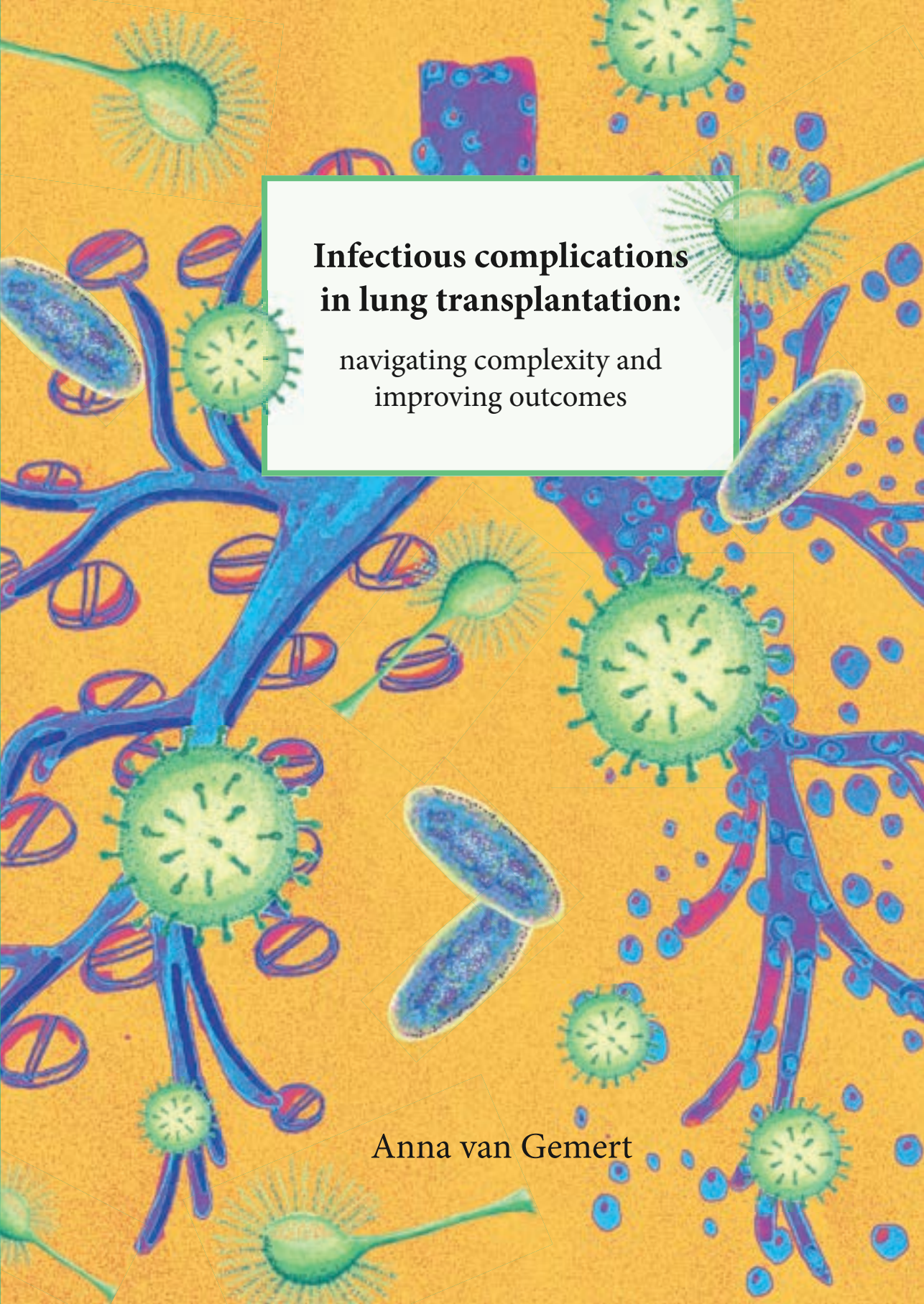

Infectious complications in lung transplantation:

navigating complexity and improving outcomes

Anna van Gemert

STELLINGEN

behorende bij het proefschrift

Infectious complications in lung transplantation: navigating complexity and improving outcomes

1. Een van de belangrijkste taken van de longtransplantatiearts is het voortdurend streven naar het behouden van een delicate balans tussen infectiebeheer en afstotingscontrole voor elke individuele longtransplantatiepatiënt. [Dit proefschrift]
2. Een effectieve behandeling van aspergillose en niet-tuberculeuze mycobacteriën-infecties bij longtransplantatiepatiënten begint met een accurate diagnose, maar het ontbreken van praktische diagnostische criteria bemoeilijkt tijdige en gerichte therapie. [Dit proefschrift]
3. Bij de behandeling van infectieziekten bij longtransplantatiepatiënten moet de focus niet alleen liggen op het bestrijden van de ziekteverwekker, maar is verder onderzoek nodig naar aanpassingen in immunosuppressie tijdens infecties. Dit is een onderbelicht gebied met potentieel voor gerichte therapeutische interventies. [Dit proefschrift]
4. De effectiviteit van vaccins na longtransplantatie is zorgwekkend laag: zelfs na vijf doses van het SARS-CoV-2-vaccin ontwikkelt slechts de helft van de longtransplantatiepatiënten een adequate antistofrespons. [Dit proefschrift]
5. Er is weinig bekend over de juiste elexacaftor/tezacaftor/ivacaftor (ETI) dosering voor Cystic Fibrose (CF) patiënten na longtransplantatie, en de huidige ‘one-dose-fits-all’-aanpak lijkt door de grote variabiliteit in farmacokinetiek van zowel ETI als calcineurineremmers niet geschikt voor alle longtransplantatiepatiënten met CF. [Dit proefschrift]

6. Gezien de beperkte levensverwachting na een longtransplantatie, is het cruciaal om de kwaliteit van leven te prioriteren en te optimaliseren. ETI levert hieraan een belangrijke bijdrage en moet daarom worden beschouwd als een waardevolle investering. [Dit proefschrift]

7. De behandeling van acute rejectie vertoont overeenkomsten met chemische oorlogsvoering: een gerichte analyse wordt gevolgd door een escalatie in therapeutische agressie, variërend van het schieten van duizendjes uit de broekzak (Methylprednisolon), dieptebommen (Anti Thymocyten Globuline) of neutronenbommen (Alemtuzumab). [Gebaseerd op uitspraken van dr. W. van der Bij]

8. *Aspergillus* is voor de longtransplantatiepatiënt allesbehalve een zegen, ondanks zijn symbolische naamgeving naar de wijwaterkwast.

9. De snelheid en mate van inclusie van patiënten in klinische studies wordt in belangrijke mate beïnvloed door de zichtbaarheid en populariteit van de onderzoeker en/of het te onderzoeken middel binnen patiëntengroepen op sociale media, zoals Facebook.

10. Een donorprocedure vereist onvermijdelijk graafwerkzaamheden, aangezien overleden donoren zich doorgaans onder de grond bevinden. [Wen Li Fokkinga, 7 jaar]

Anna van Gemert
Groningen, 24 september 2025

Infectious complications in lung transplantation: navigating complexity and improving outcomes

Anna van Gemert

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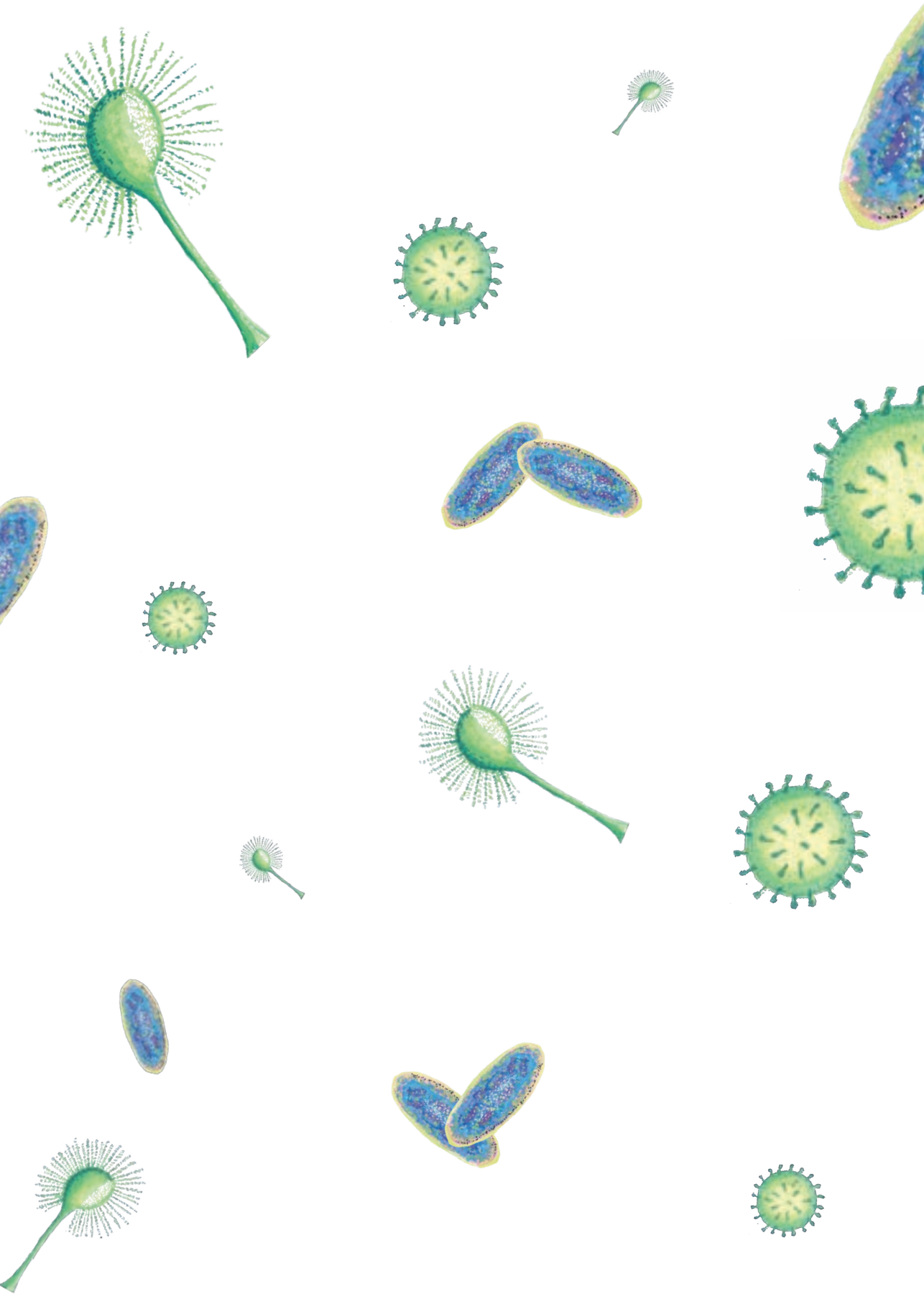
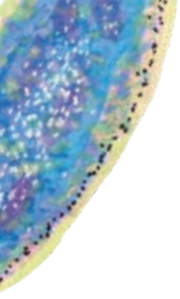
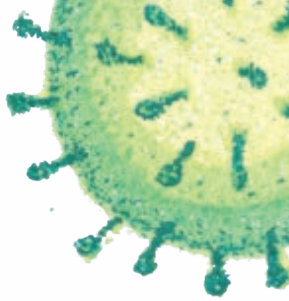
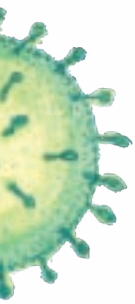
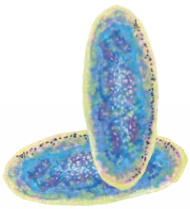
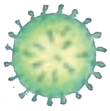






Chapter 1

Introduction and outline of this thesis



Introduction

Lung transplantation (LTx) is a treatment option for patients with severe chronic lung diseases when no other therapeutic alternatives are available, with the primary goal of extending life. The most common indications for LTx include Chronic Obstructive Pulmonary Disease (COPD), Cystic Fibrosis (CF), pulmonary fibrosis, and pulmonary hypertension. Lung transplantation can be performed as either a bilateral or unilateral procedure.

To be considered for LTx, patients must first undergo a comprehensive screening process, which involves hospitalization and extensive diagnostic evaluations to assess their suitability for this complex surgery. Once deemed eligible, patients are placed on a waiting list. The duration of this often stressful waiting period varies depending on individual urgency, as determined by the Lung Allocation Score (LAS), and donor organ availability. When suitable donor lungs become available, the patient is called in for transplantation.

Following the procedure, patients typically spend approximately five days in the intensive care unit, followed by a hospital stay on the general ward. The total hospitalization period ranges from four to six weeks, with a one-year post-transplant mortality rate of 10%.

The new donor law in the Netherlands came into effect on July 1, 2020. This law introduced an opt-out system for organ donation, meaning that all individuals aged 18 and older are automatically registered as organ donors unless they explicitly choose to opt out. This new donor law has led to an increase in the number of available donor organs.[1]

Lung transplantation history

The first human lung transplantation (LTx) was performed in 1963 in Mississippi.[2] Although the procedure itself was technically successful, the recipient died after 18 days. From then onwards in the 1960s and 1970s, 36 experimental LTx were performed, but only 2 recipients survived longer than 2 months largely due to inadequate immunosuppression.[3] These poor outcomes led to a significant decline in the number of LTx by the late 1970s. This trend reversed with better immune suppression, which significantly improved survival rates and spurred a global increase in LTx procedures.[4]

In the Netherlands, the first LTx was performed at the St. Antonius Hospital in Nieuwegein in 1989.[5] The first government-recognized LTx program had already been established in 1986 at the Academic Hospital Groningen, now University Medical Center Groningen.[6] Since then, significant advancements in the field have resulted in improved outcomes, with a median post-transplant survival of 10–11 years at the University Medical Center Groningen today (Figure 1.1).

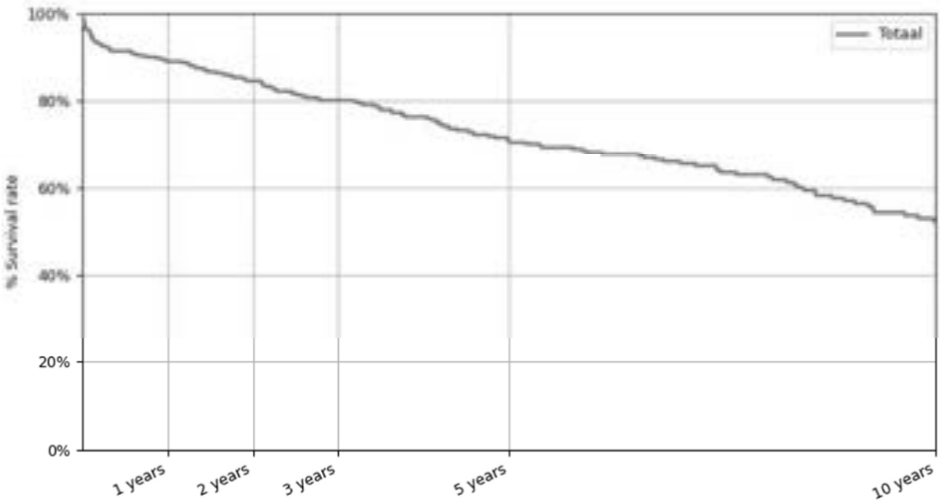


Figure 1.1: Patient survival for lung transplantation at the University Medical Center Groningen form 2010–2025.
Data obtained from the Nederlandse Transplantatie Stichting as of 4-2-2025.

Immunosuppression

As is clear from the above brief historic summary, immunosuppressive drugs are essential after LTx to prevent acute rejection and chronic lung allograft dysfunction (CLAD). Initially, immunosuppressive protocols were based on anti-thymocyte globulin, corticosteroids, and azathioprine in the 1970s. However, these protocols were associated with infectious complications, issues with the healing of bronchial anastomoses and poor survival.[7] The discovery of cyclosporine, a calcineurin inhibitor which inhibits T-cel activation, led to improved outcomes in LTx in the early 1980s.[4] Its successor, the calcineurin inhibitor tacrolimus, further reduced the incidence of CLAD and is now the first-choice calcineurin inhibitor after lung transplantation.[8]

Current immunosuppressive regimens consist of tacrolimus, azathioprine or mycophenolate mofetil, and steroids. These regimens are not allograft-specific, which increases the risk of infectious diseases (see below) and malignancies. This is because the suppression of cellular immunity through immunosuppression not only inhibits the immunological response directed against the transplanted lungs, but also impairs the immunological responses to infections and cancer.[9,10] Moreover, these agents have significant toxicity profiles, necessitating the use of combination therapies to mitigate the adverse effects of any single agent. Calcineurin inhibitor related renal toxicity is among the most critical toxic complications and is correlated with lower survival rates, decreased quality of life and high costs.[11]

Additionally, significant drug interactions occur between immunosuppressive agents and medications used to treat infectious diseases, such as antifungals, therapies for non-tuberculous

mycobacteria (NTM) pulmonary disease, and newer drugs for cystic fibrosis (CF). These interactions further complicate the management of LTx recipients.

Infectious diseases in LTx recipients

As stated above, though potent immunosuppressive agents have reduced the incidence of CLAD, they increase patients' susceptibility to opportunistic infectious diseases and the progression of community-acquired infectious diseases.[9]

Infectious diseases arise from an interplay between 3 key factors: the pathogen (agent), the host, and environmental influences.[12] Following exposure, the initial step in this process is often colonization—the adherence and initial proliferation of a microorganism at a portal of entry, such as the mucosal surfaces of the respiratory tract. Importantly, colonization alone does not constitute disease. For disease to develop, the pathogen must successfully infect the host by invading and establishing itself within host tissues. While infection inevitably disrupts host homeostasis to some extent, it does not always progress to clinically apparent disease. Disease occurs when the extent of disruption and host tissue damage crosses a threshold that manifests as both subjective symptoms and objective clinical signs.[13]

In LTx recipients, the host is particularly compromised. Immunosuppressive therapy reduces the host's ability to mount an effective immuno response against pathogens. As a result, colonization can more readily progress to infection and overt disease, potentially impairing graft function. This theses primarily focuses on infectious diseases rather than colonization; however, distinguishing between colonization and disease in clinical practice remains a considerable challenge.

The risk of infectious diseases after LTx changes over time, particularly with modifications in immunosuppression. In the early postoperative period, LTx recipients are vulnerable to intensive care unit (ICU)- or hospital-ward-acquired infectious diseases, such as central line infections, wound infections, and ventilator-associated pneumonia. Donor-derived infectious diseases including bacteria, fungi, Epstein-Barr virus, and cytomegalovirus can also occur. Preventive strategies, such as prophylactic valganciclovir for herpes viruses, antibiotics for bacterial transmission, and antifungal medications, have been implemented to address these risks.

After hospital discharge, LTx recipients remain highly susceptible to community-acquired infectious diseases, opportunistic infectious diseases, and reactivation of latent infections during the first post-transplant year, when immunosuppression is most intense. Pathogens such as cytomegalovirus, Epstein-Barr virus, fungi, and NTM may emerge as primary infec-

tious disease or from latency or reservoirs in the sinuses or thoracic cavity. After the first year, community-acquired infectious diseases predominate, although opportunistic infectious diseases can still occur.

Balancing the risk of rejection against infectious diseases is the key challenge for transplant clinicians. It requires knowledge of the clinical presentation, immunosuppression levels, and the individual LTx recipient to provide sufficient immunosuppression to preserve the allograft, while not administering too much, allowing the LTx recipient to maintain an adequate immune response against infectious pathogens.

This thesis will delve deeper into the following infectious pathogens in LTx recipients: SARS-CoV-2, NTM and *Aspergillus* species. COVID-19, which is the infectious disease caused by the virus SARS-CoV-2, is addressed because this virus has had, and continues to have, a significant impact on LTx recipients. During the first COVID-19 wave, there was also significant uncertainty regarding the effects on mortality, lung function, treatment strategies, and prevention.

NTM pulmonary disease (NTM-PD) is the infectious pulmonary disease caused by NTM. NTM-PD is discussed due to the relative scarcity of research on this topic and the substantial consequences it poses for LTx recipients and candidates. Finally, a chapter is dedicated to infectious diseases caused by *Aspergillus* (aspergillosis), as it represents a common infectious disease in LTx recipients, with treatment and prophylaxis posing challenges due to interactions between antifungal therapies and immunosuppressive regimens, as well as the associated toxicity of antifungal treatments. Other infectious diseases do not fall within the scope of this thesis.

COVID-19

The COVID-19 pandemic has taught us valuable lessons, including the heightened vulnerability of solid organ transplant recipients. Solid organ transplant recipients face a significantly higher COVID-19-related mortality compared to the general population.[14,15] Small case series suggest that COVID-19 leads to high admission (80–100%) and mortality rates (8–55%) among LTx recipient.[16–22] Furthermore, COVID-19 mortality rates vary considerably between countries, leaving the impact on the Dutch LTx population largely unknown.

In addition to the limited understanding of COVID-19's impact on Dutch LTx patients, its effects on transplant function remain insufficiently explored. Single-center studies have reported a significant association between COVID-19 and chronic lung allograft dysfunction (CLAD).[15,23] In contrast to COVID-19, the impact of other viral infectious diseases on CLAD is relatively

well-documented. For example infectious diseases caused by the community acquired respiratory syncytial virus (RSV), parainfluenza virus (PIV), and human metapneumovirus (hMPV) are increasingly associated with CLAD in LTx recipients.[24] It is therefore important to investigate whether COVID-19 is also associated with CLAD.

Prevention being preferable to cure, highly effective SARS-CoV-2 vaccines were fortunately developed during the COVID-19 pandemic.[25,26] Their implementation resulted in a reduction in infections and a decrease in severity of COVID-19 within the general population.[26–28] Most of the initial trials excluded solid organ transplant recipients. Unfortunately, solid organ transplant recipients and especially LTx recipients, are less likely to develop an antibody response to SARS-CoV-2 vaccines and have a higher risk of severe COVID-19 course and worse outcome, due to their immunosuppressed status.[29–31] Therefore, in LTx recipients optimal vaccination status must be aimed for as much as possible to protect them against severe COVID-19. LTx recipients develop poor antibody responses, even after a third SARS-CoV-2 vaccine dose (16–46%).[32] The effect on the antibody response following 4 or 5 SARS-CoV-2 vaccine doses is not yet known. Additionally, it remains unclear whether certain factors (comorbidity, immunosuppressive drugs etc.) are associated with the absence of a vaccination response. Investigating these aspects is crucial, as it could inform targeted vaccination interventions or identify those LTx recipients who may require additional vaccine doses.

A variety of therapeutic treatments are being developed or repurposed to treat COVID-19 in LTx recipients. The proposed treatment options include oral antiviral agents such as Paxlovid (which have significant interactions with calcineurin inhibitors) steroids, and IL-6 inhibitors.[33] Additionally, monoclonal antibodies, which may be effective in general, are potentially less effective against newer SARS-CoV-2 variants.[34] Treatment regimens for COVID-19 vary among different LTX centers. A survey among 180 LTX centers showed that most participating centers proposed specific antiviral regimens for COVID-19–positive LTx recipients, whereas substantial changes in the immunosuppressive regimen were mostly limited to severe cases. While treatment guidelines exist for COVID-19, there are few guidelines on how to manage immunosuppression during infectious diseases. For COVID-19, there is an ISHLT statement addressing immunosuppression management during COVID-19, but it comprises merely recommendations with little evidence.[35]

Viral diagnostics

During the COVID-19 pandemic, the use of viral diagnostics increased significantly. This shift not only impacted the diagnosis of COVID-19 but also enhanced the detection of other viruses.

Before the COVID-19 pandemic, viral diagnostics had already been used for LTx candidates arriving at the hospital for a lung offer, but they were now also applied to donors. After the pandemic, this raised the question of whether this testing leads to overdiagnosis, particularly in asymptomatic LTx candidates prior to LTx. The implications can be substantial for awaiting a LTx, as the procedure may be cancelled due to the detection of an asymptomatic respiratory virus. To date, no studies explored the post-LTx outcomes of immediately pre-LTx acquired respiratory viruses in asymptomatic recipients.

Non-tuberculous mycobacteria

NTM are a group of bacteria naturally found in water, soil and dust. NTM consist of a divers group of tuberculous mycobacterium species and subspecies, specifically excluding *Mycobacterium tuberculosis* complex, *Mycobacterium leprae* complex and *Mycobacterium ulcerans* which are not considered NTM. The most common clinical manifestation is NTM-PD, typically associated with underlying structural airway abnormalities such as CF, non- CF bronchiectasis or chronic obstructive pulmonary disease (COPD).[36] The annual prevalence of NTM-PD was estimated at 6.2/100,000 in Europe and 10–20% in people with CF.[37–39] Patients listed for LTx often have structural lung damage, bronchiectasis or CF, and therefore have an increased risk of NTM-PD. NTM-PD used to be a contra-indication for LTx, but several small studies have shown that LTx is feasible.[40–44] Currently, according to International Society for Heart and Lung Transplantation 2021 guidelines, patients with NTM-PD may be referred for LTx, but should be managed at centers with expertise and protocols for NTM-PD.[45]

NTM, especially *Mycobacterium abscessus* and *Mycobacterium avium* complex are associated with increased post-LTx mortality and the development of CLAD.[46,47] It is unclear whether patient-related factors or immunosuppressive regimens lead to worse prognosis of NTM-PD post-LTx.

To reduce the risk of post-LTx NTM-PD, it is recommended that patients listed for LTx with cultured NTM be treated pre-LTx.[48] Directly after LTx, when immunosuppression is most intense, NTM treatment might be necessary to prevent transition to pulmonary or disseminated NTM disease post-LTx. However, there is no clear guideline for this due to a lack of scientific evidence.

In general, NTM-PD is difficult to treat, and cure rates for NTM vary depending on the species, ranging from 16% to 65%.[49–51] Although culture-directed therapy for most NTM species is standardized worldwide in non-transplant patients, there is no consensus on treatment options, strategies, or duration for patients listed for LTx.[50] Post-LTx, drug interactions between

antimycobacterial agents and immunosuppressive therapy are challenging and may lead to severe side effects.

Aspergillosis

Aspergillosis is an infectious disease caused by inhalation of spores of the fungus *Aspergillus*. Invasive aspergillosis (IA) is the most severe and life-threatening form of aspergillosis, occurring almost exclusively in patients with significantly compromised immune defences, such as those after LTx. Pathologically, the fungus invades and spreads through lung tissue and frequently infiltrates blood vessels within the lungs. IA poses significant risks of morbidity and mortality for LTx recipients.[52–54] The reported incidence of fungal infectious diseases in LTx recipients ranges from 15 to 35%.[55] Managing IA in the context of LTx presents significant challenges, largely due to potential drug interactions between antifungal treatments and immunosuppressive therapies. Given the critical desirability of prevention over treatment, there is a pressing need for further investigation into the factors contributing to aspergillosis post-LTx.

Unfortunately there is considerable variation among various LTx centers in fungal prophylactic therapy, with differences in regimen, treatment duration and outcome.[56] The effectiveness of preventive measures, such as nebulized Amphotericin B and azoles, has been shown to be inconsistent.[57–61] Statins have been suggested another potential strategy for preventing aspergillosis. Statins inhibit ergosterol synthesis, a critical component of the fungal cell membrane, and therefore may play a role in fungal prophylaxis.[62] However, insufficient research has been conducted to determine whether this is effective in clinical practice.

Additionally, the relationship between *Aspergillus* colonization, IA, and CLAD remains controversial.[55,58,63–66] While the progression of CLAD likely increases the risk of *Aspergillus* colonization and IA, the data on the trajectory of lung function in LTx recipients with *Aspergillus* colonization or IA are limited.

Lung transplantation in Cystic Fibrosis

The most common indications for LTx at the University Medical Center Groningen (cohort 2013–2022), as briefly indicated above, are obstructive pulmonary disease (45%), interstitial lung disease (26%), pulmonary arterial hypertension (13%), and cystic fibrosis (7%). The remaining 9% consists of a variety of diagnoses that span the spectrum of end-stage lung disease from non-CF bronchiectasis to lymphangioleiomyomatosis to pulmonary Langerhans cell histiocytosis and graft-versus-host disease (GVHD). In cases such as chronic obstructive pulmonary disease and interstitial lung disease, the initial problems of the disease may

have been resolved, but the consequences of the chronic condition persist. Similarly, some systemic diseases with severe pulmonary involvement are also present with manifestations in other organ systems. Examples include CF, systemic sclerosis, rheumatic lung diseases etc. In these cases, post-transplant care must also focus on managing extra-pulmonary disease manifestations. This highlights the complexity and challenges of LTx care in the context of multi-organ diseases. Post-transplant care must extend beyond the focus on LTx itself to address the extra-pulmonary manifestations of the underlying disease, requiring a comprehensive and multidisciplinary approach, such as in people with CF (pwCF).

CF is a multi-organ disease arising from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.[67] In most pwCF the lungs are the most severely affected organ, making it a life-threatening condition for which, until recently, LTx was the only treatment option. LTx for CF was first performed using a combined heart–lung transplant in 1983, with the first bilateral LTx for CF performed a few years later.[68] Since then CF has become the third most common indication for LTx worldwide.[69] While survival following LTx has improved significantly over recent decades, the degree of improvement varies between countries. For instance, 5-year survival rates are 74.6% in the Netherlands, compared to 60.3% in the United States and 69.7% in Canada.[1,70] In the Netherlands, the 10-year survival rate is 57.8%.[1]

The leading causes of post-LTx mortality in pwCF are primary graft dysfunction, infections, bronchiolitis obliterans and other forms of graft failure. Infectious diseases continue to be major drivers of mortality throughout the posttransplant course, and are the leading causes of mortality in the first year after LTx in pwCF.[71] Additionally, however, other problems of CF also occur post LTx. One of the key extra-pulmonary complications after LTx in pwCF is CF-related diabetes, affecting up to 50% of pwCF and it is associated with worse outcomes. Also gastrointestinal issues, such as reflux and intestinal dysmotility, often worsen post-LTx, while distal intestinal obstruction syndrome may occur early after surgery and later on. Other complications are hepatobiliary disease, an increased risk of malignancies and chronic sinus infections which can persist post-LTx and predispose to pulmonary infections.[71] Post-transplant management of these comorbidities in pwCF remains essential.

The medical management of pwCF has changed with the development of small molecules that partially restore the function of the defective CF transmembrane conductance regulator protein and are called CFTR modulators. In 2017 these CFTR modulators became available in the Netherlands. Nowadays the most potent CFTR modulator combination, Elexacaftor/Tezacaftor/Ivacaftor, is approved in the Netherlands for those with at least one copy of

the F508del allele. CFTR modulators have substantially improved pulmonary function and health-related quality of life for pwCF. Additional extra-pulmonary benefits include improved gastrointestinal function, growth, pancreatic function and sinus symptoms.[72]

Recent advancements with CFTR modulators have dramatically reduced the number of pwCF requiring LTx, but there are still pwCF requiring or having had an LTx (Figure 1.2).[73] As the organ most affected by CF is the lung, CFTR modulators were conventionally not considered to be of value in transplanted pwCF. Additionally, CFTR modulators were not used post-LTx due to the high costs and these concerns regarding the benefits post-LTx and interactions with immunosuppressive drugs, especially calcineurin inhibitors. However, as indicated, CF affects multiple organ systems, leading to extrapulmonary symptoms post-LTx that may impact quality of life and graft function post-LTx as well. Therefore the hypothesis explored in this dissertation is that CFTR modulators may benefit post-transplant pwCF. This has not been previously investigated in a prospective multicenter study.

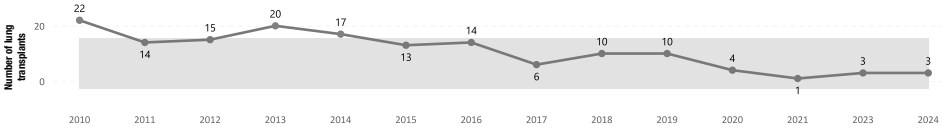


Figure 1.2: Annual number of lung transplants performed in people with cystic fibrosis in the Netherlands.
Data obtained from the Nederlandse Transplantatie Stichting as of 4-2-2025.

Collaborating on the complexities of lung transplantation

The complexity and challenges of LTx care are evident through all the aspects mentioned above. Progress in this field often requires both literal and figurative boundary-pushing, which is impossible without collaboration. This collaboration extends across borders through the establishment of international guidelines, joint research efforts, and the provision of remote patient care. Given the relatively small population of LTx recipients in individual countries, collaboration with other centers is essential to facilitate larger international studies with sufficient statistical power to improve patient care. The case report in this dissertation serves as a tribute to the importance of such collaboration

Aims of the thesis

In this thesis several aspects regarding infectious diseases and the use of CFTR modulators post-LTx were investigated.

The first aim of this thesis is to map the spectrum of infectious disease in LTx recipients, explore optimal treatment strategies, and evaluate preventive measures to reduce infection risk. Specific attention is given to viral infectious diseases, including COVID-19 (**chapters 2–4**), aspergillosis (**chapter 6**), and NTM-PD (**chapter 5**).

In **chapter 2**, the objective is to provide insights into the clinical impact and treatment of COVID-19 in LTx recipients. This retrospective national cohort study evaluates mortality and transplant function before and after COVID-19 in LTx recipients, including data from all 3 Dutch transplant centers.

SARS-CoV-2 vaccination is potentially life-saving for LTx recipients, yet their antibody response is impaired. In **chapter 3** we investigate whether this response can be enhanced. We specifically study the serological IgG antibody response after up to 5 doses of the SARS-CoV-2 vaccine using a large cohort of LTx recipients from the University Medical Center Groningen.

In **chapter 4** community-acquired respiratory virus carriage is examined in all LTx recipients at the University Medical Center Groningen immediately before transplantation. The study evaluates its impact on the early post-transplant course in asymptomatic recipients who tested positive for a community-acquired respiratory virus at the time of transplantation.

In **chapter 5** the treatment of NTM-PD pre- and post-LTx is reviewed. A systematic review is performed with the goal of providing a comprehensive oversight of the literature on treatment regimen and duration pre- and directly post-LTx, for patients with known NTM-PD pre-LTx. Additionally, we search the literature for risk factors for NTM-PD development post-LTx and for mortality.

In **chapter 6** we delve deeper into the aspergillosis dilemma following LTx. To address the problem of IA following LTx, the primary goal of this chapter is to identify factors associated with IA following LTx and to explore potential protective modalities, including nebulized Amphotericin B and statin therapy. Additionally, the impact of IA on lung function, the development of CLAD, and mortality is studied.

In **chapter 7** a case is presented that transcends borders, both in a literal and metaphorical sense. This case demonstrates what can be achieved internationally through effective collaboration.

The second aim of this thesis is to investigate the benefits of CFTR modulators post-transplant in pwCF. Therefore, in **chapter 8**, a national multi-centre prospective study (the KOALA study) is presented investigating the relevant benefits and safety of CFTR modulators for pwCF after LTx.

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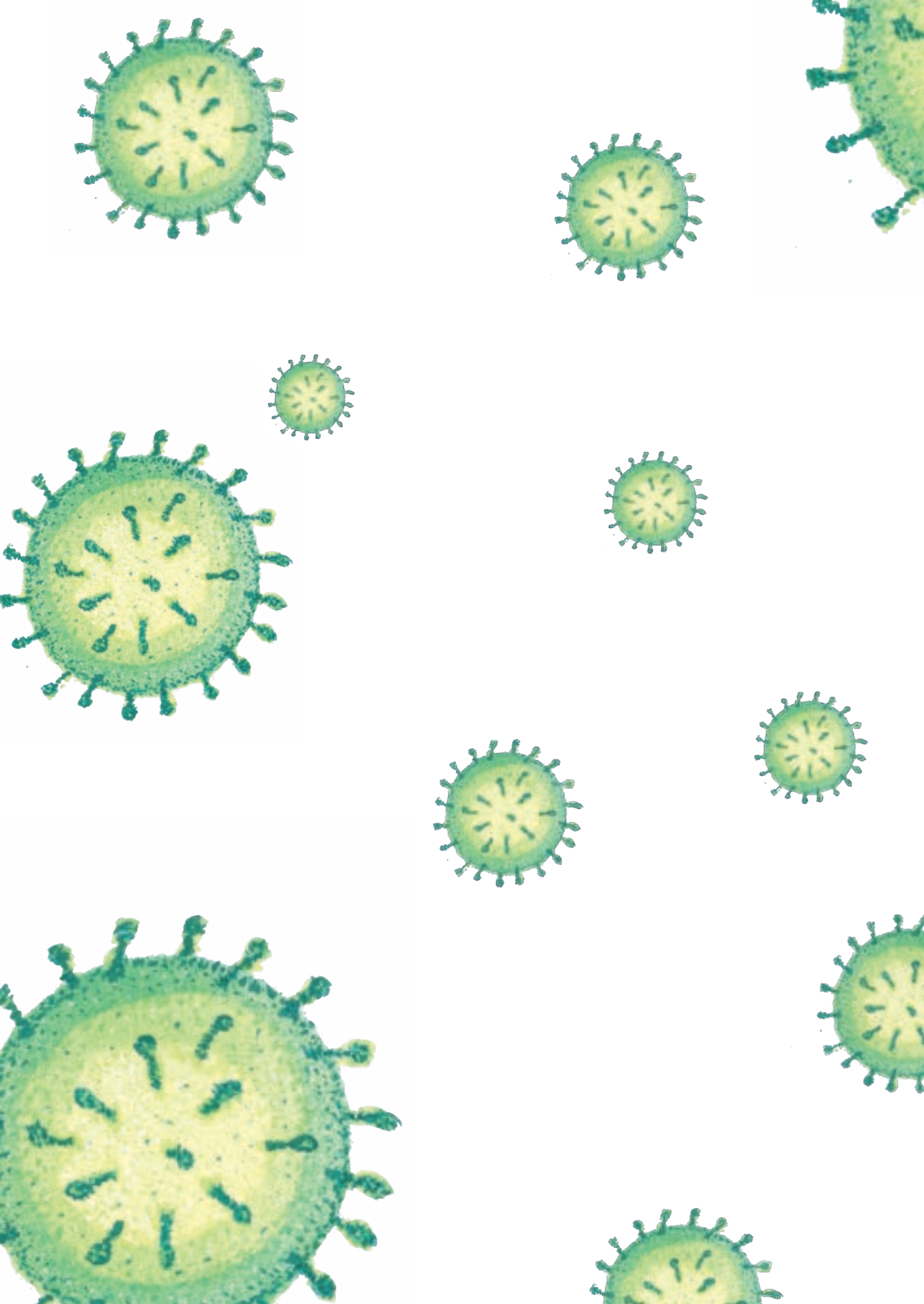
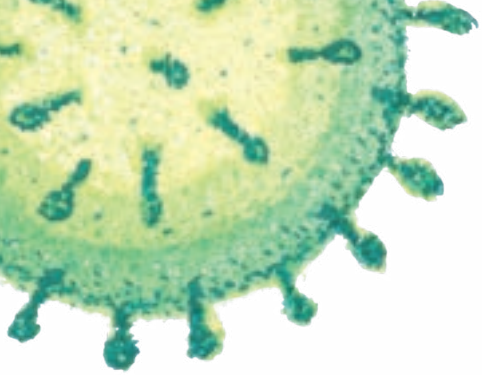
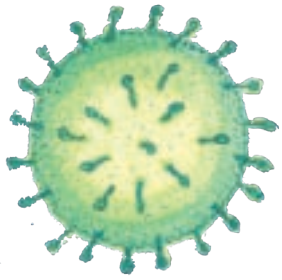
Part I

Infectious diseases and lung transplantation

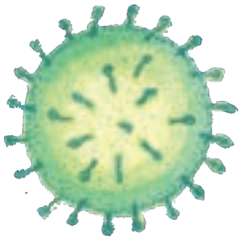






Chapter 2

The effect of COVID-19 on transplant function and development of CLAD in lung transplant patients: A multicenter experience



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Abstract

Purpose: Concerns have been raised on the impact of coronavirus disease (COVID-19) on lung transplant (LTx) patients. The aim of this study was to evaluate the transplant function pre- and post-COVID-19 in LTx patients.

Methods: Data were retrospectively collected from LTx patients with confirmed COVID-19 from all 3 Dutch transplant centers, between February 2020 and September 2021. Spirometry results were collected pre-COVID-19, 3- and 6-months post-infection.

Results: Seventy-four LTx patients were included. Forty-two (57%) patients were admitted, 19 (26%) to the intensive care unit. The in-hospital mortality was 20%. Twelve out of 19 ICU patients died (63%), a further 3 died on general wards. Patients with available spirometry (78% at 3 months, 65% at 6 months) showed a significant decline in mean FEV1 (ΔFEV1 138 ± 39 ml, $p = 0.001$), and FVC (ΔFVC 233 ± 74 ml, $p = 0.000$) 3 months post-infection. Lung function improved slightly from 3 to 6 months after COVID-19 (ΔFEV1 24 ± 38 ml; ΔFVC 100 ± 46 ml), but remained significantly lower than pre-COVID-19 values (ΔFEV1 86 ml ± 36 ml, $p = 0.021$; ΔFVC 117 ± 35 ml, $p = 0.012$). FEV1/FVC was > 0.70.

Conclusions: In LTx patients COVID-19 results in high mortality in hospitalized patients. Lung function declined 3 months after infection and gradually improved at 6 months, but remained significantly lower compared to pre-COVID-19 values. The more significant decline in FVC than in FEV1 and FEV1/FVC > 70%, suggested a more restrictive pattern.

Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is responsible for coronavirus disease 2019 (COVID-19), has emerged as a global health threat. As of 19 December 2021, over 273 million COVID-19 cases and over 5.3 million deaths have been reported globally.[1] Mortality rates from COVID-19 vary across countries, reflecting differences in population measures taken and social behaviour, as well as in case identification, death registration, access to medical care and variable thresholds for hospitalization. The overall in-hospital mortality is around 15% to 20%, but up to 40% among patients admitted to the intensive care unit (ICU).[2]

Concerns have been raised on the impact of COVID-19 on solid organ transplant (SOT) recipients, since SOT-recipients are at higher risk for severe COVID-19 as a result of chronic immunosuppression.

Several case series have been published on COVID-19 after solid organ transplantation and showed a higher mortality rate in SOT-recipients compared to non-SOT recipients.[3,4] COVID-19 leads to a high admission rate (80–100%). Reported mortality rates in small series of lung transplantation (LTx) patients with COVID-19 range from 8% to 55%.[5–11] Limited data are available regarding the transplant function outcome after COVID-19. In addition, single center studies have reported a significant association between COVID-19 and chronic lung allograft dysfunction (CLAD).[4,12] The aim of this multicenter retrospective study was to evaluate the effect of COVID-19 on the clinical course and lung function in LTx patients.

Material and methods

Patients

The study was waived by the respective Ethical Committees based on its retrospective nature (METc number 2021.283). The study complies with the ISHLT Ethics Statement. All patients provided written informed consent upon entry into the program. Data were retrospectively collected at three transplant centers in the Netherlands. All adult patients who underwent unilateral or bilateral lung transplantation with a polymerase chain reaction confirmed COVID-19 between February 27th 2020 and September 1st 2021 were eligible for inclusion.

Transplant care

Follow-up of all patients took place at least every 3 months for monitoring of transplant function. All patients received standard maintenance immunosuppression: tacrolimus, pred-

nisolone and mycophenolate mophetil. Alternative immunosuppressants were cyclosporine, azathioprine, everolimus or sirolimus (Table 2.1). Post-transplantation prophylactic therapy included cotrimoxazole for pneumocystis pneumonia and valganciclovir (5 mg/kg OD) for CMV, depending on CMV (mis)match.

Comorbidities and symptoms

For chronic kidney disease we use the Kidney Disease Improving Global Outcomes (KDIGO) staging.[13] Heart failure is defined according to the 2021 ESC guidelines. Upper respiratory tract symptoms include: sore throat, rhinorrhea, nasal congestion, smell or taste disturbances.

Virologic diagnostics

The indications for testing for SARS-CoV-2 virus in LTx patient in our cohort are: 1) fever, with or without symptoms of respiratory tract infection, e.g. cough, dyspnoea, running nose loss of sense of smell or taste; 2) close contact with a confirmed COVID-19 infected person. SARS-CoV-2 infection was defined as a positive polymerase chain reaction on a nasopharyngeal swab. Swabs were collected by municipal public health services or at the hospital.

Pulmonary function

Spirometry was performed pre-COVID-19 as part of standard care. Baseline lung function is computed as the mean of the best 2 post-operative FEV1 measurements, taken $>$ 3 weeks apart. Lung function pre-COVID-19 was taken at 6 and 3–0 months before infection. Follow-up measurements were performed at the routine visits of outpatients with COVID-19 or 2–4 weeks after discharge in patients that had been admitted for COVID-19. Second measurement took place 6 months after COVID-19. Mild lung function loss is defined as a $\leq$ 10% loss in this FEV1 or FVC 3 months post-COVID-19 compared to pre-COVID-19, whereas a $>$ 10% loss in FEV1 or FVC 3 months post-COVID-19 compared to pre-infection is classified as severe infection.[14]

Spirometry was performed according to ATS/ERS guidelines.[15] The Global Lung Function Initiative Network (GLI) reference values were used to express percentages of predicted values, the z-scores and the lower limit of normal (LLN). CLAD was defined as a persistent decline of 20% or more in FEV1 value from baseline value post transplantation, independent of a change in FVC, according to the most recent ISHLT criteria.[16]

Statistical analysis

Normally distributed continuous variables are expressed as mean (standard error of mean (SEM) or median (quartiles)) for non-normally distributed variables. Mann–Whitney U-test and

chi-squared test were used to compare patient characteristics between admitted and non-admitted patients. Paired-samples T-test were used for within-group analyses to compare FEV1 and FVC pre- and post-COVID-19. Analyses were carried out using IBM SPSS for Mac, version 24.0. A *p*-value of less than 0.05 was used as the cut-off for significance. Log-rank test and Kaplan-Meier survival curves were used to investigate relations between low-flow oxygen, high-flow nasal oxygen, invasive ventilation use and survival.

Results

Patients

Seventy-four from a total of 754 LTx patients (10%) had a confirmed COVID-19 infection at all 3 transplant centers in the Netherlands, between March 2020 and September 1st 2021 (Figure 2.1). The median follow up period was 6 months (IQR 5–8). Baseline characteristics for all LTx patients with COVID-19 are shown in Table 2.1. The median age was 59 years (IQR 48–65) and the majority of patients (45/74; 61%) were male. COPD (29/74; 39%) or pulmonary fibrosis (21/74; 28%) were the most common indications for LTx, and a bilateral LTx was performed in 64/74 (87%). The most frequent reported comorbidities were chronic kidney disease, hypertension and diabetes mellitus. Seven patients (7/74; 10%) were vaccinated before SARS-CoV-2 infection (2 vaccins). None of the patients used oxygen therapy before SARS-CoV-2 infection. The symptoms most commonly reported in LTx patients with COVID-19 were fatigue (38/74; 51%), upper respiratory symptoms (33/74; 45%); i.e. sore throat, rhinorrhea, nasal congestion, smell or taste disturbances, fever (31/74; 42%), cough (31/74; 42%), and dyspnoea (30/74; 41%). Thirty-two of 74 patients (43%) were not hospitalized and closely monitored with home spirometry and oximetry combined with telephone or video consultation by the transplant team to assess for clinical and/or respiratory worsening warranting hospital admission. Forty-two out of 74 patients (57%) were hospitalized, of whom 19/42 (45%) were admitted to the ICU (Figure 2.1). Criteria for hospitalization were: clinical symptoms of (upper) respiratory infection, fever, shortness of breath with or without oxygen requirement (peripheral oxygen saturation $<$ 92%). Hospitalized patients and patients who died, were older, had more often diabetes and chronic kidney disease and a higher BMI (Table 2.1). No other significant differences in comorbidities were found between hospitalized and non-hospitalized patients and between survivors and non-survivors (Table 2.1). Hospitalized patients more often had symptoms of dyspnoea and myalgia than non-hospitalized patients (Table 2.2). There were no significant differences in clinical characteristics and comorbidities between patients admitted to the ICU or the general ward. After discharge all patients were followed routinely in the outpatient clinic. After six months the majority (33/59) had persisting

symptoms; i.e., fatigue, shortness of breath, coughing. The timeline of lung transplant patients with COVID-19 is shown in Figure 2.2. The median time between transplantation and COVID-19 was 5 years (IQR 2–10 years), with a median 2-day delay between symptom onset and PCR test. The median interval from test to hospitalization was 6 days (IQR 3–8 days). The median length of hospital stay was 2 weeks (IQR 7–26 days) and the median time between admission and death was 24 days (IQR 19–46 days).

Table 2.1: Baseline characteristics of lung transplant patients with COVID-19

Variable	All patients	Hospitalized	Non-hospitalized	*p*-value*	Died	*p*-value**
Recipients, n (%)	74 (100)	42 (57)	32 (43)		15 (20)	
Age, years	59 (48–65)	63 (54–67)	54 (39–63)	0.010	63 (49–71)	0.010
Gender, male (%)	45 (61)	24 (57)	21 (66)	0.459	9 (60)	0.943
Non-Caucasian, n (%)	9 (12)	6 (14)	3 (9)	0.522	2 (13)	0.876
Transplant indication, n (%)						
COPD	29 (39)	18 (43)	11 (34)		9 (60)	
Fibrosis	21 (28)	13 (31)	8 (25)		2 (13)	
Pulmonary hypertension	5 (7)	2 (5)	3 (9)		0 (0)	
Cystic fibrosis	15 (20)	6 (14)	9 (28)		3 (20)	
Other	4 (5)	3 (7)	1 (3)		1 (6)	
Bilateral LTx, n (%)	64 (87)	35 (83)	29 (91)	0.363	11 (73)	0.095
Time since transplant, years	5 (2–10)	6 (2–10)	5 (1–11)	0.477	8 (2–11)	0.477
Body mass index, kg/m^2	26 (24–29)	28 (24–32)	25 (23–27)	0.036	27 (20–32)	0.036
Comorbidities, n (%)						
Hypertension	27 (37)	18 (43)	9 (28)	0.192	5 (33)	0.776
Dyslipidemia	7 (10)	2 (6)	5 (16)	0.114	1 (7)	0.679
Diabetes Mellitus	25 (34)	20 (48)	5 (16)	0.004	11 (73)	0.000
Chronic kidney disease	45 (61)	30 (71)	15 (47)	0.032	13 (87)	0.022
Atrial fibrillation	3 (4)	2 (5)	1 (3)	0.724	0 (0)	0.373
Heart failure	4 (5)	3 (7)	1 (3)	0.449	1 (7)	0.809
Immunosuppression, n (%)						
Tacrolimus	71 (96)	40 (95)	31(97)		14 (93)	
CYC	2 (3)	1 (2)	1 (3)		0 (0)	
AZA	6 (8)	3 (9)	3 (9)		1 (7)	
MMF	65 (87)	37 (88)	28 (88)		12 (80)	
mTORi	8 (11)	6 (14)	2 (6)		4 (27)	
COVID-19 vaccination, n (%)***	7 (10)	5 (12)	2 (6)		0 (0)	

Continuous variables are expressed as median (interquartile range).
AZA, azathioprine; COPD, chronic obstructive pulmonary disease; LTx, lung transplantation; MMF, mycophenalte mofetil; mTORi, mammalian target of rapamycin inhibitors (everolimus or sirolimus); CYC, cyclosporine.
* *p*-value for the difference between hospitalized patients and non-hospitalized patients.
** *p*-value for the difference between survivors and non-survivors.
*** The vaccine used was mRNA (Moderna®).

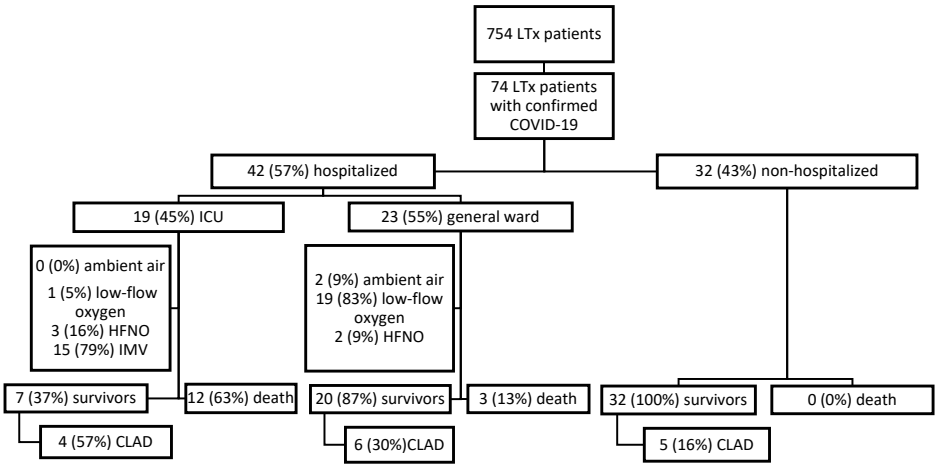


Figure 2.1: Flow diagram among lung transplant (LTx) patients.
LTx, lung transplantation; HFNO, high flow nasal oxygen; IMV, invasive mechanical ventilation; CLAD, Chronic Lung Allograft Dysfunction.

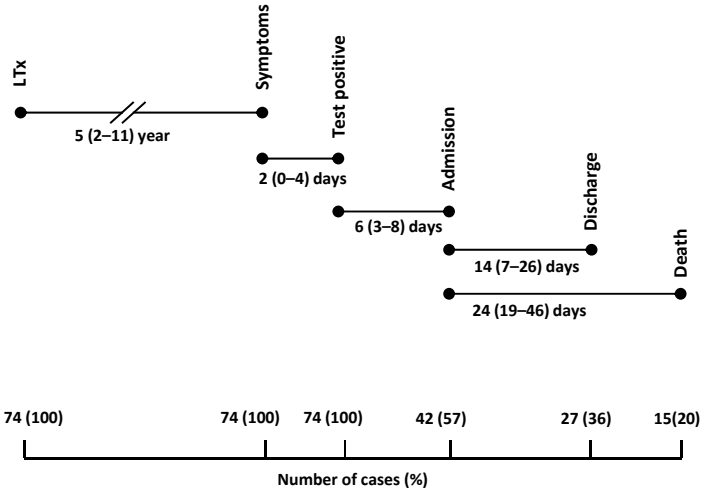


Figure 2.2: Timeline of lung transplant patients with COVID-19.
Variables are expresses as median (interquartile range).

COVID-19 management and outcome in LTx patients

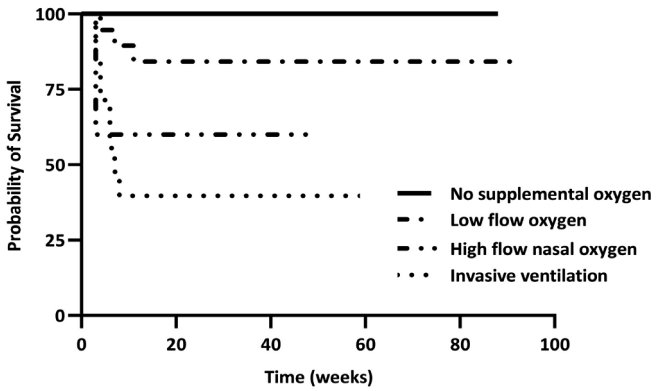
Thirty-four out of 74 patients (46%) did not require supplementary oxygen. The median length of hospital stay of those patients who did not receive supplementary oxygen, was 2 days (IQR 1–2). Twenty of the 42 (48%) hospitalized patients received non-high-flow oxygen, 5/42 (12%) patients received high-flow nasal oxygen (HFNO) and 15/42 (36%) patients needed invasive mechanical ventilation (Table 2.2). Of the fifteen mechanical ventilated patients 9 (60%) were intubated because of HFNO failure. The mortality was 60% (9/15) among mechanically

ventilated patients and 40% (2/5) in patients who received HFNO. The Kaplan-Meier curve of survival in lung transplant patients with no supplementary oxygen, non-high-flow oxygen, HFNO and invasive ventilation is shown in Figure 2.3.

Table 2.2: Clinical data lung transplant patients with COVID-19

	All patients n = 74	Hospitalized n = 42	Non-hospitalized n = 32	*p*-value	Died n = 15	*p*-value
Symptoms, n (%)						
Fatigue	38 (51)	18 (43)	15 (47)	0.434	8 (53)	0.672
Dyspnoea	30 (41)	21 (50)	9 (28)	0.047	5 (33)	0.649
Cough	31 (42)	18 (43)	13 (41)	0.779	5 (33)	0.570
Chest pain	7 (10)	4 (10)	3 (9)	0.956	1 (7)	0.730
Fever	31 (42)	19 (45)	12 (38)	0.448	5 (33)	0.570
Myalgia	13 (18)	4 (10)	9 (28)	0.042	2 (13)	0.702
Gastro-intestinal	21 (28)	14 (33)	7 (22)	0.250	7 (47)	0.051
Headache	19 (26)	11 (26)	8 (25)	0.860	2 (13)	0.265
Upper respiratory	33 (45)	15 (36)	18 (56)	0.094	4 (31)	0.844
Respiratory support						
Ambient air	34 (46)	2 (5)	32 (100)		0 (0)	
Low-flow oxygen	20 (27)	20 (48)	0 (0)		4 (27)	
High-flow oxygen	5 (7)	5 (12)	0 (0)		2 (13)	
IMV	15 (20)	15 (36)	0 (0)		9 (60)	
Treatment COVID-19						
None	31 (42)	5 (12)	26 (81)		2 (13)	
Corticosteroids	42 (57)	36 (86)	6 (19)		13 (87)	
Hydroxychloroquine	3 (4)	3 (7)	0 (0)		1 (7)	
Remdesivir	6 (8)	6 (14)	0 (0)		3 (2)	
Tocilizumab	13 (18)	13 (31)	0 (0)		5 (33)	
Convalescent plasma	8 (11)	8 (19)	0 (0)		2 (13)	
Monoclonal antibodies	2 (3)	2 (5)	0 (0)		0 (0)	
Immunosuppressive medication adjustment						
AM discontinued/lower dose	38 (51)	30 (71)	8 (25)		10 (67)	
AZA	3 (4)	1(2)	2 (6)		0 (0)	
MMF	35 (47)	29 (69)	6 (19)		10 (67)	
CI discontinued	1 (1)	1 (3)	0 (0)		1 (7)	
CI lower trough levels	6 (8)	6 (14)	0 (0)		5 (33)	
mTORi discontinued	1 (1)	1 (2)	0 (0)		1 (7)	
ICU admission, n (%)	19 (26)	19 (45)	0 (0)		12 (80)	
Died, n (%)						
COVID-19 related	13 (18)	13 (31)	0 (0)		13 (87)	
Other cause of death	2 (3)	2 (5)	0 (0)		2 (13)	

Continuous variables are expressed as median (interquartile range).
AM, antimetabolite; AZA, azathioprine; CI, calcineurin inhibitor; ICU, intensive care unit; IMV, invasive mechanical ventilation; MMF, mycophenolate mofetil; mTORi, mammalian target of rapamycin inhibitors.
* *p*-value for the difference between hospitalized patients and non-hospitalized patients.
** *p*-value for the difference between survivors and non-survivors.



Time (weeks)	0	20	40	60	80	100
Patients at risk						
No supplemental oxygen	34	33	24	6	2	1
Low flow oxygen	19	14	12	5	5	2
High flow nasal oxygen	5	3	2	-	-	-
Invasive ventilation	14	6	5	-	-	-

Figure 2.3: Kaplan-Meier curve of survival in lung transplant patients with no supplementary oxygen, non-high-flow oxygen, high flow nasal oxygen and invasive ventilation.

Survival of patients without supplemental oxygen was better when compared to low flow oxygen, HFNO and invasive ventilation ($p < 0.001$). Patients on low flow oxygen had better survival than on invasive ventilation ($p < 0.01$), but was not different from patients on HFNO ($p = 0.16$), There was no significant difference in survival between patients on HFNO and invasive ventilation ($p = 0.65$).

One patient received extracorporeal membrane oxygenation (ECMO) support. Corticosteroid dose was increased in those patients with lower respiratory tract symptoms and/or lung function decline. Patients who were not admitted with mild symptoms did not receive an increased dose of steroids.

Corticosteroids were increased in 42/74 (57%) patients, of whom 6/42 (14%) patients were treated at home and 36/42 (86%) were admitted. Additional treatment consisted of hydroxychloroquine (3/74; 4%), remdesivir (6/74; 8%), tocilizumab (13/74; 18%), convalescent plasma (8/74; 11%) or monoclonal antibodies (2/74; 3%). Dose reductions or discontinuation of antimetabolite treatment occurred among 38/74 (51%) of patients, in 6/74 (8%) calcineurin inhibitors were adjusted to lower trough levels (6–7 µg/l). In one patient the

tacrolimus was temporarily stopped and, in another patient who used tacrolimus, sirolimus and prednisolone, the sirolimus was discontinued. The median length of hospitalization was 18 days (IQR 8–29). The overall COVID-19 related mortality in the cohort was 13/74 (18%), 2/60 (3%) patients died due to another cause (metastatic breast cancer, progressive multifocal leukoencephalopathy). Twelve out of 19 ICU patients died (63%), 3 died on the general wards. They were found not eligible for intensive treatment on the ICU, because of comorbidities, and deteriorating performance status pre COVID-19. All non-hospitalized patients survived. None of the vaccinated patients died. In patients who survived 33/59 (56%) had persisting symptoms after COVID-19 infection.

Transplant function

In 58/74 (78%) patients post-COVID-19 spirometry at three months was available and in 48/74 (65%) patients spirometry was available at 6 months post-COVID-19 (Table 2.3). There was a significant decline in FEV1 (ΔFEV1 138 ± 39 ml, $p = 0.001$) and in FVC (ΔFVC 233 ± 74 ml, $p = 0.000$) at first follow-up within 3 months. There was no decline in FEV1/FVC ratio (0.72 vs 0.73) after COVID-19. Even though FEV1 (ΔFEV1 24 ± 38 ml) and FVC (ΔFVC 100 ± 46 ml) improved between the first follow-up (within 3 months) to 6 months, FEV1 and FVC remained significantly lower than pre-COVID-19 values (ΔFEV1 86 ml ± 36 ml, $p = 0.021$; ΔFVC 117 ± 35 ml, $p = 0.012$). Mean FEV1/FVC ratio after 6 months remained > 0.70. Figure 2.4 demonstrates the percentage of FEV1 and FVC decline from pre-COVID-19 at 3- and 6-months post-COVID-19. There was no difference in FEV1 decline between hospitalizes and non-hospitalized patients ($p = 0.473$). There was a trend toward a more sever FVC decline in hospitalized patients than in non-hospitalized patients (Δ 208 ± 75 ml vs Δ40 ± 49 ml; $p = 0.059$).

Table 2.3: Transplant function pre- and post-COVID-19

	Pre-COVID-19	3 months post-COVID-19	*p*-value	6 months post-COVID-19	*p*-value
FEV1, L	2.55 ± 0.10 Δ 40 ± 55/3 months	2.41 ± 0.11 Δ 138 ± 39 ml/3 months	0.001	2.47 ± 0.11 Δ 86 ml ± 36 ml/6 months	0.021
FVC, L	3.58 ± 0.14 19 ± 61/3 months	3.35 ± 0.15 Δ 233 ±62 ml/3 months	0.001	3.35 ± 0.16 Δ 117 ± 45 ml/6 months	0.012
FEV1/FVC ratio	72 ± 0.02	73 ± 0.02	0.219	73 ± 0.02	0.833
BMI, kg/m^2	26 ± 0.58			26 ± 0.58	0.163

Continuous variables are expressed as mean and standard error of mean (SEM).
FEV1, Forced expiratory volume; FVC, Forced vital capacity.
Δ = ml lung function decline of pre-COVID infection.

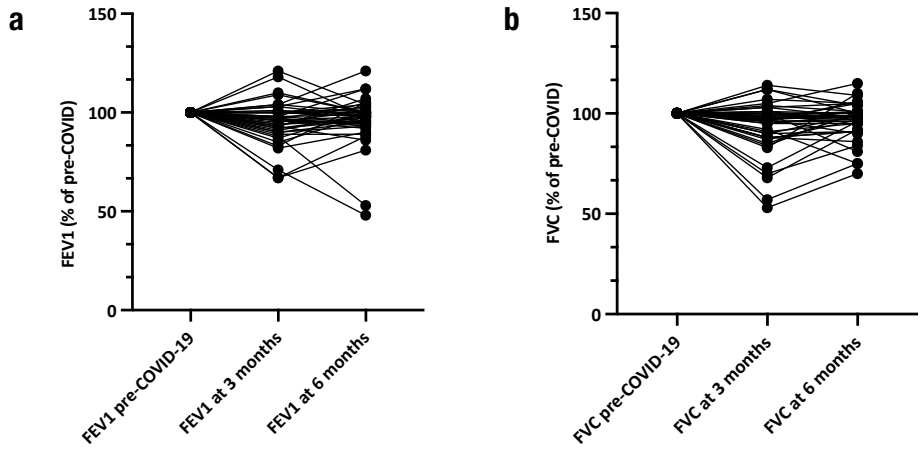


Figure 2.4: (a) FEV1 3 and 6 months after COVID-19. (b) FVC 3 and 6 months after COVID-19.

In our cohort 15/58 (26%) of the patients had a severe loss in FEV1 and 18/58 patients (31%) had a severe loss in FVC, 3 months after COVID-19. Patients with increased corticosteroid dose had more often severe lung function loss compared to those without increased corticosteroids dose (for FEV1 $p = 0.007$; for FVC $p < 0.001$). No significant differences, except from hypertension, in comorbidities and clinical characteristics were found between patients with mild and severe FEV1 or FVC loss (Supplemental Table S2.1).

Lung function pre-COVID-19 in the cohort was stable, i.e. there was no significant difference between FEV1 and FVC measurements 6 and 3 months pre-COVID (ΔFEV1 40 ml ± 55 ml, $p = 0.469$; ΔFVC 19 ± 61 ml, $p = 0.756$).

Pre-COVID-19 there were 21/74 (28%) patients with CLAD, 6 months post-COVID-19 there were 15/48 (20%) patients with CLAD and 15 patients died (Figure 2.5). Seven out of 18 (39%) patients with the bronchiolitis obliterans syndrome (BOS) phenotype died and 1 patient converted to the restrictive allograft syndrome (RAS) phenotype. There were 3 patients with new BOS and 4 patients with new RAS. Patients with RAS or BOS pre-COVID-19 who survived, remained stable. In patients with CLAD pre-COVID-19, mortality appeared higher than in patients without CLAD (33% vs 15%), but the difference was not statistically significant ($p = 0.078$). Two of the seven vaccinated patients had stable CLAD and 2 patients developed new CLAD.

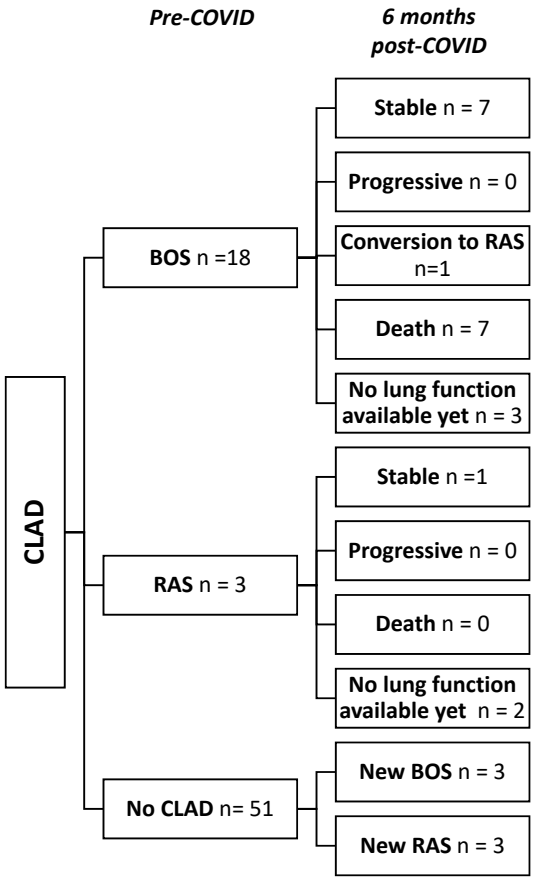


Figure 2.5: CLAD incidence pre-COVID-19 and 6 months post-COVID-19.
BOS, Bronchiolitis Obliterans Syndrome; CLAD, Chronic Lung Allograft Dysfunction; RAS, Restrictive Allograft Syndrome.

Radiology

In patients with severe FVC decline, 17/18 (94%) showed persistent changes on the x-ray or CT-scan after 3 months. In 16 of these 17 patients the extent of the radiologic abnormalities improved over 3 months. In patients with severe FEV1 decline 9/15 (60%) showed persisting but improvement of radiologic abnormalities on x-ray or CT. In patients without severe FVC decline 4/37 (11%) had abnormalities on x-ray or CT and in patients without severe FEV1 decline 12/40 (30%) show abnormalities on x-ray or CT. In summary, patients with a significant decline in FVC ($p < 0.001$) or FEV1 ($p = 0.041$) had significantly more x-ray or CT abnormalities than patients with no severe FVC or FEV1 decline.

Discussion

In this Dutch multicenter study, in which we studied LTx patients with COVID-19 for a follow-up period of 6 months, we showed that the clinical course may vary. More than half of the LTx patients required hospitalisation and nearly 40% were in need of mechanical ventilation. However, COVID-19 in LTx patients may also have a mild clinical course without need for hospitalisation, but with close monitoring at home. The older LTx patients with multiple comorbidities were hospitalized. In the hospitalized LTx patients COVID-19 results in higher mortality rate compared to the non-hospitalized. In addition, we showed that lung function decline after COVID-19 gradually improves from 3 to 6 months post-infection, but remained significantly lower and showed a restrictive pattern compared to pre-COVID-19 values. Patients with CLAD did not progress over time, but mortality appeared to be higher when compared to the non-CLAD patients.

The incidence of COVID-19 among LTx patients during the first 18-months of the global COVID-19 pandemic, was similar to the general Dutch population (10%),[17] which is remarkable since LTx patients are at higher risk for life threatening infections. This might be attributed to the number of unreported cases in the general population and the engagement of the LTx patients to the advised measures; i.e. social distancing and wearing a non-surgical mask.

Hospitalized vs non-hospitalized

The hospital admission rate in our study was high (57%), but markedly lower than in previous series on LTx patients with COVID-19 (84–100%).[5–7,18] This difference in hospitalization rate might be explained by the low threshold for testing and the shorter average home-to-hospital distance in the Netherlands, allowing close patient monitoring. Adequate outpatient management of mild COVID-19 is feasible in non-SOT patient and was shown to avoid hospitalization.[19,20] It is unknown whether this is feasible in LTx patients. Nevertheless, home monitoring of LTx patients was safe in the Dutch setting with home spirometry, close contact and a short distance between hospital and LTx patient. There were no deaths in this group.

Despite the lower admission rate, nearly half of the hospitalized LTx patients were admitted to the ICU requiring invasive mechanical ventilation, indicating more severe disease. The intubation rate in LTx patient is much higher than in the general population (20% vs 4–12%).[21] Also, the mortality rates in mechanical ventilated patients and in patients who received HFNO are higher than in the general population: 60% vs 21% in mechanical ventilated patients, and 40% vs 15% in patients who received HFNO. This might be explained by the more severe course of COVID-19 disease seen in LTx patients due to the immunocompromised state and the significant comorbidities in LTx patients.[22]

In line with COVID-19 risk factors in the general population, LTx patients hospitalized, and patients who died, were older, had more often diabetes and chronic kidney disease and a higher BMI.[23]

Management

Although the higher risk of severe disease and mortality, the management strategy among patients with LTx and COVID-19 has been similar to the general population in the Netherlands. In LTx patients treatment regiments was based on scientific evidence from studies and experience in the general non transplant population with additional international expert opinion summarized in the ISHLT consensus guideline.[24] Since the study period covers the start of the pandemic up to September 2021 treatment regiments have changed due to new insights. Therefore, different treatment regiments were used in our study according to national and regional availability of specific drugs and consensus on treatment regiments.

The majority of LTx patients were on a triple immunosuppressive regimen with calcineurin inhibitors, steroids and antimetabolites. When infected with COVID-19, most patients received an increased dosage of steroids in the case of severe symptoms, the need of supplemental oxygen or a decline in home spirometry. Antimetabolites were discontinued or reduced, which is in line with the study by Verleden et al. and Pereira et al.[8,25] However, it remains unclear which immunosuppressive has to be reduced, discontinued or continued at lower trough levels, considering the importance of hyperinflammation in the pathogenesis of severe COVID-19. Therefor more research on the optimal regime is needed in LTx patients with COVID-19.

Outcome

The COVID-19 mortality in LTx patients in our study (20%) is comparable to an 8-39% mortality rate in previous reports on SOT patients and LTx patients.[5–11] Kamp et al. suggested that the limited respiratory reserve and the substantial CLAD prevalence might contribute to poor outcomes.[26] The is supported by our data which showed that mortality among patients with CLAD was high (33%), and showed a trend towards significance when compared to the non-CLAD patients.

Lung function

There was a decline in lung function 3 and 6 months after COVID-19. Which is in line with a recent study of Mohanka et al. among 13 LTx patients with available post-COVID-19 lung function, with median follow-up of 43.5 days. They showed that 6 patients had > 10% loss in FVC or FEV1.[12] Kamp et al demonstrated a decline in TLC but not in FEV1 in LTx patients

4–12 weeks after survived SARS-CoV-2 infection.[26] However, the effect of COVID-19 on lung function at long-term follow-up is unknown. Therefore, this is the first multicenter study in LTx patients to show that lung function gradually improved from 3 to 6 months follow-up, but remained significantly lower compared to pre-COVID-19 values. This is in agreement with a long-term follow up study in non-SOT patients with COVID-19, that showed that restrictive lung function significantly improved over time, but was not resolved by 6 months.[27] Moreover, the more restrictive pattern demonstrated in these studies is in agreement with our results.[19,21] This restrictive pattern may be explained by the destruction in alveolar structure and pulmonary interstitial fibrosis found by autopsies of COVID-19.[28] It might be that patients with more sever FVC decline have more fibrosis on CT-scan post COVID-19. In our cohort, patients with severe FVC or FEV1 decline had significantly more x-ray or CT abnormalities than patients with no severe FVC or FEV1 decline ($p < 0.001$ and $p = 0.041$ respectively). However, in most of these patients the extent of the radiologic abnormalities improved over 3 months. Therefore, it seems that patients with severe lung function decline, especially in FVC, have more radiological abnormalities on CT or x-ray, but like lung function decline, these abnormalities improved over time.

Future studies are needed to explore the relationship between FVC decline and radiological changes. Other explanations for a restrictive lung function pattern might be an increase in BMI or a reduction in muscle strength. However, BMI did not change in our population and unfortunately, we have no data on muscle strength. Muscle weakness is common after ICU admission. In our cohort only 7 patients had post COVID-19 lung function measured after ICU admission. Therefore, in our cohort, FVC fall is less likely to be related to muscle strength. Furthermore, the decline in FVC gradually improved at 6 months post-COVID-19, but did not return to pre-COVID values, while there were no patients with permanent muscle weakness in our cohort.

The amount of lung function decline 6 months after COVID-19 is not severe, but still significant. In addition, the decline in lung function might be underestimated, since the patients who died did not have available post-COVID-19 spirometry results. Pre-COVID-19 the average decline per 3 months in FEV1 was 40 ± 55 ml and in FVC was 19 ± 61 ml. Therefore a FEV1 decline of 138 ml and FVC decline of 233 ml between pre-covid and 3 months post covid is indeed much more than expected in our LTx population. In addition, 26% of the patients had a severe loss in FEV1 and 31% had a severe loss in FVC, 3 months after COVID-19, which indicates that COVID-19 does have an important influence on lung function. Two LTx patients stand out in terms of their FEV1 decrease (ΔFEV1 1130 and 990 ml) as shown in Figure 2.4a. Both patients were male, 58 and 63 years, had a history of COPD and obesity

(BMI 28 and 31) without other comorbidities and were using standard immunosuppression (tacrolimus, prednisolone and mycophenolate mofetil). One of the patients had a stenosis of the right main bronchus. Both had CLAD grade 0 before COVID-19. Those two patients were not hospitalized and did not use supplementary oxygen. Only steroids were increased. In addition to age and BMI, there were no other parameters who could predict such severe lung function decline due to COVID-19.

Post infection improvement in pulmonary function has been demonstrated in non-SOT and LTx patients with non-SARS-CoV-2 viral infections.[14] De Zwart et al. showed that in LTx patients with non-COVID-19 viral infections, only 17% did not return to their pre-infection FEV1-value at 6 months post-infection.[14] Long term follow-up needs to be awaited whether lung function in LTx patients post COVID infection may recover completely.

After COVID-19, patients with CLAD did not progress over time in this study. Our findings are conflicting with the study by Kamp et al. They showed that in the subgroup of LTx recipients with pre-existing CLAD 43% had a substantial deterioration in graft function at 4–12 weeks post COVID infection.[26] Although there were no cases of progressive CLAD in our study, 33% of the patients with CLAD died. The lung function post-COVID-19 of these patients was unknown. This difference in the number of patients with CLAD progression, might also be explained by the longer follow-up period in our study. There were 3 patients with new BOS and 4 with new RAS (1 conversion from BOS to RAS). Several studies have shown the effect of non-COVID-19 respiratory virus infections on the incidence of CLAD.[14,29,30] In these studies, a restrictive pattern was less common than an obstructive pattern, which does not correspond to the more restrictive pattern seen after COVID-19 in our study. Our findings are in line with the study of Mahan et al. who showed that the 3 patients with CLAD post-COVID-19, all had RAS phenotype.[31]

Our study has some limitations. First, not all patients had available follow-up spirometry results. Second, due to evolving insights over the past 18 months, patient management has been diverse, which may have affected the clinical course and outcome. Administration of vaccines, might also have an effect on the clinical course. Third, we did not have a matched control group to compare transplant function over time. Fourth, to fulfil the definition of RAS, according to Verleden et al., TLC is needed.[16] Unfortunately, we did not have TLC results in the majority of our patients. Therefore, the findings of a restrictive pattern in lung function should be interpreted with caution.

In conclusion, COVID-19 infection in LTx patients results in high hospitalization and mortality rate. There was a decline in lung function shortly after infection which gradually improved

at 6 months post-COVID-19, but did not return to pre-COVID values and showed a more restrictive pattern. Patients with CLAD did not progress over time, but mortality appeared to be higher when compared to the non-CLAD patients.

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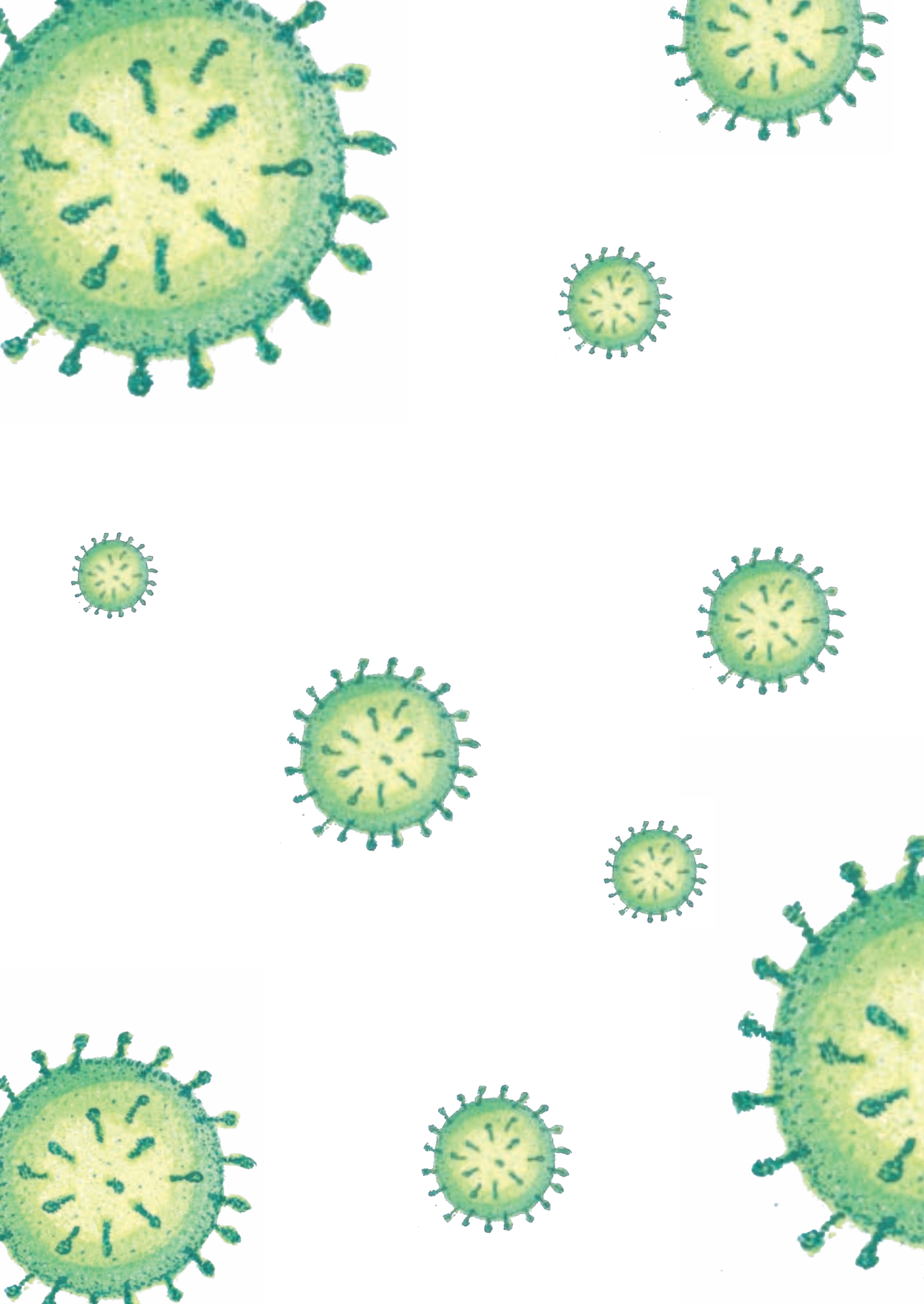
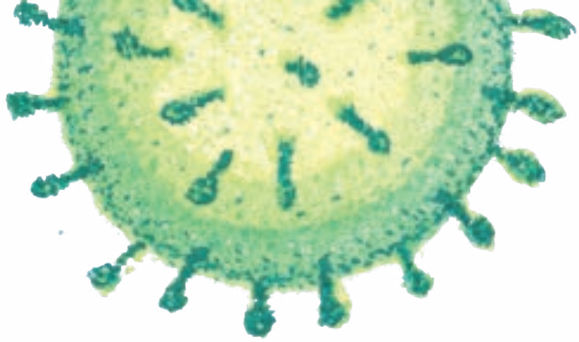
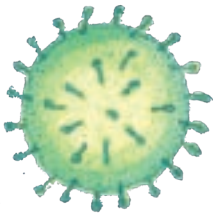
Supplemental Table S2.1: Univariate analysis of predictors for severe lung function loss: comorbidities, clinical characteristics and therapy

	Severe FEV1 decline (> 10%) n = 15	Severe FVC decline (> 10%) n = 18
Variable	*p*-value	*p*-value
Age	0.965	0.512
Gender	0.670	0.920
Race	0.273	0.111
Transplant indication	0.494	0.081
Bilateral or unilateral Ltx	0.450	0.143
Time since transplant	0.069	0.826
Body mass index	0.121	0.090
Comorbidities		
Hypertension	0.723	0.040
Dyslipidemia	0.659	0.422
Diabetes Mellitus	0.256	0.819
Chronic kidney disease	0.225	0.433
Atrial fibrillation	0.762	0.233
Heart failure	0.097	0.171
Immunosuppression	0.836	0.120
Symptoms		
Fatigue	0.179	0.457
Dyspnoea	0.353	0.063
Cough	0.299	0.969
Chest pain	0.659	0.284
Fever	0.442	0.595
Myalgia	0.158	0.081
Gastro-intestinal	0.334	0.272
Headache	0.114	0.426
Upper respiratory	0.299	0.680
CLAD pre LTx	0.334	0.252
Corticosteroids increased dosage	0.007	< 0.001
X-ray/CT abnormalities	0.060	< 0.001

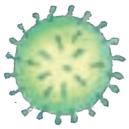




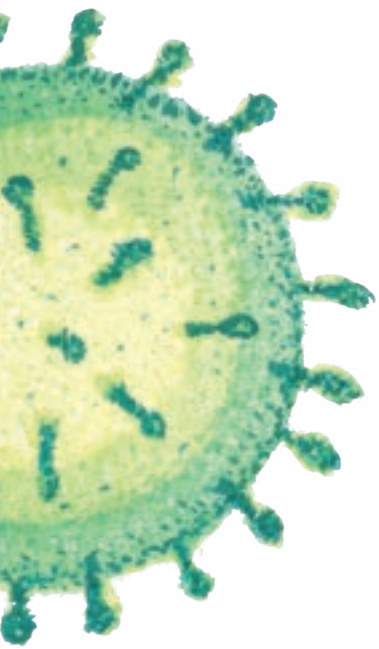


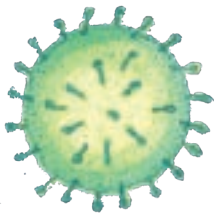
Chapter 3

Increasing antibody responses to five doses of SARS-CoV-2 mRNA vaccine in lung transplant patients



J.P. van Gemert, F.J. Steenberg, C.C. van Leer-Buter, H.A.M. Kerstjens, W.N. Steenhuis, O.W. Akkerman, E.A.M. Verschuuren, C.T. Gan





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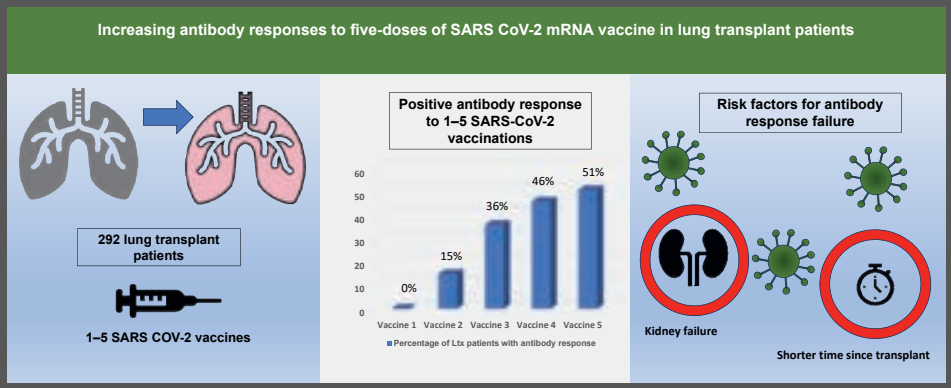
Abstract

Purpose: COVID-19 causes high mortality in Lung Transplant (LTx) patients, therefore vaccination in this population is potentially life-saving. However, the antibody response is impaired after three vaccinations in LTx patients. We questioned whether this response might be increased, and therefore studied the serological IgG antibody response across up to five doses of the SARS-CoV-2 vaccine. In addition, risk factors for non-response were investigated.

Methods: In this large retrospective cohort study, antibody responses were assessed after 1–5 mRNA-based SARS-CoV-2 vaccines in all LTx patients between February 2021 and September 2022. A positive vaccine response was defined as an IgG level ≥ 300 BAU/mL. Positive antibody responses due to COVID-19 were excluded from the analysis. Outcome and clinical parameters were compared between responders and non-responders, and a multivariable logistic regression analysis was performed to determine the risk factors for vaccine-response failure.

Results: The antibody responses of 292 LTx patients were analyzed. Positive antibody responses to 1–5 SARS-CoV-2 vaccinations occurred in 0%, 15%, 36%, 46%, and 51%, respectively. During the study period, 146/292 (50%) of the vaccinated individuals tested positive for SARS-CoV-2 infection. The COVID-19-related mortality was 2.7% (4/146), and all four patients were non-responders. Risk factors associated with non-response to SARS-CoV-2 vaccines in univariable analyses were age (p = 0.004), chronic kidney disease (CKD) (p = 0.006), and shorter time since transplantation (p = 0.047). In the multivariable analysis, they were CKD (p = 0.043), and shorter time since transplantation (p = 0.028). Conclusion: A two- to five-dose regime of SARS-CoV-2 vaccines in LTx patients increases the probability of vaccine response and results in a cumulative vaccine response in 51% of the LTx population. LTx patient antibody response to SARS-CoV-2 vaccinations is therefore impaired, especially in patients shortly after LTx, patients with CKD, and the elderly.

Conclusion: A two- to five-dose regime of SARS-CoV-2 vaccines in LTx patients increases the probability of vaccine response and results in a cumulative vaccine response in 51% of the LTx population. LTx patient antibody response to SARS-CoV-2 vaccinations is therefore impaired, especially in patients shortly after LTx, patients with CKD, and the elderly.



Introduction

Since the beginning of the COVID-19 pandemic, highly effective SARS-CoV-2 vaccines have been developed.[1,2] The rapid development and distribution of vaccines has proved to be instrumental in reducing the incidence and severity of COVID-19 in the general population.[1,3,4] However, most of the initial trials excluded solid organ transplant (SOT) patients.[1,3,5] Unfortunately, SOT patients are less likely to develop an antibody response to SARS-CoV-2 vaccines and have a higher risk of worse COVID-19 disease course and outcome, due to their immunosuppressive status and additional comorbidities.[6–8] SOT patients elicit a reduced immunogenicity to COVID-19 vaccines, because of the inhibition of lymphocyte activation, interaction with antigen-presenting cells, and decreased B-cell memory responses, depending on the type of immunosuppressive drug.[6] Therefore, these patients must reach an optimal vaccination status to protect them against severe COVID-19. Antibody response to SARS-CoV-2 vaccines was reported to vary according to the type of SOT, and the response was especially low in lung transplant (LTx) patients.[9] Antibody response in LTx patients is likely to be lower than in other SOT patients because LTx patients receive more intensive immunosuppression than other SOT patients. Several studies have investigated the development of an immune response after receiving two or three vaccines in LTx patients and have shown poor antibody response even after a third vaccine (16–46%).[10,11] We hypothesize that more LTx patients might achieve antibody response after a fourth or fifth vaccine. Therefore, the primary objective of this study is to document increasing humoral antibody responses after one to five vaccine doses. The secondary objective is to identify the patient characteristics that predict both reduced and increased humoral responses to SAR-COV-2 vaccination in LTx patients. Additionally, the study aims to investigate any potential differences in disease course between vaccine responders and non-responders.

Material and methods

Patients

Data from LTx patients were retrospectively collected at the University Medical Center Groningen, the Netherlands, between February 2021 and September 2022. All SARS-CoV-2 vaccinated adult LTx patients, with at least one measured antibody response between February 2021 and September 2022, were eligible for inclusion. Patients were excluded if they had received one or more vaccinations before the transplantation. Patients were also excluded if the number of vaccines given or the date of the vaccination or antibody measurement was unknown. Only mRNA-based SARS-CoV-2 vaccines were used in all patients, i.e.,

Moderna or Pfizer/BioNTech (Comirnaty) according to the COVID-19 treatment guidelines, special considerations in SOT patients. Furthermore, in accordance with these guidelines, the COVID-19 vaccines were offered as early as 3 months after transplantation, and after receiving the primary series or booster doses. LTx patients were advised to continue to take precautions.[12] For classifying chronic kidney disease (CKD), we used the Kidney Disease Improving Global Outcomes (KDIGO) staging[13] Heart failure was classified according to the 2021 ESC guidelines.[14]

Transplant care

Follow-up of all patients took place at least every 3 months for the monitoring of transplant function, with spirometry and blood tests. Patients received standard maintenance immunosuppression: tacrolimus, prednisolone, and mycophenolate mofetil (MMF), and induction with Basiliximab postoperatively. Alternative immunosuppressants were cyclosporine, azathioprine, everolimus, or sirolimus. Tacrolimus trough level goals were specified per protocol at 7–10 µg/mL. Post-transplantation prophylactic therapy included cotrimoxazole for pneumocystis pneumonia and valganciclovir/aciclovir for CMV, depending on CMV (mis)match. The management of COVID-19 with steroids, adjustment of immunosuppression, and COVID-19-directed therapies was according to the ISHLT Guidance.[15] Patients were urged to contact the transplant team on a low-threshold basis when infected with SARS-CoV-2.

S-specific antibody testing

Humoral responses to vaccination were measured with a quantitative IgG assay detecting antibodies against the SARS-CoV-2 Spike (s) protein, with a lower limit of detection of 50 AU/mL. Antibody responses to vaccination were measured after standard outpatient visits, at least every 3 months. The same IgG test was used for all participants, i.e., the SARS-spike-IgG quantitative test by Abbott, on the Aliniti-I platform. The assay was performed following the manufacturer's instructions.[16] A positive vaccine response was defined as an IgG level above 300 AU/mL, and a low response as an IgG level between 50 and 300 AU/mL.[17,18] Antibody responses were excluded from the analysis if they developed after a positive SARS-CoV-2 test, as these antibody responses could be due to COVID-19 and therefore could not be considered (solely) a vaccine response. Intercurrent infections were diagnosed by PCR. If PCR tests were repeatedly negative and there were clinical suspicions of a recent COVID-19 infection, antibody testing of Nucleocapsid (N) antibodies was performed using the SARS-N antibody qualitative IgG test on the Abbott Aliniti-I. platform.

Patient groups

Responders were defined as patients with a positive SARS-CoV-2 antibody (≥ 300 BAU/mL); non-responders were defined as patients without a SARS-CoV-2 antibody response (< 50 BAU/mL); low responders were defined as patients with a SARS-CoV-2 antibody response between 50 and 300 BAU/mL. Patients with a previously negative vaccine antibody response, but a positive antibody response after a positive SARS-CoV-2 antigen test were defined as "post-COVID-19 responders" and were excluded from analysis.

Patients who tested positive for SARS-CoV-2 antibodies after a given vaccine were considered to be responders to a subsequent vaccine. Patients who were non-responders after a given vaccine were considered unknown if their antibodies were not measured after a subsequent vaccine.

Statistical analysis

Analyses were carried out using IBM SPSS for Mac, version 24.0. Continuous variables are expressed as median (interquartile range). After each vaccination, the number of patients with a positive response was measured cumulatively. Wilcoxon Rank tests were used for within-group analyses to compare the number of patients with and without IgG response after 1–5 vaccines. Clinical parameters and outcomes were compared between responders and non-responders by univariable analysis (Mann–Whitney U-test and chi-squared test). All parameters with a *p*-value less than 0.10 by univariable analysis were considered to be a trend to statistical significance and were entered into a multivariable logistic regression analysis model, to identify independent risk factors for vaccine-response failure. A *p*-value of less than 0.05 was considered significant. Risk factors are expressed as odds ratios with 95% confidence intervals and *p*-values.

Results

All patients

Of the 832 patients transplanted between November 1990 and September 2022, 406 patients were alive at the beginning of the study period. Of those, 107 patients were excluded for the following reasons: vaccinated before LTx (53/107; 50%), no vaccination (23/107; 21%), number of vaccines/date of the vaccination unknown 17/107 (16%), children 9/107 (8%), no antibody measurement (3/107; 3%), loss to follow-up (2/107; 2%). A total of 292 (72%) of the alive adult LTx patients were included in the study. Baseline characteristics for all 292 LTx patients with antibody responses after 2–5 vaccinations are shown in Table 3.1. The median age was 60 years (IQR 48–66) and most patients (52%) were male. COPD (41%)

and pulmonary fibrosis (21%) were the most common indications for LTx, and a bilateral LTx was performed in 91%. The median time between LTx and the first vaccine was 8 years (IQR 4–12). The most frequently reported comorbidities were dyslipidemia (81%), CKD (70%), and hypertension (47%). During the study, 20 (7%) of the LTx patients died, 4 (1%) from COVID. Details of the responders and non-responders are shown in Table 3.1. In general, responders were younger, had a longer interval since transplantation, and less often had CKD. In Supplemental Table S3.1, clinical parameters of the excluded post-COVID-19 responders are included.

Table 3.1: Baseline characteristics of all SARS-CoV-2 vaccinated LTx patients, responders and non-responders

Variable n (%)	All patients# 292	Responders 123 (42)	Non-responders 126 (43)	*p*-value*
Age, years	60 (48–66)	58 (40–64)	62 (54–66)	0.004
Gender, male, n (%)	152 (52)	69 (56)	64 (51)	0.402
Caucasian, n (%)	281 (96)	120 (98)	124 (98)	0.549
Transplant indication, n (%)				0.106
COPD	120 (41)	51 (42)	53 (42)	
Fibrosis	63 (21)	23 (19)	32 (25)	
Pulmonary hypertension	35 (12)	11 (9)	18 (14)	
Cystic fibrosis	43 (15)	23 (19)	17 (14)	
Other	31 (11)	15 (12)	6 (5)	
Bilateral LTx, n (%)	267 (91)	118 (96)	113 (90)	0.057
Time since transplant**, years	8 (4–12)	9 (5–13)	7 (4–11)	0.047
Comorbidities, n (%)				
Hypertension	137 (47)	54 (44)	64 (51)	0.276
Dyslipidemia	236 (81)	101 (82)	100 (79)	0.583
Diabetes Mellitus	99 (34)	42 (34)	46 (37)	0.697
Chronic kidney disease	205 (70)	77 (63)	99 (79)	0.006
Obesitas, BMI > 30	33 (11)	12 (10)	14 (11)	0.727
Heart failure	27 (9)	11 (9)	12 (10)	0.874
Immunosuppression, n (%)				
Tacrolimus	245 (84)	100 (81)	105 (83)	0.372
Cyclosporine	10 (4)	5 (4)	3 (2)	0.455
Azathioprine	57 (22)	27 (22)	22 (18)	0.377
Mycophenalte mofetil	199 (76)	80 (65)	85 (68)	0.686
mTORi	12 (5)	5 (4)	6 (5)	0.781
CLAD	86 (30)	33 (27)	41 (33)	0.324
COVID-19, n (%)	146 (50)	49 (40)	54 (43)	0.629
All-cause mortality, n (%)	20 (7)	3 (2)	17 (14)	0.001
COVID-19 mortality	4 (1)	0 (0)	4 (3)	0.046

Continuous variables are expressed as median (interquartile range).
COPD, chronic obstructive pulmonary disease; LTx, lung transplantation; mTORi, mammalian target of rapamycin inhibitors (everolimus or sirolimus).
Post-COVID-19 Responders 43(15%) were excluded from analysis: These are patients with an previously negative vaccine antibody response, but a positive antibody response after a positive SARS-COV-2 antigen test.
* *P*-value for the difference between responders and non-responders.
** Time between transplant and first vaccination.

Responders

In the responders group, 56% of the 132 patients were male, the median age was 58 years (IQR 40–64), and 96% were transplanted bilateral (Table 3.1). The most common indications for LTx in the responders group were COPD (42%); fibrosis (19%), and CF (19%). The median time between LTx and the first vaccine was 9 years (IQR 5–13). The most frequently reported comorbidities were dyslipidemia (82%), CKD (63%), and hypertension (44%). During the study period, 3 (2%) of the responders died, none from COVID-19.

Non-responders

In the non-responders group, 51% of the 126 patients were male, the median age was 62 years (54–66), and 90% were transplanted bilateral (Table 3.1). The most common indications for LTx in the non-responders group were COPD (42%) and fibrosis (25%). The median time between LTx and the first vaccine was 7 years (IQR 4–11). The most frequently reported comorbidities were dyslipidemia (79%), CKD (79%), and hypertension (51%). During the study period, 17 (14%) of the non-responders died, and 4 (3%) from COVID-19.

Vaccines

In 292 SARS-CoV-2-vaccinated LTx patients, 100% received 1 vaccine, 98% received 2 vaccines, 90% received 3 vaccines, 72% received 4 vaccines, and 46% received 5 vaccines. Five patients died before the second vaccination. The median time between the first and second vaccine was 1 month (IQR 0–1), between the second and third vaccine 6 months (IQR 5–6); between the third and fourth vaccine 2 months (2–3) and between the fourth and fifth vaccine 3 months (IQR 2–3). The cumulative positive antibody response rate to SARS-CoV-2 vaccination was 0% in patients with measured antibody response after 1 vaccination, 15% (37/249) after 2 vaccinations, 36% (85/234) after 3 vaccinations, 46% (112/243) after 4 vaccinations and 51% (113/220) after 5 vaccinations (Table 3.2). The median time between vaccination and antibody measurement was 16 weeks (IQR 3–20) after the first vaccine, 15 weeks (IQR 11–18) after the second vaccine, 5 weeks (IQR 4–9) after the third vaccine, 5 weeks (IQR 4–9) after the fourth vaccine and 8 weeks (IQR 4–13) weeks after the fifth vaccine. The median number of vaccines needed before a positive antibody response (in those with a response) was 3 (IQR 2–4).

The number of patients with a positive antibody response increased after each vaccination, starting from vaccination 2 (Figure 3.1a,b). A total of 42/154 (27%) of the non-responders after the second vaccine became responders after the third vaccine; 15/97 (15%) of the non-responders after the third vaccine became responders after the fourth vaccine; 2/61

(3%) of the non-responders after the fourth vaccine became responders after fifth vaccine (Figure 3.1b). Figure 3.2 shows the responses of the non-responders and low responders after 1–5 vaccines. With the help of Figure 3.2, an attempt can be made to estimate the likelihood that a non-responder will become a responder after further vaccination. For example, a considerable percentage of low responders after 2 vaccines became responders after the third or fourth vaccine (55% and 76%). Similarly, 18 and 30% of non-responders after 2 vaccines became responders after a third or fourth vaccine. The addition of a fifth vaccine in non-responders or low responders after a fourth vaccine was only useful for a small number (3%) of LTx patients (Figure 3.1b and Figure 3.2). After 5 vaccines, 51% of the total population had an antibody response.

Risk factors for the failure to develop an antibody response after vaccination

Patient characteristics were compared between vaccine responders and non-responders (Table 3.1). Risk factors associated with non-response to SARS-CoV-2 vaccines were CKD ($p = 0.006$), older age ($p = 0.004$), and shorter time between LTx and the initial vaccine ($p = 0.047$) in univariable analysis. In a multivariable analysis, CKD ($p = 0.043$; OR = 1.9; 95% CI [1.02–3.57]) and a shorter time between LTx and the initial vaccine ($p = 0.028$; OR = 1.1; 95% CI [1.00–1.10]) were independently associated with vaccine-response failure.

Table 3.2: SARS-CoV-2 cumulative antibody response after 1–5 vaccinations

	Vaccin 1	Vaccin 1–2	Vaccin 1–3	Vaccin 1–4	Vaccin 1–5
Patients with antibody response assessed, n (%)	11/292 (4)	249/292 (85)	234/292 (80)	243/292 (83)	220/292 (75)
Patients with pos. antibody response*, n (%)	0 (0)	37 (15)	85 (36)	112 (46)	113 (51)
Patients without pos. antibody response#, n (%)	8 (73)	197 (79)	117 (50)	88 (36)	58 (26)
Non-response, n	7	159	84	55	26
Low-response, n	1	38	33	33	32
Patients with antibody response due to COVID-19, n (%)	3 (27)	15 (6)	32 (14)	43 (18)	49 (22)

* Patients who were responders after a given vaccine were considered to be responders to a subsequent vaccine.
# Patients who were non-responders after a given vaccine were considered unknown if their antibodies were not measured after a subsequent vaccine.
Vaccine response: IgG level ≥ 300 BAU/ml.
No response: IgG < 300 BAU/ml.
Low response: IgG level 50–300 BAU/ml.

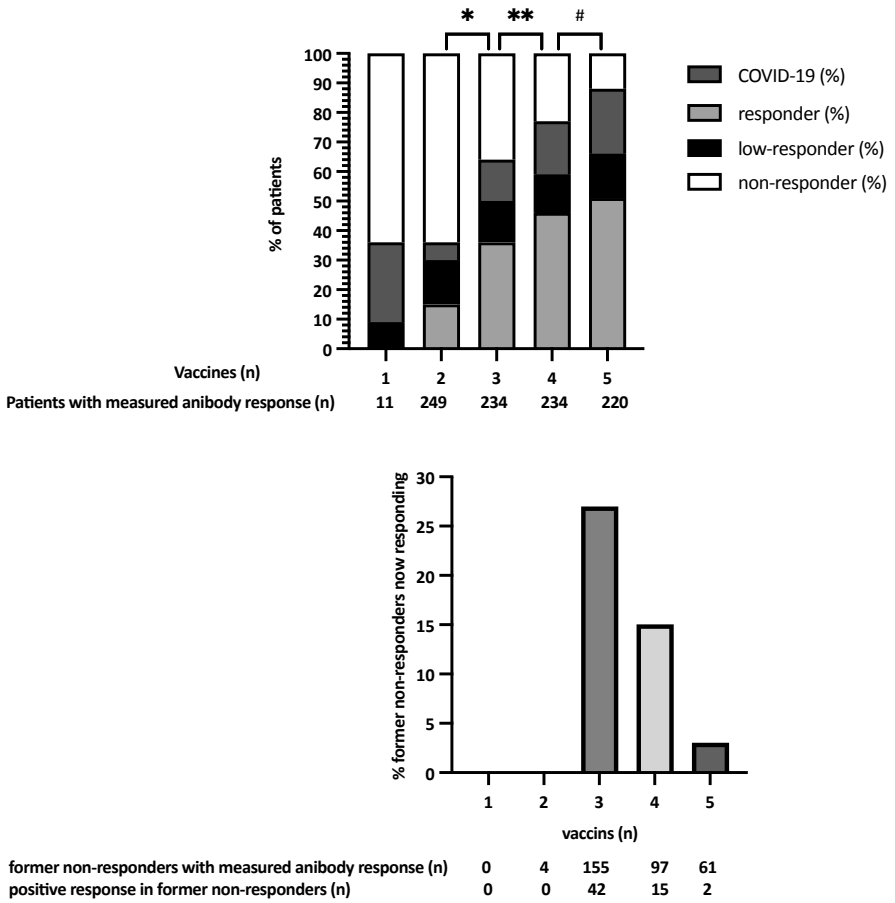


Figure 3.1: (a) SARS-CoV-2-specific cumulative antibody response after 1–5 vaccinations in LTx patients. * *p*-value < 0.001 for the difference between responders after the 2nd and 3rd vaccination. ** *p*-value < 0.001 for the difference between responders after the 3rd and 4th vaccination. # *p*-value 0.21 for the difference between responders after the 4th and 5th vaccination. **(b)** Percentage of former non-responders responding after 1–5 SARS-CoV-2 vaccines in LTx patients.

COVID

During the study period, 146/292 (50%) of the vaccinated LTx patients tested positive for SARS-CoV-2 (Table 3.1). When infected with COVID-19, 50% of the patients received an increased dosage of steroids because of severe symptoms, the need for supplemental oxygen, or a decline in home spirometry. Antimetabolites were discontinued or reduced by 48%. Tacrolimus trough levels were maintained in all patients. Of the 123 responders, 49 (40%) patients developed COVID-19 during the study period, while 54 (43%) of the non-responders developed COVID-19 ($p = 0.629$). Of the responders, 4% tested positive for SARS-CoV-2 after the first vaccine, 10% after the second vaccine, 33% after the third vaccine, 43% after the

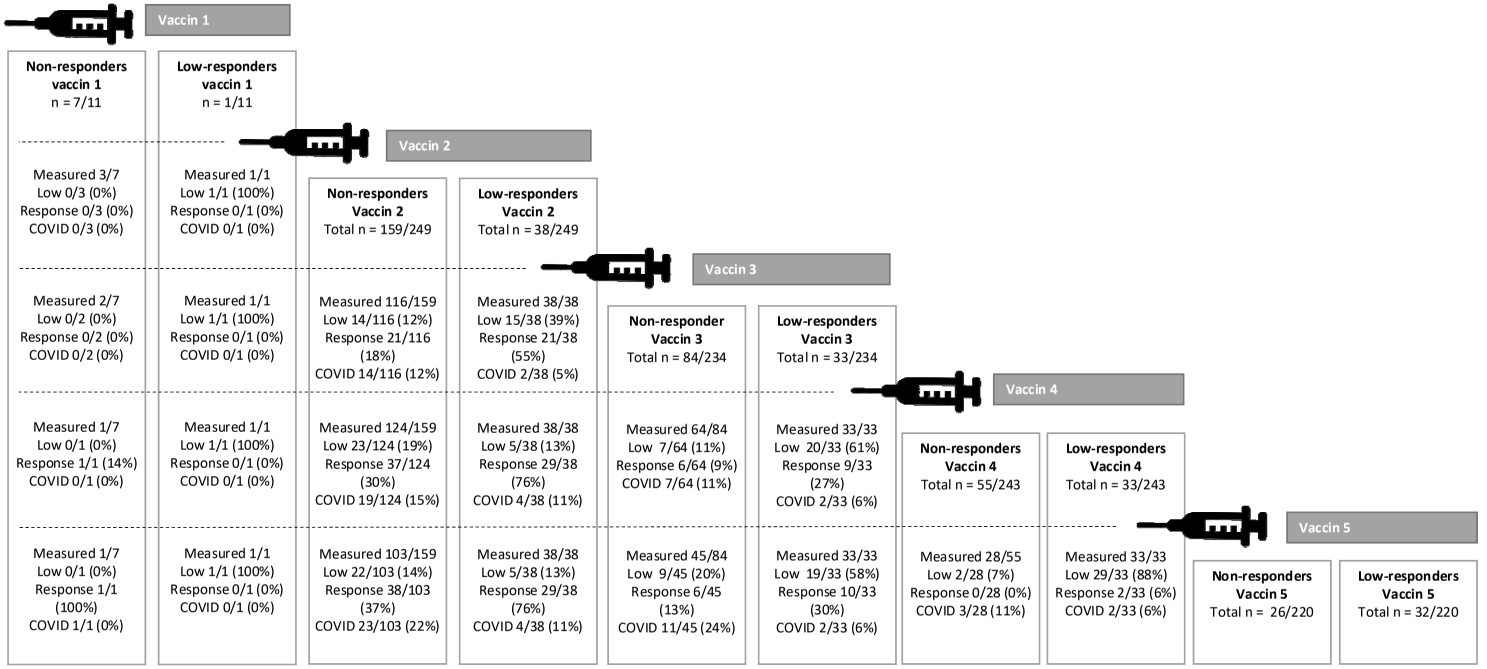


Figure 3.2: Non-responders and low-responders after 1–5 SARS-CoV-2 vaccinations in LTx patients.

fourth vaccine, and 10% after the fifth vaccine. Of non-responders, 2% tested positive for SARS-CoV-2 after the first vaccine, 15% after the second vaccine, 15% after the third vaccine, 37% after the fourth vaccine, and 31% after the fifth vaccine. The mortality rate associated with COVID-19 was 2.7% (4/146). All 4 patients who died were non-responders. Of the 146 patients with COVID, 20 (14%) were admitted. The criteria for hospitalization were clinical symptoms of (upper) respiratory infection, fever, or shortness of breath with or without oxygen requirement (peripheral oxygen saturation < 92%).[19] There was no significant difference in COVID-19-related admission rates between responders and non-responders (Table 3.3).

Table 3.3: Outcome in SARS-CoV-2 vaccine responders and non-responders with COVID-19

	Responders with COVID n = 49	Non-responders/low-responders with COVID n = 54	*p*-value
Admission, n (%)	4 (8)	9 (17)	0.168
Death, n (%)	0 (0)	4 (8)	0.046

All-cause mortality

Twenty patients died during the study period. The causes of death were cancer (7/20; 35%), CLAD (4/20; 20%), COVID-19 (4/20; 20%), renal failure (3/20; 15%), subarachnoid hemorrhage (1/20; 5%), and lung bleeding 1/20; 5%). In the 20 LTx patients who died during the study period, 35% had received 1 vaccine, 50% had received 2 vaccines, 10% had received 3 vaccines, 5% had received 4 vaccines, and none had received 5 vaccines. The all-cause mortality was significantly higher in non-responders (14%), compared to responders (2%) ($p = 0.001$).

Discussion

To our knowledge, this is the first study in a large LTx population that describes the antibody response after receiving 1–5 SARS-CoV-2 vaccines. We showed that the number of patients with a positive antibody response increases with the number of vaccines received and increases by up to 51% after five vaccines. Patients without an antibody response or with low antibodies after a second vaccination have a significant chance of developing antibodies after a third or fourth vaccination and a 3% chance after a fifth vaccine. This is consistent with the case series of Alejo et al., who showed in a small study of 18 patients with any SOT and without an antibody response after a third vaccine, 50% developed a response after the fourth vaccine. All patients with a low response after the third vaccination had a good

response after the fourth vaccination.[20] In addition, Abedon et al. showed that 18 SOT patients with a low antibody response after a fourth vaccination had more antibodies after a fifth vaccination.[21] The vaccination response in LTx patients after two vaccines remains lower than seen in liver-, heart-, and kidney-transplant patients, most likely due to the higher levels of immunosuppression used in LTx patients compared to other SOT patients.[8,18] Our large study in LTx patients, in addition to the smaller studies in SOT patients, advocates the importance of measuring an antibody response in LTx patients following SARS-CoV-2 vaccination, since there is a significant chance of developing antibodies after a third or fourth vaccine, and even some chance after a fifth vaccine in the case of a prior negative antibody response. However, the increase in response rate is smaller with each additional vaccine after the third, suggesting that a significant number of LTx patients may not develop antibodies, even when multiple doses are given.

Repeated vaccinations with SARS-CoV-2 mRNA vaccines have been shown to be safe, not causing major adverse effects in SOT patients.[1,10]

We have shown that in LTx, patient age, CKD, and a shorter time since transplantation were associated with the failure to obtain an antibody response after SARS-CoV-2 vaccination in univariable analysis. In multivariable analysis, CKD and a shorter time since transplantation were independently associated with vaccine-response failure. Age-related heterogeneity of SARS-CoV-2 vaccine-acquired immunity is not specific to LTx patients, but has also been seen in the general population.[22] Studies in SOT patients have also found that older age is associated with a failure to obtain an antibody response in SOT patients.[8,10,23,24] CKD is also a known risk factor for vaccine failure in non-SOT and SOT patients. In non-SOT CKD patients, the efficacy of vaccines might be hampered because of the alteration of immunity by the uremic milieu, vitamin D, and EPO deficiency.[22] In studies with SOT patients, CKD has been associated with a reduced immune response to SARS-CoV-2 vaccination.[25,26]

We showed that a shorter time between LTx and the first vaccine was associated with antibody response failure after SARS-CoV-2 vaccination. In the first year after LTx, immunosuppression is most intense. Patients who have had LTx more recently will therefore have a more intense immunosuppressive regimen and a probably lower chance of a vaccine response. This might explain why a shorter time after LTx is associated with vaccine-response failure. Such an association has also been shown by Hoek et al. They showed in their study that a lower torque tenovirus (TTV) load, which is associated with less-intensive immunosuppression and a longer time since transplantation, is associated with a better antibody response to SARS-CoV-2 vaccines.[18] In our study, there was no difference in the type of immunosup-

pressive drug's impact on antibody response. Other studies have shown that only MMF is significantly associated with vaccine failure.[10,24,27] MMF is a potent inhibitor of B-cell function and immunoglobulin secretion. However, we could not confirm this association in our study. This could be because most people (76%) in our study used MMF.

Although repeated SARS-CoV-2 vaccination in LTx patients results in a better antibody response, this does not prevent SARS-CoV-2 infection. The percentage of COVID-19 cases was not higher in non-responders than in responders (43% vs. 40%, $p = 0.629$). The lack of a significant difference may be due to the possibility that non-responders may protect themselves from infection better than responders using masks, good hand hygiene, social distancing, etc. Another explanation could be due to the timeframe of this study, during which various COVID variants emerged, against which the available vaccines were not equally effective. In our earlier COVID-19 study in LTx patients, when vaccines were not available and the Delta variant was endemic, the hospitalization rate was 57% and the mortality rate was 20%.[19] In our current study, admission rates and mortality due to COVID-19 are much lower (14% and 4%). This is not only due to the availability of SARS-CoV-2 vaccines, but might also be due to the less-virulent Omicron variant and more knowledge about treating COVID-19 in immunocompromised patients, such as administering monoclonal antibodies in patients without antibodies. Admission rates were not significantly higher in non-responders than in responders. However, all four patients who died from COVID-19 in this study were non-responders.

The all-cause mortality was significantly higher in non-responders, compared to responders. However, the vaccination rate among deceased patients was low because they died before their next vaccination during the study period. Therefore, deceased patients are less likely to have been vaccine responders.

This study has some limitations. Because it is a retrospective study, we did not have complete antibody assessments in all patients after each vaccination. Nevertheless, our study does provide a reliable reflection of daily practice regarding vaccination in immunocompromised patients. Additionally, we did not measure T-cell responses, which may be detectable even in patients without antibody response. Accumulating evidence has shown the importance of T-lymphocyte responses in protection against severe COVID-19.[28,29] Our finding that serological non-responders did not require hospital admission more frequently than serological responders may, to some extent, be attributable to a degree of vaccination-induced T-cell immunity which we did not measure. Lastly, we did not examine the effect of different mRNA vaccines on obtaining an antibody response.

Our data show that, in LTx patients, it is important to vaccinate repeatedly with the probability of a vaccine response increasing but reaching a plateau after about 4–5 vaccinations. In the group of LTx patients with no response after 4–5 vaccinations, who are older, have renal insufficiency, and are shorter after transplantation, the likelihood of developing a vaccine response after further vaccination is low. In these patients, the adjustment of immunosuppression to lower doses and/or lower trough levels could be considered to increase the likelihood of a vaccine response. However, further studies are needed to confirm this.

Conclusions

A two- to five-dose regime of SARS-CoV-2 vaccines in LTx patients increases the probability of vaccine response, reaching a plateau after about four to five vaccinations. There is still a group of patients who are older, have CKD, and are more recently post-transplant who are unlikely to respond even after five vaccinations. Therefore, repeated vaccination and measurement of antibody responses after each SARS-CoV-2 vaccination is important, particularly in those at risk of vaccine failure.

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Supplemental Table S3.1: Baseline characteristics of all SARS-CoV-2 vaccinated LTx patients, responders, non-responders and post-COVID-19 responders

Variable n (%)	All patients 292	Responders 123 (42)	Non-responders 126 (43)	*p*-value*	Post-COVID-19 responders# 43 (15)
Age, years	60 (48–66)	58 (40–64)	62 (54–66)	0.004	61 (49–66)
Gender, male (%)	152 (52)	69 (56)	64 (51)	0.402	19 (44)
Caucasian, n (%)	281 (96)	120 (98)	124 (98)	0.549	38 (88)
Transplant indication, n (%)				0.106	
COPD	120 (41)	51 (42)	53 (42)		16 (37)
Fibrosis	63 (21)	23 (19)	32 (25)		8 (19)
Pulmonary hypertension	35 (12)	11 (9)	18 (14)		6 (14)
Cystic fibrosis	43 (15)	23 (19)	17 (14)		3 (7)
Other	31 (11)	15 (12)	6 (5)		10 (23)
Bilateral LTx, n (%)	267 (91)	118 (96)	113 (90)	0.057	36 (84)
Time since transplant**, years	8 (4–12)	9 (5–13)	7 (4–11)	0.047	8 (5–12)
Comorbidities, n (%)					
Hypertension	137 (47)	54 (44)	64 (51)	0.276	19 (44)
Dyslipidemia	236 (81)	101 (82)	100 (79)	0.583	35 (81)
Diabetes Mellitus	99 (34)	42 (34)	46 (37)	0.697	11 (26)
Chronic kidney disease	205 (70)	77 (63)	99 (79)	0.006	29 (67)
Obesitas, BMI > 30	33 (11)	12 (10)	14 (11)	0.727	7 (16)
Heart failure	27 (9)	11 (9)	12 (10)	0.874	4 (9)
Immunosuppression, n (%)					
Tacrolimus	245 (84)	100 (81)	105 (83)	0.372	40 (93)
Cyclosporine	10 (4)	5 (4)	3 (2)	0.455	2 (5)
Azathioprine	57 (22)	27 (22)	22 (18)	0.377	8 (19)
Mycophenalte mofetil	199 (76)	80 (65)	85 (68)	0.686	34 (79)
mTORi	12 (5)	5 (4)	6 (5)	0.781	1 (2)
CLAD	86 (30)	33 (27)	41 (33)	0.324	12 (30)
COVID-19, n (%)	146 (50)	49 (40)	54 (43)	0.629	43 (100)
All-cause mortality (%)	20 (7)	3 (2)	17 (14)	0.001	0 (0)
COVID-19 mortality (%)	4 (1)	0 (0)	4 (3)	0.046	0 (0)

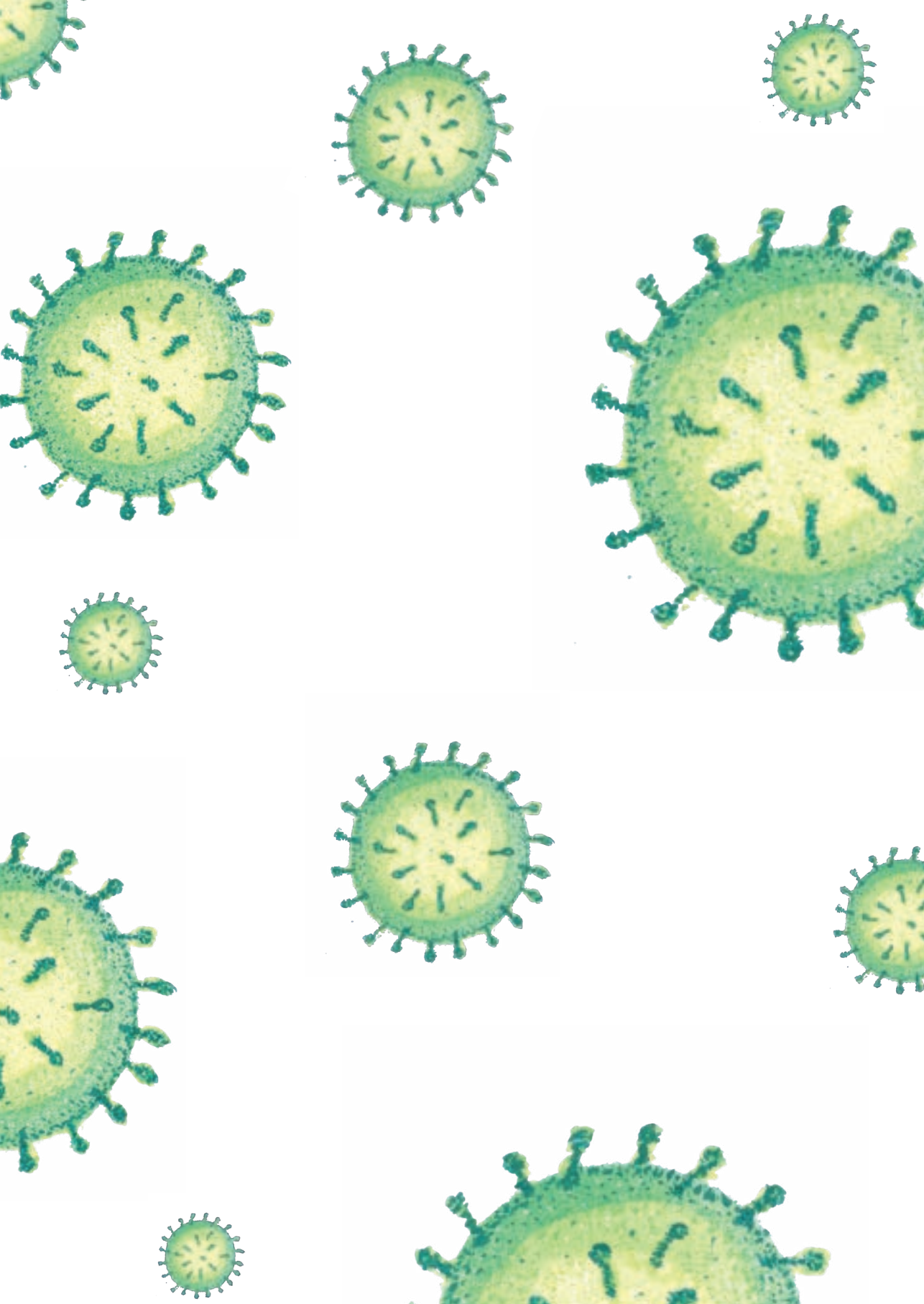
Continuous variables are expressed as median (interquartile range).

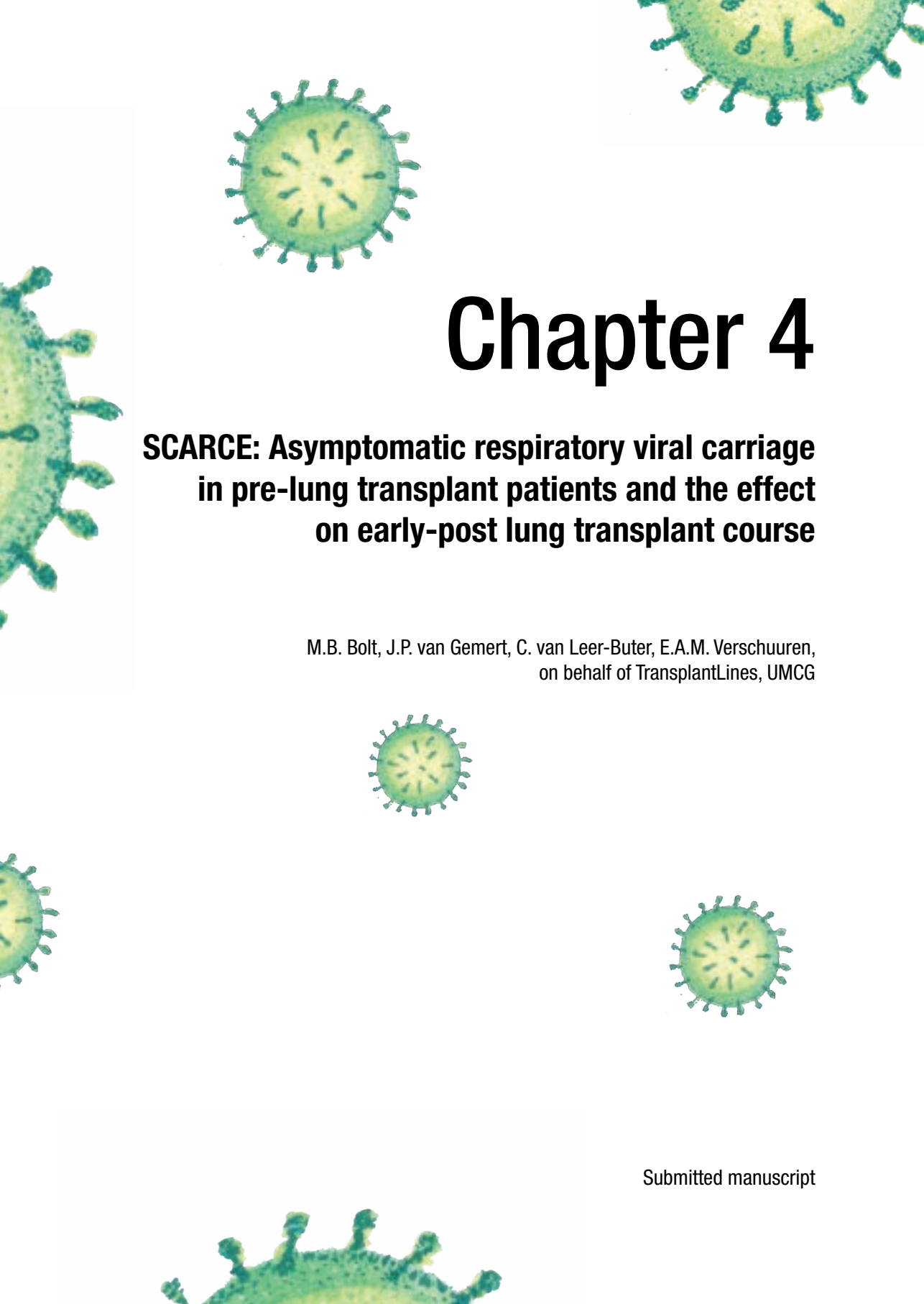
COPD, chronic obstructive pulmonary disease; LTx, lung transplantation; mTORi, mammalian target of rapamycin inhibitors (everolimus or sirolimus).

Post-COVID-19 responders: Patients with a previously negative vaccine antibody response, but a positive antibody response after a positive SARS-COV-2 antigen test.

* *P*-value for the difference between responders and non-responders.

** Time between transplant and first vaccination.





Chapter 4

SCARCE: Asymptomatic respiratory viral carriage in pre-lung transplant patients and the effect on early-post lung transplant course

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Abstract

Background: Community acquired respiratory viruses (CARV) can be found in asymptomatic persons. Lung transplant (LTx) recipients are at particular risk for CARV due to their high immunosuppressive regimen. We aim to gain insight into CARV carriage in LTx candidates immediately before transplantation and its influence on the early post-LTx course in recipients transplanted while positive for a CARV.

Methods: This retrospective cohort study included all adult LTx recipients transplanted at the UMC Groningen from January 2017 to August 2023. Routinely obtained pre-LTx viral swabs were tested for a CARV. Primary outcome was incidence of primary graft dysfunction (PGD) 72 hours post-LTx. Secondary outcomes were duration of mechanical ventilation, duration of stay on the intensive care unit (ICU), total hospital length of stay (LOS), 30-day mortality rate, 90-day mortality rate, PGD at 48 hours and rejection therapy within the first month after LTx.

Results: 23 (10.4%) out of 222 LTx candidates tested positive for a CARV pre-LTx. Most were infected with rhinovirus (n = 10). Overall, 10.8% of recipients developed PGD grade 2–3 at 72h. For recipients positive for a CARV the incidence was 9.5%, and for recipients negative for a CARV it was 10.9% ($p = 1.00$). Duration of mechanical ventilation, ICU stay, total hospital LOS, 30-day and 90-day mortality rate, occurrence of acute rejection did not differ between both groups.

Conclusion: This study showed no association between asymptomatic pre-LTx viral carriage for LTx and the early post-LTx course. This suggests that testing for a CARV in asymptomatic LTx candidates is not indicated.

Introduction

Community acquired respiratory viruses (CARV) can proceed asymptomatically.[1,2] Lung transplant (LTx) recipients are at particular risk for CARV due to their high immunosuppressive regimens.[2] Importantly, CARV has been associated with chronic lung allograft dysfunction (CLAD) in previous studies.[3,4] So far, pre-LTx viral swab results have not been studied. However, due to the heightened susceptibility immediately following LTx induced by induction therapy, CARV infections shortly after LTx may contribute to early complications or prolonged recovery times.

Primary graft dysfunction (PGD), a common complication, has been reported to occur with a 30% incidence of PGD grade 3 at any time within the first 3 days following transplantation. This results in significant mortality and morbidity both in the short- and long-term.[5–7] Furthermore, microbiological and epidemiological data have supported the role of a pro-inflammatory state in the development of PGD.[5,7] Yet, the relationship between PGD and asymptomatic CARV carriage pre-LTx, which also causes an inflammatory state,[8] remains unexplored.

Therefore, we hypothesized that LTx candidates positive for a CARV pre-LTx have a more complicated early post-LTx course than candidates transplanted while negative for a CARV.

Materials and methods

Patients

This retrospective cohort study included all adult LTx recipients transplanted at the University Medical Center Groningen (UMCG) in the Netherlands from January 2017 to August 2023. As of January 2017, pre-LTx CARV swabs were routinely performed on all candidates upon arrival for LTx. Data were retrospectively collected from the LTx database and Electronic Patient Records from the UMCG. For all LTx recipients, swab data were obtained from the electronic patient records, if available. LTx recipients were excluded if no CARV swab data were available. Immunosuppression consisted of induction with Basiliximab on day 0 and 4, tacrolimus intravenous with an initial trough level of 13–15 mcg/L, mycophenolate mofetil (MMF) 1000 mg twice daily and methylprednisolone 2x500 mg during transplantation, 3x125 mg in the first 24 hours and 0.4 mg/kg prednisolone until day 7 and 0.2 mg/kg thereafter. All LTx recipients had provided written informed consent for transplant related research and the study was approved by the local Ethics Committee (CTc 18250).

The primary outcome was the incidence of PGD at 72 hours post-LTx. PGD incidence was categorized between stage 0–1 and 2–3 according to the most recent classification of the

International Society for Heart and Lung Transplantation (ISHLT).[5] Secondary outcomes were PGD at 48 hours, duration of mechanical ventilation, intensive care unit (ICU) lenght of stay (LOS), hospital LOS, 30-day and 90-day mortality rate, and the incidence of acute rejection within the first month after LTx. LTx recipients who died within 72 hours post-LTx were excluded from all outcome variables except mortality.

Statistical analysis

Analyses were carried out using IBM SPSS, version 23.0. Continuous variables are expressed as median (interquartile range). Clinical parameters and outcomes were compared between CARV positive and negative LTx recipients by Mann–Whitney U-test, Fisher's exact or Chi square test. A *p*-value of less than 0.05 was considered significant.

Virology

Nasopharyngeal swabs, sputum or broncoalveolar lavage specimens were used to collect specimens for the detection of human metapneumovirus (hMPV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), (para)-influenza viruses (PIV) and coronaviruses including SARS-CoV-2 by polymerase chain reaction (PCR). A CARV was defined as a positive viral detection from a nasalpharyngeal swab. R tests were performed as previously described.[3]

Results

From January 2017 to August 2023 243 patients underwent a LTx at the UMCG. Of those, 21 (8.6%) recipients were excluded because no swab data were available. Of the 222 recipients, 23 (10.4%) had a positive pre-LTx CARV swab (Table 4.1 and 4.2). Rhinovirus was most frequently detected (n = 10, median Ct-value 25; range 19–37). Five recipients tested positive for SARS-CoV-2 (median Ct-value 39; range 35–41), 3 for coronavirus (229E1 or OC43) (median Ct-value 29; range 24–34), 3 for PIV (median Ct-value 32.5; range 26–39) and 2 for hMPV (1 available Ct-value 33) (Table 4.2).

Overall, 10.8% (21/195) of LTx recipients developed PGD grade 2–3 at 72h. For LTx recipients positive for a CARV the incidence was 9.5% (2/21), and for LTx recipients negative for a CARV it was 10.9% (19/174) ($p = 1.00$) (Table 4.3). Moreover, duration of mechanical ventilation, ICU stay, total hospital LOS, 30-day mortality rate, occurrence of acute rejection did not significantly differ between both groups (Table 4.3). Focusing on SARS-CoV-2, we saw a longer ventilation time and ICU stay but no higher mortality in these 5 patients (Table 4.4).

Table 4.1: Patient demographics

	All patients	Pre-LTx CARV swab		
	Total	Positive	Negative	*p*-value
Patients	222	23	199	
Gender, female (%)	105 (47.3)	9 (30.4)	98 (49.2)	0.087[2]
Age, y (IQR)	57.8 (50.1–62.2)	58.0 (51.2–60.9)	58.6 (51.9–62.7)	0.424[3]
Underlying disease, n (%)				0.747[4]
Emphysema/ COPD	116 (52.4)	11 (47.8)	105 (52.8)	
Cystic fibrosis	9 (4.1)	0 (0.0)	9 (4.5)	
Pulmonary hypertension	25 (11.3)	2 (8.7)	23 (11.6)	
Fibrosis	56 (25.2)	8 (34.8)	48 (24.1)	
Re-transplantation	5 (2.3)	1 (4.3)	4 (2.0)	
Bronchiectasis	3 (1.4)	0 (0.0)	3 (1.5)	
Other[1]	8 (3.6)	1 (4.3)	7 (3.5)	
Transplant type, n (%)				0.354[4]
Single	5 (2.3)	1 (4.3)	4 (2.0)	
Double	211 (95.0)	21 (91.3)	190 (95.5)	
Re-LTx	5 (2.3)	1 (4.3)	4 (2.0)	
Lung-liver	1 (0.5)	0 (0.0)	1 (0.5)	

Continuous data are displayed as medians with interquartile range (IQR).
Abbreviations: COPD, chronic obstructive pulmonary disease; Re-LTx, re-transplantation.
[1] Other underlying disease (histiocytosis, sarcoidosis, Stevens-Johnson syndrome, graft versus host disease, bronchiectasis, post-SARS-cov-2).
[2] *P*-value from Chi-square test.
[3] *P*-value from Mann-Whitney U test.
[4] *P*-value from Fisher's exact.

Table 4.2: CARV swab results

	Total	Ct-value, median (range)
Negative	199	
Positive	23	35 (19–41)
Rhinovirus[1]	10	25 (19–37)
Coronavirus[1]	8	39 (24–41)
SARS-CoV-2	5	39 (35–41)
229E[1]	2	34
OC43	1	24
PIV[1]	3	32.5 (26–39)
hMPV[1]	2	33

Abbreviations: hMPV, human metapneumovirus; PIV, parainfluenza virus.
[1] Missing one Ct-value.

Table 4.3: Post-LTx outcomes

	All patients	Pre-LTx CARV swab		
	Total	Positive	Negative	*p*-value
PGD 72h, n (%)				1.00[1]
Grade 0–1	174 (89.2)	19 (90.5)	155 (89.1)	
Grade 2–3	21 (10.8)	2 (9.5)	19 (10.9)	
PGD 48h, n (%)				0.476[1]
Grade 0–1	174 (88.8)	17 (85.0)	157 (89.2)	
Grade 2–3	22 (11.2)	3 (15.0)	19 (10.8)	
Duration mechanical ventilation, days (IQR)	1 (1–5)	2 (1–13)	1 (1–4)	0.318[2]
ICU stay, days (IQR)	5 (3–13)	12 (2–26)	5 (3–11)	0.434[2]
Hospital LOS, days (IQR)	33 (23–50)	37 (29–65)	32 (23–49)	0.216[2]
Rejection therapy use, n (%)	63 (28.4)	7 (30.4)	56 (28.1)	0.817[3]
30-day mortality, n (%)	11 (5.0)	0 (0.0)	11 (5.5)	0.610[1]
90-day mortality, n (%)	20 (9.0)	0 (0.0)	20 (10.1)	0.238[1]

Continuous data are displayed as medians with interquartile range (IQR).
Abbreviations: ICU, intensive care unit; LOS, length of stay; PGD, primary graft dysfunction.
[1] *P*-value from Fisher's exact.
[2] *P*-value from Mann-Whitney U test.
[3] *P*-value from Chi-square test.

Table 4.4: Post-hoc analysis of COVID-19 and post-LTx outcomes

	All patients	Pre-LTx CARV swab		
	Total	COVID-19	Negative	*p*-value
PGD 72h, n (%)				1.00[1]
Grade 0–1	160 (89.1)	5 (100.0)	155 (89.1)	
Grade 2–3	19 (10.9)	0 (0.0)	19 (10.9)	
PGD 48h, n (%)				0.476[1]
Grade 0–1	160 (88.9)	3 (75.0)	157 (89.2)	
Grade 2–3	20 (11.1)	1 (25.0)	19 (10.8)	
Duration mechanical ventilation, days (IQR)	1 (1–5)	8 (5.5–14)	1 (1–4)	0.008[2]
ICU stay, days (IQR)	5 (3–12.5)	15 (11–33.5)	5 (3–11)	0.022[2]
Hospital LOS, days (IQR)	32 (23–48.5)	42 (25–61.5)	32 (23–49)	0.620[2]
Rejection therapy use, n (%)	58 (28.4)	2 (40.0)	56 (28.1)	0.624[1]
30-day mortality, n (%)	11 (5.0)	0 (0.0)	11 (5.5)	1.00[1]
90-day mortality, n (%)	20 (9.0)	0 (0.0)	20 (10.1)	1.00[1]

Continuous data are displayed as medians with interquartile range (IQR).
Abbreviations: ICU, intensive care unit; LOS, length of stay; PGD, primary graft dysfunction.
[1] *P*-value from Fisher's exact.
[2] *P*-value from Mann-Whitney U test.

Discussion

This study shows that pre-LTx CARV carriage is common (10.4%) among asymptomatic LTx candidates. However, we found no association between asymptomatic CARV carriage and the early post-LTx course. Our findings suggest that asymptomatic CARV carriage does not impact early outcomes, as no significant differences were observed in terms of primary PGD, duration of mechanical ventilation, ICU stay, overall hospital duration, incidence of acute rejection, or mortality.

Our findings regarding the prevalence of pre-LTx CARV carriage among asymptomatic LTx candidates were in line (10.4%) with rates reported in a study conducted among the general, ambulatory adult population in New York (4.3–6%).[9] The slightly higher rates could be attributed to a higher susceptibility to CARV among LTx candidates with pre-existing pulmonary disease compared to the general population. It is important to mention that our study encompassed all seasons, whereas the previous study only performed tests over two seasons, missing data of late summer, fall and early winter season in which rates might differ. Moreover, our study's findings align with asymptomatic CARV rates reported in previous research among LTx recipients on average 3–4 years after lung transplantation.[10,11] Rhinovirus was the predominant CARV (43%) among our study cohort, consistent with prior studies.[1,10,11]

To our knowledge, this is the first study to provide data on the association between asymptomatic CARV infection and PGD development. Thus far, studies have shown a link between symptomatic infections and the risk of adverse outcomes such as CLAD or graft loss, while asymptomatic infections do not.[3,4,12] However, these studies did not focus on the early-post LTx period and therefore did not evaluate the risk of PGD. Our study extends the knowledge on asymptomatic CARV carriage to this period.

In our study, all our patients were asymptomatic upon arrival for LTx despite a positive swab. This could be due to the fact that patients called in for LTx have respiratory symptoms anyway, masking the respiratory symptoms of a CARV. On the other hand, positive viral swabs can be obtained at any point during infection, encompassing recipients who are recovering from a viral infection and persons who continue to shed the virus post-infection. This could explain the minimal viral loads detected in our study. Yet, it is important to highlight that some LTx recipients carried higher viral loads.

Among the LTx recipients, 5 individuals tested positive for SARS-CoV-2. All had low viral loads (median Ct-value 39; range 35–41) This could indicate post-infection shedding, a phenomenon well-documented in COVID-19 cases.[13] Notably, two of these patients were transplanted due

to COVID-19-induced lung fibrosis and two had recent confirmed infections. One recipient had both a positive and a negative test within 24h before transplantation. Additionally, one recipient exhibited decreasing Ct-values post-transplantation, suggesting a possible onset of COVID-19 infection around the time of LTx, although this recipient was vaccinated and recovered satisfactorily.

We conducted a post hoc analysis (Table 4.4) comparing COVID-19 presence with a negative swab group. We found a significant prolongation in mechanical ventilation and ICU duration for COVID-19 carriers, without impacting overall hospital LOS or mortality. The extended duration of mechanical ventilation may be explained by the fact that 2 out of 5 (40%) COVID-positive recipients were on mechanical ventilation before transplantation.

The suitability of LTx candidates who have SARS-CoV-2 has been a subject of controversy.[14] ISHLT guidelines suggest a case-by-case approach for end-stage lung disease patients contracting SARS-CoV-2, taking into consideration the clinical resolution of symptoms and the necessity for transplantation.[13] Our findings support their recommendation to consider LTx in patients with a high Ct-value. However, ISHLT recommends waiting at least 28 days post-infection before LTx, while one of our recipients was only 9 days after initial confirmed infection.

The main limitation is that we did not routinely collect follow-up swabs or evaluate symptom development. Consequently, we were unable to analyze changes in Ct-values or the advancement of infection. On the other hand, there was no clinical suspicion of a CARV post-LTx. Other limitations of this study are the relatively small sample size and the retrospective design. Due to the small sample size, we could not demonstrate that different CARVs exert distinct effects. Additionally, donors did not undergo routine CARV testing. Nevertheless, this absence of testing would likely impact both study groups similarly and is unlikely to significantly alter our findings.

In conclusion, our study offers valuable insights into the pre-transplant CARV carriage among asymptomatic lung transplant candidates. 10.4% of all recipients tested positive for a CARV. Our findings show no correlation between asymptomatic CARV carriage and the early post-LTx course. Moreover, it is crucial to recognize the potential drawbacks of testing. For instance, the risk of an unnecessary LTx refusal due to asymptomatic CARV infection could increase mortality rates for patients on the waiting list. Therefore, we do not recommend routinely performing CARV testing before transplantation.

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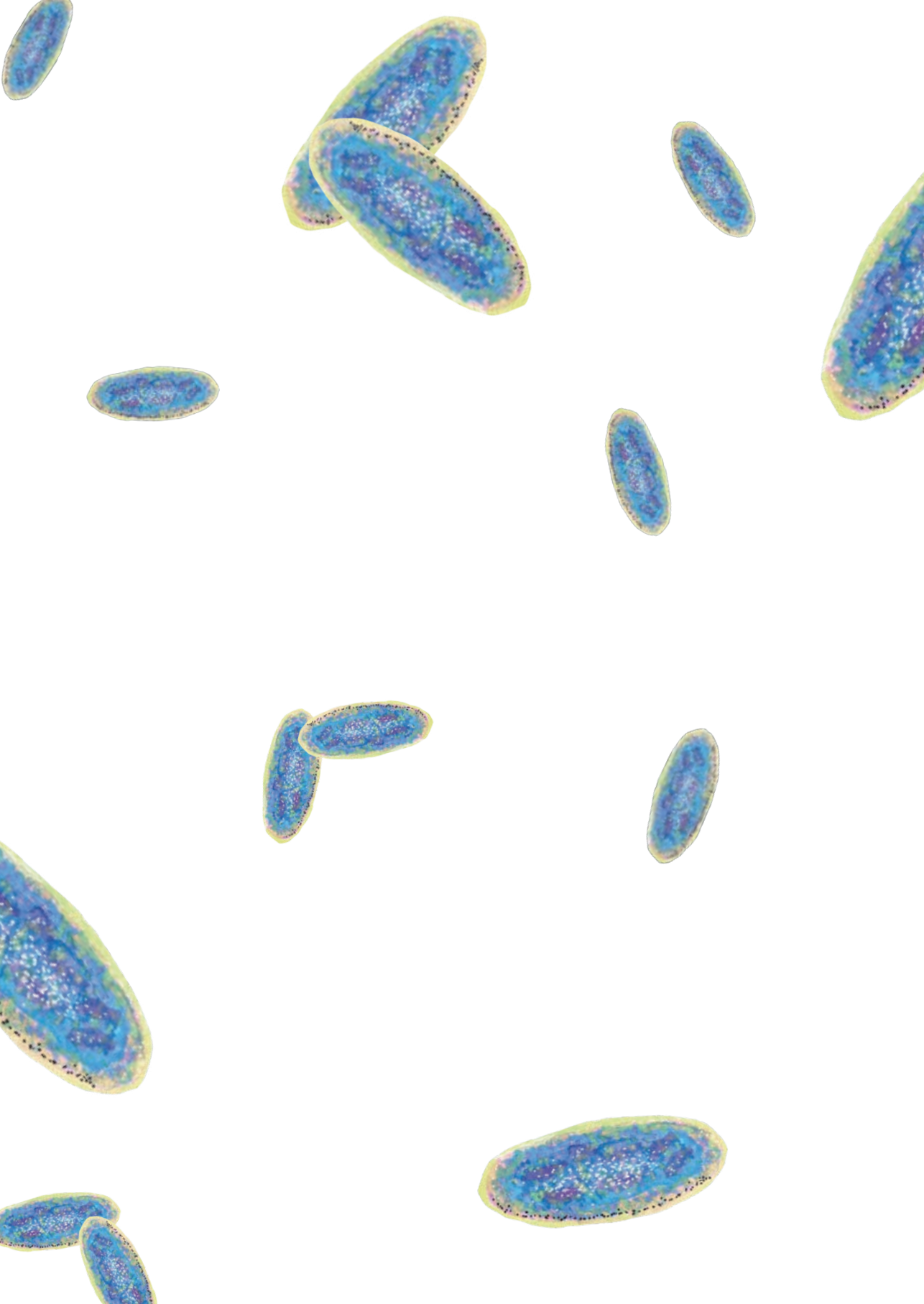

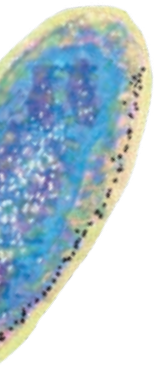


Chapter 5

Non-tuberculous mycobacteria disease pre-lung transplantation: A systematic review of the treatment regimens and duration pre- and post-transplant

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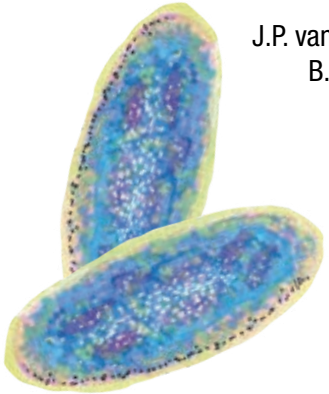
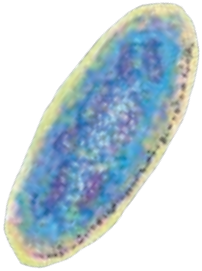

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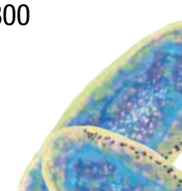

Abstract

Background: There is lack of consensus on non-tuberculous mycobacteria pulmonary disease (NTM-PD) treatment regimen and duration in patient listed for lung transplantation (LTx). We conducted a systematic review on treatment regimen and duration pre- and directly post-LTx, for patients with known NTM-PD pre-LTx. Additionally, we searched for risk factors for NTM disease development post-LTx and for mortality.

Methods: Literature was reviewed on PubMed, Embase and the Cochrane Library, for articles published from inception to January, 2022. Individual patient data were sought.

Results: Sixteen studies were included reporting 92 patients. Most frequent used agents were aminoglycosides and macrolides for *Mycobacterium abscessus* (*M. abscessus*) and macrolides and tuberculostatic agents for *Mycobacterium avium* complex (*M. avium* complex). The median treatment duration pre-LTx was 10 months (IQR 6–17) and 2 months (IQR 2–8) directly post-LTx. Longer treatment duration pre-LTx was observed in children and in patients with *M. abscessus*. Forty-six percent of the patients with NTM-PD pre-LTx developed NTM disease post-LTx, with a related mortality rate of 10%. Longer treatment duration pre-LTx ($p < 0.001$) and sputum non-conversion pre-LTx ($p = 0.003$) were significantly associated with development of NTM-disease post-LTx. Longer treatment duration pre-LTx ($p = 0.004$), younger age ($p < 0.001$) and sputum non-conversion ($p = 0.044$) were risk factors for NTM related death.

Conclusions: The median treatment duration pre-LTx was 10 months (IQR 6–17) and 2 months (IQR 2–8) directly post-LTx. Patients with longer treatment duration for NTM-PD pre-LTx and with sputum non-conversion are at risk for NTM disease post-LTx and for NTM-related death. Children were particularly at risk for NTM related death.

Introduction

Lung transplantation (LTx) is a treatment option for selected patients with life-threatening, advanced lung disease, unresponsive to other medical or surgical treatments. Non-tuberculous mycobacteria (NTM) are frequently isolated from the diseased airways of patients listed for LTx with structural lung disease, such as cystic fibrosis (CF).[1–3]

NTM pulmonary disease (NTM-PD) is defined as symptomatic patients with HRCT compatible with NTM disease and positive sputum cultures, bronchial washes or biopsies and exclusion of other causes.[4] NTM-PD pre-LTx, may lead to post-LTx NTM disease (pulmonary or disseminated disease), due to spillage of native lung contents into the pleural cavity and surgical wounds, or reinfection from spread from infected airways above the anastomoses.

NTM-PD used to be a contra-indication for LTx. However, several small studies have shown that LTx is feasible.[5–9] Currently, according to ISHLT 2021 guidelines, patients with NTM-PD may be referred for LTx, but should be managed at centers with expertise and protocols for NTM-PD.[10]

NTM, especially *Mycobacterium abscessus* (*M. abscessus*) and *Mycobacterium avium* complex (*M. avium* complex), are associated with increased post-LTx mortality and the development of chronic lung allograft dysfunction (CLAD).[8,11] To reduce the risk of post-LTx NTM disease, patients listed for LTx, should be treated pre-LTx to eradicate the NTM or, at least to reduce mycobacterial burden.[12] In general, NTM-PD is hard to treat. Cure rates for NTM are variable, for *M. avium* complex 42–67% and for *M. abscessus* 16–65%.[13–15] Directly after LTx, NTM treatment might be necessary to prevent transition from NTM-PD pre-LTx to pulmonary or disseminated NTM disease post-LTx, in the immunocompromised patient. Although treatment targeting therapy for most NTM species are standardized worldwide, there is a lack of consensus on treatment options, strategies and duration in patient listed for LTx.[14] Post-LTx, drug interactions between antimycobacterial agents and immunosuppressive therapy (especially calcineurin- and mammalian target of rapamycin (mTOR)-inhibitors) are challenging and may lead to severe side effects, which are the result of high drug blood concentrations due to drug-drug interactions, poor kidney function and high doses necessary for efficacy. This may lead to poor NTM therapy tolerability, affecting duration and effectiveness of treatment.

The primary aims of this review are to describe the different treatment regimens and duration pre- and directly post-LTx and to evaluate the effect of treatment duration on development of NTM post-LTx and on mortality. Secondary aims are to describe additional risk factors for NTM disease development post-LTx and for mortality.

Methods

The review protocol was registered in PROSPERO (CRD42022329301). The Preferred Reporting Items for Systematic Review (PRISMA) guidelines were followed in reporting of this review.[16] The literature search, was conducted on major databases including PubMed, EMBASE and Cochrane Library. The original search query is shown in Supplement 5.1. References of the included papers were reviewed for missing articles. First, the titles and abstracts of search results were screened independently by two authors, thereafter the full texts were evaluated for inclusion by J.G. and S.R. Discrepancies were solved by consensus or by consulting a third author (O.A.) (Supplement 5.2). Studies were eligible if they investigated LTx patients (adults and children), with NTM-PD pre-LTx and treated with oral, nebulized or intravenous antibiotics pre- and/or directly post-LTx. Directly post-LTx treatment was defined as treatment started directly post-LTx for pre-LTx NTM-PD, which might be continuation of the pre-LTx treatment (Supplement 5.3). Studies were excluded when investigating: 1) only post-LTx NTM disease, 2) data from animal studies or NTM isolated from explanted lungs, 3) *Mycobacterium tuberculosis*.

Data extraction

The following data were extracted from each included study using a standardized data extraction form: authors, publication date, design, objective, follow-up, patient characteristics, NTM species pre-LTx, treatment regimens pre-LTx and directly post-LTx, treatment duration, frequency of NTM disease post-LTx, mortality, graft loss during follow-up. When needed authors were contacted to add information. Papers without original data, conference abstracts, studies with duplicate data, and review papers were excluded.

Risk of bias (quality) assessment

The mixed methods appraisal tool (MMAT) was used to assess risk of bias of the papers.[17] Two reviewers were independently involved in the appraisal process (J.G. and S.R.). Discrepancies were resolved by consensus or by consulting a third author (O.A).

Outcomes

Study outcome measures in patients with pre-LTX NTM-PD included the development of post-LTX NTM disease and mortality.

Statistical analysis

All analyses were performed using SPSS (23.0; SPSS Inc., Chicago, Illinois, USA) for Mac. Variables were expressed as median (quartiles). Mann–Whitney U-test and chi-squared test were used to compare parameters between patients who developed NTM disease post-LTx and those without development of NTM disease post-LTx. Furthermore, parameters were compared between patients who survived and died due to NTM disease. A Cox proportional hazards model with time dependency was used to compare post-LTx survival between patients with and without post-LTx NTM-PD. A *p*-value of less than 0.05 was considered significant.

Results

Search results, study description and quality

Sixteen studies were included providing data on 92 cases in total (PRISMA flowchart Supplement 5.2).[5-7,9,18-29] Four of the original studies had a case-control design, 6 were cohort studies, 5 were case series/reports and 1 was a survey study (Supplement 5.4). All studies had a retrospective design. Authors of 2 studies provided additional data after being contacted.

Study quality regarding NTM-PD treatment varied and was mostly hampered by small sample size, the lack of appropriate measurements and incomplete outcome data (MMAT results in Supplement 5.5).

Patients with NTM-PD

Patient characteristics of the 92 patients are summarized in Table 5.1. The median age was 24 (IQR 18–32) years, 18 (20%) were children (age < 18 year) and 57 (57%) were female. The most frequent indications for LTx were cystic fibrosis (CF) (75/92; 82%), followed by pulmonary fibrosis (6/92; 8%), and COPD/, Alpha-1 antitrypsin deficiency (6/92; 8%). Immunosuppressive therapy was described in 11 studies. Sixty percent of the patients received induction therapy. Patients received triple maintenance immunosuppression therapy in all but one study, and consisted mainly of tacrolimus (TAC)/cyclosporine (CYC), mycophenolate mofetil (MMF)/azathioprine (AZA) and prednisolone.

NTM-PD treatment and treatment duration

Treatment data were available for 76 patients pre-LTx and for 54 patients directly post-LTx. Eighteen out of 76 (24%) patients did not receive treatment pre-LTx and 5 out of 54 patients (9%) directly post-LTx. Of the 18 patients who did not receive treatment pre-LTx, 15 were considered not to have met criteria for NTM-PD, 1 patient spontaneously cleared the *M.*

abscessus pre-LTx and for 2 patients the reason for withholding treatment is unknown. The 5 patients who did not receive treatment directly post-LTx, had not met diagnostic criteria for NTM-PD according to the authors. In 78% patients with NTM-PD pre-LTx and in 94% directly post-LTx medication was delivered intravenously.

Table 5.1: Clinical features of patients with NTM-PD pre-LTx who developed NTM disease post-LTx and/or died due to NTM disease

Variable	All patients n = 92	NTM post-LTx n = 42	*p*-value	NTM death n = 9	*p*-value
Age			0.052		< 0.001
< 18 years , n (%)	18	12 (67)		6 (33)	
> 18 years, n (%)	66	27 (41)		3 (5)	
Gender			0.607		0.678
Male, n (%)	32	16 (50)		4 (13)	
Female, n (%)	52	23 (44)		5 (10)	
Transplant indication, n (%)			0.682		0.664
Cystic Fibrosis	75	34 (45)		8 (11)	
COPD/AATD	6	4 (67)		0 (0)	
Fibrosis	6	2 (33)		1 (17)	
Other	5	2 (40)		0 (0)	
NTM species, n (%)			0.237		0.517
M. abscessus	68	34 (50)		8 (12)	
M. avium complex	18	7 (39)		1 (6)	
Other	6	1 (17)		0 (0)	
Sputum conversion, n (%)			0.003		0.044
Yes	22	5 (23)		0 (0)	
No	49	30 (61)		8 (16)	

NTM-PD, non-tuberculous mycobacteria pulmonary disease; LTx, lung transplant; NTM, Non-tuberculous mycobacteria; COPD, chronic obstructive pulmonary disease; AATD, alpha-1 antitrypsin defiency.

The median treatment duration pre-LTx was 10 months (IQR 6–17) in 14 studies (64 patients). The mean treatment duration directly post-LTx was 2 months (IQR 2–8) in 12 studies (51 patients). The effect of treatment duration and thorax irrigation on the development of NTM disease post LTx and on NTM related mortality is shown in Table 5.2. Longer treatment duration pre-LTx was significantly associated with the development of NTM disease post-LTx ($p < 0.001$) and with NTM related mortality ($p = 0.004$). Treatment duration directly post-LTx was not associated with the development of NTM disease ($p = 0.374$) or NTM related mortality ($p = 0.105$). In 22 long term survivors (> 5 year post-LTx) treatment duration pre-LTx was 7 months (IQR 6–12) and post-LTx 2 months (IQR 2–3). There was an association between *M. abscessus* and age with treatment duration: patients with *M. abscessus* and children, had significant longer treatment duration pre-LTx ($p < 0.001$ for both).

In 10 studies (70 patients) information about surgical procedures to reduce NTM load at the time of LTx was available. Pleural cavity irrigation with or without lymphadenectomy was performed in 32 patients (35%). Pleural cavity irrigation had no significant effect on the development of NTM disease post-LTx or on mortality (Table 5.2).

Table 5.2: Effect of treatment duration and thorax irrigation on the development of NTM disease post LTx and on NTM related mortality

Variable	NTM post-LTx n = 42	No NTM post-LTx n = 50	*p*-value	NTM death n = 9	Survived/ non-NTM death n = 83	*p*-value
Treatment duration						
Pre-LTx, months	12 (8–36)	6 (3–12)	< 0.001	30 (18–60)	7 (6–12)	0.004
Post-LTx, months	3 (2–12)	2 (2–6)	0.374	2 (1–2)	2 (2–8)	0.105
Thorax irrigation	18	14	0.064	5	27	0.311

NTM, Non-tuberculous mycobacteria; LTx, lung transplant.

M. abscessus disease and treatment

Supplement 5.6 shows all the NTM species identified in this review. Combining all 16 studies the most common isolated NTM species was *M. abscessus*. Pre-LTx 68 patients (74%) had *M. abscessus* NTM-PD. Of those, there was 1 patient with subspecies *bolletii*, 5 patients with subspecies *massiliense* and 7 patients with subspecies *abscessus*. In 55 patients the *M. abscessus* subspecies was not available. *M. avium* complex was isolated in 20% of the patients, *M. fortuitum* in 4%, in one patient both *M. abscessus* and *M. avium* complex were isolated and in one patients the NTM species was not specified (Supplement 5.6).

M. abscessus treatment data were available in 60 patients pre-LTX and 58 patients directly post-LTx. The most frequently used agents pre- and directly post-LTx for *M. abscessus* are shown in Figure 5.1a. The most frequent used classes of antibacterial agents used for *M. abscessus* pre-LTx and directly post LTx were aminoglycosides and macrolides (Table 5.3a). Treatment regimens for *M. abscessus* included a combination of 3 different agents pre-Ltx (IQR 3–4) and 3 (IQR 2–5) post-LTx. The median treatment duration for *M. abscessus* pre-LTx was 12 (IQR 6–20) months and directly post-LTx 2 (IQR 2–8) months. Treatment duration pre-LTx was significant longer in patients with *M. abscessus* than in patients with other NTM species ($p < 0.001$). The sputum conversion rate after pre-LTx treatment in patients with *M. abscessus* was 34%. In patients with *M. abscessus* pre-LTx 34 (50%) developed NTM disease post-LTx (Table 5.1) and 8 (12%) died due to NTM disease. Of the 9 patients who died due to NTM disease, 8 patients had *M. abscessus* pre-LTx ($p = 0.517$).

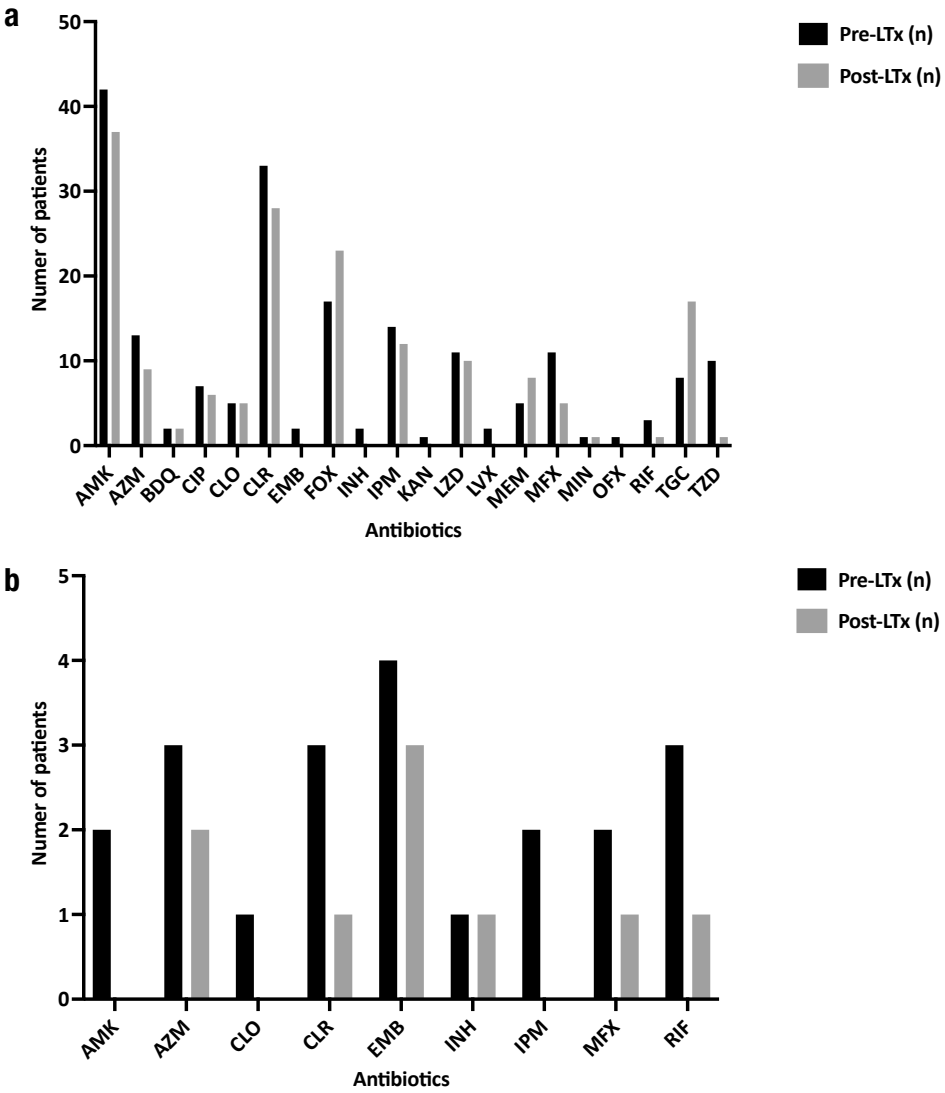

Figure 5.1: (a) Treatment for *M. abscessus* pre- and post-LTx. (b) Treatment for *M. avium* complex pre- and post-LTx. LTx, lung transplantation; AMK, amikacin; AZM, azithromycin; BDQ, bedaquiline; CIP, ciprofloxacin; CLO, clofazimine; CLR, clarithromycin; EMB, ethambutol; FOX, cefoxitin; INH, isoniazid; IPM, imipenem; KAN, kanamycin; LZD, linezolid; LVX, levofloxacin; MEM, meropenem; MFX, moxifloxacin; MIN, minocycline; OFX, ofloxacin; RIF, rifampin; TGC, tigecycline; TZD, tedizolid.

M. avium complex disease and treatment

Pre-LTx 18 patients (20%) had *M. avium* complex NTM-PD. *M. avium* complex treatment data were available in 15 patients pre-LTX and 16 patients directly post-LTx. The most frequently used agents pre- and directly post-LTx for *M. avium* complex are shown in Figure 5.1b. The

most frequent used classes of antibacterial agents used for *M. avium* complex pre-LTx and directly post LTx were macrolides and tuberculostatic agents (Table 5.3b). Treatment regimens pre-LTx and post-LTx for *M. avium* complex included a combination of 3 different agents (IQR 0–3). The median treatment duration for *M. avium* complex pre-LTx was 6 months (IQR 3–7) and directly post-LTx 3 months (IQR 1–10). The sputum conversion rate after pre-LTx treatment in patients with *M. avium* complex was 25% pre-LTx. In patients with *M. avium* complex pre-LTx 7 (39%) developed NTM disease post-LTx (Table 5.1a) and 1 (6%) died due to NTM disease. Of the 9 patients who died due to NTM disease, 1 patient with *M. avium* complex had pre-LTx. There was no difference in the risk of NTM disease post-LTx or mortality between patients with *M. avium* complex, *M. abscessus* or other species (Table 5.1)

Table 5.3a: Class of antibacterial agents used for *M. abscessus* pre-LTx and post LTx

	Pre-LTx	Post-LTx
Antibiotic class	Patients n (%)	Patients n (%)
Aminoglycosides	42 (70)	37 (64)
Cephalosporins	20 (33)	26 (45)
Tuberculostatic agents	8 (13)	3 (5)
Fluoroquinolones	20 (33)	10 (17)
Macrolides	47 (78)	37 (64)
Tetracyclines	13 (22)	17 (29)
Carbapenems	19 (32)	19 (33)
Oxazolidinones	20 (33)	11 (19)
Clofazimine	5 (8)	5 (9)

LTx, lung transplant; NTM, Non-tuberculous mycobacteria.

Table 5.3b: Class of antibacterial agents used for *M. avium* complex pre-LTx and post LTx

	Pre-LTx	Post-LTx
Antibiotic class	Patients n (%)	Patients n (%)
Aminoglycosides	2 (13)	0 (0)
Tuberculostatic agents	4 (27)	3 (19)
Fluoroquinolones	2 (13)	1 (6)
Macrolides	6 (40)	3 (19)
Carbapenems	2 (13)	0 (0)
Clofazimine	1 (7)	0 (0)

LTx, lung transplant; NTM, Non-tuberculous mycobacteria.

Development of NTM disease post-LTX and risk factors

In the 92 patients with pre-LTx NTM-PD, 42 (46%) patients developed NTM disease post-LTx (Table 5.1). The median time from LTx to NTM disease post-LTx was 2 (IQR 1–9) months. Eleven (26%) of the patients with NTM disease post-LTx had disseminated disease, 20 (48%) had pulmonary disease, 7 (17%) had surgical wound infection, 2 (5%) had both lung and surgical wound infection, 1 patient had a breast abscess and 1 patient had osteomyelitis. Nine patients (21%) with NTM disease post LTx died. The NTM species pre-LTx were the same as post-LTx, in all but one.

Clinical features of patients with NTM-PD pre-LTx who developed NTM disease post-LTx are shown in Table 5.1. Twelve of the 18 children (67%) developed NTM disease post-LTx, whereas 27 of the 66 adults (41%) developed NTM disease post-LTx ($p = 0.052$). The risk of NTM disease post-LTx was significant higher in patients without sputum conversion than in patients with sputum conversion pre-LTx (61% vs 23%, $p = 0.003$).

Survival and risk factors for NTM related death

Forty-four (48%) patients survived till follow-up, 9 (10%) patients died due to NTM disease and 39 (42%) died, not-NTM related within 97 months. Median follow up time in patients who survived was 53 (IQR 24–95) months. The median survival time from LTx to NTM related death was 3 (IQR 2–16) months, and 22 (IQR 5–52) months for non-NTM related death.

In patients who died due to NTM disease, the median time between the first positive NTM culture and LTx was 4 (IQR 2–7) year. Moreover, these patients were very young when NTM were cultured for the first time pre-LTx: median age 11 (IQR 4–13) year.

Characteristics of patients who died due to NTM disease are presented in Table 5.1. In children the NTM related mortality was significant higher than in adults (6/18 (33%) vs 3/66 (5%); $p < 0.001$). Seventeen out of 18 children had *M. abscessus* NTM-PD (94%; $p = 0.100$). The risk of NTM related death was significant higher in patients without sputum conversion than in patients with sputum conversion pre-LTx (8/49 (16%) vs 0/22 (0%), $p = 0.044$). Notably, there was an association between sputum conversion and age: sputum conversion was achieved pre-LTx in less children compared to adults (2/7 (28%) vs 26/46 (56%), $p = 0.019$).

In 83 patients the time to follow up or to death was available. The median survival time for patients with NTM disease post-LTx was 48 months and for patients without 62 months. The 6-month survival rate in patient with NTM disease post-LTx was 79% and for patients without NTM disease post-LTx 85%. Survival rates at 1 year were 70% for patients with NTM disease post-LTx and 80% for those without. There was no significant difference in survival

between patients with or without NTM disease post-LTx (Table 5.4; $p = 0.103$). In the 22 long-term survivors (> 5 years), 77% had *M. abscessus* NTM-PD pre-LTx, 27% had NTM disease post-LTx and in 32% sputum conversion was achieved pre-LTx. The overall 30-day survival rate in these 83 patients was 96% and the overall 5-year survival rate was 48%.

Table 5.4: Survival in LTx patients, with and without NTM-PD post-LTx

	Survival	
NTM-PD after LTx	Alive	Death
No, n (%)	23 (46)	27 (54)
Yes, n (%)	21 (50)	21 (50)

LTx, lung transplantation; NTM-PD, non-tuberculous mycobacteria pulmonary disease

Chronic lung allograft dysfunction

In 71 (77%) patients data about CLAD were available. Seventeen (18%) patients with pre-LTX NTM-PD developed CLAD post-LTX. There was no significant association between CLAD and NTM disease post-LTX: 28% of the patients with NTM disease post-LTx developed CLAD, whereas 21% of patients without NTM disease post-LTX developed CLAD ($p = 0.550$).

Discussion

In this systematic review treatment regimens and duration pre-LTx and directly post-LTx in patients with pre-LTx NTM-PD are described. We showed that longer treatment duration pre-LTx was significantly associated with the development of NTM disease post-LTx and with NTM related mortality.

In patients who meet the diagnostic criteria for NTM-PD, initiation of treatment rather than watchful waiting is suggested. In patients with *M. abscessus* disease, a multidrug regimen that includes at least 3 active agents (guided by in vitro susceptibility) is recommended.[4] Not all patients in this review received treatment, because these patients did not met the criteria for NTM-PD and colonization was suggested. However, the concept of NTM airway colonization has never been studies rigorously and it is generally assumed that colonization is, in fact, indolent or slowly progressive disease.[30]

The most frequently used agents for *M. abscessus* were macrolides and aminoglycosides, and for *M. avium* complex macrolides and ethambutol, which is according to the guidelines.[4] The median treatment duration for *M. abscessus* was 12 (IQR 6–20) months pre-LTx and 2

(IQR 2–8) months directly post-LTx. For *M. avium* complex the median treatment duration was 6 (IQR 3–7) months pre-LTx and 3 months (IQR 1–10) directly post-LTx. The optimal duration of therapy for *M. abscessus* and *M. avium* complex disease is not currently known, nevertheless treatment for at least 12 months after culture conversion is recommended for *M. avium* complex.[4] Drug intolerance and drug interactions complicates the optimal duration of therapy. Clinicians might prefer to initiate long-time aggressive treatment pre-LTx, because most patients are not immunocompromised pre-LTx and there is no need to take into account drug interactions with immunosuppressive agents. However, given the long time on waiting list for LTx with severe lung disease, a vulnerable state and co-infection with other bacteria or fungus, make long treatments for NTM very challenging. Moreover, waiting time for LTx varies and makes it difficult to plan therapy for a defined time until surgery. Therefore, in the difficult setting of pre- and post-LTx NTM-PD, management should be restricted to expert centers.[4]

We showed that longer treatment duration pre-LTx was associated with higher NTM disease rates post-LTx and NTM-related mortality. However, treatment duration itself, is affected by two other variables: age and *M. abscessus* disease. In younger patients and patients with *M. abscessus* NTM-PD treatment duration pre-LTx was longer. In addition, it might be that patients, with *M. abscessus* disease and younger age, needed longer treatment because of more severe NTM disease or more extensive structural lung damage and therefore were at risk for NTM disease post-LTx. Unfortunately, information about the severity of NTM disease and the extensiveness of the pre-LTx NTM-PD treatment was not available. What we do know, is that patients with NTM disease post-LTx who died, had NTM-PD long before LTx at very young age. Most of these patients had oral and IV regiments, but information about the duration of oral versus IV treatment is missing. Longer treatment duration pre-LTx did not result in a higher sputum conversion rates, which is comparable with the results of the Dutch CF foundation.[31] Zomer et al. showed that in CF patients with NTM-PD the sputum conversion rate was 50% after 1 year of treatment, which did not change after prolongation of treatment (> 1 year).[31]

In this review, the rate of NTM disease post-LTx after prior pre-LTx NTM-PD is high (46%), which underlines the need to sustain treatment rather than discontinue it directly post-LTx. However, the heterogeneity of the used agents, variable duration of treatment pre- and directly post-LTx and the unknown drug susceptibility preclude any firm recommendation regarding treatment of pre-LTx NTM-PD.

Our secondary aim was to describe additional risk factors for NTM disease development post-LTx and for mortality. Patients without sputum conversion pre-LTx did develop NTM

disease post-LTx more often than patients with sputum conversion. Therefore, it is a good goal to aim for sputum conversion before LTx.[12,32] If sputum conversion fails, it is advisable to start NTM treatment immediately post-operatively to prevent NTM disease post-LTx.

Importantly, we showed that children are at greater risk for NTM related death than adults. Most children (94%) in this review had *M. abscessus* NTM-PD. Catherinot et al. showed that the clinical presentation of NTM-PD due to *M. abscessus* is usually more severe, and the affected patients are younger with more severe CF.[33] Furthermore, Zomer at al showed that children with CF and NTM-PD had more severe pulmonary function decline than adults with CF and NTM-PD.[31] In children treatment duration pre-LTx was significantly longer than for adults. Treatment duration pre-LTx in children might be longer because these children more often had *M. abscessus* NTM-PD (94% of the children). Moreover, these children had first NTM isolation long before transplant, with longer treatment duration, probably due to eradication failure, which might have resulted in worse outcome. NTM treatment in children is challenging. Saint et. al retrospectively collected data from children with CF without LTx from 11 CF specialist centres. They reported refractory disease in 20% after treatment, drug cessation due to adverse events in 30%, and dose change in 10%.[34]

M. abscessus NTM-PD was not a direct risk factor for NTM disease post-LTx or NTM related death. However 94% of the children, who were at greater risk for NTM disease post-LTX and NTM related death, had *M. abscessus* NTM-PD. In non-LTx studies a poor prognosis is found for *M. abscessus* NTM-PD and *M. abscessus* is considered a clinically problematic pathogen which is extremely difficult to eradicate given its naturally multidrug resistant properties.[5,19,20,24,35] However, our results show that not only the aetiological NTM species, but the duration of the treatment and host factors such as age are prognostic factors for NTM disease and mortality post-LTx. Besides, the duration of the treatment depends on the bacterial load of the NTM-PD, which might be reflected by the lack of sputum conversion.[36]

This review has some limitations. The literature available for this review consists of small non-randomized case-series and cohort studies with important differences in quality of evidence, methods, sample size and follow-up time. Despite the limited data, we managed to find 92 patients for whom individual patient data were provided in 16 studies.

Conclusions

Median treatment duration pre-LTx was 10 months (IQR 6–17) and directly post-LTx 2 months (IQR 2–8). Treatment regimens for *M. abscessus* included a combination of 3 different agents

and most frequent used agents were aminoglycosides and macrolides. Treatment regimens for *M. avium* complex included a combination of 3 different agents and most frequent used agents were macrolides and tuberculostatic agents. Longer treatment duration pre-LTx was observed in children and in patients with *M. abscessus* NTM-PD and was significantly associated with the development of NTM disease post-LTx and with NTM related mortality. Patients without sputum conversion pre-LTx had a higher risk of NTM disease post-LTx and NTM related mortality. Children were particularly at risk for NTM related death.

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Supplement 5.1: Research syntax

Pubmed
("Mycobacterium Infections, Nontuberculous"[Mesh] OR "Mycobacterium"[Mesh] OR Mycobact*[tiab] OR NTM[tiab] OR MAC[tiab] OR MAB[tiab] OR kansasii[tiab] OR Fortuitum[tiab] OR gordonae[tiab] OR Xenopi[tiab] OR Lentiflavum[tiab] OR scrofulaceum[tiab] OR abscessus[tiab] OR avium[tiab] OR malmoense[tiab])

AND

("Lung Transplantation"[Mesh] OR "lung transplant*"[tiab] OR LTX[tiab] OR LOTX[tiab] OR “solid organ transplant*”[tiab] OR “thoracic transplant*”[tiab] OR SOT[tiab])

Embase
('atypical mycobacteriosis'/exp OR 'mycobacteriosis'/de OR 'Mycobacterium'/exp OR (Mycobact* OR NTM OR MAC OR MAB OR kansasii OR fortuitum OR gordonae OR xenopi OR lentiflavum OR scrofulaceum OR abscessus OR avium OR malmoense):ab,ti)

AND

('lung transplantation'/exp OR ('lung transplant*' OR LTX OR LOTX OR 'solid organ transplant*' OR 'thoracic transplant*' OR SOT):ab,ti)

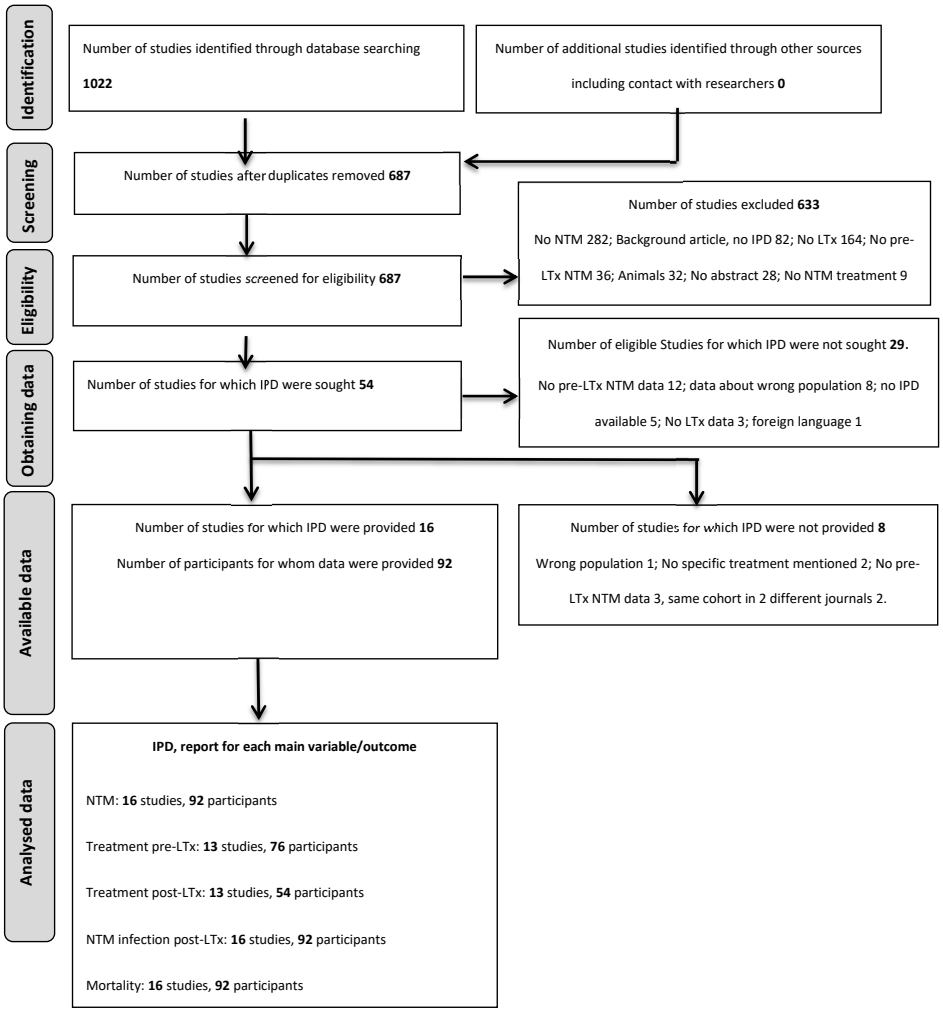
NOT

'conference abstract'/it

Cochrane
([mh "Mycobacterium Infections, Nontuberculous"] OR [mh Mycobacterium] OR Mycobact*:ti,ab OR NTM:ti,ab OR MAC:ti,ab OR MAB:ti,ab OR kansasii:ti,ab OR fortuitum:ti,ab OR gordonae:ti,ab OR xenopi:ti,ab OR lentiflavum:ti,ab OR scrofulaceum:ti,ab OR abscessus:ti,ab OR avium:ti,ab OR malmoense:ti,ab)

AND

([mh "Lung Transplantation"] OR ("lung" NEXT transplant*):ti,ab OR LTX:ti,ab OR LOTX:ti,ab OR “solid organ transplant*”:ti,ab OR “thoracic transplant*”:ti,ab OR SOT:ti,ab)



Supplement 5.2: Prisma flow chart.
LTx: lung transplant; NTM: non-tuberculous mycobacteria; IPD: individual patient data.
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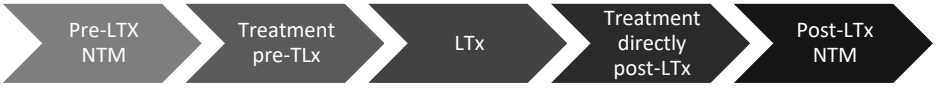


Supplement 5.3: Time line of treatment for non-tuberculous mycobacteria in patients listed for lung transplantation.
LTx: lung transplant; NTM: non-tuberculous mycobacteria.

Supplement 5.4: Studies reporting clinical characteristics, nontuberculous mycobacteria species, treatment, immunosuppression in lung transplant patients

Author, year	Design	LTx Indication (n)	NTM Species (n)	Pre-LTx agents	Agents directly post-LTx	Agents started for post-LTx NTM disease	Outcome post-LTx	Immuno-suppression	MMAT
Chalermskulrat 2006	CCS	CF (18)	*MAC* (7) *MAB* (7) *M. gordonae* + *MAC* (1) *MAB* + *MAC* (1) *M. fortuitum* + *MAC* (1) Unspecified (1)	6: NTM disease: MAB: CLR or AZM, AM, FOX. M. kansasii: INH, quinolone, RIF, EMB 12: no treatement	No	1. MAB: 48 mo AMK, AZM, FOX 2. MAB: 27 mo AMK, CLR, FOX 3. Refused 4. M. kansasii: 2mo INH, CIP, RIF, EMB	Recurrence: 7 culture pos, 4 NTM- disease. Survival mean 38,6 mo	n/a	***
Chemenko 2006	SS	Bronchiectasis (1) CF (1)	*MAB* (2)	1 y treatment not specified	n/a	AMK 4 mo, AZM 11 mo, FOX 7 mo, IPM 2 wk, CLO 5 mo, LZD 2 mo, GA 6 mo	Recurrence breast, died after 14 mo not NTM related,	n/a	***
				1 y treatment not specified	n/a	1 mo. CIP, MEM, AMK	Recurrence lung. Died after 2 mo not NTM related		
Giljam 2010	CR	CF 4	*MAB* (4)	6 mo: AZM, DOX	No	AMK, EMB, AZM, MEM surgery with skin graft. Followed by AMK iv 3 mo, neb AMK 2y, EMB 2y, macrolide 2 y. IVIG	Dis. infection surgical wound osteomyelitis. After 7 y clean, CLAD 0, CKD, hearing loss	ATG, CYC, MMF, pred	***
				4 y: AZM	AMK	AMK 6mo,EMB 5 mo, AZM 5y, IVIG	Dis. infection surgical wound. After 5 y clean, CLAD 1, CKD		
				5 y: AZM, RIF	AMK, MEM	AMK 6 mo, AZM, IVIG 3y	Surgical wound infection. After 3 y clean, CLAD 0. CKD		
				10 mo: AMK, OFX, EMC, CLR	MEM	None	No recurrence. CLAD 0		
Hamad 2019	CCS	CF (3) Bronchiectasis (1) Hyper IgE syndrome (1) IPF (1)	*MAB* (6)	No	No	8 mo: not specified	Recurrence: - Surgical site (2) - Lung (2) 2 died NTM related	Alemtuzumab/ Basiliximab, TAC, MMF, pred	****
				Not specified	n/a	9 mo: not specified			
				Not specified	19 mo: not specified	No			
				Not specified	8 mo: not specified	No			
				No	No	2 mo: not specified			
				Not specified	n/a	8 mo: not specfied			

Supplement 5.4 continues on next page.

Supplement 5.4: ***Continued***

Author, year	Design	LTx Indication (n)	NTM Species (n)	Pre-LTx agents	Agents directly post-LTx	Agents started for post-LTx NTM disease	Outcome post-LTx	Immuno-suppression	MMAT
Hirama 2021	RCS	COPD (2)	*MAC* (2)	MAC: AZM, EMB, RIF until LTX	No AZM, EMB, MXF 12 mo	12 mo: AZM, EMB, MXF 8 mo: AZM. EMB, MXF	Alive, CLAD No improvement, died, CLAD	n/a	***
Huang 2011	CCS	IPF (4) Sarcoidosis (1) COPD (1)	*M. fortuitum* (4) *MAC* (2)	4 *M. fortuitum*: 3 mo, not specified 1 MAC: 6 mo, not specified 1 MAC: no therapy	No	1 pat 6 mo unknown therapy 5 pat no therapy (culture neg)	1 post-transplant MAC other no post-LTx NTM	Basilixsimab or ATG, TAC, MMF, pred	****
Kavaliunaite 2020	RCS	CF (13) children	*MAB bolletii* (1) *MAB M. massiliense* (5) *MAB abscessus* (7)		All perioperative lympheadenectomy and thoracic cavity irrigation with AMK		8 (62%) died (1 M. massiliense 7 M. abcsessus). 9 MAB post LTX	Basilisximab, TAC, MMF, pred	***
				M. bollettii: > 12 mo. PO: RIF, EMB, CLR.	4 wk. IV: AMK, TEC ERY (CLR). LTH: PO CLR. Neb AMK	No			
				M. massiliense: > 7 mo. PO: RIF, INH, CLR. neb AMK	4 wk. IV: AMK, CIP, CLR, TEC. LTH: PO CLR, CIP, Neb AMK	No			
				M. massiliense: IV: CLR, DOX. duration n/a	7 wk. IV: MEM, TGC, TEC, CLR. LTH: PO: CLR, MFX, LZD	No			
				M. massiliense: 5 y. PO: CLR. neb AMK	20 wk. IV: IPM (MEM), AMK, TGC, TEC, CLR.	PO: CLR, MFX, neb AMK			
				M. massiliense: PO: CLR, MIN, LZD, Neb AMK 6–8 wk. NTMs IV's 2y	45 wk. IV: AMK, TGC, CIP (MXF), IPM, CLR, FOX.	IV: TGC, AMK. PO: MFX, CLO, BDQ, LZD			
				M. massiliense: 6y: PO: CLR, CIP. neb AMK, regular anti-NTM IV's	10 wk. IV: AMK, TGC, IPM, CLR, LZD, TEC, MXF. IV 10 wk	IV: TGC, AMK, IPM. PO: BDQ, CLO			
				M. abscessus: 5 mo. IV: TGC, AMK	12 wk. IV: AMK, TGC. LTH: PO: CLR, CIP. neb AMK	No			
				M. abscessus: 3y PO: CLR, CIP. neb AMK	84 wk. IV: AMK, CLR, CIP, TGC, TEC.	PO: CLR, CIP. neb AMK			
				M. abscessus: > 5y. PO: MFX, CLR, neb AMK, 3 mo. IV: MEM, TGC	81.6 wk. IV: TGC, AMK, CLR, MEM,	n/a			
				M. abscessus: >3y. PO: CLR, CIP. Neb AMK. IV: AMK	.8 wk. IV: AMK, CLR, CIP, TGC, IPM, TEC	n/a			

Supplement 5.4: *Continued*

Author, year	Design	LTx Indication (n)	NTM Species (n)	Pre-LTx agents	Agents directly post-LTx	Agents started for post-LTx NTM disease	Outcome post-LTx	Immuno-suppression	MMAT
				M. abscessus: 2y. PO: MIN, LZD. neb AMK, regular anti-NTM IV's	8.7 wk. IV: AMK, MEM, CLR, LZD, TZD, TEC.	n/a			
				M. abscessus: 2y. PO: CLR, CIP. Neb AMK.FOX, regular drugs IV	8.8 wk. IV: AMK, MEM, TGC, CLT, TEC FOX.	n/a			
				M. abscessus: 5y. PO: CLR, CIP. Neb AMK. IV: IPM	6 wk. IV: MEM, AMK, TGC.	IV: TGC, AMK, MEM, TEC, FOX BDQ. PO: AZM			
Knoll 2012	RCS	COPD (2)	*MAC* (2)	7 mo. CLR, EMB, INH	3 mo. CLR, EMB, INH	3 mo. CLR, EMB, INH	MAC recurrence. Died after 3 mo due to Aspergillus	ATG, CYC, AZA, pred	**
				No	No	No	MAC recurrence		
Lobo 2013	RCS	CF (13)	*MAB* (13)	12 mo. INH, RIF, CLR	All intra-thoracic betadine > 6 wk. IV: AMK, CLR, FOX	Pat 4. 7 y: FOX, AMK, CLR IV Pat 7. 6 mo: FOX, TGC, AZM IV Pat 12. 19 mo: FOX, CLR IV	Recurrence: 6 NTM culture + 3 MAB related complications Survival: 1 y: 77% 3 y: 64% 5 y: 50%. Non died due to MAB. Survival similar to CF patients without NTM	CYC/TAC, AZA/ MMF, pred, no induction	****
				None					
				6 mo. FOX, AMK, CLR					
				6 mo FOX, AMK, CLR Maintenance : FOX, CLR					
				18 mo. FOX, AMK, CLR Maintenance: CLR					
				1 mo. FOX, AMK, CLR. Full therapy during LTX					
				None					
				12 mo. IMP, AMK, CLR					
				6 mo. IMP, AMK, CLR Maintenance: KAN, AZM					
				12 mo. LZD, AMK, CLR Maintenance: CLR					
				1 y. FOX, AMK, neb AMK Maintenance: CLR, neb AMK					
				16 mo. FOX, TGC, AMK, LZD. Full therapy during LTX					
				15 mo. CLR, LNZ, MEM, TGC, neb AMK. Full therapy during LTX					

Supplement 5.4 continues on next page.

Supplement 5.4: ***Continued***

Author, year	Design	LTx Indication (n)	NTM Species (n)	Pre-LTx agents	Agents directly post-LTx	Agents started for post-LTx NTM disease	Outcome post-LTx	Immuno-suppression	MMAT
Osmani 2018	RCS	CF (1) COPD (1)	*MAB* (2)	CF 1y.	12 mo. AZM, FOX, LNZ	no	Died after 3 days cerebral haemorrhage. Culture-	ATG, TAC, MMF/AZA, pred	****
				COPD 9 wk.	2 mo. AMK, CLR, IPM	no	Survived 37 mo. Culture -		
Perez 2019	CCS	CF (5) CLAD (1) BO (1)	*MAB* (7)	5 mo. IV: FOX, PO: CLR, TGC, AMK 2 mo.	2 mo. FOX IV, PO CLR, TGC IV	no	Culture + (3), tissue infection (1) CLAD (2) Time to CLAD en survival similar to non-NTM CF, Died non-NTM related (1)	n/a	****
				8 mo. PO: AZR, IV: AMK, IPM	6 mo: PO: AZR. IV: AMK, IPM 3 mo.	no			
				1 y. AZR, IV: FOX, AMK, TGC. PO: LZD, neb AMK.	AMK pleural cavity. 8 mo. PO: AZR, LZD. IV: FOX, TGC,	8 mo. PO: AZR, LZD. IV: FOX, TGC medihoney incisional infection			
				11 mo. PO: AZR, IV: TGC, FOX, PO: CLO. AMK irrigation pleural cavity	1 y. PO: AZR, CLO. IV: TGC, FOX. neb AMK 6 mo.	1 y. PO: AZR, CLO. IV: TGC, FOX. neb AMK 6 mo.			
				1 y. IV: AMK, FOX 2. PO: AZR. AMK. CIP	AMK irrigation pleural cavity. 6 mo. PO AZR. IV: TGC, IPM. neb AMK.	no			
				1 y. IV: AMK, IMP. PO: CLO, AZR.	AMK irrigation pleural cavity. 5,5 mo. IV: AMK, FOX. PO CLO, AZR	5,5 mo. IV: AMK, FOX. PO CLO, AZR			
				9 mo. neb AMK, IV: TGC. PO: TZD, TZD changed to CLO. IPM	AMK irrigation pleural cavity 6 mo. neb AMK. IV: iv IMP, TGC. PO: CLO.	6 mo. neb AMK. IV: iv IMP, TGC. PO: CLO.			
Qvist 2013	RCS	CF (9)	*MAB* (7) *MAC* (2)	6 mo. PO: CLR, MFX. IV: AMK, IPM	Pat 1: Y MXF Pat 5: 36 mo Pat 7: 18 mo. PO: RXM, RIF. neb AMK, 2-3 IV: IMP, AKM	Pat 1: Y MXF Pat 5: 36 mo treatment Pat 7: 18 mo. PO: RXM, RIF. neb AMK, 2-3 IV: IMP, AKM	3 died (non NTM related) 2 NTM wound infection all culture neg patients at LTX stayed neg after LTX	CYC, pred	***

Supplement 5.4: ***Continued***

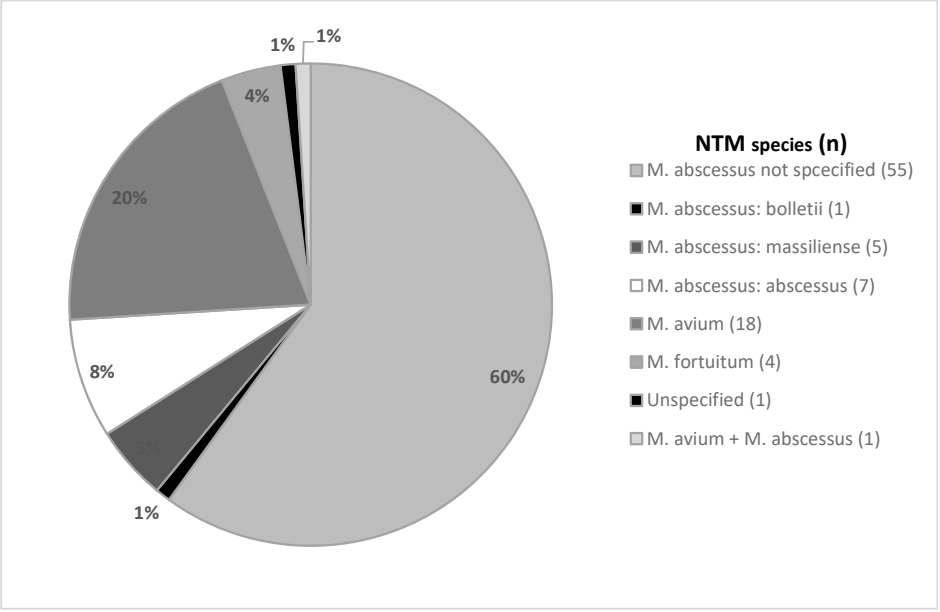
Author, year	Design	LTx Indication (n)	NTM Species (n)	Pre-LTx agents	Agents directly post-LTx	Agents started for post-LTx NTM disease	Outcome post-LTx	Immuno-suppression	MMAT
Raats 2019	CR	CF (4)	*MAB* (4)	3 mo. IV: FOX, MEM, AMK. PO: LZD, AZR, MFX	3 wk. IV: FOX, MEM, AMK, followed by 6 mo PO: MFX, AZR. neb AMK	no	1 pat died not NTM related 3 pat no recurrence	ATG, TAC/CYC, MMF/AZA, TAC target conc. reduced	***
				5 mo. IV: MEM, AMK. PO: MFX, DOX, AZR. MEM, LNZ AMK	n/a	n/a			
				IV: TGC, IPM. PO: CLO, BDQ, AZR. BDQ LNZ, AMK (neb)	14 mo IV IPM, LNZ, AMK, PO CLO, AZR. Continued by PO VLO, BDQ, AZR, neb AMK	no			
				IV: TGC, IMP, PO CLO, CLR, MFX, LNZ, AMK, BDQ.	14 mo. IV: TGC, IMP, LNZ, AMK. PO: CLO, CLR, MFX, BDQ, followed by 14 mo. PO: CLO, AZR, MIN, LNZ	14 mo. IV: TGC, IMP, LNZ, AMK. PO: CLO, CLR, MFX, BDQ, followed by 14 mo. PO: CLO, AZR, MIN, LNZ			
Taylor	CR	1 CF	*MAB* (1)	11 mo: CLR, LVX	4 wk: LZD, FOX	AMK, FOX	Breast abscess	Pred, AZA, TAC	*
Valinetz	CR	1 CF	*MAC* (1)	EMV, RIF, AZM, CLO	PO: EMB, IV: AZM; RIF	EMB, AZM, RIF, CLO, BDQ, TZD	After 4 weeks: MAC disseminated	n/a	**
Zaidi 2009	CR	CF (2) 1 child	*MAB* (2)	None	FOX , LNZ, AMK	IV: FOX, LNZ, TGC. neb AMK Mediastinum irrigation LNZ 87 days	Sternal wound infection died (1) No recurrence (1)	Basilisximab, CYC, MMF, pred	**
				7 mo. MEM, LNZ, CLR, TGC	LNZ 10 days	no			

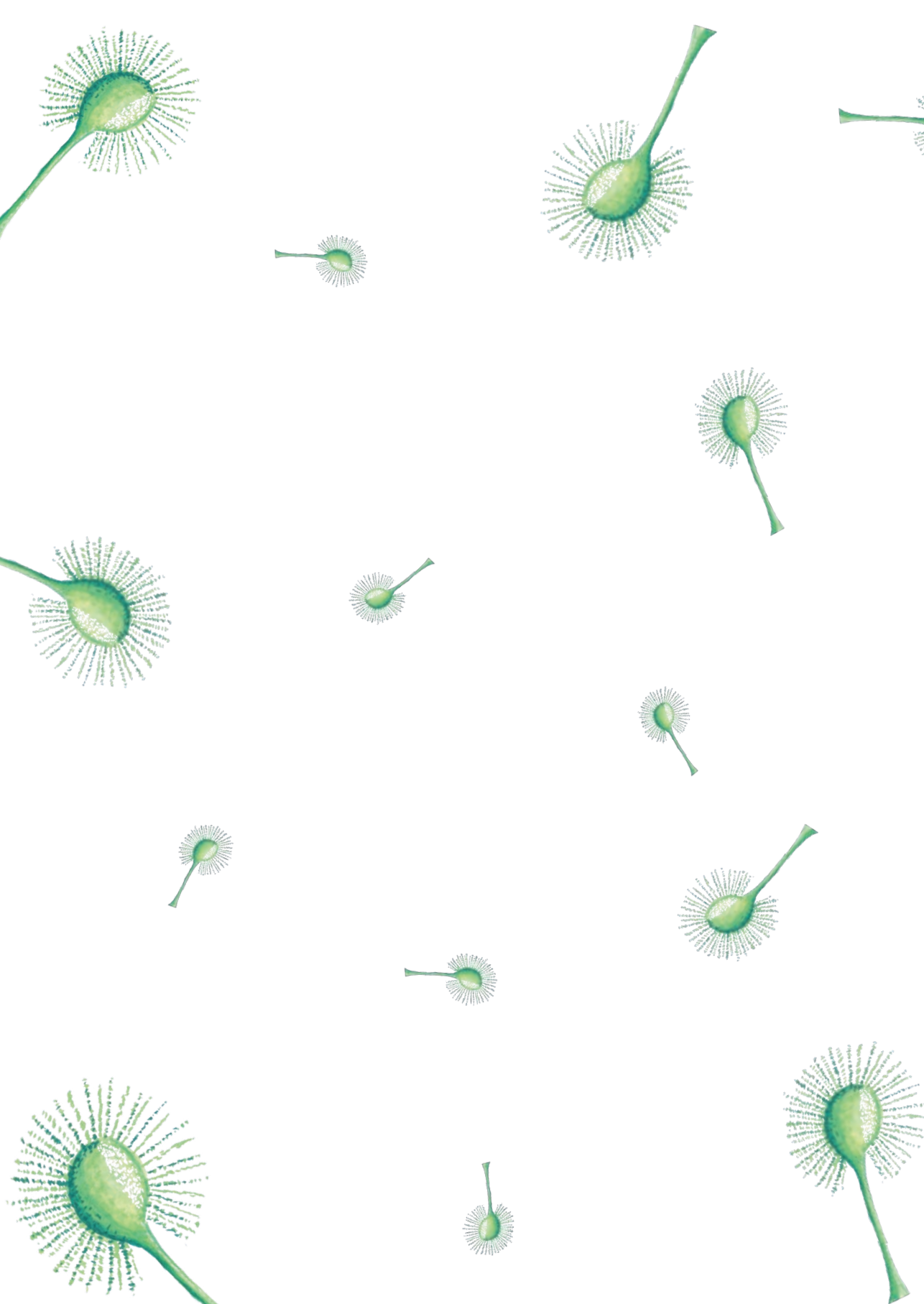
AMK: amikacin; AZM: azithromycin; AZA: azathioprine; BDQ: bedaquiline; BO: bronchiolitis obliterans; CCS: Case-control study; CF: Cystic Fibrosis; CLAD: Chronic lung allograft dysfunction; CR: case report/series, CIP: ciprofloxacin; CKD: chronic kidney disease; CLR: clarithromycin; CLO: clofazimine; CYC: cyclosporine; DOX: doxycycline; ERY: erythromycin; EMB: ethambutol; FOX: cefoxitime; FU: follow-up; GA: gatifloxacin; IPM: imipenem; INH: isoniazid; IPF: Idiopathic pulmonary fibrosis; KAN: kanamycin; LVX: levofloxacin; LTX: Lung transplantation; LZD: linezolid; MAB: Mycobacterium abscessus; MAC: Mycobacterium avium complex ;MEM: meropenem; MFX: moxifloxacin; MIN: minocycline; MMF: mycophenolate mofetil; mo: month; n/a: not available; NTM: Nontuberculous mycobacteria; OFX: ofloxacin; PO: per os; pred: prednisolone; RCS: retrospective cohort study; RIF: rifampin; RXM: roxithromycin; SS: survey study; TAC: tacrolimus; TEC: teicoplanin; TGC: tigecycline; TZD: tedizolid; y,year; wk: week.

Supplement 5.5: Mixed methods appraisal tool (MMAT) results

See https://ars.els-cdn.com/content/image/1-s2.0-S0955470X2300054X-mmc5.docx

Supplement 5.6: Non-tuberculous mycobacteria pre-LTx.
LTx, lung transplant; NTM, Non-tuberculous mycobacteria. Total: 100 NTMs. 3 patients had 2 NTMs.







Chapter 6

Aspergillus after lung transplantation: prophylaxis, risk factors, and the impact on chronic lung allograft dysfunction

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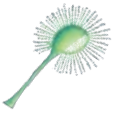

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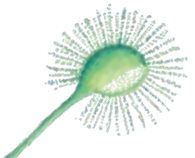


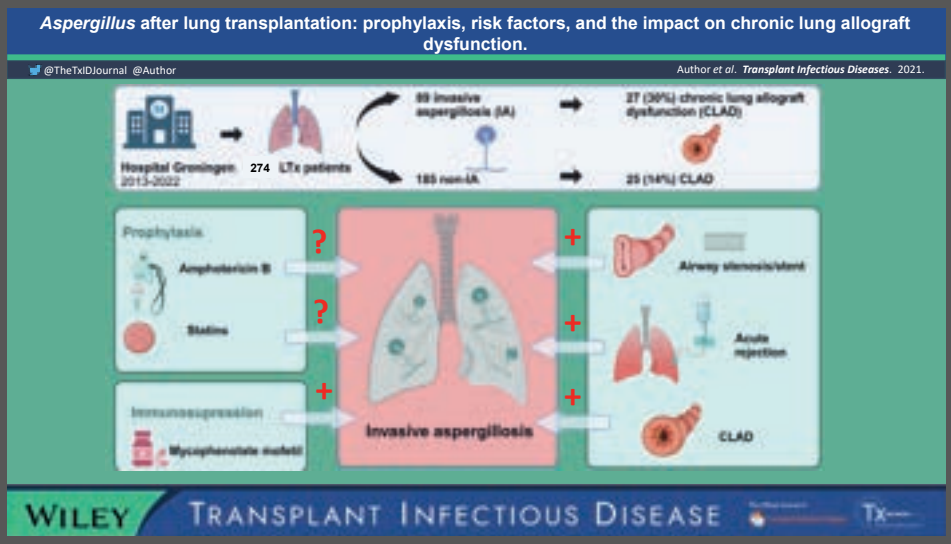

Abstract

Background: Invasive pulmonary aspergillosis (IA) poses significant challenges for lung transplant (LTx) patients, with unclear risk factors and preventive strategies. The effectiveness of nebulized Amphotericin B (AmB) or statins for IA prevention and the effect of IA on chronic lung allograft dysfunction (CLAD) and mortality remain questionable.

Methods: Data was collected from all LTx patients transplanted between 1-12-2013 and 1-1-2022 at the University Medical Center Groningen. IA, was defined according to published criteria. Pre-specified risk factors were compared between patients with and without IA post-LTx and were entered in a logistic regression model. Two additional logistic regression models were built with factors that might be associated with statin or nebulized AmB prophylaxis and IA. A matched case-control study was conducted for the association between statins and IA, with matching based on follow-up time.

Results: *Aspergillus* was cultured in 110 /274 (40%) patients post-LTx, 89/110 (81%) were classified as probable IA. MMF use, airway stenosis, *Aspergillus* cultured pre-LTx, CLAD and acute rejection (AR), were significantly associated with IA. Statin use was associated with a lower incidence of IA, while nebulized AmB prophylaxis showed no significant effect. A significant statin effect could not be confirmed by the case control analysis. There was no significant difference in all-cause mortality between patients with and without IA (34% vs 29%).

Conclusions: The high incidence of IA post-LTx necessitates more effective strategies. Key targets for intervention include prior positive cultures, airway stenosis, AR and the use of MMF. The role of statins remains unclear and requires further research.



Introduction

Invasive aspergillosis (IA) poses significant challenges for lung transplant (LTx) recipients, frequently leading to increased rates of morbidity and mortality.[1–3] The reported incidence of fungal infections in LTx patients ranges from 15 to 35%.[4] Managing IA in the LTx context is particularly complex due to potential drug-drug interactions between antifungal therapy and immunosuppressants. Recognizing the importance of prevention over treatment, there is an urgent need to delve deeper into the factors that are associated with *Aspergillus* infection.

There is considerable variation among various LTx centers in prophylactic therapy for IA, with differences in regimen and treatment duration.[5] Variable outcomes have been described regarding the efficacy of nebulized Amphotericin B (AmB) and azoles as preventive measures. A systematic review and meta-analysis from 2013, including 22 studies, did not find a significant reduction in IA or *Aspergillus* colonization with universal anti-*Aspergillus* prophylaxis.[6] However, more recent studies suggested that prophylaxis does indeed result in fewer cases of aspergillosis following LTx.[7–9] Targeted prophylaxis, for patients at high risk of IA, may be preferred over universal prophylaxis due to comparable IA rates and lower rates of adverse events.[10] Alternatively, a pre-emptive therapy approach, based on bronchoalveolar lavage (BAL) culture and galactomannan, has shown a 50% reduction in antifungal exposure compared to universal prophylaxis.[11] Another potential strategy for preventing aspergillosis could be statin therapy. Statins inhibit ergosterol synthesis, a critical component of the fungal cell membrane, and therefore may play a role in fungal prophylaxis.[12]

Additionally, it remains unclear whether *Aspergillus* colonization and IA associated with chronic lung allograft dysfunction (CLAD).[4,8,13–16] Conversely, progression of CLAD probably also leads to an increased risk of *Aspergillus* colonization and IA. However, there is limited reporting on the course of lung function in LTx patients with *Aspergillus* colonization or IA.

To address the problem of IA following LTx, the primary goals of this study were to identify factors associated with IA following LTx and to explore potential protective modalities, including nebulized AmB and statin therapy. Additionally, we evaluated the impact of IA on lung function, the development of CLAD, and mortality.

Material and methods

Patients

The study was waived by the Ethical Committee based on its observational nature using routine care data (METc number 2024/292). The study complies with the ISHLT Ethics Statement. All patients provided written informed consent upon entry into the program. Data were retrospectively collected at the University Medical Center Groningen (UMCG), the Netherlands. All adult patients who underwent unilateral or bilateral LTx between December 2013 and January 2022 in the UMCG were eligible for inclusion.

Transplant care

Follow-up of all LTx patients took place at least every 3 months for monitoring of transplant function. A protocolized bronchoscopy is performed 1–3 months after transplantation. Subsequent bronchoscopies are only performed on clinical indication. All patients received standard induction therapy with Basiliximab at day 1 and day 4 and maintenance immunosuppression mostly with tacrolimus, prednisolone, and mycophenolate mofetil (MMF). Alternative immunosuppressants were cyclosporine, azathioprine, everolimus, or sirolimus. Post-LTx prophylactic therapy included: nebulized AmB 5 mg twice daily for aspergillosis in the cohort from December 2017 to January 2022, cotrimoxazole for *Pneumocystis* pneumonia and valganciclovir (900 mg OD) or acyclovir 1000 mg for Herpes viruses, depending on cytomegalovirus (CMV) (mis) match. Nebulized AmB was administered during hospitalization immediately post-LTx (for the entire length of hospital stay, depending on tolerance and/or toxicity).

In our LTx program, statins were prescribed exclusively for hypercholesterolemia management, not as prophylaxis against aspergillosis.

Aspergillus infection criteria and definitions

In accordance with prior studies and guidelines, the diverse manifestations of aspergillosis in LTx patients were delineated for the objectives of this study as follows:[4,17–19]

Aspergillus airway colonization is characterized by the detection of *Aspergillus* in respiratory secretions (sputum or BAL) through culture, polymerase chain reaction (PCR), or galactomannan (GM) testing, in the absence of symptoms, radiologic, and endobronchial changes.[19]

Invasive pulmonary aspergillosis (IPA) is defined according to the European Organization for Research and Treatment of Cancer (EORTC) criteria.[17] Proven pulmonary IA necessitates the presence of a host factor (LTx patients), a mycological criterion (*Aspergillus* in sputum or BAL

from culture, PCR, or GM), and a clinical criterion (symptoms, radiological abnormalities or endobronchial changes). For a diagnosis of proven extra-pulmonary IA, histological evidence consistent with fungal tissue invasion is also required. Probable aspergillosis requires a host factor, a clinical criterion, and a mycological criterion. Possible aspergillosis is diagnosed when cases fulfil the criteria for a host factor and clinical criterion but lack a mycological criterion.

Tracheobronchial aspergillosis, a subtype of IA, is characterized by the presence of a host factor accompanied by a mycological criterion and a clinical criterion, which includes endobronchial changes observed during bronchoscopy or symptomatic presentation along with a decline in lung function.

Treatment response definitions

Treatment responses were defined as follows: "*Complete response*" clinical recovery and recovery of lung function, "*Intermediate response*", not fully clinically recovered and/or not fully recovered lung function, and "*Treatment failure*", lung function decline without any recovery or death.

Pulmonary function

Spirometry pre-IA was performed as part of standard care 0–3 months before infection. Baseline lung function is computed as the mean of the 2 best post-operative FEV1 values, measured > 3 weeks apart. Follow-up measurements were performed at the routine visits of outpatients with IA. Follow-up measurements took place 3 and 6 months after the onset of IA. Spirometry was performed according to ATS/ERS guidelines.[20] CLAD was defined as a persistent decline of 20% or more in FEV1 value from baseline value post transplantation, independent of a change in FVC, according to the most recent ISHLT criteria.[21] Acute rejection (AR) was clinically defined as an acute deterioration of allograft function without an obvious other cause and/or histological confirmation.

Statistical analysis

Clinical parameters were compared between patients with and without IA post-LTx using Mann–Whitney U-test or chi-squared test, as appropriate. Pre-specified risk factors and protective factors were entered into a multivariable logistic regression analysis model. Two additional logistic regression models were built to adjust for potential confounders regarding the use of statins and nebulized AmB prophylaxis. In the adjusted analysis for nebulized AmB, the following parameters were entered: nebulized AmB prophylaxis, ICU length of stay, pre-LTx *Aspergillus* cultured, stenosis, acute rejection, MMF and age. In the adjusted

analysis for statins the following variables were included: statin use, low-density lipoprotein (LDL), diabetes mellitus, age, airway stenosis, intensive care unit (ICU) length of stay, acute rejection, MMF use, and the presence of cardiovascular risk factors.

We performed a before-after analysis of patients receiving nebulized AmB prophylaxis. Here, we compared the cohort from 2013 to December 2017, in which no nebulized AmB prophylaxis was used, with the cohort from December 2017 to January 2022, in which nebulized AmB prophylaxis was used.

Due to statins being initiated at various times post-LTx, we also conducted a sensitivity analysis using a matched case-control study, where the cases were matched based on follow-up time from LTx to IA. Additionally, to assess differences in outcomes between patients with IA according to EORTC criteria or ISHLT criteria, we also conducted a sensitivity analysis only including patients with IA classified by the ISHLT criteria.

Paired sample T-tests were used for within-group analyses to compare FEV1 and FVC before, 3 months, and 6 months after the start of IA. A Cox proportional hazards model with IA as a time-dependent variable was used to compare post-LTx survival and CLAD development between patients with and without IA. A *p*-value of less than 0.05 was considered significant. All analyses were carried out using IBM SPSS for Mac, version 24.0.

Results

Patient characteristics

In total 274 patients received a LTx between December 2013 and January 2022 and were included in the study. The median follow-up time was 56 (IQR 32–88) months. Baseline characteristics and clinical parameters for all LTx patients with and without IA are shown in Table 6.1.

Aspergillus colonization and classification of IA

Of the 274 patients in the study cohort, 110 patients had a positive *Aspergillus* culture. Of those patients, 89 (81%) were classified as having probable IA, according to the EORTC criteria (Table 6.1) and 21 (19%) were only colonized with *Aspergillus* and did not have evidence of invasive disease. There were no cases of possible or proven IA in our cohort.

Of the 89 patients with probable IA, 35% were diagnosed based on a combination of host factors, culture results, and radiological findings and 62% were diagnosed based on host factors, culture results, and evidence (based on bronchoscopy or pulmonary fuction) of

Aspergillus tracheobronchitis. Three patients (3%) underwent treatment because of deep wound infections with IA.

Table 6.1: Baseline characteristics and clinical parameters of lung transplant patients with and without invasive aspergillosis

Variable	All patients	IA	Non-IA	*p*-value*
Recipients, n (%)	274 (100)	89 (32)	185 (68)	
Age at LTx, years	56 (46–61)	55 (45–61)	56 (48–61)	0.319
Gender, male (%)	137 (50)	44 (49)	93 (50)	0.897
Transplant indication, n (%)				0.183
COPD	122 (45)	44 (49)	78 (42)	
Fibrosis	72 (26)	17 (19)	55 (30)	
Pulmonary hypertension	35 (13)	11 (12)	24 (13)	
Cystic fibrosis	19 (7)	10 (11)	9 (5)	
Non-CF bronchiectasis	1 (0.4)	0 (0)	1 (0.5)	
Other	25 (9)	7 (8)	18 (9.7)	
Bilateral LTx, n (%)	254 (93)	81 (91)	173 (94)	0.360
DM, n (%)	71 (26)	23 (26)	48 (26)	0.985
Body mass index, kg/m^2	24 (21–28)	23 (21–26)	24 (21–28)	0.190
Statins, n (%)	89 (33)	19 (21)	70 (38)	0.006
Smoking, pack years	13 (0–30)	13 (0–30)	13 (0–30)	0.625
Aspergillus pre-LTx, n (%)	58 (21)	27 (30)	31 (17)	0.010
nAmB prophylaxes, n (%)	138 (50)	46 (52)	92 (50)	0.684
MMF	220 (80)	82 (92)	138 (75)	< 0.001
Acute rejection, n (%)	147 (54)	61 (69)	86 (47)	< 0.001
CLAD, n (%)	52 (19)	27 (30)	25 (14)	< 0.001
Airway stenosis, n (%)	23 (8)	15 (17)	8 (4)	< 0.001

Continuous variables are expressed as median (interquartile range). nAmB, nebulized amphotericin B; CLAD, chronic allograft dysfunction; COPD, chronic obstructive pulmonary disease; DM, Diabetes Mellitus; IA, invasive aspergillosis; LTx, lung transplantation; MMF, mycophenolate mofetil. * *p*-value for the difference between patients with invasive aspergillosis and patients without invasive aspergillosis post-LTx.

Invasive aspergillosis

The median age of patients with IA was 55 years (IQR 45–61) and 44 (49%) were male. The median time between LTx and IA was 6 months (IQR 1–18). Prior to LTx, *Aspergillus* had been cultured from sputum in 27/89 (30%) of patients. Prior to *Aspergillus* infection, 23 (26%) of the patients had diabetes and 19 (21%) were on statins. During hospitalization for LTx, 46 (52%) of patients who developed IA received nebulized AmB prophylaxis, MMF was used in 82 (92%) of the patients as part of their immunosuppressive regimen (Table 6.1). Ten of 46 patients (22%) with nebulized AmB prophylaxis experienced a breakthrough infection while on nebulized AmB prophylaxis, while the remaining 36 (78 %) developed IA after prophylaxis was discontinued.

Positive *Aspergillus* culture occurred in 34 of the 89 patients with IA after fungal treatment (38%). This could involve a recurrence of aspergillosis, colonization, or treatment failure.

Aspergillus species

The predominant cultured *Aspergillus* species was *A. fumigatus*, comprising 95 isolates (35%), followed by A. niger with 9 isolates (3%) and *A. flavus* with 4 isolates (2%) (Supplemental Figure S6.1). *Aspergillus* was isolated from BAL specimens in 51% of cases, from sputum cultures in 46%, and from wound infections in 3%. Galactomannan testing in BAL samples was performed in 33 patients (30%), yielding positive results in 20 patients (61%). Azole resistance prevalence was 15%.

Antifungal treatment

Antifungal treatment was administered in all patients with IA. The therapeutic modalities are shown in Supplemental Table S6.1. Predominantly, 61 patients (69%) received monotherapy with voriconazole, while 9 patients (10%) received combination therapy consisting of voriconazole and intravenous liposomal-AmB. Additionally, 6 patients (7%) received nebulized AmB monotherapy. The median duration of treatment was 12 weeks (IQR 6–14). Adverse events are shown in Supplemental Table S6.2. Notably, the most frequently reported adverse effects comprised of nausea/vomiting (16%), visual disturbances (9%), diarrhoea (8%), rash (8%), and hepatotoxicity (8%). Thirteen patients (15%) prematurely discontinued therapy due to adverse reactions. Complete treatment response occurred in 52/89 (58%), intermediate response in 20/89 (23%), and treatment failure in 17/89 (19%).

Table 6.2: Clinical parameters associated with invasive aspergillosis post lung transplantation

	OR	95% CI	*p*-value
Unilateral	1.69	0.52–5.52	0.401
Cystic fibrosis	1.80	0.47–7.05	0.401
Age	1.02	0.99–1.04	0.184
Pre-LTx *Aspergillus*	2.71	1.24–5.96	0.013
nAmB prophylaxis	1.01	0.55–1.88	0.968
MMF	7.02	2.57–19.19	< 0.001
Statins	0.38	0.19–0.76	0.006
Stenosis	6.2	2.16–18.00	< 0.001
Acute rejection	2.42	1.29–4.52	0.006
CLAD	3.08	1.37–6.89	0.006

nAmB, nebulized amphocytericin B; CLAD, chronic lung allograft dysfunction; LTx, lung transplantation; MMF, mycophenolate mofetil; data derived by multivariale logistic regression.

Factors associated with invasive aspergillosis

We compared predefined parameters between patients with and without IA post-LTx (Table 6.1). In multivariate analysis, MMF use (OR = 7.02; 95% CI [2.57–19.19]), airway stenosis (OR = 6.20; 95% CI [2.16–18.00]), *Aspergillus* cultured pre-LTx (OR = 2.71; 95% CI [1.24–5.96]), CLAD (OR = 3.08; 95% CI [1.37–6.89]) and acute rejection (OR = 2.42; 95% CI [1.29–4.52]), were significantly associated with IA (Table 6.2).

The additional sensitivity analysis of patients with IA classified according to the ISHLT criteria included 51 of the original 89 patients with IA. The difference was due to the absence of bronchoscopy in these patients. The analysis showed consistent findings: pre-LTx *Aspergillus* colonization, acute rejection, CLAD, airway stenosis, MMF use, and statin use were all associated with post-LTx IA. However, the associations of statins and acute rejection with IA were no longer significant, likely due to reduced statistical power as a result of the smaller patient sample size in this analysis (Supplemental Table S6.3).

Nebulized AmB prophylaxis

In total, 138 (50%) of all LTx patients received prophylaxis with nebulized AmB. In the IA group, 46 patients (52%) had received prophylaxis with nebulized Amb, and in the non-IA group 92 patients (50%). We performed a before-after analysis of patients receiving nebulized AmB prophylaxis. The median duration of nebulized AmB prophylaxis was 26 days (IQR 18–36). Nebulized AmB was not associated with a decreased risk of IA in both the crude and adjusted analysis (adjusted OR = 0.96; 95% CI [0.54–1.70]).

Statins

Eighty-nine (33%) of all patients received statins. Of those 53 (60%) were started on statins pre-LTx. The median time between LTx and the start of statins was 20 months (IQR 7.0–37.5). Statin use was associated with a decreased risk of IA in the crude and adjusted analysis (adjusted OR = 0.43; 95% CI [0.22–0.86]). However, the same association studied in the matched case-control analysis for post-LTx follow-up time, showed no significant association between statin use and IA (adjusted OR = 0.95, 95% CI 0.46–1.96) (Supplemental Table S6.4).

Lung transplant function in patients with IA

Spirometry data were available in 77 (87%), 83 (93%), and 79 (89%) patients before they developed IA, 3 months after IA, and 6 months after IA, respectively. Spirometry results are shown in Figure 6.1 and Supplemental Table S6.5. There was a significant decline in FEV1 ($p < 0.001$) but not in FVC ($p = 0.948$) at the time of IA. After antifungal treatment FEV1 and

FVC increased at first follow-up within 3 months and even more at 6 months follow up (Figure 6.31, Supplemental Table S6.5). Of the 89 patients with IA 61 (69%) had at least one episode of acute rejection (AR). Of those with AR 34 (56%) had an episode of AR prior to IA and 27 (44%) after IA. By comparison, in the 185 patients without IA the AR incidence was 47%.

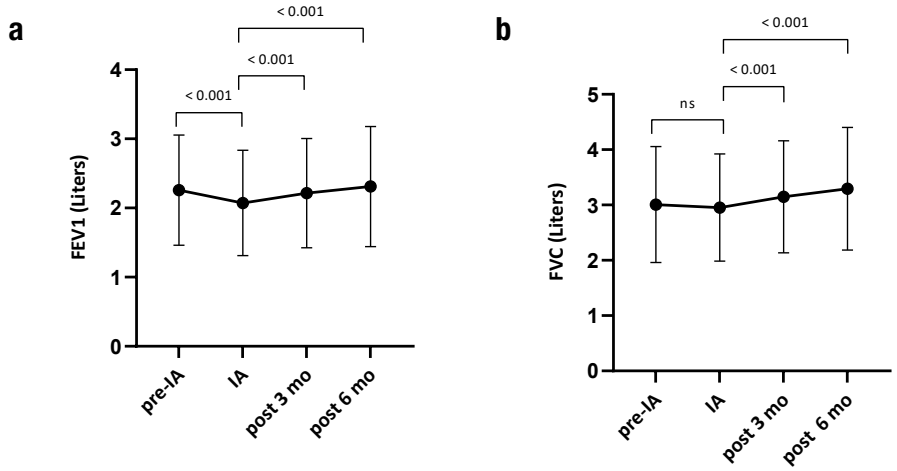


Figure 6.1: (a) FEV1 before, during and after IA. (b) FVC before, during and after IA.
FEV1, forced expiratory volume in 1 second; FVC, Forced vital capacity; IA, invasive aspergillosis.

CLAD

During the study period, 52 out of 274 patients (19%) developed CLAD. In patients with IA, 20 (22%) developed CLAD after IA, whereas 25 (14%) patients without IA developed CLAD during the study period. A Cox proportional hazards model with IA as a time-dependent covariate showed that IA was significantly associated with the development of CLAD (HR 2.712, $p < 0.001$). Among patients with IA, 27 (30%) had CLAD, compared to 25 (14%) of those without IA (Table 6.1). Twenty patients (74%) developed CLAD after IA, but 7 (26%) patients already had CLAD before the onset of IA, with 4 of them experiencing progressive CLAD (Figure 6.2). Patients with CLAD pre-IA and post-IA were both included in the logistic regression model. Therefore CLAD could not be considered a risk factor for IA. Among the 82 patients without CLAD before IA, 20 (24%) developed CLAD after the IA. In the patients with IA the time between the infection and CLAD was 17 (IQR 4–37) months.

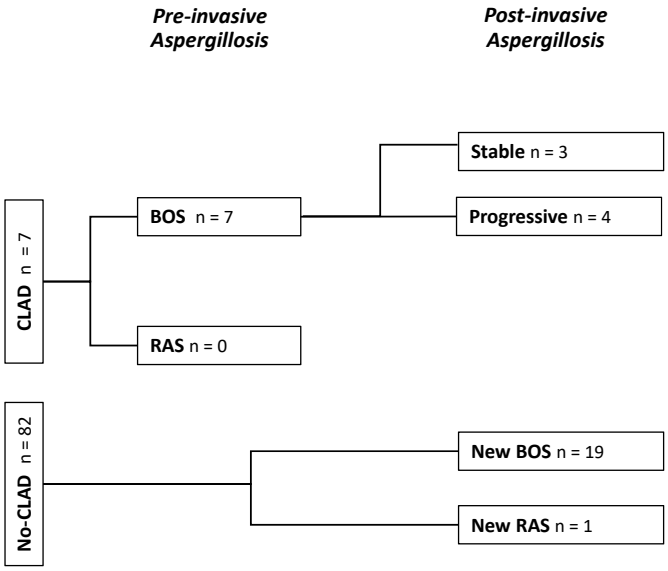


Figure 6.2: Chronic lung allograft syndrome pre- and post-invasive aspergillosis.
CLAD, chronic lung allograft syndrome; RAS, restrictive lung allograft syndrome.

All-cause mortality

All-cause mortality, during the median follow-up period of 4.6 year, was 30% (83/274). In patients with IA, the mortality rate was 34 % (30/89), compared to 29 % (53/184) in those without IA. One- and two-year survival rates in LTx patients with IA where 87% and 73%, respectively. In the Cox proportional hazards model with consideration for the time between LTx and IA, no statistically significant difference in mortality was observed between LTx patients with IA and those without IA (HR 1.547; $p = 0.067$).

Aspergillus colonisation subgroup analysis

Of the 274 included patients, 21 (19%) were classified as having *Aspergillus* colonization. A subgroup analysis was conducted to juxtapose clinical parameters, nebulized AmB prophylaxis, and the utilization of statins between patients with *Aspergillus* colonization and those with negative sputum cultures. Notably, within the *Aspergillus* colonization subgroup, a higher incidence of CLAD was observed (6/15; 40%) in comparison to the culture-negative group (19/145; 13%) ($p = 0.032$). Nonetheless, upon multivariable analysis, no significant disparities in CLAD occurrence or other parameters were determined between patients with *Aspergillus* colonization and patients who were culture-negative.

Discussion

In this single-center, observational study with a long follow-up time, we show that IA remains an important complication of LTx. We confirm the use of MMF, AR, CLAD, airway stenosis, and a positive *Aspergillus* culture pre-LTx as important factors associated IA. Furthermore, we also found a possible protective effect of statin therapy in the prevention of IA, although residual confounding cannot be completely ruled out. *Aspergillus* colonization and IA are associated with lung function decline and CLAD, while all-cause mortality does not seem to be directly related.

Of the previously reported risk factors, we could not confirm single LTx as a risk factor for IA, probably due to the low percentage of single LTx procedues (< 10%) in our center. MMF was associated independently with IA, consistent with the literature.[22] A possible explanation could be that MMF enhances *Aspergillus* fumigatus-induced oxidative burst of polymorphonuclear neutrophils, but without a corresponding increase in fungal killing.[22] AR is also associated with IA. This association could potentially be attributed to the impact of AR on the airways of LTx patients, which might favour easy colonization and growth of *Aspergillus*. However, it is also plausible that it may stem from the intensity of immunosuppression and the administration of high-dose methylprednisolone (1000 mg for 3 days in our center) as treatment for AR. From a pathophysiological perspective, high-dose methylprednisolone inhibits the release of pro-inflammatory cytokines and autophagy by alveolar macrophages. Furthermore, the use of high-dose corticosteroids is a well-established risk factor for invasive fungal disease.[23] Next, airway stenosis following LTx seems to be associated with IA. However, this observation should be interpreted with caution as it involves small numbers of patients. A correlation between *Aspergillus* infection and the subsequent emergence of clinically significant endobronchial abnormalities has been previously reported in a cohort of LTx patients.[24]

An interesting observation from our study is the possible protective effect of statins against the development of IA, although not confirmed in our sensitivity analysis. This corresponds with another observational study which suggested that statin use was independently associated with a reduced risk of IA.[12] Statins function as competitive inhibitors targeting 3-hydroxy-3-methylglutaryl coenzyme A reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, a crucial step in the synthesis of cholesterol in humans and ergosterol in fungi. Ergosterol is a crucial component of the cell membrane in fungi, and they cannot survive without it.[12,25] However, the effect of statins on IA may be influenced by timing, as statins were initiated at different points during post-transplant follow-up. Moreover, the median duration between LTx and *Aspergillus* infection was 6 months. Therefore, it could be that these patients did not have sufficient time to receive statins when compared to patients

without IA (confounding by indication). To account for this, we conducted a matched case-control analysis in which we could not confirm the protective effect of statins on IA. Yet, the drawback of this approach is that it reduces the overall follow-up duration. Consequently, we cannot conclusively determine from this study whether statins truly protect against IA. Still, considering the variable outcomes of nebulized AmB prophylaxis, the potential drug interactions of azoles with immunosuppressants, and the rationale behind the mechanism of action of statins, administering statins as antifungal prophylaxis to post-LTx patients remains an interesting hypothesis, and could be tested in a randomised controlled trial.6

In our center, we have a low threshold for treating IA with antifungal treatment for 3 months. It is possible that we may be overtreating patients. However, with this treatment strategy, we do not observe a substantial difference in mortality between patients with and without aspergillosis. Our one-year survival rates in LTx patients with IA (87% 1 year) were comparable to other studies with one-year survival rates between 81–84%.[4,13]

Despite the recovery of lung function in the first months following the IA diagnosis in LTx patients, we observed a significant increase in the development of CLAD compared to patients without IA. Of course, it is not possible to exclude other contributing factors to the development of CLAD, given the long median duration between LTx and IA (17 months).

Aspergillosis has been demonstrated as a risk factor for CLAD in multiple studies, suggesting that the presence of *Aspergillus* in the bronchi may lead to epithelial injury, followed by dysregulation of repair mechanisms ultimately responsible for chronic fibroblast proliferation and progressive allograft dysfunction.[4,8,13–15] In our study most CLAD cases (74%) occurred after the IA diagnosis instead of before. This suggests that IA might lead to CLAD, rather than CLAD leads to more IA, also described before.[23] CLAD was not a risk factor for IA in our study, as the model included not only patients who developed CLAD after IA, but also those with CLAD prior to IA. However, given that the median time to IA onset after LTx is 6 months, the likelihood of patients having CLAD prior to IA is very low (7 patients in our cohort).

Our study has several limitations. First, it remains challenging to differentiate between *Aspergillus* colonization and invasive disease. In previous studies, *Aspergillus* colonization was reported much more frequently.[4,7,19] It could be that in other transplant centers more post-transplant surveillance bronchoscopies with fungal cultures were performed, thereby increasing the likelihood of diagnosing *Aspergillus* colonization. However, given that *Aspergillus* colonization may lead to CLAD, it suggests the presence of some form of invasive disease alongside colonization.[14,15] For clinical practice, the distinction has to be based on clinical symptoms, lung function, microbiological, and imaging findings. Since bronchoscopy

may not always be feasible, clinicians may need to rely on sputum culture or antigen testing. When symptoms are significant or lung function declinces without another clear cause, it may be justified to initiate treatment in these immunocompromised patients. This decision always involves balancing the anticipated benefits against the potential toxicity and side effects.

It is also possible that Dutch LTx patients have a higher risk of developing IA due to the high population density and intensive Dutch agricultural sector, which is associated with a high prevalence of azole resistance (in our study 15%). In this study, we primarily used the EORTC criteria, instead of the ISHLT criteria, to define IA, relying on lung function decline as a surrogate marker for endobronchial involvement. This approach is practical given the high prevalence of positive *Aspergillus* cultures (40% in LTx patients in our center) and the challenges of applying ISHLT criteria, which necessitates bronchoscopy but lack clear evidence mandating it for diagnosis.[19] The referenced study underpinning these criteria do not explicitly mandate bronchoscopy for the diagnosis of IA.[26] Importantly, bronchoscopy often does not alter management, as patients with abnormal CT findings, declining lung function, and positive sputum cultures are typically treated without BAL confirmation.

This may explain the higher incidence of IA in our study, as other centers may rely on ISHLT criteria. To evaluate whether there is a difference in outcomes between patients classified by EORTC or ISHLT criteria, we conducted a sensitivity analysis selecting the patients who fulfilled ISHLT criteria for IA. No differences were found in the factors associated with IA (Supplemental Table S6.3).

A second limitation is that we compared the group of patients with IA with those without IA, because we base our decision to treat on this diagnosis. The latter group also included the 21 patients with *Aspergillus* colonization. We chose not to exclude these patients with *Aspergillus* colonisation from analysis and to include them in the group without IA because this analysis best aligns with our primary study question and is most appropriate for daily clinical practice, where a distinction is made between treated and untreated patients. Additionally, in our subgroup analysis, we observe that the group comprising patients with *Aspergillus* colonization closely resembles the group with negative cultures. Third, it is challenging to investigate the causative effects of nebulized AmB and statins on IA in an observational study, due to the possibility of residual confounding (e.g. significant variability in the duration of prophylactic nebulized AmB use, severity of IA, and confounding by indication for statin use). Finally, not all patients underwent chest CT scans or bronchoscopies for diagnosis, potentially leading to some patients being erroneously classified as having *Aspergillus* colonization.

Conclusions

The observed incidence of IA highlights the need for more effective strategies to prevent IA, and thus, the development of CLAD. Potential intervention targets include prior positive cultures, airway stenosis, the use of MMF, and possibly antifungal prophylaxis with statins. Prospective trials are urgently needed to address the effectiveness of available preventive therapies in reducing the burden of IA in LTx patients.

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Supplemental Table S6.1: Invasive aspergillosis treatment in 89 lung transplant patients

Treatment	n (%)
Voriconazole	61 (69)
AmB nebulised	6 (7)
AmB nebulised + voriconazole	4 (5)
AmB i.v.	3 (3)
AmB iv + voriconazole	9 (10)
Voriconazole + Caspofungin	4 (5)
Posaconazole	1 (1)
Itraconazole	1 (1)

AmB, Amphotericin B; LTx, lung transplantation.

Supplemental Table S6.2: Side effects of antifungal therapy in 89 LTx patients

Side effects	n (%)
Visual disturbances	8 (9)
Nausea/vomiting	14 (16)
Diarrhoea	7 (8)
Rash	7 (8)
Renal impairment	6 (7)
Hepatotoxicity	7 (8)
Dizziness	4 (5)
Neuropathy	2 (2)
Headache	6 (7)
Fever	4 (5)
Anaphylaxis	0 (0)

LTx, lung transplantation.

Supplemental Table S6.3: Clinical parameters associated with invasive aspergillosis post lung transplantation in patients with IA according tot the ISHLT criteria

	OR	95% CI	*p*-value
Unilateral	0.84	0.15–4.80	0.840
Cystic fibrosis	1.55	0.32–7.58	0.590
Age	1.00	0.98–1.04	0.542
Pre-LTx *Aspergillus*	2.65	1.05–6.71	0.040
nAmB prophylaxis	0.83	0.40–1.74	0.625
MMF	4.03	1.34–12.07	0.013
Statins	0.45	0.20–1.04	0.061
Stenosis	9.64	3.18–29.26	< 0.001
Acute rejection	1.83	0.86–3.99	0.115
CLAD	3.40	1.37–8.39	0.008

ISHLT, International Society for Heart and Lung Transplantation; nAmB, nebulized Amphocytericin B; CLAD, chronic lung allograft dysfunction; LTx, lung transplantation; MMF, mycophenolate mofetil; data derived by multivariale logistic regression.

Supplemental Table S6.4: Matched case control study, adjusted analysis for association between statins invasive aspergillosis post lung transplantation

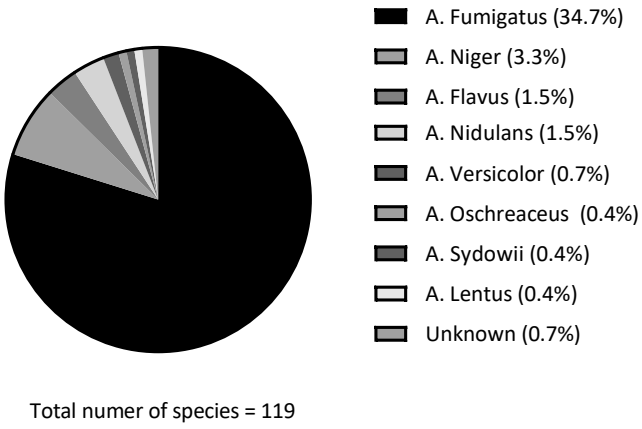
	OR	95% CI	*p*-value
Statin	0.95	0.46–1.96	0.882
LDL	0.23	0.84–0.64	0.225
Age	1.00	0.97–1.02	0.703
Stenosis	5.81	2.08–16.29	< 0.001
ICU stay	0.57	0.99–1.02	0.571
MMF			< 0.001
AR	3.08	1.69–5.59	< 0.001
CVR	1.10	0.46–2.45	0.889

AR, acute rejection, CVR, cardiovascular risk; LDL, low-density lipoproteins; ICU, intensive care unit; LTx, lung transplantation; MMF, mycophenolate mofetil; data derived from multivariable logistic regression analysis.

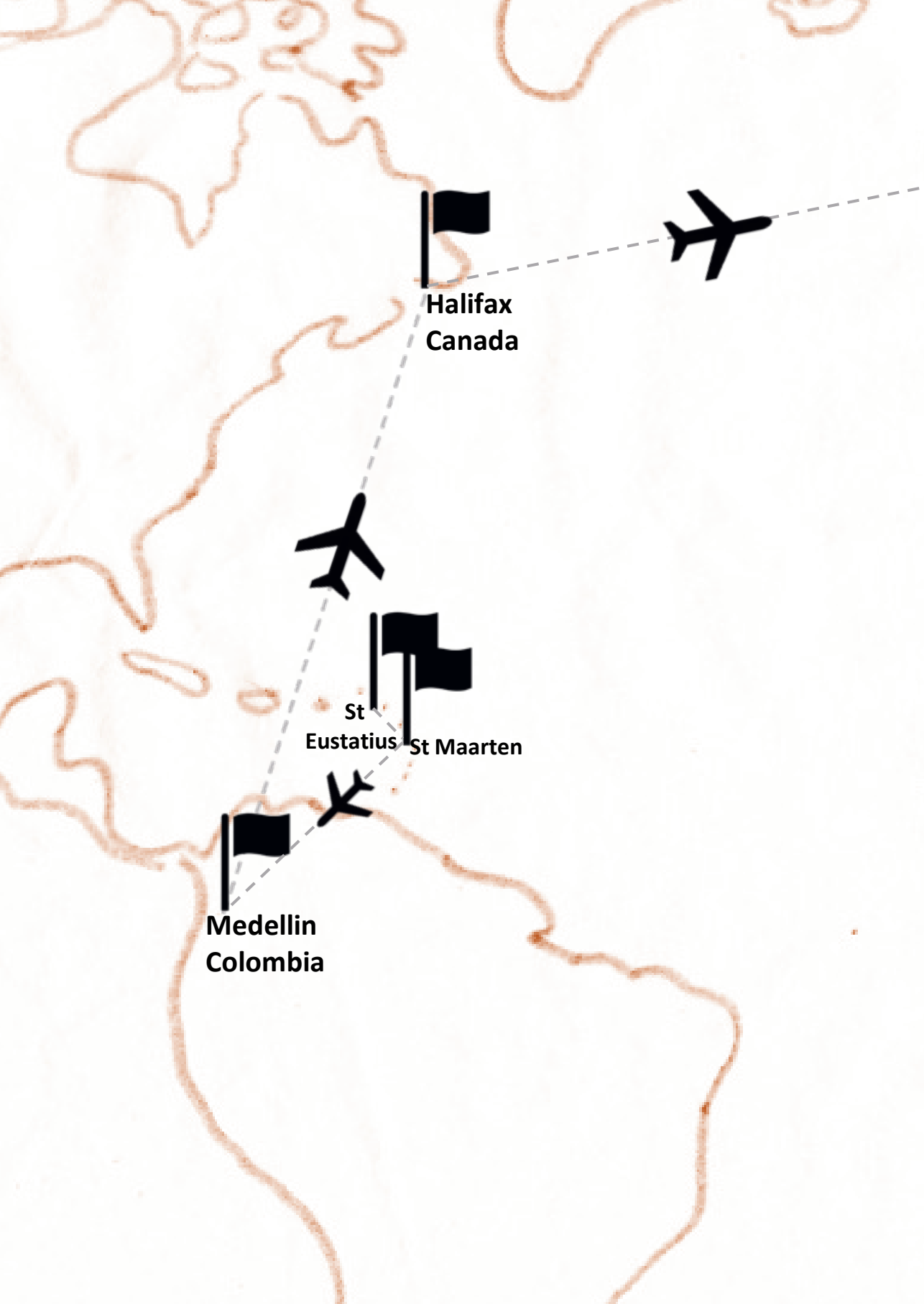
Supplement Table S6.5: The effect of invasive aspergillosis and treatment on lung function

	Pre-IA	IA	*p*-value[$]	3 months post-IA	*p*-value*	6 months post-IA	*p*-value[#]
FEV1, L (IQR)	2.16 (1.67–2.74)	1.97 (1.41–2.45)	< 0.001	2.15 (1.42–2.74)	< 0.001	2.27 (1.58–2.93)	<0.001
FVC, L (IQR)	2.94 (2.15–3.72)	2.78 (2.14–3.49)	NS	2.95 (2.20–3.86)	< 0.001	3.22 (2.35–4.12)	< 0.001

FEV1, forced expiratory volume in 1 second; FVC, Forced vital capacity; IA, invasive aspergillosis.
[$] *p*-value for the difference in lung function pre-IA and during IA.
* *p*-value for the difference in lung function during IA and 3 months post IA.
[#] *p*-value for the difference in lung function during IA and 6 months post IA.



Supplemental Figure S6.1: *Aspergillus* species (%)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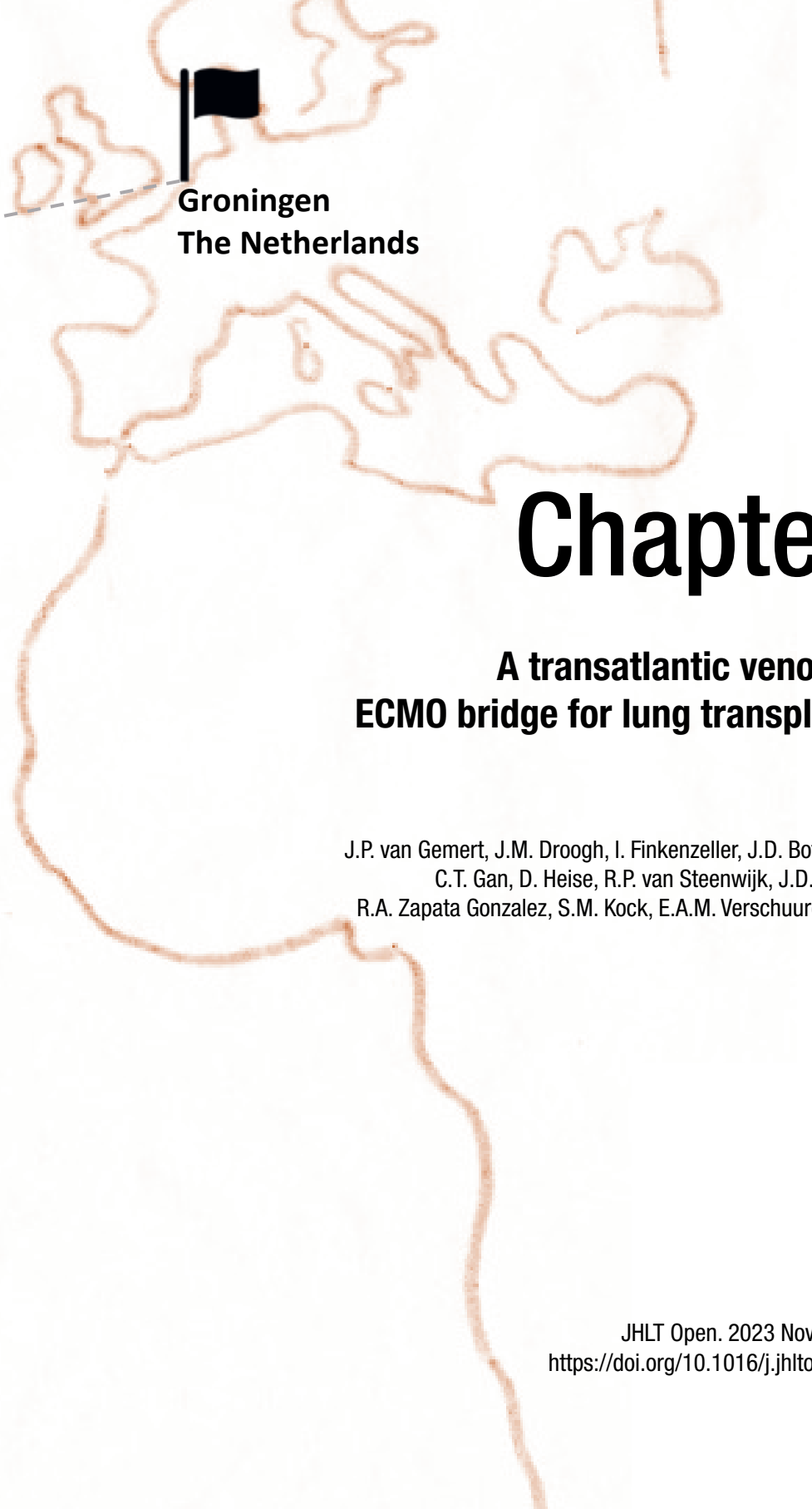

Chapter 7

A transatlantic veno-venous ECMO bridge for lung transplantation

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Abstract

This report details the challenging journey of a 58-year-old male with a progressive fibrotic lung disease who needed a lung transplantation (LTx). Residing on the remote island of St. Eustatius, access to specialized medical care was limited, but he was entitled to receive appropriate medical care in the Netherlands. Collaborative efforts among medical teams across the world resulted in a successful transatlantic transport of the patient on awake veno-venous Extracorporeal Membrane Oxygenation (vv-ECMO) to the Netherlands. A successful LTx was performed without complications. This case shows that vv-ECMO flight transport, when conducted by an experienced team, is a safe procedure for highly selected lung transplant candidates.

Case

This case report presents the challenging journey of a 58-year-old male with sarcoidosis and progressive fibrotic lung disease since 2013 (Figure 7.1). Living on the small Dutch Caribbean island of St. Eustatius, he faced limited access to specialized medical care.

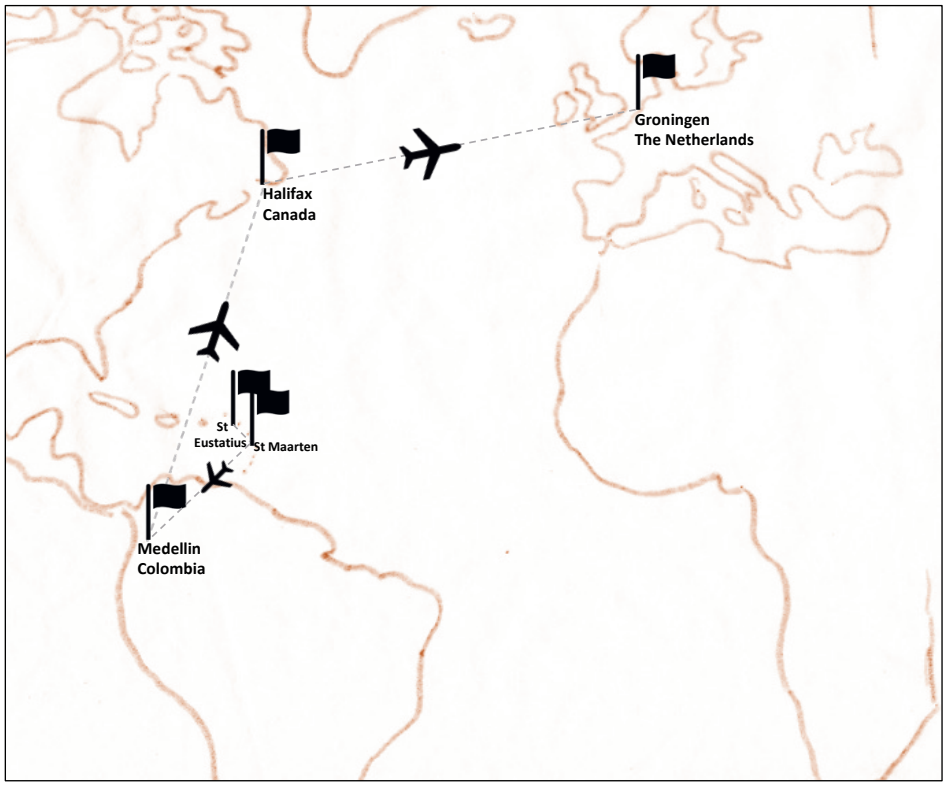

Figure 7.1: At first the flight from St. Eustatius to St. Maarten. Thereafter, the 13-hour vv-ECMO flight from Medellin, Colombia, with a refueling stop in Canada, to Groningen, the Netherlands.

In 2019 he presented with progressive fibrotic lung disease with a restrictive lung function and significant diffusion limitation. He was referred to a neighbouring island, Sint Maarten, for further evaluation and had been managed with steroids and methotrexate. However, fibrosis progressed, which required long-term oxygen therapy at home. A Dutch pulmonary physician, temporarily assigned to Hospital Fundashon Mariadal in Bonaire as part of specialist medical care support from Amsterdam University Medical Center, was well-informed about the patient's critical condition and conducted frequent video consultations with the patient. He asked our lung transplant (LTx) team from the University Medical Centre Groningen (UMCG), the Netherlands, if the patient could be a LTx candidate. Due to concerns about

safe transport by plane and insufficient information about his cardiac function, we could not accept him for LTx. To obtain a comprehensive cardiac workup, the patient was transferred to Colombia (Medellin, Clinica CardioVID), where he underwent a coronary angiogram (CAG), cardiac ultrasound, CT scan, and PET scan (health insurance policies on St. Eustatius cover medical referrals to Colombia for medical examinations that cannot be performed on St. Maarten or other islands of the Kingdom of the Netherlands). Upon returning to St. Eustatius, he experienced severe respiratory distress caused by a pneumothorax, necessitating the insertion of a chest tube. He was promptly transported to St. Maarten since there was no pulmonary physician available on St. Eustatius to manage the pneumothorax. As there were no pulmonary physicians available on the island, he was once again evacuated to Colombia by plane for pleurodesis (Medellin, Colombia, Clinica CardioVID, elevation above sea level 4900 ft). At arrival in Colombia, his oxygen levels were critically low, and he required 15l/min of oxygen by Venturi Mask. CT scans, CAG, and cardiac ultrasound findings were sent to the UMCG, and the patient was referred for LTx once again. Coronary disease was ruled out, and the PET scan showed no active sarcoidosis or malignancy. The CT scan indicated severe fibrosis with bullae and a pneumothorax (Figure 7.2), along with an ineffective chest drain placement, likely within a bulla.

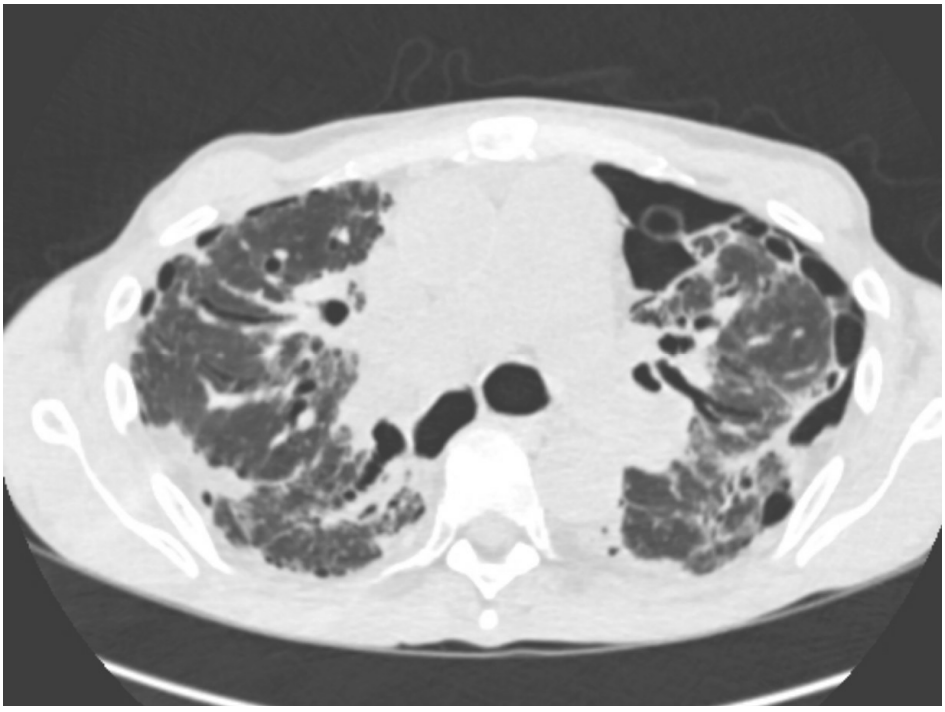

Figure 7.2: CT scan of the patient, showing fibrosis, bullae and a pneumothorax.

Right heart catheterization demonstrated pulmonary hypertension (pulmonary artery pressure 70/30; mean 43). He was accepted for bilateral LTx in the Netherlands, on the condition that safe transport could be arranged. Because he was accepted for a lung transplant, pleurodesis was no longer performed.

Transport

At the time of the request for transport, the patient was hospitalised in the Intensive Care Unit (ICU) of Clinica CardioVID in Medellin, Colombia. A teleconference with the treating team in Medellin, the UMCG, and the transport team was arranged to discuss options and limitations of a long-range air ambulance transport of the patient from Medellin, Colombia, to Groningen, the Netherlands for a possible LTx (great cicle transport distance 5550 mls). The option of transport by sea would take too long, given the condition of the patient. It would also be less safe. Air transport was planned using a Bombardier Challenger 604, with a two-sector flight plan (Medellin – Halifax, flight time 05:55h; refuelling 00:45h, Halifax – Groningen, flight time 06:05h). During aeromedical transport at 41,000-49,000 ft, patients experience a reduction in cabin pressure, which leads to a linear decrease in arterial oxygen pressure (PaO_2) unless the fraction of inspired oxygen (FiO_2) is increased. Changes in cabin pressure can be avoided if the cruising altitude is restricted to 25,000 ft, allowing the cabin to be pressurized to sea level (760 mmHg). However, in this case, a sea-level cabin pressure was not deemed necessary because the patient was already at an elevation of 4,900 ft in Medellin, and it was not feasible due to the long overall distance (lower flight levels significantly increase aircraft fuel consumption, requiring additional refuelling stops).

Although the patient did not oxygenate well, before transport, with an arterial PaO_2 of 46 mmHg despite 15 l/min via a venturi mask, intubation and positive pressure ventilation appeared to carry excessive risks due to the severely fibrosed lung with pulmonary hypertension, a pneumothorax and extensive bullae in both lungs. After considering the risk-benefit balance of all available options, cannulation for veno-venous Extracorporeal Membrane Oxygenation (vv-ECMO) in an awake patient was considered the most favourable option. The patient was cannulated in a femoral-jugular configuration by the Clinica CardioVID team the day before transport. On the day of transfer, the transport team (two physicians and a flight nurse) switched the vv-ECMO to a Getinge/Maquet Cardiohelp console (Cardiohelp, Getinge, Göteborg, Sweden). A second system was kept on standby as a backup during transport. The vv-ECMO was run with a sufficiently high blood flow rate of 3.5 L/min, ensuring adequate oxygenation and reduced interference from pressure alarms. Additionally, the high blood flow minimizes

the need for extensive anticoagulation of the patient. Blood gas analysis, ACT and INR/PT testing capabilities were available in-flight via an ISTAT PoC device (iSTAT, Abbott Point of Care, Chicago IL, USA). A minimal sweep gas flow of 1–2 L/min 100% oxygen was used to ensure spontaneous breathing. Blood gas analysis was used regularly to optimize vv-ECMO settings. At a cabin pressure equal to 6,000 ft above sea level, PaO_2 was recorded at 60 mmHg and $PaCO_2$ at 47 mmHg. Automated continuous monitoring of ECG, SpO_2, arterial blood pressure, and respiratory rate was used. Ultrasound with linear scan probe and colour Doppler was available to check and adjust vascular access if needed (Butterfly IQ+, Butterfly Network, Burlington, MA, USA). The patient was transported in a supine position (self-mobilisation) with elevated head rest, in good mood throughout the flight (Figure 7.3). The transport was carried out without complications or equipment failure, and the patient arrived well at Groningen, after a 13-h transport on awake vv-ECMO.

Figure 7.3: The patient awake on for veno-venous Extracorporeal Membrane Oxygenation (vv-ECMO) for transport.

Transplantation

In Groningen, the patient was admitted to the Intensive Care Unit (ICU) with vv-ECMO. Vv-ECMO support was initiated solely to facilitate transport. Additionally, it can take weeks for donor lungs to become available, and the use of ECMO is known to carry significant risks and complications. Therefore, the vv-ECMO support was discontinued on the same day of arrival, and the cannulas were removed. Subsequently, the patient was placed on high flow nasal oxygen therapy with a flow rate of 30 L/min and an FiO_2 of 65%. The patient was listed for LTx, with a high Lung Allocation Score (LAS). Fortunately, a suitable lung offer was received shortly after, and the LTx was successfully performed the following day without any complications. In the postoperative period, the patient experienced acute kidney injury that resolved within a few days. Additionally, he encountered difficulties in expectorating sputum, partly attributed to a right-sided diaphragm paresis. After one week, he was able to be extubated and subsequently transferred to the pulmonary ward for further care. After six weeks of hospitalization in the Netherlands, the patient was discharged. Being a Dutch citizen with appropriate insurance coverage, he was able to secure his own apartment. Family and friends from both St. Eustatius and the Netherlands came to visit him during this time. Currently, the patient's clinical condition is still moving forward, with ongoing improvement in lung function (Figure 7.4). One year after LTx, a joint decision will be made between the patient and healthcare providers to determine whether it is safe for him to return to St. Eustatius. Regular video call conversations will be conducted for check-ups and monitoring.

Discussion

This unique case-report demonstrates the safety and feasibility of vv-ECMO transportation during a 13-hour flight for a high-risk LTx candidate without the need for intubation and sedation. Our findings are consistent with a systematic review conducted by Han Yao et al., which included 647 ECMO cases, however, most of those covering transport distances of less than 1,000 mls (regional transports).[1] Although few complications during transport were reported in the literature, some instances of hemodynamic instability and bleeding were attributed to deficiencies in medical team knowledge. Hence, the involvement of experienced staff and thorough preparation prior to transport are crucial.[2] Moving a critically ill patient out of an intensive care unit into a resource limited environment, such as an air ambulance, always involves risks. These risks can be mitigated to some extend by meticulous preparation, coordination, active case management and experienced and well equipped teams, as we have described elsewhere.[3] Nevertheless, transport risk needs to be counterbalanced

by a tangible patient benefit, which in this case was access to LTx that was not available locally.

It is important to note that international air ambulance transportation, specifically those involving ECMO incurs significant costs up to 250,000 USD, depending on the distance and type of aircraft used. Therefore, this procedure should be reserved for highly selected patients. In the present case, the patient resides on a Dutch island and, being a Dutch citizen, is entitled to receive appropriate medical care in the Netherlands. The BES healthcare insurance, which covers medical expenses in Bonaire, St. Eustatius, and Saba, includes the cost of air transport to the Netherlands. It also covers the patient's stay in the Netherlands for up to a year after the transplant. However, given the high costs associated with the ECMO flight, as well as the subsequent LTx care and extended stay in the Netherlands, it is crucial to carefully select suitable candidates for this procedure. Selection criteria should encompass not only the patient's suitability for LTx and their likelihood of surviving the flight but also their willingness to immigrate to the Netherlands and leave behind friends and family. A comprehensive decision-making process involving the referral center, transplant center, ECMO team, social work, and insurance providers is necessary to arrive at an appropriate decision. In this particular case, the patient had previously resided in the Netherlands and had two sons living there. His strong motivation to undergo transplantation, along with a high position on the waiting list (due to severe lung disease), made him a suitable candidate for LTx.

In conclusion, vv-ECMO flight transport, when conducted by an experienced team, is a safe procedure for highly selected lung transplant candidates.

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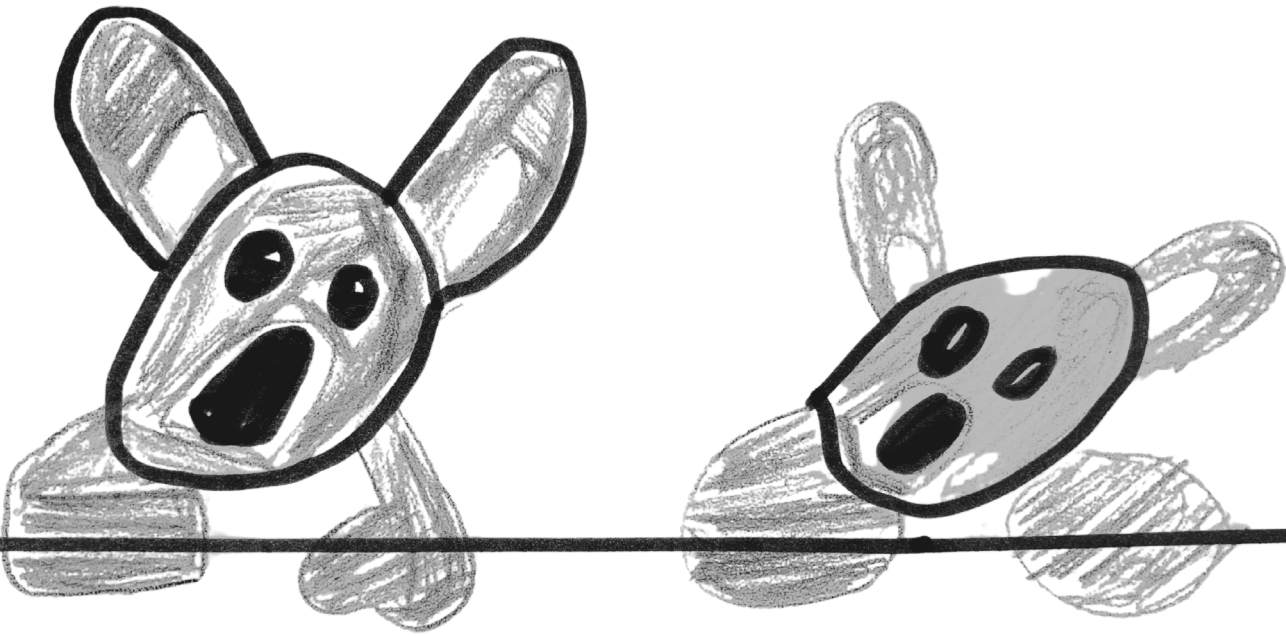
Part II

Cystic fibrosis transmembrane conductance regulator modulator therapy after lung transplantation



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Chapter 8

Evaluation of Elexacafor/Tezacaftor/Ivacaftor therapy after lung transplantation in cystic fibrosis: The Dutch national KOALA study

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Abstract

Background: Elexacaftor/Tezacaftor/Ivacaftor (ETI) for people with CF (pwCF) after lung transplantation (LTx) has been restrained due to uncertainties regarding efficacy and drug interactions. Given the persistence of extrapulmonary symptoms post-LTx, this prospective study aims to investigate the benefits and safety of ETI for pwCF post-LTx.

Methods: Between Nov 2022–Nov 2023 ETI was offered to pwCF post-LTx with at least one F508del mutation in 3 Dutch LTx centers. PwCF were considered eligible if they had either a BMI ≤ 19 kg/m², chronic rhinosinusitis (CRS), uncontrolled diabetes or gastrointestinal (GI) symptoms. BMI, HbA1c, SNOT-22 score, GI Symptom Tracker, CF Questionnaire-Revised (CFQ-R), FEV1, creatinine, changes in calcineurin inhibitor (CNI) doses and levels were compared between baseline and 3 months follow-up.

Results: Fifty-five pwCF post-LTx were included, of whom 5 were excluded because of ETI discontinuation due to side effects, within 3 months follow-up. Three months results showed a decrease in SNOT-22 score ($p < 0.001$) and GI symptoms (all 4, $p < 0.05$), an increase in BMI ($p = 0.012$) and CFQ-R (6 domains, $p < 0.05$). Median CNI daily dose had to be reduced from 6 to 4 mg ($p < 0.001$), to maintain stable CNI trough levels. Creatinine increased from 110 (87–141) to 115 (92–145) umol/L ($p = 0.002$).

Conclusion: ETI for pwCF post-LTx shows favourable effects on CRS, GI symptoms, and quality of life, but not on HbA1c. Due to its high cost, careful consideration and further studies are required. Monitoring renal function and CNI trough levels is recommended.

Introduction

Cystic fibrosis (CF) arises from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.[1] CF is a life-threatening disease that was previously a common indication for lung transplantation (LTx). The CFTR modulator Elexacaftor/Tezacaftor/Ivacaftor (ETI) shows markedly enhanced pulmonary function and reduced exacerbation frequency in people with CF (pwCF).[1–3] Consequently, this improvement in pulmonary function and number of exacerbations reduced the LTx indication in pwCF.[4] Historically, pwCF post-LTx were not considered eligible for ETI treatment due to uncertainty regarding direct benefits and potential drug-drug interactions with immunosuppressive therapy, primarily calcineurin inhibitors (CNI). However, CF affects multiple organ systems, leading to extrapulmonary symptoms post-LTx that may impact quality of life and graft function post-LTx as well.

While most research on ETI has primarily focused on pulmonary functions, earlier studies in pwCF without LTx show improvements in extrapulmonary conditions including Body Mass Index (BMI), chronic rhinosinusitis (CRS), CF-related diabetes (CFRD), pancreatic insufficiency, and CF-associated liver disease.[5] Importantly, Ramos et al. demonstrated in a retrospective cohort study in pwCF post-LTx that ETI improved HbA1c, increased haemoglobin levels in those with anaemia, and reduced antibiotic prescription frequency. However, a substantial proportion of pwCF (42%) discontinued ETI therapy at a median of 56 days.[6] Therefore, to further explore the benefit of ETI post-LTX, it is necessary to conduct further prospective studies on the benefits and to evaluate drug-drug interactions and side effects.

The primary aim of the prospective Dutch multicenter KOALA study is to investigate the potential benefits on BMI, gastro intestinal (GI) complaints, DM regulation, CRS, pulmonary function and quality of life of ETI for pwCF post-LTx. Secondary objectives include assessing adverse events (AE), the effect of ETI on CNI trough levels and determining the required CNI dose adjustments to maintain stable trough levels. In this manuscript, we will present the three-month results.

Material and methods

Study patients

All Dutch pwCF post-LTx were eligible for inclusion if they had at least one F508del mutation in the CFTR gene and one of the following: underweight (BMI ≤ 19 and/or enteral nutrition), CRS (CF-related CRS management, history of surgery), poor control of CFRD (> 50 EH insulin/day and/or HbA1C > 60) or GI manifestations (history of distal intestinal obstruction syndrome

(DIOS), use of laxatives). Patients were excluded if they had issues with medication adherence or if they had severe liver cirrhosis (Child-Pugh C), confirmed by liver ultrasound

CF care

In the Netherlands, there are 7 CF centers and 3 LTx centers. All 3 LTx centers also provide CF care. Post-transplant care is conducted collaboratively by both the transplantation physicians and the CF specialists. This collaboration is characterized by thorough communication and close coordination between healthcare providers. ETI may be prescribed in the Netherlands to all pwCF with one F508del mutation and is not restricted to study settings.

Transplant care

Follow-up of all LTx patients took place every 3 months for monitoring of transplant function as part of standard care with lab results and pulmonary function tests (PFT). All patients received maintenance immunosuppression: tacrolimus, prednisolone and mycophenolate mofetil (MMF). Alternative immunosuppressants were cyclosporine, azathioprine, everolimus or sirolimus. Post-transplantation prophylactic therapy included: cotrimoxazole for pneumocystis pneumonia and acyclovir for Herpes viruses and valganciclovir depending on Cytomegalovirus (mis)match.

Study medication

Patients started with the full ETI dosage: in the morning, 2 tablets containing ivacaftor 75 mg/tezacaftor 50 mg/elexacaftor 100 mg, and in the evening, 1 tablet of 150 mg ivacaftor. However, in patients with liver cirrhosis, confirmed by liver ultrasound, or those who have undergone a liver transplant, the dosage was adjusted to alternate between 2 tablets of ivacaftor 75 mg/tezacaftor 50 mg/elexacaftor 100 mg one day and 1 tablet of ivacaftor 75 mg/tezacaftor 50 mg/elexacaftor 100 mg the next day. The additional evening dose of ivacaftor was also omitted. When used concurrently with azoles, the dosage was adjusted to 2 tablets of ivacaftor 75 mg/tezacaftor 50 mg/elexacaftor 100 mg twice a week, with no additional ivacaftor in the evening. The above dosage recommendations were made based on the product information for ETI.[7] All patients were instructed to take their ETI with fat-containing food.

Study design

The KOALA study is a Dutch national prospective multicenter study. The study was granted a waiver by the respective Ethical Committees, as pwCF post-LTx were to receive ETI as part of their standard care. The study was conducted exclusively in the 3 LTx centers and not in

the CF centers that are not LTx centers. All participants provided written informed consent prior to enrolment in the study. The study complies with the International Society for Heart and Lung Transplantation (ISHLT) Ethics Statement.

Patients were recruited at one of the 3 Dutch LTx centers, where their follow-up also took place. Regular online meetings were held between the centers, and the study data were anonymously entered into a shared RedCap database. The principal investigator, based at one of the centers, compiled the data, conducted the analyses, and coordinated the study. A study agreement was signed by all 3 participating LTx centers.

In all 3 centers, a multidisciplinary team meeting was held to review which patients were eligible to enrol in the study according to the inclusion criteria as mentioned above. These patients were invited to participate in the study and were sent informational materials. Patients could be included between November 1, 2022, and November 1, 2023. Before starting ETI, patients were seen at the outpatient clinic during their standard LTx follow-up appointment by the transplant- or CF physician. During this visit, they received an explanation about the ETI, possible AE of the ETI, and the study visits. If they agreed to participate, baseline parameters were collected and a sweat test was conducted. Baseline parameters were gathered as part of standard care for LTx: BMI, blood tests (HbA1c, kidney function, liver enzymes, CNI trough levels) and pulmonary function tests (PFT). Spirometry was performed according to ATS/ERS guidelines.[8] Additionally, three questionnaires to asses extrapulmonary parameters were utilized, including the Sino-Nasal Outcome Test (SNOT-22) for CRS, the Gastro-intestinal (GI) Symptom Tracker for GI symptoms and the CF Questionnaire-Revised (CFQ-R) for quality of life. Follow-up visits, with lab result, PFT and the questionary's took place 3 months after starting ETI. The sweat test was only repeated after three months. For safety reasons, kidney function, liver enzymes, and CNI trough levels were measured at 2, 4, and 8 weeks after start of ETI and were discussed with the patient over the phone or at the clinic. Dose adjustments of the CNI were documented at every visit.

AE were assessed at every study visit. Patients were questioned about the potential occurrence of suspected AE of special interest including GI symptoms, headache, itching, upper airway infections, psychological side effects, decrease in FEV1 at home monitoring, weight and ear, nose and throat (ENT) symptoms.

Questionnaires

The CFQ-R is a patient-reported multiple-domain questionnaire specific for pwCF, to measure health-related quality of life. The score consists of 9 quality of life domains (physical func-

tioning, role functioning, vitality, emotional functioning, social functioning, body image, eating disturbances, treatment burden, health perception) and 3 symptom scales (weight, respiratory, and digestion). Responses of the 12 items are standardized from 0 to 100, with higher scores indicating better quality of life.[9] The Sino-Nasal Outcome Test (SNOT-22) is a patient-reported measure of outcome developed for use in CRS with or without nasal polyposis. The SNOT-22 contains 22 individual questions, with higher score indication more severe disease (Supplement 8.1).[10]

The Dutch GI Symptom Tracker (Supplement 8.2) is a standardized measure of GI symptoms, enzyme and nutrition adherence, demonstrating good reliability and validity.[11–13] It consists of 4 domains: Eating Challenges (4 items), Stools (8 items), Adherence Challenges (5 items), and Abdominal Symptoms (7 items). Scores are standardized on a 0-to-100 scale with higher scores indicating more symptoms.

Study end-points

Previous studies on ETI in pwCF used lung function as the primary outcome. Since this is not a suitable outcome measure for LTx patients, we selected extrapulmonary manifestations as the primary outcome measure. Consequently, the primary outcome consists of several parameters, namely: BMI, HbA1c, SNOT-22 score, GI Symptom Tracker score, CFQR score and FEV1 pre- and 3 months post-ETI. Secondary outcomes were, the effect of ETI on CNI trough levels, kidney function and liver enzymes, the CNI dose adjustment needed to maintain stable trough levels and AE. Primary and secondary outcomes were measured at each visit as described in the study calendar (Supplement 8.3).

Statistical analysis

Analyses were carried out using IBM SPSS for Mac, version 24.0. Continuous variables are expressed as median (interquartile range). Wilcoxon signed-rank test were used for within-group analyses, to compare the study parameters before initiation of ETI and at 3 months follow-up. A *p*-value of less than 0.05 is used as the cut-off for significance. The CNI trough levels were compared between baseline and 2, 4, 8 and 12 weeks follow-up.

Three-month results

Patients

Between November 2022 and November 2023, 55 pwCF post-LTx were included in the study in the 3 Dutch LTx centers. The flowchart detailing the inclusion and exclusion criteria is provided in Supplement 8.4. Of those, 7 pwCF received an adjusted dosage due to liver cirrhosis (n = 5) or liver transplantation (n = 2), and 1 received an adjusted dosage due to concurrent use of azoles. Five pwCF (9%) were excluded due to discontinuation of ETI, because of side effects before the 3-month follow-up. These side effects were: psychological side effects (n = 2), headache (n = 1), lung function decline (FEV1) (n = 1), muscle cramps in legs (n = 1). Consequently, 3-month follow-up parameters were available for 50 pwCF. Baseline characteristics are presented in Table 8.1. The median age was 42 years (IQR 34–49), and 31/50 patients (62%) were male. Bilateral transplant was performed in all of the pwCF. The median time post-LTx was 11 years (IQR 7–15 years). More than half (34/50; 68%) of the pwCF were homozygous for the F508del mutation. The median BMI was 22 kg/m^2 (IQR 22–24). Additionally, 41/50 (82%) of the pwCF had both endocrine and exocrine pancreatic insufficiency. GI symptoms were present in 30/50 (60%) of the pwCF, cirrhosis (Child-Pugh A or B) in 7/50 (14%), and CRS in 40/50 (80%). CLAD was present in 12/50 (24%) of the pwCF.

Table 8.1: Baseline characteristics of the 50 people with cystic fibrosis after lung transplantation

Variable	
Age, years	42 (34–49)
Time after LTx, years	11 (7–15)
Gender, male (%)	31 (62)
F508del mutation 1 copy, n (%)	50 (100)
F508del mutation 2 copies, n (%)	34 (68)
Bilateral LTx, n (%)	50 (100)
BMI, kg/m^2	22 (22–24)
Pancreas insufficiency	
Exocrine only, n (%)	8 (16)
Endocrine and exocrine, n (%)	41 (82)
GI symptoms*, n (%)	30 (60)
CF related cirrhosis, n (%)	7 (14)
CRS, n (%)	40 (80)
CLAD, n (%)	12 (24)
Sweat chloride, mmol/L	117 (106–138)

Continuous variables are expressed as median (interquartile range).
LTx, lungtransplantation; BMI, Body Mass Inex, GI, gastrointestinal; CF, cystic fibrosis, CRS, chronic rhinosinusitis, CLAD, chronic lung allograft dysfunction.
* GI symptoms: history of distal intestinal obstruction syndrome (DIOS) and/or use of laxatives.

The median sweat chloride level at baseline was 117 mmol/L (IQR 106–138 mmol/L). Of the pwCF post-LTx 48/50 (98 %) used CNI as part of their immunosuppressive therapy post-LTx.

Primary outcomes

The 3-month results are shown in Table 8.2. Sweat chloride significantly decreased from 117 mmol/L (IQR 106–138) before the start of ETI to 66 mmol/L (IQR 48–75) after 3 months of ETI treatment ($p < 0.001$). There was a significant but no clinically relevant increase in BMI from 21.7 kg/m^2 (19.8–23.6) to 21.8 kg/m^2 (19.7–24.1) ($p = 0.012$). In 11 patients with a BMI less than 19, there was no significant rise in BMI after 3 months of ETI (before ETI 16.9 kg/m^2, after 3 months 17.8 kg/m^2; $p = 0.208$).

Table 8.2: Parameters at baseline and 3 months after starting Elexacaftor/Tezacaftor/Ivacaftor

Parameter	Baseline	3 months	*p*-value
Sweat Chloride, mmol/L	117 (106–138)	66 (48–75)	< 0.001
BMI, kg/m^2	21.7 (19.8–23.6)	21.8 (19.7–24.1)	0.012
HbA1c, %	6.8 (6.1–7.5)	7.0 (6.2–7.7)	0.312
SNOT-22 sum score	31.5 (16.8–49.0)	12.5 (5.3–19.8)	< 0.001
GI Symptom Tracker score			
Abdominal symptoms	48 (38–61)	36 (32–43)	< 0.001
Stools	58 (47–66)	47 (37–54)	< 0.001
Eating challenges	38 (33–50)	31 (25–38)	0.002
Adherence challenges	32 (25–40)	25 (25–35)	0.011
FEV1, L	2.7 (2.1–3.3)	2.7 (2.1–3.2)	0.076
Creatinine, µmol/L	110 (87–141)	115 (92–145)	0.002
EGFR, ml/min/1.73 m^2	68 (48–85)	63 (43–79)	0.010
Liver enzymes			
AST, U/L	18 (13–28)	20 (14–30)	0.388
ALT, U/L	23 (15–35)	24 (18–31)	0.485
ALP, U/L	89 (67–115)	82 (67–112)	0.023
GGT, U/L	26 (17–51)	25 (18–43)	0.436
LDH, U/L	205 (183–230)	221 (193–239)	0.051
CNI trough level, µg/ml	7.0 (6.3–8.6)	7.6 (6.1–9.1)	0.077
CNI dose, mg/day	6.0 (4.0–7.1)	4.0 (3.0–7.0)	< 0.001

Continuous variables are expressed as median (interquartile range).
BMI, Body Mass Index; SNOT, Sino-Nasal Outcome Test; GI, gastrointestinal; FEV1, Forced Expiratory Volume; EGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, Alanine transaminase; ALP, Alkaline phosphatase, GGT, Gamma-glutamyltransferase; LDH, Lactate dehydrogenase; CNI, calcineurin inhibitor.

HbA1c remained unchanged and insulin usage did not significantly decrease after ETI treatment. Similarly, in the group with diabetes, HbA1c did not change significantly after 3 months of ETI. There was a significant decrease in SNOT-22 score from 31.5 (IQR 16.8–49) at baseline to 12.5 (IQR 5.3–19.8) at 3 months follow-up ($p < 0.001$). The percentage of

patients who achieved the Minimal clinically important differences (MCID) SNOT-22 score threshold of 8 was 69%.[14] There was a significant improvement in all four domains of the GI symptom tracker (Table 8.2 and Figure 8.1). Six out of the 12 domains of the CFQ-R significantly improved (Figure 8.2). The domains that showed improvement were: eating disturbances, vitality, emotional functioning, respiratory symptoms, digestive symptoms and health perceptions. FEV1 remained unchanged, 2.7 L (IQR 2.1–3.3) at baseline and 2.7 L (IQR 2.1–3.2) at 3 months of ETI treatment.

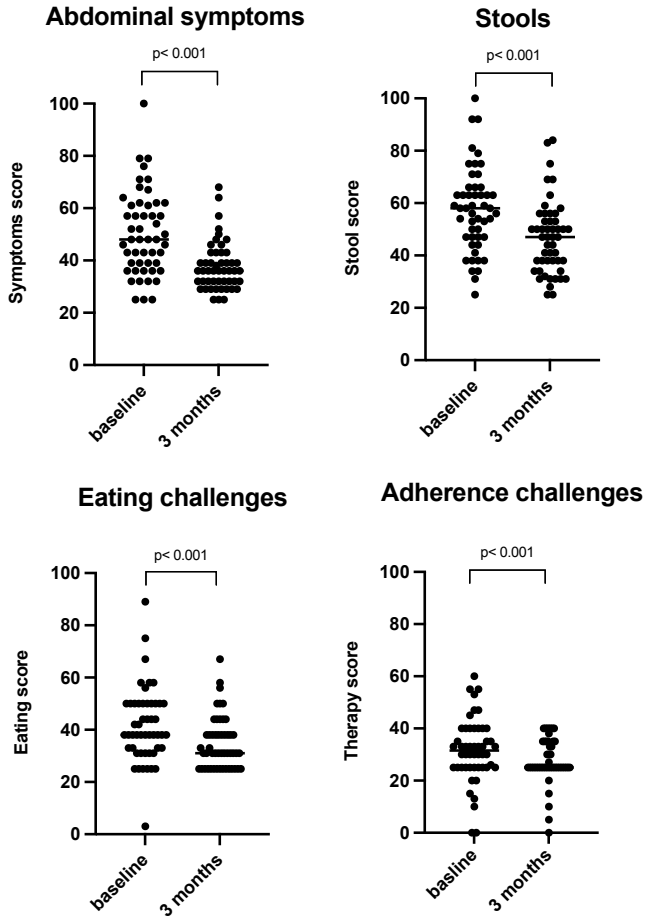

Figure 8.1: Results of the Gastrointestinal Symptom Tracker at baseline and 3 months follow-up.
Gastrointestinal symptom tracker results were available for all 50 patients at baseline and 3 months follow-up.

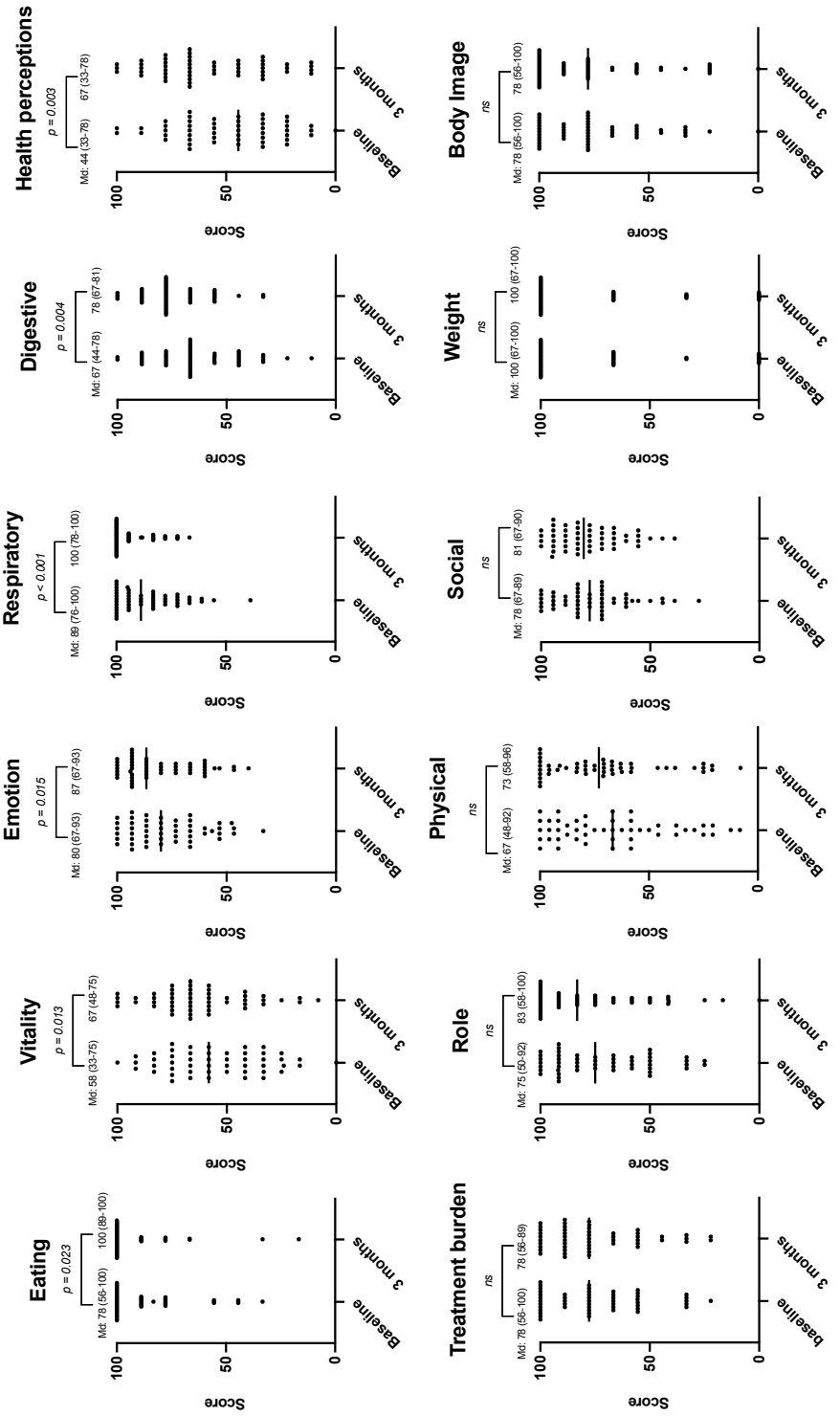

Figure 8.2: Results of the CF Questionnaire-Revised at baseline and 3 months follow-up.
CF Questionnaire-Revised results were available for 49 patients at baseline and for 50 patients 3 months follow-up. NS, not significant; Md, median with interquartile range.

Pharmacology

CNI trough levels rose directly after the start of the ETI from 7.0 to 9.1 ug/L but stabilised after dose adjustment to 7.6 ug/L (Figure 8.3b). CNI dose decreased from 6 mg/day before start of ETI to 4 mg/day after 3 months of ETI treatment (Figure 8.3a). Therefore, ETI necessitated a dose reduction of 33% to maintain stable CNI through levels. The CNI concentration/dose ratio significantly increased from 1.16 ug/L* 1/mg to 1.75 ug/L* 1/mg after 3 months of ETI treatment (Figure 8.3c).

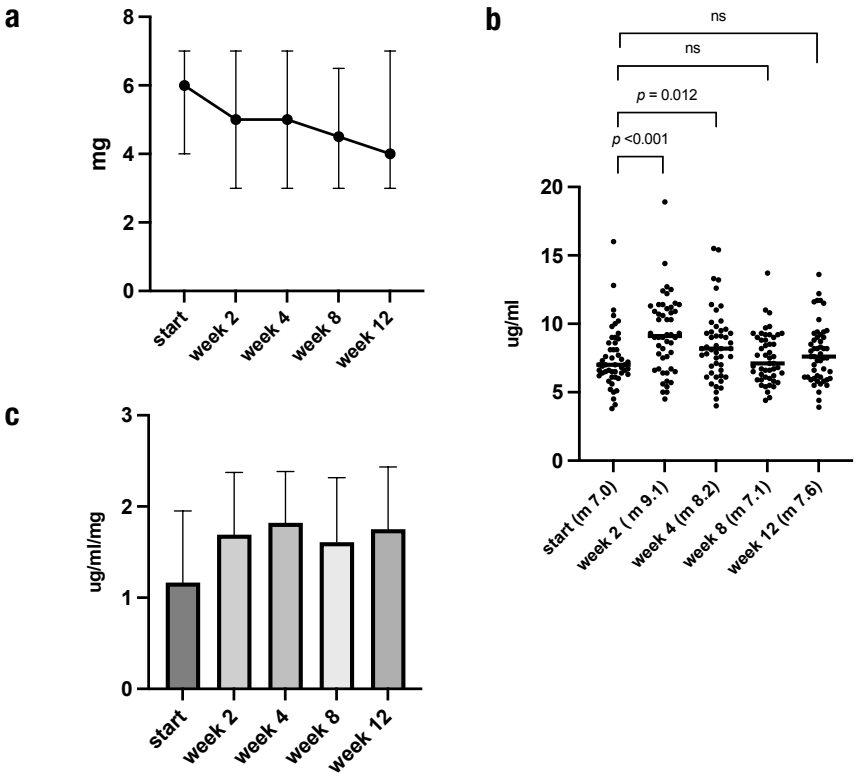

Figure 8.3: Calcineurin inhibitor trough levels dosage and concentration/dose concentration at baseline and 3 months follow-up. (a) CNI dose. **(b)** CNI trough levels. **(c)** CNI concentration/dose ratio.
CNI, calcineurin inhibitor; ns, not significant; m, median.

Adverse events

There was no effect of ETI on liver enzymes, except for ALP which declined from 89 U/L (IQR 76–115) to 82 U/L (IQR 67–112). Creatinine increased after the start of ETI but stabilised after CNI dose adjustment, although remained higher than at baseline (Table 8.2, Figure 8.4).

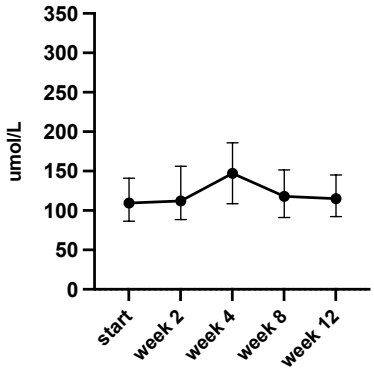

Figure 8.4: Creatinine at baseline and 2, 4, 8 and 12 week follow-up.

As a result of ETI, 46 AE occurred in 22/50 (46%) pwCF. The most common AE were GI symptoms, headache, upper respiratory infections, pruritus, and liver enzyme abnormalities. In 77% of cases, these adverse events resolved spontaneously after approximately 2 weeks (Table 8.3).

There was 1 hospitalization due to a viral upper airway infection and no deaths related to ETI during the study period.

Table 8.3: Side effects of Elexacaftor/Tezacafor/Ivacaftor in people with cystic fibrosis after lung transplantation

	ENT	GI	UAI	Liver	Headache	LF decline	Rash	Psychiatric	Fever	Weight gain
2 weeks	5	10	1	4	7	0	6	1	1	0
4 weeks	2	3	2	1	0	2	1	1	0	0
8 weeks	1	4	0	1	2	0	1	3	0	0
12 weeks	0	2	2	1	1	0	1	3	0	1

ENT, ear/nose/throat; GI, gastrointestinal; UAI, upper airway infections; LF, lung function.

Discussion

The KOALA study is the first prospective multicenter study examining the effect of ETI in people pwCF post-LTx. Our findings indicate that ETI has favourable effects on CRS, GI symptoms, and overall quality of life. These results are consistent with three small retrospective studies (total n = 27), which all demonstrated improvements in weight, CRS, and GI symptoms following ETI treatment in pwCF.[15–17] However, Ramos et al. reported, in a larger study cohort in 94 pwCF post-LTx, no effect of ETI on BMI, but did find improvements on HbA1c levels, increased Haemoglobin levels in those with anaemia, and a reduced frequency of antibiotic prescriptions.[6]

Contrary to Ramos et al., our study did not observe significant effects on HbA1c. Additionally, insulin usage among pwCF in our study did not significantly decrease after ETI treatment. The lack of an effect on HbA1c may also be due to the fact that the post-transplant immunosuppressive use of steroids and tacrolimus can also induce diabetes.

Remarkably, almost 42% of the pwCF discontinued ETI in the study of Ramos et al.[6] In our study, only 5/55 (9%) pwCF discontinued ETI before the 3-month follow-up period. Many of our patients (46%) experienced AE, but our findings showed that these typically resolve spontaneously after a few weeks. As a result, it is crucial to provide pwCF with adequate information regarding this matter with our gained knowledge about AE in pwCF without transplantation. Another important thing to realize is that pwCF post-LTx do not anticipate the same benefits of ETI as non-transplanted pwCF, such as prolonged life expectancy. Consequently, they might be less willing to tolerate ETI related AE. Additionally, pwCF post-LTx are highly protective of their transplanted lungs and may fear that ETI could harm their transplanted lungs. Fortunately, our study demonstrated that ETI has no detrimental effect on pulmonary function, as previously observed in the study by Benninger et al.[16]

Given the high costs of ETI, it is important to weigh whether the benefits outweigh these costs. CF is a heterogeneous multi-organ disease, in which patients after LTx may still suffer from many debilitating conditions such as distal intestinal obstruction syndrome (DIOS), CRS, uncontrolled diabetes, pancreatitis, and more. The burden of care for pwCF post-LTx is high and includes regular local care of sinuses, routine clinical and diagnostic check-ups. CRS can result in local and systemic complications, sometimes associated with hospitalization for prolonged intravenous antibiotic treatment of the upper and lower airways and airway colonization with pathogens such as *Pseudomonas*.[18] Frequent infections, especially *Pseudomonas* infections, may lead to CLAD.[19] However, we do not yet know whether ETI can also prevent CLAD. We will have to wait for the long-term results of the KOALA study to determine if ETI has an impact on CLAD development. Although the results of ETI appear promising for questionnaire-based endpoints, unfortunately, there is no effect on measurable values such as BMI and HbA1c. Given the high cost of ETI, a careful consideration is required, and further studies with cost-benefit analyses and more concrete measurable endpoints are needed to strike the balance. In addition, it is important to determine which pwCF post-LTx benefit the most from ETI. In the study by Ramos et al., prescription practice patterns varied across LTx programs, reflecting variability at the provider level.[6] The inclusion criteria we used to determine whether or not to administer ETI were clear to all centers and prescribers. Future study results hopefully give answer to which patients benefit the most from ETI.

In our study, we showed that ETI necessitated a CNI dose reduction of 33% to maintain stable trough levels. This is in line with the study by Dolaginsky et al., which demonstrated a required dose reduction of 50% and with van der Meer et al., who showed that five dose adjustments were performed in 4 liver transplant patients in order to attain tacrolimus target range.[15,20] In contrast, Benninger et al. showed no significant impact on the immunosuppressive drug doses.[16] The dose reduction needed can be explained by several reasons. First, both ETI and CNI are substrates of permeability glycoprotein (P-gp). P-gp is an efflux transporter present on the apical side of the intestinal epithelium that prevents intracellular accumulation of CNI and ETI by increasing their efflux to the intestinal lumen.[21] In theory, if more than one substrate competes to bind with P-gp, it decreases the likelihood of the drugs being pumped out of the cell, resulting in accumulation and higher trough levels. The second reason might be that the effect of ETI on the GI tract increases the GI absorption of CNI. With the standard dosage of ETI, the variability between pwCF in exposure as measured by AUC or Cmin is high.[7] Third, metabolization of ETI can be influenced by medication that are CYP3A4 liver enzyme inductors or inhibitors such as azoles. The latter can influence treatment start decision and increases side effects. To better understand the relationship between ETI exposure and the level of drug-drug interaction with CNI we are planning to determine trough levels of ETI in all pwCF included in our study.

Notably, in our study, renal function declined immediately after the initiation of ETI. Although this stabilized after reducing the CNI dose, it did not return to baseline. Since ETI is minimally cleared renally, the decline is most likely due to rising CNI levels immediately after starting ETI. It is possible that this decline in renal function can be prevented by adjusting the CNI dose by approximately 30% at the start of ETI. Additionally, the long-term effects of ETI on renal function should be examined over a longer follow-up period.

There are some limitations to our study that should be considered when interpreting our findings. This was a nationwide prospective observational cohort study. The small sample size, and the lack of a comparison group limits the reliability of the results. Unfortunately, the low prevalence of pwCF post-LTx constrains patient studies and data collection, which is a common reality in LTx research. Secondly, not all patients started with a full dose of ETI, potentially leading to an underestimation of ETI's effects. Third, our study showed a favorable safety profile on the short term but longer term safety profile needs to be elucidated. Fourth, the median time between LTx and start of the ETI was 11 years in our study. Therefore, our results might not be applicable to LTx patients shortly after LTx who start ETI. Fifth, we used validated questionnaires that are inherently subjective and may not be suitable for the post-transplant group of pwCF. Furthermore, the MCID is only established for the respiratory

domain of the CFQR, while it remains unavailable for the other domains.[22] As a result, we were unable to apply the MCIDs, although they are crucial for enhanced clinical interpretation of the results. Also, for sinus disease evaluation we did not perform a CT scan of the sinuses because it would have subjected patients to additional tests and radiation, which did not align with our non-WMO application. Finally, some patients may have taken their ETI together with tacrolimus and fat-containing food, while others may have taken tacrolimus at a different time than the ETI. This could potentially result in differences in drug absorption between patients.

Conclusion

This is the first prospective multicenter study to investigate the effect of ETI in pwCF post-LTx. We showed that ETI post-LTx has favorable effects on CRS, GI symptoms and quality of life. However, careful monitoring of renal function and CNI levels are recommended.

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Supplement 8.1: SNOT score

Neus-keel-oorziekten - Hoofd- Halschirurgie
Dr.Carl Van Laer - Dr. Jorn Potvin
www.nkokleinbrabant.be

Neusklachten Vragenlijst (Sino-Nasal Outcome Test-22 Questionnaire)

Hieronder vindt u een lijst met symptomen en sociale/emotionele gevolgen van de aandoening van uw neus. Gelieve volgende vragen zo goed mogelijk te beantwoorden. Er zijn geen juiste of foute antwoorden, en alleen u kan ons deze informatie geven. Kan u een score geven aan uw problemen zoals u ze de laatste tijd ondervond?

Geef een nummer aan elk van de onderstaande klachten. →	**Geen probleem**	**Zeer licht probleem**	**Licht of weinig probleem**	**Matig probleem**	**Ernstig probleem**	**Kan niet slechter**
1. Nood tot het snuiten van de neus	0	1	2	3	4	5
2. Niezen	0	1	2	3	4	5
3. Loopneus/neusloop	0	1	2	3	4	5
4. Hoest	0	1	2	3	4	5
5. Postnasale drip (neusloop achteraan in de keel))	0	1	2	3	4	5
6. Taaie neusloop	0	1	2	3	4	5
7. Volheidsgevoel in oren	0	1	2	3	4	5
8. Duizeligheid	0	1	2	3	4	5
9. Oorpijn/druk in het oor	0	1	2	3	4	5
10. Gelaatspijn/druk	0	1	2	3	4	5
11. Moeilijkheid in slaap vallen	0	1	2	3	4	5
12. 's nachts wakker worden	0	1	2	3	4	5
13. Gebrek aan goede nachtrust	0	1	2	3	4	5
14. Vermoeid wakker worden	0	1	2	3	4	5
15. Vermoeidheid overdag	0	1	2	3	4	5
16. Verminderde productiviteit	0	1	2	3	4	5
17. Verminderde concentratie	0	1	2	3	4	5
18. Frustratie/rusteloos/prikkelbaar	0	1	2	3	4	5
19. Neerslachtig	0	1	2	3	4	5
20. Beschaamdheid	0	1	2	3	4	5
21. Smaakzin/reukzin	0	1	2	3	4	5
22. Verstopte neus	0	1	2	3	4	5

SUBTOTAAL: _____ _____ _____ _____ _____

TOTAAL: _____

Supplement 8.2: GI symptom tracker

A. Please indicate the strength of the enzyme you are currently taking: _____

How many enzymes does your health care provider tell you to take with:
B. Each meal _____ C. Each snack _____

During the past week, indicate how often:

	Almost Always	Often	Sometimes	Never
1. Your appetite was poor	❍	❍	❍	❍
2. You had to rush to the bathroom because of GI problems	❍	❍	❍	❍
3. You felt bloated	❍	❍	❍	❍
4. You had to stay on the toilet for a long time	❍	❍	❍	❍
5. You had acid reflux (heartburn)	❍	❍	❍	❍
6. You skipped a meal	❍	❍	❍	❍
7. You forgot to take your enzymes	❍	❍	❍	❍

During the past week, how *bothered* were you by:

	A Great Deal	Somewhat	A Little	Not At All
8. Fatty or greasy foods	❍	❍	❍	❍
9. Taking enzymes in front of others	❍	❍	❍	❍
10. Constipation	❍	❍	❍	❍
11. Stomach ache	❍	❍	❍	❍
12. Cramping	❍	❍	❍	❍

How often in the past week have you had:

	Almost Always	Often	Sometimes	Never
13. Gas	❍	❍	❍	❍
14. Loose stools	❍	❍	❍	❍
15. Bad-smelling stools	❍	❍	❍	❍
16. Greasy stools	❍	❍	❍	❍
17. Diarrhea	❍	❍	❍	❍

During the past week:
18. How many stools did you have per day? (Please provide number or range): _____
During the past week, indicate how often:

	Almost Always	Often	Sometimes	Never
19. You were embarrassed about using a public bathroom	❍	❍	❍	❍

	Almost Always	Often	Sometimes	Never	Does not apply
20. You forgot to take medications to help your digestive system (e.g., antacids, laxatives)	❍	❍	❍	❍	❍

	Almost Always	Often	Sometimes	Never
21. You had enough time to eat	❍	❍	❍	❍
22. You missed school, work, or daily activities because of GI problems	❍	❍	❍	❍
23. You forgot to bring your enzymes with you	❍	❍	❍	❍
24. You relied on "fast food" or snacks like soda, chips, or candy to boost your calories	❍	❍	❍	❍
25. You had GI problems (stomach ache, loose stools) because you may have missed your enzymes	❍	❍	❍	❍

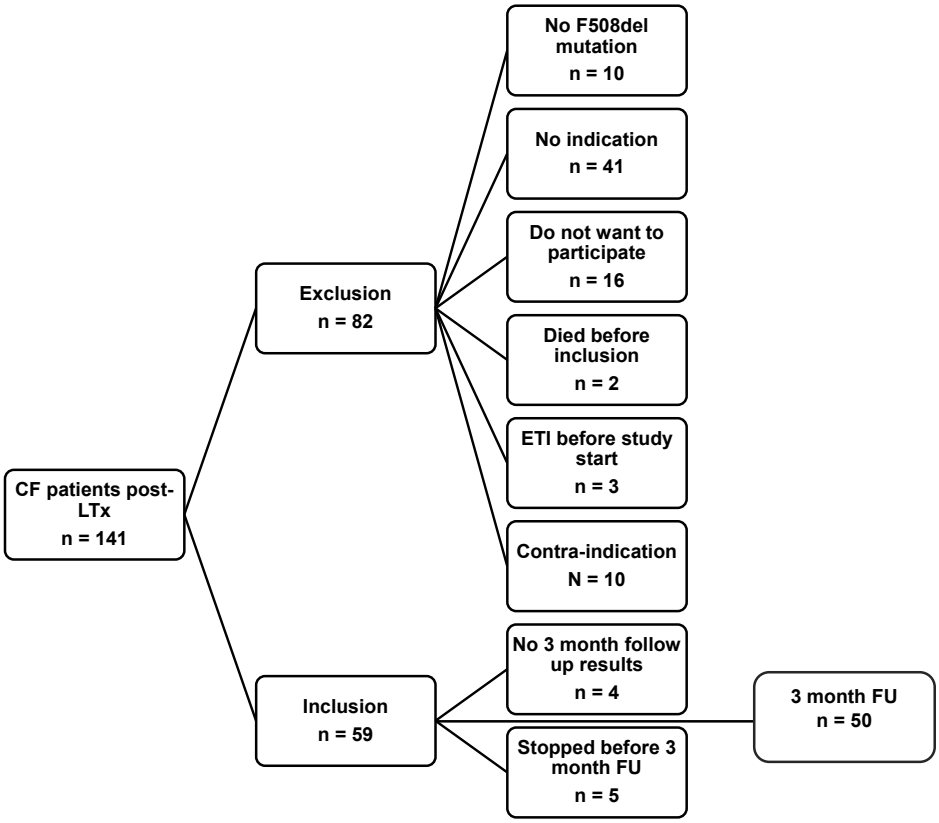
The GI Symptom Tracker is intended for informational purposes only and should not be used as a substitute for advice provided by your doctor or other healthcare professionals.

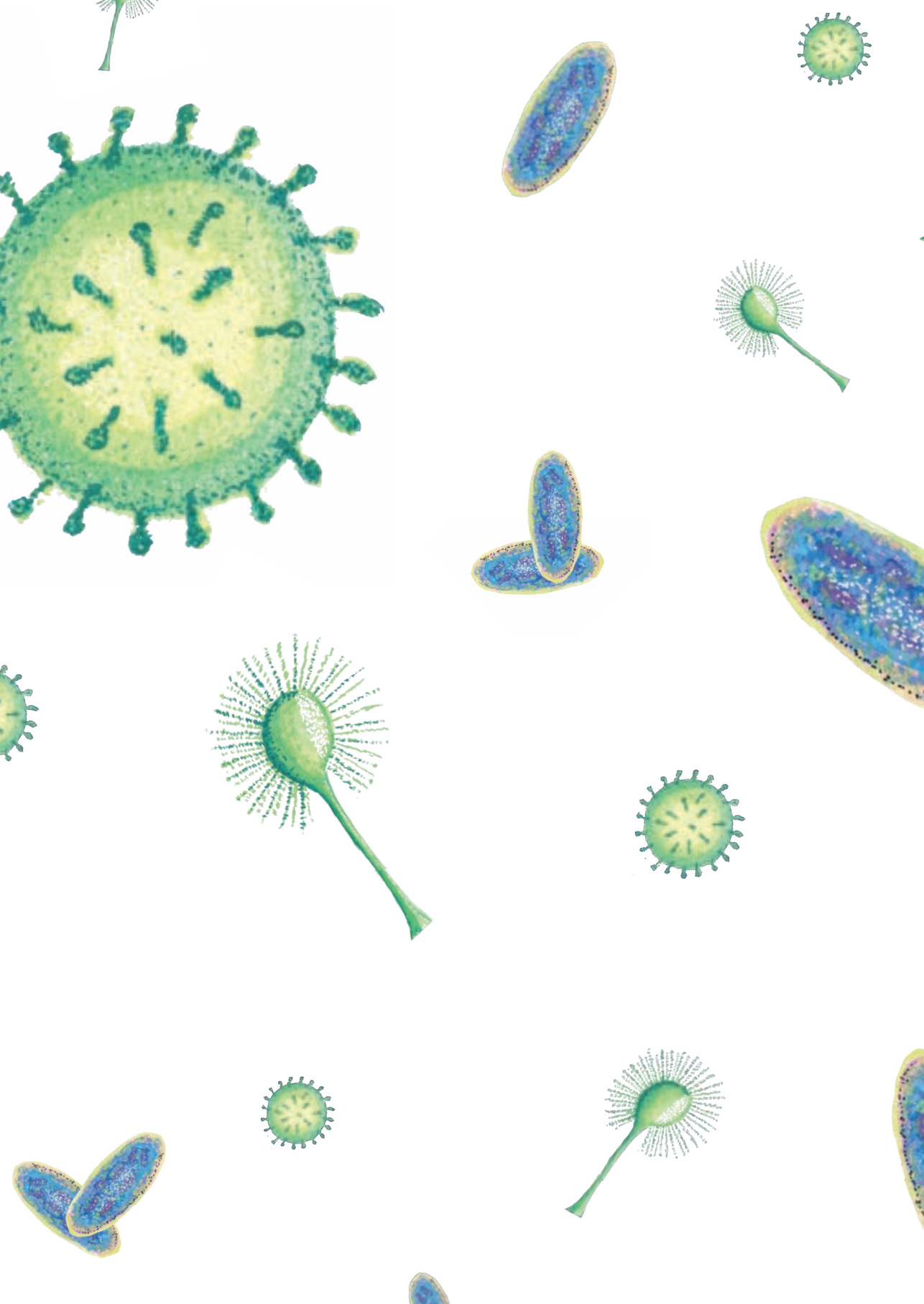
Supplement 8.3: Study calendar

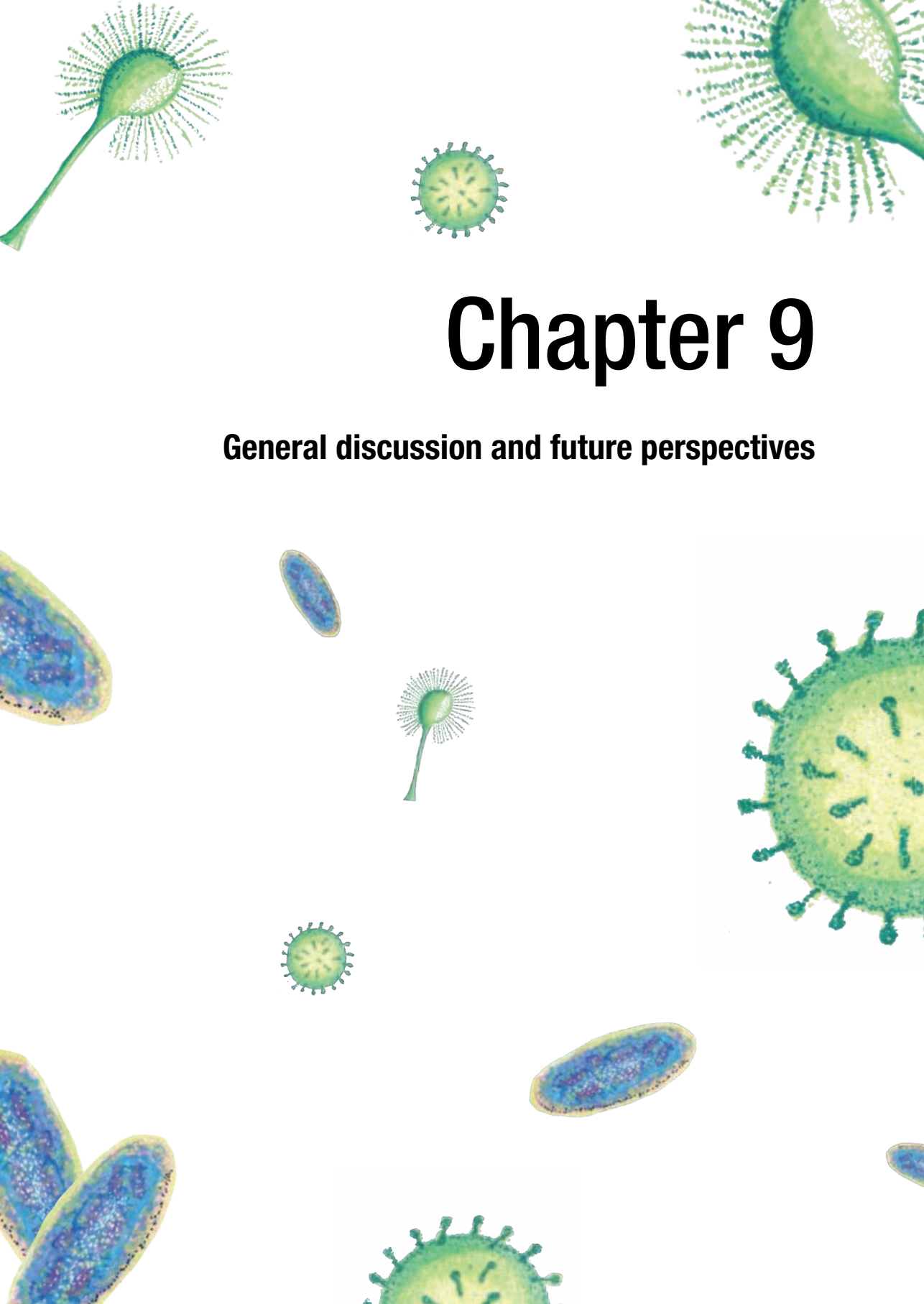
	Visit 1 Start	Visit 2 2 weeks	Visit 3 4 weeks	Visit 4 8 weeks	Visit 4 8 weeks	Visit 6 1/2 year	Visit 7 1 year
Weight	X	X	X	X	X	X	X
Lab results		X	X	X	X	X	X
• Creatinine	X	X	X	X	X		
• Liver enzymes	X	X	X	X	X		
• CNI trough level	X	X	X	X	X		
• HbA1c	X				X		
PFT	X				X	X	X
Sweat test	X				X		
Side effects		X	X	X	X	X	X
Questionnaires • SNOT score • Quality of live • GI Symptom Tracker	X				X	X	X

CNI, calcineurin inhibitor; PFT, pulmonary function tests, SNOT, Sino-Nasal Outcome Test; GI, gastrointestinal.

Supplement 8.4: Study flowchart of the KOALA study







Chapter 9

General discussion and future perspectives

In this thesis two key topics encountered by lung transplant (LTx) patients during their journey before and after transplantation are addressed.

The first part of the discussion focuses on infectious diseases, with special attention for COVID-19, non-tuberculous mycobacteria pulmonary disease (NTM-PD) and aspergillosis, which all pose significant challenges throughout the transplantation trajectory. These infectious diseases carry a higher risk in patients with severe underlying lung diseases with or without immunosuppression before transplantation, and in immunosuppressed patients after LTx. Additionally, they have the potential to impact graft function.

The second part examines the effects of novel cystic fibrosis transmembrane conductance regulator (CFTR)-modulators, for cystic fibrosis (CF), which has had a profound impact on the non-transplanted CF community. Notably, this thesis provides the first insights into the effects of CFTR-modulators on people with CF (pwCF) post-LTx.

Part 1

During the initial counselling session provided to all potential LTx candidates, patients are informed about the risks of infectious diseases post-LTx. They are made aware that the lifelong immunosuppressive medication, required to prevent rejection, renders them particularly vulnerable to infectious diseases. Education is provided on protective measures, including hygiene practices and vaccinations. Additionally, it is emphasized that the lung is a uniquely vulnerable organ due to its direct exposure to the external environment, unlike the heart, kidneys, or liver. After transplantation, LTx recipients are also instructed to immediately contact their medical team if they fall ill or develop a fever, as infectious diseases in LTx recipients can have a severe course.

As described earlier, infectious diseases have a profound impact on the lives of LTx recipients. This thesis explores the influence of COVID-19, NTM-PD and aspergillosis on the transplantation trajectory of LTx recipients, as well as on the daily clinical practice of transplant physicians. While patients frequently contend with infectious diseases, transplant physicians are continually striving to maintain a delicate balance between infection management and rejection control for each individual LTx recipient.

In this discussion, the studies on the aforementioned infectious diseases could be addressed individually. However, it is more insightful to focus on the overarching challenges related to infectious diseases. Prevention forms the cornerstone of infectious disease management, and as such, a portion of the discussion will be dedicated to this topic. Subsequently, the discussion will address the critical importance of accurate infectious disease diagnosis, as

this directly influences clinical decision-making. Finally, the treatment of infectious diseases under immunosuppression will be explored, along with the consequences of infectious diseases on graft function.

Preventive measures of infectious disease in LTx recipients

In the field of infectious diseases, prevention plays a crucial role, especially for immunocompromised organ transplant recipients. Prevention of infection is crucial not only to reduce the likelihood of attracting infectious disease, but also to deter a more severe course of disease.

In chapter 2 of this thesis, it was observed that during the first endemic of COVID-19 in the Netherlands, LTx recipients experienced a high mortality rate (20%). To prevent severe COVID-19, effective vaccination is essential.[1,2] Therefore in the study described in chapter 3 we investigated the vaccination response among LTx recipients. This study showed that, even after receiving 5 doses of SARS-COV- vaccine, only half of LTx recipients developed an adequate antibody response. This outcome highlights an alarmingly low efficacy of vaccination in this vulnerable population.

This raises the question how to improve the vaccination response in LTx recipients. To address this, in chapter 3 we explored the factors contributing to poor SARS-CoV-2 vaccine responses in LTx recipients. These included older age, chronic kidney disease, and a shorter time since transplantation. This is in line with other studies.[3,4] Currently, no adjustments are being made to immunosuppression for vaccination purposes to assess whether this improves the vaccination response. However, in elderly LTx recipients with chronic kidney disease, this may be considered. In LTx recipients immunosuppression is at its highest shortly after transplantation, but reducing it poses significant risks due to the elevated likelihood of rejection in the early post-transplantation period.[5]

One potential approach to address this involves using Torque Teno Virus (TTV) load as a biomarker to evaluate the extent of immunosuppression in these LTx recipients. TTV is a commensal non-pathogenic virus which has been proposed as marker of functional immunity: higher loads correspond to over-immunosuppression, and lower loads to under-immunosuppression.[6] Therefore TTV load might provide an indirect measure of immune function, enabling tailored adjustments to immunosuppressive therapy while balancing the risk of rejection and infection. Hoek et al. showed in their study that a lower TTV load is associated with a better antibody response to SARS-CoV-2 vaccines.[7] If the TTV load prior to vaccination is indeed a reliable predictor of the immune function, it could serve as a potential tool for optimizing vaccination regimens in LTx recipients.

When focussing on immunosuppression, Mycophenolate Mofetil (MMF) is associated with fewer serological SARS-COV-2 vaccination responses, in lung-, heart- and kidney transplant recipients.[7–9] One potential vaccination strategy could involve assessing TTV load to identify LTx recipients with a low likelihood of mounting an immune response post-vaccination. In such LTx recipients, temporary discontinuation, or at least reduction, of MMF could be considered. Studies conducted in liver and kidney transplant patients have demonstrated that discontinuation of MMF prior to vaccination is both safe and effective in those patients without an antigen response after prior vaccinations.[10,11] This strategy has not been studied in LTx recipients and carries inherent risks, as reducing immunosuppression increases the likelihood of transplant rejection. Moreover, the optimal duration for discontinuing MMF before vaccination remains uncertain. Although pharmacokinetic studies have shown a half-life of 6–12 hours and a secondary peak suggesting enterohepatic circulation, the duration of lymphocyte recovery after MMF cessation is unknown.[12] Although discontinuation of MMF based on TTV load may enhance the immune response to SARS-CoV-2 vaccination in solid organ transplant recipients, further research is required in LTx recipients. Studies should specifically examine the effects of MMF discontinuation and its duration on TTV load. Subsequently, research should assess whether MMF reduction, guided by TTV load, enhances vaccine response while also evaluating the associated risk of rejection.

The flip side of the coin is described in chapter 4, where it is shown that being too cautious can also have adverse consequences. Due to the impact of the COVID-19 pandemic, and particularly on LTx recipients, there has been an increasing reliance on viral diagnostics. For example, potential lung donors are now routinely tested for COVID-19, whereas prior to the pandemic donors were not tested for the presence of respiratory viruses. And in our center, LTx recipients are routinely subjected to a viral swab upon arrival at the hospital for a lung offer. It is however questionable if such testing is necessary.

Chapter 4 is about investigating the relevance of viral diagnostics in asymptomatic LTx recipients at the time of a lung offer. The study presented in this chapter reveals that the presence of a community-acquired respiratory virus in asymptomatic LTx recipients was not associated with the development of primary graft dysfunction, length of hospital stay, or mortality. This is a significant finding, not only because skipping viral diagnostics in asymptomatic recipients can save costs, but more importantly this prevents recipients from being declined for LTx. This ensures they do not face prolonged waiting times for another lung offer, which could otherwise increase their risk of mortality while on the waiting list. Therefore, it might be wise to avoid respiratory virus testing in asymptomatic LTx recipients.

Looking ahead, a potential recommendation for the future could be to conduct a study on the necessity of COVID-19 testing in donors. Often, such testing generates more concern than actionable insights.

Finally, In chapters 5 and 6, attention is also devoted to the prevention of invasive aspergillosis (IA) and NTM-PD. NTM and *Aspergillus* species are often already present in the recipient prior to transplantation and may reoccur after LTx. For example, in chapter 6 we showed that LTx candidates with a positive *Aspergillus* culture prior to LTx have a higher change of IA post-LTx. In addition, chapter 5 shows that failure of sputum conversion of NTM-PD pre-LTx is associated with a worse outcome of NTM-PD post-LTx. The likelihood of recurrence for NTM and aspergillosis after transplantation is quite high due to colonization of the upper airways and spillover of the pathogen into the thoracic cavity during LTx.

The question of how to prevent NTM-PD post-LTx remains unanswered. The review in chapter 6 demonstrates that prolonged treatment of NTM before LTx provides no additional benefit and is actually associated with a higher risk of mortality due to NTM-PD after LTx. This effect may be explained by the fact that the sickest patients pre-LTx are treated for the longest duration and are therefore at the greatest risk of mortality. Alternatively, it could be that patients with resistant strains were treated longer, or that resistance developed as a result of prolonged treatment. Therefore, it would be advisable to limit treatment to a single course for a positive NTM culture, avoiding prolonged regimens, as they do not impact post-LTx NTM-PD related mortality. It is also important to note that the waiting time for LTx is never fixed. This makes it very challenging to tailor the treatment duration to the time on the waiting list. Post-LTx, recipients are immunocompromised, particularly in the immediate post-transplantation period. So, aggressive pre-emptive treatment and performing a control bronchoalveolar lavage (BAL) immediately post-LTx could well be considered to be implemented as a routine.

A similar approach applies to aspergillosis. A single eradication course prior to transplantation may be beneficial, but prolonged treatment, with the associated risks of toxicity, (azole) resistance and the uncertainty of the waiting time until transplantation, is not advisable. Prophylaxis strategies immediately after LTx remain debatable. In chapter 6 we demonstrated that nebulizing amphotericin B (AmB) may not be sufficiently effective in preventing IA post-LTx. Statins, due to their potential mechanism of action, may be a promising alternative. This corresponds with another observational study which suggested that statin use was independently associated with a reduced risk of IA.[13] Statins function as competitive inhibitors targeting 3-hydroxy-3-methylglutaryl coenzyme A reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, a crucial step in the synthesis of cholesterol in

humans and ergosterol in fungi. Ergosterol is a crucial component of the cell membrane in fungi, and they cannot survive without it.[13,14] However, not all analyses conducted in the *Aspergillus* study in chapter 6 confirm this. An alternative but not very attractive option is the prophylactic use of azoles, which introduce interactions with calcineurin inhibitors (CNI), increasing the risk of nephrotoxicity. Therefore, in collaboration with other centers, we are working on a multicenter study to compare different preventive treatments for aspergillosis in LTx recipients. In addition to exploring pathogen-directed preventive measures, research should also focus on adjusting immunosuppression in LTx candidates at risk for NTM-PD or aspergillosis post-LTx.

Diagnosing infectious disease in lung transplant patients: distinguishing between disease and colonisation

To develop a treatment plan for infectious diseases following LTx, it is crucial to first determine whether the condition represents colonization or disease. This distinction is essential to avoid both under- and overtreatment. Undertreatment may result in the progression and dissemination of infection, while overtreatment may lead to adverse effects, drug–drug interactions, toxicity, and the development of resistance.

For the diagnosis of IA, the European Organization for Research and Treatment for Cancer (EORTC) definitions are available; however, these definitions were established for cancer, stem cell, and solid organ transplant patients, but are not specifically tailored for LTx recipient.[15] Additionally, the International Society for Heart and Lung Transplantation (ISHLT) guidelines provide a definition of IA established by an international and multidisciplinary panel of experts, in the absence of clinical trials.[16] While the EORTC criteria are not specific for LTx patient, the ISHLT criteria mandates bronchoscopy for the diagnosis IA, while the supporting reference does not support this.[17] The ISHLT criteria might be well-suited for research purposes, their implementation in clinical practice poses challenges, particularly because they specify the need for bronchoscopy. In many cases, an IA diagnosis can be confidently established based on symptoms, sputum culture results and CT imaging alone, without the need for bronchoscopy (chapter 6). Furthermore, in our clinical experience, performing a bronchoscopy does not often alter patient management. Patients with typical CT findings, declining lung function, and a positive sputum culture are typically treated for aspergillosis regardless of BAL confirmation. Routine bronchoscopies in patients with positive sputum cultures may not provide additional benefit and can be burdensome for patients, as well as for bronchoscopy resources, especially given the high prevalence of aspergillosis among LTx recipient. Chapter 6 shows indeed, in a large Dutch cohort of 274 LTx recipients, 40% had

positive *Aspergillus* cultures at some point post-LTx. In addition, CT analysis for detecting IA demonstrates a high sensitivity of 90.3% and a specificity of 89.5%, making it highly useful even in the absence of bronchoscopy.[18] On the other hand, without routinely performing a BAL, it remains unknown whether systematic testing would alter the policy. Unfortunately, there is little literature available on this topic in LTx recipients.

The same applies to NTM-PD. Its diagnosis relies on the American Thoracic Society/Infectious Diseases Society of America statement, which, however, is not specific to transplant recipients and not specifically tailored for LTx recipients. Clinical, radiographic, and microbiologic criteria are equally important, and all must be met to establish a diagnosis of NTM-PD.[19] However, the symptoms and CT criteria are not specific to NTM-PD, and in LTx recipients, other potential causes (including alternative pathogens) often cannot be excluded due to the frequent presence of polymicrobial sputum cultures.

To conclude, it is critically important to conduct research to establish the most reliable criteria for diagnosing IA and NTM-PD in LTx recipients in clinical practice.

Treatment of infectious disease post-LTx

Treating infectious diseases post-LTx depends on the pathogen, its susceptibility, and the host. In this context, the host specifically refers to the immunocompromised LTx recipient. The use of immunosuppressants, which predisposes patients to frequent infectious diseases, is a daily concern for every LTx physician. While treatment guidelines exist for the pathogens discussed in this thesis, there are few guidelines on how to manage immunosuppression during infectious diseases.[17,20,21] For COVID-19, the ISHLT has issued a statement on managing COVID-19 in LTx recipients. It recommends the use of remdesivir and monoclonal antibodies (effective against circulating variants), while cautioning about significant drug-drug interactions between calcineurin inhibitors (CNI) and nirmatrelvir-ritonavir. The guidelines also discuss managing immunosuppression during COVID-19 but emphasize that these are only recommendations, as adjustments to immunosuppression in LTx recipients have not been well studied.[21]

In our center, and likely in more transplant centers, it is common practice to discontinue MMF in LTx recipients with infectious diseases, including COVID-19. This approach is based on the hypothesis that reducing the immunosuppressive effects of MMF, may facilitate more effective clearance of the infectious diseases. However, MMF in vitro also exhibits antiviral properties against various viruses, such as parainfluenza virus, influenza viruses, and Middle East respiratory syndrome coronavirus.[22–24] From a virological perspective, it might therefore

be more logical to reduce doses of CNIs than of MMF. Nevertheless, CNIs are the cornerstone of immunosuppression in LTx recipients, and reduced or fluctuating trough levels of CNIs are associated with an increased risk of chronic lung allograft dysfunction (CLAD).[25,26] This risk is one of the primary reasons MMF is discontinued rather than CNIs, despite the limited evidence supporting this practice. Studies in solid organ transplant recipients with COVID-19 suggest that those on MMF are at a higher risk of hospitalization compared to patients not receiving MMF.[27] Additionally, chapter 2 highlights that in the Dutch post-LTx COVID-19 population, antimetabolites were reduced in 57% of cases. However, the true benefit of this intervention, and the specific patient populations that might benefit, remain uncertain. Only isolated case reports suggest that reducing or discontinuing MMF may result in shorter hospitalization periods for solid organ recipients with COVID-19.[27] On the other hand, cohort studies focusing on LTx recipients found no association between MMF dosage and the development of CLAD.[28,29] Given these uncertainties, further research is needed to evaluate immunosuppression adjustments during infections. This remains an underexplored area with significant potential for targeted therapeutic interventions, and potential positive effects on transplant function.

Guidelines for the treatment of NTM-PD are already available; however, they do not specifically address issues related to LTx. A thoracic transplantation Delphi panel has recommended initiating treatment for *Mycobacterium abscessus* at least 3 months prior to listing for LTx. However, the panel did not evaluate the optimal duration of therapy post-LTx or the adjustments required for immunosuppression or induction therapy. Additionally, other mycobacterial species were not included in the survey.[30] In chapter 5, we present a systematic review on the treatment of NTM-PD. This review reveals a high rate of NTM-PD post-LTx in patients with a history of pre-LTx NTM-PD (46%), emphasizing the importance of continuing treatment rather than discontinuing it immediately after LTx. However, the heterogeneity in the agents used, the variable duration of post-LTx treatment, and limited information on drug susceptibility make it challenging to establish definitive recommendations for managing post-LTx NTM-PD. Notably, patients who did not achieve sputum conversion prior to LTx were more likely to develop NTM-PD post-LTx than those who achieved sputum conversion. This highlights the importance of aiming for sputum conversion before transplantation. If sputum conversion is not achieved, initiating NTM treatment immediately post-LTx is advised to prevent post-LTx NTM disease. For drug selection, combination therapy guided by drug susceptibility testing in accordance with existing NTM guidelines is recommended.[31] Further research is essential in this area, and given the rarity of NTM-PD, collaboration between transplantation centers is crucial to advance our understanding and improve patient outcomes.

Treatment for IA is described in chapter 6. Most patients are treated with monotherapy with voriconazole which is in accordance with Dutch and international guidelines.[16,32] In these, voriconazole is recommended as first-line therapy for acute IA with proven voriconazole susceptibility. To date, no randomized trials of combination therapy for IA in LTx recipients have been performed.[16] Therefore combination therapy should be reserved for LTx recipients with azole resistant IA and those admitted to the intensive care unit.[32] The limited antifungal therapeutic options post-LTx are further challenged by drug–drug interactions between CNIs and azoles which may result in adverse events. The development of additional antifungal drug options remains a critical need. Over the past 2 decades, no new classes of antifungal agents have been introduced into clinical practice. Encouragingly, several novel antifungal drugs are currently in development, with promising candidates including fosmanogepix (a novel Gwt1 enzyme inhibitor), ibrexafungerp (a first-in-class triterpenoid), olorofim (a novel dihydroorotate dehydrogenase enzyme inhibitor), opelconazole (a triazole optimized for inhalation), and rezafungin (an echinocandin designed for once-weekly dosing).[33]

One open-label, randomized phase IIb study (OPERA-S) assessing the safety and tolerability of inhaled opelconazole for the prophylaxis and treatment of IA in LTx recipients has been completed, and phase III results are pending.[28] Rezafungin, an echinocandin with a robust safety profile and promising in vitro efficacy, also holds potential in LTx recipients. As a prophylactic agent, it may provide protection against invasive fungal diseases caused by *Candida*, *Aspergillus*, and *Pneumocystis jirovecii* species, potentially replacing multidrug prophylactic regimens.[34,35] Furthermore, its limited drug–drug interactions could be advantageous, particularly during the early post-transplantation period.[33] Despite the promise of these emerging antifungal agents, ongoing research and the development of drugs from novel antifungal classes remain essential to expand and sustain the antifungal armamentarium in LTx recipients.

Effect of infectious diseases on chronic lung allograft dysfunction

Ultimately, the prevention, diagnosis, and treatment of infectious diseases aim to prevent CLAD. In his thesis, de Zwart demonstrates that viral respiratory infectious diseases increase the risk of CLAD by 2–3 fold.[36] Chapter 2 explores whether COVID-19 also impacts CLAD in a cohort of 274 Dutch LTx recipients. Although lung function declined 3 months after infection and gradually improved by 6 months, only 7 LTx recipients developed new-onset CLAD. Of these, 3 LTx recipients were diagnosed with obstructive CLAD, and 4 with restrictive CLAD. In studies on non-COVID respiratory infectious diseases, restrictive CLAD phenotype is less common compared to obstructive phenotype. However, the predominance of restrictive

CLAD observed in COVID-19 LTx recipients was similarly reported by Mahan et al.[37] The seemingly lower risk of CLAD in LTx recipients with COVID-19 may be influenced by the fact that approximately 20% of the LTx recipients in the study died, precluding the availability of lung function parameters for these individuals (chapter 2). Among LTx recipients who had pre-existing CLAD prior to COVID-19, there was no progression of CLAD observed, although their mortality rate was higher compared to non-CLAD patients. The study presented in chapter 2 was conducted at the time when the COVID-19 Delta variant was present, when 20% of LTx recipients admitted with COVID-19 died. Currently, milder SARS-CoV-2 variants are circulating, and LTx recipients are better protected through vaccination or prior SARS-CoV-2 infection. Therefore, it would be valuable to reassess the impact of COVID-19 on CLAD in the context of the current SARS-COV-2 era.

In addition to viral infections, other infectious diseases may also be associated with the development of CLAD.[38–41] In chapter 6 we describe a clear association between IA and CLAD which is in line with other studies.[41–44] Furthermore, in the systematic review presented in chapter 5 we demonstrate that LTx recipients with NTM-PD pre-LTX who developed NTM-PD post-LTx had a higher incidence of CLAD compared to those who did not develop NTM-PD post-LTx (28% vs. 20%). However, this difference was found not to be statistically significant. Notably, this review exclusively analysed patients with NTM-PD diagnosed prior to LTx, a population that may already have an elevated baseline risk. Additionally, the time to CLAD onset in this group of LTx recipients was not clearly defined. Other studies showed that especially *Mycobacterium abscessus* and *Mycobacterium avium* complex are associated with CLAD.[38,39]

These findings, as described in chapters 2, 5, and 6, suggest that fungal, viral, and bacterial infectious diseases are all associated with the development of CLAD. Consequently, they warrant the attention of transplant physicians in terms of prevention, diagnosis, and treatment.

Part II

The second part of this thesis focuses on the effects of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)-modulator Elexacaftor/Tezacaftor/Ivacaftor (ETI) in people with CF (pwCF) after lung transplantation (LTx). In chapter 8 we describe a national multicenter prospective study investigating the effects of ETI post-LTx (the KOALA study) in pwCF. After a 3-month follow-up, ETI demonstrated positive effects on chronic rhinosinusitis (CRS) complaints, gastrointestinal (GI) complaints, and quality of life.

Several questions arise that merit discussion. First, one might question whether a 3-months follow-up period is sufficient and what can be expected in the long run. Second, are the side effects of ETI acceptable? Third, it is worth considering whether the benefits of ETI justify its costs? Finally, there are pharmacological concerns regarding the interactions of ETI with immunosuppressive therapy, therapeutic drug monitoring, and the minimally required dosage. These topics will be addressed in the discussion below.

Short and long term follow-up of ETI

The KOALA study measured the effect of ETI on extrapulmonary symptoms after 3 months, long-term results are not yet available. However, previous research indicates that, separate from LTx, the effects of ETI, for example on lung function and weight, reach their maximum at 3 months and show no significant improvement beyond six months.[45] CRS symptoms improvements may occur within 7 days and plateaus after 3 months of therapy.[46] The RECOVER study showed that GI symptoms improve rapidly, being evident in 1 month, peaking in 2 months and stabilizing thereafter.[47] This does make it plausible that the results we found after 3 months may represent the maximum outcomes. Nevertheless, the long-term outcomes of ETI in pwCF post-LTx will need to be awaited.

An intriguing area for investigating long-term outcomes would be to examine the impact of ETI on CLAD. CLAD has been identified as the most common cause of poor survival. Half of LTx recipient develop CLAD within 5 years.[48,49] A direct effect of ETI on CLAD is not likely, as the transplanted lungs do not have CF. However, there might be an indirect effect on CLAD through mechanisms such as the effective management of CRS and GI symptoms, potentially leading to reduced pulmonary infections and aspiration. CLAD is considered a multifactorial syndrome rather than being solely linked to chronic rejection. The transplanted lung is exposed to the external environment and is susceptible to external stimuli, including infection, CRS, and aspiration.[50–55] Aspiration has been identified as a factor associated with CLAD in LTx literature.[52,53] Similarly, CRS is also recognised to be associated with CLAD.[54,55] It can be hypothesised that the alleviation of GI symptoms and CRS through ETI treatment may result in a reduction of the incidence of CLAD or a delay in the onset of CLAD. Investigating this issue within our cohort is challenging, as the median time to initiate ETI was already 11 years post-transplant. In our cohort CLAD was present in 24% of the pwCF before starting ETI. In addition, the necessity for a matched control group with pwCF post-LTx not treated with ETI is imperative, which is not a feasible undertaking.

Adverse events of ETI

In addition to the positive effects, ETI appears to be safe and well-tolerated. Despite the potential for interactions between ETI and immunosuppressive medications post-LTx, as well as the possibility that medication may not be well-tolerated due to transplant-related comorbidities such as delayed gastric emptying, the incidence of adverse events (AEs) is comparable to that observed in pwCF without LTx. Approximately half of pwCF, regardless of LTx status, experiences at least one therapy-related AE. These AEs are typically mild to moderate in severity and resolve spontaneously or with dose adjustments.[56–58]

The most common AEs in pwCF without LTx include GI complaints, dermatological issues, mental health decline, and transaminitis, which were similarly reported in pwCF post-LTx in the KOALA study.[56–58] While no patients discontinued ETI therapy in most clinical trials involving pwCF without LTx, one survey reported that 5% of respondents discontinued ETI.[59] In contrast, in the only other study of Ramos et al. examining ETI post-LTx in pwCF, 42% of patients discontinued ETI due to the aforementioned AEs.[60]

In the KOALA study, 5 pwCF (9%) discontinued ETI within three months of follow-up due to AEs, which included psychological symptoms (n = 2), headache (n = 1), a decline in lung function (n = 1), and leg muscle cramps (n = 1). The difference in ETI discontinuation rates between the study of Ramos et al and the KOALA study may be attributed to differences in study design. The study by Ramos et al. was a retrospective cohort study based on electronic health records. Prescription practices varied across LTx programs, reflecting variability at the provider level.[60] In contrast, the KOALA study was a prospective multicenter study in which pwCF post-LTx were required to meet uniform inclusion criteria and received structured guidance. Patients were thoroughly informed about potential AEs, including their tendency to resolve spontaneously, and had 24/7 access to consultation for questions or concerns. This structured approach likely contributed to a lower discontinuation rate in the KOALA study. It is therefore essential to provide pwCF post-LTx with adequate information about AEs based on insights gained from studies in non-transplanted pwCF.

Due to differences in the perceived importance of medication, post-LTx pwCF may be more inclined to discontinue ETI than non-transplanted patients. For non-transplanted pwCF, the goal of ETI therapy is to prevent transplantation, extend survival, and improve or stabilize lung function. In contrast, for transplanted patients, the primary goal of ETI therapy is to alleviate extra-pulmonary symptoms and improve quality of life rather than prolong life expectancy. Consequently, they may be less inclined to tolerate ETI-related adverse events. Furthermore, pwCF post-LTx often have heightened concerns about protecting their transplanted lungs

and may fear that ETI could adversely impact their graft function. However, the KOALA study showed that there was no negative effect on pulmonary function of ETI.

Cost-benefits of ETI in pwCF after LTx

Although the KOALA study demonstrates many positive effects on CRS symptoms, GI complaints, and quality of life, the question remains whether these benefits outweigh the costs of ETI. In the Netherlands, the cost of ETI is €300 per day. These costs are justifiable in non-transplanted pwCF as it can prevent the need for LTx and increase life expectancy.[61–63] However, it is uncertain whether the reduction of extra-pulmonary symptoms post-LTx justifies the expense. Nevertheless, despite undergoing LTx, pwCF continue to require comprehensive management for their multi-organ disease. Post-LTx, these patients frequently encounter significant challenges associated with extra-pulmonary symptoms, which can markedly affect their quality of life. Among these complications, distal intestinal obstruction syndrome (DIOS), CRS, CF-related diabetes, and eating difficulties are particularly notable. CRS, for example, persists in over 60% of pwCF following LTx. It has been hypothesized that the upper airways may serve as a reservoir for microbial colonization of the graft, potentially increasing the risk of rejection.[64,65] This underscores the need for vigilant upper airway management to mitigate risks associated with microbial colonization. GI complications are also common after LTx in pwCF. The incidence of DIOS post-LTx is approximately 20%, yet no evidence-based regimen exists for its optimal management in this setting.[65,66] Furthermore, delayed gastric emptying, often observed in pwCF post-LTx, increases the risk of complications such as gastroesophageal reflux disease and micro-aspiration.[65,67] These issues are particularly concerning, as aspiration is a known contributor to the development of CLAD.[52,53] Despite its clinical significance, there are currently no validated strategies to guide the optimal management of these GI complications in pwCF post-LTx.[53] The high prevalence and multifaceted nature of extra-pulmonary complications post-LTx in pwCF emphasize the critical importance of comprehensive post-LTx care to optimise outcomes and improve quality of life. The KOALA study demonstrates that these extra-pulmonary manifestations are effectively treatable with ETI contributing to improved quality of life. Considering the limited life expectancy post-LTx (10 years survival 57.8%), it is particularly crucial to prioritize and optimize quality of life during this relatively short lifespan.[68] ETI provides a significant contribution to this improvement and might be a worthwhile investment. However, a comprehensive cost-benefit analysis would be valuable and is recommended for future research. Such an analysis should also evaluate the impact of ETI on chronic CLAD, as delaying the onset of CLAD could further enhance the benefits of ETI.

The KOALA study aims to encourage other countries to provide pwCF post-LTx the opportunity to receive treatment with ETI. It is essential to address disparities in health care in the global CF community to ensure optimal care for all pwCF including those after LTx. Additionally, the patent for ETI will eventually reach its expiry date. This will result in a reduction in costs and greater accessibility for all pwCF.

In the future, fewer pwCF will require LTx, and those who are transplanted will likely have mutations for which ETI is not yet approved. In addition, there will always be patients who do not respond well to ETI and ultimately undergo a LTx.[62] If these patients benefit from ETI for their extra-pulmonary symptoms, it could be continued after transplantation. The KOALA study has provided valuable insights into managing ETI post-transplantation and understanding its interactions with immunosuppressants.

Pharmacology of ETI

One of the main reasons ETI was initially not prescribed to pwCF post-LTx is the concern about interactions between ETI and immunosuppressive medications, especially CNIs which are the fundamental component of immunosuppression post-LTx. The extent to which the interactions between ETI and CNIs were significant, and their implications for the patient, are not well understood. Only a few small studies have described these interactions, yielding varying results.[69–71] The theoretical underpinnings of these interactions, however, are well established. At first, both ETI and CNIs are substrates of permeability glycoprotein (P-gp). P-gp is an efflux transporter present on the apical side of the intestinal epithelium that prevents intracellular accumulation of CNIs and ETI by increasing their efflux to the intestinal lumen.[72] In theory, if more than one substrate competes to bind with P-gp, it decreases the likelihood of the drugs being pumped out of the cell, resulting in accumulation of the drugs. Second, ETI may increases the GI absorption of CNIs. Third, metabolization of ETI can be influenced by medication that are CYP3A4 liver enzyme inductors or inhibitors such as CNIs. The KOALA study has provided greater insight into the interactions between ETI and CNIs. One of the key findings from the study is the recommendation to reduce CNI dosage by 30% when initiating ETI while closely monitoring CNI through levels and kidney function. Notably, in the KOALA study, renal function declined immediately after the initiation of ETI. Although this stabilized after reducing the CNI dose, it did not return to baseline. Since ETI is minimally cleared renally, the decline is most likely due to rising CNI levels immediately after starting ETI. It is possible that this decline in renal function can be prevented by adjusting the CNI dose by approximately 30% at the start of ETI. Additionally, the long-term effects of ETI on renal function should be examined over a longer follow-up period.

Finally, little is known about the required ETI dosage after LTx. The same ETI dose is recommended for all adult patients, irrespective of their weight, age, or whether they have undergone a LTx. As the interindividual variability in pharmacokinetics for both ETI and CNIs seems large, this "one-dose-fits-all" strategy may not be applicable to all pwCF after LTx.[73,74]

In non-transplanted pwCF, studies are already exploring whether lower doses of ETI can achieve similar effects.[75,76] Additionally, exposure of ETI may be monitored by assessment of the minimum blood plasma concentration(Cmin) levels.[73] Once more is known about reference values, therapeutic drug monitoring of ETI could be a useful tool to increase efficacy and decrease the risk of adverse effects in pwCF after LTx who use CNIs.

Summary of future perspectives

In summary, to effectively prevent CLAD caused by infectious diseases and to deepen our understanding of the role of ETI in the extra-pulmonary manifestations of CF in LTx recipients, significant progress is still required, necessitating continued research and collaborative efforts.

Preventive measures for infectious disease in LTx recipients

When discussing preventive measures for infectious diseases in LTx recipients one of the key points it to improve vaccination response to SARS-COV-2 in LTx recipients. Obviously, this is most easily achieved by vaccination prior to transplantation. In patients post-LTx, one potential vaccination strategy could involve assessing TTV load to identify patients with a low likelihood of mounting an immune response and temporary lower immunosuppression before vaccination. Specifically, studies should investigate the impact of MMF discontinuation on vaccine response in LTx recipients with high TTV load, determine the optimal duration of MMF discontinuation prior to vaccination, and evaluate the associated risk of rejection.

To gain a better understanding of the optimal prophylactic strategy for invasive aspergillosis, we are in the process of establishing a multicenter study to compare different prophylactic approaches, namely nebulized Amphotericin B, azoles, and the absence of prophylaxis.

For the prevention of all infectious diseases, in addition to exploring pathogen-directed preventive measures, research should also focus on adjusting immunosuppression in LTx recipients at risk, since there are no current guidelines on this matter.

Diagnosis of infectious diseases in LTx recipients

There is a lack of robust practical diagnostic criteria for NTM and IA in LTx recipients. It is critically important to conduct research to establish the most reliable criteria for diagnosing IA and NTM-PD in clinical practice.

Treatment of infectious disease in LTx recipients

In order to treat LTx patients with infectious diseases, the focus should not only be on treatment for the pathogen, but further research is needed to evaluate immunosuppression adjustments during infectious diseases. This remains an underexplored area with significant potential for targeted therapeutic interventions.

For IA ongoing research on new developed drugs from novel antifungal classes remains essential to expand and sustain the antifungal armamentarium in LTx recipients. It may be necessary to collaborate with other organ transplant physicians to set up clinical trials with novel antifungal therapies to include more patients.

Infectious disease and CLAD

Viral, fungal and bacterial infectious diseases are all associated with CLAD. For COVID-9 this is unclear. It would be valuable to reassess the impact of COVID-19 on CLAD in the context of the current SARS-COV-2 era.

ETI in pwCF post-LTx

An intriguing area for investigating long-term outcomes would be to examine the impact of ETI on CLAD. There would likely be no direct effect of ETI on CLAD, as the transplanted lungs do not have CF. However, there might be an indirect effect on CLAD through mechanisms such as the effective management of CRS and GI symptoms.

Considering the limited life expectancy post-LTx, it is particularly important to prioritize and optimize quality of life during this relatively short lifespan. ETI provides a significant contribution to quality of life and might therefore be considered a worthwhile investment. However, a comprehensive cost-benefit analysis would be valuable and is recommended for future research.

The same ETI dose may not be applicable to all pwCF after LTx. Ultimately, measuring Cmin levels of ETI might contribute to personalised medicine of CF-post LTX, which might increase efficacy and decrease the risk of adverse effects.

In the future, the need for LTx among pwCF will decrease. However, some patients will still require LTx due to an inadequate response of pulmonary function to ETI. In these cases, ETI could be continued post-transplantation to manage extra-pulmonary manifestations of CF. The KOALA study has provided valuable insights into managing ETI post-transplantation and understanding its interactions with immunosuppressants.

The KOALA study aims to encourage other countries to provide pwCF post-LTx access to treatment with ETI. It is essential to address disparities in health care in the global CF community to ensure optimal care for all pwCF including those after LTx. Additionally, the patent for ETI will eventually reach its expiry date. This will result in a reduction in costs and greater accessibility for all pwCF.

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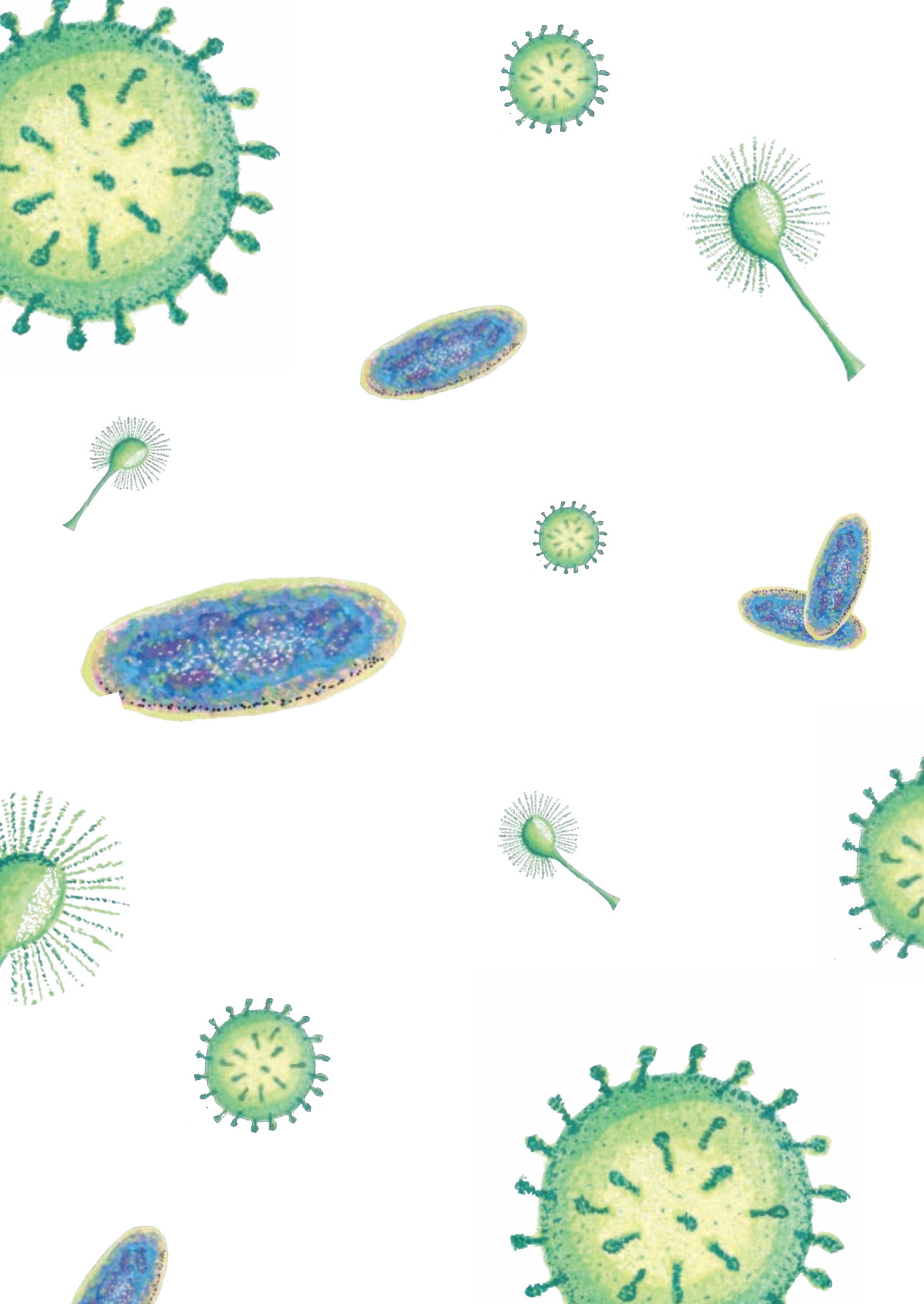

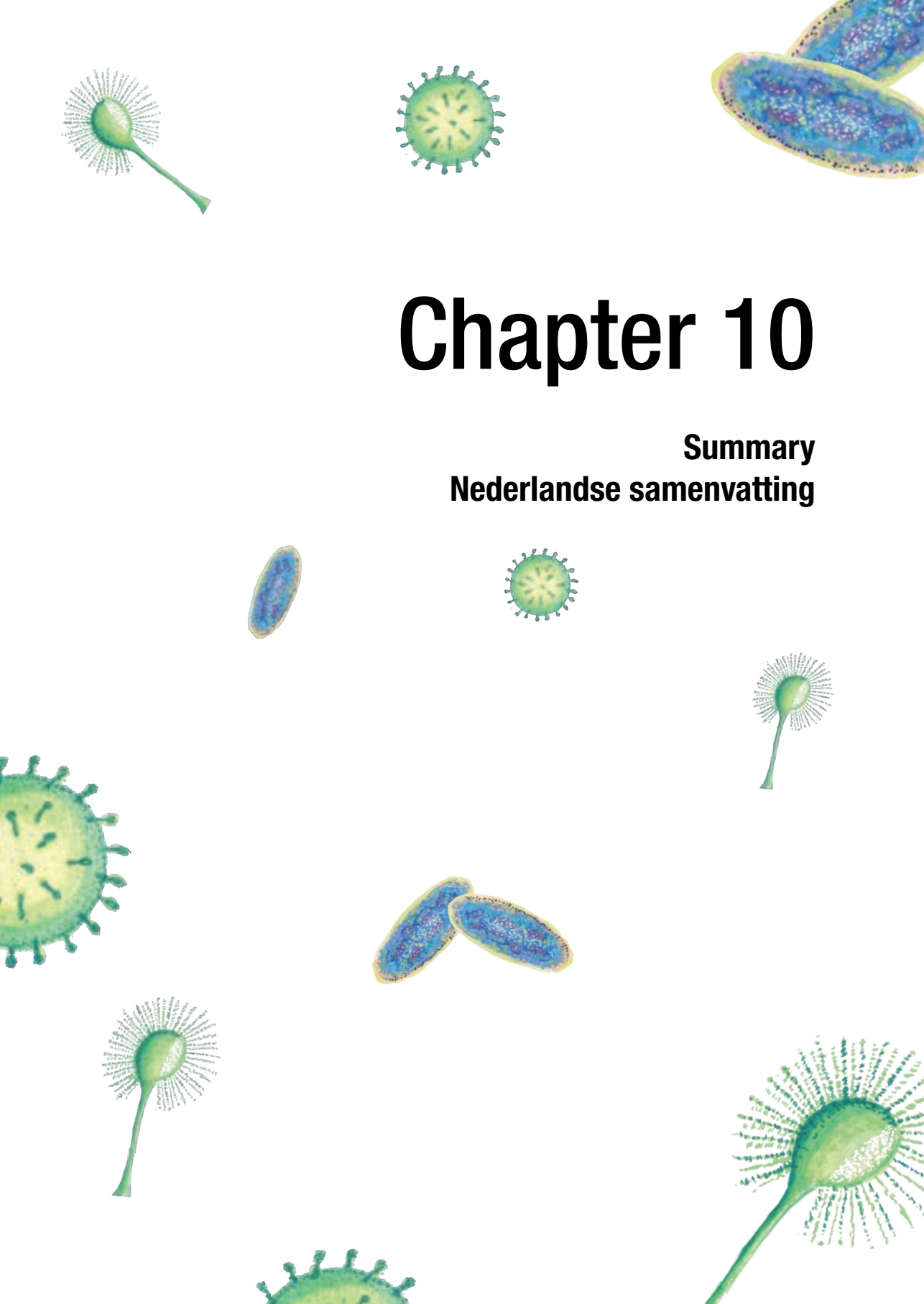

Chapter 10

Summary
Nederlandse samenvatting

Summary

The first aim of this thesis was to map the spectrum of infectious diseases in lung transplant (LTx) recipients, explore optimal treatment strategies, and evaluate preventive measures to reduce the risk of infectious diseases post-LTx. Specific attention is given to infectious diseases caused by SARS-CoV-2, *Aspergillus* and nontuberculous mycobacteria (NTM).

The aim of the study described in **chapter 2** was to evaluate mortality and the transplant function pre- and post-COVID-19 in LTx recipients of all 3 Dutch transplant centers. We found that, the in-hospital mortality and intensive care unit (ICU) mortality were 20% and 63% respectively in 74 LTx recipients admitted due to COVID-19. Mortality appeared higher in patients with chronic lung allograft dysfuncion (CLAD) than in patients without CLAD. Lung function declined 3 months after infection and gradually improved at 6 months, but remained significantly lower compared to pre-COVID-19 values. The more significant decline in FVC than in FEV1 and FEV1/FVC > 70% suggested a more restrictive CLAD pattern.

In **chapter 3**, we studied the serological IgG antibody response across up to five doses of the SARS-CoV-2 vaccine in LTx recipients. In addition, risk factors for non-response were investigated. Therefore the IgG antibody responses of 292 LTx recipients were analysed. Positive antibody response to 1–5 SARS-CoV-2 vaccinations occurred in 0%, 15%, 36%, 46%, and 51%, respectively, showing that the antibody response to SARS-CoV-2 vaccinations is impaired in LTx recipients. Factors associated with non-response to SARS-CoV-2 vaccines were chronic kidney disease and shorter time since transplantation.

The objective of **chapter 4** was to ascertain the impact of asymptomatic community-acquired respiratory viruses in LTx candidates immediately prior to transplantation on the early post-transplant course. Therefore routinely obtained pre-LTx viral swabs were tested for respiratory viruses. Of the 222 LTx candidates tested, 10.4% had a community acquired respiratory virus. We found no influence of asymptomatic pre-LTx viral carriage on primary graft dysfunction, duration of mechanical ventilation, ICU stay, 30-day mortality rate and the occurrence of acute rejection after LTx. This suggests that testing for respiratory viruses in asymptomatic LTx candidates is not indicated.

In **chapter 5** we described a systematic review on treatment regimen and duration pre- and directly post-LTx, for patients with known NTM pulmonary disease (NTM-PD) pre-LTx. Additionally, risk factors for NTM-PD development post-LTx and for mortality were searched. Sixteen studies were included reporting 92 patients. Of the patients with NTM-PD pre-LTx 46% developed NTM disease post-LTx and the related mortality rate was 10%. Most frequently used

agents were aminoglycosides and macrolides for *Mycobacterium abscessus* (*M. abscessus*) and macrolides and tuberculostatic agents for *Mycobacterium avium complex* (*M. avium* complex). The median treatment duration pre-LTx was 10 months (IQR 6–17) and 2 months (IQR 2–8) directly post-LTx. Longer treatment duration pre-LTx and sputum non-conversion pre-LTx were significantly associated with development of NTM-disease post-LTx and NTM related death. Children were particularly at risk for NTM related death.

The primary goals of the study in **chapter 6** were to identify factors associated with invasive aspergillosis (IA) following LTx and to explore potential protective modalities, including nebulized amphotericin B (AmB) and statin therapy. Additionally, the impact of IA on lung function, the development of CLAD, and mortality was evaluated. *Aspergillus* was cultured in 110 /274 (40%) patients post-LTx; of these 81% were classified as having probable IA. Mycophenolate mofetil (MMF) use, airway stenosis, *Aspergillus* cultured pre-LTx and acute rejection, were significantly associated with an increased risk of IA. Statin use was associated with a decreased risk, while nebulized AmB prophylaxis was not. IA post-LTx was associated with CLAD but not with mortality.

In **chapter 7** we report details about the challenging journey of a 58-year-old male with a progressive fibrotic lung disease who needed a LTx. Residing on the remote island of St. Eustatius, access to specialized medical care was limited, but he was entitled to receive appropriate medical care in the Netherlands. Collaborative efforts among medical teams across the world resulted in a successful transatlantic transport of the patient on awake veno-venous extracorporeal membrane oxygenation (vv-ECMO) to the Netherlands. A successful LTx was performed without complications. The case in chapter 7 shows that vv-ECMO flight transport, when conducted by an experienced team, is a safe procedure for highly selected LTx candidates.

In **chapter 8** we show the 3 months results of the KOALA study. This Dutch multicenter study aimed to evaluate the he benefits and safety of Elexacaftor/Tezacaftor/Ivacaftor (ETI) for people with CF (pwCF) after LTx. The study included 55 pwCF post-LTx, of whom 5 were excluded because of ETI discontinuation due to side effects within 3 months follow-up. Three months results showed favourable effects on chronic rhino sinusitis complaints, gastro-intestinal symptoms, and quality of life, but not on body mass index and HbA1c. Median calcineurin inhibitor (CNI) daily dose had to be reduced from 6 to 4 mg to maintain stable CNI trough levels, due to interactions with ETI. Creatinine increased slightly due to higher CNI through levels directly after start of ETI. Although ETI seems to have positive effect on extra-pulmonary CF symptoms and is safe in pwCF post-LTx, monitoring renal function and CNI trough levels is recommended.

Nederlandse samenvatting

Inleiding

Longtransplantatie (LTx) is een behandeling voor patiënten met ernstige chronische longziekten, wanneer er geen andere therapeutische alternatieven beschikbaar zijn, met als primair doel het verlengen van het leven. De meest voorkomende indicaties voor LTx zijn chronische obstructieve longziekte (COPD), cystic fibrosis (CF), longfibrose en pulmonale hypertensie. Een LTx kan worden uitgevoerd als een enkele of dubbelzijdige procedure.

Om in aanmerking te komen voor een LTx, moeten patiënten eerst een uitgebreid screeningsproces ondergaan gedurende een ziekenhuisopname, om hun geschiktheid voor deze complexe operatie te beoordelen. Zodra patiënten geschikt worden bevonden, worden zij op de LTx wachtlijst geplaatst. De wachttijd tot een LTx varieert afhankelijk van de individuele urgentie, die wordt bepaald door de Lung Allocation Score (LAS), en de beschikbaarheid van donororganen. Wanneer geschikte donorlongen beschikbaar komen, wordt de patiënt opgeroepen voor de transplantatie.

Na de procedure verblijven patiënten doorgaans ongeveer vijf dagen op de intensive care, gevolgd door een ziekenhuisverblijf op de verpleegafdeling afdeling gedurende vier tot zes weken. Het percentage dat overlijdt tijdens het eerste jaar na transplantatie is in Nederland 10%[1]. Na de transplantatie moeten de patiënten levenslang medicatie slikken om te voorkomen dat de nieuwe longen afgestoten worden. Deze medicatie remt het afweersysteem van de patiënt en noemt men immuunsuppressie.

De nieuwe donorwet in Nederland is op 1 juli 2020 in werking getreden. Deze wet introduceerde een opt-out systeem voor orgaandonatie, wat betekent dat alle personen van 18 jaar en ouder automatisch geregistreerd staan als orgaandonor, tenzij ze expliciet kiezen om zich af te melden. Deze nieuwe donorwet heeft geleid tot een toename van het aantal beschikbare donororganen.[1]

Geschiedenis van longtransplantatie

De eerste humane LTx werd uitgevoerd in 1963 in Mississippi.[2] Hoewel de procedure technisch succesvol was, stierf de ontvanger na 18 dagen. Vanaf dat moment werden in de jaren ’60 en ’70 36 experimentele LTx uitgevoerd, maar slechts 2 ontvangers overleefden langer dan 2 maanden, grotendeels vanwege inadequate immunosuppressie.[3] Deze slechte resultaten leidden tegen het einde van de jaren ’70 tot een aanzienlijke daling van het aantal LTx. Nadien verbeterde de immunosuppressieve medicatie waardoor de patiënten langer overleefden en er meer LTx werden uitgevoerd.[4]

In Nederland werd de eerste LTx uitgevoerd in het St. Antonius Ziekenhuis in Nieuwegein in 1989.[5] Het eerste door de overheid erkende LTx-programma werd al in 1986 opgestart in het Academisch Ziekenhuis Groningen, nu het Universitair Medisch Centrum Groningen. Later werden de LTx-programma's van Nieuwegein, Utrecht en Rotterdam ook erkend.[6] Sindsdien hebben aanzienlijke vooruitgangen in het veld geleid tot verbeterde resultaten, met heden een mediane overleving na transplantatie van 10–11 jaar in het Universitair Medisch Centrum Groningen.[1]

Immuunsuppressie en infectie

Eerder werd al benoemd dat immunosuppressieve medicatie essentieel is na LTx om acute afstoting, maar ook chronische afstoting te voorkomen. Chronische afstoting, ook wel chronische long allograft disfunctie (CLAD) genoemd, is wat de longtransplantatiepatiënt het meeste bedreigt en de overleving na longtransplantatie beperkt. De immunosuppressieve medicatie heeft echter ook nadelen. Omdat het afweerremmers betreft, hebben zij een afweeronderdrukkend effect, waardoor LTx-patiënten geen goede afweer hebben tegen infecties. Een simpele virusinfectie kan voor een LTx-patiënt grote gevolgen hebben. Het virus kan moeilijk door de afweer opgeruimd worden en kan daardoor schade brengen aan het transplantaat, wat ook weer kan leiden tot CLAD. Ook de COVID-19 pandemie had grote gevolgen voor longtransplantatiepatiënten. Ook hebben LTx-patiënten veel meer last van andere infecties die bij gezonden mensen niet ziekmakend zijn of niet voorkomen. Goede voorbeelden hiervan zijn schimmelinfecties, zoals aspergillose en infecties met bacteriën zoals non-tuberculeuze mycobacteriën (NTM).

Cystic fibrosis

Cystic fibrosis (CF) is een aangeboren aandoening, waarbij de slijmvormende cellen in het lichaam niet goed werken. Hierdoor is het slijm taaier dan bij mensen zonder CF en wordt de ziekte ook wel taaislijmziekte genoemd. CF is een levensbedreigende aandoening waarbij de longen het meest aangetaste orgaan zijn. Tot voor kort was LTx hiervoor de enige behandelingsoptie. Na transplantatie hebben de longen geen afwijkende slijmvormende cellen meer. Echter, de andere organen hebben wel nog afwijkende slijmvormende cellen, waardoor CF-patiënten ook veel last hebben van hun bijholtes en hun darmen. Daarnaast hebben zij vaak suikerziekte en ondergewicht. Na een longtransplantatie zijn de longproblemen over, echter blijven de problemen van de andere organen bestaan.

Gelukkig zijn er nieuwe medicijnen op de markt voor CF. Elexacaftor/Tezacaftor/Ivacaftor (ETI), verbetert de werking van het CF-eiwit dat door het hele lichaam een belangrijke rol speelt in o.a. de soepelheid van het slijm. Deze medicijnen hebben niet alleen de longfunctie van

CF-patiënten verbeterd, maar ook de klachten van de darmen, bijholtes en suikerziekte.[7] Hierdoor heeft het grote merendeel van de CF-patiënten geen longtransplantatie meer nodig.[8] CF-patiënten die al een LTx hebben ondergaan, kwamen niet in aanmerking voor ETI, zij hebben namelijk al nieuwe longen. Met het oog op de vele niet-longklachten die na de transplantatie blijven, hebben wij in dit proefschrift onderzocht of ook na longtransplantatie het wel geven van ETI meerwaarde heeft bij CF-patiënten.

Het doel van dit proefschrift

Het doel van dit proefschrift is tweeledig. Het eerste doel is om het spectrum van infectieziekten bij LTx-patiënten in kaart te brengen, optimale behandelingsstrategieën te onderzoeken en preventieve maatregelen te evalueren, om daarmee het infectierisico te verminderen. Er wordt specifieke aandacht besteed aan virale infectieziekten, waaronder COVID-19 (hoofdstukken 2–4), aspergillose (hoofdstuk 6) en NTM (hoofdstuk 5).

Het tweede doel is om de voordelen van ETI na LTx bij mensen met CF te onderzoeken. Daarom wordt in hoofdstuk 8 een nationale multicenter prospectieve studie (de KOALA-studie) gepresenteerd, waarin de voordelen en veiligheid van ETI voor mensen met CF na LTx worden onderzocht.

Samenvatting van de resultaten

In **hoofdstuk 2** worden de gevolgen van COVID-19 voor LTx-patiënten beschreven. Dit werd onderzocht in alle 3 de LTx-centra van Nederland. We vonden dat als LTx-patiënten vanwege COVID-19 werden opgenomen in het ziekenhuis, de kans op overlijden 20% was. Als er zelfs opname op de intensive care nodig was, was de kans op overlijden zelfs 63%. De longfunctie nam als gevolg van COVID-19 af en verbeterde geleidelijk na 6 maanden, maar bleef significant lager in vergelijking met de waarden van vóór COVID-19.

In **hoofdstuk 3** beschrijven we het effect van SARS-CoV-2-vaccins bij 292 LTx-patiënten. Dit effect maten we door antistoffen te meten bij deze patiënten na de vaccinatie. We vonden dat na 5 vaccinaties slechts 51% van de patiënten antistoffen had gevormd, hetgeen erg weinig is. Factoren die geassocieerd waren met het niet ontwikkelen van antistoffen na SARS-CoV-2-vaccins waren chronische nierziekte en kortere tijd sinds transplantatie.

Het doel van **hoofdstuk 4** was om de gevolgen van de aanwezigheid van luchtwegvirussen bij patiënten zonder klachten die voor een dooraanbod kwamen te onderzoeken. Als een

patiënt voor een longaanbod komt, wordt hij/zij eerst op de spoedeisende hulp onderzocht om te kijken of de patiënt op dat moment de operatie kan ondergaan. Hierbij wordt standaard een test op luchtwegvirussen gedaan. We vonden dat van de 222 geteste LTx-kandidaten 10,4% een luchtwegvirus had zonder klachten. We vonden geen invloed van deze virussen pre-LTx op het verloop van de transplantatie gedurende de eerste 30 dagen. Dit suggereert dat testen op luchtwegvirussen bij asymptomatische LTx-kandidaten niet geïndiceerd is.

In **hoofdstuk 5** beschrijven we een systematische review over het behandelingsregime en de behandelduur voor patiënten met bekende non-tuberculeuze mycobacteriën-longziekte (NTM-PD) pre-LTx. Daarnaast werden risicofactoren voor de ontwikkeling van NTM-PD na LTx en voor mortaliteit onderzocht. Er werden 16 studies gevonden met hierin 92 patiënten met NTM-PD pre-LTx. Van de patiënten met NTM-PD pre-LTx ontwikkelde 46% NTM-ziekte na LTx en het gerelateerde sterftecijfer was 10%. De mediane behandelingsduur van NTM-PD vóór LTx was 10 maanden (IQR 6–17) en 2 maanden (IQR 2–8) direct na LTx. Langere behandelingsduur pre-LTx en het niet verdwijnen van de NTM uit het sputum pre-LTx, waren significant geassocieerd met de ontwikkeling van NTM-ziekte na LTx en met NTM-gerelateerde sterfte. Kinderen liepen een hoger risico op NTM-gerelateerde sterfte.

De primaire doelen van de studie in **hoofdstuk 6** waren om factoren te identificeren die geassocieerd zijn met invasieve aspergillose na LTx en om potentiële preventieve medicatie te onderzoeken, waaronder vernevelen met amfotericine B (AmB) en het gebruik van statines (cholesterol remmers). *Aspergillus* werd gekweekt bij 110/274 (40%) patiënten na LTx, waarvan 81% een invasieve infectie hadden. Het gebruik van mycofenolaatmofetil (MMF), luchtwegstenose, *Aspergillus* gekweekt pre-LTx en acute afstoting waren significant geassocieerd met een verhoogd risico op invasieve aspergillose. Het gebruik van statines leek beschermend te werken, terwijl AmB-profylaxe niet beschermend leek te werken. Invasieve aspergillose leidde in deze studie tot chronische afstoting, maar niet tot meer sterfte.

In **hoofdstuk 7** rapporteren we details over de uitdagende reis van een 58-jarige man met een progressieve fibrotische longziekte die een LTx nodig had. Deze patiënt was woonachtig op het afgelegen eiland St. Eustatius waar toegang tot gespecialiseerde medische zorg beperkt is. Echter als Nederlands staatsburger had hij recht op passende medische zorg in Nederland. Samenwerking tussen medische teams over de hele wereld resulteerde in een succesvol transatlantisch transport van de patiënt aan een hart-longmachine, per vliegtuig naar Nederland. Na 1 dag op de wachtlijst werd de LTx uitgevoerd zonder complicaties.

In **hoofdstuk 8** tonen we de 3-maanden resultaten van de KOALA-studie. Deze Nederlandse multicenterstudie had tot doel de voordelen en veiligheid van ETI voor patiënten met CF na LTx

te evalueren. Vijfenvijftig patiënten deden mee aan de studie. De resultaten na drie maanden toonden gunstige effecten op chronische bijholte klachten, darmklachten en kwaliteit van leven. Ook bleek deze medicatie veilig. Wel bleek dat de dosering van de immunosuppressieve medicatie verlaagd moest worden vanwege interacties met ETI.

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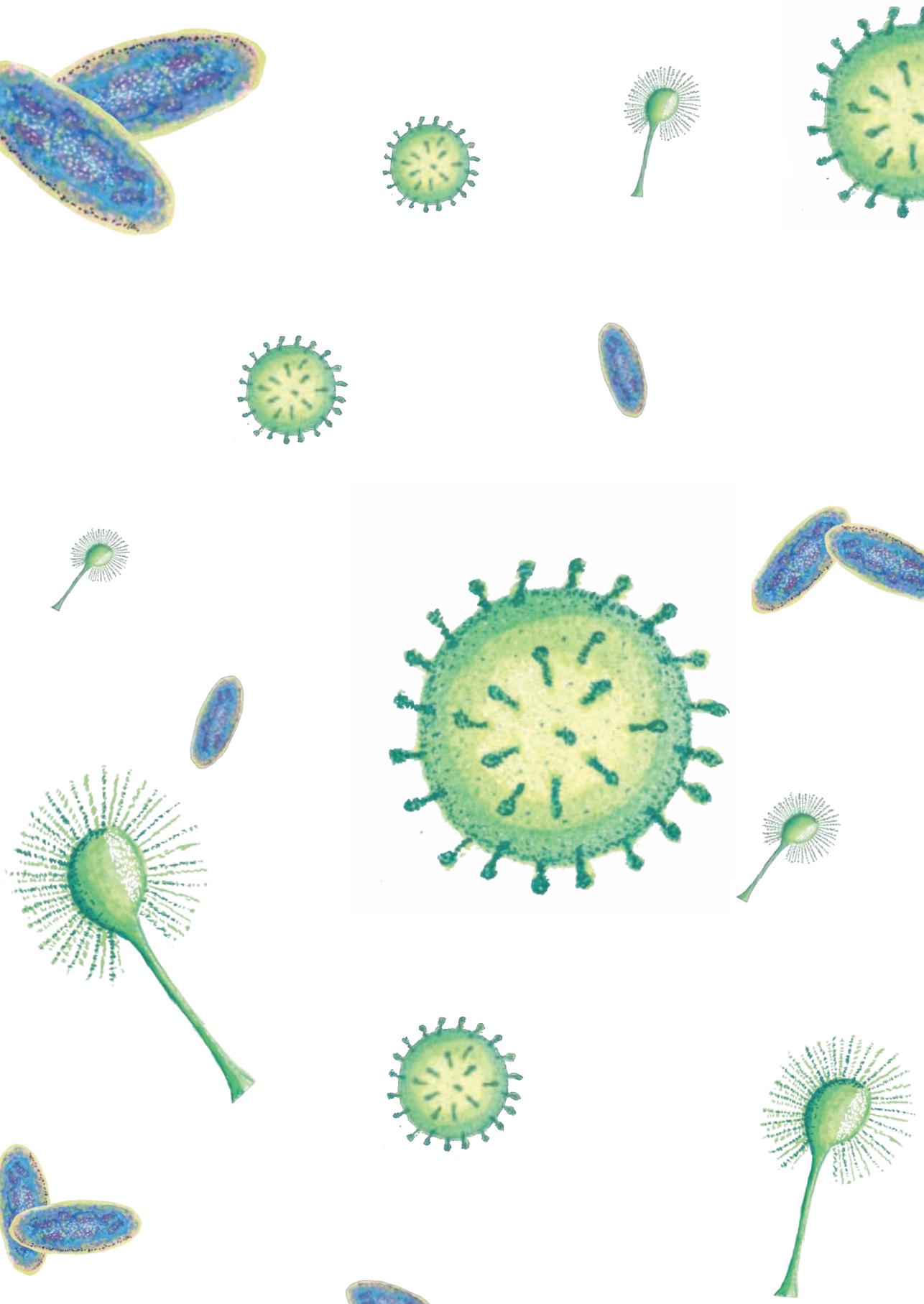
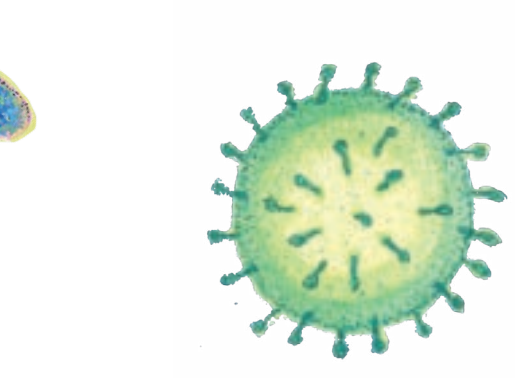
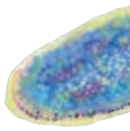
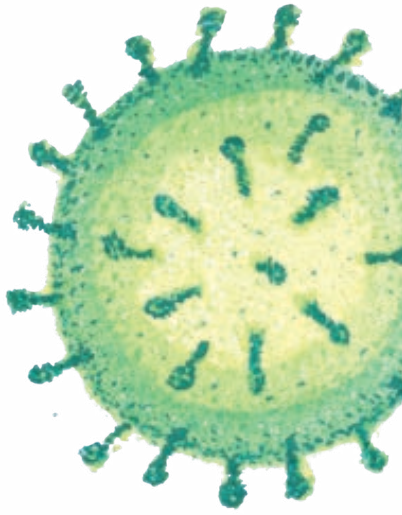


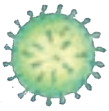
Chapter 11

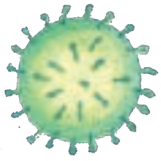
Dankwoord











Dankwoord

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