history (12 months prior to NTM detection)	Categorial
Microbiological findings	
Species of NTM	Categorial
Sampling site	Categorial
Date of detection	Date
Mode of detection	Culture/PCR
Susceptibility for each antibiotic (if applicable)	Susceptible/intermediate/resistant (+MIC if applicable)

Medical treatment	
Antibiotics prescribed	Categorial
Start of treatment	Date
End of treatment	Date
Route of medical administration	Categorial
Dosage of antibiotics	mg/day
Reason for discontinuation of treatment	Categorial
Adverse drug reactions	Categorial
Hospitalisation	
Date of hospitalisation	Date
Date of admission	Date
Reason for hospitalisation	Categorial
Level of care	General ward/intermediate care/intensive care
Outcome	
Clinical cure	Yes/no
Microbiological cure	Yes/no
Treatment failure	Yes/no
Recurrence	Yes/no
Reinfection	Yes/no
Death until 01.06.2024	Yes/no
Date of Death (if applicable)	Date
Cause of Death (if applicable)	International Statistical Classification of Diseases and Related Health Problems (ICD)-10 code
Laboratory results (0, 3, 6, 12 and 24 months after detection of NTM)	
Haemoglobin	g/dL
Thrombocytes	G/L
Leukocytes	G/L
Lymphocytes	G/L

Neutrophils	G/L
Monocytes	G/L
Natrium	mmol/L
Kalium	mmol/L
Creatinine	mg/dL
Glomerular filtration rate (GFR)	mL/min/1.73m^2
Bilirubin (total)	mg/dL
Albumin	g/L
Aspartate transaminase (AST)	U/L
Alanine aminotransferase (ALT)	U/L
Alkaline phosphatase (AP)	U/L
Gamma-glutamyltransferase (GGT)	U/L
Lactate dehydrogenase (LDH)	U/L
C-reactive protein (CRP)	mg/dL
Ferritin	µg/dL
IL-6	pg/mL
Procalcitonin	ng/mL
HIV-1/2 antigen/antibody	Positive/negative
Helper/inducer T cells	G/L
Hepatitis-A-Virus Immunoglobulin G	Positive/negative
Hepatitis-A-Virus Immunoglobulin M	Positive/negative
Hepatitis-B-Virus-core-antigen	Positive/negative
Hepatitis-B-Virus-surface-antibody	Positive/negative
Hepatitis-B-Virus-core-antibody	Positive/negative
Hepatitis-C-Virus-antibody	Positive/negative

2.3 Definitions

Clinical cure: Patient-reported and/or objective improvement of symptoms during antimycobacterial treatment that is sustained at least until the end of treatment, but no cultures available to prove culture conversion or microbiological cure.

Microbiological cure: Multiple consecutive negative but no positive cultures with the causative species from (respiratory) samples after culture conversion and until the end of antimycobacterial treatment.

Recurrence: The re-emergence of at least one positive culture with the causative species from samples after cessation of antimycobacterial treatment.

Reinfection: New infection caused by a different strain of mycobacterium after the initial infection has been cleared

Treatment failure: The re-emergence of multiple positive cultures or persistence of positive cultures with the causative species from samples after $\geq$12 months of antimycobacterial treatment, while the patient is still on treatment.

2.4 Ethics approval

Ethical approval was obtained from the ethics committee of the Medical University of Graz (Ethics committee number 1206/2024).

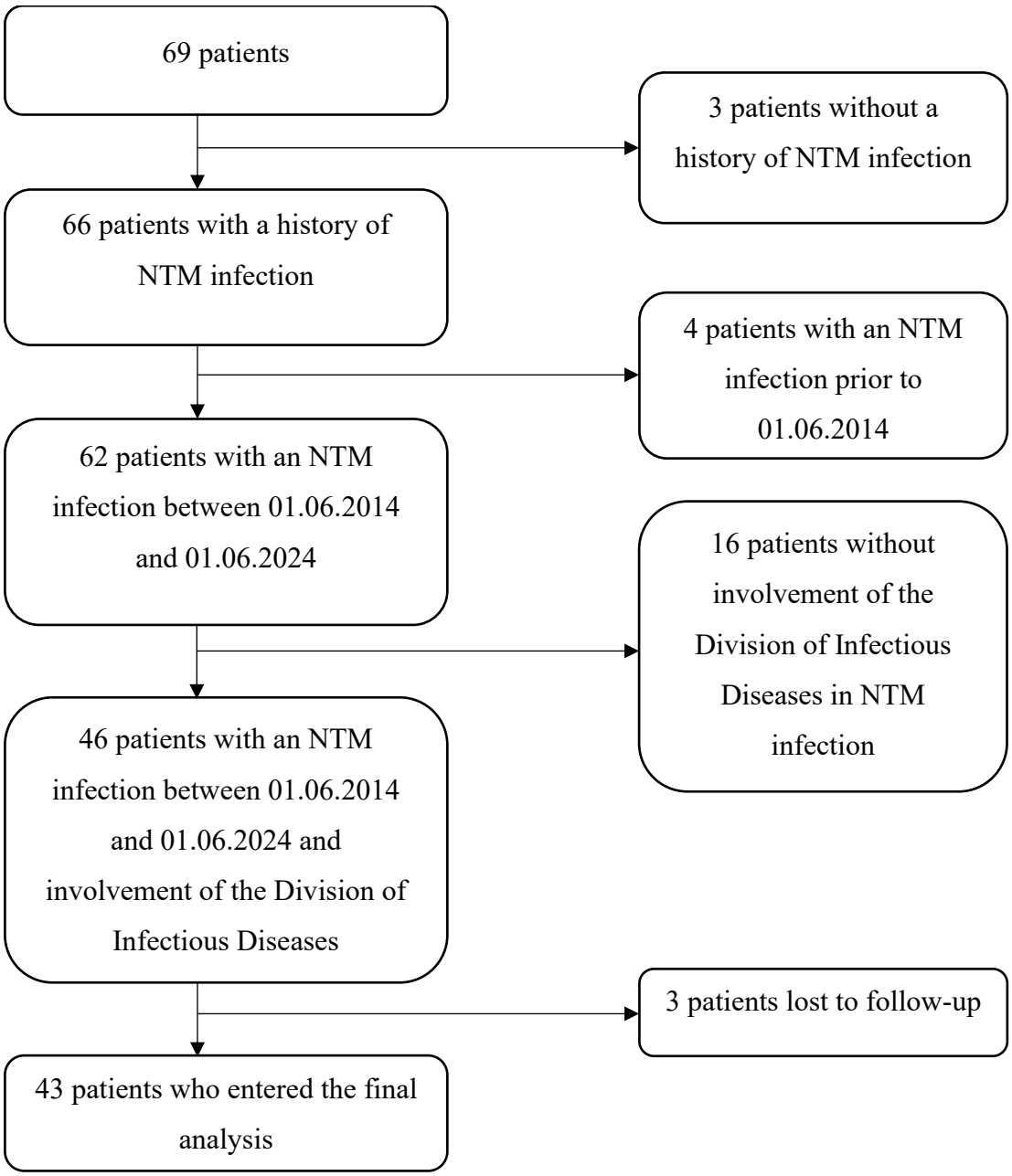
2.5 Statistics

The collected data were analysed by using the software IBM SPSS Statistics Version 29. Metric data was described as mean value $\pm$ standard deviation and range. For description of nominal data, absolute and relative frequencies were used. Depending on the assumptions, either the Fisher's Exact Test or the Chi-Square Test was used to determine correlations. To compare treatment duration between pulmonary and extrapulmonary infections, an independent t-test was performed. Statistical significance was considered at a p-value smaller than 0.05. Recurrences and reinfections were not included in the analysis of the initial infections presented in the text and tables but are discussed in the outcome section.

3 Results

A list of the potentially eligible patients was retrieved from the hospital information system (openMEDOCS) and included 69 patients. Forty-three of them were included in the final study cohort (figure 1). Data collection was completed on 27.01.2025.

Figure 1: Flow chart of the study cohort



3.1 Baseline characteristics

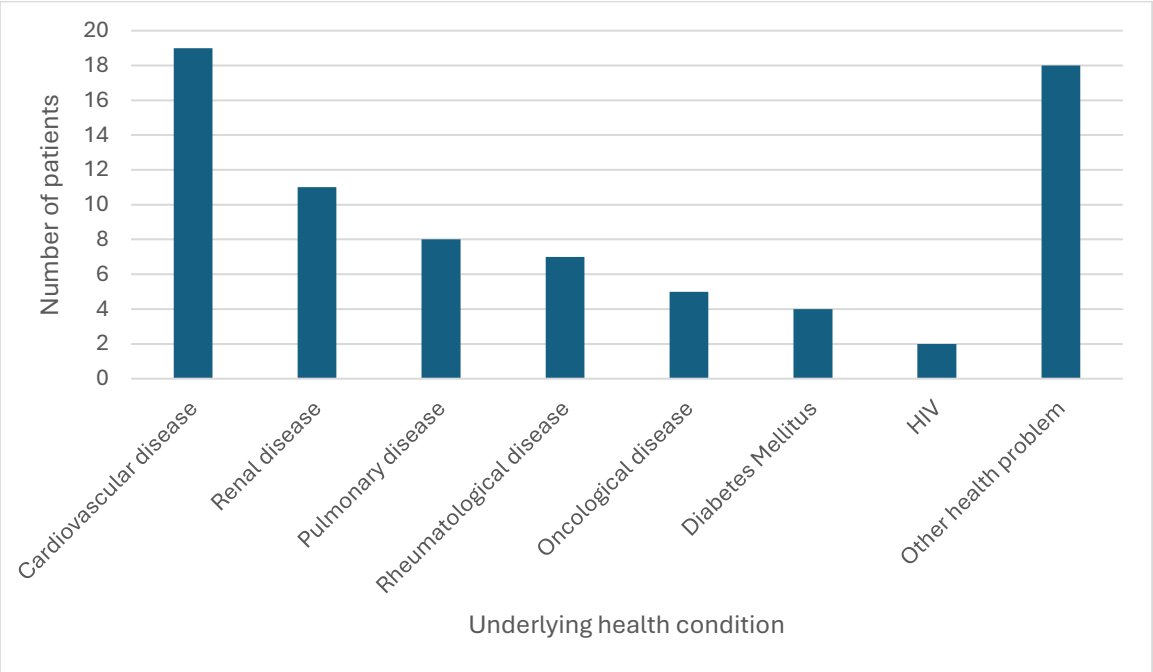
Out of the 43 patients included in this study, 26 (60.5%) were male and 17 (39.5%) were female. The mean age was 56.1 years (±17.9), with the youngest patient being 5 years old and the oldest being 80 years old. A total of 22 patients were either smoking at the time of NTM diagnosis or had smoked previously. Further demographic data is displayed in table 4.

Table 4: Demographic data of the study cohort (N=43)

Category	Mean value and standard deviation	Range
Age in years	56.1 ± 17.9	5-80
BMI	24.0 ± 5.1	13.5-35.7
Pack Years (N=22*)	25.6 ± 17.1	5-60
	Frequency	
Sex	Male: 26 (60.5%) Female: 17 (39.5%)	
Current smoker (N=37*)	13 (35.1%)	
Former smoker (N=37*)	9 (24.3%)	
Alcohol abuse (N=23*)	1 (4,3%)	
Nationality	Austria: 38 (88.4%) France: 1 (2.3%) Ghana: 1 (2.3%) Greece: 1 (2.3%) Romania: 1 (2.3%) South Africa; 1 (2.3%)	
Travel history in the last year (N=5*)	Croatia: 1 (20%) Greece: 1 (20%) Romania: 1 (20%) Spain: 1 (20%) Tropics: 1 (20%)	
*N= Number of patients where information regarding smoking (and pack years), alcohol abuse or travel history was available.		

A total of 33 patients (76.7%) had an underlying health condition. Cardiovascular diseases were most common, followed by renal diseases. Diabetes Mellitus and HIV have only been noted in a small number of patients. The exact numbers of underlying diseases are shown in figure 2.

Figure 2: Underlying health conditions



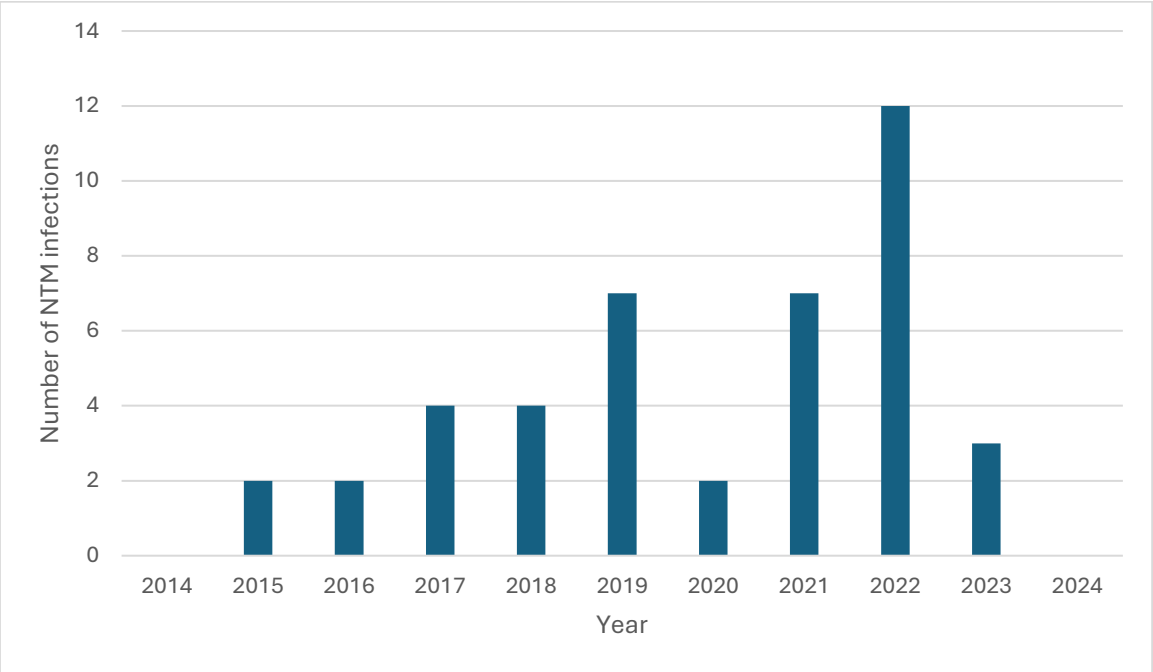
A total of seven patients (16.3%) were immunosuppressed with systemic corticosteroids and/or other immunosuppressants at the time of NTM diagnosis. Four patients (9.3%) were on systemic prednisolone as a long-term medication. The daily dose was 5 mg in three of the patients and 12.5 mg in one patient. Two of the mentioned patients had additional immunosuppressant drugs. Three patients had no systemic corticosteroid as a long-term medication but took other immunosuppressants.

3.2 Annual data on NTM infections

In this study, no patients were diagnosed with an NTM infection in 2014 or 2024. The year with the most registered NTM infections was 2022, with a total of 12 cases. In the other years between 2015 and 2023, the number of annual infections that were diagnosed varied

between two and seven. The annual infections for each year in the study period are shown in figure 3.

Figure 3: Annual NTM infections



3.3 Isolated species and site of infection

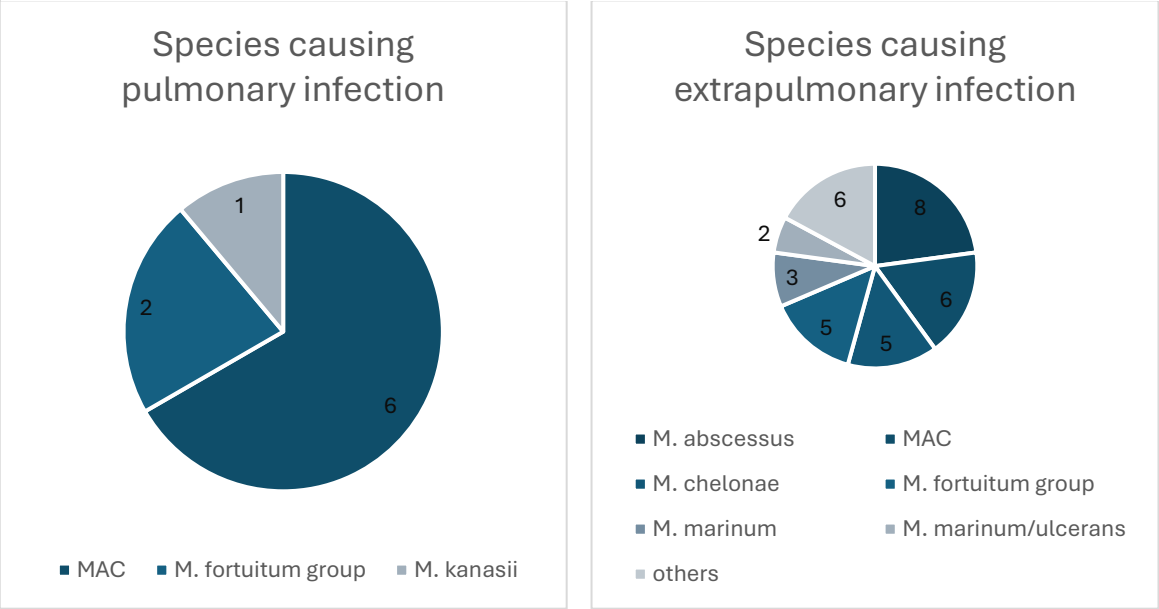
In the 43 patients, a total of 44 NTM were isolated, because one patient had two different species at the same time. MAC was most common and was found in 12 cases (27.3%). *M. abscessus* was found in eight cases (18.2%) and in seven cases (15.9%) a species attributed to the M. fortuitum group was detected. All isolated NTM are listed in table 5. MAC was associated with pulmonary infections (p=0.013), while no significant correlation between species and site of infection was found regarding the other NTM.

Table 5: List of isolated NTM (N=44)

NTM	Number of isolates	Percentage (%)
MAC	12	27.3%
M. abscessus	8	18.2%
M. fortuitum group	7	15.9%
M. chelonae	5	11.4%
M. marinum	3	6.8%
M. kanasii	2	4.5%
M. marinum/ulcerans	2	4.5%
M. chelonae/abscessus	1	2.3%
M. chelonae/immunogenum	1	2.3%
M. gordonae	1	2.3%
M. haemophilum	1	2.3%
M. mucogenicum	1	2.3%
M. fortuitum group: *M. alvei, M. boenickei, M. conceptionense, M. farcinogenes, M. houstonense, M. fortuitum, M. neworleansense, M. peregrinum, M. porcinum, M. senegalense, M. septicum, M. setense* (89)		

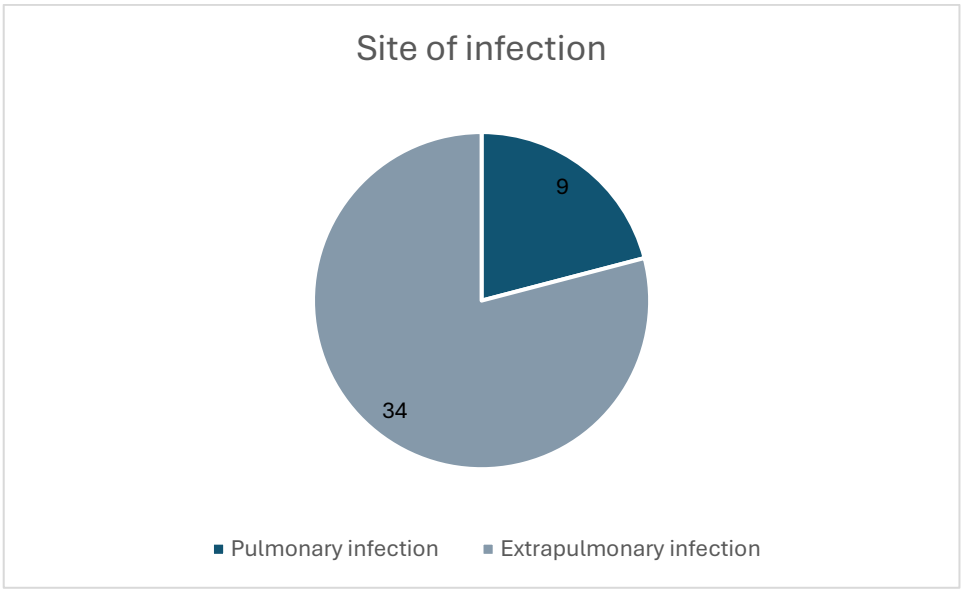
From pulmonary samples, three different NTM have been isolated. Most frequent NTM was MAC, with six cases (66.7%), followed by the M. fortuitum group (22.2%) and *M. kanasii* (11.1%). In extrapulmonary infection, a total of 35 NTM have been found. *M. abscessus* was most common with eight cases (22.9%), followed by MAC (17.1%), *M. chelonae* (14.3%) and the M. fortuitum group (14.3%). The different species isolated from pulmonary sites in comparison to the species isolated from extrapulmonary sites are displayed in figure 4.

Figure 4: NTM responsible for pulmonary vs. extrapulmonary infections



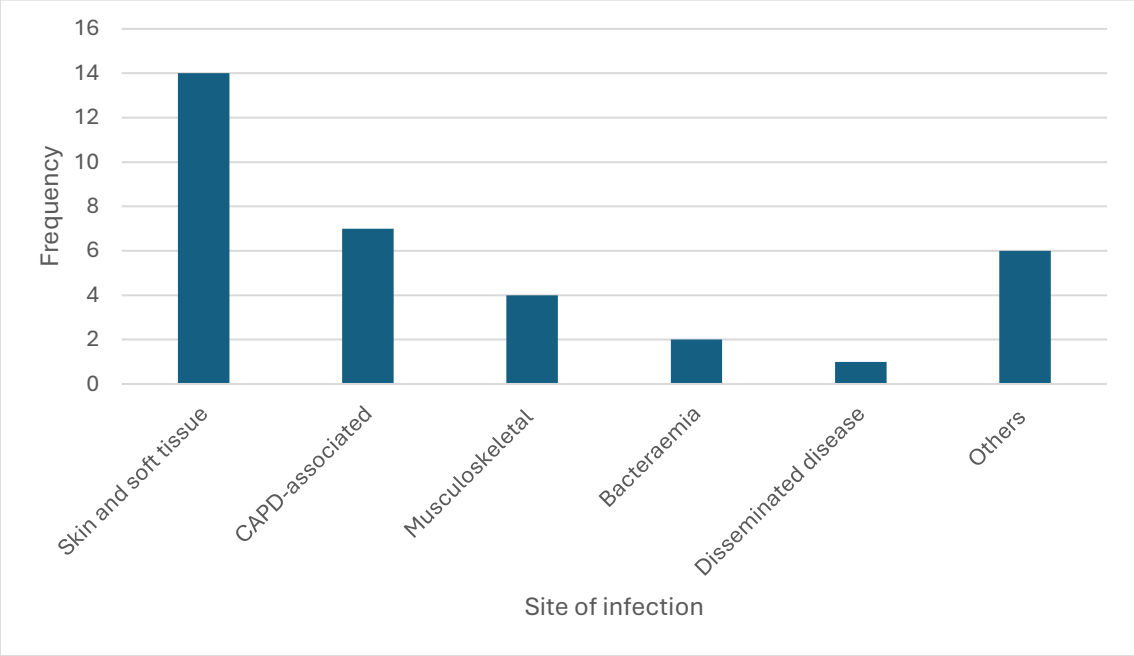
With 79.1%, most NTM infections in this study were localised extrapulmonary. Only nine patients (20.9%) had a pulmonary infection. It is important to note, that only seven of them had pulmonary disease, according to the ATS/ERS/ESCMID/IDSA criteria (table 2). The distribution of pulmonary and extrapulmonary infections is displayed in figure 5.

Figure 5: Pulmonary versus extrapulmonary infections



Amongst the extrapulmonary infections, skin and soft tissue infections were found in 14 patients (41.2%). Seven patients (20.6%) had CAPD-associated infections. Out of these seven infections, CAPD peritonitis occurred in two patients. The frequencies of extrapulmonary infections by site are displayed in figure 6. Infections of the aortic valve, kidney, nasal mucosa, nasal septum and pelvis are summarized as “others”.

Figure 6: Localisation of extrapulmonary infections



3.4 Inflammatory markers and genetic mutations

Mean leukocytes at the time of diagnosis ± 14 days were 8.64 G/L (±5.14) and ranged from 0.87 G/L to 26.51 G/L. CRP ranged from 0.05 mg/dL to 27.49 mg/dL, with the mean being 3.94 mg/dL. Genetic analysis was only performed in one patient and showed an Interferon gamma receptor polymorphism. This patient was tested, because he already had his third NTM infection.

3.5 Treatment

Out of the 43 patients, 41 (95.4%) received medication for their NTM infection. One of the 41 individuals was a patient with multicentric Morbus Castleman. In this case, NTM treatment was only preventive, as the treatment of the underlying condition with Siltuximab

was necessary. One patient (2.3%) was treated surgically only and another patient did not receive any therapy due to questionable infection. 26 patients (60.5%) were hospitalized at least once for the treatment of NTM infection. The median duration of treatment was 179 days (range 21 to 1358 days). Mean treatment duration did not differ significantly between pulmonary and extrapulmonary infections, but was shorter in extrapulmonary infections.
In the 41 patients that received medical treatment, macrolides were the most frequently used antiinfective group and were prescribed to 37 individuals (90.2%). Fluoroquinolones were used in 19 cases (46,3%), antituberculosis drugs in 18 cases (43.9%), aminoglycosides in 15 cases (36.6%), tetracyclines in 10 cases (24.4%) and oxazolidinones in seven cases (17%). 10 patients (24.4%) received other antibiotics. The different antibiotics used for each patient and the treatment duration can be seen in table 6.

Table 6: NTM treatment for each patient

Patient	Macrolides	Fluoroquinolones	Aminoglycosides	Tetracyclines	Oxazolidinones	Antituberculosis drugs	Other antibiotics	Treatment duration (days)
1	AZM	-	AMK	-	-	-	-	218
2	CLR	-	-	-	TDZ	-	-	193
3	CLR	MXF	-	-	-	EMB RFB	-	179
4*	-	-	-	-	-	-	-	-
5**	AZM	-	-	-	-	-	-	136
6	AZM	-	-	-	-	EMB RIF	-	195
7	CLR	-	-	DXY	LZD	-	-	127
8	AZM CLR	-	AMK	TGC	-	-	CFZ MER	217

9	CLR	-	-	-	-	EMB INH RIF	-	518
10	-	LVX	-	-	-	-	CFM MER TMP	259
11***	-	-	-	-	-	-	-	-
12	AZM	-	AMK	-	-	AMB RFB	-	337
13	AZM	-	-	-	LZD	-	IPM	116
14	AZM	-	-	-	-	EMB RIF	-	176
15	CLR	CIP	-	-	-	EMB INH RIF	-	311
16	CLR	-	-	-	-	EMB	-	96
17	AZM	-	-	-	-	EMB RIF	-	200
18	AZM CLR	MXF	-	-	-	EMB RIF	-	1358
19	AZM	MXF	-	DXY	-	-	-	171
20	AZM CLR	MXF	TOB	DXY	LZD	-	CFZ IPM	335
21	AZM	CIP	-	-	-	RIF	-	365
22	CLR	LVX	AMK	-	LZD	-	IPM	21
23	CLR	-	-	-	-	EMB	-	47
24	CLR	-	-	-	-	EMB	-	63
25	AZM	-	AMK	-	-	-	CFZ IPM	239

26	AZM CLR	-	AMK	-	-	EMB RIF RFB	-	Still on treatment
27	AZM CLR	CIP	AMK	-	-	-	-	380
28	AZM CLR	CIP MXF	AMK	DXY MINO	-	-	IPM	241
29	AZM CLR	-	AMK	DXY	-	-	CFZ	-
30	AZM CLR	CIP	-	-	-	-	-	160
31	-	CIP	-	-	-	-	-	41
32	CLR	-	-	DXY	-	RIF	-	263
33	-	LVX	-	-	LZD	-	-	33
34	AZM	-	AMK	-	-	EMB RIF	-	433
35	-	CIP	-	MINO	-	-	-	98
36	CLR	MXF	AMK	MINO TGC	-	-	IPM MER	42
37	AZM CLR RXT	MXF	-	-	-	-	-	89
38	CLR	MXF	-	-	-	-	-	60
39	CLR	-	-	-	-	RIF	-	-
40	AZM	MXF	AMK	-	-	EMB RIF	CFZ	795
41	AZM	-	AMK	-	LZD	-	-	69

42	AZM	MXF	AMK	-	-	EMB RFB	-	Still on treatment
43	AZM CLR	-	-	DXY	-	-	-	142

Abbreviations: AMK=Amikacin, AZM=Azithromycin, CIP= Ciprofloxacin, CFM=Cefepim, CFX= Clofazimin CLR=Clarithromycin, DXY=Doxycyclin, EMB=Ethambutol, INH=Isoniazid, IPM=Imipenem, LVX=Levofloxacin, LZD=Linezolid, MER=Meropenem, MINO=Minocyclin, MXF=Moxifloxacin, RIF=Rifampicin, RFB=Rifabutin, RXT=Roxithromycin, TDZ=Tedizolid, TGC=Tigecyclin, TOB=Tobramycin

* No therapy due to questionable infection

**Preventive treatment because of Siltuximab therapy

***Surgical removal of nasal polyp

16 out of the 41 patients who received medication (39%) reported drug associated side effects. Gastrointestinal side effects were reported by six patients (14.6%) and skin reactions occurred in five patients (12.2%). The frequencies of all side effects are displayed in table 7.

Table 7: Frequency of different side effects in patients who received medication (N=41)

Side effect	Absolute frequency	Percentage (%)
Gastrointestinal side effects	6	14.6
Skin reactions	5	12.2
Tinnitus/Hearing loss	3	7.3
Thrombocytopenia/Pancytopenia	2	4.9
Vision impairment	1	2.4
Others	5	12.2

All cases of tinnitus/hearing loss occurred during treatment with amikacin (p=0.043) and the two cases of thrombocytopenia/pancytopenia happened during treatment with linezolid (p=0.018). For the other side effects, no significant correlation between antibiotic and side effect was found.

3.6 Outcome

Out of the 41 patients that were treated for NTM infection, clinical cure was achieved in 27 patients (65.9%) and five patients (12.2%) had microbiological cure. Only one treatment failure was noted, but it is uncertain whether this patient took medication as prescribed. Recurrence happened in three of the 41 treated patients (7.3%), while reinfection only occurred in one patient who also had recurrence. NTM found in case of recurrence were MAC, the M. fortuitum group and *M. abscessus*. *M. chelonae* was the causative species for reinfection. Two recurrences happened after CAPD-associated infection and were localised at the same site. Recurrence/reinfection was localised in skin and soft tissue in the patient who had disseminated disease previously. Frequencies of different outcomes are listed in table 8. The different outcomes did not show a significant correlation with different species or sites of infection.

Table 8: Outcome of patients treated for NTM infection (N=41)

Outcome	Absolute frequency	Percentage (%)
Clinical cure	27	65.9
Microbiological cure	5	12.2
Loss to follow-up	3	7.3
Recurrence	3	7.3
Treatment discontinuation	3	7.3
Still on treatment	2	4.9
Reinfection	1	2.4
Treatment failure	1	2.4

4 Discussion

The aim of this study was to investigate NTM infections diagnosed and/or treated at the Division of Infectious Diseases at the LKH-University Hospital of Graz (Medical University of Graz) over the last 10 years. Results show that most NTM infections were localised in extrapulmonary sites, with the most frequent species being MAC, followed by *M. abscessus*, the M. fortuitum group and *M. chelonae*. 60.5% of patients were male and the mean age at the time of sampling was 56.1 (±17.9) years. In 78.1% of the patients treated for NTM infection either clinical or microbiological cure was achieved, while recurrence, reinfection and treatment failure were rare.

While the age at time of NTM diagnosis found in this study is supported by other studies, the gender distribution found by others is more balanced.(90–92) The fact, that 60.5% of patients in this study were male, might be due to the small patient cohort. Data regarding the smoking history of individuals with NTM infection are mostly found in studies regarding pulmonary NTM infections. In this study, only 22.2% of patients with pulmonary infections were never smokers. That is much less than the numbers reported by studies from Helsinki (42%) and Japan (64.1%).(93,94) Lim et al. analysed patients with pulmonary and extrapulmonary NTM infections in Singapore and found, that 61% were never smokers, which is distinctly more than the 40.5% of never smokers in our study.(95)

The increase in NTM infections, that is reported worldwide, could not be confirmed in this study. (8,11,17,22) However, it is conspicuous, that the annual detected NTM infections decreased in 2020 and increased drastically in the two following years, reaching the peak in 2022 with 12 detected infections. A possible explanation for that is, that due to coronavirus disease 2019 (COVID19) access to hospitals was difficult in 2020. When the numbers of COVID19 infections decreased, the NTM infections that had not been detected before, might have been detected with a delay. It is important to note, that in Slovakia no significant decrease of detected NTM infections was noted in 2020, which weakens this hypothesis.(91)

In this study, only nine cases (20.9%) of NTM infections were localised in the lung. Seven of which (77.2%) fulfilled the ATS/ERS/ESCMID/IDSA criteria for pulmonary disease (table 2) and were treated. One patient only had one positive sputum culture, making

pulmonary disease unlikely. Another patient was diagnosed with NTM sampled by BAL, but had Castleman disease, which made it impossible to tell, if radiologic features were caused by the underlying health condition or NTM infection. The patient received preventive treatment due to Siltuximab therapy. The rate of pulmonary infections in this study is much lower than the numbers reported by other studies.(2,22,25,96) Cassidy et al. found, that 77.2% of NTM cases detected in Oregon between 2005 and 2006 were pulmonary cases. In their study, only 51% of pulmonary cases fulfilled the ATS criteria for pulmonary disease, which is much less than in our study. The ATS criteria for pulmonary disease at that time were only slightly different.(2) Jarchow-MacDonald et al. reviewed NTM isolates in Scotland from 20011 to 2019 and found, that 81.1% of all infections were pulmonary infections.(96) Hermansen et al. investigated NTM infections in Denmark between 1991 and 2015 and state, that 69.1% of all positive samples collected were from pulmonary sites.(25) From all NTM isolated in England, Northern Ireland and Wales, even 90.9% of isolates were from pulmonary sites.(22) The lower rate of pulmonary infections in this study might be explained by the fact that NTM pulmonary disease is often treated by the Division of Pulmonology at the LKH-University Hospital of Graz and in a nearby hospital, which is specialized in tuberculosis, without involvement of the Division of Infectious Diseases.
Regarding extrapulmonary infections, this study supports the findings of others, that skin and soft tissue infections are most common.(97,98) Conspicuous is the high number of CAPD-associated infections, which account for 20.5% of extrapulmonary infections in this study. A possible explanation for that is that the Division of Nephrology at the LKH-University Hospital of Graz is a large centre for ambulatory dialysis and therefor has a high number of CAPD patients.

As studies from other European studies have shown, MAC was also the most isolated NTM in Graz over the study period.(22,90,91,99) While *M. abscessus* was found in 17.8% in this study, it was not as frequently isolated in other studies. In Italy only 6.6% of isolates were *M. abscessus* and in Slovakia even less (3.1%).(91,99) The three different NTM isolated from pulmonary samples in this study are found frequently and the fact that MAC is the most popular NTM causing pulmonary disease has also been shown by others.(22,100)
Out of the six MAC pulmonary diseases that were treated, five patients (83.3%) received the recommended antibiotics. The other patient died prior to definite detection of MAC. It is

important to note, that two patients were only treated for six and ten months, which is shorter than recommended by the ATS/ERS/ESCMID/IDSA guidelines.(6) Especially in extrapulmonary disease, many patients were not treated for the recommended six months.(41) Some patients were only treated for a few weeks, but nevertheless, outcomes in most patients were good.

This study has several limitations. First, the patient cohort is relatively small, with only 43 patients included. In addition to that, the study analyses only NTM infections that were diagnosed and/or treated at the Division of Infectious Diseases at the LKH-University Hospital of Graz. The number of actual infections in Graz over the last years is higher, due to patients that never had contact to the Division of Infectious Diseases regarding their NTM infection. This might also be the reason for the uncharacteristic proportion of pulmonary and extrapulmonary infections. Furthermore, many patients only had one positive sample for NTM and contamination cannot completely be ruled out. Because of the retrospective nature, flawless documentation might not be ensured.

In conclusion, this study has shown that most cases of NTM infections diagnosed and/or treated at the Division of Infectious Diseases at the LKH-University Hospital of Graz (Medical University of Graz) over the past 10 years were extrapulmonary. With only 44 mycobacteria isolates, NTM infections remain rare and no clear increase in incidence was noted. Medical treatment was prescribed in almost all cases and led to successful outcomes. To get a better understanding of NTM infections in Graz, or Austria in general, further research is needed and should include data from all relevant microbiological laboratories in the region.

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