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CLINICAL AND IMMUNOLOGICAL STUDIES IN PATIENTS WITH ATYPICAL MYCOBACTERIAL ISOLATIONS

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ACADEMIC DISSERTATION

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To my family

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on four original publications, referred to in the text by their Roman numerals I–IV:

I Kotilainen H, Valtonen V, Tukiainen P, Poussa T, Eskola J, Järvinen A. Clinical symptoms and survival in non-smoking and smoking HIV-negative patients with non-tuberculous mycobacterial isolation. Scand J Infect Dis 2011;43:188–196.

II Kotilainen H, Valtonen V, Tukiainen P, Poussa T, Eskola J, Järvinen A. Prognostic value of American Thoracic Society criteria for non-tuberculous mycobacterial disease: A retrospective analysis of 120 cases with four years of follow-up. Scand J Infect Dis 2013;45:194–202.

III Kotilainen H, Valtonen V, Tukiainen P, Poussa T, Eskola J, Järvinen A. Clinical findings in relation to mortality in non-tuberculous mycobacteria infections patients with *Mycobacterium avium* complex have better survival than patients with other mycobacteria. Eur J Clin Microbiol Infect Dis 2015;34:1909–1918, DOI 10.1007/s10096-015-2432-8.

IV Kotilainen H, Lokki M-L, Paakkanen R, Seppänen M, Tukiainen P, Meri S, Poussa T, Eskola J, Valtonen V, Järvinen A. Complement C4 deficiency – a plausible risk factor for non-tuberculous mycobacteria (NTM) infection in apparently immunocompetent patients. PLoS ONE 2014;9(3): e91450.

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ABBREVIATIONS

AIDS	acquired immunodeficiency syndrome
ATS	American Thoracic Society
AFB	acid fast bacilli
BCG	bacillus Calmette-Guérin
BMI	body mass index, kg/m^2
C4	complement component 4
C4A	acidic isotype of complement component 4
C4B	basic isotype of complement component 4
CNV	copy number variation
CT	computer tomography
CT*ins*	CT insertion mutation
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
OR	odds ratio
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HR	hazard ratio
HRCT	high resolution computer tomography
IL-12	interleukin-12
IFN-γ	Interferon-γ
IRIS	immune reconstitution inflammatory syndrome
NTM	non-tuberculous mycobacteria, atypical mycobacteria
MAC	*Mycobacterium avium* complex
MHC	major histocompatibility complex
MOTT	mycobacteria other than tuberculosis
MTB	*Mycobacterium tuberculosis*
SNP	single nucleotide polymorphism
TB	tuberculosis, a disease caused by *Mycobacterium tuberculosis*
TNF-α	Tumor necrosis factor-α

ABSTRACT

Non-tuberculous mycobacteria (NTM) have become more common than *M. tuberculosis* (MTB) in many countries. NTM are ubiquitous and all people are exposed to them but only a few people may catch a NTM infection. Most infections due to NTM are pulmonary, thereafter cutaneous and disseminated infections and some NTM strains may cause lymphadenitis. The most common NTM strain in clinical samples is *Mycobacterium avium* complex (MAC) which is responsible for more than half of clinical cases.

The reasons why some people will develop a clinical NTM disease are still poorly known. Chronic lung-disease patients and non-smoking healthy elderly females have been described as the main risk groups. NTM infections are common opportunistic infections in acquired immunodeficiency syndrome (AIDS) patients. Rare genetic defects with disseminated NTM infection have also been described in families. Immunological defects behind pulmonary NTM infections have not been found except a local defect in nitric oxide production and ciliary beat of airway epithelium.

In this doctoral thesis, the underlying factors and clinical picture of NTM infections in Finland have been studied. The clinical picture and smoking as a risk factor for NTM infection have been investigated in Study I. The American Thoracic Society (ATS) has published criteria to discern patients with a clinical disease from those of airway colonization with NTM. The prognostic value of these criteria has been studied (II). The clinical picture and prognosis of patients infected with MAC has been compared to patients with other NTM infections (III). Genetic susceptibility to pulmonary tuberculosis has been linked to major histocompatibility complex (MHC) class I, II and III regions on chromosome 6p21.3. The complement system has a host defense role in innate immunity, thus deficiencies of complement components *C4A* or *C4B* that are encoded by major histocompatibility complex (MHC) were studied in NTM and tuberculosis patients (IV).

Materials and methods. Altogether 120 adult non-HIV patients with at least one NTM isolation during 1990–1998 were included in Studies I and II. Their symptoms, clinical findings, comorbidities, laboratory findings, medical therapy, and survival were retrieved from medical records comprising at least four years to 8th June 2006. The patients were classified as smokers or never smokers and categorized according to fulfillment of ATS 2007 criteria. In study III, 167 patients with at least one positive NTM isolation including patients from Studies I–II and IV were included and data was retrieved as described above. Study IV consisted of 50 adult NTM patients and 31 patients with MTB infection who were admitted

to hospital between August 2004 and December 2009. Their clinical picture was retrieved and they gave blood samples for analysis of *C4A* and *C4B* genotype and phenotype frequencies of the C4 allotypes, serum immunoglobulin, and complement levels. Controls were comprised of 149 healthy, unselected Finnish people.

Results. Overall 42% of patients had never smoked. In the group of non-smokers, 72 % were female, while in the group of smokers, only 30% of smokers were female (I). MAC comprised 72% of isolates among non-smokers and 41% among smokers. No potentially fatal underlying diseases were found in 82% of non-smokers but only in 59% of smokers. Smokers had higher risk of mortality than non-smokers but no difference was observed after adjusting for underlying diseases. Symptoms had started within a year of positive NTM isolation in 48% of patients suggesting rapid development of symptoms. Only half of patients with a NTM isolation fulfilled the 2007 ATS criteria. ATS positive cases were, more often, female and had less frequent fatal underlying diseases as compared to ATS-negative cases. No significant difference was seen in median survival time or symptoms between ATS-positive and -negative cases except in fatigue which was more common in ATS-positive patients. ATS criteria fulfillment was a weak prognostic marker. MAC was isolated in 59% of cases and MAC patients were more often female, had more frequent bronchiectasis, and presented fewer fatal underlying diseases than patients with other NTM. There was no difference in ATS 2007 criteria fulfillment between MAC and other NTM patients. The other NTM patients (54%) had suffered from symptoms less than a year as compared to MAC patients (34%). MAC patients had significantly lower risk of death and longer survival time than other NTM patients. Finnish NTM patients (72%) had significantly more frequent *C4* deficiencies (*C4A* or *C4B*) as compared to unselected healthy control subjects (56%) and MTB (35%). Especially, *C4* deficiencies were common in female NTM patients (81%) as compared to female controls (55%).

Conclusion. Smokers and non-smokers had different risk factors for NTM infection. ATS 2007 criteria had a weak prognostic value in finding patients with risk of fatal outcome. Patients with MAC had a longer survival than patients with other NTM. About half of the patients had suffered from symptoms for less than a year, suggesting a more rapid disease progression than previously emphasized. Complement *C4* deficiency may be a risk factor for NTM infection, especially in elderly female patients.

1 INTRODUCTION

The genus mycobacterium is assumed to have originated over 150 million years ago. *Mycobacterium tuberculosis* (MTB) is the best known representative of this genus. Tuberculosis (TB) was documented in Egypt more than 5000 years ago. Typical skeletal abnormalities of tuberculosis, including characteristic Pott's deformities in bones, have been found in Egyptian mummies (Daniel 2006).

The disease caused by *M. tuberculosis* was well known in classical Greece, where it was called "phthisis". Hippocrates clearly recognized tuberculosis and understood its clinical presentation (Daniel 2006). TB reached epidemic proportions in Europe and North America during the 18th and 19th centuries (Daniel 2006).

Tuberculosis also played a major role in the development of modern microbiology. In 1882, Robert Koch made his famous presentation on the etiology of tuberculosis to the Berlin Physiological Society. Koch demonstrated the tubercle bacillus which he had identified and posted at the same time as his famous postulates. Koch`s four postulates established a causal relationship between a microorganism and a disease and also provided a basis for describing microbes as pathogens, which led to the development of modern infectious and communicable diseases etiology (Fredriks and Relman 1996).

Even today, tuberculosis (TB) remains one of the world's deadliest communicable diseases. In 2013, the World Health Organization (WHO) estimated that 9.0 million people developed TB and 1.5 million died from the disease (WHO 2014).

Late in the 19th century, it was recognized that a micro-organism different from *Mycobacterium tuberculosis* (MTB) caused "tuberculosis" in chickens (Thorel et al. 1997). This micro-organism was later shown to be Mycobacterium *avium*. It was first thought that it would not cause disease in humans but during the 1950s, *M. avium* was demonstrated to be able to act as a human pathogen (Wolinsky 1979). Since the 1950s new mycobacteria have been characterized with increasing speed (Wolinsky 1979, Brown-Elliot et al. 2010).

The term "atypical mycobacteria" originated from the earlier belief that they were unusual compared to *M. tuberculosis* (Falkinham 1996). As understanding of mycobacteria grew, those less pathogenic "mycobacteria other than tuberculosis (MOTT)" were found to be ubiquitous in the environment; in natural waters, drinking waters, and soils (Falkinham 1996). Thus, a new term "nontuberculous mycobacteria" was created and it included those mycobacterial species that were not members of the *Mycobacterium tuberculosis* complex (*M. tuberculosis, M. africae, M. bovis*) or *M. leprae*. When it became well-known that *M. tuberculosis* could be transmitted through aerosols from person to person, infection of NTM proved to

be acquired from environment by the inhaled aerosolized droplets containing NTM or exposure to them through to skin abrasions (Falkinham 1996). Pulmonary NTM infections were first related to pre-existing pulmonary diseases or heavy occupational exposure to them as was seen in gold miners (Maliwan et al. 2005). However, more than 35 years ago they were found to cause disease in previously healthy persons, often females, as well (Prince et al. 1989, Kubo et al.1998, Huang et al. 1999).

The clinical importance and interest in NTM exploded when they were observed as one of the most important opportunistic infections in patients with Acquired Immune Deficiency Syndrome (AIDS) (Lowell et al.1986, Horsburgh et al. 2001). NTM infections associated to human immunodeficiency virus (HIV) profoundly revealed the pathogenic potential of nontuberculous mycobacteria (Benson and Ellner 1993). Simultaneously, however, the focus of NTM disease was changed to severely immunosuppressed patients. In HIV patients, NTM and mainly *M. avium* caused a disseminated disease first when the cell-mediated immune response was severely affected (Benson and Ellner 1993). The mechanisms of disseminated NTM infection were revealed but the interest in NTM infections in immunocompetent people was halted for almost 30 years. In Finland, NTM infections have been less studied, yet the number of publications on NTM infections has increased dramatically over the last years in Scandinavia, Europe and Asia.

The incidence of TB is slowly declining each year and it is estimated that 37 million lives were saved between 2000 and 2013 through effective diagnosis and treatment (WHO 2014). However, over 120 new species of mycobacteria have been discovered and advances have been made in diagnosis and treatment (Falkinham 2010). Further, the incidence of NTM isolations has been increasing in many countries (Cassidy et al. 2009, Andréjak et al. 2010, Marras et al. 2013). In Finland, the annual incidence of all NTM isolations has increased between 1995–2014 from 6.45/ 100 000 person-years to 11.98/ 100 000 person-years (National Institute for Health and Welfare, National Infectious Disease Register in Finland 2015). Concurrently, the incidence of tuberculosis in Finland has decreased from 12.93/ 100 000 person-years in 1995 to 4.42/ 100 000 person-years in 2014 (National Institute for Health and Welfare, National Infectious Disease Register in Finland 2015).

2 REVIEW OF THE LITERATURE

2.1 NTM MYCOBACTERIA

2.1.1 NON-TUBERCULOUS MYCOBACTERIA (NTM) SPECIES

Mycobacterium is the only genus in the family *Mycobacteriaceae*, which consists of over 120 species (Tortoli 2006). The more pathogenic group entails *Mycobacterium tuberculosis* complex, which consists of eight subgroups *(M. tuberculosis, M. bovis, M. africanum, M. microti, M. canettii, M. caprae, M. pinnipedii, M. mungi)* and *M. leprae* which was found in mammals (Maartens and Wilkinson 2007, van Ingen et al. 2012). However, various mycobacteria have later been found in environmental sources, most abundantly in aqueous spaces (Falkinham 1996). Most of these environmental NTM species have been discovered since 1950 (Wolinsky 1979).

Only 10 NTM species were known in 1948 (Griffith et al. 2002). The speed of detection of new species seems to be increasing, because 42 new NTM species have been detected from clinical samples since 1990 (Tortoli 2003). Thereafter, only 28 NTM species have been identified during the years 2003–2006 (Tortoli 2006). Out of 120 presently characterized NTM species, approximately 60% are probably connected to disease in humans (Tortoli 2006).

2.1.2 CHARACTERISTICS OF MYCOBACTERIA

Mycobacteria are aerobic, straight, germless rods (Brennan and Nikaido 1995), which possess a cell wall with a high lipid content; up to 60–70% of cell wall weight. The cell wall is held together by three layers of macromolecules: a mycolic acid containing layer, a polysaccharide layer with arabinogalactan, and an inner peptidoglycan layer in connection with the cell membrane (Brennan and Nikaido 1995, Daffe et al. 1998). High lipid content makes the cell wall highly hydrophobic and protects mycobacteria against phagocytosis and even against physical stress like acid, alcohol, heavy metals and antimycobacterial medication (Falkinham 2010). The cell wall impermeability constitutes one of the main characteristics of mycobacteria; they are acid-fast and capable of intracellular living (Primm et al. 2004). These common characteristics make NTM and *Mycobacterium tuberculosis* indistinguishable in microscopic appearance where they both are characterized as gram-positive rods and they also are indistinguishable in acid-fast (Ziehl-Neelsen) stain (Woods 2002).

Several physiologic characteristics contribute NTM to overcome in nature. Mycobacteria are considered obligate aerobes but numbers of microaerophilic mycobacteria have been isolated from low-oxygen habitats (Falkinham 2010). NTM grow in acidic environments with pH values 5.0–6.5 and there is only little growth in alkaline environments, where pH is above 7.5. Most NTM are able to grow in temperatures ranging from 10°C to 45°C (Falkinham 2003). In nature NTM grow in fresh, brackish, and salty water (Falkinham 2003). Most NTM species are thermoresistant and some species like *M. avium, M. intracellulare, M. scrofulaceum,* and *M. xenopi* have considerably higher resistance in hot water heaters than *Legionella pneumophila* (Falkinham 2010). The most heat resistant species, *M. xenopi*, has been associated with epidemics through hot water distribution systems (Brown-Elliot 2002, Falkinham 2010). In contrast, low temperatures are optimal to NTM species that cause skin infections like *M. marinum* (optimal growth at 30°C) and *M. haemophilium* (32 °C) (Falkinham 2010).

The growth of NTM in environmental reservoirs even low in organic matter is a result of metabolic characters of NTM. They are able to use carbon and nitrogen for nutrition (Falkinham 2010). Further, mycobacteria are able to degrade hydrocarbon pollutants even in clean water sources, which are used for drinking water (Primm et al. 2004). Moreover, the hydrophobicity and impermeability of cell membranes protect NTM in the environment. Conversely, the slow growth rate and delayed metabolism represent disadvantages (Primm et al. 2004). Biofilm formation supports growth and persistence in nature but increases the virulence of NTM in foreign bodies like vascular catheters of patients (Falkinham 2010).

2.1.3 NTM SPECIES AND THEIR CLASSIFICATION

Since the 1950s, NTM have been categorized into four types based on their growth rate and pigmentation (Timpe and Runyon 1954) (Table 2.1.3). According to this classification, types I, II, III grow slowly in culture over 7 days whereas type IV grows in less than 7 days. Further, these types were classified according to their colony pigmentation (Table 2.1.1).

Table 2.1.3. NTM classification according to Timpe and Runyon based on NTM growth and colony pigment formation. Modified according to: Yeager and Farah 2006, Wallace Jr 1994

Group	Morphology	Examples
I Photochromogens	Producing pigment on exposure to light	*M. kansasii, M. marinum*
II Scotochromogens	Producing pigment in the dark	*M. scrofulaceum, M. gordonae*
III Nonchromogens	Producing no pigment	*M. avium* complex, *M. malmoense, M. nonchromogenicum*
IV Rapid growers	Grows in less than 7 days and any of the above pigment types	*M. abscessus, M. fortuitum, M. chelonae*

Due to the development of genetic techniques, several new species of both slowly and rapidly growing NTM have been found during 1990–2000. Many of these NTM species were found from clinical human samples but were not found in the environment (Brown-Elliot 2002, Tortoli 2003).

2.2 EPIDEMIOLOGY

2.2.1 NTM ARE UBIQUITOUS IN THE ENVIRONMENT

Reservoirs of NTM are natural and municipal water sources, soil, food, protozoans, and other animals (Falkinham 2002, Primm et al. 2004). NTM have also been recovered from potting soils and even cigarettes (Eaton et al. 1995, Falkinham 2002). Aqueous environments are the main source of NTM and they have been isolated from lakes and ponds and especially from acid brown-water swamps in USA and peat land in Finland (Falkinham 2002). In Finland during 1990–1993, mycobacterial species were measured from brook waters and collected from 53 drainages located in a linear belt crossing Finland at 63° (Iivanainen et al. 1993). The majority of environmental isolates represented unknown mycobacterial species, but 15% of the isolated species were also common in human infections like *M. avium, M. avium complex, M. scrofulaceum, M. branderi, M. terrae, M. gordonae, M. xenopi* (Iivanainen et al. 1993, Torkko et al. 2003). MAC, *M. kansasii, M. malmoense, M. xenopi*, and RGM have been found from drinking water, and MAC has been discovered also in public bath water, hospital water, and water supplies of hemodialysis centers (Falkinham 2002). No NTM has been found in ground water and bottled water, however (Falkinham 2010, Mello 2013). NTM may escape

water filtration and they are resistant to chlorine and biocides (Falkinham 2002). In hospitals, NTM resistant to disinfectants have been recovered in instruments and in contaminated bronchoscopes (Falkinham 2002). Similarly, in household water and plumbing, *M. avium* and *M.intracellulare* may be concentrated in biofilm (Falkinham 2010, Mello 2013).

NTM have rarely been found in food but they may be found, for example, in vegetables and eggs. However, raw milk samples have been reported to contain *M. kansasii, M. avium, M. intracelluare, and M. fortuitum* (Falkinham 2002). Environmental exposure to NTM may lead to disease pictures distinct from classical infections.

2.2.2 NTM TRANSMISSION INTO HUMAN AND ANIMALS

NTM are opportunistic pathogens of humans and animals, with variable virulence and geographical distribution (Falkinham 2002, Primm et al. 2004). In nature, MAC may live in symbiosis with amoebae, which may increase its virulence (Cirillo et al. 1997). Various NTM have been isolated from wild and domestic animals, e.g. macaques and mesenteric lymph nodes of pigs (Falkinham 2002). *M.avium*, *M.fortuitum*, and *M.genavense* have been recovered from birds (Falkinham 2002).

M. tuberculosis is a known airborn disease, where the aerosol transmission occurs from person to person. In contrast, infection of NTM is assumed to be acquired from the environment by inhaled aerosolized droplets, ingestion or exposure to skin abrasions (Falkinham 1996). In that way inhaled shower water droplets may create airborn exposure leading to respiratory NTM diseases (Wallace et al.1997). *M. avium* has been isolated from water and also from biofilm sedmiment of showerheads (Falkinham 2007), which may support NTM transmission via shower droplets. The transmission of NTM is not well defined, however, because both environmental exposure and the host defense may be the major factor (Dirac et al. 2012).

The evidence of human-to-human or animal-to-human transmission has not been discovered (Griffith et al. 2007). Even NTM is suspected to be acquired from the environment by airway exposure or by ingestion; however, the specific source of infection in individual cases cannot usually be identified (Wallace et al.1997, Criffith et al. 2007). In addition, regional differences also appear to exist in distribution of mycobacteria in household water systems according to a study in Japan (Ichijo et al. 2014).

Cervical lymphadenitis in children at age 1–5 years is caused by NTM, probably due to childrens habitual contact with natural water (Falkinham 2003) or ingestion of soil (Griffith et al. 2007). Ingestion may be an important infection route also in patients with AIDS related lymphadenitis or disseminated *M. avium*, which

are thought to arise through gastrointestinal colonization (Wallace et al.1997). *M. avium* in drinking water has been proved to cause infections in AIDS-patients and high incidence of *M. avium* infection in Finnish AIDS patients correlated with the concentration of *M. avium* in their household drinking water (von Reyn et al.1993).

Some mycobacteria cause mainly cutaneous infections through transmission via small cutaneous erosions. Fishing and aquaculture may lead to exposure to *M. marinum*. A potter may be exposed to MAC through skin abrasion. Leisure activities like gardening or peat-rich potting soils may lead exposure to NTM through the skin wounds (Falkinham 2002).

2.2.3 GEOGRAPHICAL DISTRIBUTION

Various NTM species are observed in geographically different locations, however MAC is the most frequently isolated species in pulmonary infections worldwide (Field et al. 2006).

In Australia, up to 91% of non-HIV pulmonary NTM diseases are reported to be caused by MAC (OBrien 2003). In USA, MAC was responsible for 80% of NTM cases in New York and Portland (Bodle et al. 2008, Cassidy et al. 2010). In France, among 262 HIV-negative patients with NTM lung disease, 80% was caused by MAC (Dailloux et al. 2006). In Asia, MAC caused over 43% and in Japan up to 81% of pulmonary lung diseases (Simons and van Ingen 2011).

In Europe, *M. xenopi* has been the second after MAC as a cause of lung disease in France, the Netherlands and Italy and in the south-east United Kingdom (Cook 2010). In Ontario, Canada, *M. xenopi* is common but it is rarely found in the USA (Varadi and Marras 2009, Marras et al. 2007).

In northern Europe, *M. malmoense* has been reported to be the second after pulmonary MAC in Sweden (Petrini et al. 2006), in Finland (Katila and Brander 1991), and in Scotland (Russel et al. 2014). Interestingly, *M. malmoense* was initially the fourth most common species after MAC in Denmark (Thomsen et al. 2002, Andréjak et al. 2010). *M. malmoense* has been found in the Netherlands, Italy (Tortoli 1997), and the UK (Henry et al. 2004), but it is rare in the USA (Butcholds et al. 1998, Cook 2010).

M. kansasii pulmonary diseases have been found as the second most common species after MAC in the Central USA (Bloch et al. 1998), South America (Mello et al. 2013), England, Wales, and France (Dailloux et al. 2006, Cook 2010). Moreover, among South African miners, *M. kansasii* has accounted for 68% of isolates in pulmonary infections (Corbett[a] et al. 1999). In Asia, RGM have been described to be equally clinically relevant as MAC species during 1971–2007 (Simons and van Ingen 2011). *M. abscessus* has caused 35%, *M.*

chelonae 31%, and *M. fortuitum* 3%, of pulmonary NTM infections in Asia (Simons and van Ingen 2011). However, in some regions in Asia, like in India, Taiwan, and South Korea, RGM have caused more than 30% of pulmonary NTM infections (Simons and van Ingen 2011).

Most recent laboratory data comes from NTM-Network European Trials Group (NET) that reported the prevalence of NTM isolations in the world in 2008. Altogether 30 countries from six continents participated in the study (Hoefsloot et al. 2013). In Europe and in Scandinavia, MAC represented 67%–33% of all NTM pulmonary isolations. Moreover, MAC constituted 50%–70% of all NTM respiratory isolations in the world. Other NTM strains in clinical findings were all clearly less common (Hoefsloot et al. 2013). Table 2.2.3.

Table 2.2.3. NTM geographical distribution. Modified according to Hoefsloot et al. 2013.

	All NTM pulmonary isolates % in Europe (Hoefsloot et al. 2013)					
	MAC	*M. kansasii*	*M. xenopi*	*M. malmoense*	RGM	*M.gordonae*
Finland	38	1	0	1	15	15
Sweden	67	4	0	4	12	1
Norway	55	5	2	5	15	15
Denmark	54	4	3	4	10	18
Germany	55	6	3	2	12	19
UK	22	11	10	1	44	10
Netherland	34	7	3	2	17	16
France	38	5	8	1	16	29
Italy	33	2	22	2	13	24
	All NTM respiratory isolates % in the world (Hoefsloot et al. 2013)					
N-America	52	1	12	rare	20	12
S-America	31	20	rare	rare	20	17
Europe	37	5	14	1	16	17
Asia	50	3	rare	rare	30	6
S-Africa	50	3	1	rare	7	5
Australia	70	4	rare	rare	15	2

2.2.4 INCIDENCE AND PREVALENCE OF NTM

The incidence of NTM in clinical samples is reported to have increased during the last decade (Cassidy et al. 2009, Marras 2013). However, comparison of incidence rates is complicated by the different ways to report them. In some countries, incidence is given only on ATS criteria positive cases, i.e. NTM disease whereas in other countries incidence of NTM isolations is reported. NTM is not a notifiable condition in most of the European countries and in the USA. Therefore, the reported incidence estimates of NTM are not comparable. In Finland, NTM is notifiable. Moreover, the epidemiological methods (i.e. standardization) are different between studies.

Indeed, the incidence estimates vary markedly from each other in recent studies: 0.73 cases of NTM pulmonary diseases per 100,000 person-years in France in 2002 (Dailloux et al. 2006). In Denmark in 1997–2008, the annual incidence rate of at least one NTM-positive specimen was 2.44 per 100,000 person-years and 1.36 of NTM colonization (Andréjak et al. 2010). Further, the annualized isolation rate in Ontario, Canada was 11.4 per 100,000 person-years in 1998 and increased even up to 22–25 per 100,000 person-years in 2008–2010 (Cassidy et al. 2009, Marras et al. 2010).

The incidence of all NTM isolates in Finland has been increased from 1995 to 2014, from 6.45/ 100,000 person-years up to 11.98/ 100,000 person-years (National Institute for Health and Welfare, National Infectious Disease Register in Finland 2015). In contrast, the incidence of tuberculosis has been decreased from 1995 to 2014 from 12.93/ 100,000 person-years to 4.42/ 100,000 person-years (National Institute for Health and Welfare, National Infectious Disease Register in Finland 2015).

2.3 RISK FACTORS FOR NTM INFECTIONS IN IMMUNOCOMPETENT PERSONS

2.3.1 AGE AND GENDER

Pulmonary NTM infections have been predominantly described in older individuals and the risk for disease seems to increase along with age (Mirsaeidi[b] et al. 2014). NTM disease may, however, affect at any age which was observed in the Danish population-based study where the age of patients with NTM isolation ranged from 15 to 96 years (Andréjak et al. 2010). However, age of patients with NTM diseases of the skin and soft tissue has been variable and these infections may affect at any age. Notably, patients with M. ulcerans are often young adults (Piersimoni 2012). Further immunocompetent patients with NTM lymphadenitis constitute children, for the most part, of day-care age (Griffith et al. 2007). Male gender and underlying pulmonary diseases were the first risk factors connected to NTM infection

(Piersimoni and Scarparo 2008, Varadi and Marras 2009). However, in the more previous literature the gender differences have been changed or a slight female predominance has been described: 59% of 634 Japanese patients with ATS positive pulmonary disease were female (Hyashi et al. 2012). In contrast, in the Danish nationwide survey on all NTM isolations a slight male predominance (55%) was seen (Andréjak et al. 2010). Pulmonary NTM diseases have been revealed to form two main groups. These groups associate to age and gender, which predispose to NTM infection. Notably, it has been revealed that NTM disease affects immunocompetent female and male differently for unknown reasons (Cassidy et al. 2009, Andréjak et al. 2010). Furthermore, there has been a different predominance of age and gender during last decades. At first, pulmonary NTM occurred predominantly in men until 1980 (Field et al. 2004). The men aged 60–80 years (Piersimoni and Scarparo 2008, Waller 2006), have been traditionally more frequent smokers, with destructive pulmonary and radiological findings. The treatment of outcome has been poor with relapsing infections (Piersimoni and Scarparo 2008).

In the second risk group, since 1990, pulmonary MAC diseases have been considered to demonstrate a 60% predominance for elderly women (Prince et al. 1989, Dailloux et al. 2006, Cassidy et al. 2009). Pulmonary MAC in elderly, non-smoker women with bronchiectasis typically ranged in age: 55–75 years (Piersimoni and Scarparo 2008, Field et al 2006).

The third group, both male and female, exposed by environmental or vocational risk factors, will recover after removal from source alone. However, this age group includes patients with hereditary cystic fibrosis and pulmonary MAC (Piersimoni and Scarparo 2008).

2.3.2 UNDERLYING PULMONARY DISEASES

Bronchiectasis has been suggested as one of the most important risk factors, but also as a consequence, of pulmonary NTM infection (Fuijta et al. 2003, Field and Cowie 2006). Especially women with bronchiectasis and with low BMI (body mass index, kg/m^2) have been found to be at high risk for NTM infection in both older and more recent studies (Prince et al. 1998, Chan and Iseman 2010, Mirsaeidi et al. 2013). Furthermore, elderly women with pulmonary MAC have been reported to have a higher incidence of bronchiectasis than other patients with pulmonary NTM, although they did not have underlying pulmonary diseases (Kim et al. 2008, Obayashi et al. 1999, Chan and Iseman 2010, Mirsaeidi et al. 2013). Pulmonary MAC has been thought to be a chronic, slowly-progressive disease and in the disease course MAC might first be a colonizing or indolent but thereafter it might gradually affect the lower pulmonary lobes. Persistent inflammation and mucus plugging

could contribute to bronchiectasis formation and both MAC and M. abscessus have been shown to cause bronchiectasis (Wickremasinghe et al. 2005). Chronic obstructive pulmonary disease (COPD) is associated with persistent inflammation and mucus plugging in the bronchi which could predispose to NTM infection. COPD and corticosteroid treatment were found to be strong risk factors for NTM disease due to MAC, M. kansasii, M. malmoense, and M. xenopi (Andréjak et al. 2013). Furthermore, COPD has been related to higher mortality in pulmonary NTM disease (Henry et al. 2004).

Many other pre-existing lung diseases have been linked to increased risk for pulmonary NTM infection. Silicosis and coal workers pneumoconiosis have been linked to M. kansasii infection (Field et al. 2006). Coal workers pneumoconiosis, silicosis, smoking, and prior tuberculosis contribute to NTM diseases by disrupting the normal mucosa and contribute NTM to adhere to damaged mucosa (Middleton 2004). Also, idiopathic pulmonary fibrosis has been associated with increased risk for NTM infection (Al-Anazi et al. 2014). However, it seems that the role of underlying pulmonary diseases might be decreasing as the proportion of women with pulmonary NTM but no previous pulmonary comorbidity has increased since the 1980s from 25% up to 70% in the last decade (Prince et al. 1989, Cassidy et al. 2008, Kim et al. 2008).

In addition, women with NTM infection have been observed to be, in general, taller and leaner and 50% of them had scoliosis, 11–27% pectus excavatum, and 9% mitral valve prolapse (Kim et al. 2008). Due to this morphotype, it was suggested that they might have an associated mucociliary, immunological, or epithelial defect (Kim et al. 2008, Chan and Iseman 2010).

2.3.3 OTHER UNDERLYING DISEASES RELATIVE RISK FACTORS

Mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene might be more common among NTM patients than in the general population. CFTR mutations have been found in recent studies among 36–50% of pulmonary NTM patients (Ziedalski et al. 2006). Most of these pulmonary NTM patients were heterozygotes with normal sweat chloride levels and presented only mild clinical signs of clinical cystic fibrosis at most (Ziedalski et al. 2006, Kim et al. 2008).

Mucociliary clearance is retarded both in cystic fibrosis disease and in primary ciliary dyskinesia (Knowles and Boucher 2002). Fowler et al. 2013 found that retarded airway ciliary beat, reduced nasal nitric oxide output and a disorder in response to Toll- like- receptor might be risk factors to pulmonary NTM patients (Fowler et al. 2013). Reduced nitric oxide production could further hamper the

opsonization of macrophages and thereby also mycobacterial killing (Fowler et al. 2013).

Gastro-esophageal reflux and chronic aspiration have been related to MAC and *M. fortuitum* pulmonary diseases (Thomson 2007). However, neither Marfan's syndrome with bronchiectasis nor α1-antirypsin deficiency with bronchiectasis have been found to clearly predispose to pulmonary NTM (Kim et al. 2008, Sexton et al. 2008). Recently, rheumatoid arthritis was also linked to increased risk for pulmonary NTM infection (Al-Anazi et al. 2014).

2.4 PATHOGENESIS

The hydrophobic capsular lipid layer of mycobacteria is very protective against host defenses and supports mycobacteria evasion of phagocytosis and lysosomes (Fernando and Britton 2006, Astarie-Dequeker et al. 2010). Thus, mycobacteria are able to adapt and multiply in the intracellular environment of macrophages (Gupta et al. 2012, Maartens and Wilkinson 2007).

Rather few NTM species have a sufficient virulence to cause pulmonary NTM disease to immunocompetent patients with MAC as the best characterized group. MAC is a commonly used acronym for *MAC* which includes both *M. avium* and *M. intracellulare*. These two species are usually not reported separately by clinical microbiological laboratories, although *M. intracellulare* has been suggested to be more pathogenic than *M. avium* (Corbet et al. 1999, Han et al. 2005). Other species that have been definitely linked to pulmonary disease are *M. kansasii, M. malmoense, M. xenopi,* and some rapidly growing mycobacteria (RGM), including *M. abscessus, M. fortuitum,* and *M. chelonae* (Taiwo and Glassroth 2010). Of these, *M. kansasii* has been regarded to be the strongest pulmonary pathogen and *M. abscessus* causes the most cases of pulmonary RGM infection (Corbet[a] et al. 1999, Esteban et al. 2008). *M. gordonae* is usually considered a contaminant species, however, even in ATS 2007 criteria, *M. gordonae* has been reported to cause infections especially in patients with an underlying predisposition or immunosuppression such as AIDS, steroid therapy, or prostata and pulmonary carcinoma, in addition to patients undergoing peritoneal dialysis and transplant recipients (Eckburg et al. 2000, Griffith et al. 2007, Pinho et al. 2009). *M. marinum* and *M. ulcerans* has not been reported to cause pulmonary NTM infections (Griffith et al. 2007), which is interesting, because *M. marinum* is closely related to *M. tuberculosis*. *M. ulcerans* is a well-known skin disease. The painless tropical, ulcerative cutaneous infection with extensive tissue damage in the absence of an acute inflammatory response is caused by mycolactone toxin (George et al. 2000).

The infectious dose of NTM infection is largely unknown (Mirsaedi[a] et al. 2014). The potential of different NTM species to cause a clinical infection is variable and the mechanisms are still not well understood (Fernando and Britton 2006). The development of NTM infection into symptoms and signs most probably depend not only on the infecting species but on local factors on the infection site and perhaps even more on the capability of host immune system to clear the infection before symptom onset. When aerosol droplets of NTM are inhaled by patients, most probably a disorder of innate local host defenses, mainly impaired mucociliary clearance, damaged respiratory mucosa or weakened cough response, will be behind a persisting infection and clinical symptoms in most cases (McGarvey and Bermudez 2002). Many of these factors remain unknown, but the universal existence of NTM in the environment suggests that infection, i.e. contact with NTM alone, is not sufficient for clinical symptoms. Further, the pathogenesis of pulmonary NTM evidently differs from those of the disseminated gastrointestinal NTM infections (Guide and Holland 2002, Griffith et al. 2007). Patients' ability to resist transmission of NTM depends on local and systemic host defense. The local pulmonary defects in the host might explain the oldest recognized risk factors for NTM infection, including: bronchiectasis, COPD, prior tuberculosis and persistent smoking (Mirsaedi[b] et al. 2014). These risk factors are associated with pulmonary tissue destruction, mucus plugging, and immunocompromise via disease or drugs, which partly explain the pulmonary pathogenesis (Mirsaedi[b] et al. 2014). Mucus plugging and suppression of cough was suggested to be associated with pulmonary NTM infection and this phenomenon was labeled "Lady Windmere infection syndrome" (Reich and Johnson 1992, Chalermskulrat et al. 2002). Among lung NTM patients, low nasal nitric oxide output and inhibition of ciliary movement as local defects have been observed in pulmonary NTM patients and have been suggested as the main or as some of the main underlying factors for infection (Fowler et al. 2013).

The underlying, hereditary pulmonary diseases, such as cystic fibrosis with reduced ciliary movement, alpha-1 antitrypsin (AAT) defect, and Marfanoid syndrome, predispose to pulmonary NTM, most probably through bronchiectasis and causing local barrier damage (Chan et al. 2007). In this process, fibronectin is thought to attach NTM on damaged mucosa (Middleton et al. 2000, Middleton et al. 2004). In contrast, the female, slender, nonsmokers with special morphotype have been suspected to have an immunological defect, which predisposes them to NTM infection (Guide and Holland 2002).

2.4.1 GENETIC SUSCEPTIBILITY TO NTM

When mycobacteria have been phagocytized by macrophages, the intracellular infection of mycobacteria will activate the pro-inflammatory cytokine pathways and thus stimulate cytokine interleukin 12 (IL-12) and tumor necrosis factor- α (TNF-α) production (Haverkamp et al. 2006, Sexton et al. 2008). Interferon- γ (IFN- γ) stimulates innate and adaptive immunity, and coordinates major histocompatibility complex (MHC) class II expression on the surface of macrophages (Haverkamp et al. 2006). Moreover, it activates immunoglobulin (Ig) production by B-lymphocytes, contributing to the maturation of T-helper lymphocytes (Th) into the Th1 phenotype (Haverkamp et al. 2006, Field et al. 2006, Sexton et al. 2008, Guide and Holland 2002). The complement system is stimulated by immunoglobulins and is involved in innate and acquired immune responses (Janeway et al. 2005). The complement system, an enzyme protein cascade, is regulated by complement genes, which are located in major histocompatibility complex (MHC) (Walport 2001, Fernando and Britton 2006, Senbagavalli et al. 2011). An activated complement opsonizes mycobacteria and may present mycobacteria to macrophages for engulfing (Walport 2001). In the case of complement-protein malfunction, opsonization will be hampered and positive feedback of IL-12/IFN-γ on macrophages will be delayed (Gupta et al. 2012). INF-γ is essential in host defense against intracellular infection like mycobacteria and some salmonella species (Glosli et al. 2008). The possible malfunction of complement proteins are a result of defects of the complement genes due to mutations (Gupta et al. 2012), and malfunction may contribute to intracellular NTM survival.

Knowledge of human genetic susceptibility to mycobacteria is based primarily on pulmonary tuberculosis, which has been linked to major histocompatibility complex MHC class I, II, and III regions on chromosome 6 (Fernando and Britton 2006, Senbagavalli et al. 2011). Studies on pulmonary tuberculosis have suggested that susceptibility to tuberculosis is associated to some human leukocyte antigen (HLA) class I and II genes in several populations with variably and moderately increased risk for pulmonary tuberculosis (Yuliwulandari et al. 2010, Shi et al. 2011). Defects in MHC complement genes *C2, C3*, factor *B (FB)*, and *C4* have been studied in India and complete *C4A* deficiency, *BF*FA* and *C3*F* have been associated to pulmonary tuberculosis (Senbagavalli et al. 2011, Singh et al. 2007). Further, complement genes C2, C4 (*C4A, C4B*) and factor B (FB) are located in the MHC class III region between the class I (HLA-A, -C, -B) and the class II (HLADR,-DQ, -DP) (Fernando and Britton 2006, Senbagavalli et al. 2011). Defects in complement genes will produce quantitatively less active complement proteins, which will thus hamper the opsonization and positive feedback of IL-12/IFN- γ of the macrophages and mycobacteria will evade phagocytosis (Fernando and Britton 2006, Maartens and Wilkinson 2007). Rare data on genetic variation in the MHC region or on defects

in the complement cascade in NTM infections exists, but the data on tuberculosis make them interesting as study objects.

Disseminated NTM infection is clearly another entity than local pulmonary or cutaneous NTM infections. Some important observations have been made on NTM pathogenesis and host defense of disseminated NTM infection during the last three decades. Disseminated diseases have increased our knowledge on the principal components of the immune system for NTM defense. In immunocompromised HIV-patients, disseminated MAC infection has been thought to have its onset in entry of MAC through gastrointestinal mucosa to intestinal lymph nodes (McGarvey and Bermudez 2002). However, dissemination is usually seen only in HIV-patients with CD4+ T-lymphocyte number below 0.05×10^9/L, reflecting the important role of specific T-cell populations or activity (Orme and Ordway 2014).

While disseminated NTM infections are common among AIDS patients, pulmonary NTM infections are reported in less than 2.5% of AIDS patients (Kalayjian et al. 1995). Moreover, defects in T-cell mediated immunity have also been linked to disseminated NTM infections in solid organ or stem-cell transplantation patients (Doucette et al. 2004). However, neutropenia and humoral immune defects have not been linked to disseminated NTM infections (Kalayjian et al. 1995). Patients with immune modulatory agents such as TNF-α antagonist, biological medication, including monoclonal antibodies such as infliximab and adalimumab among rheumapatients, have been reported to be risk factors for disseminated NTM, in some cases (Salvana et al. 2007, Sexton et al. 2008). Disseminated NTM infections have been linked to rare familial genetic defects (Sexton and Harrison 2008, Guide and Holland 2002): a few mutations in IFN-γ and IL-12 production in critical points leading to disseminated infections. The defects in IFN-γ and IL-12 production have been reported to be a critical pathway for host defense; MAC infections have been fatal (Foote 1999, Dorman and Holland 2000, Casanova and Abel 2002, Guide and Holland 2002, Haverkamp et al. 2006, Fernando and Britton 2006, Glosli et al. 2008). The genetic mutations are reducing cytokine signals of IL-12 or IFN-γ as consequence they are causing severe disseminated NTM and also BCG (bacillus Calmette-Guérin) or *Salmonella* infections (Guide and Holland 2002). Mutations in IFN-γ receptor 1 and 2 have been identified in children and childhood with autosomal recessive patterns in 1994. These receptor deficiencies have caused severe disseminated MAC, RGM and *Salmonella* infections (Sexton and Harrison 2008, Guide and Holland 2002). However, these defects have not been found among pulmonary NTM patients (Fernando and Britton 2006, Holland 2001). In addition neutralizing auto-antibodies against IFN-γ were observed in Asian adult patients with disseminated NTM infections. The patients with these antibodies had multiple opportunistic infections, which included disseminated RGM infections. The symptoms in this adult-onset immunodeficiency resembled

advanced HIV (Browne et al. 2012) To what extent the genetic defects IFN-γ and IL-12 pathways may be involved in local NTM infections has not been elucidated, but there are some studies on familial clustering of pulmonary NTM infections, suggesting a defect in the immune system (Colombo et al. 2010).

The most common cutaneous NTM infections are caused worldwide by RGM, *M. marinum*, and and further in the tropics by *M. ulcerans* (Griffith et al. 2007). *M.ulcerans* and *M. marinum* affect host immune response differently. *M. ulcerans* is an extracellular bacterium and inhibits inflammatory response, whereas *M. marinum* is an intracellular pathogen and causes a strong inflammatory responses (Stamm and Brown 2004). *M. ulcerans* is causing tropical ulcerative skin disease (Boyd et al.2012). Painless tropical ulcerative cutaneous infection with extensive tissue damage in the absence of an acute inflammatory response is caused by mycolactone toxin (George et al. 2000, Stamm and Brown 2004). Mycolactone of *M. ulcerans* is able to induce a cytopathic effect and apoptosis including neutrophils and macrophages explains the absence of inflammation (George et al. 2000, Stamm and Brown 2004, Boyd et al.2012). *M. marinum* causes infection resulting in a subcutaneous granulomatous response (Stamm and Brown 2004). Pathologically cutaneous granulomas are like granulomas in lungs caused by *M. tuberculosis* (Stamm and Brown 2004). *M. marinum* skin infection may have a potential local spread and systemic dissemination in patients with rheumatoid arthritis or Crohn's disease receiving TNF-α inhibitors (Ramos et al. 2010, Fallon et al. 2008).

Extrapulmonary NTM infections like lymphadenitis in children were primarily related to *M. scrofulaceum* in the 1970s.Thereafter, during the past 30 years, *M. avium* has been most often associated with children with lymphadenitis. The reason for this change is unknown (Falkinham 2002).

2.5 CLINICAL MANIFESTATIONS

Over 80% of NTM infections are pulmonary (Falkinham 1996, Griffith et al. 2007, Piersimoni and Scarparo 2009), but cutaneous (Berliner 2015), soft tissue (Song et al. 2012, Hamade et al. 2014), lymph nodal (Penn et al. 2011), and bone infections (Wang et al. 2011, Park et al.2014) are also described. Rare infections in the central nervous system, cornea, and otitis media have also been reported (Griffith et al. 2007). Lymphadenitis caused by *M. scrofulaceum* is currently rare, because lymphadenitis is primarily caused by MAC, worldwide (Lindeboom et al. 2005). In contrast, the most common reason for lymphadenitis in the UK and Scandinavia is *M. malmoense*. Rare skin, bone, and soft tissue infections may occur both in immunocompetent and immunocompromised patients as a result of occlusive injury, postoperative wound infections (Brickman et al. 2005, Dessy et

al. 2006, Kim et al. 2014, Cadena et al. 2014), contaminated tattooing (Kennedy et al. 2012), or nail manicuring (Winthrop et al. 2004). Disseminated NTM infections manifest as blood or bone marrow NTM-positive cultures and have been found mainly among HIV-patients with low CD4-count but also recently among severely immunosuppressed patients like solid-organ transplant patients (Doucette et al. 2004, Al-Anazi et al. 2014). Disseminated NTM infections form an entity of their own and are not dealt with more in detail in this publication. However, very rare NTM bloodstream infections in immunocompetent patients have been associated with contaminated intravenous catheters in hospitals (Helou et al. 2013).

2.5.1 PULMONARY NTM INFECTIONS

It has been estimated that more than 80% of pulmonary NTM infections are due to MAC; these are discussed separately in this chapter (Piersimoni and Scarparo 2009). In the 1950`s pulmonary NTM infections were reported in typical male smokers with alcohol abuse and an underlying lung disease. In radiological findings they had a cavitation in the upper lobes resembling *M. tuberculosis*.These first reported clinical cases of NTM pulmonary disease were caused largely by MAC and by *M. kansasii* and less often by *M. malmoense* or *M. xenopi*. Later reticulonodular appearances caused by MAC and *M. kansasii* were decripted by computer tomography (CT) (McGrath 2008). Radiological findings had a predilection for apical and posterior segments resulting in "tuberculosis–like disease" characterization (McGrath 2008). Furthermore, multiple lung lobes have been shown to be affected (Taiwo and Glassroth 2010). Cavitations had thick walls without air fluid level and in contrast to tuberculosis, pleural effusion was rare (Taiwo and Glassroth 2010). The symptoms were similar to TB with productive cough, haemoptysis, fever, sweats, and weight loss. The nodular MAC changes may be difficult to discern from adenocarcinoma, which is also common in this risk group of male ex-smokers (Kobashi et al. 2004). Symptoms were described similar to TB with productive cough, hemoptysis, fever, sweats, and weight loss. Table 2.5.1.

In 1989, Prince et al. described the second prototype of pulmonary MAC disease affecting mainly immunocompetent females (Prince et al. 1989). These patients with persistent cough were non-smokers or ex-smokers without underlying pulmonary diseases (Prince et al. 1989).

Radiological findings of bronchiolar inflammation in early disease process showed "tree-in-bud" pattern, which may progress slowly to fibronodular bronchiectasis without cavitation (Wickremasinghe et al. 2005). Radiological changes are most commonly observed in the right middle lobe and lingua associated with a productive cough. In 1992, Reich and Johnson created a theory on habitually suppressed cough

that would lead into collection of secretions and infection in the right middle lobe or lingual and they described this clinical picture as "Lady Windmere Syndrome" (Reich and Johnson 1992). These female patients were described to have a typical body/morphotype: a lean body with bone deformities as scoliosis and pectus excavatum, and mitral prolapse (Guide and Holland 2002). These characteristic features also promoted the idea of a common immunological defect in these patients. Table 2.5.1.

The third pattern is rare, largely caused by MAC, and referred to as a hypersensivity syndrome which was first described after exposure to contaminated hot tubs (Mangione et al. 2001). Some vocational exposure to hot tubs that contain MAC caused hypersensitivity pneumonitis in lifeguards working at indoor swimming pools (Field et al. 2006) and aerosols that contain *M. immunogenum* in metal grinding workers have caused hypersensitivity pneumonitis (Field et al. 2006). The symptoms of "hot tub lung" consist of subacute or acute dyspnea, fever and cough. NTM findings in sputum and inflammatory products support pathogenesis (Marchetti et al. 2004).The radiological findings are not typical, in CT or the high-resolution computed tomography (HRCT) which may show alveolar or interstinal process with patchy or ground glass infiltrates, thickened interlobular septae, or interstitial nodules (Pham et al. 2003). Table 2.5.1.

RGM cause both pulmonary infections, but more often skin and soft tissue infections. Especially *M. abscessus* has been reported in 80% of RGM pulmonary diseases in the USA (Griffith et al. 1993). The classification of M. abscessus to subspecies *M. abscessus, M. massiliense*, and *M. bolletii* is clinically useful, especially in the case of treatment (Benwill and Wallace 2014). *M. fortuitum* and *M. chelonae* consist of remaining RGM pulmonary infections in the USA. In the *M. abscessus* pulmonary diseases, radiological findings demonstrate primarily nodular bronchiectasis, which rarely includes cavitation (Griffith et al. 1993). Gastroesophageal reflux has been reported to associate with RGM disease (Winthrop 2010). Patients with cystic fibrosis are affected by *M. abscessus* in all ages, whereas MAC only presents in older patients (Wickremasinghe et al 2005). Table 2.5.1.

Radiological findings in *M. malmoense* pulmonary infections are reported as fibrocavitary changes associated with underlying pulmonary diseases such as COPD. Cavitary findings are typically a large cavity with a diameter of more than 6 cm and an air-fluid level is often present. Symtoms and signs resemble tuberculosis (Piersimoni and Scarparo 2008, Hoefsloot et al. 2009).

M. xenopi is usually found in elderly male smokers with underlying pulmonary diseases. Their radiological findings usually consist of fibrocavitary findings (Varadi and Marras 2009). Both respiratory symptoms (such as cough and haemotphysis) and systemic symtoms (like body-weight loss, low BMI, anemia, hypoalbuminemia, and elevation of inflammatory markers) are prominent manifestations (Hayashi et al. 2012). Table 2.5.1.

M. kansasii is less often reported in Europe, in contrast to the USA where it is the second most common NTM isolation. *M. kansasii* is found in two subtypes while subtype 1 is common among immunocompetent and subtype 2 among immunocompromised patients (Maliwan 2005, Piersimoni and Scarparo 2008). Radiological findings are characterized as typically tuberculous fibrocavitary lesions in the upper lobes and on the other hand nodular bronchiectasis without cavities (Arend et al. 2004). Table 2.5.1. *M. gordonae* has been found in patients with underlying pulmonary (e.g. COPD) diseases (Henry et al 2004, Griffith et al. 2007).

Table 2.5.1. NTM pulmonary infections and classification according to clinical manifestation. Modifed according to Piersimoni and Scarparo 2008, Griffith et al.2007.

NTM pulmonary disease	Grow-rate	Clinical manifestation
Common		
M. avium complex	Slowly	Elderly male 60–80 years, heavy smokers, pre-existing pulm disease :bilateral disease, usually cavitary or fibrocavitary Elderly female predominance 55–75 years, non-smokers without pre-existing pulmonary diseases: nodular infiltrates with cylindrical bronchiectasis. Male/ female predominance average age 36 years:hot tub lung: diff use diseases: nodular infiltrates with cylindrical bronchiectasis due to MAC.
M. kansasii, subtype I and II	Slowly	Elderly men with upper lobe cavitations (subtype I). Immunocompromised patients (subtype II).
M. malmoense	Slowly	Middle age or elderly men with fibrocavitary findings
M. xenopi	Slowly	Elderly men, heavy smokers, with upper lobe cavitations and nodules
M. abscessus	Rapidly	Elderly female with multilobar interstitial and nodular lesions, rarely cavitations.
Uncommon		
M. szulgai	Slowly	Elderly men with upper lobe cavitations and nodules
M. simiae complex, (*M. simiae, M. lentiflavum, M. triplex)*	Slowly	Elderly men with upper lobe cavitations and nodules
*M. celatum**	Slowly	Elderly patients with upper lobe cavitations and nodules
M. chelonae	Rapidly	Infiltrates, nodules
M. fortuitum	Rapidly	Infiltrates, nodules

2.5.2 EXTRAPULMONARY NTM INFECTIONS

Lymphadenitis occurs typically among children, most often aged 1–5 years (Griffith et al. 2007). In contrast, TB is a common cause of lymphadenitis in immunocompetent adults. In children, at day-care age, lymphadenitis due to *M. tuberculosis* consists of only 10–20% of all mycobacterial lymphanode infections (Falkinhamn 2003). NTM lymphadenitis in children is painless and unilateral involvement of submandibular, submaxillary, cervical, or preaurical lymphnodes (Wolinsky 1995). The disease has insidious course without systemic symptoms and the child is afebrile. Lymph nodes may be swollen for weeks or months, then they may finally rapidly soften and rupture, forming a draining sinus. MAC has been the main NTM in lymphadenitis of children followed by *M. scrofulaceum*, *M. malmoense,* and *M. hemophilum* (Lindeboom et al. 2005). In adults, NTM lymphadenitis is a rarity and most cases are due to M. *tuberculosis*. In AIDS patients, NTM lymphadenitis may be associated with a disseminated MAC infection (Falkinhamn 2003).

Most commonly skin and soft tissue NTM infections are caused by RGM like *M. fortuitum, M. chelonae,* and *M. abscesssus* and more rarely *M. marinum* and *M. ulcerans* (Esteban and Ortiz-Pe´rez 2009). The skin and soft tissue RGM infections may show a slightly different clinical picture depending on the causative species. Most often, *M. fortuitum*, appears in immunocompetent persons after penetrating trauma or surgery (Piersimoni and Scarparo 2009). Clinical cutaneous findings are nodular or ulcerating with slight exudate and reddish blue discolorations. *M. chelonae* and *M. abscessus* clinical manifestations appear as multiple and disseminated lesions as a result of bacteremic spread, and they are often associated with immunosuppressive underlying disease, transplant organ, or steroid-treatment (Piersimoni and Scarparo 2009). However, the spectrum of diseases due to *M. chelonae* and *M. abscessus* is wide and cutaneous infections like folliculitis and postsurgical infections like after mammoplasty or even in wounds after soil contamination, may be seen as was described in many casualities of the Thailand tsunami 10 years ago (Groote and Huitt 2006). Outbreaks related to contaminated equipment or dye have been reported related to skin punctures as tattoo or manicure saloons (Groote and Huitt 2006). Symptoms and signs might appear in cutaneous infections as early as 3 weeks but may delay up to 4 months (Piersimoni and Scarparo 2009). Cutaneous infection caused by MAC after cutanous injection, trauma, or surgery reveals skin ulceration and abscesses. Further, MAC skin infection with indolent erythematous dermatitis may mimic *Lupus vulgaris* (Piersimoni and Scarparo 2009).

M. marinum infections have been associated to fish tanks, home aquaria, and swimming pools. Infection may also be obtained from skin-penetrating traumas from fish fins or bites (Piersimoni 2012). In *M. marinum* skin infections, 50%–80% of patients have had an aquarium at home or at a work place, or a swimming pool contact or fish contact at work (Piersimoni 2012). A cutaneous disease in peripheral

extremities may cause “fish tank granuloma” or “swimming pool granuloma” and may migrate to other areas, especially joints or bones (Escalonilla et al. 1998, Aubry et al. 2002).

After inoculation, small nodules begin to grow in 2–3 weeks making skin abscesses. *M. marinum* infection may spread proximally along the lymphatics producing additional “sporotrichoid spread” nodules. Sometimes, rarely, it may cause disseminated infections (Streit et al. 2006). *M. marinum* skin infection has been reported in rare cases to spread locally or experience systemic dissemination in patients with rheumatoid arthritis or Crohn’s disease receiving TNF-α inhibitors (Ramos et al. 2010, Fallon et al. 2008).

M. ulcerans inoculates the skin through a cut or wound contaminated with water, soil, or vegetation (Esteban and Ortiz-Pe´rez 2009). Clinically cutaneous nodules and ulcers affect 62% in a lower limb and 30% in an upper limb (Asiedu et al. 2000). The incubation time is generally under 3 months. Affected patients are often children under 15 years-of-age (Piersimoni and Scarparo 2009). Spontaneous healing usually takes 4–6 months and involves extensive scar formation, resulting in severe deformity with joint contracture, subluxation, muscle atrophy, or distal lymph edema (Asiedu et al. 2000). Multiple lesions represent the most severe form of the disease; a high percentage of cases are osteomyelitis, often leading to amputation or even death. Disabilities are frequent after *M. ulcerans* infection and have been estimated in 25% to 58% of cases (Piersimoni et Scarparo 2009, Asiedu et al. 2000).

Biopsies of NTM skin infections have demonstrated suppurative granuloma and abscesses with necrosis, but without caseation and acid fast stains may be positive (Piersimoni and Scarparo 2009). Symptoms and signs may appear in cutaneous infections as early as 3 weeks but may delay up to 4 months (Piersimoni and Scarparo 2009). NTM infection may involve the visceral organs and *M. avium*, subspecies paratuberculosis has been described in some human cases to relate intestinal Crohn’s disease (Falkinham 1996).

2.5.3 DISSEMINATED NTM INFECTION IN IMMUNOCOMPROMISED PATIENTS

Disseminated NTM diseases are seen in patients with impaired cellular immunity, HIV patients with low CD4 + T-cell counts, transplant recipients, hematological leukemia patients, patients with autoimmune disease treated with a biological drug or chronic corticosteroid medication, and a few genetic disorders of interferon gamma production and function (Griffith et al. 2007, Piersimoni 2012, Doucette and Fishman 2004, Dorman and Holland 2000, Casanova 2002).

Over 90% of HIV disseminated cases are caused by MAC, largely due to *M. avium* (Griffith et al. 2007). Infection with NTM is a rare event in forms of

immunosuppression other than advanced HIV, but infections have been reported after organ transplantation, in connection to hematological malignancies, and also in patients receiving to chronic corticosteroid medication or TNFα-antagonist (Doucette 2004, Cordonnier 2004, Griffith et al. 2007).

Patients undergoing solid-organ or stem-cell transplantation are at moderate risk for NTM infection, especially during the second through sixth post-transplant months as a result of cumulative immunosuppression. NTM infections due to 20 different species (both slowly growing NTM and RGM and also species with low virulence such as *M. genavense* and *M. haemophilum*) have been reported after transplantation (Piersimoni 2012, McGarth et al. 2008).

Infection risk seems to correlate with immunosuppression needed after transplantation and an incidence of 0.16–0.38% after renal transplantation, 0.24–2.8% after heart, and 0.46–2.3% after lung transplantation have been reported (Piersimoni 2012).

The most common forms of NTM infection after organ transplantation are cutaneous lesions of the extremities, tenosynovitis, arthritis with a causative agent, *M. fortuitum, M. abscessus, and M. chelonae,* moreover half of these NTM infections are associated to disseminated infection (Piersimoni 2012). The clinical picture of the skin infections are painful, erythematous or violaceous nodules progressing to abscess draining fluid. Sometimes they might enlarge to necrotic cellulitis and require surgical treatment. Notably, the systemic symptoms like fever, weight loss, night sweats, and leukocytosis are absent. Among lung and heart transplant recipients, pleuropulmonary NTM diseases appear in 26 %–82 % of reported cases and they are often caused by *M. kansasii, M. avium, M. abscessus,* and *M. xenopi* (Piersimoni 2012). Among lung transplant patients, pulmonary MAC and *M. abscessus* infections are the most common with symptoms involving persistent cough, dyspnea, and sputum production (McGarth et al. 2008). It is worth observing that over half of these pulmonary cases also include skin, joint or soft tissue infections (Piersimoni 2012). In contrast, in kidney transplantations pulmonary NTM lung infection has rarely been reported, instead in renal transplantation NTM infections at postsurgical sites cause the majority of dissemination due to *M. chelonae* and *M. abscessus* (McGarth et al. 2008). Both types of infection occur in later years after transplantation and a median time of onset of 48 months after transplantation has been reported. For kidney, time onset has been described to be 24 mo, for lung 15 mo, and for heart 30 mo (Piersimoni 2012). Life-time immunosuppression after organ transplantation seems to increase the cumulative risk for NTM infection (Doucette and Fishman 2004). Consequently, decreased immunosuppressive medication may protect patients against NTM infections.

Patients with rheumatoid arthritis receiving TNF-α-antagonists (infliximab, toshilitsumab) have been reported to possess an increased risk of tuberculosis

reactivation and the treatment can thus be thought to render them more sensitive to NTM infections. TNFα-antagonist impairs granuloma formation. In addition, nitric oxidase synthase decreases affecting macrophage defense mechanisms (Raychaudhuri et al. 2009).

2.5.4 HEALTH CARE RELATED NTM INFECTIONS

RGM, especially *M. fortuitum* and *M. abscessus* have been reported in health-care-associated outbreaks. The reason for outbreaks is primarily that patients are exposed to NTM infected liquid, e.g. tap water given the resistance of mycobacteria to chlorine, formaldehyde, alkaline glutaraldehyde, and other commonly used disinfectants (Groote and Huitt 2006, Helou et al. 2013). Contaminated tap water, used for rinsing surgical devices after disinfection, was identified as the source of the outbreak in 1993 when 58 cases of *M xenopi* spinal infection were identified after microsurgery operation (Astagneau 2001). Catheter-related RGM bloodstream infections were identified altogether in 116 patients with hematologic or solid cancer. A treatment response was moderate in 92 patients (96%), but three patients had complications: one complication with endocarditis in a mitral valve caused by *M. chelonae* and two patients had complications with thrombophlebitis (Helou et al. 2012). NTM outbreaks have been associated with cardiac surgery (primarily median sternotomy wounds and cardioplegia solution), injections, plastic surgery, liposuction, dialysis-related outbreaks, and central intravenous catheters (Philips and von Reyn 2001, Griffith et al. 2007). Recently, *M. chimaera* caused a prolonged outbreak to 6 adult patients that had valve endocarditis or vascular graft infection 1.5-3.6. years after postoperative open-chest heart surgery in Europe. Epidemilogical and microbiological findings linked heater-cooler water tanks used in cardiac surgery, which was contaminated with *M. chimaera* which transferred to patients during open–heart surgery. All 6 patients were treated with prolonged antimycobacterial therapy. Three of the patients were reoperated for valve replacement surgery, two others died (Sax et al. 2015).

In hospitals RGM bloodstream infections in humans are caused by about 20 species of RGM, largely through catheter-related infections. RGM are capable of creating biofilm and colonizing intravascular catheters. Catheter-related bloodstream infection symptoms are systemic; fever, chills, and local infection. RGM infections in cardiac implantable devicies or prosthetic valves are usually subacute, but the mortality rate may be up to 25% (Helou et al. 2013).

2.6 DIAGNOSIS OF NTM INFECTION

2.6.1 LABORATORY DIAGNOSIS OF NTM

NTM and *Mycobacterium tuberculosis* appear to be similar under the microscope. In the laboratory, however, the growth rates and colony pigmentation of NTM provide practical means for grouping. Modern culture techniques and molecular techniques provide more rapid molecular diagnostic systems. As such, NTM have been classified into three groups: slowly, intermediately, and rapidly growing. Rapidly-growing NTM reach maturation within 7 days and are classified in the laboratory according to pigmentation and genetic relatedness. Intermediately growing mycobacteria need 7 to 10 days to mature. Some slowly growing NTM may require nutrional supplementation, because they are growing more than 7 days with one exception. The cultivation of *M. ulcerans* requires up to several months to grow and identification by molecular detection is most useful. In the laboratory, clinical microbiological samples, except blood, are first stained with flourochrome dye (Woods 2002). To prevent overgrowth of Pseudomonas aeruginosa and other bacteria, the specimens are treated with sodium hydroxide and N-acetylcysteine. Thereafter the samples are cultured in both solid and liquid media. On solid media the growing takes 3–6 weeks for slowly growing NTM, but is shortened to 1–2 weeks in liquid media. The most modern techniques are used to differentiate mycobacteria specimens: high-performance liquid chromatography, restriction fragment length polymorphism analysis amplification techniques, DNA sequencing, and the use of DNA probes (Petrini 2006).

Cultures positive for acid-fast bacilli are identified by conventional biochemical tests; MTB is differentiated from NTM through its catalase, nitrate reductase, and niacin production. Nucleic acid amplification techniques, hybridization with DNA probes (AccuProbe; Gen-Probe, San Diego, CA, USA), distinguish NTM from MTB. In case cultures negative for *M. tuberculosis* and MAC complex, NTM are identified by amplification and sequencing of the 16S rRNA gene (Petrini 2006).

2.6.2 DIAGNOSTIC ATS CRITERIA

Emanuel Wolinsky suggested the use of acid fast bacilli (AFB) sputum cultures in NTM lung diagnosis and thereby recognized NTM cases grew. In 1979, Wolinsky published the first review of NTM diseases, including diagnosing NTM lung disease, describing "chronic pulmonary disease resembling tuberculosis" and noted *M. kansasii* and MAC to be the most common pulmonary pathogens (Wolinsky 1979). To distinguish pulmonary NTM from pulmonary tuberculosis, more frequent positive NTM culture findings were necessary and the microbiolgical findings

constituted the cornerstone of diagnosis. These concepts suggest the repetitive isolation of an NTM species in culture with radiological cavitation findings mimicking *M. tuberculosis*. In 1981, the American Thoracic Society (ATS) published the first diagnostic guidelines for *M. kansasii* and *M.avium* complex, including radiological findings with cavitations or infiltrates, and isolations of mycobacteria from biopsy tissue together with histopatological changes. Later, noncavitary MAC lung disease was revealed and the modification of the diagnostic criteria for MAC and *M. kansasii* was suggested, including specific radiological and microbiological criteria. This was included in the publications in 1990 by ATS: In the presence of a noncavitary infiltrate, NTM lung disease is considered to be present when at least two sputums are AFB smear-positive and/or moderate to heavy growth on culture (Wallace et al. 1990). The typical patient with infection caused by MAC was a heavy smoking, immunocompetent elderly white man with a chronic lung disease. The signs and symptoms in NTM lung disease were similar: cough, shortness of breath, fatigue, fever, weight loss, and decreased appetite (Piersimoni and Scarparo 2008; Kubo et al. 1998). Soon after these opportunistic bacteria revealed to be an important illness in patients with severe, immunocompromised underlying disease, AIDS, among HIV-patients (Benson 1994). Later on NTM were found more frequently in other immunocompromised patients, such as hematologic and cancer patients (Doucette and Fishman 2004, Cordonnier et al. 2004).

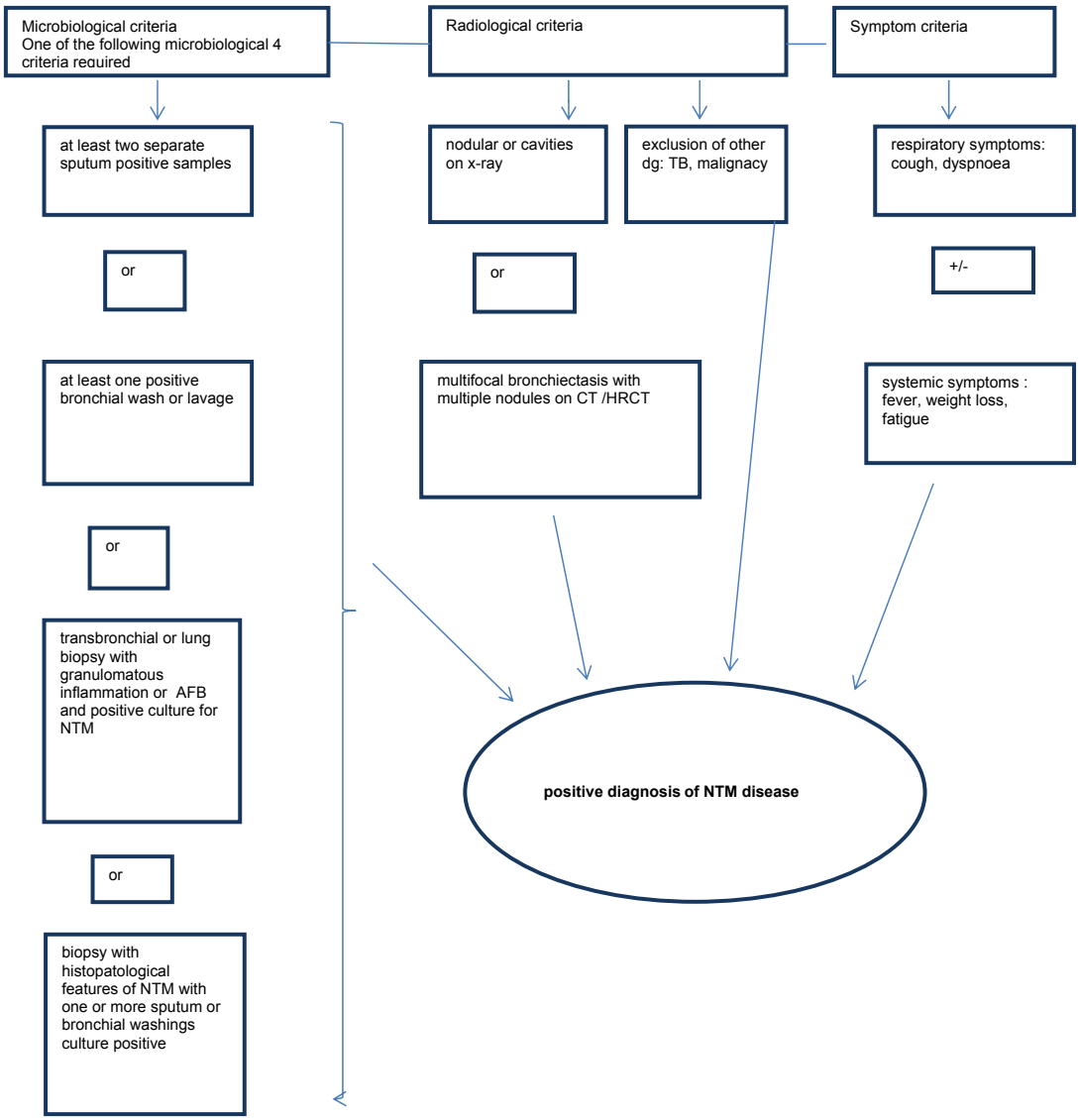
Moreover in the late 1980s, NTM diseases in patients without underlying diseases came up in non-smoking, eldery white women with noncavitary MAC diseases. Prince et al described 21 patients with MAC lung disease: eldery women without known predisposing factors or lung disease (Prince et al. 1989). Most of them had slowly progressive nodular opacities, 29% had some cavitary abnormalities, and some developed cavitary during the course of disease. The important aspect was the understanding of noncavitary MAC disease, namely progressive; nodularic MAC lung disease could even be fatal. The modern radiological CT-scanning revealed obvious noncavitary MAC lung disease, small nodules, and fibrosis. A Japanese study confirmed these findings and emphasized CT as a diagnostic tool (Tanaka et al. 1997). CT-scanning was included into the ATS criteria in 1997. The radiographic criteria included the lung findings: infiltrate, nodular, or cavitary disease found by x-ray or multifocal bronchiectasis and/or multiple small nodules found by HRCT (Wallace et al. 1997). Microbiological criteria fit best with M. avium complex, M. abscessus, and M. kansasii. Three positive NTM cultures with negative AFB smear results from sputum/bronchial wash during the last 12 mo or appropriately two positive cultures and one positive smear finding. When those findings were nondiagnostic or another disease was unable to exclude positive NTM findings, transbronchial or lung biopsy fulfilled the criteria. Alternatively, biopsy findings with granulomatous inflammation and/or AFB and one or more sputum or bronchial washings are

positive for an NTM even in low numbers. When only one bronschial wash was available, one positive culture with a 2+,3+,4+ AFB smear or 2+,3+,4+ growth on solid media fulfilled microbiological criteria (Wallace et al. 1997).

Colonization of pulmonary NTM in immunocompetent and immunocompromised patients has been discussed, because there has been discrepancy between sputum findings and symptoms (Field and Cowie 2006). NTM might be found in patients with clinical symptoms without causal relation to symptoms. Airway colonization might be difficult to separate from a clinical disease. Treatment of colonization might lead to unnecessary adverse effects. As a result, the existence of a low-grade NTM infection is unclear. It has been proposed that "colonization" be replaced with the term "indolent disease" (Piersimoni and Scarparo 2008, Field and Cowie 2006).

This aspect has been taken into consideration in the ATS criteria 2007: at least two positive NTM sputum cultures or histopathological findings are required. Moreover radiographic findings and pulmonary symptoms fit for NTM disease are required. See Table 2.6.2 (Griffith et al. 2007). The challenge in diagnosing NTM lung disease is the variability in clinical symptoms and findings depending on the causative species, underlying diseases, and difference in immunocompetent and immunocompromised patients. Cough is a common symptom, but other symptoms like malaise, fatigue, sputum production, dyspnoea, weight loss, fever, or haemoptysis are seen variably (Griffith et al. 2007). Radiologically and clinically, NTM resembles pulmonary tuberculosis and pulmonary cavities resembling those seen in tuberculosis have been described to be associated to *M. kansasii* in heavy-smoking men (Ellis et al. 2002). The cavitations are a less frequent manifestation of disease due to MAC, where bronchiectasis with nodules has been frequently reported (Piersimoni and Scarparo 2008, Griffith et al. 2007, Kubo et al. 1998). However, disease forms with nodular or fibrotic changes are also common and none of the radiological findings can be considered pathognomonic for an NTM infection (Griffith et al. 2007). If ATS criteria 2007 are not fulfilled, the follow-up of suspected NTM patients is emphasized because the nature of NTM is slowly progressive (Griffith et al. 2007). However, ATS 2007 criteria have been evaluated only in a few modified criteria studies, if the criteria have the prognostic value or the response to NTM treatment (Andréjak et al. 2010, Hayashi et al. 2012).

Table 2.6.2. ATS guidelines 2007 on the diagnosis of NTM disease.



2.7 TREATMENT OF NTM INFECTIONS

2.7.1 TREATMENT OF NTM AMONG IMMUNOCOMPETENT PATIENTS

Even though NTM and tuberculosis are both mycobacteria infections, their treatment is different. The therapy of tuberculosis has defined protocols, susceptibility testing, and a less complicated regimen (McGrath et al. 2008). To optimize the clinical cure for pulmonary NTM patients, the clinican must first estimate whether NTM culture findings represent true pathogenesis or colonization. Further benefits of the treatment versus side effects to the patients must be evaluated (Piersimoni and Scarparo 2008, Esteban et al. 2009, Al-Anazi et al. 2014). The aim of the drug treatment might be definitive eradication or be suppressive minimizing of the symptoms. The drug combinations with surgery have to take priority in cases where the NTM disease is pulmonary unilateral or locally cutaneous (Esteban et al. 2009). It is unclear if patients need different regimens, when clinical manifestations, risk factors and patient's characteristics are different, as in the case of cavitary and nodular bronchiectatic pulmonary NTM diseases (Ingen et al. 2013).

It is important to consider the differences between pulmonary and disseminate NTM pathogenesis when treating NTM cases. Namely, pulmonary NTM infection due to MAC and other slow growing NTM tends to occur in immunocompetent patients both with and without underlying lung disease; e.g. bronchiectasis, COPD, prior tuberculosis, and cancer. The treatment of co-morbidities have to be considered when evaluating the period of NTM therapy, patients compliance, and surgery requirement (Esteban et al. 2009). However, even with modern laboratory diagnoses and new antimicrobial drugs, treatment outcomes of NTM are typically poor (Piersimoni and Scarparo 2008) and unfortunately treatments according guidelines do not necessary improve the prognosis of immunocompetent NTM patients (Andréjak et al. 2013). A valid combination of several drugs should give a synergistic effect and avoid drug resistance. Moreover if surgery has to be considered, good collaboration between microbiologists, clinicians and surgeons is important (Esteban et al. 2009, Griffith et al. 2007, Al-Anazi et al. 2014).

The treatment of slowly growing NTM (MAC, *M. malmoense, M. xenopi*) includes the first-line antituberculous drugs rifampin and ethambutol combined to clarithromycin or to an aminoglycoside. Resistance to isoniazid is common and is not advised (Brown-Elliot et al. 2012). Macrolides have been the most important advancement in the treatment of MAC pulmonary disease (Wallace et al.1996, Tanaka et al. 1999, Griffith et al. 2007). In contrast, RGM are intrinsically resistant to first-line antituberculosis drugs. Therefore the treatment combinations consist of a macrolide and/or a quinolone with amikacin. Further, other antibiotics (cefoxitin, doxycycline, cotrimoxazole) may be valid (Griffith et al. 2007).

According to ATS 2007 guidelines, macrolides form the cornerstone of antimycobacterial treatment of MAC. The treatment of *M avium* complex pulmonary disease is advised to be treated with a combination of a macrolide (clarithromycin or azithromycin), ethambutol, and rifampicin avoiding macrolide monotherapy, which leads to rapid resistance and treatment failure (Griffith et al. 2007, Piersimoni and Scarparo 2008, Brown-Elliot et al. 2012). An intermittent thrice-weekly regimen is recommened only for patients with limited disease or nodular bronchiectatic disease (Taiwo and Glassroth 2010). Treatment should be continued until sputum cultures have been negative for at least 12 months. During treatment, sputum samples are advised to be collected monthly (Griffith et al. 2007). Daily therapy is recommended to MAC patients with cavitary disease or extensive bronchiectatic MAC, or patients with previous treatment failure and further in that case, a parenteral aminoglycoside are combined like streptomycin or amikacin for the first 8 to 12 weeks (Griffith et al. 2007, Piersimoni and Scarparo 2008, Taiwo and Glassroth 2010). Surgery is recommended in pulmonary MAC, in case there is a localised fibrocavitary disease or macrolide resistance (Griffith et al. 2007, Piersimoni and Scarparo 2008, Taiwo and Glassroth 2010).

M. xenopi treatment according to ATS 2007 guidelines are including clarithromycin, rifampin, isoniazid, and ethambutol (Griffith et al. 2007). The regimen is recommended for 18–24 months. Out of quinolones, moxifloxacin may be effective, but experience is limited (Taiwo and Glassroth 2010, Brown-Elliot et al. 2012). *M. malmoense* treatment with rifampicin, ethambutol with or without macrolide or quinolone should be up to 24 months (Griffith et al. 2007). The growth of *M. malmoense* may be poor and therefore *in vitro* susceptibility is difficult (Brown-Elliot et al. 2012). Symptoms relapse and progressive lung destruction determined radiographically are signs to overweight resectional surgery, provided that extensive lung involvement is excluded (Taiwo and Glassroth 2010).

RGM pulmonary infections are caused mainly (80%) by *M. abscessus*. *M. abscessus* subsp. *massiliense* is macrolide susceptible and treatment is successful. Instead the treatment challenge is *M. abscessus* subsp. *abscessus*, because 80% of isolates are macrolide resistant due to the erm- gene (Benwill and Wallace 2014). The treatment is difficult and rarely curable in patients with underlying pulmonary diseases (Taiwo and Glassroth 2010). Immunocompetent patients require up to 24 months multidrug treatment with macrolide, amikacin, cefoxitin or imipenem, and even so relapses are usual (Jeon et al. 2009, Taiwo and Glassroth 2010, Brown-Elliot et al. 2012, Beltrame et al. 2013). Co-infection with MAC has been reported in 15% of cases (Taiwo and Glassroth 2010). Surgical resection is necessary in claritromycin resitant MAC and *M. abscessus* pulmonary infections (Griffith et al. 2007, Taiwo and Glassroth 2010, Brown-Elliot et al. 2012, Kang et al. 2015). In a retrospective observational study over 2001–2008, 107 non-smoking women with

bronchiectasis carried a pulmonary *M. abscessus* infection and half of the patients had MAC co-infection. The patients were treated with antimycobacterial drug, with or without surgical intervention, and patients were followed for 34 months. The treatment outcome was poor but similar in both groups. As a result, 29% of patients remained culture positive, 23% had relapses, and mortality rate was 16%. Surgical resection showed some success; sputum cultures converted to negative in 48% of patients (Jarand et al. 2011). In another retrospective review, a total of 70 adult patients with pulmonary NTM underwent pulmonary operation due to poor response to drug therapy, cavitary lesions, or severe bronchiectasis or hemoptysis. A negative sputum culture was achieved and maintained in 57 (81%) patients. A complication rate of 21% was rather high due to usual complication bronchopleural fistula and moreover one patient died (Kang et al. 2015). Today, indications for surgery are unclear and careful patient selection is important (Mirsaeidi[a] et al. 2014). The adjunctive treatment of patients with inhaled interferon- γ in functional IFN-γ deficiency with pulmonary NTM disease has been found controversial (Hallstrand et al. 2004, Lam et al. 2006) and treatment of inhaled amikacin has been reported without any outcome benefit (Ingen et al. 2013).

Localized cutaneous infections are associated often to skin, soft tissues, lymph nodes, tenosynovitis, and bones. Ocular or otitis, or even central nervous system diseases, are rarely reported (Piersimoni and Scarparo 2009). Cutaneous MAC infection requires frequent surgical depridement with 6–12 months therapy with at least 3 drugs, usually clarithromycin, rifampin, and ethambutol (Piersimoni and Scarparo 2009). RGM cutaneous infections are needed to treat 4–6 months. *M. fortuitum* infection in surgical sites or after penetrating skin trauma treatment may include amikacin, cefoxitin, fluoroquinolones, sulfonamides, and imipenem. *M. abscessus* and *M. chelonae* are susceptible to clarithromycin, amikacin, imipenem, and also linezolid (Brown-Elliot et al. 2012). Surgery is useful to assess severe cutaneous infections. *M. fortuitum* cellulitis, *M. abscessus*, *or M. chelonae* abscesses or osteomyelitis (Piersimoni and Scarparo 2009, Brown-Elliot et al. 2012). In *M. marinum* infections, two antimicrobial claritromycin with rifampin or ethambutol 3–6 months is usually sufficient (Piersimoni and Scarparo 2009, Brown-Elliot et al. 2012). At least three drugs are needed in deeper soft tissue and tenosynovitis infections: macrolide with rifampin, ethambutol, and fluoroquinolones with or without amikacin or linezolide for 12–18 months. Moreover, surgical debridement is necessary for deep abscesses (Aubry et al. 2002).

In lymphadenitis of NTM, excision seems to be the treatment of choice (Piersimoni and Scarparo 2009). In a retrospective study of 1986 to 1998, of 57 children with NTM lymphadenitis over a 12-year period, it was revealed that excision gave a higher rate of healing and re-operations were needed less often (Flint et al. 2000). In another study of 100 children with NTM lymphadenitis, surgery was

more effective than antimycobacterial medications with a cure rate of 96% vs 66% (Lindeboom et al. 2007).

2.8 PROGNOSIS OF NTM INFECTION

2.8.1 PROGNOSIS OF PULMONARY NTM INFECTION

In general, NTM infection carries a poor prognosis. In a Danish population-based study, 40% of the patients with a definitive NTM disease had died within 5 years of NTM isolation (Andréjak et al. 2010). The prognosis was no different in patients who were regarded to have NTM colonization only. Overall 5-year mortality of ATS-criteria-positive MAC-pulmonary-infection patients was 24%, and 10-year mortality 47 % (Hyashi et al. 2012). In the latter study, MAC-infection related mortality was estimated to be only 5.4% within 5 years and 15.7% within 10 years, respectively. In contrast, *M. kansasii* pulmonary infection was reported to cause death only in 8% of patients (Maliwan et al. 2005). Five-year mortality in *M. xenopi* pulmonary disease was 15% in a review of cases during 1960–2008 (Varadi and Marras 2009). Five-year mortality in *M. malmoense* pulmonary infections has been reported to be 11–13% (Hoefsloot et al. 2009, Henry et al. 2008). Patient age and underlying comorbidities in *M. xenopi* pulmonary NTM associated up to 69% with mortality in 3-year surveillance in France (Andréjak et al. 2009). In a recent study of all adults in Denmark with at least one NTM-positive pulmonary specimen during 1997–2008, *M. xenopi* and *M. malmoense* had the worst prognosis with up to 51% mortality rate in 5-year surveillance. The impact of NTM disease on mortality seems to be increasing as suggested by death-certificate data from the USA. A significant increase in the annual number of NTM-related deaths was observed in 1999–2010 (Mirsaeidi[b] et al. 2014). However, when adjusted for age, no increase during this period in fatal cases related to NTM isolation was seen. These studies fail to point out the immediate impact of NTM infection on mortality but merely show that NTM isolation is a sign of poor prognosis. In ATS criteria, positive MAC pulmonary disease, MAC-specific mortality, was estimated to be only 5% (Hayashi et al. 2012).

2.8.2 PROGNOSTIC FACTORS IN PULMONARY NTM INFECTION

Infections due to different NTM species seem to have a variable prognosis (Gommans et al. 2015). *M. xenopi* and *M. malmoense* had higher 5 year and 10 year mortality rates than MAC in a Danish study, but other non-rapidly growing mycobacteria and RGM had a better prognosis than MAC (Andréjak et al. 2010, Gommans et al. 2015). Cutaneous NTM infections may spread locally but fatal outcome is a rare

exception. No death was observed from 63 *M. marinum* cases, although treatment failure was observed in 13% of patients (Aubry et al. 2002).

ATS criteria were developed to find patients who would be in need of antimycobacterial medication, however no difference in prognosis was seen between patients who did and did not fulfil the modified ATS criteria (Andréjak et al. 2010). There seems to be somewhat contrasting results on the effect of gender on outcome. In a Danish study on all NTM infections, women were observed to have significantly better prognosis than men but in a similar analysis from the USA, no gender bias regarding mortality was seen (Andréjak et al. 2010, Mirsaeidi[b] et al. 2014). Male gender was, however, a prognostic factor predicting poor outcome in MAC pulmonary disease (Hyashi et al. 2012). Older age has also invariably been a prognostic marker for poor outcome (Andréjak et al. 2010, Hyashi et al. 2012, Commans et al. 2015). The extent and type of pulmonary infection, as well as underlying pulmonary disease, have been related to poor prognosis. Smear-positive cases, where the NTM load is probably higher than in smear negative cases, had a poorer 5-year survival (Andréjak et al. 2010). Fibrocavitary MAC disease was observed to have poorer prognosis than nodular-brochiectatic disease (Hyashi et al. 2012). However, underlying pulmonary diseases like fibrocavitary bronchiectasis have been associated to poor prognosis (Kim et al. 2008, Hyashi et al. 2012). In addition, COPD, bronchiectasis, interstitial lung diseases, and smoking were frequently found in deceased cases with NTM (Mirsaeidi[b] et al. 2014). Various underlying diseases are common and they have generally been strongly associated as risk factos for fatal outcome. It is known that pre-exiting pulmonary diseases are risk factors and predispose to NTM disease, e.g. bronchiectasis, COPD, prior tuberculosis, and frank immunosuppressive states (Piersimoni and Scarparo 2008, Fowler et al. 2006, Wickremasinghe et al. 2005). Furthermore, studies indicate that particular NTM species, risk factors such as underlying comorbidities, advanced age, and male sex had a prognostic factor among immunocompetent patients (Taiwo and Glassroth 2010, Hoefsloot et al. 2009). Low BMI has also been a poor prognostic factor (Hyashi et al. 2012).

2.8.3 PROGNOSIS OF DISSEMINATED NTM INFECTION

Patients with disseminated NTM infections with underlying immunosuppressive diseases, congenital deficiencies, or genetic mutations are known to have a poor outcome (Al-Anazi et al. 2014, Holland et al. 2002). In contrast, immunosuppressive patients with lower immunosuppressive level, receiving treatment regimen, and catheter removal experience a better prognosis (Al-Anazi et al. 2014, Helou et al. 2013).

3 AIMS OF THE STUDY

The purpose of the present study was to investigate the clinical symptoms and underlying diseases in non-HIV patients with an isolation of NTM.

The specific aims were:

I To study the role of smoking as a risk and prognostic factors among patients with NTM infection by comparing underlying diseases, clinical symptoms, and survival.

II To compare clinical picture, symptoms, and survival according to fulfilment of The American Thoracic Society 2007 (ATS) diagnostic criteria and evaluate the prognostic value of the ATS criteria in HIV-negative NTM patients.

III To evaluate differences in underlying diseases, symptoms and signs, radiological findings, survival, and prognostic factors among patients with MAC and patients with other NTM categorized according to the 2007 American Thoracic Society (ATS) NTM case definition.

IV To assess the genetic deficiency of complement components C4A or C4B encoded by major histocompatibility complex as a plausible risk factor in patients with pulmonary NTM infections and in *M. tuberculosis* patients.

4 MATERIALS AND METHODS

4.1 PATIENTS (STUDY I–IV)

Studies I–II were based on retrospective laboratory-based surveillance data on adult patients with at least one positive culture finding for NTM. All adult non-HIV patients from Southern Finland were retrieved from 1990 to the end of 1998 from the Microbiological Central Laboratory of Helsinki City, later Helsinki University Central Hospital Laboratory (HUSLAB) analyses.

Patients with NTM isolates matched with their medical records by national identity code. Data of records collected on paper retrospectively from hospital archives at Helsinki University Central Hospitals Meilahti, Peijas, Porvoo, and Laakso Helsinki city Hospital. Data of each patient was collected from a period of at least 4 years from the first culture positive NTM finding and the date of death was ascertained from the Population Register Centre records at 8 June 2006. The collected population consisted of 121 patients and patient data on paper format collected to electronic format. One patient with a positive finding of both tuberculosis and *M. avium*, was excluded. After exclusion, a total of 120 patients were identified (Table 4.1).

Study III included both study I–II and study IV patients with NTM**.** Study III based on retrospective laboratory-based surveillance data on 120 NTM mycobacteria patients collected from 1990 to the end of 1998 as declared in study I-II above. Their survival was followed until 8 June 2006. A prospective data was collected of study IV population of 50 NTM patients with at least one positive NTM finding, collected from 2004 to the end of 2009 among patients, who were admitted to the Division of Lung Disease or to the Division of Infectious Diseases, at Helsinki University Central Hospital clinics. Patients matched with their medical records by national identity code and patient data was collected retrospectively in electronic and paper format. The medical records of these patients were reviewed and survival followed until 31 December 2011. The date of death was ascertained from patients' records or the Population Register Centre records. The collected population consisted of 120 retrospectively collected patients (study I–II) and 50 prospective NTM patients (study IV). Two of the 50 prospective NTM patients were excluded as they were already included in the 1990–98 study group and one patient of 50 prospective NTM patients was excluded due to negative culture findings. After exclusion, a total of 167 patients were identified (Table 4.1).

Table 4.1. Patients with NTM study design and data collection.

Study	Study design	Number of patients	Patients available	Patients data collection	Surveillance time end point	Inclusion criteria	Exclusion criteria
Patients		n	n	years			
I-II	retrospective	121	120	1990–1998	8 June 2006	At least one culture positive finding for NTM.	Concomitant infection with *M. tuberculosis* and NTM, HIV, age under 16 year.
III	retrospective	121	120	1990–1998	8 June 2006		
	retrospective	50	47	2004–2009	31 December 2011		
IV	prospective	52 NTM 32 MTB	50 NTM 31 MTB	2004–2009	31 December 2011	At least one culture positive finding for NTM	Lack of consent, pregnant, handicapped, prisoners, under 18 years of age, conscripts and patients with HIV or CVI (common variable immunodeficiency). Concomitant infection with M. tuberculosis and NTM
Controls							
IV		149	149			Healthy, voluntary had been recruited in a health survey of the Helsinki region	

Study IV comprised adult patients at Helsinki University Central Hospital, Division of Lung Diseases or Division of Infectious Diseases with a NTM isolation. Data from patients with a *M. tuberculosis* isolation will also be presented in this thesis (unpublished), which was not included in published Study IV. Complement C4 and immunoglobulin levels in NTM and MTB patients will be presented (IV and unpublished results).

Patients who were admitted to these clinics with at least one positive culture with NTM or *Mycobacteria tuberculosis* finding, were eligible between August 2004 and December 2009. Out of 257 patients with a NTM isolation, 52 patients were recruited, and out of 180 patients with a *M. tuberculosis* finding, 32 patients were recruited. Two NTM patients were excluded due to missing consent. One MTB patient was excluded because of a missing laboratory specimen. After exclusion criteria were enforced, 50 patients with NTM and 31 patients with Mycobacteria tuberculosis were included to study IV (Table 4.1).

In study IV, patients were excluded when they were pregnant, handicapped, prisoners, under 18 years-of-age, conscripts, and if they had HIV or CVI (common variable immunodeficiency).

The control cohort (n = 149) consisted of healthy 100 females and 49 males, who had been recruited in a health survey from Helsinki region and used in a previous study (Seppänen M, Suvilehto J, Lokki M-L, Notkola I-L, Järvinen A, et al. (2006) Immunoglobulins and complement factor C4 in adult rhinosinusitis. Clin Exp Immunol 145: 219–227). The Ethics Committee of the Department of Medicine, Hospital District of Helsinki and Uusimaa approved the study.

4.2 ETHICS

Patients gave written informed consent for study IV and The Ethics Committee of the Department of Medicine, Hospital District of Helsinki and Uusimaa approved all study protocols (I–IV). Permissions were acquired from the Ministry of Social Affairs and Health to retrieve patient records outside the Helsinki University Central Hospital (Studies I–III).

4.3 DATA COLLECTION

Study I–II. Data from the patient records collected on the paper form. Relevant patient data reviewed at the time of the first positive NTM culture and at patient visits closest (+/- 6 months) and 1, 2, and 4 year thereafter.

At the time of the first positive NTM culture and patient visit (+/- 6 months), the following patient data was collected. Demographic characteristics (gender, age, body weight, height, occupation) were collected. Data on smoking habits were retrieved from both the medical records and pulmonary function test questionnaires. Alcohol abuse was retrieved from medical records. Underlying pulmonary diseases (bronchiectasis, COPD, prior tuberculosis, lung fibrosis, asthma, other pulmonary disease, pulmonary or other malignancy, no previous pulmonary diseases) were collected. Further other underlying diseases (sinusitis, scoliosis or thoracic abnormality, hypertonia, coronary artery disease, atrial fibrillation, myocardial infarction, palpitation, systemic corticosteroid treatment over 1 month, inhalation steroid treatment over 1 month, immunosuppressive therapy) were collected. Concomitant (underlying) diseases were classified as described by McCabe and Jackson (McCabe and Jackson 1962), as follows: (1) healthy, i.e. no other diseases; (2) chronic non-fatal diseases; (3) ultimately fatal diseases with a life-expectancy of 5 y maximum, such as carcinoma with local spreading and uncompensated hepatic cirrhosis; (4) rapidly fatal diseases with expected survival of no more than 6 months, such as carcinoma with widespread metastases.

Pulmonary symptoms (cough, dysphnea, haemothpysis) and systemic symptoms (fatigue, fever >37.5 ° C, weight loss, decreased appetite, night sweats, lymphadenitis, skin nodules) were collected.

From the microbiological findings, the numbers of subsequent NTM positive or negative mycobacterial smears, cultures, and sampling sites were recorded. Other laboratory findings (hemoglobin, hematocrit, thrombocytes, leukocytes, lymphocytes, eosinophils, CRP, sedimentation rate, creatinine, ALT, AST, alkaline phosphatas) were all reviewed at the time of first positive NTM culture.

The radiological findings of chest X-ray and CT scans were collected from the original radiologist's statements relevant to ATS criteria and classified as infiltrates, nodules, cavities, or bronchiectasis according to the 2007 ATS criteria. Further pulmonary function tests (FVC %, FEV 1 %, MEF 50 %) were collected.

During retrospective surveillance, data was collected from 1, 2, and 4 years following the first positive NTM culture. Similarity patient symptoms, the microbiological data, the radiological findings, pulmonary function tests and smoking habit were reviewed and collected at the time of 1, 2 and 4 years during patient visit (+/- 6 months).

Concurrent medication and the previous (6 months) immunosuppressive treatments were reviewed. Systemic or inhalation corticosteroid treatment was recorded when continued for longer than 1 month. The anti-mycobacterial medication and the results of *in vitro* sensitivity tests were recorded. The use of at least 2 potentially effective agents, based on the existing literature, for at least 3 months was required to classify the patient as having received anti-mycobacterial

medication treatment. Therefore, the anti-mycobacterial medication was categorized into the following subgroups: (1) less than 3 months; (2) 3–5 months; (3) 6–11 months; (4) 1–2 y; (5) 2–3 y; (6) at least 3 y (I-II).

According to symptoms, microbiological, and radiological findings, the patients were classified according to the 2007 ATS (Griffith et al. 2007) NTM case definition into positive and negative groups (Table 4.2).

Table 4.2. Patients with NTM and ATS 2007 criteria positive definitions (Griffith et al. 2007).

Symptoms compatible with NTM	Pulmonary symptoms: Cough, dyspnoea, haemoptysis. Systemic symptoms: Fatigue, fever >37.5 °C, weight loss, decreased appetite, night sweats, lymphadenitis, skin nodules.
Microbiological criteria	At least 2 separate positive sputum cultures, or 1 positive culture from a bronchoscopic sample (lavage or brush) or from transbronchial/ other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM. One positive culture from a skin or lymphatic tissue biopsy.
Radiological criteria	Nodular or cavitary opacities on chest radiographs or bronchiectasis with multiple small nodules on CT/ HRCT and appropriate exclusion of other diagnoses.

Study III. Data from study I–II patients was collected at the time of the first positive NTM culture (+/- 6 months) and one year thereafter as described above (I–II). From study IV, NTM patient records were collected on the electronic form and reviewed at the time of the first positive NTM culture and at patient visits closest (+/- 6 months) and one year thereafter. Relevant patient data for demographic characteristics, smoking habits and alcohol abuse data collected. The microbiological data (NTM positive or negative mycobacterial smears, cultures, and sampling sites) were recorded. Other laboratory findings, and pulmonary function tests were reviewed at the time of positive NTM culture and at patient visits closest (+/- 6 months) to one year thereafter. Further, the radiological findings of chest X-ray and CT scans were collected at the time of positive NTM culture and at patient visits closest (+/- 6 months) to one year thereafter.

Concomitant underlying diseases were categorized as decribed for study I–II (McCabe and Jackson 1962). According to symptoms, in addition to NTM microbiological and radiological findings, patients were classified according to the 2007 ATS NTM case definition into positive and negative groups (Griffith et al. 2007).

In **Study IV,** peripheral blood samples of 35 ml were drawn from patients with NTM and MTB on average during one month of admission to hospital before any antimycobacterial therapy. Plasma and serum were stored frozen at -70°C until analyzed. Data from patients with NTM and MTB, including demographic characteristics, underlying diseases classified according to McCabe and Jackson (McCabe and Jackson 1962), symptoms, laboratory findings, and radiological examinations, were reviewed at the time of recruitment or closest +/-6 months. NTM patients categorized to the 2007 ATS criteria NTM case definition into positive and negative groups (Griffith et al. 2007) (Table 4.2).

4.4 DEFINITIONS

4.4.1 NTM INFECTION AND DISEASE ACCORDING TO ATS CRITERIA 2007

In study I–IV, NTM isolation was defined as NTM infection if patients had at least one positive NTM culture finding from sputum, pulmonary, extarpulmonary, or blood isolate specimens.

In study I–IV, NTM infection was defined as NTM disease if patients were ATS-positive, fulfilling the ATS criteria 2007 from the first positive NTM culture isolation within 1 year of surveillance. Otherwise the patient was categorized as ATS-negative (Table 4.2). Fever was defined when axillary temperature was over 37.5 °C. Weight loss was defined at least 5 kg in 6 months. BMI was defined as underweight when less than 18.5 kg /m^2 and 18.5–24.9 kg /m^2 was defined as normal and over 25.0 kg /m^2 was defined as overweight. In study I, (smoking) patients were divided into non-smokers (those who had never smoked) and smokers (those who were previous or current smokers). Four patients whose smoking history was unclear were categorized as smokers, because probably they have been ex-smokers in their youth according to patient data. Occupations were divided to blue and white collar. The blue collar included farmers, fishermen, vendors, and industrial workers and the white collar included civil servants, office workers, enterprise, business, and industrial administration personal.

4.4.2 C4 DEFICIENCY ANALYSES

In study IV, patients with NTM infection or with NTM disease had C4 deficiency if C4 deficiency was exposed less than two copies of C4A or C4B.

4.4.3 RADIOLOGICAL FINDINGS

The chest X-ray and CT scan findings were classified as infiltrates, nodules, cavities, or bronchiectasis. Further, the findings were categorized to each lobe of the lungs separately, or to diffuse findings in both lungs.

4.4.4 LABORATORY FINDINGS

CRP was defined as elevated when over 10 mg/ml. Sedimentation rate was elevated when over 20 mm/h. Leukocytosis was defined elevated if white blood counter was over 12 x 10^{9}/L. Thrombocytopenia was defined to be when blood platelet count was less than 100 x 10^{9} /L. Alanine aminotransferase (ALT) > 120 U/L in females and > 150 U/L in males, aspartate aminotransferase (AST) >120 U/L in females and > 150 U/L in males, and alkaline phosphatase >300 U/L were defined as elevated liver enzyme conditions. Haemoglobin lower than 120 g/L was defined as anemia. In pulmonary function tests, FVC % (forced vital capacity, % from the predicted value) was defined as obstruction when less than 80%. FEV 1% (forced expiratory volume in 1 s, % from the predicted value) was defined as obstruction when less than 80%. MEF 50% (maximum mid-expiratory flow rate, measured during expiration of 50% of the vital capacity, % from the predicted value) was defined as obstruction when less than 62% (Sovijärvi 2011).

4.5 MICROBIOLOGICAL METHODS

4.5.1 IDENTIFICATION OF MYCOBACTERIA

In study I–IV, patient findings for NTM were detected by the Central Microbiology Laboratory of Helsinki City, later Helsinki University Central Hospital Laboratory (HUSLAB). Clinical samples, except blood samples, were stained with auramine-O-fluorochrome dye and examined microscopically for acid-fast bacilli (AFB). Cultures positive for acidfast bacilli were identified by conventional biochemical tests and hybridization with DNA probes (AccuProbe; Gen-Probe, San Diego, CA, USA). Cultures negative for Mycobacterium tuberculosis and MAC complex were identified by amplification and sequencing of the 16S rRNA gene. Those NTM patients with a positive culture finding for NTM from 2004 to the end of 2009, cultures positive

for acid-fast bacilli were identified by DNA strip assays (GenoType Mycobacterium CM/AS, Hain Lifescience, Nehren, Germany). Nomenclature was changing during the study period and for that reason MAC, *M. avium*, and *M. intracellulare* were all classified as MAC.

4.5.2 IDENTIFICATION OF C4 DEFICIENCY

Blood samples for immunoglobulin, the deficiencies of complement, C4A and C4B gene copy numbers, and phenotype frequencies of the C4 allotypes were analyzed. The numbers of *C4A* and *C4B* genes and allotypes of C4A and C4B proteins were determined, as previously published (Paakkanen et al. 2012), in the HLA Laboratory with international EFI-accreditation (European Federation for Immunogenetics) a division of the Transplantation Laboratory at the Haartman Institute, University of Helsinki. All samples were kept frozen at –70°C. Out of *C4A* genes, CT insertion (a C4-silencing mutation), was reduced. Less than two copies of *C4A* or *C4B* was defined as C4 deficiency.

4.5.3 IDENTIFICATION OF LOW IMMUNOGLOBULIN LEVELS

Levels of IgA, IgM, IgG, and IgG subclasses, C3, C4 levels and CH100 were ascertained.

Plasma IgA, IgM, IgG (Dade Behring BN ProSpec, Marburg, Germany), and IgG subclasses 1–4 (PeliClass BN, Sanquin Reagents, Amsterdam, the Netherlands) were measured by nephelometry according to the manufacturers' instructions. We used the manufacturer's reference values for levels below two standard deviations from the mean to define low immunoglobulin levels.

Plasma concentrations of C4, C3 (Behringwerke AG, Marburg/Lahn, Germany), and CH 100 (Quidel Corporation, San Diego, CA, USA) were analyzed. Low immunoglobulins were defined as Ig G < 6.8.g/l, IgM < 0.6 g/l, IgG1 < 4.9g/l, IgG2< 1.5g/l , IgG3<0.2g/l, IgG4<0.08g/l. Low complements were defined as C3 < 0.50 g/l, C4< 0.12g/l, CH100 < 74%.

4.6 STATISTICAL METHODS

Study I–III.

Patient groups were compared using the independent samples *t* -test for continuous variables. Variables that were skewed to the right were logarithmically (ln)

transformed before analysis (I-II). Mann-Whitney U test was used if the assumption of normality was not achieved using transformations (Study III). The Chi-squared test and Fisher's exact test were used to analyse the dichotomous variables.

The Kaplan–Meier method was used to estimate the median survival times with 95% confidence intervals (CI).

Cox regression multivariable analyses were performed to adjust the survival times for other explanatory factors (CI) (I). The forward stepwise multivariate Cox proportional hazards model was run to find the most important prognostic factors and stepwise model as potential explanatory variables and third, adjusted survival time comparisons were performed using 2 multivariate Cox proportional hazards models (II). The univariate Cox proportional hazards model was used to compare the survival times. The forward stepwise (criterion for entry $p<0.05$) multivariate Cox proportional hazards model for explanatory variables (III). All results were given as hazard ratios (HR) with 95% confidence intervals. Study I data were analyzed using SPSS statistical software (version 15.0; SPSS Inc., Chicago, IL USA), study II using SPSS statistical software (version 17.0; SPSS Inc., Chicago, IL, USA) and study III data using IBM SPSS Statistics for Windows (version 21.0, Armonk, NY, USA, IBM Corp).

Study IV.

All statistical tests were performed using IBM SPSS statistics software (version 20, NY). Differences in proportions between groups were tested by the Chi-square test or by Fisher's exact two tailed test, according to which was appropriate. Continuous variables that were not normally distributed were transformed as such by using logarithmic transformation. The continuous variables were compared using the Student's t-test. Laboratory parameters were assessed as continuous variables and as abnormal values, according to the reference values of the performing Laboratory (HUSLAB).

The Mantel-Haenszel method and the Breslow-Day test were used to test the homogeneity of the odds ratios between males and females. The sample size calculation was based on the prevalence previously reported; 58% prevalence of C4 deficiency in healthy controls and an assumption of C4 deficiency in 80% of NTM patients (Seppänen et al. 2006). A two group c^2 test with a 0.05 two-sided significance level will have 80% power to detect the difference between these prevalences (odds ratio of 2.90) resulted in sample sizes of 47 NTM patients and 141 healthy controls, respectively. P-values of < 0.05 were considered statistically significant.

5 RESULTS

5.1 DEMOGRAPHIC CHARACTERISTICS OF PATIENTS WITH A NTM ISOLATION (I–III)

The demographic characteristics of the patient population in Studies I–III are shown in Table 5.1.1. The average age of patients was 66 years and there was no difference in age between patients with MAC or other isolated NTM. No difference was seen in the age of non-smokers and smokers, but ATS-negative patients were significantly older than ATS-positive patients (70 vs 63 years; p= 0.003, I–II, Table 5.1.1). Whereas smoking did not differ between ATS-negative and -positive patients smoking was significantly more common in patients with other NTM (61%) than in MAC group (61% vs 42%, p= < 0.001, II, III, Table 5.1.1).

In total, 55% of all NTM patients were female. There was a significant overrerpresentation of female gender among non-smokers, ATS-positive, and MAC patients as compared to smokers, ATS-negative, and other NTM patients (I–III, Table 5.1.1). BMI was on an average 21.3 ± 4.2 (kg/m^2, mean ± SD) among all NTM patients. Patients with MAC were significantly leaner as compared to patients with other NTM (20 vs 23 BMI, p= 0.001, III, Table 5.1.1).

Table 5.1.1. Characteristics of 167 patients with non-tuberculous (NTM) isolation categorized according to smoking (Study I), ATS 2007 criteria fulfilment (Study II), and MAC vs other NTM (Study III) (Studies I–III).

		Age Mean (SD)	[c]BMI[d] Mean (SD)	Female	Alcohol abuse	Non-smoker	ATS 2007 positive
	n[b]	years	kg/m^2	%	%	%	%
Study I							
Non-smokers	50	64.1 (17.3)	19.9 (3.0)	72	0	42	58
Smokers	70	68.0 (10.5)	21.4 (4.2)	30	17	0	46
Total	120	66.4 (13.8)	20.8 (3.8)	48	10	42	51
p[a]		0.156[c]	0.061	<0.001[c]	0.001[e]		0.184[a]
Study II							
ATS 2007 Positive	61	62.7 (15.0)	20.3 (3.1)	57	7	48	100
ATS 2007 Negative	59	70.1 (11.3)	21.3 (4.5)	37	14	36	0
Total	120	66.4 (13.8)	20.8 (3.8)	48	10	42	51
p[a]		0.003	0.24	0.03	0.20	0.18	
Study III							
MAC	99	65.6 (14.4)	20.3 (3.7)	70	5	58	61
Other NTM	68	66.7 (13.3)	22.9 (4.3)	34	12	29	49
Total	167	66.0 (13.9)	21.3 (4.2)	55	8	46	56
p[a]		0.624[c]	0.001[c]	<0.001[a]		<0.001[a]	0.123[a]

[a] Chi-squared test
[b] Values are expressed as n (%), unless otherwise stated
[c] *t*-Test for independent samples
[d] BMI, body mass index kg/m^2
[e] Fisher's exact test.

5.2 UNDERLYING DISEASES AMONG PATIENTS WITH A NTM ISOLATION (I–III)

Patients who were non-smokers, ATS positive, or had MAC had significantly less fatal underlying diseases as compared to smokers, ATS negative, and other NTM, respectively (I–III, Table 5.2.1). In total, 67% of all NTM patients lacked any potentially fatal underlying diseases (III, Table 5.1.2). Bronchiectasis was significantly more common among non-smokers (28%) and MAC patients (25%) as compared to smokers (4%) and other NTM patients (5%), but there was no difference in bronchiectasis according to ATS criteria fulfillment (I–III, Table 5.2.1). Only 25% of all NTM patients had no previous pulmonary diseases with no difference according to smoking history or ATS criteria fulfillment (III, Table 5. 2.1). While 25% of ATS-negative patients had a history of asthma, only 10% of ATS-positive patients were asthmatic ($p = 0.02$) but no difference in previous asthma was observed according smoking status or NTM strain (I–III, Table 5.2.1).

Table 5.2.1. Underlying diseases in 167 patients with non-tuberculous (NTM) isolation categorized according to smoking, ATS 2007 criteria fulfilment and MAC vs other NTM strain.

		Underlying diseasec		Underlying pulmonary diseases									
		Healthy or nonfatal disease	Ultimately or rapidly fatal disease	Bronchiectasies	COPD[d]	Prior tuberculosis	Lung fibrosis	Asthma	Other pulmonary disease	Malignancy	No previous pulmonary diseases	Systemic corticosteroid >1 month	Corticosteroid inhalation >1 month
	n[b]	%	%	%	%	%	%	%	%	%	%	%	%
Study I													
Non-smokers	50	82	18	28	4	16	8	18	26	12	28	22	16
Smokers	70	59	41	4	43	26	17	17	23	23	19	30	17
Total	120	68	32	14	27	22	13	18	24	18	23	27	17
p[a]		0.007		<0.001	<0.001	0.203	0.146	0.903	0.692	0.130	0.223	0.329	0.868
Study II													
ATS 2007 Positive	61	77	23	18	20	16	10	10	33	16	26	20	11
ATS 2007 Negative	59	59	41	10	34	27	17	25	15	20	19	34	22
Total	120	68	32	14	27	22	13	18	24	18	23	27	17
p[a]		0.04		0.22	0.08	0.15	0.25	0.02	0.02	0.58	0.32	0.08	0.12
Study III													
MAC	99	77	23	25	23	16	8	14	16	12	27	26	27
Other NTM	68	53	47	7	31	19	13	16	28	22	23	27	13
Total	167	67	33	18	26	17	10	15	21	16	25	26	21
p[a]		0.001[a]		0.003[a]	0.270[a]	0.620[a]	0.279[a]	0.717[a]	0.066[a]	0.087[a]		0.846[a]	0.043[a]

[a] Chi-squared test (or t-test for independent samples
[b] Values are expressed as n (%), unless otherwise stated.
[c] Underlying diseases classified according to the criteria of McCabe classification (McCabe and Jackson 1962) :
1) healthy i.e. no other diseases
2) non-fatal chronic diseases
3) ultimately fatal diseases with expected life expectancy of maximally 5 years (i.e. carcinoma with local spreading)
4) rapidly fatal diseases with expected survival for no more than 6 months (i.e. carcinoma with wide spread metastases
[d] COPD, chronic obstructive pulmonary diseases.

5.3 SYMPTOMS AT THE TIME OF NTM ISOLATION (I–III)

All patients had symptoms suitable for NTM infection at the time of NTM isolation. In almost half (48%) of the patients' symptoms had appeared during the year before a positive NTM culture finding (I–II, Table 5.3.1). Patients with other NTM had symptoms for a shorter time as compared to patients with MAC (54% vs. 34%, p= 0.001, III, Table 5.3.1).

Fatigue was significantly more common in ATS-positive patients than in ATS-negative patients (56% vs. 37%, p= 0.04, II, Table 5.3.1). Fever was significantly more common among MAC than other NTM patients (48% vs. 31%, p = 0.023, III, Table 5.3.1). Instead there was no difference in the occurrence of various symptoms between the groups divided according to smoking status, ATS criteria fulfillment, or MAC vs. other NTM (I-III, Table 5.3.1) like cough, which was the most common symptom and it was reported by two thirds of all patients (III) and dyspnoea, the second most common symptom, which occurred in about half of the patients (III). About one fifth of the patients had haemoptysis and about one third experienced weight loss, whereas non-specific general symptoms like night sweats were fairly uncommon (I–III, Table 5.3.1). Clinically the early presenting symptoms were cough, dyspnoea upon physical exercise and fatigue, but we did not analyze the appearance order or time of different symptoms .

Table 5.3.1. Symptoms and signs at the time of the first positive NTM isolation from 167 patients.

	Duration of symptoms					Respiratory symptoms				Nonspecific symptoms					
		<1 year	1–2 years	3–10years	>10 years	Cough	Dyspnoea	Hemoptysis	Fatigue	Fever >37.5 ºC	Weight loss	Palpitation	Night sweats	Lymphadenitis	Chest pain
	n[b]	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Study I															
Non-smokers	50	50	22	16	12	76	52	28	54	46	36	16	6	8	16
Smokers	70	46	36	6	12	73	51	21	41	39	30	16	4	3	14
Total	120	48	30	10	12	74	52	24	47	42	33	16	5	5	15
p[a]		0.194[c]				0.698[d]	0.951[d]	0.407[d]	0.174[d]	0.416[d]	0.489[d]	0.966[d]	0.693[d]	0.233[d]	0.795
Study II															
ATS 2007 Positive	61	47	36	8	8	75	51	21	56	48	36	11	8	7	13
ATS 2007 Negative	59	48	24	12	16	73	53	27	37	36	29	20	2	3	17
Total[f]	120	48	30	10	12	74	52	24	47	42	33	16	5	5	15
p[a]		0.17[c]				0.75	0.85	0.46	0.04	0.18	0.40	0.18	0.21[d]	0.68[d]	0.56
Study III															
MAC	99	34	36	18	12	80	54	25	47	48	3	12	7	4	14
Other NTM	68	54	26	15	4	72	47	19	32	31	25	13	1	6	15
Total	167	43	32	16	9	77	51	23	41	41	29	13	5	5	14
p[a]		0.01[c]				0.245	0.411	0.353	0.051	0.023	0.307	0.831	0.144[d]	0.717[d]	0.91

[a] Chi-squared test
[b] Values are expressed as, n (%) of patients with valid information unless otherwise stated.
[c] The categories were combined (£2 years vs. [3]3 years) before analysis (Study I, II).
[c] The categories 1-2 y, 3-10 y and > 10 y were combined before analysis (Study III).
[d] Fisher's exact test.
[f] Missing information: Study II: 3 patients (ATS 2007 positive two patients, ATS 2007 negative one patients.),

5.4 MICROBIOLOGICAL FINDINGS (I-III)

Of all 167 patients, MAC was found in 60% (III). It was found in 72% of non-smokers and 41% of smokers (p = 0.001), but no difference was observed according to ATS criteria fulfillment; 61% of isolates in ATS 2007 positive and 47% in ATS 2007 negative patients (p = 0.15, II).

Of the 167 NTM patients, combinations of two different strains were cultured from five patients. These patients were recorded in accordance with their first isolated strain. Patients with these combinations were as follows: MAC + *M. malmoense*, *M. avium* +*M. lentiflavum*, *M. intracellulare* + *M. fortuitum*, *M. abscessus* + *M. fortuitum*, and *M. triplex* + *M. gordonae*.

Smears were negative for a total of 62% of the all patients. A positive sputum culture was obtained from 145 patients out of all 167 patients. NTM was cultured from pulmonary biopsy from seven patients and from bronchoscopic samples from five patients. In four patients, positive NTM culture was found only in a culture from a lymph node (MAC) or blood (*M. chelonae*). Skin culture was positive in six patients: two patients had *M. marinum* and four patients had RGM.

After the first positive NTM culture, a subsequent second positive culture was obtained in 54% of non-smokers and 51% of smokers, in 80% of ATS 2007 positive patients but only in 22% of ATS 2007 negative patients during the following year (Study I Table IV, Study II Table IV).

Table 5.4.1. Microbiological findings in 167 patients with at least one NTM culture positive isolation

		MAC[b]	Other NTM total	M.malmoense	M.fortuitum	M.chelonae	M.abscessus	M.xenopi	M.gordonae	M.marinumd	M.terrae	M.triplex	M.paraniffinicum	Other non-spesificed NTM
	n	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**	**%**
Study I														
Non-smokers	50[a]	72	28	8	4	2	0	2	8	2	0	2	0	0
Smokers	70[a]	41c	59	11	13	6	1	1	17	0	3	0	1	4
Total	120[a]	54		10	9	4	1	2	13	1	2	1	1	3
Study II														
ATS 2007[f] Positive	61[a]	61c	39	13	5	5	2	2	5	2	2	2	0	3
ATS 2007[f] Negative	59[a]	47	53	7	14	3	0	2	22	0	2	0	2	2
Total	120[a]	54	46	10	9	4	1	2	13	1	2	1	1	3
Study III														
MAC	99	59												
Other NTM	68	-	41	22	21	9	23	6	24	3	3	2	2	7
Total	167	59	41	9	8	4	1	2	10	1	1	1	1	3

[a] Values are expressed as n (% within each group)
[b] MAC, Mycobacterium avium complex; NTM, non-tuberculous mycobacteria
[b] MAC includes M. avium, M.avium complex and M. intracellulare
[c] p = 0.001 as compared to non-smokers, Chi-squared test.
[c] p=0.15 as compared to negative ATS 2007, Chi-squared test
[d] skin infection
[f] ATS, American Thoracic Society.

5.5 RADIOLOGICAL FINDINGS (I–III)

Only one smoker had a normal chest X-ray, whereas all other patients who had an X-ray taken had findings suitable for NTM infection. Computer tomographies were performed for 55% of the patients and all demonstrated findings related to NTM disease. Nodules and bronchiectasis were significantly more common among non-smokers, ATS positive patients, and in MAC patients as compared to smokers, ATS negative patients, and other NTM patients, respectively (I-III, Table 5.5.1). Infiltrates were found in 30–40% of patients in groups without significant differences between the groups (I–III, Table 5. 5.1). Cavities were a rare finding and they were observed in only 8% of patients (III, Table 5. 5.1).

The radiological findings were most common in right upper (35% of patients) and right middle lobes (27%), but they were also observed in all other parts of the lung (left lower lobe in 21%, right lower lobe in 17%, left upper lobe in 16% of patients) (I–III, Table 5. 5.1). No difference was seen in the localization of radiological changes between the groups, but diffuse findings were more common in MAC (29%) compared to other NTM (15%) patients (III).

Pulmonary function tests were made only on 53–60% of patients in the various studies. Obstructive impairment observed as a decreased FEV 1% and MEF 50% but no differences between the groups in pulmonary function tests were found (Study I Table V, Study II Table V).

CRP level was elevated in 90% of patients and ESR (mm/h) was elevated in 84% of patients without differences between the patient groups (Study I Table V, Study II Table V). Haemoglobin was an average normal without clear anemia (Study I Table V, Study II Table V). Leukocyte count was an average close to upper normal limit (Study I Table V, Study II Table V).

Table 5.5.1. Radiological findings during the first year after NTM isolation categorized according to smoking, the ATS 2007 criteria fulfillment and MAC vs other NTM

		Radiographical examinations[g,h,i]		Radiographical findingse				Location of radiographical findingse					
		Abnormal X-ray	Abnormal CT	Infiltrates	Nodules	Cavities	Bronchiectasis	Right upper lobe	Left upper lobe	Right middle lobe	Right lower lobe	Left lower lobe	in both lungs
	n	%	%	%	%	%	%	%	%	%	%	%	%
Study I[g]													
Non-smokers	50	84	50	33	33	10	31	21	13	21	21	35	27
Smokers	70	91	41	38	7	4	6	37	22	27	13	21	15
Total	120	88	45	36	18	7	16	30	18	24	16	27	20
p[a]		0.211	0.352	0.630	<0.001	0.270[f]	<0.001	0.066	0.188	0.485	0.276	0.075	0.100
Study II													
ATS 2007 Positive	61	92	59	42	27	12	23	33	15	26	21	28	21
ATS 2007 Negative	59	88	30	30	9	2	9	27	22	22	11	26	18
Total	120	90	45	36	18	7	16	30	18	24	16	27	20
p[a]		0.46[c]	0.002	0.18	0.01	0.06[f]	0.03	0.52	0.32	0.58	0.13	0.77	0.67
Study III													
MAC	99	94	56	42	34	10	31	38	14	28	18	21	29
Other NTM	68	90	53	40	12	6	13	31	19	25	15	22	15
Total	167	92	55	41	26	8	24	35	16	27	17	21	23
p[a]				0.842	0.003	0.478	0.009	0.417	0.315	0.697	0.638	0.812	0.034

[a] Chi-squared test
[b] Values are expressed as n (%) of patients with valid information
[d] CT, computer tomography,
[e] Multiple locations were possible
[f] Fisher's exact test
[g] Missing information I-II: Radiographical examinations 2 patients. Radiographical findings 3 patients. Location of radiographical findings: 4patients
[h] Missing information study III: Radiographical findings: 6 MAC patients, 6 other NTM patients (no x-ray 12 patients). Location of radiographical findings: 4 patients.
[i] No-x-ray 11 patients Study I-II (Study I: 7 non-smokers and 4 smokers. Study II: 5 ATS –positive and 6 ATS -negative). No-x-ray 12 patients Study III (6 MAC and 6 other NTM patients).

5.6 PROGNOSTIC FACTORS

5.6.1 SMOKING AS A PROGNOSTIC FACTOR (I)

Smokers had a significantly higher risk of death as compared to non-smokers (HR 1.64, 95% CI 1.00–2.69, $p = 0.049$, Study I, Figure 1, panel A). The median survival time for non-smokers was 155 months (=12.9 years) which was significantly longer than that for smokers (64 months i.e. 5.3 years) ($p = 0.047$, Study I, Figure 1) and (Table 5.6.1). Severe underlying diseases (McCabe classes 3–4) were found to be the most important predictor of mortality (HR 4.69, 95% CI 2.82–7.79, $p < 0.001$, Study I, Figure 1, panel C). After adjusting for underlying diseases, smoking was not significantly associated with higher mortality (HR 1.18, 95% CI 0.71–1.97, $p = 0.529$, Study I, Figure 1, panel C). The median survival time for smokers with McCabe classes 3–4 was only 1.7 years as compared to 2.0 years in non-smokers with McCabe classes 3–4 (I, Table 5.6.1).

5.6.2 ATS 2007 CRITERIA AS A PROGNOSTIC FACTOR (II)

ATS 2007 criteria fulfillment was not observed to be a significant predictor of fatal outcome. The median survival time for ATS-positive patients was 7.4 years (95% CI 0.2–14.6) as compared to 5.3 years (95% CI 3.0–7.6) for ATS-negative patients (HR 0.68, 95% CI 0.43–1.09, $p = 0.11$, Study II, Figure 1, Table 5.6.1). ATS criteria fulfillment was not a significant explaining factor for fatal outcome in the multivariate Cox proportional hazards model in which McCabe class was found to be a significant predictor for mortality (HR 4.76, 95 % CI 2.90-7.81, p>0.001, Study II, Figure 2). Figure 2 (Study II) shows that no difference in mortality was observed between the ATS-positive and ATS-negative groups when they were categorized according to McCabe classes. The median survival time for patients in McCabe classes 3–4 and ATS 2007 criteria positivity was 1.9 (1.5–2.3) years and 1.9 (0.9–3.0) years also in ATS 2007 negative patients (HR = 0.82, 95% CI 0.51–1.33, $p = 0.42$, Study II, Figure 2, Table 5.6.1).

5.6.3 ANTI-MYCOBACTERIAL MEDICATION AS A PROGNOSTIC FACTOR (II)

Out of all 120 patients (Studies I–II) anti-mycobacterial medication with at least 2 potentially effective drugs for at least 3 months was given to 60% (72/120) of patients. In 57% of patients, the medication was started within 6 months of the first NTM isolation. Treatment for 3 months was given to 20% of patients whereas 8% of patients received medication for 6 months, 20% for at least 12 months, and 12%

for over 12 months. Only 13% of patients who received any medication were given drugs that were not effective against the isolated NTM strain. Medication was given to 72% (44/61) of ATS-positive and 47% (28/59) of ATS-negative patients (Study II). Patients who received antimycobacterial medication in ATS 2007 positive and ATS 2007 negative groups did not have a difference in mortality as compared to corresponding patients without treatment (HR 1.14, 95% CI 0.69–1.87, p = 0.61, Study II, Figure 3, Table 5.6.1).

5.6.4 MAC AS A PROGNOSTIC FACTOR (III)

The median survival time was 13.0 years (95% CI 5.9–20.1) for all MAC patients and 5.2 years (2.3–8.1) for all other NTM patients (III, Table 5.6.1). In the subgroup analysis patients with pulmonary MAC had a significantly lower risk of death as compared to patients with pulmonary infection due to other NTM [HR 0.50 (95% CI 0.33–0.77), p = 0.002, Study III, Fig. 1, panel A] or a subgroup of other slowly growing NTM (*M. malmonese, M. xenopi, M. paranifficum, M. terrae, M. triplex*) [(HR 0.55 (95% CI 0.31–0.99), p = 0.048), Study III, Fig.1, panel B] or as rapidly growing NTM [(HR 0.47 (95% CI 0.25–0.87), p = 0.02), Study III, Fig. 1, panel C]. Median survival time was 13.0 years (95% CI 5.9–20.1) for pulmonary MAC patients but 4.6 years (95% CI 3.4–5.9) for pulmonary infections due to other NTM (Study III, Fig.1, panel A).

For all patients, when fulfillment of ATS 2007 criteria and fulfillment of MAC were predictors in the same multivariate Cox proportional hazards model, the survival among the MAC group was significantly higher than that of the other NTM group (HR = 0.57, 95% CI 0.37–0.88, p = 0.01, Study III, Fig. 2, panel A). Furthermore, survival among the ATS 2007 positive patients tended to be higher than among the ATS 2007 negative patients [HR = 0.66, 95% CI 0.44–1.01, p = 0.06, Fig. 2, panel A] (III, Table 5.6.1.). The effect of ATS 2007 criteria fulfillment was non-significant in reducing the mortality risk (HR = 0.66, 95% CI 0.44–1.01, p = 0.06, Study III, Fig. 2, panel A). When fatal underlying diseases (McCabe 3–4) and fulfillment of MAC were predictors in the same multivariate Cox proportional hazards model, the survival among MAC group was significantly higher than in other NTM group [HR = 0.65, 95% CI 0.42–1.00, p = 0.048, Study III, Fig.2, panel B], (III; Table 5.6.1). The effect of McCabe 3–4 as a risk factor for fatal outcome was also significant (HR = 3.33, 95% CI 2.16–5.12, p < 0.001, Study III, Fig. 2, panel B, Table 5.6.1).

Table 5.6.1. Survival of NTM patients (Studies I, II, and III). Results are given in groups according to smoking status, ATS 2007 criteria fulfillment, and MAC or other NTM isolation, and further divided into subgroups by McCabe classification and treatment with antimycobacterial medication for at least 3 months.

			Survival time 1 (years)	Survival rates (%) 1		
		N	Median (95% CI)	1 yr	5 yrs	10 yrs
Study I (Smoking)						
Smoking +		70	5.3 (4.3–6.3)	82.9	54.3	35.1
Smoking –		50	12.9 (4.8–21.0)	96.0	68.0	56.0
Overall		120	6.1 (2.9–9.3)	88.3	60.0	43.7
Smoking and ATS 2007						
smoking +	ATS 2007 +	32	5.3 (4.7–5.9)	90.6	59.4	37.2
smoking +	ATS 2007 –	38	4.6 (1.8–7.4)	76.3	50.0	33.5
smoking –	ATS 2007 +	29	12.9 *	100.0	75.9	62.1
smoking –	ATS 2007 –	21	5.9 (0.0–12.0)	90.5	57.1	47.6
Smoking and McCabe						
smoking +	McCabe 1–2	41	15.6 (4.9–26.4)	92.7	78.0	50.5
smoking +	McCabe 3–4	29	1.7 (1.0–2.4)	69.0	20.7	13.8
smoking –	McCabe 1–2	41	12.9 *	100.0	78.0	65.9
smoking –	McCabe 3–4	9	2.0 (1.8–2.1)	77.8	22.2	11.1
Smoking and medication (>3 mo)						
smoking +	medication –	27	5.6 (4.2–7.0)	81.5	63.0	25.4
smoking +	medication +	43	4.6 (3.0–6.3)	83.7	48.8	37.2
smoking –	medication –	21	*	100.0	71.4	52.4
smoking –	medication +	29	12.9 (4.4–21.4)	93.1	65.5	58.6
Study II (ATS 2007)						
ATS 2007 +		61	7.4 (0.2–14.6)	95.1	67.2	49.1
ATS 2007 –		59	5.3 (3.0–7.6)	81.4	52.5	38.0
Overall		120	6.1 (2.9–9.3)	88.3	60.0	43.7
ATS 2007 and smoking						
ATS 2007 +	smoking +	32	5.3 (4.7–5.9)	90.6	59.4	37.2
ATS 2007 +	smoking –	29	12.9	100.0	75.9	62.1
ATS 2007 –	smoking +	38	4.6 (1.8–7.4)	76.3	50.0	33.5
ATS 2007 –	smoking –	21	5.9 (0.0–12.0)	90.5	57.1	47.6
ATS 2007 and McCabe						
ATS 2007 +	McCabe 1–2	47	*	97.9	76.6	59.5
ATS 2007 +	McCabe 3–4	14	1.9 (1.5–2.3)	85.7	35.7	14.3
ATS 2007 –	McCabe 1–2	35	10.2 (6.0–14.4)	94.3	80.0	55.2
ATS 2007 –	McCabe 3–4	24	1.9 (0.9–3.0)	62.5	12.5	12.5
ATS 2007 and medication						
ATS 2007 +	medication –	17	*	100.0	76.5	52.3
ATS 2007 +	medication +	44	7.0 (0.5–13.6)	93.2	63.6	47.7
ATS 2007 –	medication –	31	5.9 (3.0–8.7)	83.9	61.3	29.0

ATS 2007 –	medication +	28	4.0 (2.5–5.5)	78.6	42.9	42.9
Study III (MAC)						
MAC +		99	13.0 (5.9–20.1)	94.9	73.6	53.8
MAC –		68	5.2 (2.3–8.1)	82.4	52.7	33.9
Overall		167	8.2 (5.1–11.2)	89.8	65.1	46.3
Mac and smoking						
MAC +	smoking +	42	7.5 (0.6–14.4)	90.5	69.0	49.2
MAC +	smoking –	57	*	98.2	77.0	57.1
MAC –	smoking +	48	4.6 (2.7–6.5)	79.2	47.9	27.6
MAC –	smoking –	20	12.9	90.0	64.6	52.6
Mac and McCabe						
MAC +	McCabe 1–2	76	*	97.4	80.2	60.2
MAC +	McCabe 3–4	23	5.3 (1.2–9.4)	87.0	52.2	33.2
MAC –	McCabe 1–2	36	12.9 (8.0–17.8)	94.4	80.3	50.1
MAC –	McCabe 3–4	32	1.9 (1.1–2.7)	68.8	21.9	15.0
Mac and medication						
MAC +	ATS 2007 +	60	*	96.7	78.3	55.0
MAC +	ATS 2007 –	39	10.2 (4.2–16.2)	92.3	66.5	52.2
MAC –	ATS 2007 +	33	7.4 (3.4–11.3)	93.9	60.2	46.2
MAC –	ATS 2007 –	35	4.3 (1.4–7.2)	71.4	45.7	21.2

[1] Kaplan-Meier method was used to estimate the median survival time and the survival rates at 1, 5, and 10 years after the date of NTM diagnosis.

* The median survival time or 95% CI cannot be estimated because high proportion of cases at risk are censored.

5.7 COMPLEMENT C4 AND IMMUNOGLOBULIN LEVELS IN NTM AND MTB PATIENTS (IV AND UNPUBLISHED RESULTS OF MTB PATIENTS)

5.7.1 NTM AND MTB PATIENT CHARACTERISTICS AND MYCOBACTERIAL STRAINS

Complement C4 levels and phenotype and serum immunoglobulin levels in 50 patients with a NTM isolation were compared to 31 patients with tuberculosis (MTB) and to 149 healthy controls. The data from NTM patients and healthy controls has been published in Study IV but the results of tuberculosis patients have not been published elsewhere. All NTM infection patients (50/81, 62%) were of European descent. Forty-four patients had pulmonary NTM infection whereas six had extra pulmonary NTM infection. The MTB patient group accounted for 31/81 (38%) of the study patients. Of these MTB patients, the pulmonary samples were culture positive in 30 patients and a lymphatic node culture positive in the remaining one patient. Two MTB patients were of African origin, whereas all others were of European descent (Table 5.7.1).

There were significantly more females in the NTM patient group (72%) compared with the MTB patients (29%). The MTB patients were predominantly male and significantly younger than patients of the NTM group (Table5.7.1). Notably, NTM patients had a higher incidence of ultimately or rapidly fatal disease than the MTB patients (McCabe classes III–IV, 18/50[36%] vs. 3/31 [10%], respectively, $p = 0.009$, Table 5.7.1). The NTM patients had a higher incidence of bronchiectasis than the MTB patients (15/50 [30%] vs. 3/31 [10%], $p = 0.032$). The NTM patients showed a significantly higher proportion of rheumatoid arthritis (8/50 [16%] vs. 0/31[0%], $p = 0.021$, Table 5.7.1) than MTB patients.

MAC comprised 36/50 (72%) and RGM (M. fortuitum, M. chelonae, M. abscessus) was found in (6/50) 12% of all NTM isolations. *M. xenopi* was found in two patients, and *M. malmoense* in three patients. Other NTM had two patients and *M. marinum* was found in one patient. NTM and M. tuberculosis (MTB) concomitant infections were not found. All 31 MTB patients were *Mycobacterium tuberculosis* culture positive and 24 were also sputum smear positive.

Table 5.7.1. Characteristics and underlying diseases of 81 patients with a nontuberculous mycobacteria (NTM) or *Mycobacterium tuberculosis* (MTB) isolation. The data only partly published in Study IV Values are expressed as no (%) of patients with valid information unless otherwise stated.

	NTM n=50		MTBd n=31		Totald n=81		p a
	no.	(%)	no.	(%)	no.	(%)	
Age median (IQR) yr	65 (23–90)		50 (19–80)				<0.001
Female	36	(72)	9	(29)	45	(56)	<0.001
Pulmonary mycobacterial infection	44	(88)	30	(97)	74	(91)	0.077 b
Mycobacterial infection in lymph nodes	1	(2)	1	(3)	2	(2)	1.00 b
Mycobacterial infection cutaneous	5	(10)	0	(0)	5	(6)	0.151 b
Underlying diseases c							
Healthy or non-fatal diseases (1–2)	32	(64)	28	(90)	60	(74)	0.009
Ultimately or rapidly fatal diseases (3–4)	18	(36)	3	(10)	21	(26)	
Underlying pulmonary diseases							
Bronchiectasis	15	(30)	3	(10)	18	(22)	0.032
COPD	13	(26)	7	(23)	20	(25)	0.729
Prior tuberculosis	3	(6)	5	(16)	8	(10)	0.249 b
Asbestosis	2	(4)	3	(10)	5	(6)	0.366 b
Pulmonary fibrosis	1	(2)	0	(0)	1	(1)	1.00f b
Asthma	4	(8)	2	(6)	6	(7)	1.00 b
Pulmonary or other malignancy	6	(12)	2	(6)	8	(10)	0.704 b
No previous pulmonary diseases	16	(32)	13	(42)	29	(36)	0.365
Rheumatoid arthritis	8	(16)	0	(0)	8	(10)	0.021b
Other autoimmune disease	3	(6)	0	(0)	3	(4)	0.282 b

[a] Chi-squared test for MTB vs NMT.
[b] Fisher ' s exact test.
[c] Underlying diseases classified according to the criteria of the McCabe classification (viitetiedot):
1) healthy i.e. no other diseases
2) non-fatal chronic diseases
3) ultimately fatal diseases with expected life expectancy of maximally 5 years
4) rapidly fatal diseases with expected survival for no more than 6 months.
[d] Unpublished MTB patients data

5.7.2 COMPLEMENT ANALYSES

C4 deficiency (*C4A or C4B*) was more common among the NTM patients than healthy controls (36/50 [72%] vs 83/149 [56%], OR = 2.05, 95% CI = 1.019–4.105, p = 0.042). *C4* deficiency was also more prevalent in the NTM patients than in the MTB patients 36/50 [72%] vs 11/31 [35%], OR=4.071, 95% CI=1.574–10.533, p = 0.003, unpublished, Table 5.7.2). When the deficiencies of *C4A* and *C4B* were assessed individually, *C4A* deficiency seemed to account for the difference observed in patients with the NTM vs the MTB (13/50 [26%] vs 2/31 [6%], OR 5.095 95%CI=1.06- 24.39, p = 0.028, respectively, unpublished, Table 5.7.2). In contrast, there was no statistically significant difference in the frequency of the *C4B* deficiency.

As the majority of the NTM patients were female, *C4* deficiencies were assessed in females only. The females in the healthy control group were compared with females in the NTM patient group, but not in the MTB group due to low female number in the MTB patient group (Study IV, Table 3). Female NTM patients had more frequent C4 deficiencies compared to controls (29/36 [81%] vs 55/100 [55%], OR = 3.39, 95% CI = 1.358–8.460, p = 0.007, Study IV). This difference in C4 deficiency was especially seen in *C4B* deficiency (20/36 [56%] female NTM patients vs 38/100 [38%] in female controls, p = 0.027, respectively, Study IV Table 3). No difference was seen between female NTM and control patients in the frequency of *C4A* deficiency.

Table 5.7.2. The *C4* deficiencies in patients with nontuberculous mycobacteria (NTM), Mycobacterium tuberculosis (MTB) and in healthy control population (H). The data is only partly publsihed in Stady IV. Values are expressed as no. (%) of patients, unless otherwise stated.

	NTM n=50		MTB[d] n=31		H n=149		NTM vs MTB[d]		NTM vs H	
Type of deficiency	**no.**	**(%)**	**no.**	**(%)**	**no.**	**(%)**	**OR (95 % CI)**	**p [a]**	**OR a (95 % CI)**	**p [a]**
C4A <2	13	(26)	2 b	(6)	24	(16)	5.095 (1.06–24.39)	0.028	1.83 (0.85–3.95)	0.120
C4B <2	25	(50)	10	(32)	61	(41)	2.100 (0.824–5.350)	0.117	1.44 (0.76–2.75)	0.263
C4A or C4B < 2	36c	(72)	11	(35)	83	(56)	4.071 (1.574–10.533)	0.003	2.05 (1.019–4.105)	0.042
C4 < 4	22	(44)	5	(16)	56	(38)	4.086 (1.35–12.37)	0.010	1.31 (0.85–1.47)	0.421

OR = odds ratio.
CI= confidence interval
[a] p values for differences between groups.
[b] CT insertion is deleted.
[c] Two patients had both C4A <2 and C4B<2 deficiency, one patient had a total C4A deficiency
[d] Unpublished MTB patients

In the phenotype frequencies of the C4 allotypes, the A3, Q0 allotype was found in 12/50 (24%) of the NTM patients but in only 2/31 (6%) of the MTB patients (p = 0.042), which reflected the increased frequency of C4A deficiency in NTM patients (Table 5.7.3) (Table 4). We observed that the C4 allotype, A3, 3 was less common in the NTM patients (11/50, 22%) as compared to the healthy controls (58/149 [39%], p = 0.030, Study IV, Table 5) but not different from the MTB patients (data not shown). The allotype B1, 2 was found in only 6% of the NTM patients whereas it was seen in 20% of the healthy controls (p = 0.020, Study IV, Table 5).

Table 5.7.3. The most common C4 phenotypes in patients infected with a nontuberculous mycobacteria (NTM), *Mycobacterium tuberculosis* (MTB) and Healthy group (H). The data has only been partly published in Stady IV.

	NTM=50 [a]		MTB[e] n=31[a]		Total[e] n=81[a]		p [b]	H n= 149		p [b]
	no.	(%)	no.	(%)	no.	(%)		no.	(%)	
C4A phenotype [c]										
3	12	(24)	2	(6)	14	(17)	0.042	21	(14)	0.103
3,2	3 n	(6)	1	(3)	4	(5)	1.000 [d]	9	(6)	1.000 [d]
3,3	11	(22)	11	(35)	22	(27)	0.185	58	(39)	0.030
3,3,2	8	(16)	6	(19)	14	(17)	0.698	18	(12)	0.477
3,3,3	7	(14)	6	(19)	13	(16)	0.547 [d]	17	(11)	0.626
C4B phenotype										
0	6	(12)	2	(6)	8	(10)	0.704 [d]	15	(10)	0.700
1	15	(30)	7	(23)	22	(27)	0.466	37	(25)	0.464
1,1	16	(32)	11	(35)	27	(33)	0.746	52	(35)	0.708
1,2	3	(6)	2	(6)	5	(6)	1.000 [d]	30	(20)	0.020
1,3	2	(4)	3	(10)	5	(6)	0.366 [d]	3	(2)	0.601[d]
2	3	(6)	1	(3)	4	(5)	1.000 [d]	6	(4)	0.694 [d]
2,2	0	(0)	2	(6)	2	(3)	0.144 [d]	5	(3)	0.333 [d]

[a] Values are expressed as no. (%) of patients with valid information unless otherwise stated.
[b] p Chi-squared test
[c] One patient with C4A total deficiency
[d] Fisher's exact test.
[e] Unpublished MTB patients

A C4 deficiency (C4A or C4B deficiency) was found in 31/44 (70%) of the NTM patients with pulmonary infection and in 22/29 (76%) of those pulmonary NTM patients who met all ATS 2007 criteria. C4B deficiency was observed in all five patients with a cutaneous NTM infection fulfilling the ATS 2007 criteria. Interestingly, 67% of patients, who did not fulfill the radiological criteria but who gave several positive NTM sputum culture samples, had a C4 deficiency.

5.7.3 IMMUNOGLOBULIN CONCENTRATIONS AND OTHER LABORATORY MEASUREMENTS

Nobody had a low IgA concentration. The number of patients with low IgG and IgM levels was too low for statistical comparison (data not shown). The NTM patients had slightly lower plasma C3 concentrations compared with the MTB patient group. The CH100 was slightly higher in the NTM patients than in the MTB patients (data not shown). Serum C-reactive protein (CRP) levels, reviewed at the time of recruitment or closest +/- 6 months were significantly lower in the NTM patients than in the MTB patients (median [IQR] 6.50 [16%] vs 28.0 mg/l [46%], respectively, p = 0.008). In contrast, no difference in erythrocyte sedimentation rate was observed between groups.

6 DISCUSSION

6.1 GENERAL DISCUSSION

We evaluated clinical and immunological findings in Finnish NTM patients. The strength of this study is due to its material; samples for mycobacterial cultures were analyzed in the same microbiological laboratory by the Central Microbiology Laboratory of Helsinki City, later Helsinki University Central Hospital Laboratory (HUSLAB). All data relevant to NTM infection was collected carefully from patient records. The NTM patient population was homogenous and there were no cases with a family history of a mycobacterial infection. The median (min-max) follow-up time of these patients was 7.0 years. By using this method we were able to form an opinion of clinical symptoms and signs, whereas previous studies have consisted of mostly nationwide registers without clinical findings of patients' symptoms. Moreover, a statistician was included to the study group and statistical approaches were considered.

Both non-smoking and smoking patients revealed new information about NTM infections. Symptom onset commenced within a year before the first isolation of NTM, which revealed that the course of NTM disease may actually be quick. MAC pulmonary disease and bronchiectasis were more common among non-smoking elderly women without underlying pulmonary diseases compared to smokers. No significant association was found between smoking and high mortality, instead, severe underlying diseases were the most important predictors of mortality.

We evaluated the "gold standard" ATS 2007 criteria for the first time. Many patients with NTM infection fail to meet the criteria. Obviously categorical methods are also leading to insufficient diagnosis of pulmonary NTM. Thus, the ATS 2007 criteria seemed to be a weak prognostic factor. Namely, patients with MAC had three-times-longer survival than patients with other NTM.

The clinical signs suggested immunological deficiency. Therefore we investigated the association between NTM infections and complement C4 deficiency, which has not been studied previously. We found complement C4 deficiency to be a plausible risk factor for NTM infection.

A weakness of this study includes small patient numbers, which may cause bias and therefore larger studies to confirm the current findings are obviously required. Nonetheless, the results of this study are generalizable only to other areas in Finland or in Scandinavia. This study might assist clinicians and students in infectious and lung departments with NTM-disease patients, because these patients regularly visit the clinics and require intensive healthcare and frequent hospitalizations.

To find NTM-infection patients for timely diagnosis, ATS 2007 criteria alone are insufficient. Namely, patients with NTM other than MAC have a poor prognosis. Smoking alone as a risk factor fails to account for poor prognosis. Moreover, those NTM patients without risk factors seemed to have a local immunodeficiency.

Patients with NTM infections are in need of multi-center studies, where timely clinical symptoms and signs, as well as microbiological findings, are observed in proportion to radiological findings. We demonstrated that preliminary treatment with the anti-mycobacterial agents had no impact on prognosis. Therefore, treatment of NTM infections requires deeper insight, where severe underlying diseases and local immunological defects will be taken into consideration.

6.2 RISK FACTORS FOR NTM INFECTION

6.2.1 SMOKING AS A RISK FACTOR FOR NTM INFECTION

The smoking group had more smoking-related lung diseases like COPD and also more other serious underlying diseases than non-smokers. In this study, life-time smoking abstinence was a requirement for non-smokers status. In fact, altogether more than half of smokers in this study had smoked for longer than 30 years and only one fifth had smoked for less than 10 years. This suggests that long-lasting smoking seems to be at least a contributing factor for NTM infection as suggested also in other studies (Sonnenberg et al. 2000, Maliwan et al. 2005, Varadi and Marras 2009).

Smoking with COPD, pneumoconiosis, bronchiectasis, prior tuberculosis, alcoholism or pulmonary alveolar proteinosis have been classified as risk factors for pulmonary MAC infection (Falkinham 1996). Although, smoking related diseases and other pulmonary diseases are well recognized risk factors for NTM infection, the data on the effect of smoking itself on the risk of acquiring a NTM infection is scarce and may be biased by the fact that in many studies previous smokers have been regarded as non-smokers (Prince et al. 1989, Kubo et al. 1998, Huang et al. 1999). COPD with inhaled corticosteroid therapy was found to be strong risk factor for NTM pulmonary disease even in a recent study (Andréjak et al. 2013).

Smoking may not be the main underlying factor for pulmonary NTM infections, however, because less than half of all NTM patients had never smoked. This is consistent with another patient cohort where 68% of the patients with NTM were lifetime non-smokers (Kim et al. 2008). Non-smokers also had less often previous pulmonary diseases. More than fourth quarter of non-smokers in our study and less than half of patients in a previous study showed no previous pulmonary diseases (Prince et al. 1989, Kubo et al.1998, Huang et al. 1999, Henry et al. 2004 Kim et al. 2008). The most common pre-existing pulmonary disease among non-smokers was

bronchiectasis which was found in more than a quarter of them but very rarely of smokers. Bronchiectasis has been found more often in non-smoking female patients with chronic obstructive lung disease (Wittram et al. 2002, Wickremasinghe et al. 2005). Non-smokers also had serious underlying diseases less often and their infection was more often due to MAC than other NTM species. MAC has also been observed more often in previously healthy persons (Kubo et al. 1998, Huang et al. 1999, Field et al. 2004). These differences suggest that NTM infection among smokers and non-smokers might often be different in pathogenesis and underlying factors. However, no difference in symptom duration, occurrence of various symptoms, or other clinical parameters were seen except some more peculiar differences which might also point out the differences in infection between smokers and non-smokers. Non-smokers had a tendency for lower BMI and they had higher serum CRP and alkaline phosphatase levels. Non-smokers also presented a three fold higher incidence of nodular findings when compared to smokers.

Interestingly, there was a striking gender difference in relation to smoking status. Namely, men constitute majority of smokers, but less than a quarter of non-smokers. Also according to recent national statistics 77% of never smokers are women among persons over 65 years-of-age and the corresponding figures for people between 45–64 years were 37% males (Varis and Virtanen 2013). In conclusion, smoking related pulmonary diseases predispose men for NTM infection and seems to shorten their survival. Smokers with COPD and severe underlying diseases are included to the other NTM group, which had significantly shorter median survival than MAC group.

6.2.2 AGE AND GENDER AS RISK FACTORS FOR NTM INFECTION

Pulmonary NTM infections are encountered in middle-aged or older adults. The mean age of all patients with NTM infection was 66.0 years. There were only 12 patients that were under 50 years-of-age and had a pulmonary NTM isolation. There is no data whether the occurrence of NTM infections in middle-aged or older is due to local pulmonary changes or immunological factors, but younger individuals seem to be largely protected from NTM infection. However, host defense and immunological aging have been suggested as a predisposing reason for NTM infection (Beerman et al. 2010).

NTM isolations were quite evenly distributed between males and females when all NTM isolations were reviewed. Nonetheless, a majority of patients with MAC were female and a majority of MAC patients in this study had no underlying pulmonary diseases. The striking female predominance among healthy patients with a NTM infection has also puzzled researchers in many previous studies where female proportion has ranged from 21% up to 95% of all patients (Prince

et al. 1989, Reich and Johnson 1991, Huang et al 1999, Kim et al. 2008). Elderly females have long been recognized as a risk group for pulmonary MAC infection for unknown reasons (Prince et al. 1989, Huang et al. 1999, Guide and Holland 2002, Kim et al. 2008). Moreover, elderly, slender female patients with NTM infection have often had bronchiectasis (Kim et al. 2008, Piersimoni and Scarparo 2008). Bronchiectasis and nodules were more common in ATS-positive as compared to ATS-negative patients. The classical patterns of the NTM disease with cavitary disease in middle-aged smoking men and fibronodular disease in elderly, non-smoking women were uncommon both in our patient material and in other recent reports (Prince et al. 1989, Kubo et al. 1998, Dailloux et al. 2006, Piersimoni and Scarparo 2008). Although, the reason for this female predominance with a specific clinical morphotype is unclear, it has been suggested that females with pulmonary NTM might form a specific group with as yet undefined predisposing factors (Guide and Holland 2002, Taiwo and Glassroth 2010).

In conclusion, the men with NTM other than MAC, and who reported severe smoking-related diseases more frequently, had poor outcome. These gender and age findings are consistent with recent studies, in which *M. kansasii* pulmonary infection was found in two third of cases among middle age smoking males with COPD and who had 11% mortality within 40 years (Maliwan et al. 2005). Equally, *M. xenopi* pulmonary infection was found in two thirds of cases among smoking males with COPD (van Ingen et al. 2008) and *M. malmoense* pulmonary infection in two thirds of cases among middle-aged males with 13–15% mortality (Hoefsloot et al. 2009). In a retrospective cohort study of 124 patients, the majority was smoking males with *M. malmoense* or *M. xenopi* pulmonary infection which was related in a quarter of observed mortalities (Commans et al. 2014). Also, in a large register-based Danish study, male patients had worse outcome than females and *M. malmoense* and *M. xenopi* pulmonary infections were related to half of mortality as compared to infections due to other NTM species (Andréjak et al. 2010).

6.2.3 PREVIOUS PULMONARY DISEASES AS A RISK FACTOR FOR NTM INFECTION

In this study, two thirds of patients in both MAC and other NTM groups had a previous pulmonary disease which also points out those NTM infections are mainly seen in patients with at least some degree of previous pulmonary pathology. COPD was also the most common previous pulmonary disease and it was observed in a quarter of all patients. A quarter of patients, however, had no previous pulmonary diseases. Asthma was diagnosed in less than a quarter of patients, and together with COPD it explains the use of corticosteroids which was observed with systemic dosing

in one quarter and by inhalation in one fifth of patients. Pre-existing lung diseases, such as chronic bronchitis with emphysema or bronchiectasis have been traditionally related to pulmonary NTM infections (Field and Cowie 2004, Wickremasinghe et al. 2005, Piersimoni and Scarparo 2008). Even in the latest large Danish population based-study, COPD was strongly linked to pulmonary NTM infection (Andréjak et al. 2013).

Bronchiectasis was the second most common pulmonary disease seen at the time of diagnosis. Both bronchiectasis and nodules were found for the most part in the non-smoking group and in ATS-positive patients. Further, bronchiectasis were concentrated to MAC group who had suffered from symptoms of NTM disease for longer and who also had longer mean survival time than patients with other NTM species. These facts would comply for the impression that MAC would be a slowly progressive process and bronchiectasis would be formed during it (Huang et al. 1999, Barker 2002, Griffith 2010). It seems unclear, however, whether bronchiectasis is a cause of NTM disease or a predisposing factor. This is substantiated by findings in this study where two thirds of non-smokers were female and over two thirds of non-smokers had experienced symptoms for less than 2 years, and yet bronchiectasis was observed in more than one fifth of them.

In line with previous studies one fifth of patients had a prior history of pulmonary tuberculosis (Dailloux et al. 2006, Varadi and Marras 2009, Billinger et al. 2009, Winthrop et al. 2010, Ito et al. 2015). Higher prevalence of tuberculosis has been reported in African gold miners with *M. kansasii* and MAC infection with half of prior pulmonary tuberculosis cases (Sonnenberg et al. 2000). Structural deformities like scoliosis or pectus excavatum have previously been linked to NTM infection but they were rare in this study (Guide and Holland 2002). In conclusion, bronchiectasis was concentrated to the MAC group, and related to longer survival compared to other NTM group. This suggests that bronchiectasis in MAC patients is a slowly progressive disease, which is in line with other studies (Huang et al. 1999, Barker 2002, Griffith 2010). In contrast, COPD, prior tuberculosis, malignancies and other pulmonary diseases included to severe underlying diseases and concentrated to smokers, to ATS 2007 negative patients and to other NTM group with short survival. These findings should be taken into account in diagnosing and in the treatment of NTM patients.

6.2.4 SEVERE UNDERLYING DISEASES AS RISK FACTORS FOR NTM INFECTION

In this study, the classification of McCabe and Jackson (McCabe and Jackson 1962) was used mainly to be able to analyze the importance of diseases with potential lethal outcome on prognosis. Severe underlying diseases classified either as ultimately or

rapidly fatal were quite common and were observed in one third of patients. The severe underlying diseases were more common in half of the patients with other NTM as compared to one fifth of MAC isolations, in half of smokers as compared to one fifth of non-smokers, and in ATS-2007-criteria-negative patients (41 %) as compared to ATS-positive patients (23 %). These figures seem to be quite comparable to those observed with another classification, Charlson comorbidity index, in the study by Andréjak et al. (2010). They found that 17% of patients had a high Charlson comorbidity index and 40–50% had a medium index. Hayashi et al (2012) found comorbidities in 66.4% of patients and they observed in a multivariate model that both systemic (40%) and respiratory (38%) comorbidities were negative prognostic factors for all-cause mortality in HIV–negative patients with MAC lung disease. Various other underlying diseases have been observed to be common in NTM infected patients (Skogberg et al. 1995, Kim et al. 2008, Billinger et al. 2009, Lai et al. 2011, Yeh[a] et al. 2014, Mirsaeidi[a] et al. 2015, Gommans et al. 2015, Ito et al. 2015). Recently, malignant diseases and immunosuppressive conditions have been increasingly taken up as underlying factors for local or even disseminated NTM infection (Cordonnier et al. 2004, Doucette and Fishman 2004, Sexton et al. 2008, O`Connel et al. 2012, Browne et al. 2012, Al-Anazi et al. 2014, Gommans et al. 2015). A malignant disease was observed in less than one fifth of patients in this study and rare patients had immunosuppressive therapy, suggesting that these factors would not have common underlying factors for pulmonary NTM infection. Pulmonary, gastroenteric, and hematological malignancies are those most often related to NTM infection (Lai et al. 2102). At the highest end of immunosuppression, pulmonary NTM infections seem uncommon. The incidence of pulmonary NTM infection among stem-cell-transplant recipients between 2004 and 2013 was low (0, 95%) (Kang et al. 2015). In the USA, it has been observed that patients with rheumatoid arthritis have increased risk for NTM infections (Winthrop et al. 2013). In a large study on 29,131 rheumatoid arthritis patients they reported a 4.2 fold greater risk for NTM infection than non-rheumatoid patients (Yeh[b] et al. 2014). Among peritoneal-dialysis patients, NTM peritonitis has been reported recently (Song et al.2012, Miyashita et al. 2014).

6.2.5 C4 DEFICIENCY AS A RISK FACTOR FOR NTM INFECTION

This study suggested that C4 complement deficiency might be a risk factor for NTM infection. We observed that the deficiencies of C4 in the majority of NTM-infected patients were significantly more common than in one third of the MTB patients or in more than half of the healthy controls. Especially, *C4A* deficiency was observed to be more common in the NTM patients than in the MTB group.

Further, our study suggested that *C4* susceptibility might be a risk factor for NTM infection in a specific patient group of elderly female patients of whom 81% had a *C4* deficiency. However, this data must be viewed in light of the fact that complement C4 deficiencies are common in the Finnish population; 17% lack one of the two C4A alleles and 38% lack one of the two C4B alleles (Seppänen et al. 2006). Equal frequencies have been observed in European decent North Americans, of whom 13% had a deficiency one of the two C4A alleles and 18% lacked one of the two C4B alleles (Blanchong et al. 2000).

This study was the first to suggest that complement C4 deficiencies might have some role in NTM infections. C4A deficiency has previously been linked to increased tendency to upper respiratory infections and various autoimmune diseases but also to pulmonary tuberculosis (Seppänen et al. 2006, Singh et al. 2007, Senbagavalli et al. 2011, Kainulainen et al. 2012). In contrast, C4B deficiency has been reported to have an association to another mycobacterial disease; leprosy (Messias et al. 1993). Furthermore, pulmonary tuberculosis has been associated to HLA class I and II genes in several populations (Yuliwulandar et al. 2010, Shi et al. 2011, Dubaniewicz et al. 2000 Sriram et al. 2001, Lombard et al. 2006). The susceptibility to *Mycobacterium tuberculosis* (MTB) disease has been found to be variable among different ethnic groups although in real life the strength of exposure to it may play a big role of unknown reason respiratory infections (Dubaniewicz et al. 2000, Sriram et al. 2001). Together this data suggests that genetic defects or factors may play a role in who will get a mycobacterial infection. Today, only the background of genetic defects of rare disseminated NTM infections have been clarified in some patients. Genetic defects in them have been characterized in T-cell mediated immunity and in cytokine signaling in families with a few mutations in IFN-γ and IL-12 signaling (Dorman and Holland 2000, Holland 2001, Casanova and Abel 2002, Guide and Holland 2002, Haverkamp et al. 2006, Fernando and Britton 2006). The detailed genetic background of pulmonary NTM infections still waits to be solved. In recent studies, up to two-thirds of NTM infections were found in female patients without predisposing lung diseases (Cassidy et al. 2009, Thomson 2010, Iseman and Marras 2008). The reason behind this female predominance has been hypothesized to be immunological defects making them more susceptible for NTM infections (Guide and Holland 2002). This study revealed that sufferers of one immunological defect, namely 81% of them, had a deficiency in *C4* genes. Another distinct feature in this female group of patients is their well characterized high frequency of bronchiectasis (Field and Cowie 2004, Piersimoni and Scarparo 2008, Kim et al. 2008).

Although, the exact link between C4 deficiency and mycobacterial infections is not verified, some data support a link between them. The polymorphisms of the complement and its receptor genes have been associated with pulmonary tuberculosis (Fernando and Britton 2006). It has been suggested, that complement

proteins are involved in a mechanism through which mycobacteria enter the cell and may thereby evade the adaptive immune response (Gupta et al. 2012). The *C4* deficiency found might partly prevent the complement activation and thus hamper the opsonization mechanisms and the positive feedback of IL-12/ IFN-γ in macrophages. The influence of aging may hamper the immune system, which might slow the manifest in both cellular and humoral immune responses. Those changes might predispose postmenopausal women to autoimmune diseases and NTM infections (Gameiro 2010, Kartalilja et al. 2013). Further, it has been suggested that while the immune system is getting older, hematopoietic stem cells have age-dependent functional alterations and the aged hematopoietic system might not be able to return to normal capacity after stress or injury (Beerman et al. 2010). In conclusion we observed that a majority of NTM patients with bronchiectasis fulfilling ATS criteria, mostly women, had a deficiency in *C4*. Although the pathogenetic link has not been revealed, this suggests that *C4* deficiency together with bronchiectasis might be indicative of the immunologic defect in pulmonary NTM infections of elderly females.

6.3 PROGNOSTIC FACTORS IN NTM INFECTIONS

6.3.1 SMOKING AS A PROGNOSTIC FACTOR

More than half of our patients were smokers and they had significantly higher mortality and shorter median survival time as compared to non-smokers. In a study from the USA, the immediate death causes of 2,990 people with NTM infection were reviewed (Mirsaedi et al. 2014). In this analysis from the USA, deceased NTM infected patients were 2.5 fold more likely to have COPD, 3.7 fold more likely to have bronchiectasis, 1.63 fold more likely to have interstitial lung disease and twice as common tobacco use as compared to deceased patients with tuberculosis. COPD was found to increase the risk of mortality in NTM patients already in an earlier study where as high as 55% mortality within 2 years of diagnosis 1995–1999 among COPD patients was reported (Henry et al. 2004). Less than half of the smokers had a diagnosis of COPD. The smokers also experienced severe underlying diseases more often, but mortality of the smokers did not differ from that of non-smokers. This suggests that at least in some smokers, the infection might be different from that of non-smokers, which is further supported by the lower frequency of MAC among the smokers as compared to non-smokers. Yet, severe underlying diseases could not be the main explanation of fatal outcome as two thirds of patients had no severe underlying diseases. The interplay of NTM infection and smoking-related COPD might be more complicated as it has also been suggested that NTM infection

might be one reason for rapid decline in pulmonary function in COPD disease (Huang et al. 2012).

Less than half of patients were non-smokers and they did not differ from smokers in their clinical picture or in laboratory abnormalities, but in only a few points. Smokers had more often severe underlying diseases, higher serum CRP, and alkaline phosphatase levels and, unexpectedly, lower incidence of bronchiectasis. Lower incidence of bronchiectasis is in contrast with that previously reported among smokers. One of the classical forms of NTM-infections has been described as a tuberculosis-like fibrocavitary pattern of disease which often affects older male smokers with COPD, bronchiectasis, pneumoconiosis, or prior tuberculosis and has been associated to *M. kansasii*, *M. xenopi*, *M.malmoense*, and *M. abscessus* (Corbett[a] et al. 1999, The Research Committee of the British Thoracic Society 2003, Arend et al. 2004, Hoefsloot et al. 2009, Varadi and Marras 2009, Andréjak et al. 2010). The other is a pattern of nodular bronchiectasis with or without fibrocavitary pulmonary changes and has been described mainly in connection to MAC infection and they have been found classically in middle-aged or older women who had never smoked (Wickremasinghe 2005, Glassroth 2008, Taiwo and Glassroth 2010). Prognosis in nodular form has been described as better than in fibrocavitary disease (Field et al. 2004, Waller et al. 2006, Huang et al. 1999, Taiwo and Glassroth 2010). These classical disease forms were not clearly evident in our patient population although the division according to smoking status would suggest it. Nodules were an infrequent finding and they were observed only in one third of non-smokers and rarely of smokers. However, MAC was a significantly more common finding among non-smokers than smokers. Bronchiectasis was more common in non-smokers than in smokers. Bronchiectasis has been associated to poor outcome (Field and Cowie 2006, Kim et al. 2008, Hyashi et al. 2012, Mirsaeidi 2013). Either the low prevalence of bronchiectasis in our non-smokers or the early assessment of their presence at the time of positive NTM culture made it seem that they failed to affect the outcome of non-smokers. In conclusion, smokers had significantly higher mortality and shorter median survival time as compared to non-smokers.

6.3.2 ATS CRITERIA AS A PROGNOSTIC FACTOR

ATS criteria were designed to help the clinician in treatment decisions and to guide which patients might benefit from antimycobacterial treatment (Griffith et al. 2007). Their prognostic value was not evaluated here. When all NTM isolations in HIV-negative patients during the study period were collected, only half of the patients with a NTM isolation fulfilled the ATS criteria and this is line with other studies

(Bodle et al. 2008, Billinger et al. 2009). When ATS-positive and -negative patients were compared, ATS criteria fulfillment was not a clear predictor of mortality. The median survival time of patients fulfilling the ATS criteria was not different from that of the ATS-negative patients. Similar results have also been obtained in a larger register-based study where half of the patients with a NTM isolation were classified as colonized, one fifth as having possibly a NTM disease, and quarter as having a definitive NTM disease and no difference was observed in five year outcome of the patients in these groups (Andréjak et al. 2010).

ATS criteria were designed with, especially, MAC infections in mind (Griffith et al. 2007). Interestingly, patients who met the ATS criteria had better prognosis as compared to ATS-negative patients, both in MAC and other NTM groups. ATS-negative and -positive patients demonstrated no difference in most of the clinical signs or symptoms from each other. Nodules were slightly more common among ATS-positive patients (27%) as compared to ATS negative (9%), and the same was observed with fatigue (56 vs 37% of patients, respectively). Severe underlying diseases, male gender, and previous pulmonary diseases were more common in ATS-negative patients in our patient cohort and in some other studies (Andréjak et al. 2010, Hyashi et al. 2012). Presence of severe underlying diseases was a significant predictor of poor outcome in this study and in that of Andréjak et al (2010). In the latter study, male gender was also associated to higher mortality. This data suggests that more studies are needed to find clinical signs that might define patients at risk of poor outcome or in need of medication. Close follow-up of patients could be recommended and recently poor prognosis has been reported in patients with hemoptysis and consolidation in radiological imaging (Commans et al. 2015). Among ATS negative patients, 65% did not meet radiological criteria: most were COPD patients with positive NTM sputum findings with high mortality due to severe COPD. By using only ATS criteria 2007, these COPD patients can be overlooked. This suggests that the ATS criteria were a poor predictor of case-fatality. This is consistent with recent study from Denmark, which revealed that colonized patients had almost as high mortality as patients with definitive NTM diseases (Andréjak et al. 2010).

6.3.3 NTM STRAIN AS A PROGNOSTIC FACTOR

MAC comprised more than half of all NTM isolations and RGM (*M. fortuitum, M. chelonae, M. abscessus*) were the most other NTM isolations consisting less than one fifth of all NTM isolations and *M. malmoense* almost less than one fifth (and the species distribution corresponded that observed in northern Europe previously) (Thompsen et al. 2002). In line with some other reports, we observed

that pulmonary MAC infection was associated with significantly better prognosis with a median survival time of 13.0 years as compared to patients with another pulmonary NTM infection with a median survival time of 4.6 years (Andréjak et al. 2010, Hyashi et al. 2012). Although our patient material did not allow detailed analysis across all the different species, no difference in mortality during the first three or five years was observed between RGM, *M. gordonae*, or *M. malmoense*. It is evident that the prognosis of NTM infection may depend on the NTM species, but the mortality rate in various studies has also been very variable. Overall 5-year mortality of patients with ATS-criteria positive MAC pulmonary infection was 24% and 10-year mortality was 47% (Hayashi et al. 2012). However, MAC-infection related mortality was estimated to be only 5.4% within 5 years and 15.7% within 10 years. In another study, three year mortality of 33% and five year mortality of 40% was associated to MAC isolation (Andréjak et al. 2010). In contrast, *M. kansasii* pulmonary infection was reported to cause death only in 8% of patients (Maliwan et al. 2005). *M. xenopi* infection was reported to carry only 15% overall mortality but in another study, mortality of up to 69% was reported (Andréjak et al. 2009, Varadi and Marras, 2009). *M. xenopi* and *M. malmoense* isolations were associated with about 40% three year and 50% five year case fatality rates in one study, but only 13% mortality was reported in another study for *M. malmoense* (Hoefsloot et al. 2009).

M. gordonae has commonly been regarded as a colonizer only (Piersimoni and Scarparo 2008), but symptomatic infections due to *M. gordonae* infection have also been reported recently in patients with immunosuppression or malignancies (Eckburg et al. 2000, Griffith et al. 2007, Pinho et al. 2009). Our patients with *M. gordonae* isolation also had a malignancy. However, the similar and poor prognosis associated with M. gordonae isolation suggests that it should not be overlooked.

Severe underlying diseases were also one of the most important predictors of mortality in this analysis. Interestingly, among patients without any severe underlying diseases, the survival curves were almost identical in MAC and other NTM groups. Even in the group of patients who had severe underlying diseases, MAC patients seemed to have a better prognosis as compared to patients with other NTM. There were also other differences between MAC and other NTM patients in their clinical picture and underlying characteristics. Patients with MAC had lower BMI, were more often female and non-smokers, and had more bronchiectasis, factors which together may all have affected the survival. These findings are in context with those previously reported (Kim et al. 2008, Obayashi 1999, Chan and Iseman 2010, Mirsaeidi et al. 2013). Notably, in recent studies MAC patients with both bronchiectasis and low BMI had more rapid progression of their pulmonary disease and higher mortality (Hayashi et al. 2012, Mirsaeidi et al. 2013, Yamakawa et al. 2013). This data suggests that MAC and other NTM species might hit various

patient groups and apart from geographical differences the host differences should probably be looked for more carefully. Within the groups of each NTM species there might also be different subgroups of patients with variable underlying and prognostic factors. In a recent study from Japan, MAC pulmonary infection with fibrocavitary or nodulary-bronchiectatic radiographic changes had mortality of 41% in 5-years and 75% in 10-years, but the corresponding mortality rates for nodular or bronchiectatic disease forms were only 18% and 35%, respectively (Hayashi et al. 2012).

Less than half of patients have suffered from symptoms for less than a year and additional one third less than two years. NTM infections have generally been regarded as slowly progressing processes which this finding challenges (Rosenzweig 1979, Prince et al.1989, Huang et al. 1999, Field and Cowie 2006, Griffths et al. 2007, Kim et al. 2008, Piersimoni and Scarparo 2008). In previous studies which have reported the median time between the onset of symptoms and NTM isolation it has varied from 4 months up to five-six years (Prince et al. 1989, Huang et al. 1999, Arend et al. 2004). Time from symptoms to NTM isolation was significantly longer among MAC patients than other NTM patients. Furthermore, MAC patients were significantly more likely to have fatigue and fever and also had a lower BMI, which would all together fit in a longer disease process. A rapid course of NTM infection is also indicated by the finding of appearance of respiratory failure within the first 6 months after NTM isolation, especially among COPD patients (Yeh et al. 2014[a]). Furthermore, median survival of less than a year has been reported for *M. xenopi* and *M. malmoense* infections, both in apparently immunocompetent and in solid-organ transplanted patients (Gommans et al. 2015). These findings suggest that frequent follow up of patients with NTM isolation could be encouraged at least soon after microbiological results have been obtained, because the symptom onset revealed rather rapid disease course and therefore timely diagnosis and evaluation of the predictors of mortality are useful. Namely, pulmonary MAC patients with bronchiectasis associated with significantly better prognosis as compared to patients with another pulmonary NTM with severe underlying disease.

In future perspectives, further investigation with a larger study to confirm the current immunological findings and finding the prognostic factors are obvious. Further data on the effect of treatment on outcome would be needed.

7 SUMMARY AND CONCLUSIONS

The main results of the present study were:

I Nearly half of patients with a NTM isolation had never smoked. MAC comprised a majority of isolates among non-smokers and 41% among smokers. Non-smokers were significantly more often females than smokers. Over 80% of non-smokers but only half of smokers had no potentially fatal underlying diseases. Smokers had higher risk of mortality than non-smokers, but no difference was observed after adjusting for underlying diseases. Smokers and non-smokers seemed to have different risk factors for NTM infection. Symptoms had started within a year of positive NTM isolation in nearly half of patients suggesting rapid development of symptoms.

II We found that half of patients with a NTM isolation fulfilled the 2007 ATS criteria. ATS-positive cases were more often female and had less often fatal underlying diseases as compared to ATS-negative cases. No significant difference was seen in median survival time or symptoms between ATS-positive and -negative cases except in fatigue, which was more common in ATS-positive patients. ATS-criteria fulfillment was a weak prognostic marker.

III MAC was isolated in half of cases and MAC patients were more often female and had more often bronchiectasis and less often fatal underlying diseases than patients with other NTM. There was no difference in ATS-2007-criteria fulfillment between MAC and other NTM patients. A higher proportion of other NTM patients had suffered from symptoms less than a year as compared to MAC patients. MAC patients had significantly lower risk of death and longer survival time than other NTM patients. Even after adjustment for underlying diseases, other NTM was still associated with poorer outcome than MAC.

IV A majority of Finnish NTM patients had significantly more frequent C4 deficiencies (C4A or C4B) as compared to unselected healthy control subjects and MTB. Especially, a majority of female NTM patients demonstrated more frequent C4 deficiencies as compared to female controls. The data suggest C4 deficiency to be a plausible risk factor for NTM infection especially in aged female patients.

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9 REFERENCES

Al-Anazi KA, Al-Jasser AM, Waleed Khalid Al-Anazi, WK: Infections caused by non-tuberculous mycobacteria in recipients of hematopoietic stem cell transplantation. Frontiers in oncology 4:1–11, 2014.

Andréjak C, Lescure FX, Douadi, Laurans G, Smail A, Duhaut P, Jounieaux V, Schmit JL: Non-tuberculous mycobacteria pulmonary infection: management and follow-up of 31 infected patients. J Infect 55:34–40, 2007.

Andréjak C, Lescure FX, Pukenyte E, Douadi Y, Yazdanpanah Y, Laurans G, Schmit JL, Jounieaux V: Mycobacterium xenopi pulmonary infections: a multicentric retrospectivestudy of 136 cases in North East France. Clinical and radiological features, treatment and outcome. Thorax 64:291–296, 2009.

Andréjak C, ThomsenVØ, Johansen IS, Riis A, Benfield TL, Duhaut P, Sørensen HT, Lescure F-X, Thomsen RW: Nontuberculous pulmonary mycobacteriosis in Denmark. Incidence and prognostic factors. Am J Respir Crit Care Med 181:514–521, 2010.

Andréjak C, Nielsen R, Thomsen V, Duhaut P, Sørensen HT, Thomsen RW: Chronic respiratory disease, inhaled corticosteroids and risk of non-tuberculous mycobacteriosis. Thorax 68:256–262, 2013.

Arend SM, Palou EC, Haas P, Janssen R, Hoeve MA, Verhard EM, Ottenhoff THM, Soolingen D, Dissel JT. Pneumonia caused by Mycobacterium kansasii in a series of patients without recognised immune defect. Clin Microbiol Infect 10:738–748, 2004.

Asiedu K, Scherpbier R, Raviglione M: Buruli ulcer. Mycobacterium ulcerans infection. Geneva, World Health Organization, WHO/CDS/CPE/GBUI/2000.1, 2000.

Astarie-Dequeker C, Nigou J, Passemar C, Guilhot C: The role of mycobacterial lipids in host pathogenesis. Drug Discovery Today: Disease Mechanisms 7:33–40, 2010.

Astagneau P, Desplaces N, Vincent V, Chicheportiche V, Botherel A, Maugat S, Lebascle K, Léonard P, Desenclos J, Grosset J, Ziza J, Brücker G: Mycobacterium xenopi spinal infections after discovertebral surgery: investigation and screening of a large outbreak. Lancet 358:747–51, 2001.

Aubry A, Chosidow O, Caumes E, Robert J, Cambau E: Sixty-three cases of Mycobacterium marinum infection: clinical features, treatment, and antibiotic susceptibility of causative isolates. Arch Intern Med16:1746–52, 2002.

Barker AF: Bronchiectasis. N Engl J Med 346:1383–1393, 2002.

Beerman I, Maloney WJ, Irving, Weissmann L, Derrick J Rossi DJ: Stem cells and the aging hematopoietic system. Curr Opin Immunol 22:500–506, 2010.

Beltrame A, Cattani G, Screm MC, Brillo F, Merelli M, Scarparo C, Como G, Tortoli E, Matteelli A, Bassetti M: Successful antibiotic treatment of mycobacterium abscessus pulmonary disease in an immunocompetent individual: Infectious Diseases and Therapy 1: 1–3, 2013.

Benson CA, Ellner JJ: MAC infection and AIDS: advances in theory and practice. Clin Infect Dis 17:7–20, 1993.

Benwill JL, Wallace Jr RJ: Mycobacterium abscessus: challenges in diagnosis and treatment. Curr Opin Infect Dis 27:506–510, 2014.

Berliner JG, Aldabagh B, Mully T, Yu SS, Schwartz BS, Berger TG: Non-tuberculous mycobacterial infections following cosmetic laser procedures: acase report and review of the literature. J Drugs Dermatol 14:80–83, 2015.

Billinger ME, Olivier KN, Viboud C, Montes de Oca R, Steiner C, Holland SM, Prevots DR: Nontuberculous Mycobacteria– associated Lung Disease in Hospitalized Persons,United States, 1998–2005: Emerg Infect Dis 15: 1562–1569, 2009.

Blanchong CA, Zhou B, Rupert KL, Chung EK, Jones KN, Sotos JF, Zipf WB, Rennebohm RM, Yu CY: Deficiencies of human complement component C4A and C4B and heterozygosity in length variants of *RP-C4-CYP21-TNX*(RCCX) modules in caucasians: the load of RCCX genetic diversity on major histocompatibility complex–associated disease. J Exp Med 191: 2183–2196, 2000.

Bloch KC, Zwerling L, Pletcher MJ, Hahn, JA, Gerberding JL, Ostroff SM,Vugia DJ, Reingold AL: Incidence and clinical implications of isolation of *Mycobacterium kansasii:* Results of a 5-year, population-based study. Ann Intern Med 129:698–704, 1998.

Bodle EE Cunningham JA, Phyllis Della-Latta P, Schluger NW, Saiman L: Epidemiology of Nontuberculous Mycobacteria in Patients without HIV Infection, New York City. Emerg Infect Dis 14: 390–396, 2008.

Boyd SC, Athan E, Friedman ND, Hughes A, Walton A, Callan P, McDonald A, O'Brien DP: Epidemiology, clinical features and diagnosis of *Mycobacteriumulcerans* in an Australian population. Med J Aust 196:341–4, 2012.

Brennan PJ, Nikaido H: The envelope of mycobacteria. Annu Rev Biochem 64:29–63, 1995.

Brickman M, Parsa AA, Parsa FD: Mycobacterium cheloneae infection after breast augmentation. Aesth Plast Surg 29:116–118, 2005.

Brown-Elliott BA, Griffith DE, Wallace Jr RJ: Newly described or emerging human species of nontuberculous mycobacteria. Infect Dis Clin North Am 16: 187–218, 2002.

Brown-Elliott BA, Nash KA, Wallace Jr RJ: Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with Nontuberculous mycobacteria. Clin Microbiol Rev 25: 545–582, 2012.

Brown-Elliot BA, Wallace Jr RJ: Infections due to nontuberculous mycobacteria other than mycobacterium avium-intracellulare. In: Mandell GL, Bennet JE, Dolin R, eds. Mandell, Douglas and Bennet`s Principles and Practice of Infectious Diseases. 7th ed. Vol. 2. Philadelphia: Churchill Livingstone, 2010:3191–3198.

Browne SK, Burbelo PD, Chetchotisakd P, Suputtamongkol Y, Kiertiburanakul S, Shaw PA, Jennifer L. Kirk JL, Jutivorakool K, Zaman R, Din L, Hsu AP, Patel SY, Olivier KN, Lulitanond V, Mootsikapun P, Anunnatsiri S, Angkasekwinai N, Sathapatayavongs B, HsuehPR, Shieh CC, Margaret R. Brown MR,Thongnoppakhun W, Claypool R, Sampaio EP, Theptha C, Waywa D, Dacombe C, Reizes Y, Zelazny AM, Saleeb P, Rosen LB, Mo A: Adult-Onset Immuno deficiency in Thailand and Taiwan. N Engl J Med 367:725–734, 2012.

Buchholz UT, McNeil MM, Keyes LE: *Mycobacterium malmoense* Infections in the United States, January 1993 through June 1995. Clin Infect Dis 27:551–558, 1998.

Cadena G, Wiedeman J, Boggan JE: Ventriculoperitoneal shunt infection with Mycobacterium fortuitum: a rareoffending organism. J Neurosurg Pediatr 14:704–7, 2014.

Casanova J-L, Abel L: Genetic dissection of immunity to mycobacteria: the human model. Annu Rev Immunol 20:581–620, 2002.

Cassidy PM, Hedberg K, Saulson A, McNelly E, Winthrop KL: Nontuberculous Mycobacterial disease prevalence and risk factors: A changing epidemiology. Clin Infect Dis 49:124–129, 2009.

Chan ED, Kaminska AM, Gill W, Chmura K, Felman NE, Bai X, Floyd CM, Fulton KE, Huitt GA, Strand MJ, Iseman MD, Shapiro L: Alpha-1-antitrypsin (AAT) anomalies are associated with lung disease due to rapidly growing mycobacteria and AAT inhibits Mycobacterium abscessus infection of macrophages. Scand J Infect Dis 39:690–696, 2007.

Chan ED, Iseman MD: Slender, older women appear to be more susceptible to nontuberculous mycobacteria lung disease. Gend Med 7:5–18, 2010.

Chae DR, Kim YI, Kee SJ, Kim YH, Chi SY, Ban HJ, Kwon YS, Oh IJ, Kim KS, Kim SO, Kim YC, Lim SC: The impact of the 2007 ATS/IDSA diagnostic criteria for nontuberculous mycobacterialdisease on the diagnosis of nontuberculous mycobacterial lung disease. Respiration 82:124–129, 2011.

Chalermskulrat W, Gilbey JG, Donohue JF: Nontuberculous mycobacteria in women, young and old. Clin Chest Med 23: 675–686, 2002.

Cirillo JD, Falkow S, Tompkins LS, Bermudez LE: Interaction of *Mycobacterium avium* with environmental amoebae enhances virulence. Infect Immun 65:3759–3767, 1997.

Colombo RE, Hill SC, Claypool RJ, Holland SM, Olivier KN: FamilialClustering of Pulmonary Nontuberculous Mycobacterial Disease. Chest 137: 629–634, 2010.

Cook JL: Nontuberculous mycobacteria: opportunistic environmental pathogens for predisposed hosts. Br Med Bull 96:45–59, 2010.

Corbett[a] EL, Churchyard GJ, Clayton T, Herselman P, Williams B, Hayes R, Mulder D, Cock KM: Risk Factors for Pulmonary Mycobacterial Disease in South African Gold Miners. Am J Respir Crit care Med 159:94–99, 1999.

Corbett[b] EL, Hay M, Churchyard GJ, Herselman P, Clayton T, Williams BG, Hayes R, Mulder D, De Cock KM: Mycobacterium kansasii and M. scrofulaceum isolates from HIV-negative South African gold miners: incidence, clinical significance and radiology. Int J Tuberc Lung Dis 3:501–507, 1999.

Cordonnier C, Martino R, Trabasso P, Held TK, Akan H, Ward MS, Fabian K, Ullmann AJ, Wulffraat N, Ljungman P, Alessandrino EP, Pretnar J, Gmür J, Varela R, Vitek A, Sica S, Rovira M, and European Blood and Marrow Transplant Group Infectious Diseases Working Party: Mycobacterial infection: a difficult and late diagnosis in steam cell transplant recipients. Clin Infect Dis 38:1229–36, 2004.

Dailloux M, Abalain M.L, Laurain C, Lebrun L, Loos-Ayav C, Lozniewski A,Maugein J and the French Mycobacteria Study Group: Respiratory infections associated with nontuberculous mycobacteria in non-HIV patients. Eur Respir J 28:1211–1215, 2006.

Daniel TM: The history of tuberculosis. Historical review: Respir Med 100:1862–1870, 2006.

Daffe M, Draper P: The envelope layers of mycobacteria with reference to their pathogenicity. Adv Microb Physiol 39:131–203, 1998.

Dessy LA, Mazzocchi M, Fioramonti P, Scuderi N: Conservative management of local Mycobacterium chelonae infection after combined liposuction and lipofilling. Aesth Plast Surg 30:717–722, 2006.

Dirac MA, Horan KL, Doody DR, Meschke JS, Park DR, Jackson LA, Weiss N, Winthrop KL, Cangleosi GA: Environment or host? A case–control study of risk factors for Mycobacterium avium complex lung disease. Am J Respir Crit Care Med 186:684–691, 2012.

Dorman SE, Holland SM: Interferon gamma and interleukin-12 pathway defects and human disease. Cytokine Growth Factor Rev 11:321–333, 2000.

Doucette D K, Fishman JA: Non-tuberculous mycobacterial infection in haematopoietic stem cell and solid organ transplant recipients. Clin Infect Dis 38:1428–44, 2004.

Dubaniewicz A, Lewko B, Moszkowska G, Zamorska B, Stepinski J: Molecular subtypes of the HLA-DR antigens in pulmonary tuberculosis. Int J Infect Dis 4:129–133, 2000.

Eaton T, Falkinham JO, von Reyn CF: Recovery of *Mycobacterium avium* from cigarettes. J Clin Microbiology 33:2757–2758, 1995.

Eckburg PB, Buadu EO, Stark P, Sarinas PSA, Chitkara RK, Kuschner WG: Clinical and chest radiographic findings among persons with sputum culture positive for mycobacterium gordonae. Chest 117:96–102, 2000.

Ellis SM, Hansell DM: Imaging of Non-tuberculous (Atypical) Mycobacterial Pulmonary Infection. Rev. Clin Radiol 57:661–669, 2002.

Escalonilla P, Esteban J, Soriano ML, Fariña MC, Piqu E, Grilli R, Ramírez JR, Barat A, Martín L, Requena L. Cutaneous manifestations of infection by nontuberculous mycobacteria. Clin Exp Dermatol 23:214–21, 1998.

Esteban J, Martin-de-Hijas N, Kinnari TJ, Ayala G, Fernández-Roblas R, Gadea I: Biofilm development by potentially pathogenic non-pigmented rapidly growing bacteria. BMC Microbiol 8:184, 2008.

Esteban J, Ortiz-Pe´rez A: Current treatment of atypical mycobacteriosis. Expert Opin Pharmacother 10:2787–2799, 2009.

Falkinham JO III: Epidemiology of infection by nontuberculous mycobacteria. Clin Microbiol Rev 9:177–215, 1996.

Falkinham JO III: Nontuberculous mycobacteria in the environment. Clin Chest Med 23:529–551, 2002.

Falkinham JO III: The changing pattern of nontuberculous mycobacterial disease. Can J Infect Dis 14:281–286, 2003.

Falkinham JO III: Impact of human activities on the ecology of nontuberculous mycobacteria. Future Microbiol 5:951–60, 2010.

Fallon JC, Patchett S, Gulmann C, Murphy GM: Mycobacterium marinum infection complicating Crohn's disease, treated with infliximab. Clin Exp Dermatol 33: 43–45, 2008.

Fernando SL, Britton WJ: Genetic susceptibility to mycobacterial diseasein humans. Rev. Immunol Cell Biol 84: 125–137, 2006.

Field SK, Cowie RL: Lung disease due to the more common nontuberculous mycobacteria. Chest 129:1653–1672, 2006.

Flint D, Mahadevan M, Barber C, Grayson D, Small R.: Cervical lymphadenitis due to non-tuberculousmycobacteria: surgical treatment and review: International Journal of Pediatric Otorhinolaryngology53: 187–194, 2000.

Foote S: Mediating immunity to mycobacteria. Nat Genet 21:345–346, 1999.

Fowler CJ, Olivier KN, Leung JM, Smith CC, Huth AG, Root H, Kuhns DB, Logun C, Zelazny A, Frein CA, Dau J, Haney C, Shelhamer JH, Bryant CE, Steven Holland SM:Abnormal nasal nitric oxide production, ciliary beat frequency, and toll-like receptorresponse in pulmonary nontuberculous mycobacterial disease epithelium. Am J Respir Crit Care Med 187:1374–1381, 2013.

Fredericks DN, Relman DA: Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin Microbiol Rev 9:118–331, 1996.

Fujita J, Ohtsukib Y, Shigetoc E, Suemitsud I, Yamadorid I, Bandoha S, Shiodee M, Nishimuraf K, Hirayamaf T, Matsushimag T, Fukunagah H, Ishida T: Pathological findings of bronchiectases caused by Mycobacterium avium intracellulare complex. Respir Med 97:933–938, 2003.

Gameiro C, Romano F: Changes in the immune system during menopause and aging. Rev. Maturitas 67:316–320, 2010.

George KM, Pascopella L, Welty DM, Small PL: A Mycobacterium ulcerans toxin, mycolactone, causes apoptosis in guinea pig ulcers and tissue culture cells. Infect Immun 68:877–83, 2000.

Cirillo JD ja Sahar H. Entry mechanisms of mycobacteria. Frontiers in Bioscience 6:737–747, 2001.

Glosli H, Stray-Pedersen A, Brun AC, Holtmon LW, Tønjum T, Chapgier A, Casanova JL, Abrahamsen TG: Infections due to various atypical mycobacteria in a Norwegian multiplex family with dominant interferon-gamma receptor deficiency. Clin Infect Dis 46:23–7, 2008.

Gommans EPAT, Even P, Linssen CFM, van Dessel H, van Haren E, de Vries GJ, Dingemans AMC, Kotz D, Rohde GGU: Risk factors for mortality in patients with pulmonary infections with non-tuberculous mycobacteria: a retrospective cohort study. Respir Med 109:137–145, 2015.

Guide SV, Holland SM: Host susceptibility Factors in mycobacterial infection. Genetics and body morphotype. Infect Dis Clin North Am 16:163–185, 2002.

Gupta A, Kaul A, Tsolaki AG, Kishore U, Bhakta S: Mycobacterium tuberculosis: immune evasion, latency and reactivation. Immunobiology 217: 363–374, 2012.

Griffith DE, Girard WM, Wallace RJ Jr: Clinical features of pulmonary diseasecaused by rapidly growing mycobacteria. An analysis of 154 patients. Am RevRespir Dis 147:1271, 1993.

Griffith DE, MD, BarbaraA.Brown-Elliott MD, WallaceJr. RJ: Diagnosing nontuberculous mycobacterial lung disease. Infect Dis Clin North Am 16:235–249, 2002.

Griffith DE, Aksamit T, Brown-Elliot BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburg R, Huitt G, Iademarco MF, Iseman M, Oliver K, Ruoss S, von ReynCF, Wallace RJ Jr, Winthorp K: American Thoracic Society: diagnosis, treatment, and prevention of nontuberculous mycobacterial disease. Am J Respir Crit Care Med 175: 367–416, 2007.

Griffith DE: Nontuberculous mycobacterial lung disease. Curr Opin Infect Dis 23:185–90, 2010.

Groote MA, Huitt G: Infections due to rapidly growing mycobacteria. Clin Infect Dis 42:1756–63, 2006.

Hallstrand TS, Ochs HD, Zhu Q, Liles WC: Inhaled IFN-γ for persistent nontuberculous mycobacterial pulmonary disease due to functional IFN-γ deficiency. Eur Respir J 24: 367–370, 2004.

Hamade A, Pozdzik A, Denis O, Tooulou M, Keyzer C, Jacobs F, Khabbout J, Nortier JL: Mycobacterium fortuitum and Polymicrobial Peritoneal Dialysis-Related Peritonitis: A case report and review of the literature. Case Rep Nephrol 2014:323757, 2014.

Han XY, Tarrand JJ, Infante R, Jacobson KL, Truong M: Clinical significance and epidemiologic analyses of Mycobacterium avium and Mycobacterium intracellulare among patients without AIDS. J Clin Microbiol 43:4407–4412, 2005.

Haverkamp MH, van Dissel JT, Holland SM: Human host genetic factors in nontuberculous mycobacterial infection: lessons from single gene disorders affecting innate and adaptive immunity and lessons from molecular defects in interferon-γ-dependent signaling. Microbes Infect 8:1157–1166, 2006.

Hayashi M, T akayanagi N, K anauchi T, M iyahara Y, Yanagisawa T, Sugita Y: Prognostic factors of 634 HIVnegative patients with Mycobacterium avium complex lungdisease. Am J Respir Crit Care Med 185:575–83, 2012.

Helou G, Viola GM, Hachem R, Han XY, Raad II: Rapidly growing mycobacterial bloodstream infections. Lancet Infect Dis 13:166–74, 2013.

Henry MT, Inamdar L, O' R iordain D, Scweiger M, Watson JP: Non-tuberculous mycobacteria in non-HIV patients: epidemiology, treatment and response. Eur Respir J 23:741–746, 2004.

Hoefsloot W, van Ingen J, Andréjak C, Ängeby K, Bauriaud R, Bemer P, Beylis N, Boeree MJ, Cacho J, Chihota V, Chimara E, Churchyard G, Cias R, Daza, R, Daley CL, Dekhuijzen R, Domingo D, Drobniewski F, Esteban J, Fauville-Dufaux M, Folkvardsen B, Gibbons N, Go´mez-Mampaso E, Gonzalez R, Hoffmann H, Hsueh P-R, Indra A,Jagielski T, Jamieson F, Jankovic M, JongE, Keane J, Koh W-J, Lange B, Leao S, Macedo R, Mannsa`ker T, Marras TK, Maugein J, Milburn MJ, Mlinko´ T, Morcillo N, Morimoto K, Papaventsis D, Palenque E, Paez-Pen˜a M, Piersimoni C, Polanova M, Rastogi N, Richter E, Ruiz-Serrano MJ, Silva A, Pedro da Silva M, Simsek H, van Soolingen D, Szabo´ N,Thomson R, Fernandez TT, Tortoli E, Totten SE,Tyrrell G, Vasankari T, Villar M, Walkiewicz R, Winthrop KL, Wagner D: The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. Eur Respir J 42:1604–1613, 2013.

Hoefsloot W, van Ingen J, de Lange WCM, Dekhuijzen PNR, Boeree MJ, van Soolingen D: Clinical relevance of *Mycobacterium malmoense* isolation in the Netherlands. Eur Respir J 34:926–931, 2009.

Holland SM: Nontuberculous Mycobacteria. Am J Med Sci 321:49–55, 2001.

Horsburgh, Jr., Jill Gettings J, Alexander LN, Lennox JL: Disseminated *Mycobacterium avium* Complex Disease among Patients Infected with Human Immunodeficiency Virus, 1985–2000. Clin Inf Dis 33:1938–43, 2001.

Huang H, Kao P, Adi V, Ruoss S: Mycobacterium aviumintracellulare pulmonary infection in HIV-negative patients without preexisting lung disease. Chest 115:1033–40, 1999.

Huang CT, Tsai YJ, Wu HD, Wang JY, Yu CJ, Lee LN, Yang PC: Impact of non-tuberculous mycobacteria on pulmonary function decline in chronic obstructive pulmonary disease. Int J Tuberc Lung Dis. 16:539–45, 2012.

Iivnanainen EK, Martikainen PJ, Vaananen PK, Katila M-L: Environmental factors affecting the occurrence of mycobacteria in Brook waters. Appl environ microbial 59:398–404, 1993.

Ingen J, Fero BE, Hofsloot W, Boeree MJ, van Soolongen D: Drug treatment of pulmonary nontuberculous mycobacterial disease in HIV-negative patients: the evidence. Expert Rev Anti Infect Ther 11:1065–1077, 2013.

Ingen J, Rahim Z, Mulder A, Boeree MJ, Simeone R, Brosch R, Soolingen D: Characterization of mycobacterium orygis as M. tuberculosis complex subspecies. Emerg Infect Dis 18:653–655, 2012.

Iseman MD, Buschman DL, Ackerson LM: Pectus excavatum and scoliosis: thoracic anomalies associated with pulmonary disease caused by Mycobacterium avium complex. Am Rev Respir Dis 144:914–916, 1991.

Iseman MD, Marras TK: The importance of nontuberculous mycobacterial lung diseas. Am J Respir Crit Care Med 178: 999–1001, 2008.

Ichijo T, Izumi Y, Nakamoto S, Yamaguchi N, Nasu M: Distribution and respiratory activity of mycobacteria in household water system of healthy volunteers in Japan. PLoS One 9(10):e110554, 2014.

Ito Y, Hirai T, Fujita K, Maekawa K, Niimi A, Ichiyama S, Mishima M: Increasing patients with pulmonary Mycobacterium avium complex disease and associated underlying diseases in Japan. J Infect Chemother 1:1–5 ePub, 2015.

Janeway CA Jr, Travers P, Walport M, Shlomchik MJ: Innate immunity. Part I. Chapter 2. In: Janeway CA Jr, Travers P, Walport M, Shlomchik MJ, Lawrence E (ed), Catherall E (ed), Reandi S (ed), Bushell G (ed), Morales M (ed), Goatly B (ed). Immmunobiology the immune system in health and disease. 6th ed, Garland Science Publishing, New York, NY, 2005: 37–95.

Jeon K, Kwon OJ, Lee NY, Kim B-J, Kook Y-H, Lee S-H, ParkY-K, Kim CK, KohW-J: Antibiotic treatment of mycobacterium abscessus lung disease a retrospective analysis of 65 Patients. Am J Respir Crit Care Med 180: 896–902, 2009.

Jarand J, Adrah Levin A, Lening Zhang L, Gwen Huitt G, John D. Mitchell JD, Daley CL: Clinical and microbiologic outcomes in patients receiving treatment for Mycobacterium abscessus pulmonary disease. Clin Infect Dis 52:565–571, 2011.

Kainulainen L, Peltola V, Seppänen M, Viander M, He Q, He Q, Lkki M-L, Ruuskanen O: C4A deficiency in children and adolescents with recurrent respiratory infections. Hum Immunol 73:498–501, 2012.

Katila ML, Brander E, Jantzen E, Huttunen R, Linkosalo L: Chemotypes of Mycobacterium malmoense based on glycolipid profiles. J Clin Microbiol 29:355–8, 1991.

Kang JY, Ha JH, Kang HS, Yoon HK, Kim HJ, Lee S, Lee DG, Jung JI, Kim SC, Kim YK: Clinical significance of nontuberculous mycobacteria from respiratory specimensin stem cell transplantation recipients. Int J Hematol Feb 8:[Epub ahead of print], 2015.

Kalayjian RC, Toossi Z, Tomashefski JF Jr, Carey JT, Ross JA, Tomford JW, Blinkhorn RJ: Pulmonary disease due to infection by Mycobacterium avium complex in patients with AIDS. Clin Infect Dis 20:1186–94, 1995.

Kang HK, Park HY, Kim D, Jeong B-H, Jeon K, Cho JH, Kim HK, Choi YS, Kim J, Koh W-J: Treatment outcomes of adjuvant resectional surgery for nontuberculous mycobacterial lung disease. BMC Infectious Diseases 15:1–9, 2015.

Kartalilja M, Fantuzzi G, Thomas J, Strand MJ, Bai X, Ramamoorthy P, Rothman MS, Nagabhushanam V, McDermott M, Levin AR, Frazer-Abel A, Giclas PC, Korner J, Iseman MD, Shapiro L, Chan ED: Patients with nontuberculous mycobacterial lung disease exhibit unique body and immune phenotypes. Am J Respir Crit Care Med 187:197–205, 2013.

Kennedy BS, Bedard B, Younge M, Tuttle D, Ammerman E, Ricci J, Doniger AS, Escuyer VE, Mitchell K, Noble-Wang JA, O'Connell HA, Lanier WA, Katz LM, Betts RF, Mercurio MG, Scott GA, Lewis MA, Goldgeier MH: Outbreak of Mycobacterium chelonae infection associated with tattoo ink. N Engl J Med 367:1020–4, 2012.

Kim RD, Greenberg DE, Ehrmantraut ME, Guide SV, Ding L, Shea Y, Brown MR, Chernick M, Steagall WK, Glasgow CG, Lin JP, Jolley C, Sorbara L, Raffeld M, Hill S, Avila N, Sachdev V, Barnhart LA, AndersonVL, Claypool R, Hilligoss DM, Garofalo M, Fitzgerald A, Anaya-O'Brien S, Darnell D,DeCastro R, Heather M. Menning HM: Pulmonary nontuberculous mycobacterial disease prospective study of a distinct preexisting syndrome. Am J Respir Crit Care Med 178:1066–1074, 2008.

Kim HR, Yoon ES, Kim DW, Hwang NH, Shon YS, Lee BI, Park SH: Empirical treatment of highly suspected nontuberculous mycobacteria infections following aesthetic procedures. Arch Plast Surg 41:759–67, 2014.

Knoll BM, Kappagoda S, Gill RR, Goldberg HJ, Boyle K, Baden LR, Fuhlbrigge AL, Marty FM: Non-tuberculous mycobacterial infection among lung transplant recipients: a 15-year cohort study. Transpl Infect Dis 14:452-460, 2012.

Knowles MR, Boucher RC: Mucus clearance as a primary innate defensemechanism for mammalian airways. J Clin Invest 109:571–577, 2002.

Kobashi Y, Yoshida K, Miyashita N, Niki Y, Matsushima T: Pulmonary Mycobacterium avium disease with a solitary pulmonary nodule requiring differentiation from recurrence of pulmonary adenocarcinoma. Intern Med 43:855–60, 2004.

Kubo K, Yamazaki Y, Hachiya T, Hayasaka M, Honda T, Hasegawa M and Sone S: Mycobacterium avium-intracellulare pulmonary infection in patients without known predisposing lung disease. Lung 176:381–91, 1998.

Lai C-C, Tan CK, Lin SH, Liu WL, Liao CH, Huang YT, Hsueh PR: Clinical significance of nontuberculous mycobacteria isolates in elderly Taiwanese patients. Eur J Clin Microbiol Infect Dis 30:779–783, 2011.

Lamprecht B, McBurnie MA, Vollmer WM, Gudmundsson G, Welte T, Nizankowska-Mogilnicka E, Studnicka M, Bateman E, Anto JM, Burney P, Mannino DM, Sonia A. Buist and for the BOLD Collaborative Research: COPD in Never Smokers : Results From the Population-Based Burden of Obstructive Lung Disease Study. Chest 139:752–763, 2011.

Lindeboom JA, Prins JM, Bruijnesteijn van Coppenraet ES, LindeboomR, Kuijper EJ: Cervicofacial lymphadenitis in children caused by *Mycobacterium haemophilum*. Clin Infect Dis 41:1569–75, 2005.

Maartens G, Wilkinson RJ: Tuberculosis. Rev. Lancet 370: 2030–2043, 2007.

Maliwan N, Zvetina JR: Clinical features and follow up of 302 patientswith Mycobacterium kansasii pulmonary infection: a 50 year experience.Postgrad Med J 81:530–533, 2005.

Mangione EJ, Huitt G, Lenaway D, Beebe J, Bailey A, Figoski M, Michael P. Rau MP, Kurt D. Albrecht KD, Yakrus MA: Nontuberculous mycobacterial disease following hot tub exposure. Emerg Infect Dis 7:1039–1042, 2001.

Marchetti N, Criner K, Criner GJ: Characterization of functional, radiologic andlung function recovery post treatment of hot tub lung. A case report and reviewof the literature. Lung 182:271–7, 2004.

Marras TK, Chedore P, Ying AM, Jamieson F: Isolation prevalence of pulmonary nontuberculous mycobacteria in Ontario, 1997–2003. Thorax 62:661–666, 2007.

Marras TK, Mendelson D, Marchand-Austin A, May K, Jamieson FB: Pulmonary Nontuberculous Mycobacterial Disease, Ontario, Canada, 1998–2010. Emerg Inf Dis 19:1889–1891, 2013.

McGarvey JM, Bermudez LE: Pathogenesis of nontuberculous mycobacterial infections. Clin Chest Med 23:2002, 2002.

McGrath EE, McCabe J, P. Anderson PB: Guidelines on the diagnosis and treatment of pulmonarynon-tuberculous mycobacteria infection. Int J Clin Pract 62:12, 1947–1955, 2008.

McCabe WR, Jackson GG: Gram-negative bacteremia. ArchIntern Med 110:847–55, 1962.

Mello KG, Mello FC, Borga L, Rolla V, Duarte RS, Sampaio EP, Holland SM, Prevots DR, Dalcolmo MP: Clinical and therapeutic features of pulmonary nontuberculous mycobacterial disease, Brazil, 1993–2011. Emerg Infect Dis 19:393–399, 2013.

Messias IJ, Santamaria J, Brenden M, Reis A, Mauff G: Association of C4B deficiency (C4B*Q0) with erythema nodosum in leprosy. Clin Exp Immunol 92:284–287, 1993.

Miyashita E, Yoshida H, Mori D, Nakagawa N, Ohta H, Seki M, Tomono K, Hashii Y, Ozono K: *Mycobacterium avium* complex-associated peritonitis with CAPD after unrelated bone marrow transplantation. Pediatrics International 56:96–98, 2014.

Middleton AM, Chadwick MV, Nicholsona AG, Dewara A, Grogerb RK, Brownc EJ, T.L. Ratliffd TL, R. Wilsona R: Inhibition of adherence of mycobacterium avium complex and mycobacterium tuberculosis to fibronectin on the respiratory mucosa. Respir Med 98:1203–6, 2004.

Middleton AM, Chadwick MV, Nicholson AG, Dewar A, Groger RK, Brown EJ, Wilson R: The role of mycobacterium avium complex fibronectin attachment protein in adherence to the human respiratory mucosa. Mol Microbiol 38:381–91, 2000.

Mirsaeidi M, Hadid W, Ericsoussi B, Rodgers D, Ruxana T. Sadikot RT. Non-tuberculous mycobacterial disease is common in patients with non-cystic fibrosis bronchiectasis. Int J Inf Dis 17:1000–1004, 2013.

Mirsaeidi[a] M, Farshidpour M, Allen MB, Ebrahimi G, Falkinham JO3rd: Highlight on advances in nontuberculous mycobacterial disease in North America. Rev. BioMed Research International 2014, Article ID 919474:1-10, 2014.

Mirsaeidi[b] M, Machado R F, Garcia J G N, Schraufnagel D E: Nontuberculous mycobacterial disease mortality in the United States, 1999–2010: a population-based comparative study. PlosOne 9:e91879, 2014.

National Institute for Health and Welfare, National Infectious Disease Register in Finland 2015. Available at: https://www.thl.fi/fi/web/infektiotaudit/seuranta-ja-epidemiat/tartuntatautirekisteri. Accsessed March 30 2015.

O'Brien DP, Currie BJ, Krause VL: Nontuberculous Mycobacterial Disease in Northern Australia: A case series and review of the literature. Clin Infect Dis 31:958–68, 2000.

O'Connell ML, Birkenkamp KE, Kleiner DE, Folio LR, Holland SM, Olivier KN: Lung manifestations in an autopsy based series of pulmonary or disseminated nontuberculous mycobacterial disease. Chest 141:1203e9, 2012.

Obayashi Y, Fujita J, Suemitsu I, Kamei T, Nii M, Takahara J: Successive follow-up of chest computed tomography in patients with *Mycobacterium avium-intracellulare* complex. Respir Med 93:11–15, 1999.

Orme IM, Ordway DJ: Host response to nontuberculous mycobacterial infections of current clinical importance. Infect Immun 82:3516–3522, 2014.

Paakkanen R, Vauhkonen H, Eronen KT, Järvinen A, Seppänen M, Lokki M-L: Copy number analysis of complement *C4A*, *C4B* and *C4A* silencing mutation by real-time quantitative polymerase chain reaction.PLoS One 7:e38813, 2012.

Park JW, Kim YS, Yoon JO, Kim JS, Chang JS, Kim JM, Chun JM, Jeon IH: Non-tuberculous mycobacterial infection of the musculoskeletal system: pattern of infection and efficacy of combined surgical/antimicrobial treatment. Bone Joint J 96:1561–1565, 2014.

Penn R, Steehler MK, Sokohl A, Harley EH: Nontuberculous mycobacterial cervicofacial lymphadenitis--a review and proposed classification system. Int J Pediatr Otorhinolaryngol 75:1599–603, 2011.

Petrini B: Non-tuberculous mycobacterial infections. Rev. Scand J Infect Dis 38:246–255, 2006.

Pham RV, Vydareny KH, Gal AA: High-resolution computed tomography appearance of pulmonary Mycobacterium avium complex infection after exposure to hot tub: case of hot-tub lung. J Thorac Imaging 18:48–52, 2003.

Philips MS, von Reyn: Nosocomial Infections due to nontuberculous mycobacteria. Clin Inf Dis 33: 1363–1374, 2001.

Piersimoni C, Scarparo C: Extrapulmonary Infections Associated with Nontuberculous Mycobacteria in Immunocompetent Persons. Emerg Infect Dis 15: 1351–1358, 2009.

Piersimoni C, Scarparo C: Pulmonary infections associated with non-tuberculousmycobacteria in immunocompetent patients. Lancet Infect Dis 8: 323–34, 2008.

Piersimoni C: Nontuberculous mycobacteria infection in solid organtransplant recipients. Eur J Clin Microbiol Infect Dis 31:397–403, 2012.

Pinho L, Santos J, Oliveira G, Pestana M: Mycobacterium gordonae urinary infection in a renal transplant recipient. Transpl Infect Dis 11:253–256, 2009.

Primm TP, Lucero CA, Falkinham JO III: Health impacts of environmental mycobacteria. Clin Microbiol Rev 17:98–106, 2004.

Prince DS, Peterson DD, Steiner RM, Gottlieb JE, Scott R, Israel ML, Figueroa WG, Fish JE: Infection with Mycobacterium avium complex in patients without predisposingconditions. N Engl J Med 321:863–868, 1989.

Ramos JM, Garcı´a- Sepulcre MF, Juan C. Rodrı´guez JC, Padilla S and Gutie´rrez F: Mycobacterium marinum infection complicated by anti-tumour necrosis factor therapy. J Med Microbiol 59:617–62, 2010.

Raychaudhuri SP, Nguyen CT, Raychaudhuri SK, Gershwin EM: Incidence and nature of infectious disease in patients treated with anti-TNF agents. Autoimmunity Reviews 9:67–81, 2009.

Reich JM and Johnson RE: Mycobacterium avium complex pulmonary disease presenting as an isolated lingular or middle lobe pattern.The Lady Windermere syndrome. Chest 101:1605–1609, 1992.

von Reyn CF, Waddell RD, Eaton T, Arbeit RD, Maslow JN, Barber TW, Brindle RJ, C F Gilks CF, Lumio J, Lähdevirta J, Ranki A, Dawson D, Falkinham JO: Isolation of mycobacterium avium complex from water in the United States, Finland, Zaire, and Kenya. J Clin Microbiol 31:3227–3230, 1993.

Robert P, Kay V, Anthony G. High-Resolution Computed Tomography Appearance of Pulmonary Mycobacterium Avium Complex Infection After Exposure to Hot Tub: Case of Hot-Tub Lung. Journal of Thoracic Imaging 18:42–52, 2003.

Rosenzweig DY: Pulmonary mycobacterial infections due to Mycobacterium intracellulare- avium complex. Clinical features and course in 100 consecutive cases. Chest 75;115–119, 1979.

Russell, CD, Claxton P, Doig C, Seagar A-L, Rayner A, Laurenson IF: Non-tuberculous mycobacteria: a retrospective review of Scottish isolates from 2000 to 2010. Thorax 69:593–595, 2014.

Salama C, Policar M, Venkataraman M: Isolated pulmonary Mycobacteriumavium complex infection in patients with human immunodeficiency virus infection: case reports and literature review. Clin Infect Dis 37:e35–40, 2003.

Salvana EM, Cooper GS, Salata RA: Mycobacterium other than tuberculosis(MOTT) infection: an emerging disease in infliximab-treated patients. J Infect 55:484–7, 2007.

Sax H, Bloemberg G, Hasse B, Sommerstein R, Kohler P, Achermann Y, Rössle M, Falk V, Kuster SP, Böttger EC, Weber R: Prolonged outbreak of Mycobacterium chimaera infection after open-chest heart surgery. Clin Infect Dis 3:1–9, ebup, 2015.

Senbagavalli P, Kumar N, Kaur G, Mehra NK, Geetha C, Ramanathan VD: Majorhistocompatibility complex class III (C2, C4, factor B) and C3 gene variants inpatients with pulmonary tuberculosis. Hum Immunol 72:173–178, 2011.

Sexton P, Harrison AC: Susceptibility to nontuberculousmycobacterial lung disease. Eur Respir J 31:1322–1333, 2008.

Shi GL, Hu XL, Yang L, Rong CL, Guo YL, Song CX: Association of HLA-DRB alleles and pulmonary tuberculosis in North Chinese patients. Genet Mol Res 10:1331–1336, 2011.

Singh M, Balamurugan A, Katoch K, Sharma SK, Mehra NK: Immunogenetics of mycobacterial infections in the North Indian population. Tissue Antiqens 69 (Suppl 1) 228–230, 2007.

Simons S, van Ingen J, Hsueh P-R, Van Hung N, Dekhuijzen R, Boeree MJ, van Soolingen D: Nontuberculous mycobacteria in respiratory tract infections, Eastern Asia. Emerg Infect Dis 17:343- 349, 2011.

Skogberg K, Ruutu P, Tukiainen P, Valtonen VV: Nontuberculous mycobacterial infection in HIV-negative patients receiving immunosuppressive therapy. Eur J Clin Microbiol Infect Dis 14:755–63, 1995.

Song Y, Wu J, Yan H, Chen J: Peritoneal dialysis-associated nontuberculous mycobacterium peritonitis: a systematic review of reported cases. Nephrol Dial Transplant 27:1639–1644, 2012.

Sriram U, Selvaraj P, Kurian SM, Reetha AM, Narayanan PR: HLA-DR2 subtypes and immune responses in pulmonary tuberculosis. Indian J Med Res 113: 117–124, 2001.

Streit M, Bo¨hlen LM, Hunziker T, Zimmerli S, Tschaener GG, Nivergelt H, Bodmer T, Braathen LR: Disseminated Mycobacterium marinum infection with extensive cutaneous eruption and bacteremia in an immunocompromised patient. Eur J Dermatol 16:79–83, 2006.

Seppänen M, Suvilehto J, Lokki M-L, Notkola I-L, Järvinen A, Jarva H, Seppälä I, Tahkokallio O, Malmberg H, Meri S, Valtonen V: Immunoglobulins and complement factor C4 in adult rhinosinusitis. Clin Exp Immunol 145:219–227, 2006.

Stamm LM, Brown EJ: *Mycobacterium marinum*: the generalization and specialization of a pathogenic mycobacterium. Rev. Microbes and Infection 6:1418–1428, 2004.

Tanaka E, Amitani R, Niimi A, Suzuki K, Muryama T, Kutze F: Yield of computed tomography and bronchoscopy for the diagnosis of Mycobacterium avium complex pulmonary disease. Am J Repir Crit Care Med 155:2041–046, 1997.

Taiwo B, Glassroth J: Nontuberculous mycobacterial lung diseases. Infect Dis Clin North Am 24:769–789, 2010.

The Research Committee of the British Thoracic Society: Pulmonarydisease caused by *Mycobacterium avium-intracellulare* in HIV-negativepatients: five-year follow-up of patients receiving standardised treatment. Int J Tuberc Lung Dis 67:628–634, 2002.

The Research Committee of the British Thoracic Society: Pulmonary disease caused by M. malmoense in HIV negative patients: 5-yr follow-up of patients receiving standardized treatment. Eur Respir J 21:478–82, 2003.

Thomsen VO, Andersen AB, Miorner H: Incidence and clinical significance of non-tuberculous mycobacteria isolated from clinical specimens during a 2-y nationwide survey. Scand J Infect Dis 34:648–653, 2002.

Thompson RM, Amstrong JG, Looke DF: Gastroesophageal reflux disease, acid suppression, and Mycobacterium avium complex pulmonary disease. Chest 131:1166–72, 2007.

Thorel MF, Huchzermeyer H, Weiss R, Fontaine JJ: Mycobacterium avium infections in animals. Vet Res.28:439–47, 1997.

Timpe A, Runyon EH: The relationship of atypical acid-fast bacteria to human disease. J Lab Clin Med 44:202–209, 1954.

Torkko, Katila, Kontro. Gas-chromatographic lipid profiles in identificationof currently known slowly growing environmental mycobacteria. Journal of Medical Microbiology 52, 315–323, 2003.

Tortoli E, Piersimoni C, Bartoloni A, Burrini C, A. Callegaro P, Caroli GC, Colombrita D, Goglio A, Mantella A,Tosi CP, Simonetti T: *Mycobacterium malmoense* in Italy: The modern Norman invasion? Eur J Epidemiol 13:341–346, 1997.

Tortoli E: Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. Clin Microbiol Rev 16: 319–354, 2003.

Tortoli E: The new mycobacteria: an update. FEMS Immunol Med Microbiol 48:159–178, 2006.

Varadi RG, Marras TK: Pulmonary *Mycobacterium xenopi* infection in non-HIV-infected patients: a systematic review. Int J Tuberc Lung Dis 13:1210–1218, 2009.

Varis T, Virtanen S: Tobacco statistics 2012. Health 2013. National Institute for Health and Welfare (www.thl.fi), Statistical Report OSF 27/2013: 1–77, 2013.

Ziedalski TM, Kao PN, Henig NR, Jacobs SS, Ruoss SJ: Prospective analysis of cystic fibrosis transmembrane regulator mutations in adults with bronchiectasis or pulmonary nontuberculous mycobacterial infection. Chest 130:995–1002, 2006.

Wallace RJ Jr, O'Brien R, Glassroth J, et al: Diagnosis and treatment of disease caused by nontuberculous mycobacteria. American Review of Respiratory Disease 42:940–53, 1990.

Wallace R Jr, Glassroth J, Griffith DE, Olivier KN, Cook JL, Gordin F: American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am J Respir Crit Care Med 156:1–25, 1997.

Wallace Jr RJ: Recent changes in taxonomy and disease manifestation of the rapidly growing mycobacteria. Eur J Microbiol Infect Dis 13:953–60, 1994.

Waller EA, Roy A, Brumble L, Khoor A: The Expanding Spectrum of Mycobacterium avium Complex-Associated Pulmonary Disease. Chest 1301234–124, 2006.

Walport MJ: Complement first of two parts. N Engl J Med 344: 1058–1066, 2001.

Wang SX, Yang CJ, Chen YC, Lay CJ, Tsai CC : Septic arthritis caused by Mycobacterium fortuitum and Mycobacterium abscessus in a prosthetic knee joint: case report and review of literature. Intern Med 50:2227–2232, 2011.

Wickremasinghe M, Ozerovitch LJ, Davies G, Wodehouse T, Chadwick MV, Abdallah S, Shah P, Wilson R: Non-tuberculous mycobacteria in patients with bronchiectasis. Thorax 60:1045–1051, 2005.

Winthrop KL, Albridge K, South D, Albrecht P, Abrams M, Samuel MC, Leonard W, Wagner J, Vugia DJ : The clinical management and outcome of nail salon-acquired *Mycobacterium fortuitum* skin infection. Clin Infect Dis 38:38–44, 2004.

Winthrop KL, McNelley E, Kendall B, Marshall-Olson A, Christy Morris C, Cassidy M,Saulson A, Hedberg K: Pulmonary nontuberculous mycobacterial diseaseprevalence and clinical features. Am J Respir Crit Care Med 182:977–982, 2010.

Wittram C, Weisbrod GL: Mycobacterium avium complex lung disease in immunocompetent patients: radiography – CT correlation. Br J Radiol 75:340–344, 2002.

Wolinsky E: State of the art: nontuberculous mycobacteria and associated diseases. Am Rev Respir Dis 119:107–59, 1979.

Wolinsky W: Mycobacterial lymphadenitis in children a prospective study of 105 nontuberculous cases with longterm follow –up. Clin Infect Dis 20: 954–963, 1995.

Woods GL: The mycobacteriology laboratory and new diagnostic techniques. Infect Dis Clin North Am 16:127–144, 2002.

World Health Organization: Global tuberculosis report. WHO Library Cataloguing-in-Publication data: 1–147, 2014.

Yamakawa H, Takayanagi N, Ishiguro T, Kanauchi T, Hoshi T, Sugita Y: Clinical investigation of nontuberculous mycobacterial lung disease in Japanese patients with rheumatoid arthritis receiving biologic therapy. J Rheumatol 40:1994–2000, 2013.

Yeager H, Farah Jr, KE: Non-tuberculous mycobacterial syndromes. In: Schlossberg D eds. Tuberculosis and non-tuberculous mycobacterial infections. 5th. Philadelphia: McGraw-Hill Medical publishing Division, 2006: 413–418.

Yeh[a] J-J, Wang Y-C, Lin C-L, Chou C, Yeh T-C, Wu B-T, Sung F-C, Kao C-H: Nontuberculous mycobacterial infection is associated with increased respiratory failure: a nationwide cohort study. PLoS ONE 9: e99260, 2014.

Yeh[b] J-J, Wang YC, Sung FC, Kao CH: Rheumatoid arthritis increases the risk of nontuberculosis mycobacterial disease and active pulmonary tuberculosis. PLoS One 9(10):e110922, 2014.

Young LS, Inderlied CB, Berlin OG, Gottlieb MS: Mycobacterial Infections in AIDS patients, with an Emphasis on the *Mycobacterium avium* Complex. Clin Infect Dis 8: 1024–1033, 1986.

Yuliwulandari R, Sachrowardi Q, Nakajima H, Kashiwase K, Hirayasua K, Mabuchi A, Sofro ASM, Tokunaga K: Association of HLA-A, -B, and -DRB1 with pulmonary tuberculosis in western Javanese Indonesia. Hum Immunol 71:697–701, 2010.