The Prevalence and Risk Factors for Nontuberculous Mycobacterial Infection in Lung Transplant Patients and Its Impact on Patient Survival and Graft Function

by

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Abstract

Nontuberculous mycobacteria (NTM) are environmentally ubiquitous bacteria and frequent colonizers of immunocompetent and immunocompromised patients. NTM infections have been described in patients with chronic lung disease and post-lung transplantation. However, the risk factors for infection and its consequence on patient and graft outcomes are not consistently reported in the literature. NTM species are geographically diverse, and to date, there is a paucity of Canadian data, particularly in the transplant population. We sought to analyze data from a high-volume Canadian transplant centre to better characterize the local epidemiology of NTM infection, assess risk factors for infection, and determine the association between infection and patient and graft outcome.

We performed a retrospective cohort study of 375 adult lung transplant recipients at the University of Alberta Hospital (Edmonton, Canada) between 2005 and 2014 to assess NTM epidemiology and risk factors. All positive NTM culture results in this cohort were extracted from the Provincial Laboratory database. The impact of NTM infection and colonization on patient and graft survival was tested by multivariate Cox regression analysis.

NTM species were cultured from 26 (7%) patients before and 17 (4.5%) patients after transplant. The most commonly isolated species were *Mycobacterium avium* complex (55%) and *Mycobacterium abscessus* (20%). Five-year mortality was significantly higher in those infected with NTM after transplant ($p=0.016$), but there was no difference in chronic lung allograft dysfunction (CLAD) at 5 years ($p=0.999$). Cystic fibrosis and lower body mass index were both associated with pre-transplant, but not post-transplant, NTM infection.

In this cohort, NTM isolation was associated with an increased risk of death but not CLAD onset at 5 years.

Preface

This thesis is a final work submitted for partial fulfillment of the requirements for a Master of Science in the Department of Medicine at the University of Alberta, Edmonton, Canada. Parts of this thesis have been published as Friedman DZP, Cervera C, Halloran K, Tyrrell G, Doucette K. Non-tuberculous mycobacteria in lung transplant recipients: Prevalence, risk factors, and impact on survival and chronic lung allograft dysfunction. *Transplant Infectious Disease*. December 2019:e13229. doi:10.1111/tid.13229. I was responsible for data collection and manuscript composition. C. Cervera[1] was involved in concept formation and statistical analysis. K. Halloran[2] was involved in concept formation and provided pulmonary function data and algorithm for computing grade of chronic lung allograft dysfunction. G. Tyrrell[3] was involved in concept formation and providing microbiological data. K. Doucette[1] was the supervisory author and was involved with concept formation. All authors reviewed, edited, and approved the manuscript.

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List of Abbreviations

AIDS, acquired immunodeficiency syndrome	**IFNγ,** interferon-gamma
ALTP, Alberta Lung Transplant Program	**IL,** interleukin
ATG, antithymocyte globulin	**IVIG,** intravenous immunoglobulin
ATS, American Thoracic Society	**IQR,** interquartile range
BMI, body-mass index	**LOS,** length of stay
BOS, bronchiolitis obliterans syndrome	**MAC,** *Mycobacterium avium* complex
CF, cystic fibrosis	**MMF,** mycophenolate mofetil
CI, confidence interval	**MOTT,** mycobacteria other than tuberculosis
CLAD, chronic lung allograft dysfunction	**NRAD,** neutrophilic reversible allograft dysfunction
COPD, chronic obstructive pulmonary disorder	**NTM,** nontuberculous mycobacteria
FEV1, forced expiratory volume after 1 second	**RAS,** restrictive allograft syndrome
FVC, forced vital capacity	**RGM,** rapidly growing mycobacteria
HIV, human immunodeficiency virus	**SD**, standard deviation
ICU, intensive care unit	**SGM,** slowly growing mycobacteria
IDSA, Infectious Disease Society of America	**TNFα,** tumour necrosis factor-alpha

Chapter 1 – Introduction

1.1 Study rationale

Lung transplantation provides a therapeutic option for many patients with medically refractory end-stage lung disease. The outcomes of lung transplantation in Canada and internationally continue to improve, in part due to optimized pre-transplant patient selection, novel surgical techniques including *ex vivo* lung perfusion, and increased understanding and prevention of rejection[1]. However, chronic lung allograft dysfunction (CLAD) remains a significant cause of morbidity and mortality following lung transplant.

Several factors have been implicated in the development of CLAD, and respiratory infections are thought to play a crucial role[2]. Nontuberculous mycobacterial (NTM) infections are especially challenging in lung transplantation as their effects on mortality and CLAD development are inconsistently reported in the literature. Furthermore, the number of reported cases worldwide is likely underreported, which also complicates our understanding. Therefore, we sought to examine the rates of infection and their outcomes in our sizable lung transplantation population.

1.2 Organization of thesis

This thesis is divided into 6 chapters. Following this first introductory chapter, which introduces the topic and outline of the thesis, I present a literature review highlighting historical and technical aspects of lung transplantation; microbiology of NTM; and the clinical aspects of NTM infections in transplant recipients.

In Chapters 3-5, I highlight the research project's components, discussing first the project objectives and hypotheses, followed by an outline of the methodology and main results. To conclude, I discuss the impacts of our results, limitations and strengths of the study, and future insights.

Chapter 2 – Background and literature review

2.1 Lung transplantation in Canada

2.1.1 Historical aspects of lung transplantation in Canada

Since the first reported case in 1963[3], lung transplantation provides a therapeutic option for patients suffering from end-stage lung disease. Over the subsequent fifteen years, 38 lung transplants (single-lung or lobar transplant) were performed worldwide but were associated with 2-week postoperative mortality higher than 80%[4]. By the early 1980s, cardiothoracic surgeons at the Toronto General Hospital, Toronto, Canada, published their new experience with double-lung transplantation with increasing success[5]. The following decade saw the rates of lung transplantation performed in Canada double, with almost 130 transplants performed in 2001. This increase was associated with improving long term survival, with 70-80% of recipients surviving at least three years[6]. This trend continued, and in 2016-2017, the number of lung transplants performed in Canada exceeded 500, and the 5-year survival approached 70%[7]. In Alberta, now over 60 lung and heart-lung transplants are performed each year. The Alberta Lung Transplant Program (ALTP), which operates at the University of Alberta Hospital, Edmonton, Canada, provides assessments and care to adults and children from Alberta, British Columbia, Saskatchewan, Manitoba, and parts of the Canadian territories[8]. From 1986 to 2016, 2595 patients have been referred to the ALTP, with 1272 patients going on to listing; 59% of listed patients continued on to transplantation[8].

2.1.2 Chronic lung allograft dysfunction

Graft failure remains the leading cause of morbidity and mortality following lung transplantation. From January 1990 to June 2016, graft failure and bronchiolitis obliterans syndrome (BOS) accounted for 37.3-47.8% of deaths beyond the first post-transplant year[9]. In Alberta, chronic lung allograft dysfunction (CLAD) accounts for 39% of deaths in lung transplant recipients[8]. Previously, BOS was considered a synonym of chronic rejection; however, in 2010, Woodrow et al. published data of chronic allograft restriction as evidenced by a drop in the forced vital capacity distinguishing this phenotype from its obstructive counterpart[10]. Now the term chronic lung allograft dysfunction encompasses three phenotypic syndromes: the obstructive phenotype (BOS), restrictive phenotype (restrictive allograft syndrome [RAS]), and a reversible subphenotype of BOS (neutrophilic reversible allograft dysfunction [NRAD]), which is associated with partial improvement in lung function with the use of azithromycin[11]. These phenotypes are associated with different outcomes—median survival following the diagnosis of BOS is 3-5 years and 1-2 years following the diagnosis of RAS[12]. Outcomes are similar in paediatric lung transplant recipients [13]

There are no proven therapies to cure CLAD aside from repeat lung transplantation, and therefore risk mitigation is tantamount[14]; however, the mechanisms that result in irreversible lung injury are not entirely understood. Multiple studies have investigated the allograft microarchitecture, inflammasome and non-invasive biomarkers to differentiate BOS and RAS at a cellular level. We do know that CLAD is not entirely a consequence of chronic rejection, a term that implies alloimmunity. Although the recognition of donor epithelial epitopes does contribute to the development of CLAD, other non-immune insults can occur. For example,

gastroesophageal reflux and various chronic and acute infections may directly injure lung epithelia, possibly in tandem with triggering alloimmunity.[15–17].

2.1.3 Immunosuppression following lung transplantation for the prevention of allograft rejection

The lung allograft is considerably immunogenic[18]. Disrupted bronchial circulation at the time of transplant, denervation with loss of ciliary function, as well as exposure of the allograft to inhalation of pathogens, air pollutants, and allergens, can trigger an influx of immune cells with adverse consequences on allograft function[15]. To prevent episodes of acute rejection and CLAD, intense pharmacotherapeutic suppression of the recipients' immune system is necessary.

Immunosuppressive therapy occurs in 3 phases. The *induction phase* begins in the perioperative time and is characterized by the use of agents that will rapidly reduce the number and function of recipient lymphocytes to prevent hyperacute cellular rejection and subsequent early allograft dysfunction. This is commonly achieved by using antithymocyte globulin (ATG) or an interleukin(IL)-2 antagonist, such as basiliximab. The *maintenance phase* continues lifelong and involves the use of two or three drugs to suppress different lymphocyte activation and proliferation pathways. As the time from transplant extends, the goal is to use the lowest possible doses of immunosuppressants to minimize the risk of opportunistic infections and malignancies, while preventing chronic and acute allograft dysfunction. Commonly used agents are corticosteroids, calcineurin inhibitors (tacrolimus or cyclosporine) and anti-metabolites (mycophenolate or azathioprine). The *rescue phase* occurs when there is suspicion of acute cellular rejection or antibody-mediated rejection and entails re-augmentation of immune

suppression with basiliximab or ATG, or other immunosuppressants, such as high-dose corticosteroids, a B-lymphocyte depleting agent (rituximab), intravenous immunoglobulin and plasma exchange[19]. Although these induction and maintenance immunosuppressive agents target different metabolic pathways, they share the critical function of reducing lymphocyte activity and cell-mediated immune function, which is integral in determining the future risk of infectious complications[20].

2.2 Infectious complications of transplant-associated immunosuppression

First described by Fishman and Rubin, the *net state of immunosuppression* describes the multifaceted function that evolves at various rates following solid organ transplantation[21]. The anti-rejection medications alone do not determine the patient's net state immunosuppression nor the subsequent infectious risk. Previous immunomodulating therapies (for example, biologics, chronic corticosteroids, and chemotherapy), defects to mucocutaneous barriers (for example, lines and drains), postoperative complications, drug-induced leukopenia, and other metabolic conditions all affect the patient's risk[22]. Other alterations in the innate and humoral immune systems have also been described following transplantation[23–25].

Although variable, specific infections occur predictably after transplantation [26]. In the first month post-transplant, the recipient spends a considerable time convalescing in hospital, and therefore most infections are nosocomial bacterial or fungal infections, infections related to surgical complications, catheter-related bacteremia, *Clostridioides difficile* colitis, and early donor-derived infections. Over the following year, rates of reactivated latent infections, such as herpesviruses, and opportunistic infections increase; however, with the use of antimicrobial

prophylaxis, such as trimethoprim-sulfamethoxazole for *Pneumocystis jirovecii* and valganciclovir for cytomegalovirus, the rates of these opportunistic infections have decreased, or they occur once prophylaxis is discontinued[27]. As the effects of induction immunosuppression and hospital-associated factors wane near the end of the first year, community-acquired bacterial and viral infections prevail[22].

Patients with cystic fibrosis and other suppurative lung diseases have additional risks of infectious complications to consider. In this population, extensive pre-transplant colonization with multidrug-resistant organisms, including *Pseudomonas aeruginosa*, *Burkholderia cepacia*, methicillin-resistant *Staphylococcus aureus*, saprophytic moulds, and NTM, can increase the rates of these infections following lung transplantation[28]. In some centres, colonization with *Burkholderia cenocepacia* or *Mycobacterium abscessus* is a relative or absolute contraindication for lung transplantation[29].

2.3 Nontuberculous mycobacterial (NTM) infections

2.3.1 Microbiology of NTM

The genus *Mycobacterium* comprises a large group of mycolic acid-containing aerobic bacteria that range in degree of pathogenicity and transmissibility. Obligate pathogens include the causative species of tuberculosis, the *Mycobacterium tuberculosis* complex, and of leprosy, *M. leprae*, *M. lepromatosis*, and *M. lepraemurium* (the latter causing disease only in murine hosts). The other hundreds of mycobacterial species not included in these complexes are known collectively as nontuberculous mycobacteria. NTM are ubiquitous environmental bacteria that, for the most part, are opportunistic pathogens or commensal bacteria, although exceptions do

exist. To date, over 150 NTM species have been recognized, and due to the advent of molecular techniques, such as 16S rRNA and heat shock protein sequencing, new species are being rapidly identified[30,31].

One of the first methods of characterizing NTM species dates back to 1959 and focuses on colony growth characteristics. This Runyon classification categorizes species by the colonies' growth rate, morphology, and pigmentation pattern (Table 1). While newer molecular diagnostic techniques are being utilized to speciate NTM rapidly, the speed of colony growth remains a significant clinical distinction. Rapidly growing mycobacteria (RGM), such as *M. abscessus*, *M. fortuitum*, and *M. chelonae*, typically produce visible colonies on solid media within seven days. On the other hand, slowly growing mycobacteria (SGM) may take up to 2-3 weeks. Common SGM species include those in the *M. avium* complex (MAC), *M. kansasii*, *M. xenopi*, and *M. haemophilum.* Intermediate-growing mycobacteria, for example, *M. marinum* and *M. gordonae*, represent a small subgroup of SGM that requires anywhere from 7 to 10 days for colony production. Although the ability for a colony to produce pigment in both light and dark environments (scotochromogens), in light only (photochromogens), or not at all (non-chromogens), may help further differentiate mycobacterial species, this property is seldom used to identify species in modern microbiology laboratories.

Table 1 - Runyon classification of representative NTM species

Rapidly growing	Slowly and intermediate growing		
	Photochromogens	**Scotochromogens**	**Non-chromogens**
M. abscessus *M. fortuitum* *M. chelonae* *M. smegmatis*	*M. marinum* *M. kansasii* *M. simiae*	*M. gordonae* *M. xenopi* *M. scrofulaceum*	*M. avium* complex *M. ulcerans* *M. haemophilum* *M. genavense*

The virulence factors and reasons for variable pathogenicity of NTM species are not entirely understood, although studies have identified which species are more likely to produce invasive pulmonary disease. In a multicenter Belgian cohort of 384 patients with pulmonary NTM infections, there was a spectrum of the likelihood that a particular species would cause pulmonary disease[32]. Highly pathogenic species associated with pulmonary disease in over 60% of infections included *M. abscessus*, *M. malmoense*, *M. intracellulare*, and *M. kansasii*. *M. gordonae* was amongst the most common non-pathogenic species. *M. fortuitum* was occasionally implicated in pulmonary disease, whereas *M. avium* and *M. xenopi* cause pulmonary disease approximately 50% of the time.

Some species, such as *M. abscessus* and *M. avium*, display two colonial morphotypes. The smooth morphotype has been associated with increased bacterial motility and biofilm formation, allowing them to persist in the environment. The rough morphotype is associated with severe or disseminated infections, likely due to mechanisms that bypass macrophage activity. The ability to transition between these two morphotypes may, in part, explain some species' increased pathogenicity[33–35]. *M. abscessus* can also be highly resistant to macrophages' bactericidal activity, allowing it to persist and multiply intracellularly. This, too, sets it apart

from less pathogenic species, whose ability to divide and survive in a host are readily halted by the macrophage[33].

2.3.2 Epidemiology of NTM infections

The prevalence of NTM infections is likely underestimated, primarily due to asymptomatic colonization and non-universal requirements to report infections to public health authorities[36]. There is a significant variation in the geographic distribution of species globally. MAC species are the most common species from clinical isolates worldwide and account for more than half of all infections in North America[36–38]; geographic variation also exists within the complex, with *M. avium* being more prevalent in Europe and the Americas and *M. intracellulare* more prevalent in Australia and South Africa[38].

North American data are limited mainly to publications from the United States (Table 2). In a recent review of almost 6 million patients across the United States from 2009-2013, Spaulding et al. found that 0.13% of patients had a positive respiratory culture for an NTM species[39]. The only published data from Canada to date stem from several groups in Ontario that have reported the annual rate of NTM isolation in the general population as 14.1–22.2 per 100 000[40–43]. Rates of reported cases of NTM infections are increasing in North America, likely due to growing awareness of NTM, improvement in current diagnostic techniques, increase use of immunosuppressive agents for autoimmune diseases, and jurisdictions implementing decrease use of household water and temperature of water coolers[31,44–46].

Table 2 - Geographic variation of common NTM species in North America

Region	Years	Species Distribution, %					Reference
		M. avium complex	*M. chelonae/ abscessus*	*M. fortuitum*	*M. kansasii*	*M. xenopi*	
Canada							
Ontario	1997-2013	58.6-63.0	2.1-2.6‡	2.6-4.7‡	1.3-1.7	23.9-26.5	[41–43]
United States							
New York	2000-2003	83.6	5.7	3.2	2.4	2.6	[53]
North Carolina	2006-2010	48.3	17.9	6.3	1.9	NR	[54]
Pennsylvania, Colorado, Washington, California	2004-2006	80.1	12.1	5.6	5.5	1.7	[55]
Oregon	2005-2012	69.3-87.5	3.0-6.2	0.5-1.9	0.5-0.6	0.3-0.5	[56–59]
Hawaii	2005-2013	63.7	20	24	2.0	0	[60]
New England†	2009-2013	86	4	NR	NR	NR	[39]
Middle Atlantic†	2009-2013	83	5	NR	NR	NR	
East South Central†	2009-2013	91	2	NR	NR	NR	
South Atlantic†	2009-2013	78	9	NR	NR	NR	
West South Central†	2009-2013	61	18	NR	NR	NR	
Mountain†	2009-2013	80	4	NR	NR	NR	
Pacific†	2009-2013	74	11	NR	NR	NR	
East North Central†	2009-2013	78	7	NR	NR	NR	
West North Central†	2009-2013	64	10	NR	NR	NR	

NR = Not reported

†States included in each region are defined in [39]

‡ In [41], *M. chelonae*, *M. abscessus*, and *M. fortuitum* were combined to 13.0%

NTM species are typically non-pathogenic in patients with intact cell-mediated immunity. In immunocompetent hosts, pulmonary infections can occur in instances of local defects in immune response, as seen in structural airway diseases, cystic fibrosis and bronchiectasis. Skin and soft tissues NTM infections in otherwise immunocompetent adults can occur in those with saltwater exposures[47,48] and those who have received cosmetic surgery, particularly surgeries related to medical tourism[49,50].

Risk factors for the development of severe or disseminated infections include acquired immune deficiency syndrome (AIDS), prolonged use of tumour necrosis factor-alpha (TNFα) inhibitors, immunosuppression following solid organ and hematopoietic stem cell transplants, and genetic disruptions of the IL-12/interferon-gamma (IFN-γ) pathways[36,51]. Although most IL-12/IFN-γ pathway defects present in childhood, two increasingly recognized diseases that predispose adults to the development of extrapulmonary and disseminated NTM infections are GATA2 mutations and anti-IFNγ autoantibody syndrome, the latter being more common in Southeast Asian adults[52].

The prevalence of NTM infections in solid organ transplant recipients is markedly higher than the general population with the highest rates occurring in heart and lung transplant recipients (0.24–2.8% and 0.46–8.0%, respectively)[61,62]. Rates amongst renal and liver transplant recipients are lower, but have been reported as high as 0.16-0.38% and 0.04% respectively[61,62]. Some identified variables associated with the development of infection following SOT include episodes of acute rejection, chronic kidney disease and cystic fibrosis[63].

2.3.3 Clinical presentation of NTM infections

Clinical manifestations of NTM infections are highly variable due to species-specific virulence factors, route of infection and the host's immune status[64]. Pleuropulmonary infections are the most common presentation in the general population and in lung transplant recipients[61]. Two predominant radiographic patterns exist—apical fibrocavitary disease, which is common to patients with underlying structural lung disease (such as pneumoconiosis or chronic obstructive pulmonary disease [COPD]), and nodular bronchiectatic disease[64]. The latter has been associated with the *Lady Windermere Syndrome*, a bronchiectatic pulmonary MAC infection, which typically occurs in thin, middle-aged, Caucasian women without any apparent predisposing factor[65,66].

Skin and soft tissue infections are often associated with the RGM species, particularly following trauma or surgery. *M. abscessus* and *M. chelonae* are associated with surgical site infections that can be progressive and associated with high mortality[67]. With the exception of lung transplant recipients, where they account for only 10% of NTM infections, cutaneous disease and surgical site infections are the predominant presentations following SOT and hematopoietic stem cell transplant[61,68]. Other important dermatologic presentations include chronic ulcers caused by *M. marinum* following exposure to fish tanks and Buruli ulcers caused by *M. ulcerans*[69,47]. Other NTM, similar to *M. marinum*, can cause sporotrichoid lesions or deep-space limb infections (such as tenosynovitis or septic arthritis) following dirt inoculums, aquaria or saltwater exposures [70–72].

Lymphadenitis, especially cervicofacial adenitis, is the most common presentation of NTM infection in children and may range from a painless, firm, enlarged lymph node to fistulization and chronic drainage[31,73]; seldom is an underlying immune defect demonstrated

in children with NTM lymphadenitis. In the absence of infection with human immunodeficiency virus (HIV), isolated lymphatic disease is rare in adults[31].

Although sporadic cases of disseminated infection had been noted in the early 1980s, rates dramatically increased with the HIV pandemic; by the end of 1990, approximately 8% of American patients with AIDS were diagnosed with disseminated MAC infections[74]. In patients with HIV, slow-growing mycobacteria, especially MAC, predominate as causes of disseminated infection; in non-HIV-infected immunosuppressed patients, RGM species are more commonly implicated [31]. Symptoms of disseminated disease are non-specific and typically include fever, night sweats, weight loss, lymphadenopathy and diarrhea[31].

2.3.4 Management of NTM infections

The most crucial step in managing NTM infections is determining whether the presence of infection represents colonization or disease; the latter may require therapy, whereas the former often does not. Isolation of species known to be pathogenic from sterile spaces or histopathologic evidence of invasive disease supports the decision to treat. Pulmonary infections can be challenging to categorize, and therefore guidelines have been established to aid in decision-making[31,75,76]. The diagnosis of pulmonary disease is supported by repeated, good-quality, respiratory cultures; compatible symptoms or objective pulmonary dysfunction; and abnormal radiography (Table 3).

Table 3 - ATS/IDSA criteria for pulmonary NTM disease

Clinical *(Both criteria required)*
1. Pulmonary symptoms (chronic cough, dyspnea, or hemoptysis), nodular or cavitary lesions on chest x-ray, or multifocal nodular bronchiectasis on high-resolution CT scan 2. Exclusion of other diagnoses
Microbiological *(At least one criterion required)*
1. Positive culture results of the same species from 2 separate expectorated sputums OR 2. A positive culture from at least one bronchoscopic specimen OR 3. Lung biopsy with histopathologic features consistent with mycobacterial infection with a positive culture for an NTM species from the biopsy, sputum or bronchoscopic specimen

Adapted from [31].

The choice and duration of therapy are dependent on the organism, *in vitro* susceptibility pattern (where validated), site of infection and degree of immunosuppression. Although the details of treatment are beyond the scope of this thesis and are outlined in guidelines[31,75,76], some important concepts are highlighted below. The cornerstone of therapy includes the use of combination antimycobacterial chemotherapy, often including macrolides, rifamycins, ethambutol, isoniazid, fluoroquinolones, linezolid or aminoglycosides. The duration of antimicrobial therapy is generally on the scale of months to years, depending on the location and severity of the infection. Several of these antimicrobials, particularly rifamycins, can interact with immunosuppressive therapy, and therefore close attention to drug levels may be required. There are other notable concerns specific to SOT recipients related to long term use of these antimycobacterial agents. Aminoglycosides can potentiate calcineurin-associated nephrotoxicity[77]; linezolid has been associated with cytopenias, especially thrombocytopenia, in SOT recipients[78,79]; and fluoroquinolones can increase the risk of tendinopathies in SOT recipients, especially those on chronic corticosteroids[80]. In select cases, surgical debridement or resection should also be considered. In solid organ transplant recipients and others with

iatrogenic immunosuppression, decreasing the degree of immunosuppression may be required to effectively eradicate complicated infections[81].

2.3.5 Outcomes of NTM infections following lung transplant

In individuals with impaired cell-mediated immunity, as in recipients of solid organ or hematopoietic stem cell transplants, NTM can cause life-threatening disease[36,51]. Lung transplant recipients are at particular risk for NTM lung infections due to a combination of factors, including immune suppression; local defects in the transplanted allograft resulting in abnormal ciliary function; bronchial devascularization, denervation, and lymphatic insufficiency post-transplant; propensity for pre-transplant airway colonization with NTM; and the nature of the lung itself with constant environmental exposure. In some studies, rates of NTM infections in lung transplants have been as high as 8%[61,62,66].

Data are conflicting regarding the impact of NTM infections on patient survival and graft outcomes following lung transplantation. In a cohort of 201 lung transplant recipients, Huang et al. found that all infections with NTM after lung transplant was associated with over twice the incidence of mortality. This was significant in both those with colonization and disease. There was no significant association with the development of BOS[82]. In a cohort of 1301 lung transplant recipients, Hamad et al. analyzed 22 cases of *M. abscessus* infection in the first year post-transplant, concluding that pulmonary disease was associated with a significant increase in 1-year mortality, but similarly not BOS, compared to those who were colonized[83]. Longworth et al. compared a cohort of 18 SOT recipients with NTM disease diagnosed within the first year following transplant to matched controls; similarly, they found an association of early NTM

disease with 3-year mortality, although no significant increase in mortality was seen with *M. abscessus* compared to the other NTM species (which was against the authors' initial hypothesis) [84].

On the other hand, several studies have found no increase in mortality associated with NTM infections following transplant. Over a 15-year analysis of 237 lung transplant recipients, Knoll et al. found no association with NTM infection and increased post-transplant mortality[85]. In a small group of CF patients with *M. abscessus* lung disease following lung transplant, Perez et al. found no difference in survival or graft outcomes when compared to uninfected CF patients.[86]. George et al. found in their cohort of 553 lung transplant recipients, those with NTM infection did no experience increased mortality; although, a small subset of those with disease did[87]. In a cohort of 208 lung transplant recipients, those with bronchopulmonary NTM disease post-transplant had higher hazard of BOS, but not mortality[88].

Chapter 3 – Objectives

3.1 Epidemiology of NTM infections

Our first objective was to estimate the local incidence of NTM infections in patients undergoing lung transplant. Specifically, we aimed to define species distribution and identify possible risk factors for acquiring NTM infection before and after transplant.

3.2 Effect of NTM infection on post-transplant mortality and graft function

Our second objective was to evaluate the relationship of post-transplant NTM infection on graft function and patient survival. We hypothesized that having an NTM infection following lung transplant increased the likelihood of early mortality and development of CLAD.

Chapter 4 – Methods

4.1 Study Design

This study was a retrospective cohort analysis of all patients, 17 years and older, undergoing lung or heart-lung transplant at the University of Alberta Hospital (Edmonton, Alberta, Canada) between January 01, 2005 and December 31, 2014. Patients were identified using our prospectively collected Alberta Lung Transplant Program database. We extracted data on positive NTM cultures on these patients from the Public Health Laboratory (ProvLab), the only lab in the province that performs mycobacterial culture. Notably, a pre-transplant bronchoscopy is performed, however otherwise surveillance sputa and post-transplant bronchoscopies are not performed at our centre. Specimens were collected for mycobacterial assessment at the attending physician's discretion based on clinical symptoms. We compared cases with positive cultures pre- and post-transplant to those without. Regular follow-up data were collected on all patients until November 01, 2016 or death.

4.2 Definitions

NTM infection was defined as isolation of any NTM species from culture of skin, respiratory or sterile body specimen. Pulmonary NTM disease was defined according to the American Thoracic Society (ATS)/Infectious Disease Society of America (IDSA) guidelines (Table 3). If a pulmonary infection did not meet these criteria, it was considered NTM colonization.

CLAD was defined according to criteria previously published by the International Society for Heart and Lung Transplantation, as a decrease in forced expiratory volume (FEV1) to

below 80% of the patient's previous baseline after transplant, calculated by averaging two FEV1 values measured at least three weeks apart[89].

The diagnosis of CLAD was further subclassified by phenotype as restrictive or obstructive by the presence or absence, respectively, of a coexistent decrease in the forced vital capacity (FVC) from baseline (average of the two values corresponding to the maximum FEV1 values). Sufficient total lung capacity values were not available for phenotyping based on that parameter. CLAD severity was classified as mild, moderate or severe if FEV1 was 66-80% of baseline, 51-65% of baseline, or ≤50% of baseline, respectively. Calculations were performed with Microsoft Excel (Redmond, WA), Version 16.0 and were reviewed manually for errors.

4.3 Statistical Analysis

Categorical variables are expressed as percentages and compared using chi-square or Fisher's exact test. Continuous variables are expressed as means (and standard deviation [SD]) or medians (and interquartile range [IQR]) according to their distribution and compared using the Student's T-test or Mann-Whitney test, respectively. Prevalence is expressed as a percentage. We analyzed survival and CLAD-free survival, or time to CLAD onset or death. To evaluate the influence of NTM infection on CLAD-free survival, we performed death-censored univariate and multivariate Cox-regression analyses and included co-variables previously defined to influence graft loss and patient mortality[31,66,84], including the cause of end-stage lung disease and body-mass index (BMI), as well as others, such as duration of ICU stay after transplant and induction with ATG. NTM infection was introduced in the model as a time-dependent variable (days to NTM infection). To evaluate risk factors associated with NTM infection, we performed

multivariate Cox analysis including previously described covariates associated with higher risk in addition to NTM colonization prior to lung transplantation. A two-sided p-value of less than 0.05 was considered significant. For all hazard ratios calculated, the 95% confidence interval (CI) was calculated. All statistics were performed using SPSS (Chicago, IL), Version 23.

4.4 Ethics

We received research ethics approval from the University of Alberta Research Ethics Board, Project Name "The prevalence and risk factors of nontuberculous mycobacterial infection in lung transplant patients and its impact on patient survival and graft function", No. Pro00064699, April 29, 2016.

Chapter 5 – Results

5.1 Patient characteristics

Three hundred seventy-five patients underwent lung- or heart-lung transplant between January 01, 2005, and December 31, 2014, at the University of Alberta Hospital, Edmonton, Alberta, Canada. Patient demographics are summarized in Table 4. The most common indications for lung transplantation were interstitial lung disease (n=149), obstructive lung disease (including emphysema, α-1-antitrypsin deficiency, and other unspecified obstructive lung disease, n=134), and cystic fibrosis (CF) (n=59). Among this cohort, 26 patients (6.9%) had NTM first isolated from respiratory samples before transplant at a median of 660 days (IQR 249-1776 days) beforehand; one patient had NTM isolated from an abscess (site unspecified in clinical and laboratory databases) in conjunction with respiratory infection. Patients with CF (p=0.003) and BMI less than 22 kg/m^2 (p=0.008) were at a significantly higher likelihood of having NTM infection before transplant (Table 5).

Figure 1a summarizes the frequency of NTM species isolated before transplant—the two most frequently isolated species were MAC (n=18; 55%) and *M. abscessus* (n=17; 21%). Five patients (19%) had two different species, and 1 patient (4%) had three species isolated. All patients had pulmonary infection; three patients had evidence of disseminated infection evidenced by the same NTM species isolated from the airway and in the blood (n=2) or skin abscess drainage (n=1).

Table 4 - Patient demographics (N=375)

Patients with NTM infection, N (%)	38 (10)
Patients with infection before transplant	26 (7)
Patients with infection after transplant	17 (4.5)
Patients with infection before and after transplant with same species	4 (1.1)
Patients with infection before and transplant with different species	1 (0.3)
Male, N (%)	252 (67)
Average time on waitlist, days (range)	298 (2-3404)
Average age at transplant, years (range)	52 (17-71)
Average length of stay in hospital, days (range)	44 (3-534)
Induction immunosuppressants, N (%)	
Anti-thymocyte globulin	216 (58)
Interleukin-2 antagonist	147 (39)
None	11 (3)
Maintenance immunosuppressants, N (%)	
Azathioprine	8 (2)
MMF	370 (99)
Prednisone	367 (98)
Cyclosporine	94 (25)
Tacrolimus	327 (87)
Transplant type, N (%)	
Single (left)	6 (2)
Single (right)	10 (3)
Double	339 (90)
Heart/Lung	15 (4)
Living lobar	5 (1)
Donor type, N (%)	
Living	5 (1)
Donation after cardiac death	7 (2)
Donation after neurologic death	363 (97)

Table 5 - Comparison of patient characteristics of those with and without history of pre-transplant NTM infection

	History of NTM infection (N=26)	No history of NTM infection (N=349)	p-value
Mean age (years)	48.2	51.8	0.243
Male gender, N (%)	18 (69)	232 (67)	0.774
Diagnosis, N (%)			**0.001**
CF	10 (38)	49 (14)	
Obstructive lung disease	12 (46)	121 (35)	
Interstitial lung disease	1 (4)	134 (38)	
Other	3 (12)	45 (13)	
Diagnosis of CF, N (%)	10 (38)	49 (14)	**0.003**
Mean BMI (kg/m^2)	22.0	24.7	**0.008**

BMI = body-mass index, CF = cystic fibrosis

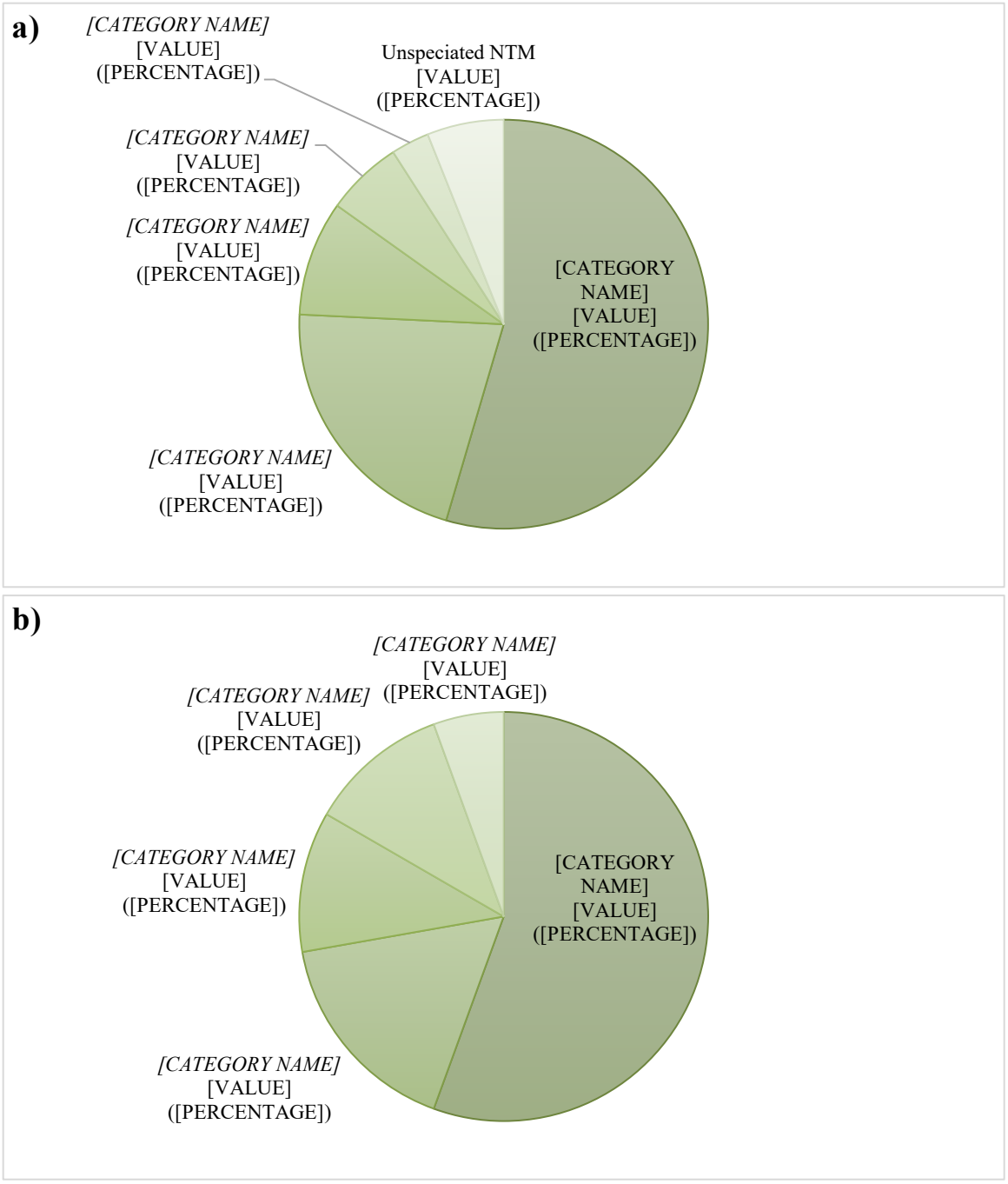

Figure 1 - Frequency of isolated NTM species a) before transplant and b) after transplant

After receiving a transplant, 17 patients had NTM isolated from respiratory samples at a median time of 253 days post-transplant (IQR 6-542 days). While 4 of these recipients had the same species of NTM isolated both before and after transplant, the other 13 recipients had a *de novo* NTM infection after transplant. No predictors of post-transplant NTM infection were noted (Table 6). MAC was the most commonly isolated species after transplant accounting for 56% of the isolates (n=10). The remaining species distribution is summarized in Figure 1b. All cases had pulmonary NTM infection; 3 patients, however, had disseminated disease as evidence by positive blood cultures (n=2; one blood culture was positive for *M. abscessus* and one culture was positive for MAC in two separate patients) or positive culture from a skin biopsy (n=1; culture was positive for MAC) in conjunction with a positive respiratory culture of the same species.

Table 6 - Comparison of patient characteristics of those with and without NTM infection after transplant

	Infected (N=17)	Not Infected (N=358)	p-value
Mean age (years)	46.2	51.8	0.376
Male gender, N (%)	11 (65)	241 (67)	0.857
Diagnosis, N (%)			0.324
Cystic fibrosis	5 (29)	54 (15)	
Obstructive lung disease	7 (41)	126 (35)	
Interstitial lung disease	5 (29)	130 (36)	
Other	0 (0)	48 (13)	
Diagnosis of cystic fibrosis, N (%)	5 (29)	54 (15)	0.294
Mean BMI (kg/m^2)	24.7	24.7	0.591
Mean duration of ICU stay post-transplant (days)	16.4	12.3	0.308
Induction with anti-thymocyte globulin, N (%)	9 (53)	208 (58)	0.575

BMI = body-mass index, ICU = intensive care unit

The mean number of positive respiratory specimens per patient was 3.47 (SD=4.31). This represents a mean of 2.28 positive sputums (SD=3.23) and 1.19 bronchoscopic specimens (SD=2.01) per patient.

5.2 NTM infection and impact on survival

During the follow-up period to November 1, 2016, nine patients infected with NTM exclusively pre-transplant (43%) died with a median survival of 4.1 years. After transplant, 11 patients with post-transplant NTM infection (65%) died at a median of 5.3 years and 129 non-infected patients (36%) died with a median survival of 5.3 years and 4.8 years, respectively. Eight of these infected patients died within the first 5 years following transplant. The cause of death was known in 7 of these patients; 2 were deemed to be NTM-attributable (both due to sepsis from disseminated *M. abscessus* infection). The other causes of death were invasive aspergillosis (n=1), CLAD (n=2), myocardial infarction (n=1) and end-stage renal disease (n=1). By multivariate Cox regression, NTM infection after transplant was significantly associated with all-cause 5-year mortality (p=0.016), after accounting for patient age, BMI, previous episode of acute rejection and underlying diagnosis (Table 7). After excluding *M. gordonae* from the Cox-regression analysis, the hazard ratio for 5-year mortality was 2.068 (95% CI 1.007-2.247; p = 0.048).

Table 7 - Univariate and multivariate analysis of predictive variables associated with 5-year mortality after lung transplantation

		Univariate Analysis		Multivariate Analysis	
	Mortality at 5 years	**Hazard Ratio (95% CI)**	**p-value**	**Adjusted Hazard Ratio (95% CI)**	**p-value**
NTM infection post-transplant, N (%)					
Yes	8/16† (50)	2.510 (1.217-5.178)	**0.013**	2.458 (1.187-5.093)	**0.016**
No	102/359 (28)				
Diagnosis of cystic fibrosis, N (%)					
Yes	11/59 (19)	0.640 (0.359-1.142)	0.131	1.019 (0.467-2.223)	0.962
No	94/316 (30)				
Acute rejection in 5 years, N (%)					
Yes	50/137 (37)	1.329 (0.913-1.934)	0.138	1.312 (0.899-1.916)	0.160
No	60/238 (25)				
Mean age at transplant (years)					
Alive at 5 years	50.7	1.018 (1.002-1.034)	**0.024**	1.017 (0.996-1.038)`	0.106
Dead at 5 years	53.7				
Mean body-mass index at transplant (kg/m^2)					
Alive at 5 years	24.2	1.028 (0.989-1.069)	0.407	1.011 (0.968-1.057)	0.614
Dead at 5 years	25.0				

† One of the 17 infected patients was diagnosed with NTM infection after 5 years and, therefore, was considered non-infected for this analysis

5.3 NTM infection and impact on CLAD

During the follow-up period, CLAD developed in 143 patients following lung transplant (38%) at a median of 27.7 months; 110 of these patients developed CLAD within the first 5 years after transplant. Based on pulmonary function tests, at the time of diagnosis, 58 patients (41%) had mild CLAD, 38 patients (27%) had moderate CLAD and 47 patients (33%) had severe CLAD. The obstructive phenotype was almost twice as common as the restrictive. Table 8 summarizes predictive risk factors for the development of CLAD. NTM infection after transplant was not significantly associated with the development of CLAD within 5 years ($p = 0.999$); only 4 transplant recipients (4%) had NTM infection preceding the diagnosis of CLAD. After excluding *M. gordonae* from the Cox-regression analysis, the hazard ratio for CLAD within 5 years was 1.404 (95% CI 0.518-3.808; $p = 0.505$) for 5-year mortality.

Table 8 - Univariate and multivariate analysis of predictive variables associated with development of CLAD at 5 years.

		Univariate Analysis		Multivariate Analysis	
	CLAD at 5 years	**Hazard Ratio (95% CI)**	**p-value**	**Adjusted Hazard Ratio (95% CI)**	**p-value**
NTM infection post-transplant, N (%)					
Yes†	4/12 (33)	1.225 (0.451-3.326)	0.691	1.001 (0.366-2.736)	0.999
No	109/363 (30)				
Diagnosis of cystic fibrosis, N (%)					
Yes	15/59 (25)	0.693 (0.402-1.194)	0.186	0.778 (0.377-1.606)	0.497
No	98/316 (31)				
Acute rejection in 5 years, N (%)					
Yes††	67/126 (53)	3.239 (2.224-4.717)	**<0.0001**	3.215 (2.195-4.709)	**<0.0001**
No	46/249 (19)				
Mean age at transplant (years)					
No CLAD at 5 years	51.4	1.007 (0.993-1.022)	0.326	1.006 (0.987-1.025)	0.529
CLAD at 5 years	51.9				
Mean body-mass index at transplant (kg/m^2)					
No CLAD at 5 years	24.5	1.005 (0.967-1.045)	0.800	0.978 (0.935-1.023)	0.330
CLAD at 5 years	24.4				
Induction with anti-thymocyte globulin, N (%)					
Yes	73/217 (34)	1.284 (0.872-1.889)	0.205	1.127 (0.760-1.670)	0.552
No	40/158 (25)				

† Includes only those with NTM infection diagnosed prior to CLAD diagnosis
†† Includes only those with acute rejection occurring prior to CLAD diagnosis

Chapter 6 – Discussion

6.1 Insights into the epidemiology of NTM infections

This study is the first to explore the epidemiology of NTM infections in lung transplant recipients in Western Canada. Over the 10-year study period, pre-transplant NTM isolation was relatively common with a 7% prevalence in lung transplant recipients consistent with literature demonstrating higher rates of NTM airway infection in this population compared to the general population[66]. The most commonly isolated species in our patients before transplant were MAC and *M. abscessus*. This is similar to previous studies from the Northwest United States[56–59], but unlike data from Ontario, Canada, citing MAC and *M. xenopi* as the more frequent isolates[41–43]. The only patient characteristics found to be associated with pre-transplant NTM infection were a diagnosis of CF and lower BMI.

6.2 Impact of NTM infections on patient survival and lung allograft function following lung transplantation

The association between pulmonary NTM infections and patient survival has been conflicting in the literature. As is the case in our cohort, Huang et al. found a significant increase in all-cause mortality in patients with NTM infection, colonization, and disease; NTM infection was not associated with a statistically significant increase in the development of bronchiolitis obliterans syndrome[82]. Similarly, Longworth et al. showed NTM infection in the first year following solid organ transplantation was strongly associated with increased mortality at 3 years[63].

The findings of Shah et al. show an opposite pattern of increasing development of BOS in those infected with NTM following transplant with no impact on long-term survival[88]. However, in our cohort, fewer cases of CLAD developing NTM-infected patients were present.

Traditionally, *M. gordonae* is considered to be non-pathogenic[31]; however, we included it in our analysis as previous cohort studies in lung transplantation have also included it[85,88]. Furthermore, after excluding *M. gordonae* from the Cox-regression analysis, our conclusions were unchanged.

6.3 Strengths and limitations

Our study has several strengths. We collected data on a large sample of lung transplant recipients, and the diagnosis was via a single provincial mycobacteriology laboratory, allowing for complete microbiology to be recovered for all patients. Our diagnosis of CLAD included both obstructive and restrictive phenotypes, the latter of which may have been unaccounted for in previous studies.

Our approach also had several limitations, however. The relationship between the development of CLAD and NTM infection is challenging to assess, because either condition can plausibly predispose to the other; only four patients in our cohort had NTM infection before the diagnosis of CLAD, making the ability to make a strong statistical inference challenging. Second, even with a sizable population of lung transplant recipients at our institution, the number of NTM cases was small. Importantly, colonization could not consistently and reliably be differentiated from disease, and co-infections and antimicrobial treatment could not be assessed from the available databases; both factors may impact patient outcomes.

6.4 Future directions

The results of this study contribute to the ongoing debate of NTM infections' impact on survival and graft function following lung transplantation, strengthening the argument that NTM infection contributes to all-cause mortality. Prospective multicenter studies of the impact of NTM colonization and disease on lung transplant recipients are unlikely to be feasible given the relative paucity of cases worldwide. As such, the increasing number of retrospective observational studies continue to build our understanding of these important infections and support the development of international guidelines and decision-making in transplantation.

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