Optimizing drug therapy in liver transplantation

For better patient outcomes

Midas Berend Mulder

OPTIMIZING DURG THERAPY IN LIVER TRANSPLANTATION

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Optimaliseren van geneesmiddel therapie in levertransplantatie
Voor betere patiëntuitkomsten

OPTIMIZING DRUG THERAPY IN LIVER TRANSPLANTATION
For better patient outcomes

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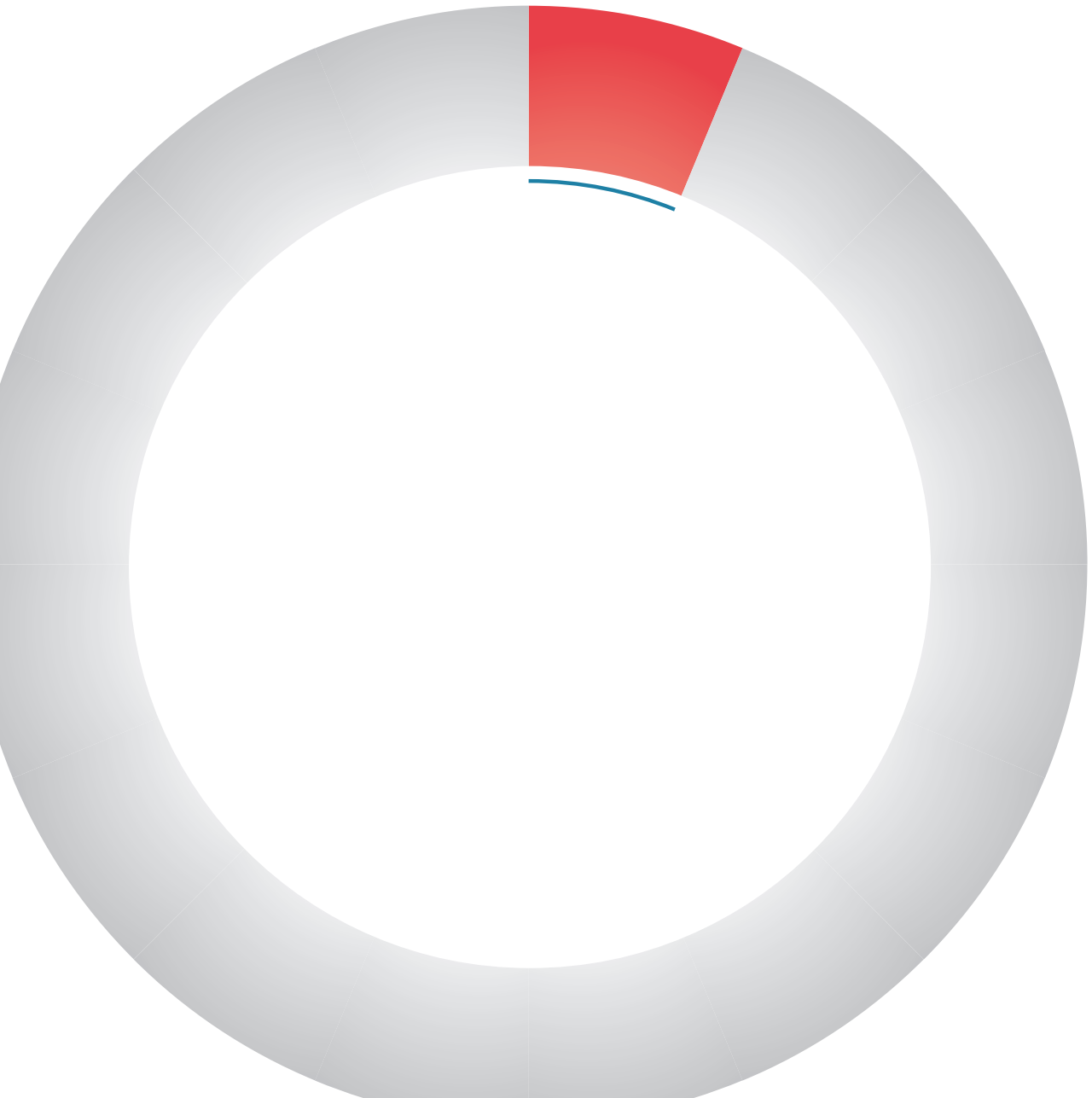
Table of Contents

Part I

General introduction



Chapter 1

General introduction and outline of this thesis

Midas B. Mulder

Liver transplantation

Liver transplantation (LT) is the preferred treatment in patients with end-stage liver disease and hepatocellular carcinoma with 1-year patient survival exceeding 80%. However, survival rates gradually decline over time with 5-year and 10-year patient survival rates of respectively 71 and 61%.(1) Since 1986, approximately 1600 liver transplantations have been performed in 1450 adult patients at the Erasmus University Medical Center, Rotterdam, the Netherlands. Currently, over 750 LT recipients are being seen annually at the outpatient clinic.

An effective immunosuppressive regimen is essential to reduce graft loss due to acute or chronic rejection. However, too much immunosuppression could cause amongst others severe infections. The development of a severe infection in the first years after transplantation is the leading cause of death after LT. Other important complications and causes of death due to the immunosuppressive agents are the development of renal failure, malignancies or cardiovascular events.(2)

General aspects of immunosuppression in liver transplantation

Over the years many different drugs have been developed to achieve adequate immunosuppression and optimal outcomes in LT recipients as shown in figure 1. After transplantation the immunosuppression agents could be divided into agents used in the induction or maintenance phase. Induction agents used in liver transplantation are IL-2 receptor antibodies (e.g., basiliximab) and intravenous corticosteroids. During the maintenance phase calcineurin inhibitors (tacrolimus or cyclosporine), antiproliferative agents (mycophenolic acid or azathioprine) or a mTOR-inhibitor (everolimus or sirolimus) can be used.(3, 4)

The preferred CNI during the maintenance phase is tacrolimus. Tacrolimus is a macrolide antibiotic with immunosuppressive properties and isolated from Streptomyces tsukubaensis, a gram-positive bacteria.(5) Tacrolimus was first investigated and approved for the use in LT by the Food and Drug Administration in 1994.(6) It has a greater immunosuppressive potency compared to cyclosporine and became the cornerstone in immunosuppressive protocols.(7)

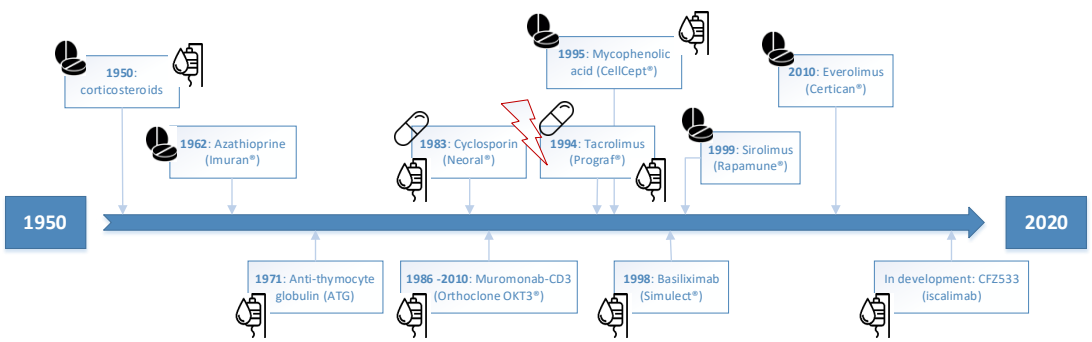


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Figure 1. Development of immunosuppressive agents in liver transplantation

Lightning represents a marker in the development of immunosuppressive agents. With the introduction of calcineurin inhibitors (cyclosporine and tacrolimus), the 1-year graft survival increased from 50% to 90%. Tacrolimus is now the cornerstone in solid organ transplantation. Tablets, capsules and infusions represent the currently available formulations in the clinical and outpatient setting.

Unfortunately, tacrolimus has several side effects as shown in figure 2. For many years research has been done to limit the side effects and toxicity of tacrolimus. Since kidney insufficiency is one of the main limiting side effects on the long term after LT, the last couple of years many studies have been performed to reduce the tacrolimus exposure to preserve the kidney function. The ReSpECT study investigated the delayed introduction of tacrolimus on renal function in LT recipients.(8) In line with this study, tacrolimus as part of the current immunosuppressive protocol of the LT program at the Erasmus MC is postponed and introduced on the fifth day post-transplant in order to prevent for nephrotoxicity in the early days after transplantation. Furthermore, several studies have investigated the combination of low-dosed tacrolimus with another class of immunosuppressive agents, e.g. everolimus or mycophenolic acid and even studies have been performed in which tacrolimus has been stopped.(9-11) In the current clinical practice in LT, if the kidney function of a patient deteriorates to below 50 ml/min/1.73m2 the immunosuppressive protocol for that patient is changed from monotherapy tacrolimus to the combination of low-dosed tacrolimus and another class of immunosuppressive agents.

Besides kidney insufficiency, several other side effects, shown in figure 2, are modifiable risk factors and need long-term management and follow-up by a multidisciplinary LT team. It is known that factors as pre-LT cardiovascular disease, the development of new onset diabetes after transplantation (NODAT), post-LT hypertension and an impaired renal function at 1-year are predictive for developing CVD after LT.(12) Furthermore, a meta-analysis by Konerman *et al.* showed an incidence of CV events of approximately 22% in the first 6 months post LT and 12% in LT recipients more than 6 months post LT.(13) Therefore, prevention of the development and treatment of metabolic syndrome is essential on the long-term after transplantation. Finally, an important side effect on the long-term is the development of malignancies. The most common malignancies directly related to immunosuppression are nonmelanoma skin cancers and post-transplant lymphoproliferative disorder.(14) In 2017 the COMMIT guidance report has been published with practical recommendations for the long-term management of adverse effects related to immunosuppression.(15)

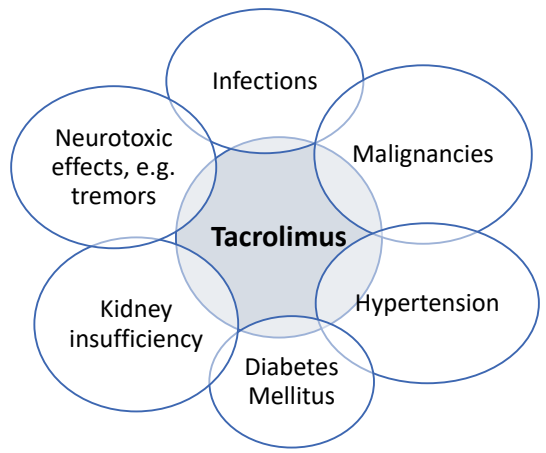

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Figure 2. Side effects of tacrolimus

Quality of life and optimization of drug therapy in LT recipients

After a LT, patients experience a rapid progression in their physical and mental condition. Important factors contributing to the well-being of a LT recipient at the long-term are health-related quality of life (HRQoL) and the severity of fatigue. Quality of life is a multidimensional construct reflecting the physical, mental or psychological, and social dimensions of health.(16) Various studies describe a substantial benefit in the HRQoL after LT, whereas fatigue remains a major factor at the long-term.(17, 18) Due to the development of comorbidities of which some caused by the use of immunosuppressive agents, LT recipients will usually end up with multiple drugs over the years. Since every drug can cause side effects, the addition of drugs might have a negative impact on the well-being of a LT recipient at the long-term.

Adherence to immunosuppressive medication and the avoidance of contra-indicated drugs are two potential modifiable risk factors to improve long-term outcome in LT recipients.(15) Over 30 years of experience, we learned that medication errors contribute to a substantial number of unplanned hospitalizations.(19, 20) LT recipients regularly use over 10 drugs per day which indicates that the involvement of a pharmacist might be useful in order to improve medication safety. However, in the Netherlands and European Union it is very uncommon that pharmacists are directly involved in the post-transplant care, whereas in the Anglo-Saxon countries clinical pharmacists have been involved in the direct patient care in transplantation since the early 1970s.(21)

A gamechanger which showed the relevancy of optimizing drug therapy in LT recipients was the appearance of the new viral infection severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) at the end of 2019. LT recipients have an increased risk of acquiring coronavirus disease 2019 (COVID-19) and for a complicated course because of their immunocompromised state.(22, 23) Several vaccines were developed in only one year after the appearance of SARS-CoV-2 and vaccination is strongly recommended in all LT recipients with no preference for either mRNA or vector-based vaccines.(24). However, studies have shown that the immunogenicity to SARS-CoV-2 vaccines in LT recipients is reduced, with detectable antibodies ranging from 30% - 65%.(25, 26) Optimizing the humoral response to SARS-CoV-2 vaccines in relation to the immunosuppressive agents used is an important aspect for the treating physician and clinical pharmacist in the recent and newly upcoming pandemics.

Chronic Hepatitis E virus infection

Hepatitis E virus (HEV) infection is one of the most common causes of acute viral hepatitis in the world. Although the exact number of human infections due to HEV worldwide is unclear, several studies have shown that a higher number of HEV infections have been diagnosed the last decade due to an increased awareness.(27-30) In immunocompetent individuals, HEV is normally self-limiting and spontaneously cleared.(31) However, in solid organ transplant (SOT) recipients HEV can lead to chronic hepatitis and, if untreated, to cirrhosis. The EASL (European Association for the Study of the Liver) current clinical practice guidelines on HEV infection recommend starting with a dose reduction of the immunosuppressive drugs in transplant recipients with a chronic HEV infection. If this is not possible or unsuccessful, clinicians start a 3-month course of ribavirin (RBV) monotherapy.(31)

The off-label use of RBV as treatment for chronic HEV infection in immunocompromised patients was suggested by several studies.(32-35) Debing *et al.* showed *in vitro* that RBV inhibits HEV replication.(36) Kamar *et al.* showed in vivo that first-line RBV therapy was associated with a sustained virologic response (SVR) in 81.2% of 255 patients.(37) The use of RBV is limited by its side effects: (severe) hemolytic anemia, mood disturbances, sleeping disorders, neuropathy and a decrease in glomerular filtration rate. The most significant side effect in hepatitis C virus (HCV) infected patients treated with the combination RBV and (peg)interferon is dose-dependent hemolytic anemia necessitating dose reduction or discontinuation of therapy.(38-40) Until now, the optimal dose, therapeutic window and treatment duration of ribavirin in transplant recipients is unknown.

Population pharmacokinetic (PK) and pharmacodynamics (PD) modeling is a way to describe and record clinical experience with the behavior of a drug in a certain group of patients. Insights into a drug's PK and PD are used to inform the drug development program and are critical for guiding input and decision-making by regulatory authorities like the European Medicines Agency (EMA) and U.S. Food and Drug Administration. In PK/PD analysis blood levels and clinical aspect of single patients are collected and pooled in order to investigate PK/PD parameters of interest as a population.(41) Non-linear mixed-effect modeling (NONMEM) is applied to estimate PK/PD parameters. In the modeling process a combination of fixed and random effects are estimated in which the fixed effect describes the mean population PK/PD parameters and the random effects describe the variability between or within subjects. Variability in a model could be explained by introducing covariates in the models (e.g. kidney function, body weight or age).(42, 43) The computer software NONMEM® has been used to investigate the optimal dose, therapeutic window and treatment duration of ribavirin.

Aims and outline of this thesis

The overall aim of this thesis is to optimize drug therapy for LT recipients in order to improve patient outcomes. Many studies in this thesis are a collaboration between several centers in the Netherlands and other countries in Europe. Part I focusses on optimizing immunosuppressive therapy and the quality of life in LT recipients. Part II focusses on optimizing therapy for viral complications after transplantation. In part III the addition of a clinical pharmacist in the liver transplant care will be evaluated. Finally, in part V the results of this thesis are summarized and discussed.

Part II – Optimizing immunosuppressive therapy in liver transplant recipients.
Chapter 2 aimed to investigate whether the combination of low-dose sirolimus and low-dose extended-release tacrolimus (interventional group) compared to normal-dose extended-release tacrolimus (control group) resulted in a difference in the renal function and comparable rates of rejection, graft and patient survival at 36 months after transplantation (LOLIII study). Subsequently in **chapter 3**, we investigated the impact of the immunosuppressive regimens in the LOLIII study on the health-related quality of life and the severity of fatigue. Finally, **Chapter 4** of this thesis aimed to evaluate the cardiovascular morbidity and mortality in a subset of the LOLIII study.

In **chapter 5** we investigated whether the Life Cycle Pharma-tacrolimus formulation compared to the extended-release tacrolimus formulation resulted in a difference in the prevalence of post-transplant diabetes mellitus, hypertension and chronic kidney disease at 12 months after liver transplantation in an open-label, multicenter, randomized controlled study (MOTTO study). Next, in **chapter 6** we evaluated the health-related quality of life and severity of tremors in the MOTTO study.

Part III – Optimizing therapy for viral complications after transplantation.

Chapter 7 aimed to define the therapeutic range for ribavirin in transplant recipients with chronic hepatitis E virus infection in a retrospective, multicenter, cohort study. Subsequently, in **chapter 8** we modelled ribavirin plasma concentrations versus virologic response and hemoglobin concentrations. The model was used to select a suitable ribavirin dosing regimen considering efficacy (decrease in viral load) and safety (hemoglobin).

In the beginning of 2021, the first severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) vaccines became available in the Netherlands. In **chapter 9** we evaluated the effect of immunosuppressive blood levels on the IgG SARS-CoV-2 anti-spike antibody response after SARS-CoV-2 vaccination. **Chapter 10** of this thesis aimed to investigate the immunogenicity in liver transplant recipients in relation to mycophenolic acid (the active substance of mycophenolate mofetil) blood levels after a third, fourth or fifth mRNA SARS-CoV-2 vaccination.

Part IV – Addition of a clinical pharmacist in the liver transplant care.

In 2018 a newly established 20-minute face-to-face consultation for liver transplant recipients with the clinical pharmacist was added to the annual check-up of these patients. The consultation consisted of medication reconciliation and a conversation about medication, adherence, adverse drug reactions and drug use. **Chapter 11** aimed to investigate the prevalence, types and severity of medication-related problems and interventions initiated by a clinical pharmacist in a cohort of liver transplant recipients in the outpatient setting. In addition, in **chapter 12** of this thesis we aimed to compare the prevalence and types of medication-related problems and interventions in liver transplant recipients with and without an outpatient medication consultation by a clinical pharmacist as well as the satisfaction with information about medicines and medication adherence.

Chapter 13 presented a case of an African American woman who underwent a liver transplantation in which adequate tacrolimus levels were difficult to accomplish due to differences in cytochrome P450 3A4/5 polymorphisms of the transplant recipient and the donor liver graft. Finally in **chapter 14** we presented three cases in which mycophenolic acid exposure severely decreased after oral antibiotic co-administration.

Part V – Summary and general discussion.

In this part of the thesis, the results and conclusions from the studies described in this thesis are summarized and discussed. Furthermore, in **chapter 15** the future perspectives on the optimization of drug therapy for LT recipients and clinical recommendations will be discussed.

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Part II

Optimizing immunosuppressive therapy in liver transplant recipients

Chapter 2

Three-year results of renal function in liver transplant recipients on low dose sirolimus and tacrolimus: a multicenter randomized, controlled trial.

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Abstract

The aim of this study was to investigate whether the combination of low-dose sirolimus (SRL) and low-dose extended-release tacrolimus (TAC) compared to normal-dose extended-release TAC results in a difference in the renal function and comparable rates of rejection, graft and patient survival at 36 months after transplantation. This study was an open-label, multicenter randomized, controlled trial. Patients were randomized to once daily normal-dose extended-release TAC (control group) or once daily combination therapy of SRL and low-dose extended-release TAC (interventional group). The primary endpoint was the cumulative incidence of chronic kidney disease (CKD) defined as grade ≥3 (eGFR <60 mL/min/1.73m^2) at 36 months after transplantation. In total, 196 patients were included. CKD at 36 months was not different between the control and interventional group (50.8%, 95% confidence interval (CI) 39.7% – 59.9%) versus 43.7%, 95% CI: 32.8% - 52.8%). Only at six months after transplantation, the eGFR was higher in the interventional group compared to the control group (mean eGFR 73.1±15 versus 67.6±16 mL/min/1.73m^2, p=0.02) in the intention to treat population. No differences in the secondary endpoints and the number of serious adverse events were found between the groups. Once-daily low-dose SRL combined with low-dose extended-release TAC does ultimately not provide less CKD grade ≥3 at 36 months compared to normal-dose extended-release TAC.

Introduction

Liver transplantation (LT) is the preferred treatment in patients with end-stage liver disease and hepatocellular carcinoma (HCC), with 1-year patient survival exceeding 80%. After LT, calcineurin inhibitors (CNIs) are the cornerstone of the immunosuppressive regimen, specifically tacrolimus (TAC).(1, 2) The use of TAC has substantially decreased the risk of acute rejection and improved short-term outcomes.(3) However, prolonged use of TAC is associated with significant short- and long-term toxicity, such as nephrotoxicity, diabetes mellitus and hypertension.(4-6) Allen *et al.* and Tapirdamaz *et al.* showed that three years after transplantation an overwhelming majority (>50%) of LT recipients develop chronic kidney disease defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73m^2.(7, 8)

The impact of CNIs on renal function after LT resulted in several strategies to minimize CNI exposure. Several studies have shown that renal function can be effectively preserved by means of a delayed introduction of and reduced exposure to CNI agents in combination with a mammalian target of rapamycin (mTOR) inhibitor.(9-13) A meta-analysis by Lin *et al.* showed that the eGFR increased by 10.2 mL/min (95% CI: 2.75-17.8) in patients using the mTOR inhibitor, everolimus, and low-dose CNI compared to normal-dose CNI at 12 months after the start of this combination. (14)

To date, the combination of TAC and sirolimus (SRL), an mTOR inhibitor, has not been extensively studied on the long-term toxicity. Most studies evaluating the effect of SRL on renal function were small, short-term or initially not designed for this evaluation.(15, 16) Furthermore, an advantage of SRL is the fact that SRL is dosed once daily compared to the twice daily dosing regimen of everolimus. Therefore, the aim of this study was to investigate whether the combination of low-dose SRL and extended-release TAC compared to normal-dose extended-release TAC results in a difference in the renal function and comparable rates of rejection, graft survival and patient survival at 36 months after transplantation.

Materials and Methods

Study design and participants

This study was an open-label, multicenter randomized, controlled trial. Patients were enrolled between February 2011 - March 2018 and prospectively followed for three years or until death. Patients were randomized between 80 and 100 days after LT to 1) once daily normal-dose extended-release TAC (control group) or 2) once daily combination therapy of low-dose SRL and low-dose extended-release TAC (interventional group) (Figure 1). The immunosuppressive therapy could be switched to local practice in cause of patient safety, medical need or preference of treating physician. In the Netherlands, tacrolimus monotherapy is the first line of immunosuppression after liver transplantation. In case of deterioration of the kidney function tacrolimus monotherapy is switched to mycophenolic acid (MPA) in combination with low-dose tacrolimus. Included were adult patients, between 18 and 70 years, after a primary LT or an early (within 14 days after the first LT) retransplantation with a patent hepatic artery, closed abdominal wound and transplanted in one of the three liver transplant centers in the Netherlands. All participants gave written informed consent before any study-related activity. Main exclusion criteria were: multi organ transplantation, biopsy proven rejection two weeks prior to randomization, estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m2, hyperlipidemia refractory to optimal medical therapy (cholesterol >9 mmol/l and/or triglycerides >8.5 mmol/L), signs of recurrent or de novo malignancies or non-HCC malignancies within the past five years, known hypersensitivity to SRL and the use of mycophenolic acid.

The study was performed at three centers in the Netherlands: Erasmus University Medical Center Rotterdam, University Medical Center Groningen and Leiden University Medical Center. The study was approved by the institutional Ethical Committees at these institutions, registered in the EudraCT database (EudraCT: 2009-017843-32) and conducted in accordance with the principles of the declaration of Helsinki.

Study Endpoints

The primary endpoint was the cumulative incidence of chronic kidney disease (CKD) defined as grade ≥3 (eGFR <60 mL/min/1.73m2) at 36 months post-LT. The renal function was measured by serum creatinine and the estimated glomerular filtration rate was calculated using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation.(17) Secondary endpoints included: treated biopsy proven acute rejection (tBPAR), retransplantation, mean eGFR, incidence of de novo diabetes mellitus (NODAT), incidence of and time to de novo or recurrent malignancy, tolerability and safety of the combination SRL and extended-release TAC.

NODAT is defined according to the definition of diabetes mellitus by the World Health Organization (i.e., fasting plasma glucose value of 7.0 mmol/L measured at least on two different occasions or HbA1C >65) and excludes the diagnosis of diabetes prior to liver transplantation. (18, 19)

Randomization and masking

Participants were randomly assigned (1:1) to either the intervention group or the control group according to a computer-generated randomization list. Stratification was done by center, to ensure an equal distribution of both arms in the three participating centers. Blinding of participants and physicians was not applied.

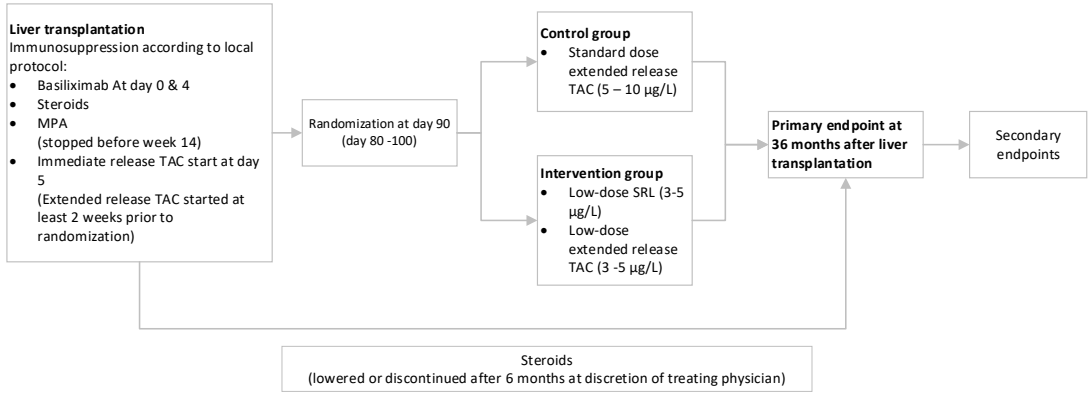

Figure 1. Overview of study design

Abbreviations: TAC, tacrolimus; SRL, sirolimus; MPA, mycophenolic acid

Procedures

Participants were screened within 7 days before randomization. At the time of randomization mycophenolic acid (MPA) had to be discontinued. During the study, dose adjustments of extended-release TAC and SRL resulting in lower trough levels were allowed in case of severe side effects. Furthermore, according to common practice, in the control group higher TAC trough levels were aimed in the first 3 months after transplantation and gradually declined thereafter with a threshold of 5 ng/ml.

Control group: Participants were treated with extended-release TAC with trough levels: 5 – 10 µg/L and 7.5 mg prednisone. Steroids were lowered or discontinued after 180 days at the discretion of the treating physician.

Intervention group: Participants were treated with once daily combination therapy of SRL and low-dose extended-release TAC with trough levels: 3–5 µg/L for both SRL and TAC and 7.5 mg prednisone. Steroids were lowered or discontinued after 180 days at the discretion of the treating physician.

Data collection

Variables collected included recipient socio-demographic, clinical and transplantation parameters, donor details, the quality of life and fatigue severity score, serious adverse events and trough levels of SRL and extended-release TAC.

Statistical analysis

A sample size of 196 patients was planned for this study. On the basis of our preliminary data, the percentage of LT recipients with an eGFR <60 mL/min/1.73m^2 in the control group at 3 years

was estimated at 26.4%. The percentage of LT recipients with an eGFR <60 mL/min/1.73m^2 in the interventional group is estimated to be 15% lower compared to the control group with an alpha (2-sided) of 0.05 and a power of 80%.

Variables were described using counts (%) for nominal and ordinal variables and mean (standard deviation, SD) or median (inter-quartile range, IQR) for the continuous variables, depending on the shape of the distribution.

The primary end point was evaluated with Kaplan–Meier analysis and the log-rank test. Secondary endpoints were analyzed using the student's t-test and Pearson's Chi-square test. For all statistical tests, a two-sided p-value of <0.05 was considered to indicate statistical significance.

A generalized mixed effect model was fitted to examine kidney function over the course of the study. The model additionally included covariates shown to be relevant in previous studies: visit, study group, tacrolimus trough levels, type of donation, recipient age and sex, lab MELD, initial cold and warm ischemic time and the usage of antihypertensive drugs as well as the interaction between visit and the study group. Participant specific random intercepts were included to account for correlation among repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. Missing data were considered as missing completely at random. To visualize the estimated associations, the expected kidney function across the course of the study was calculated while fixing the values of all other covariates to the median or reference category.

Results

Table 1 presents the baseline characteristics of the ITT population at randomization. A total of 196 patients were included (figure 2) and the majority of the patients were transplanted because of HCC (67/196, 34.2%), primary sclerosing cholangitis (37/196, 19.9%) or (non)alcoholic steatohepatitis (31/196, 15.8%). At baseline, the mean eGFR in the control and interventional group was 70.2 ± 16 and 71.8 ± 15 ml/min/1.73m^2, more patients with NODAT were included in the control group compared to the interventional group (15.3% versus 7.1%) and more patients in the interventional group experienced tBPAR (5.1% versus 2%).

During the three-year follow-up a switch in immunosuppressive therapy occurred in 48.9% (48/98) of the patients in the control group and in 44.9% (44/98) of the patients in the interventional group. In the control group the main reason for the switch in immunosuppressive therapy was deterioration of the kidney function (43/48, 89.6%). The other reason for a switch was recurrence of autoimmune hepatitis (5/48, 10.4%). In the interventional group multiple reasons for switching applied. The main reason for a switch were side effects of sirolimus and/or deterioration of the kidney function (29/44, 65.9%). The side effects consisted of pancytopenia (7/29, 24.1%), malaise (6/29, 20.7%), skin problems (n=5/29, 17.2%), anemia (2/29, 6.9%), oedema (2/29, 6.9%), hyperlipidemia (2/29, 6.9%), liver enzyme abnormalities (2/29, 6.9%), hypertension (1/29, 3.4%), proteinuria (1/29, 3.4%) and deep vein thrombosis (1/29, 3.4%). Other reasons for a switch were preference of treating physician with another immunosuppressive agent in case of deterioration of the kidney function (8/44, 18.2%), recurrence of viral infections (5/44, 11.4%) and recurrence of autoimmune hepatitis (2/44, 4.5%).

	TAC (n=98)	TAC+SRL (n=98)
Recipient demographics at randomization		
Age, year (median, IQR)	57.00 (49.50 - 62.00)	54.50 (48.00 - 62.75)
Gender, male (n, %)	72 (73.5%)	72 (73.5%)
Body mass index, kg/m^2 (mean ±SD)	26.54 ± 4.03	25.88 ± 4.01
Ethnicity (n, %)		
Caucasian	85 (86.7%)	81 (82.7%)
Other§	8 (8.2%)	12 (12.3%)
Unknown	5 (5.1%)	5 (5.1%)
Primary Disease (n, %)		
Hepatocellular carcinoma	35 (35.7%)	32 (32.7%)
(Non)alcoholic steatohepatitis	16 (16.3%)	15 (15.3%)
Primary sclerosing cholangitis	21 (21.4%)	16 (16.3%)
Acute liver failure	5 (5.1%)	10 (10.2%)
Cryptogenic cirrhosis	4 (4.1%)	4 (4.1%)
Metabolic disease	5 (5.1%)	4 (4.1%)
Viral Hepatitis	3 (3.1%)	7 (7.1%)
Other‡	9 (9.2%)	10 (10.2%)
Hematology lab		
Hemoglobin, mmol/L (mean ± SD)	7.69 ± 0.89	7.56 ± 0.81
Leucocytes, 10^9/L (mean ± SD)	7.40 ± 2.71	7.17 ± 2.37
Neutrophil granulocytes, 10^9/L (mean ± SD)	5.64 (2.41)	5.32 ± 1.85
Platelets, 10^9/L (mean ±SD)	177.23 ± 67.55	189.10 ± 74.43
Prothrombin, sec (median, IQR)	13.00 (12.00 - 14.25)	13.00 (12.00 - 14.20)
Chemistry lab		
Albumin, g/L (mean ± SD)	44.01 ± 3.96	44.38 (3.83)
Bilirubin, µmol/L (median, IQR)	8.00 (6.00 - 12.00	8.00 (6.00 - 11.00)
Creatinine, µmol/L (mean ± SD)	98.79 ± 21.37	95.33 ± 20.98
eGFR, ml/min/1.73m^2 (mean ± SD)	70.23 ± 15.51	71.77 ± 14.86
Cholesterol total, mmol/L (mean ± SD)	4.76 ± 1.25	4.84 ± 1.11
Glucose, mmol/L (median, IQR)	7.30 (5.90 - 8.90)	6.95 (5.77 - 9.50)
HbA1c, mmol/mol (median, IQR)	38.00 (33.55 - 44.00)	39.00 (34.02 - 45.50)
HD lipoproteïn, mmol/L (mean ± SD)	1.45 ± 0.45	1.40 ± 0.53
LD lipoproteïn, mmol/L (mean ± SD)	2.81 ± 1.05	2.84 ± 0.92
Blood pressure		
Diastolic, mmHG (mean ± SD)	86.49 ± 10.72	82.28 ± 11.76
Systolic, mmHG (mean ± SD)	141.64 ± 20.82	136.67 ± 15.06
Heart rate, beats per minute (mean ± SD)	75.82 ± 11.22	77.70 ±11.06
tBPAR, Yes (%)	2 (2.0%)	5 (5.1%)
New onset Diabetes after Transplantation, Yes (n, %)	15 (15.3%)	7 (7.1%)
Cholesterol medication use, Yes (n, %)	5 (5.1%)	2 (2.0%)
Antihypertensive medication use, Yes (n, %)	33 (33.7%)	28 (28.9%)
Mycophenolic acid use, Yes (n, %)	14 (14.3%)	4 (4.1%)
Tacrolimus blood level, µg/L (mean ± SD)	7.9 ± 2.6	7.5 ± 2.8
Recipient demographics pre-transplantation		
Lab MELD (median, IQR)	16.00 (10.00, 21.75)	17.00 (11.00, 22.00)
High Urgency, Yes (n, %)	7 (7.1%)	11 (11.2%)
Pre-existing Diabetes, Yes (n, %)	16 (16.3%)	26 (26.5%)
Donor demographics		
Age, year (median, IQR)	53.00 (39.25 - 60.00)	52.50 (42.00 - 63.00)
Gender, male (n, %)	51 (52.0%)	50 (51.0%)
Type Donation		
Donation after brain death (n, %)	61 (62.2%)	61 (62.2%)
Donation after circulatory death (n, %)	37 (37.8%)	36 (36.7%)
Living (n, %)	-	1 (1.0%)
Perioperative parameters		
Cold Ischemia time, min (mean ± SD)	417.56 ± 108.29	406.54 ± 131.05
Warm Ischemia time, min (median, IQR)	29.00 (25.00 - 37.00)	27.00 (24.00 - 38.00)

▲

Table 1. Baseline characteristics of the intention-to-treat population at randomization (90 days after transplantation)

Abbreviations: TAC, tacrolimus; SRL, sirolimus; eGFR, estimated glomerular filtration rate; tBPAR, treated biopsy proven acute rejection; SD, standard deviation; IQR, interquartile range

§Other includes: Asian and Afro-American

‡Other includes: primary biliary cirrhosis, secondary biliary cirrhosis, autoimmune cirrhosis, polycystic liver disease

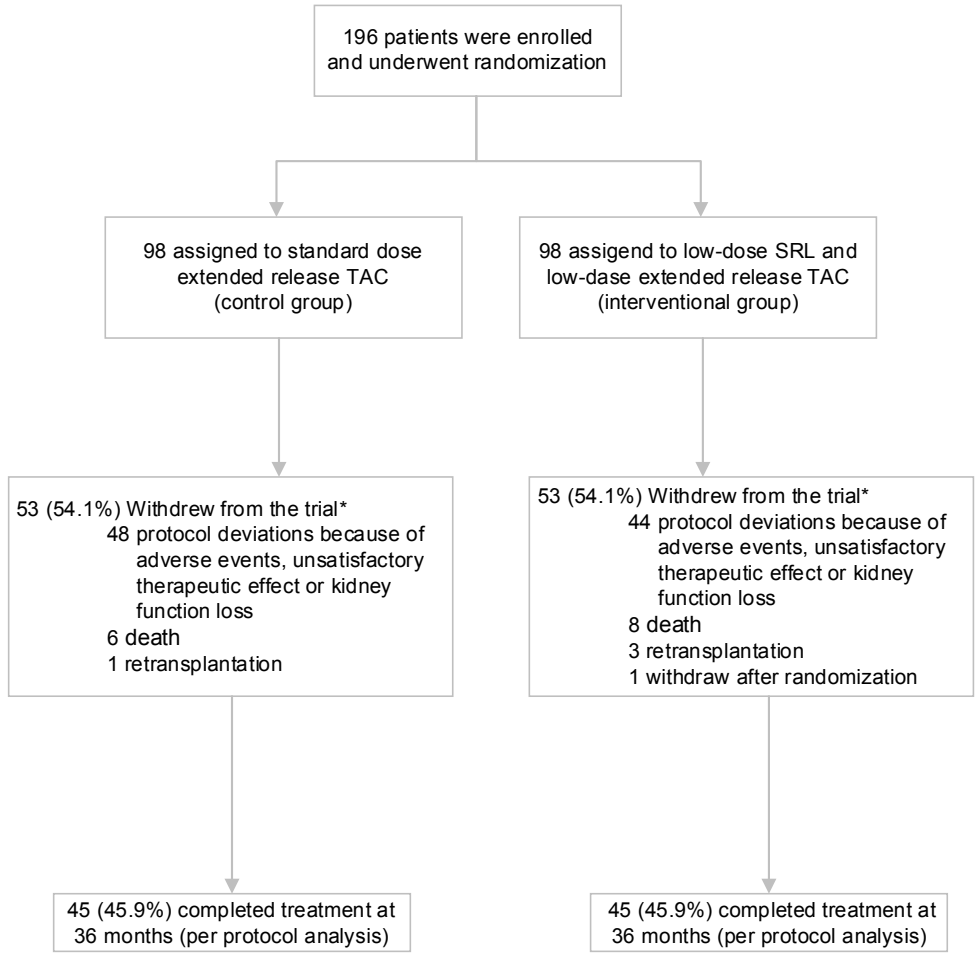

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Figure 2. Enrollment, Randomization, and Follow-up.

Abbreviations: TAC, tacrolimus; SRL, sirolimus

*Some LT recipients experiencing protocol deviations died or had a retransplantation.

Immunosuppression

During the study, mean trough levels for TAC and SRL were within the target range for both groups (table 2). At six months post-LT, the TAC trough levels in the control group were 7.1 (±2.5) µg/L and in the interventional group 5.0 (±1.9) µg/L. At the end of the study, the TAC trough levels in the control group were 5.0 (±2.3) µg/L and in the interventional group 3.9 (±1.5) µg/L. Most LT recipients in the control group in the ITT and PP population had TAC trough levels within the target range (5-10 µg/L). Whereas most LT recipients in the interventional arm in the ITT and PP population had TAC trough levels above or under the target range (3 – 5 µg/L). Over the period of three-year follow-up, TAC and SRL trough levels above the target range of LT recipients in the interventional arm of the ITT and PP analysis varied between 10% and 40%.

After one and three years, corticosteroids were used in 25.5% (25/98) and 8.2% (8/98) of the patients in the control group and 29.6% (29/98) and 10.2% (10/98) of the patients in the interventional group. During the study, several switches in the immunosuppressive therapy in both groups were performed. In the interventional group: started were mycophenolic acid (27 patients), everolimus (4 patients) and azathioprine (2 patients) and discontinued were sirolimus (42 patients) and tacrolimus (9 patients). In the control group: started were mycophenolic acid (40 patients), everolimus (6 patients), sirolimus (2 patients), azathioprine (5 patients) and cyclosporine (1 patient) and discontinued was tacrolimus (6 patients). None of these patients was switched back during the study period.

Table 2. Secondary endpoints

	Month 6		Year 1		Year 2		Year 3		Overall at end of study period		p-value*
	TAC control (n=98)	low-dose TAC + SRL (n=98)	TAC control (n=96)	low-dose TAC + SRL (n=97)	TAC control (n=95)	low-dose TAC + SRL (n=95)	TAC control (n=92)	low-dose TAC + SRL (n=92)	TAC control (n=92)	low-dose TAC + SRL (n=92)	
Malignancy, Yes (n, %)	-	-	1 (1.1)	2 (2.1)	2 (2.2)	5 (5.4)	3 (3.3)	2 (2.3)	6 (6.5)	9 (9.8)	0.59
NODAT, Yes (n, %)	1 (1.0)	2 (2.0)	-	-	1 (1.1)	1 (1.1)	-	1 (1.1)	17 (18.5)	11 (11.9)	0.31
Recovery NODAT, Yes (n, %)	1 (1.0)	-	2 (2.1)	-	5 (5.4)	3 (3.3)	3 (3.3)	2 (2.3)	11 (11.9)	5 (5.4)	0.19
tBPAR, Yes (n, %)	-	1 (1.0)	2 (2.1)	-	1 (1.1)	1 (1.1)	-	1 (1.1)	5 (5.4)	8 (8.7)	0.57
Drop out during study period											
Death, Yes (n, %)	2 (2.1)	-	1 (1.1)	-	2 (2.2)	2 (2.1)	1 (1.1)	6 (6.5)	6 (6.5)	8 (8.7)	0.78
Retransplantation, Yes (n, %)	-	-	-	2 (2.1)	1 (1.1)	1 (1.1)	-	-	1 (1.1)	3 (3.3)	0.62
Withdraw after randomization, yes (n, %)	-	1 (1.0)	-	-	-	-	-	-	-	1 (1.1)	-
Immunosuppressive drug trough levels											
Tacrolimus, µg/L (mean (SD))	7.1 (2.5)	5.01 (1.9)	6.5 (2.8)	5.3 (2.5)	5.6 (2.4)	4.4 (1.9)	5.0 (2.3)	3.9 (1.5)	-	-	-
Number of recipients in target range tacrolimus (n, %)	70 (71.4)	39 (39.8)	58 (60.4)	37 (38.1)	59 (62.1)	40 (42.1)	46 (50)	38 (41.3)	-	-	-
Number of recipients above target range tacrolimus (n, %)	11 (11.2)	43 (43.9)	10 (10.4)	42 (43.3)	3 (3.2)	28 (29.5)	5 (5.4)	20 (21.7)	-	-	-
Sirolimus, mg/L (mean (SD))	-	4.1 (1.5)	-	4.9 (1.9)	-	4.6 (1.8)		4.2 (2.1)	-	-	-
Number of recipients in target range sirolimus (n, %)	-	36 (36.7)	-	22 (22.7)	-	25 (26.3)	-	24 (26.1)	-	-	-
Number of recipients above target range tacrolimus (n, %)	-	23 (23.5)	-	26 (26.8)	-	18 (18.9)	-	10 (10.9)	-	-	-

Abbreviations: TAC, tacrolimus; SRL, sirolimus; eGFR, estimated glomerular filtration rate; NODAT, new onset diabetes after transplantation; tBPAR, treated biopsy proven acute rejection; SD, standard deviation

*p-value for testing differences in column overall at end of study period based on Pearson's Chi-square test.

Renal function: Intention-to-treat population

The cumulative incidence of eGFR <60 mL/min/1.73m^2 at 36 months post-LT was 50.8% (95% confidence interval (CI): 39.7% – 59.9%) and 43.7% (95% CI: 32.8% - 52.8% of the patients in the control and interventional group (p=0.19, figure 3A). At six months, one year and two years, no evidence was found for a significant difference in the proportion of patients with eGFR <60 mL/min/1.73m^2 in the interventional group compared to the control group.

Figure 4A visualizes the individual kidney function measurements, the observed means per group, and the estimated group trajectories based on the linear mixed-effect model across the study period. The results of the model are shown in supplementary table 1. After transplantation the eGFR was significantly improved in the interventional group compared to the control group at six months (mean eGFR 73.1±15 versus 67.6±16 mL/min/1.73m^2, p=0.02). No evidence for a significant difference in the eGFR was shown between the interventional group and the control group at one year (mean eGFR 70.1±17 versus 65.9±16 mL/min/1.73m^2, p=0.08), two years (mean eGFR 69.6±17 versus 67.4±16 mL/min/1.73m^2, p=0.39) and three years (mean eGFR 67.7±17 versus 68.5±17 mL/min/1.73m^2, p=0.77). Consistent with these results, the linear mixed-effect model did not identify significant differences in the kidney function across the study period.

Renal Function: Per Protocol population

The cumulative incidence of eGFR <60 mL/min/1.73m^2 at 36 months post-LT was 33.3% (95% CI: 18% - 45.8%) and 28.9% (95% CI: 14.3% – 41.0%) of the patients in the control and interventional group (p=0.56, figure 3B). At six months, one year and two years, no evidence was found for a significant difference in the proportion of patients with eGFR <60 mL/min/1.73m^2 in the interventional group compared to the control group.

Figure 4B visualizes the individual kidney function measurements, the observed means per group, and the estimated group trajectories based on the linear mixed-effect model across the study period. The results of the model are shown in supplementary table 1. No relevant differences in the eGFR between the interventional group and the control group were found at six months (mean eGFR 77.4±13 versus 72.4±12 mL/min/1.73m^2, p=0.07), one year (mean eGFR 75.5±15 versus 71.7±12 mL/min/1.73m^2, p=0.20), two years (mean eGFR 74.5±16 versus 73.9±11 mL/min/1.73m^2, p=0.84) and three years (mean eGFR 73.5±15 versus 73.3±13 mL/min/1.73m^2, p=0.96). The linear mixed-effect model had results consistent with this.

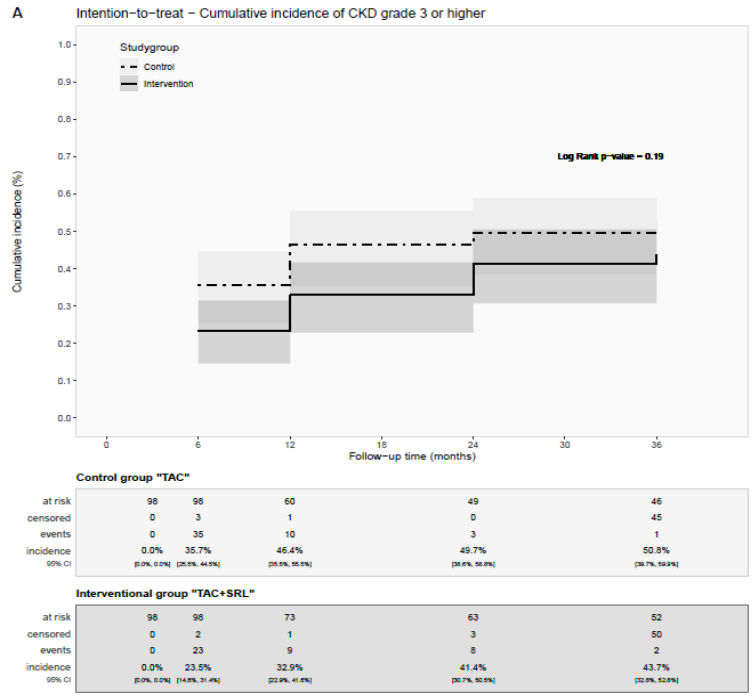

Figure 3. Overall cumulative incidence of chronic kidney disease grade ≥3 in the intention to treat and per protocol population

Abbreviations: TAC, tacrolimus; SRL, sirolimus

A) Shown is the cumulative incidence of chronic kidney disease grade ≥3 in the intention to treat population.

B) Shown is the cumulative incidence of chronic kidney disease grade ≥3 in the per protocol population.

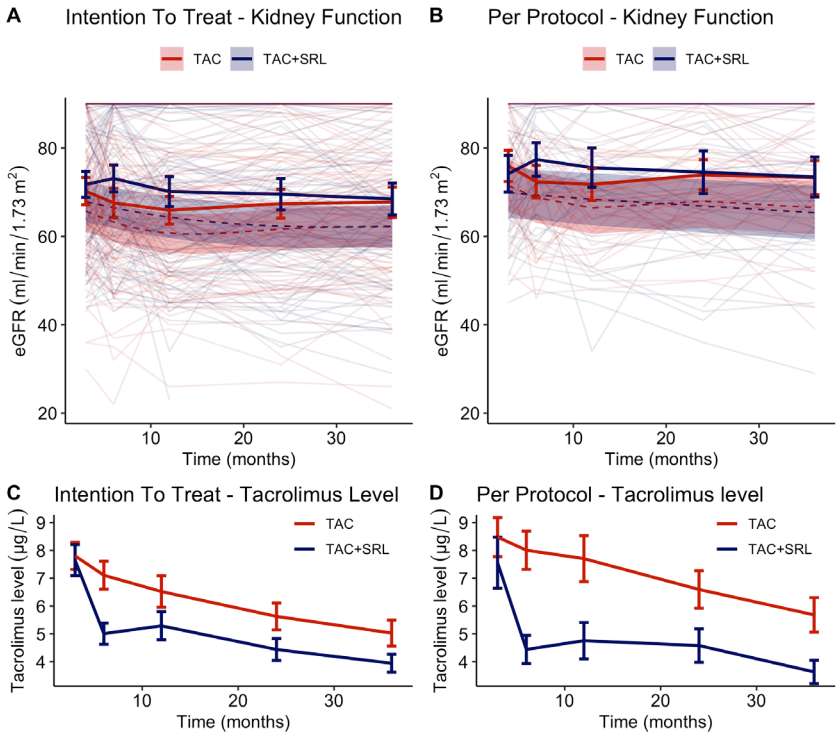

Figure 4. Kidney function and tacrolimus levels in the intention to treat and per protocol population

A) Individual eGFR trajectories (CKD-EPI formula) and group-wise mean with 95%-confidence interval (CI) during the course of the study of the intention to treat (ITT) population represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, type of donation, recipient age and sex, lab MELD, initial cold and warm ischemic time and the usage of antihypertensive drugs were set to the population median or reference category). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. Missing data were considered as missing completely at random.

B) Individual eGFR trajectories (CKD-EPI formula) and group-wise mean with 95%-CI during the course of the study of the per protocol (PP) population represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, type of donation, recipient age and sex, lab MELD, initial cold and warm ischemic time and the usage of antihypertensive drugs were set to the population median or reference category). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. Missing data were considered as missing completely at random.

C) Mean tacrolimus level (µg/L) with 95%-CI during the course of the study of the ITT population.

D) Mean tacrolimus level (µg/L) with 95%-CI during the course of the study of the PP population.

Secondary endpoints

Table 2 shows the incidence of death, retransplantation, malignancies, NODAT and tBPAR for the intention to treat population. No significant differences were demonstrated between the two groups.

Safety

Table 3 shows the Serious Adverse Events (SAEs) according to the Medical Dictionary for Regulatory Activities (MedDRA) and the severity of the SAEs during the study period. In total, 191 SAEs were reported: 50.8% (97/191) in the control group and 49.2% (94/191) in the interventional group. SAEs most frequently reported were fever (23%, 44/191), infections (17.8%, 34/191) and cholangitis and bile duct obstruction (16.2%, 31/191). More patients in the control group experienced a SAE due to fever (25.8%) compared to the interventional group (10.6 %). No differences in proteinuria and cardiovascular events were found. Hepatic artery thrombosis did not occur in both study groups during the study period.

	TAC (n= 97)		**TAC+SRL (n = 94)**	
	No. of patients with event	*No. of events (%)*	*No. of patients with event*	*No. of events (%)*
Serious adverse events				
Death	6	6 (6.2)	8	8 (8.5)
Cholangitis and bile duct obstruction	13	18 (18.6)	8	15 (15.9)
*Fever**	12	25 (25.8)	6	10 (10.6)
Infections§	19	21 (21.4)	20	22 (23.4)
Liver transplant rejection	1	1 (1.0)	4	4 (4.3)
Renal failure	2	3 (3.1)	1	2 (2.1)
Other	18	23 (23.7)	27	33 (35.1)
SEVERITY				
Mild	2	2 (2.1)	3	3 (3.2)
Severe	42	88 (90.7)	38	80 (85.1)
Life threatening	7	7 (7.2)	10	11 (11.7)

Table 3. Serious Adverse Events according to the Medical Dictionary for Regulatory Activities (MedDRA)

Abbreviations: TAC, tacrolimus; SRL, sirolimus

*Fever with an unspecified cause and no overlap with the SAEs for cholangitis or infections.

§Infections includes every viral or bacterial infection occurred during the study period excluding cholangitis.

Discussion

In this 36-month randomized, controlled trial we demonstrated that once daily low-dose SRL combined with low-dose extended-release TAC compared to normal-dose extended-release TAC does not result in an improvement of the kidney function at the long term. The mean eGFR in both study groups did not differ between the moment of randomization and the end of the study. Low-dose SRL combined with extended-release TAC could be a valuable strategy to minimize TAC exposure in LT recipients, with rates of rejection, graft survival and patient survival that are comparable in both arms. The combination significantly improved the renal function at 6 months after transplantation. However, this combination did ultimately not provide a better renal function at 36 months compared to normal-dose extended-release TAC.

Our results are in line with the findings of Buchholz *et al.*, who evaluated the effect of an SRL-based immunosuppressive regimen in combination with CNI minimization on renal function as a subset of the SiLVER study.(16, 21) They showed that an SRL-based immunosuppressive regimen protects renal function in the short-term, i.e. for 3 months after LT.

Our findings contradicts the studies of Fischer *et al.* and Sterneck *et al.*, in which the mTOR-inhibitor everolimus showed a clinically relevant renal benefit at 36 months compared to normal-dose TAC.(13, 22) In these studies patients were randomized after four weeks whereas in our study patients are randomized at day 90. Furthermore, Fischer *et al.* and Sterneck *et al.* aimed for TAC trough levels between 6 – 12 μg/L, whereas we aimed for TAC trough levels between 5 – 10 μg/L. An explanation for the difference in clinically relevant renal benefit could be the height of the TAC trough levels in the control groups. The TAC trough levels at 36 months were approximately 1.6 μg/L higher in the control groups in the studies of Fischer *et al.* and Sterneck *et al.*, compared to the control group in our study. Another explanation for the difference in clinically relevant renal benefit could be the lower difference in TAC trough levels between the study groups in our study compared to both other studies. In the interventional group of the study by Sterneck *et al.* TAC was tapered and discontinued and the study by Fischer *et al.* aimed for TAC trough levels of 3 - 5 μg/L corresponding to our target levels.(13, 22) The difference in mean TAC trough levels between the study groups in the studies by Fischer *et al.* and Sterneck *et al.* was > 3 μg/L at the end of the study period, whereas we had a difference in mean TAC trough levels between the study groups at the end of the study period of approximately 1 μg/L. CNI-induced nephrotoxicity is thought to be irreversible in the long-term due to interstitial fibrosis and glomerular sclerosis in the kidney.(23) Several studies show a prevalence of >50% of the LT recipients with a CKD defined as an eGFR of <60 mL/min/1.73m^2.(7, 8) Our ITT and PP analysis showed lower rates with a prevalence of 30 – 50% of the LT recipients having an eGFR of <60 mL/min/1.73m^2. This difference could be explained by the height of the TAC trough levels. We demonstrated that in the control group further progression of the CNI-induced nephrotoxicity could be prevented for by reducing the TAC trough levels to eventually 5 μg/L after 36 months in this study. In the past, higher TAC trough levels were aimed for resulting in too much immunosuppression and progressive CNI-induced nephrotoxicity. Reducing the TAC trough levels will prevent deterioration of the kidney function for the majority of the LT recipients. Moreover, for some LT recipients a CNI-free dosing regimen might be considered in case of severe deterioration of the kidney function.

Another important finding in our study is that we did not find a higher risk of hepatic artery thrombosis or increased mortality in the SRL-based group. This is in contrast with the FDA statement.(24) Our finding also contradicts the results of a study by Teperman *et al.* that showed an increased risk of rejection in patients treated with an SRL-based regimen without CNIs compared to patients treated with a CNI-based regimen. However, we combined SRL with low-dose extended-released TAC and randomized the LT recipients after 90 days, whereas Teperman *et al.* randomized LT recipients at 4 to 12 weeks (median: 54 days).(12) This might explain the fact that we did not find a higher risk of hepatic artery thrombosis or increased mortality in the SRL-based group. The introduction of a SRL-based regimen after three months might for some patients be a valuable addition to the existing immunosuppressive strategies and more patient-friendly compared to everolimus because of the once daily dosing regimen.

Interestingly, in the first year more LT recipients in the interventional arm had TAC trough levels above the target range than within the target range. This could have resulted in more TAC-induced nephrotoxicity and as a consequence the kidney function in the interventional arm might have been higher in the first year when more LT recipients had TAC trough levels within the target range. We have confidence that low TAC trough levels of (3 – 5 μg/L) in combination with another immunosuppressive agent in the first year are feasible. In our LT population, in the first year after transplantation we experience the most problems with infections and bile duct problems and very little early or late graft rejection. In addition, the study by Fischer *et al.* showed that the study group treated with everolimus and reduced TAC (3 – 5 μg/L) did not experience more tBPAR compared to the control TAC group (6 – 10 μg/L).(13)

SRL could also be a valuable addition in the immunosuppressive strategy to increase the antibody response after SARS-CoV-2 vaccines in the current pandemic. Several studies show that mycophenolic acid (MPA) use is a strong predictor of a low antibody response to SARS-CoV-2 vaccines regardless the height of the MPA trough levels.(25, 26) MPA inhibits both T and B lymphocytes proliferation, whereas mTOR-inhibitors deplete only the T lymphocytes and indirectly the B lymphocytes resulting in higher antibody formation after vaccination.

There are several strengths to note in this study. First, this is the first randomized controlled trial testing the effect of the combination of low-dose SRL and extended-release TAC on renal function and safety. Secondly, the study had a long follow-up and clinicians were allowed to lower the dose of TAC in the control group, which reflects the clinical practice setting.

There is one major limitation to our study, namely the fact that almost half of the patients in both groups switched immunosuppressive therapy because of deterioration of the kidney function, side effects or preference of the treating physician. This is a significant deviation and the high number of patients switching the immunosuppressive regimen could introduce selection bias and therefore difficulties with interpreting the ITT and PP results. Overall, the results in our ITT analysis might be underestimating the actual effect of the interventional regimen. Since a large proportion in the control group switched to combination therapy, where after lower TAC levels were aimed for, less TAC-induced nephrotoxicity might been experienced resulting in higher kidney functions in the control group. The selection bias is a consequence of the use of immunosuppressive agents in a study with long-term follow-up and has been addressed in several other studies.(27, 28) Patients consistent with their randomized immunosuppressive regimen at the end of the follow-up are not necessarily representative of the total study population since these patients experience less severe renal insufficiency. Although, the ITT and PP analysis needs to be cautiously interpreted, our results are consistent in the ITT and PP analysis supporting the null hypothesis that a once-daily SRL-based regimen does not result in a difference in the renal function in LT recipients on the long term.

In conclusion, in this study once-daily low-dose SRL combined with low-dose extended-release TAC does ultimately not provide less grade ≥3 chronic renal dysfunction at 36 months compared to normal-dose extended-release TAC. However, the combination improves the renal function at the short term after transplantation and could be a valuable strategy to minimize TAC exposure in LT recipients.

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	Model for ITT population (n=845)		Model for PP population (n=427)	
Fixed effects	**Estimate**	**95%-CI**	**Estimate**	**95%-CI**
Intercept	116.8	100.6 – 132.9	111.3	91.6 – 131.0
ns (Visit, df=3)1	-1.99	-5.48 – 1.53	-0.092	-4.61 – 4.46
ns (Visit, df=3)2	-9.22	-13.45 – -4.96	-10.34	-15.89 – -4.77
ns (Visit, df=3)3	0.48	-1.82 – 2.81	-0.54	-3.56 – 2.49
Study group	2.15	-1.85 – 6.15	-1.56	-6.33 – 3.22
TAC trough level	-0.76	-1.04 – -0.48	-0.98	-1.33 – -0.62
Type of donation	-0.78	-4.79 – 3.23	2.48	-1.99 – 6.96
Recipient age	-0.77	-0.96 – -0.58	-0.61	-0.84 – -0.38
Recipient sex	8.92	4.65 – 13.18	6.78	1.69 – 11.87
lab MELD	-0.15	-0.39 – 0.09	-0.15	-0.44 – 0.13
Initial cold ischemic time	-0.006	-0.022 – 0.011	-0.002	-0.19 – 0.015
Initial warm ischemic time	-0.006	-0.21 – 0.19	0.093	-0.12 – 0.31
Usage of antihypertensive drugs	-1.08	-3.11 – 0.90	0.50	-1.96 – 2.96
Interaction between Visit and study group (df =3)1	-3.08	-8.09 – 1.93	-2.391	-8.90 – 4.09
Interaction between Visit and study group (df =3)2	-0.494	-5.42 – 6.47	5.046	-2.95 – 13.06
Interaction between Visit and study group (df =3)3	-3.915	-7.17 – -0.67	-3.160	-7.39 – 1.-05
Random effects	**Variance**	**SD**	**Variance**	**SD**
Subject intercept	144.22	12.009	86.39	9.295
Residual	60.95	7.807	54.25	7.366

▲

Supplementary table 1. Results of the generalized mixed effect models

Abbreviations: CI, confidence interval; ITT, intention to treat; MPA; mycophenolic acid; PP, per protocol; TAC, tacrolimus; SD, standard deviation

Two generalized mixed effect models were fitted, investigating the association between the kidney function during the course of the study (values for the covariates: tacrolimus trough levels, type of donation, recipient age and sex, lab MELD, initial cold and warm ischemic time and the usage of antihypertensive drugs were set to the population median or reference category). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. A total of 845 kidney function measurements of LT recipients in the intention to treat analysis and a total of 427 kidney function measurements of LT recipients in the per protocol analysis were included in the models. To take into account that the kidney function may not only be independently associated with the visit and the study group, the model included the product (=interaction) of the visit and study group as independent variable./

Chapter 3

Health-related quality of life and fatigue in liver transplant recipients receiving tacrolimus versus sirolimus-based immunosuppression: results from a randomized trial.

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Abstract

Background

The impact of different immunosuppression regimes on the health-related quality of life (HRQoL) and the severity of fatigue in liver transplant recipients is largely unknown. We investigated the impact of a sirolimus (SRL) based regimen compared to a tacrolimus (TAC) based regimen on the HRQoL and the severity of fatigue.

Methods

In this multi-center open-label, randomized, controlled trial, 196 patients were randomized 90-days after transplantation to 1) once daily normal-dose TAC or 2) once daily combination therapy of low-dose SRL and TAC. HRQoL was measured with the EQ-5D-5L questionnaire, the EQ-VAS and the severity of fatigue questionnaire (FSS). The EQ-5D-5L scores were translated to the societal values. We examined the HRQoL and the FSS over the course of the study by fitting generalized mixed effect models.

Results

Baseline questionnaires were available for 87.7% (172/196) of the patients. Overall, patients reported the least problems in the states of Self-Care and Anxiety/Depression and the most problems in the states of Usual Activities and Pain/Discomfort. No significant differences in HRQol and FSS were seen between the two groups. During follow-up, the societal values of the EQ-5D-5L health states and the patient's self-rated EQ-VAS score were a little lower than those of the general Dutch population in both study arms.

Conclusions

The HRQoL and FSS was comparable in the 36 months after liver transplantation in both study groups. The HRQoL of all transplanted patients approximated that of the general Dutch population, suggesting little to no residual symptoms in the long-term after transplantation.

Introduction

Liver transplantation (LT) is a life-saving therapy and life expectancy after LT is increasing with 5-year survival rates of over 70%.(1) After a LT, patients experience a rapid improvement of their physical and mental condition. Important factors contributing to the well-being of a LT recipient at the long-term are the health-related quality of life (HRQoL) and the severity of fatigue.

Various studies describe a substantial benefit in the HRQoL after LT.(2) Quality of life is a multidimensional construct reflecting the physical, mental or psychological, and social dimensions of health.(3) A review by Yang *et al.* showed that the HRQoL and general health perception of LT recipients improved to a similar level as the general population, except for physical functioning.(4)

Fatigue in patients after LT is a major issue at the long-term. Van Ginneken *et al.* reported high rates of fatigue in LT recipients and Lin *et al.* showed that fatigue is strongly associated with insomnia, anxiety and depression.(5,6) Furthermore, several studies showed higher fatigue scores reported by LT recipients compared to the general population.(7,8)

The impact of different immunosuppression regimes on the HRQoL and the severity of fatigue in LT recipients is largely unknown. Benzing *et al.* investigated the impact of three different immunosuppression regimes on the HRQoL following orthotopic liver transplantation.(9) In their observational study in 275 LT recipients they compared calcineurin inhibitors, mTOR inhibitors and mTOR inhibitors combined with calcineurin inhibitors. The authors conclude that mTOR inhibitor-based regimens have beneficial effects on HRQoL, especially after an early conversion. A major drawback of this study is the retrospective nature of this study. Therefore, a prospective, randomized trial comparing the combination of low-dose sirolimus (SRL) and extended-release tacrolimus (TAC) to normal-dose extended-release TAC on the HRQoL and the severity of fatigue could be instrumental to evaluate this presumed beneficial effect of mTOR-inhibitor based regimens on HRQoL after liver transplantation.

Materials and Methods

Study design and participants

An extensive description of the LOLIII study design has been published previously.(10) In brief, the LOLIII study randomized patients 90-days after transplantation in a 1:1 ratio to 1) once daily normal-dose TAC with target trough levels 5–10 μg/L (control group) or 2) once daily combination therapy of SRL and low-dose TAC with target trough levels 3–5 μg/L for both SRL and TAC (interventional group). During the three-year follow-up a switch in immunosuppressive therapy occurred in 48.9% (48/98) of the patients in the control group and in 44.9% (44/98) of the patients in the interventional group. In the control group the main reason for the switch in immunosuppressive therapy was deterioration of the kidney function (43/48, 89.6%).10 In the interventional group multiple reasons for switching applied. The main reasons for a switch were side effects of sirolimus and/or preference of treating physician with another immunosuppressive agent in case of deterioration of the kidney function (29/44, 65.9%). The side effects consisted mainly of pancytopenia, malaise and skin problems.10 The majority of the LT recipients were switched within the first year after transplantation (69/92, 75%) to mycophenolic acid.

The study was performed at three centers in the Netherlands: Erasmus University Medical Center Rotterdam, University Medical Center Groningen and Leiden University Medical Center. The study was approved by the Ethical Committee of the Erasmus University Medical Center (MEC-2010-247), registered in the EudraCT database (EudraCT: 2009-017843-32) and conducted in accordance with the principles of the declaration of Helsinki. All participants gave written informed consent before any study-related activity. The inclusion period ran from February 2011 until August 2018.

Patient-reported outcomes

The evaluation of HRQoL and the severity of fatigue comprised a secondary objective of the LOLIII study. The LOLIII study was initially designed to investigate whether the combination of low-dose SRL and extended-release TAC compared to normal-dose extended-release TAC results in a difference in the renal function and comparable rates of rejection, graft survival and patient survival at 36 months after transplantation.

HRQoL and Severity of Fatigue assessments

HRQoL was assessed with the validated Dutch version of the EQ-5D-5L questionnaire (a generic HRQoL instrument) and the severity of fatigue questionnaire (a domain specific HRQoL instrument), the latter using the Fatigue Severity Score (FSS). The questionnaires were distributed at the moment of randomization and every year during the study until end of follow up, death or withdrawal due to any reason. At the start of the study, the SF-36 questionnaire (a generic HRQoL instrument) was used for the assessment of the HRQoL and in November 2011 a switch to the EQ-5D-5L questionnaire was made. Scores of the SF-36 questionnaire of 13 LT recipients were dropped.

The EQ-5D-5L questionnaire is based on a descriptive system that defines health in terms of 5 states: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression.(11) Each dimension has 5 response categories corresponding to no problems, slight problems, moderate problems, severe problems, and extreme problems. EQ-5D-5L scores were transformed to societal values based on the Dutch tariff for the EQ-5D-5L established by Versteegh *et al.*.(12)

In the EQ-5D-5L questionnaire, the respondents' overall health (patient's self-rated HRQoL scores) on the day of the interview was rated on a 0–100 hash-marked, vertical visual analogue scale (EQ-VAS). The threshold for the minimally important difference (MID), indicating a clinical meaningful improvement, in the EQ-VAS score was defined as ≥ 7 points.(13)

The severity of fatigue questionnaire is a nine-question, self-administered questionnaire with answers ranging from 1 ("strongly disagree") to 7 ("strongly agree").(14) For each patient, the mean question score ranged from 1 ("no signs of fatigue") to 7 ("most disabling fatigue"). Based on a study by van den Berg-Emons *et al.*, patients were classified as "severely fatigued" with FSS scores ≥2 standard deviations (SD) above the mean score for healthy individuals (FSS ≥ 5.1). Patients were classified as "fatigued" for FSS scores ≥1 SD above the mean score for healthy individuals (4.0≥ FSS <5.1).(15)

Data collection

Variables collected included recipient socio-demographic, clinical and transplantation parameters, the HRQoL and fatigue severity score and trough levels of SRL and extended-release TAC.

Statistical analysis

The HRQoL analysis included all patients within the LOLIII study who responded to at least one questionnaire, according to the intention-to-treat (ITT) principle. The EQ-5D-5L and FSS questionnaire included in the analysis missed < 5% based on the total number of measurements across all patients and questions. The missing data were considered as missing completely at random.

Variables were described using counts (%) for nominal and ordinal variables and mean (standard deviation, SD) or median (inter-quartile range, IQR) for continuous variables, depending on the shape of the distribution.

Three generalized linear mixed effect models were fitted to examine the HRQoL (EQ-VAS and the societal values of the EQ-5D-5L) and the severity of fatigue over the course of the study.

The models included covariates shown or suggested to be relevant: visit number, study group, tacrolimus trough concentrations, kidney function, hemoglobin, recipient age and sex, primary disease, the usage of antihypertensive and antidiabetic drugs and the usage of corticosteroids as well as the interaction between visit and the study group. Additionally, the model examining the severity of fatigue included the covariate usage of MMF. Participant specific random intercepts were included to account for correlation among repeated measurement nested within each participant. Natural cubic splines were used to model the potentially nonlinear trajectories of the EQ-VAS, societal values of the EQ-5D-5L and severity of fatigue over time. The need for these splines was evaluated using likelihood-ratio tests. Splines provide a convenient non-parametric way to flexibly model (potentially) non-linear associations in regression models. Instead of using one polynomial (e.g., a quadratic or cubic function) that spreads over the whole range of the covariate, splines use a set of several polynomial functions that are defined over smaller intervals. This allows the resulting fit to be more flexible than when using a single polynomial. To visualize the estimated associations, the expected HRQoL and severity of fatigue across the course of the study was calculated while fixing the values of all other covariates to the median or reference category.

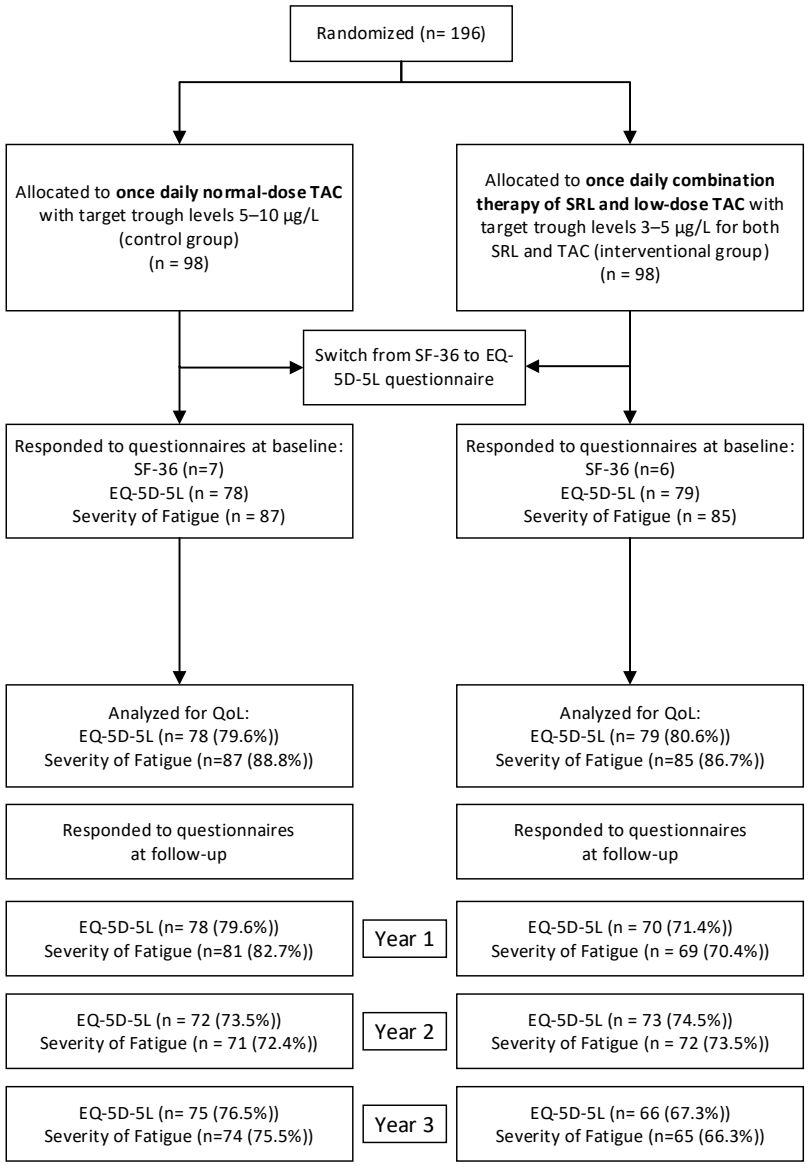
Data were approached in an intention-to-treat (ITT) and per protocol (PP) analysis. Patients with protocol violations in immunosuppressive therapy, a retransplantation or death were excluded in the per protocol analysis. Differences in the proportion of responses by level of severity for EQ-5D-5L dimensions were tested using the Chi-squared test. A p-value of <0.05 was considered as statistically significant. All data were collected in the Dutch Organ Transplantation Registry (NOTR) and analysis were performed using R software (version 3·6·2).(16)

Results

Patient and treatment characteristics

A total of 196 patients were included and randomized in the LOLIII study. In total, 157 (80.1%) patients responded to the EQ-5D-5L baseline questionnaire; 78 (79.6%) patients in the control group and 79 (80.6%) patients in the interventional group. A total of 172 (87.7%) patients responded to the baseline severity of fatigue questionnaire; 87 (88.8%) in the control group and 85 (86.7%) in the interventional group. The response rate decreased during follow up to a minimum of 66.3% at the 3-year questionnaire (figure 1).

Table 1 shows the baseline characteristics for the ITT population. No relevant differences in any of the baseline characteristics between the two groups in either questionnaire was shown.



▲

Figure 1. Enrollment, Randomization, and Follow-up.

Abbreviations: TAC, tacrolimus; SRL, sirolimus; QoL, Quality of Life

	EQ-5D-5L		Severity of Fatigue	
Recipient demographics	**TAC (n=78)**	**TAC+SRL (n=79)**	**TAC (n=87)**	**TAC+SRL (n=85)**
Age, year (median, IQR)	56.5 (49.5 - 62)	55 (48 - 63)	56 (49 – 62)	54 (48 – 63)
Gender, male (n, %)	56 (71.8%)	53 (67.1%)	63 (72.4%)	60 (70.6%)
Primary Disease (n, %)				
Hepatocellular carcinoma	27 (34.6%)	28 (35.4%)	30 (34.5%)	28 (32.9%)
(Non)alcoholic steatohepatitis	11 (14.1%)	12 (15.2%)	14 (16.1%)	14 (16.5%)
Primary sclerosing cholangitis	17 (21.8%)	15 (19%)	19 (21.8%)	15 (17.6%)
Acute liver failure	4 (5.1%)	9 (11.4%)	5 (5.7%)	10 (11.8%)
Cryptogenic cirrhosis	4 (5.1%)	3 (3.8%)	4 (4.6%)	4 (4.7%)
Metabolic disease	5 (6.4%)	4 (5.1%)	5 (5.7%)	4 (4.7%)
Viral Hepatitis	2 (2.6%)	3 (3.8%)	2 (2.3%)	4 (4.7%)
Other‡	8 (10.3%)	5 (6.3%)	8 (9.2%)	6 (7.1%)
NODAT, Yes (n, %)	12 (15.4%)	5 (6.3%)	13 (14.9%)	5 (5.9%)
Pre-existing Diabetes, Yes (n, %)	11 (14.1%)	22 (27.8%)	12 (13.8%)	25 (29.4%)
Lab MELD (median, IQR)	16 (10 - 22)	16 (9.5 - 22)	16 (10 – 21.5)	18 (11 – 23)
Hemoglobin, mmol/L (mean ± SD)	7.6 ± 0.9	7.5 ± 0.8	7.6 ± 0.9	7.5 ± 0.8
eGFR, ml/min/1.73m^2 (mean ± SD)	69 ± 16	72 ± 15	69 ± 16	71 ± 15
Tacrolimus, µg/L (mean ± SD)	7.7 ± 2.5	7.5 ± 2.9	7.7 ± 2.5	7.6 ± 2.9
Age, year (median, IQR)	54 (40 - 60)	52 (42 – 61.5)	54 (38.5 – 60)	53 (42 – 63)
Gender, male (n, %)	45 (57.7%)	43 (54.4%)	47 (54%)	44 (51.8%)
Type Donation				
Donation after brain death (n, %)	46 (59%)	49 (62%)	54 (62.1%)	53 (62.4%)
Donation after circulatory death (n, %)	32 (41%)	29 (36.7%)	33 (37.9%)	31 (36.5%)
Living (n, %)	-	1 (1.3%)	-	1 (1.2%)
Cold Ischemia time, min (mean ± SD)	415 ± 107	413 ± 129	419 ± 110	410 ± 134
Warm Ischemia time, min (median, IQR)	29 (25 - 36)	27 (24 - 38)	29 (25 – 36)	27 (24 – 37.5)
Antihypertensive drugs, Yes (n, %)	29 (37.2%)	23 (29.1%)	31 (35.6%)	23 (27.1%)
Corticosteroids, Yes (n, %)	73 (93.6%)	74 (93.7%)	82 (94.3%)	80 (94.1%)
EQ-5D-5L score				
VAS (mean ± SD) [ref: 0 – 100]	74 ± 15	74 ± 15	-	-
Societal values of the EQ-5D-5L based the Dutch tariff for the EQ-5D-5L (median, IQR) [ref: -0.466 - 1]	0.85 (0.75 – 1.00)	0.84 (0.75 – 1.00)	-	-
Severity of Fatigue				
Question score (mean ± SD) [ref: 1 – 7]	-	-	4.0 ± 1.38	3.7 ± 1.44

▲

Table 1. Baseline characteristics of the EQ-5D-5L and Severity of Fatigue at randomization

Abbreviations: eGFR, estimated glomerular filtration rate; IQR, interquartile range; MELD, model for end-stage liver disease; NODAT, new onset diabetes after transplantation; TAC, tacrolimus; SD, standard deviation; SRL, sirolimus; VAS, visual analogue scale

‡Other includes: primary biliary cirrhosis, secondary biliary cirrhosis, autoimmune cirrhosis, polycystic liver disease

Health-related quality of life Outcomes

Figure 2 shows the proportion of responses by level of severity for EQ-5D-5L dimensions during the study period for the ITT population. Overall, patients reported the least problems in the states of Self-Care and Anxiety/Depression and the most problems in the states of Usual Activities and Pain/Discomfort. No evidence for significant differences between the study groups in any of the five states were found. Patients reported significantly more often "no problems" in the states of Usual Activities (p=0.04) and Pain/Discomfort (p=0.02) at year 3 compared to the moment of randomization in both study groups. No differences in the response categories over time in the other states were found during the follow-up in both study groups.

The likelihood-ratio tests indicated non-linear patient specific trajectories of HRQoL scores but not of the societal values of the EQ-5D-5L. There was no evidence for between-group differences over the course of the study in the mixed effect models. Recipient age was significantly associated with a higher EQ-VAS score (suppl. Table 1). Figures 3A and 3B visualize the expected HRQoL scores and societal values of the EQ-5D-5L together with the corresponding observed values per time point and study group for the ITT population.

At 36 months after transplantation, for both arms the societal values of the EQ-5D-5L appoximate those of the general Dutch population. This also applied to the patient's self-rated HRQoL scores as expressed with the EQ-VAS. The per protocol analysis showed comparable results (suppl. figure 1A and 1B). In the ITT population, LT recipients in the interventional arm approached the threshold for a clinical meaningful improvement (6.47 points) in the EQ-VAS score at 36 months. LT recipients in the control group did not get near the threshold for a clinical meaningful improvement (4.98 points) in the EQ-VAS at 36 months. In the PP population, LT recipients in the interventional arm reached the threshold for a clinical meaningful improvement (7.25 points) in the EQ-VAS score at 36 months. LT recipients in the control group did not get near the threshold for a clinical meaningful improvement (4.30 points) in the EQ-VAS at 36 months. A subgroup analysis was performed in LT recipients in the ITT population without diabetes mellitus. LT recipients without diabetes mellitus in the interventional group had a clinical meaningful improvement in the EQ-VAS score at 36 months (8.1 points).

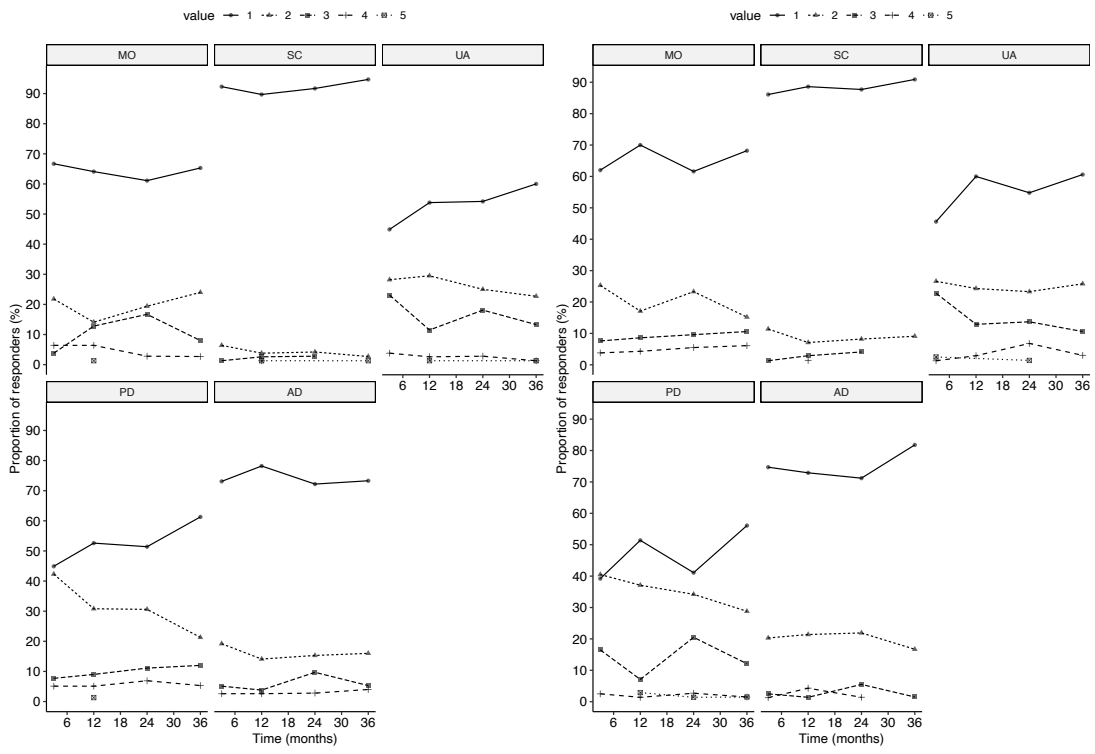


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Figure 2. Proportion of responses by level of severity for EQ-5D-5L dimensions during the study period

A) Tacrolimus group (control group)
1, no problems; 2, slight problems; 3, moderate problems; 4, severe problems; 5, Extreme problems
B) Tacrolimus + Sirolimus group (interventional group)
1, no problems; 2, slight problems; 3, moderate problems; 4, severe problems; 5, Extreme problems

Abbreviations: TAC, tacrolimus; SRL, sirolimus; MO, mobility; SC, self-care; UA, usual activities; PD, pain / discomfort; AD, anxiety / depression.

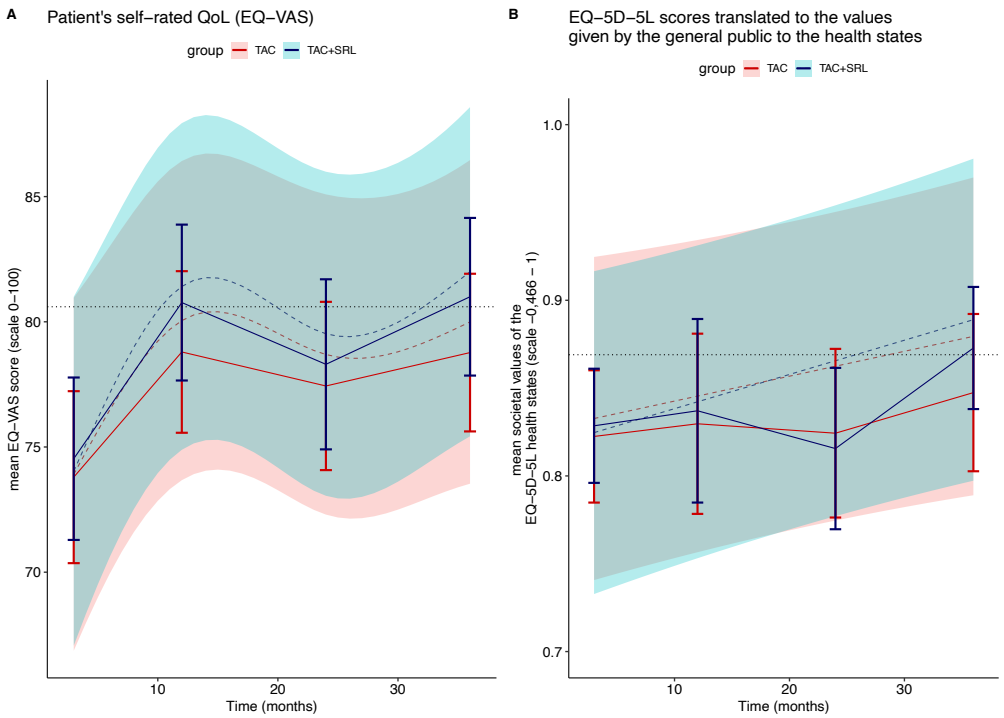

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Figure 3. EQ-VAS score and EQ-5D-5L scores on the dimensions translated to the societal values for the intention-to-treat population

A. Patient's self-rated QoL (EQ-VAS)
Group-wise mean EQ-VAS with 95%-confidence interval (CI) during the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, kidney function, hemoglobin, recipient age and sex, primary disease, the usage of antihypertensive and antidiabetic drugs and the usage of corticosteroids as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the EQ-VAS was investigated using natural cubic splines. Splines provide a convenient non-parametric way to flexibly model (potentially) non-linear associations in regression models. Instead of using one polynomial (e.g., a quadratic or cubic function) that spreads over the whole range of the covariate, splines use a set of several polynomial functions that are defined over smaller intervals. This allows the resulting fit to be more flexible than when using a single polynomial. Missing data were considered as missing completely at random. Dotted black line indicates the mean self-reported EQ-VAS score by the general Dutch population.12

B. EQ-5D-5L scores translated to the values given by the general public to the health states
Group-wise mean of the societal values of the EQ-5D-5L health states with 95%-confidence interval (CI) during the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, kidney function, hemoglobin, recipient age and sex, primary disease, the usage of antihypertensive and antidiabetic drugs and the usage of corticosteroids as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The likelihood-ratio test indicated no need for a nonlinear association structure. Missing data were considered as missing completely at random. Dotted black line indicates the mean EQ-5D-5L score given by the general Dutch population to the health states.12

Abbreviations: TAC, tacrolimus; SRL, sirolimus; QoL, quality of life; VAS, visual analogue scale

Severity of Fatigue

During the study, in the ITT and PP population, patients included in the interventional group did not report significantly lower fatigue scores compared to the control group (95%-CI for the ITT population -0.71 – 0.20 and 95%-CI for the PP population -0.93 – 0.47). In the ITT population, the average score of the LT recipients in the control group reached clinical levels for fatigue, FSS ≥ 4.0 (figure 4). These results persisted in the PP analysis (suppl. figure 2). In the ITT analysis, a minor decrease in the FSS over the course of the study is shown for both groups. Recipient age and hemoglobin concentration were significantly associated with a lower FSS in ITT analysis (suppl. Table 1)

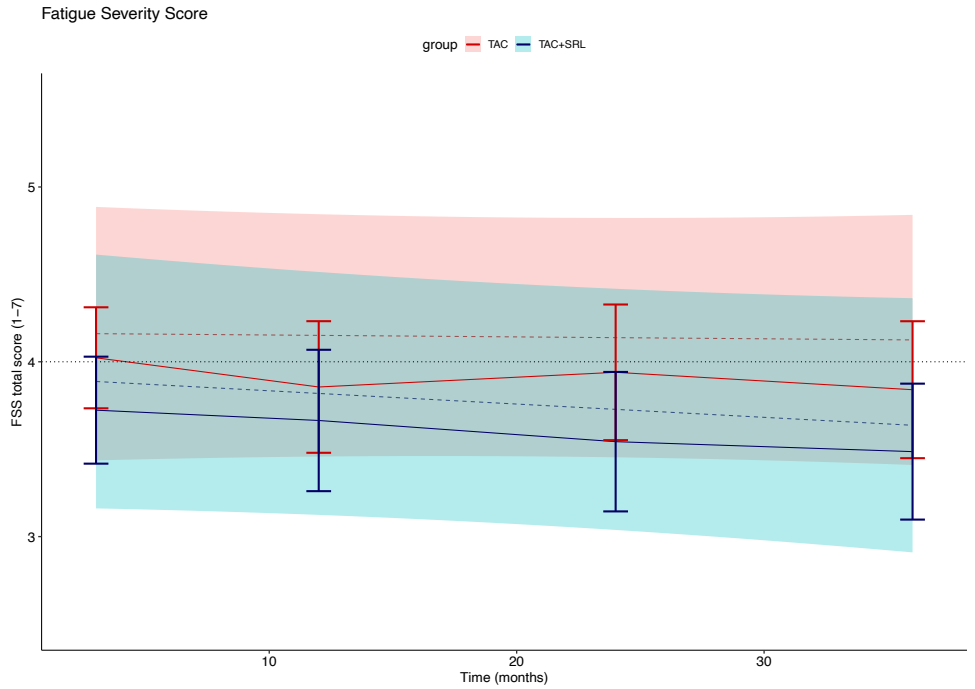

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Figure 4. Severity of Fatigue for the intention-to-treat population

Group-wise mean of the FSS with 95%-confidence interval (CI) durin
g the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, kidney function, hemoglobin, recipient age and sex, primary disease, the usage of antihypertensive and antidiabetic drugs, the usage of corticosteroids and the usage of MMF as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The likelihood-ratio test indicated no need for a nonlinear association structure. Missing data were considered as missing completely at random. Dotted black line indicates the clinical level for fatigue.15

Abbreviations: FSS, fatigue severity score, TAC, tacrolimus, SRL, sirolimus

Discussion

In this study we found no evidence for differences between the HRQoL and the severity of fatigue for the combination of low-dose SRL and extended-release TAC compared to normal-dose extended-release TAC during 36 months after transplantation. We could not confirm the beneficial effects of mTOR-inhibitor-based regimens with an early conversion on HRQoL after LT, as suggested by Benzing *et al.*.(9) The HRQoL of the transplanted patients approximates that of the general population at 36 months after transplantation. Furthermore, LT recipients using the combination of low-dose SRL and extended-release TAC approached the threshold for a clinical meaningful improvement in the EQ-VAS score at 36 months after transplantation.

In general, our HRQoL results are consistent with several reviews showing that the overall HRQoL does improve after LT to a similar level as the general population.(2,4,17) Li *et al.* showed in kidney transplant recipients comparable results for the 5 states of the EQ-5D-5L questionnaire with self-care and anxiety/depression as the states with the least problems and usual activities, pain/discomfort and mobility the most problems.(18)

In contrast with the previously published results by van Ginneken *et al.* we show an improvement in the mean FSS at three years compared to baseline for both groups.5 Van Ginneken *et al.* showed at baseline a mean FSS of 4.5±1.6, whereas we find at baseline a mean FSS for the control group of 4.0±1.4 and for the interventional group of 3.7±1.4. At two years van Ginneken *et al.* showed no improvement in the mean FSS (4.5±1.8) compared to the baseline levels. Since the study by van Ginneken *et al.* has been performed in 2010, these differences in findings likely originate from the improvements in patient care that have been made over the last 10 years. A possible explanation might be the lowering of the immunosuppressive dosages and the introduction of a lifestyle outpatient monitoring program. However, at the long-term fatigue remains with a negative effect on the quality of life of LT recipients.

Until now no universal MID has been described for the EQ-VAS.(13,19,20) Since no research has been done to describe the MID for the transplant population, we used common threshold in the oncology research.(13) In the per protocol population, LT recipients in the interventional arm showed a clinical meaningful improvement in the EQ-VAS score at 36 months. Interestingly, at 24 months after transplantation LT recipients in both study groups (interventional and control) reported a lower EQ-VAS score compared to the score at 12 months in the PP and ITT analysis. A possible explanation might be that LT recipients in the second year after transplantation are confronted with the fact that not all health problems after LT will be resolved. LT recipients being three years after transplantation have accepted their new life and therefore report a higher score on their EQ-VAS score.

The HRQoL for LT recipients in both arms approximate that of the general Dutch population. This observation needs to be considered in the context of the indication for transplantation and the fact that many LT recipients develop comorbidities as diabetes mellitus after transplantation. At the end of the study, 23.2% (46/198) of the LT recipients used diabetes medication compared to 32.3% (64/198) at baseline. Several studies showed that diabetes mellitus is associated with lower HRQoL scores in kidney transplant recipients and the general population.(18,21) As shown in our subgroup analysis for the ITT population, LT recipients without diabetes mellitus in the interventional group had a clinical meaningful improvement in the EQ-VAS score at 36 months

(8.1 points). The improvement in this subgroup was higher than the improvement in the ITT (6.47 points) or PP (7.25 points) population. The majority (54/67, 80.6%) of the LT recipients transplanted because of hepatocellular carcinoma (HCC) had received MELD-exception points. These HCC patients and primary sclerosing cholangitis (PSC) patients are representing groups that generally report better HRQoL scores.(22,23) The reduction in patients using diabetes mellitus medication and the fact that the majority (>50%) of the patients included in this study were transplanted because of HCC or PSC might have contributed to a high HRQoL and approximating that of the general Dutch population.

Tremor is the most important side effect of tacrolimus affecting health dimensions of the EQ-5D-5L. This neurological side effect is dependent of the plasma concentration of tacrolimus with higher tacrolimus plasma concentrations resulting in more frequent and intense tremors.(24) Based on the fact that, in the interventional arm, the tacrolimus plasma concentration is halved at randomization one might expect that this could result in less problems in the health dimensions mobility and usual activities in the interventional arm. However, we do not find differences between the study groups for these health dimensions. Overall, health dimensions in the EQ-5D-5L are influenced by many different factors such as lifestyle, revalidation and physiotherapy and personal characteristics (e.g. coping strategies). Therefore, translating the EQ-5D-5L scores on the health dimensions to the societal values represents the HRQoL of the LT recipients the best.

This is the first randomized controlled trial in liver transplantation investigating patient reported outcomes in the context of immunosuppressive drugs. Furthermore, the EQ-5D-5L scores on the dimensions were translated to the societal values and we achieved a high response rate. Several limitations have to be addressed. First, in both study groups almost half of the LT recipients had protocol violations in immunosuppressive therapy which could have caused an under- or overestimation of the HRQoL and severity of fatigue. However, we believe this does not affect the interpretation of the results since the HRQoL for both groups is high, half of the patients in both groups switched immunosuppressive therapy and the changes in immunosuppressive regimens reflects the daily clinical practice. Furthermore, we performed a PP analysis which showed comparable results to the ITT analysis. Next, we did not control for the use of medication, such as pain medication or the use of anxiolytic drugs, that might influence the response on the corresponding states in the EQ-5D-5L questionnaire. Unfortunately, it is not possible to determine to what extent these medications might have contributed to the results in both study groups.

In conclusion, in this study the HRQoL and the severity of fatigue did not differ for a SRL-based regimen compared to a TAC-based regimen during 36 months after transplantation. The HRQoL of all transplanted patients in this trial approximated that of the general Dutch population, suggesting little to no residual symptoms at the long-term after transplantation.

Acknowledgements

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	Model for EQ-VAS (n=559)		*Model for the societal values of the EQ-5D-5L health states (n=555)*		*Model for FSS (n=576)*	
Fixed effects	**Estimate**	**95%-CI**	**Estimate**	**95%-CI**	**Estimate**	**95%-CI**
Intercept	53.2	35.1 – 70.6	0.67	0.42 – 0.90	7.5	5.6 – 9.5
ns (Visit, df=3)1	0.89	-4.2 – 5.9	-	-	-	-
ns (Visit, df=3)2	12.5	4.4 – 20.6	-	-	-	-
ns (Visit, df=3)3	1.2	-2.3 – 4.7	-	-	-	-
Visit	-	-	0.0014	-0.000093 – 0.0029	-0.0011	-0.015 – 0.013
Study group	0.12	-4.1 – 4.3	-0.0096	-0.066 – 0.047	-0.25	-0.71 – 0.20
TAC trough level	0.012	-0.43 – 0.45	-0.00013	-0.0054 – 0.0052	0.00029	-0.048 – 0.050
Kidney function	0.053	-0.04 – 0.45	0.00087	-0.00037 – 0.0022	-0.0052	-0.016 – 0.0056
Recipient age	0.23*	0.039 – 0.42	0.0014	-0.0013 – 0.0042	-0.027*	-0.048 – -0.0058
Recipient sex	-1.79	-6.0 – 2.4	-0.02	-0.081 – 0.039	-0.021	-0.49 – 0.45
Hemoglobin	0.56	-0.78 – 1.9	0.0028	-0.013 – 0.020	-0.19*	-0.34 – -0.043
Primary disease ALF	-3.1	-10.4 – 4.2	-0.024	-0.13 – 0.082	0.16	-0.64 – 0.97
Primary disease Cryptogenic cirrhosis	1.1	-7.9 – 10.2	-0.037	-0.17 – 0.096	0.24	-0.78 – 1.2
Primary disease HCC	-1.7	-6.9 – 3.4	-0.012	-0.087 – 0.063	-0.11	-0.68 – 0.45
Primary disease metabolic disease	8.4	-0.11 – 16.9	0.070	-0.055 – 0.19	-0.77	-1.7 – 0.18
Primary disease other‡	-2.2	-9.4 – 4.8	-0.092	-0.19 – 0.012	0.0048	-0.78 – 1.2
Primary disease PSC	-2.6	-8.4 – 3.2	0.017	-0.067 – 0.10	-0.16	-0.81 – 0.48
Primary disease viral hepatitis	-4.4	-13.8 – 4.9	-0.050	-0.19 – 0.087	0.067	-0.98 – 1.1
Usage of insulin	-0.27	-4.2 – 3.6	-0.014	-0.068 – 0.038	0.16	-0.26 – 0.59
Usage of oral antidiabetics	-1.1	-4.7 – 2.6	0.0057	-0.044 – 0.053	0.25	-0.16 – 0.66
Usage of antihypertensive drugs	2.4	-0.3 – 5.1	0.0030	-0.030 – 0.038	-0.15	-0.45 – 0.15
Use of corticosteroids	2.5	-0.4 – 5.6	0.026	-0.0056 – 0.0058	-0.22	-0.51 – 0.070
Usage of MMF	-	-	-	-	-0.089	-0.42 – 0.0089
Interaction between visit and study group (df =3)1	-0.44	-7.0 – 6.1	-	-	-	-
Interaction between visit and study group (df =3)2	2.9	-5.3 – 10.9	-	-	-	-
Interaction between visit and study group (df =3)3	0.8	-3.8 – 5.4	-	-	-	-
Interaction between visit and study group	-	-	0.00053	-0.0011 – 0.0022	-0.0065	-0.022 – 0.0089
Random effects	**Variance**	**SD**	**Variance**	**SD**	**Variance**	**SD**
Subject intercept	105.5	10.3	0.025	0.16	1.3	1.1
Residual	91.4	9.6	0.013	0.11	1.2	1.1

▲

Supplementary table 1. Results of the three generalized mixed effect models for the intention to treat population

Abbreviations: ALF, acute liver failure; CI, confidence interval; FSS, fatigue severity score; HCC, Hepatocellular carcinoma; MMF; mycophenolic mofetil; PSC, Primary sclerosing cholangitis; TAC, tacrolimus; SD, standard deviation; SRL, sirolimus; VAS, visual analogue scale; *indicates statistical significance

‡Other includes: primary biliary cirrhosis, secondary biliary cirrhosis, autoimmune cirrhosis, polycystic liver disease

Three generalized mixed effect models were fitted, investigating the association between the EQ-VAS, the societal values of the EQ-5D-5L health states and the FSS during the course of the study (values for the covariates: tacrolimus trough levels, kidney function, hemoglobin, recipient age and sex, primary disease, the usage of antihypertensive and antidiabetic drugs, the usage of corticosteroids and the usage of MMF as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the EQ-VAS was investigated using natural cubic splines. The coefficients of the spline do not have a direct interpretation (see the figure 3A for interpretation). Missing data were considered as missing completely at random.

	Model for EQ-VAS (n=270)		*Model for the societal values of the EQ-5D-5L health states (n=268)*		*Model for FSS (n=278)*	
Fixed effects	**Estimate**	**95%-CI**	**Estimate**	**95%-CI**	**Estimate**	**95%-CI**
Intercept	51.7	24.2 – 77.7	0.6	0.2 – 0.9	5.56	2.54 – 8.69
ns (Visit, df=3)1	-3.4	-10.8 – 4.02	-	-	-	-
ns (Visit, df=3)2	6.7	-5.8 – 19.9	-	-	-	-
ns (Visit, df=3)3	0.087	-4.7 – 4.9	-	-	-	-
Visit	-	-	0.0001	-0.0019 – 0.0023	0.0016	-0.018 – 0.021
Study group	0.76	-5.3 – 7.0	-0.047	-0.13 – 0.036	-0.23	-0.93 – 0.47
TAC trough level	0.23	-0.40 – 0.83	-0.00019	-0.0073 – 0.0077	-0.019	-0.087 – 0.054
Kidney function	0.098	-0.04 – 0.25	0.0014	-0.00036 – 0.0036	-0.0069	-0.025 – 0.010
Recipient age	0.29*	0.016 – 0.58	0.0028	-0.00094 – 0.0069	-0.021	-0.054 – 0.012
Recipient sex	-2.9	-9.2 – 3.5	-0.046	-0.14 – 0.041	0.050	-0.70 – 0.81
Hemoglobin	0.35	-1.5 – 2.3	0.0066	-0.016 – 0.034	0.022	-0.21 – 0.24
Primary disease ALF	-10.4	-21.1 – 0.28	-0.010	-0.16 – 0.14	-0.11	-1.36 – 1.15
Primary disease Cryptogenic cirrhosis	-3.6	-20.9 – 13.7	0.013	-0.23 – 0.25	0.54	-1.49 – 2.58
Primary disease HCC	-6.5	-14.4 – 1.4	-0.036	-0.14 – 0.073	-0.11	-1.04 – 0.81
Primary disease metabolic disease	7.5	-5.3 – 20.2	0.044	-0.13 – 0.22	-0.89	-2.39 – 0.61
Primary disease other‡	-18.2	-29.1 – 7.4	-0.33*	-0.48 – -0.18	0.78	-0.48 – 2.06
Primary disease PSC	-4.2	-12.2 – 3.8	0.011	-0.099 – 0.12	-0.35	-1.29 – 0.59
Primary disease viral hepatitis	-6.9	-17.9 – 4.1	-0.055	-0.21 – 0.098	-0.21	-1.50 – 1.09
Usage of insulin	2.7	-3.9 – 8.7	-0.05	-0.14 – 0.028	0.27	-0.41 – 0.99
Usage of oral antidiabetics	1.8	-3.5 – 7.1	0.029	-0.040 – 0.10	-0.16	-0.79 – 0.47
Usage of antihypertensive drugs	4.6	0.9 – 8.2	-0.00011	-0.047 – 0.046	-0.24	-0.68 – 0.18
Use of corticosteroids	0.87	-4.0 – 6.1	0.016	-0.029 – 0.062	-0.07	-0.53 – 0.35
Interaction between visit and study group (df =3)1	3.2	-5.8 – 12.1	-	-	-	-
Interaction between visit and study group (df =3)2	6.0	-5.4 – 16.9	-	-	-	-
Interaction between visit and study group (df =3)3	2.9	-3.0 – 8.9	-	-	-	-
Interaction between visit and study group	-	-	0.0017	-0.00067 – 0.0039	-0.0034	-0.025 – 0.019
Random effects	**Variance**	**SD**	**Variance**	**SD**	**Variance**	**SD**
Subject intercept	116.2	10.8	0.023	0.15	1.58	1.26
Residual	77.7	8.8	0.012	0.11	1.19	1.09

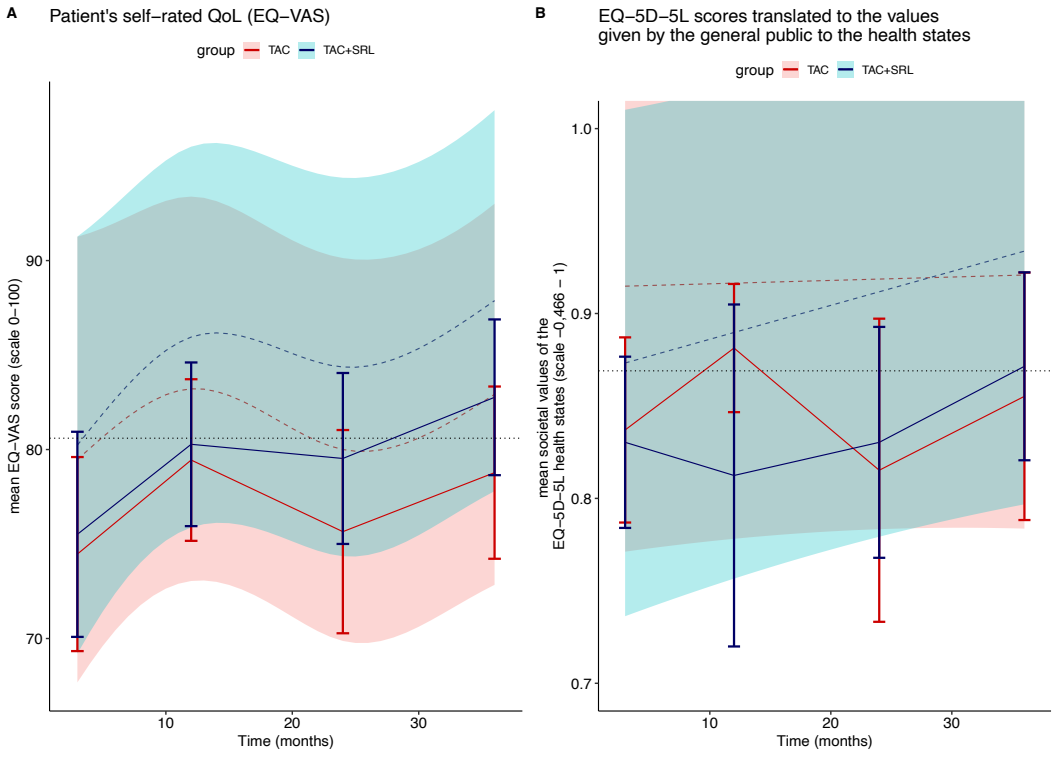
▲

Supplementary table 2. Results of the three generalized mixed effect models for the per protocol population

Abbreviations: ALF, acute liver failure; CI, confidence interval; FSS, fatigue severity score; HCC, Hepatocellular carcinoma; MMF; mycophenolic mofetil; PSC, Primary sclerosing cholangitis; TAC, tacrolimus; SD, standard deviation; SRL, sirolimus; VAS, visual analogue scale; *indicates statistical significance

‡Other includes: primary biliary cirrhosis, secondary biliary cirrhosis, autoimmune cirrhosis, polycystic liver disease

Three generalized mixed effect models were fitted, investigating the association between the EQ-VAS, the societal values of the EQ-5D-5L health states and the FSS during the course of the study (values for the covariates: tacrolimus trough levels, kidney function, hemoglobin, recipient age and sex, primary disease, the usage of antihypertensive and antidiabetic drugs and the usage of corticosteroids as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the EQ-VAS was investigated using natural cubic splines. The coefficients of the spline do not have a direct interpretation (see the supplementary figure 1A for interpretation). Missing data were considered as missing completely at random.



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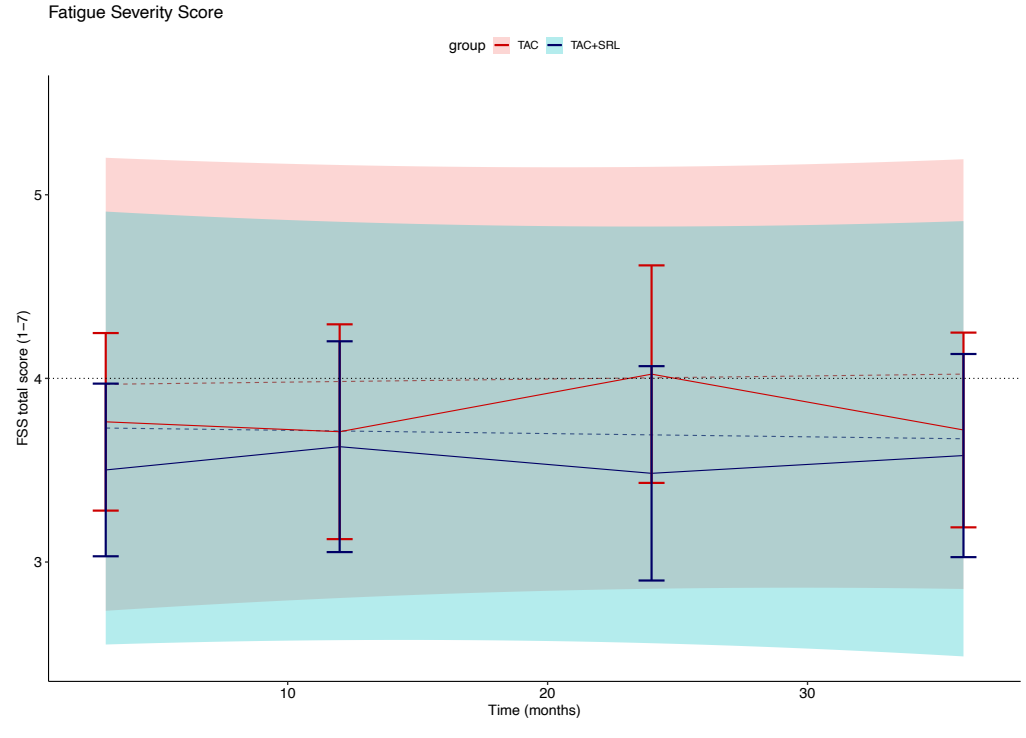
Supplementary figure 1. EQ-VAS score and EQ-5D-5L scores on the dimensions translated to the societal values for the per protocol population

A) Patient's self-rated QoL (EQ-VAS)
Group-wise mean EQ-VAS with 95%-confidence interval (CI) during the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, kidney function, hemoglobin, recipient age and sex, primary disease, the usage of antihypertensive and antidiabetic drugs and the usage of corticosteroids as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the EQ-VAS was investigated using natural cubic splines. Splines provide a convenient non-parametric way to flexibly model (potentially) non-linear associations in regression models. Instead of using one polynomial (e.g., a quadratic or cubic function) that spreads over the whole range of the covariate, splines use a set of several polynomial functions that are defined over smaller intervals. This allows the resulting fit to be more flexible than when using a single polynomial. Missing data were considered as missing completely at random. Dotted black line indicates the mean self-reported EQ-VAS score by the general Dutch population.12

B) EQ-5D-5L scores translated to the values given by the general public to the health states
Group-wise mean of the societal values of the EQ-5D-5L health states with 95%-confidence interval (CI) during the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, kidney function, hemoglobin, recipient age and sex, primary disease, the usage of antihypertensive and antidiabetic drugs and the usage of corticosteroids as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The likelihood-ratio test indicated no need for a nonlinear association structure. Missing data were considered as missing completely at random. Dotted black line indicates the mean EQ-5D-5L score given by the general Dutch population to the health states.12

Abbreviations: TAC, tacrolimus; SRL, sirolimus; QoL, quality of life; VAS, visual analogue scale



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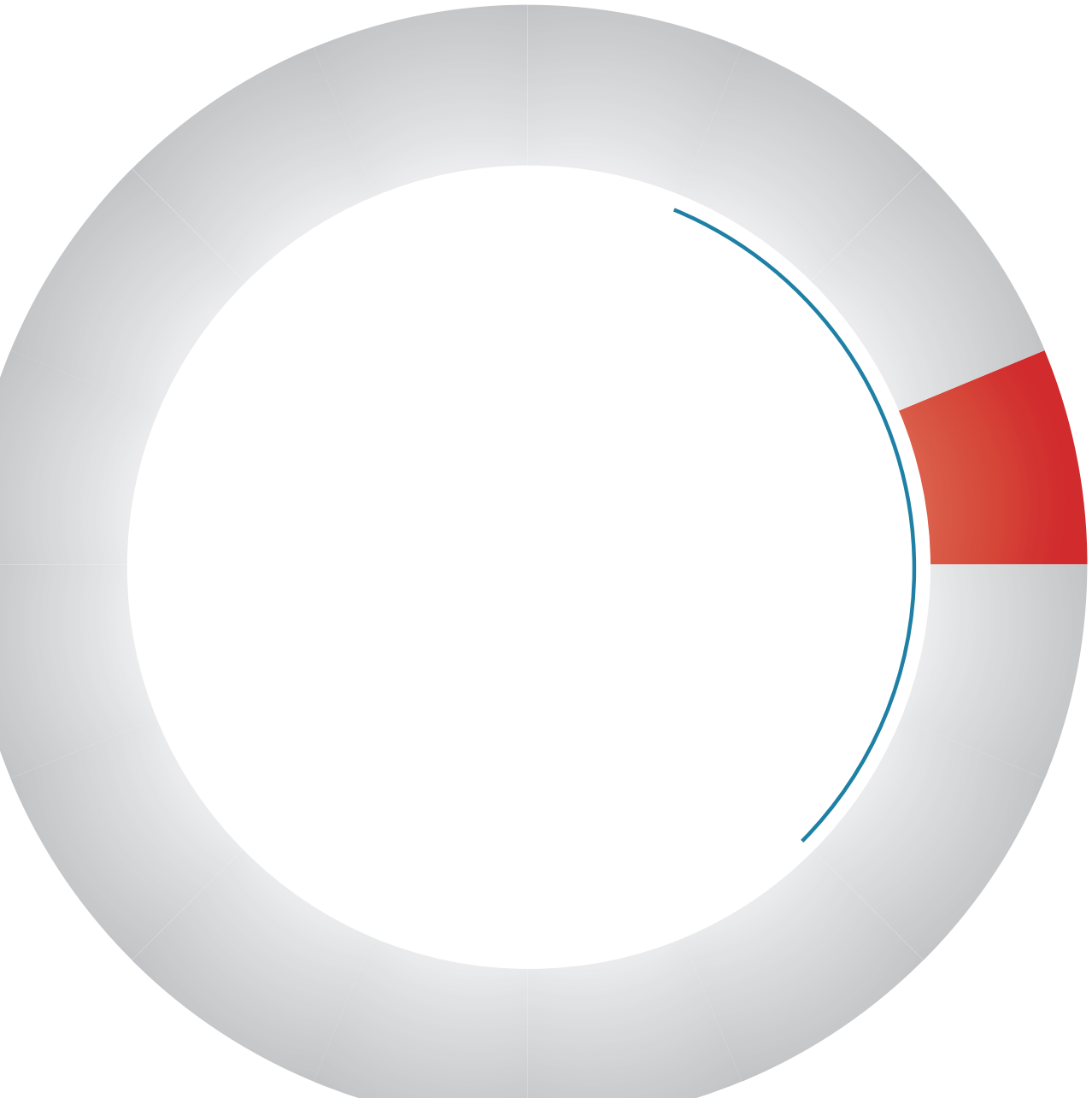
Supplementary figure 2. Severity of Fatigue for the per protocol population

Group-wise mean of the FSS with 95%-confidence interval (CI) during the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, kidney function, hemoglobin, recipient age and sex, primary disease, the usage of antihypertensive and antidiabetic drugs and the usage of corticosteroids as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The likelihood-ratio test indicated no need for a nonlinear association structure. Missing data were considered as missing completely at random. Dotted black line indicates the clinical level for fatigue.15

Abbreviations: FSS, fatigue severity score, TAC, tacrolimus, SRL, sirolimus



Chapter 4

Cardiovascular morbidity and mortality in liver transplant recipients on low-dosed sirolimus: subset study of a randomized controlled trial.

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To be submitted

Abstract

Aim

This study aimed to evaluate the cardiovascular (CV) morbidity and mortality in a subset of a multicenter, randomized, controlled trial in de novo liver transplant (LT) recipients comparing normal-dosed tacrolimus (control) with the combination of low-dosed tacrolimus and sirolimus (intervention).

Methods

LT recipients were enrolled between 2011 and 2018 and prospectively followed for three years or until death/re-transplantation. LT recipients were randomized between 80 and 100 days after LT to 1) once daily normal-dose extended-release TAC (control group) or 2) once daily combination therapy of low-dose SRL and low-dose extended-release TAC (interventional group). The primary endpoint was the cumulative incidence of any major CV event at 36 months after transplantation. The secondary endpoint was to assess the development of CV risk factors.

Results

In total, 122 LT recipients were included. No difference in the cumulative incidence of any major CV event at 36 months after transplantation was found. Significantly less LT recipients in the control group suffered from hyperlipidemia compared to the interventional group; year 1 30% [18/60], versus 50.9% [30/59]; risk difference: -0.208; 95%CI -0.378– -0.021; p=0.025) and year 2 (40.3% [23/57] versus 63.2% [36/57]; risk difference: -0.228; 95%CI -0.402– -0.032; p=0.024. The prevalence of hypertension was significantly lower in the control group compared to the interventional group; 42.1% [24/57] versus 64% [32/50]; risk difference: -0.219; 95%CI -0.399– -0.016 p=0.033).

Conclusion

A sirolimus-based regimen resulted in comparable CV morbidity and mortality among LT recipients at 36 months compared to monotherapy tacrolimus. However, after the first year post-LT significantly more hypertension and hyperlipidemia occurred in the sirolimus-based regimen.

Introduction

Liver transplantation (LT) is the best treatment in patients with end-stage liver disease and hepatocellular carcinoma (HCC). Over the past decades, the survival rates have steadily improved with 1-year patient survival exceeding 80%.(1) The improvement of survival rates is probably due to better surgical and anesthesiologic techniques, improved patient selection and developments in the efficacy and safety of the immunosuppressive medication.(1-3) While survival rates have been rising, cardiovascular (CV) morbidity and mortality have become more relevant among patients following LT.(1, 3, 4)

LT recipients have a high prevalence of CV risk factors, exceeding that of the general population. (3, 5) D'Avola *et al.* showed that the prevalence of obesity, hypertension, hyperlipidemia and diabetes among LT recipients is high and that these patients have a high risk for CV events due to preoperative and postoperative factors.(7) A meta-analysis by Konerman *et al.* identified an incidence of CV events of approximately 22% in the first 6 months post LT and 12% in LT

recipients > 6 months post LT.(6) Current immunosuppressive regimens have been associated with worsening of the preoperative risk factors and the appearance of de novo postoperative risk factors in LT recipients.(2)

All patients must take life-long immunosuppressive agents. Calcineurin inhibitors (CNIs) are the cornerstone of the immunosuppressive regimen, specifically tacrolimus (TAC).(7, 8) The use of TAC has substantially decreased the risk of acute rejection and improved short-term outcomes.(9) However, prolonged use of TAC is associated with significant toxicity, such as renal dysfunction, diabetes mellitus and hypertension.(10-12) Gansevoort *et al.* stated that in a normal population CV mortality was about twice as high in patients with stage 3 chronic kidney disease and three times as high at stage 4 than that in individuals with normal kidney function.(13) This makes individuals with chronic kidney disease one of the highest-risk groups for CV disease. D'Avola *et al.* showed that pre-existing diabetes is one of the strongest predictors of CV mortality after LT and Hjelmesaeth *et al.* identified that the 8-year cumulative incidence of major CV events was 21% in patients with diabetes before transplantation and 7% in patients without diabetes.(5, 14) New onset diabetes mellitus (NODAT) can be provoked using immunosuppressive drugs and is also associated with an increased risk of CVD.(14)

Several strategies have been proposed to reduce TAC related toxicity in LT recipients and consequently reduce the risk on CV events. (15) One strategy involves the reduction in exposure to TAC using an adjunct immunosuppressant, as the mammalian target of rapamycin (mTOR)-inhibitor, sirolimus (SRL). Due to concerns about vascular thrombosis in the post-operative period, SRL has not been licensed for the use in LT recipients.(16) Despite the concerns, SRL is used off-label in immunosuppressive drug regimens after LT.

So far, little is known with regards to the effect of different immunosuppressive regimens on the CV morbidity and mortality. Therefore, the primary aim of this study was to evaluate the cumulative incidence of CV morbidity and mortality in a large Dutch randomized, controlled trial in de novo LT recipients comparing normal-dosed TAC with the combination of low-dosed TAC and SRL over a period of 3 years. The secondary aim was to investigate the prevalence of CV risk factors in de novo LT recipients.

Materials and methods

Study design and participants

This is a subset study of the open label, multi-center, randomized, controlled trial (RCT) comparing the effect of the combination of low-dose SRL and low-dose extended-release TAC on the long-term renal function versus normal-dose extended-release TAC in de novo LT recipients (LOLIII study). An extensive description of the LOLIII study design has been published previously.(17) We included participants randomized at the Erasmus University Medical Center, Rotterdam, The Netherlands, which represent 62% of the total study population.(17) LT recipients were enrolled between February 2011 and March 2018 and prospectively followed for three years or until death/re-transplantation. LT recipients were randomized between 80 and 100 days after LT to 1) once daily normal-dose extended-release TAC (control group) or 2) once daily combination therapy of low-dose SRL and low-dose extended-release TAC (interventional group).

Data collection

In addition to the data collected in the LOLIII-study, data with regards to CV morbidity and mortality were retrospectively collected. Patient's medical records were reviewed from the date of LT until their last follow-up visit or death. The following variables were collected: smoking, age, family history of CVD, MELD-score, indication of LT, presence of CV risk factors before LT (diabetes, hypertension, obesity, previous CV diseases), the presence of NODAT, hyperlipidemia, hypertension and obesity. Furthermore, information with regards to the occurrence of any major CV event including atrial fibrillation, heart failure, stroke, venous thromboembolism, cardiac arrest and coronary artery disease was collected.

Study Endpoints

The primary endpoint was the cumulative incidence of any major CV event at 36 months after transplantation. Major CV events were: atrial fibrillation, heart failure, stroke, venous thromboembolism, cardiac arrest and coronary artery disease.

The secondary endpoint was to investigate the prevalence of CV risk factors during the study period. CV risk factors were: diabetes mellitus, hyperlipidemia, hypertension, obesity and smoking. Additionally, LT recipients lost to follow-up due to re-transplantation or death were stated.

Definitions

Diabetes mellitus was defined, according to the World Health Organization (WHO), as a fasting plasma glucose of 7.0 mmol/L measured on at least two different occasions, a HbA1c > 65 or the need of antidiabetic treatment. (18) Hyperlipidemia was defined as low-density lipoprotein (LDL) > 3 mmol/L, according to Dutch hospital guidelines. (19) Hypertension was defined, according to the WHO, as an average blood pressure of >140/90 mmHg as measured on at least two different occasions or the need of antihypertensive treatment. (5, 20) Overweight and obesity were defined as a body mass index (BMI) ≥ 25 kg/m2 and ≥ 30 kg/m2 according to the WHO definition. Obesity was divided into stage I (30-35 kg/m2) and stage II (≥ 35 kg/m2). (5, 21) The eGFR was calculated using the CKD-EPI formula and classified according to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines. (22)

Statistical analysis

Patient characteristics are summarized using counts (%) for nominal and ordinal variables and mean (SD) or median (inter-quartile range, IQR) for the continuous variables, depending on the shape of the distribution. Confidence intervals for proportions were calculated using the Wilson Score method.

All analyses were conducted in the intention-to-treat population that included all LT recipients who underwent randomization and in whom treatment was initiated. The primary endpoint was evaluated with Kaplan–Meier analysis and the log-rank test. Differences in CV risk factors were tested using the Chi-squared test. A p-value of <0.05 was considered as statistically significant. The analysis were performed using R software (version 3.6.2). (23)

Ethics

A waiver was given for this study by the Medical Ethics Committee of the Erasmus University Medical Center (MEC-2021-0942). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Patient data were sampled and stored in accordance with privacy regulations.

Results

A total of 122 liver transplant recipients were included in this study. Table 1 presents the baseline characteristics of the study population at randomization. No differences were identified between both study groups. Most frequent indications for transplantation were hepatocellular carcinoma and primary sclerosing cholangitis. At randomization, 45.9% (56/122) of the LT recipients were smokers or had a history of smoking, 39.3% (48/122) of the LT recipients had hyperlipidemia and 44.3% (54/122) of the LT recipients had hypertension. Moreover, 41.8% (51/122) of the LT recipients were classified as overweight or obese with a BMI above 25 kg/m2.

	TAC (n = 61)	TAC+SRL (n = 61)
Age (median [IQR])	56.00 [49.00, 62.00]	53.00 [48.00, 63.00]
Sex, male (n, %)	41 (67.2)	43 (70.5)
Ethnicity (n, %)		
Caucasian	56 (91.8)	53 (86.9)
Other*	5 (8.2)	8 (13.1)
Primary disease (n, %)		
(N)ASH	10 (16.4)	9 (14.8)
ALF	3 (4.9)	8 (13.1)
Cryptogenic	2 (3.3)	3 (4.9)
HCC	19 (31.1)	15 (24.6)
Metabolic disease	2 (3.3)	4 (6.6)
PSC	18 (29.5)	10 (16.4)
Viral Hepatitis	3 (4.9)	4 (6.6)
Other¶	4 (6.6)	8 (13.1)
LAB MELD (median [IQR])	17.00 [11.00, 22.00]	20.00 [14.00, 24.00]
Family history of CVD (n, %)	10 (16.4)	12 (19.7)
Previous CV events (n, %)		
Atrial fibrillation	3 (4.9)	3 (4.9)
Cerebrovascular event	-	2 (3.3)
Deep venous thrombosis	-	2 (3.3)
Ischemic heart disease	3 (4.9)	2 (3.3)
Peripheral artery disease	1 (1.6)	-
None	54 (88.5)	52 (85.2)
Diabetes mellitus pre-LT, Yes (n, %) [a]	11 (18.0)	13 (21.3)
Hyperlipidemia, Yes (n, %) [b]	24 (39.9)	24 (39.9)
Hypertension, Yes (n, %) [c]	29 (47.5)	25 (41.0)
BMI (n, %)		
< 25 kg/m^2	31 (50.8)	34 (55.7)
25-30 kg/m^2	24 (39.3)	18 (31.1)
30-35 kg/m^2	1 (1.6)	4 (6.6)
≥ 35 kg/m^2	2 (3.3)	1 (1.6)
Unknown	3 (4.9)	3 (4.9)
Smoking (%)		
Active smoker	9 (14.8)	8 (13.1)
Ex-smoker	21 (34.4)	18 (29.5)
No	21 (34.4)	23 (37.7)
Unknown	10 (16.4)	12 (19.7)
eGFR < 60 ml/min/1.73m^2, Yes (n, %) [d]	15 (24.6)	17 (27.9)

Table 1. Baseline characteristics

* Other includes: Asian and African

¶ Other includes: autoimmune cirrhosis, primary biliary cirrhosis, secondary biliary cirrhosis, polycystic liver disease;

[a] Diabetes mellitus was defined according to the World Health Organization (18);

[b] Hyperlipidemia was defined as LDL > 3 mmol/L (19);

[c] Hypertension was defined according to the World Health Organization (20);

[d] eGFR was classified according to KDIGO Guidelines (22);

Abbreviations: TAC, tacrolimus; SRL, sirolimus; (N)ASH, (non)alcoholic steatohepatitis; ALF, acute liver failure; HCC, hepatocellular carcinoma; PSC, primary sclerosing cholangitis; MELD, model for end-stage liver disease; CVD, cardiovascular disease, CV, cardiovascular; BMI, body mass index

Primary endpoint: CV morbidity and mortality

The cumulative incidence of LT recipients with a major CV event at 36 months was 8.2% (95% confidence interval [CI], 1.0 – 14.8) in the interventional group and 8.3% (95% CI, 1.1 – 14.9) in the control group (p = 1 by log-rank test) (figure 1). The prevalence of the primary end-point events during the study period are listed in Table 2. The majority (90%) of the CV events occurred within the first year after LT and 50% (5/10) of the events were venous thromboembolisms. During the study period 10 LT recipients were excluded for further follow-up due to death or re-transplantation.

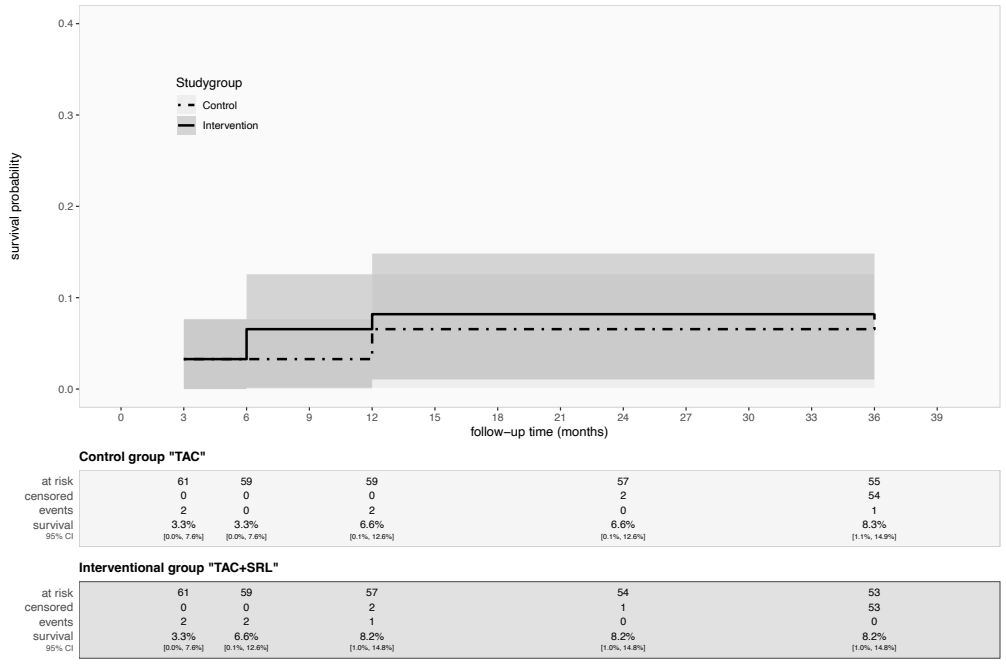

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Figure 1. Overall cardiovascular morbidity and mortality after liver transplantation.

Shown is the Kaplan-Meier estimate of the overall cardiovascular (CV) morbidity and mortality after liver transplantation. LT recipients were assigned to receive either low-dose sirolimus (SRL) combined with low-dose extended-release tacrolimus (TAC) or normal-dosed extended-release TAC. LT recipients were followed for 3 years or until death/re-transplantation. CV events included: atrial fibrillation, heart failure, stroke, venous thromboembolism, cardiac arrest and coronary artery disease. The percentage of LT reciepients with a major CV event at 36 months was 8.2% (95% confidence interval [CI], 1.0-14.8) in the interventional group and 8.3% (95% CI, 1.1-14.9) in the control group (p = 1, log-rank test).

Abbreviations: TAC, tacrolimus; SRL, sirolimus.

	Pre LT		Month 3		Month 6		Year 1		Year 2		Year 3		Overall at the end of study	
	TAC control (n=61)	Low-dose TAC + SRL (n=61)	TAC control (n=61)	Low-dose TAC + SRL (n=61)	TAC control (n=61)	Low-dose TAC + SRL (n=61)	TAC control (n=60)	Low-dose TAC + SRL (n=59)	TAC control (n=58)	Low-dose TAC + SRL (n=58)	TAC control (n=57)	Low-dose TAC + SRL (n=55)	TAC control (n=61)	Low-dose TAC + SRL (n=61)
Overall CV events, Yes (n, %)	6 (9.8)	8 (13.1)	2 (3.3)	2 (3.3)	-	2 (3.3)	2 (3.3)	1 (1.7)	-	-	1 (1.7)	-	5 (8.2)	5 (8.2)
Atrial fibrillation, Yes (n, %)	3 (4.9)	3 (4.9)	-	1 (1.6)	-	-	-	-	-	-	-	-	-	1 (1.6)
Heart failure, Yes (n, %)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stroke, Yes (n, %)	-	2 (3.3)	-	-	-	2 (3.3)	-	-	-	-	-	-	-	-
VTE, Yes (n, %)	-	2 (3.3)	2 (3.3)	1 (1.6)	-	-	1 (1.7)	1 (1.7)	-	-	-	-	3 (4.9)	2 (3.3)
Cardiac arrest, Yes (n, %)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CAD, Yes (n, %)	3 (4.9)	1 (1.6)	-	-	-	-	1 (1.7)	-	1 (1.7)	-	1 (1.7)	-	2 (3.3)	-
Re-LT, Yes (n, %)	-	-	-	-	-	-	-	2 (3.4)	1 (1.7)	-	-	-	1 (1.6)	2 (3.3)
Death, Yes (n, %)	-	-	-	-	-	-	1 (1.7)	-	1 (1.7)	1 (1.7)	1(1.7)	3 (5.5)	3 (5.5)	4 (6.6)

◄

Table 2. Detailed overview of the primary endpoints

Abbreviations: LT, liver transplantation; TAC, tacrolimus; SRL, sirolimus; CV, cardiovascular; VTE, venous thromboembolism; CAD, coronary artery disease

Secondary endpoint: Development of CV risk factors after LT

Figure 2 shows the development of CV risk factors after LT, including diabetes mellitus, hyperlipidemia, hypertension and obesity (figure 2A) and smoking (figure 2B). No significant differences were found between the study groups in the prevalence of diabetes mellitus, obesity and smoking. A decline in the prevalence of diabetes mellitus was observed in both study groups during the study period. For both groups, at the moment of randomization (i.e. 3 months after transplantation) approximately 45% of the LT recipients had diabetes mellitus compared to approximately 30% of the LT recipients after 3 years. Pre-LT approximately 20% of the LT recipients have diabetes mellitus. In the control group, significantly less LT recipients suffered from hyperlipidemia compared to the interventional group at year 1 (30% [18/60], 95%CI 19.9-42.5% versus 50.9% [30/59], 95%CI 37.7-62.3%; risk difference: -0.208; 95%CI -0.378– -0.021; p=0.025) and year 2 (40.3% [23/57], 95%CI 28.6-53.3% versus 63.2% [36/57], 95%CI 50.2-74.5%; risk difference: -0.228; 95%CI -0.402– -0.032; p=0.024). Significantly less LT recipients experienced hypertension in the control group compared to the interventional group at year 3 (42.1% [24/57], 95%CI 30.2-55.0% versus 64% [32/50], 95%CI 50.1-75,.9%; risk difference: -0.219; 95%CI -0.399– -0.016 p=0.033). The number of active smokers was equal in both study groups over the entire study period.

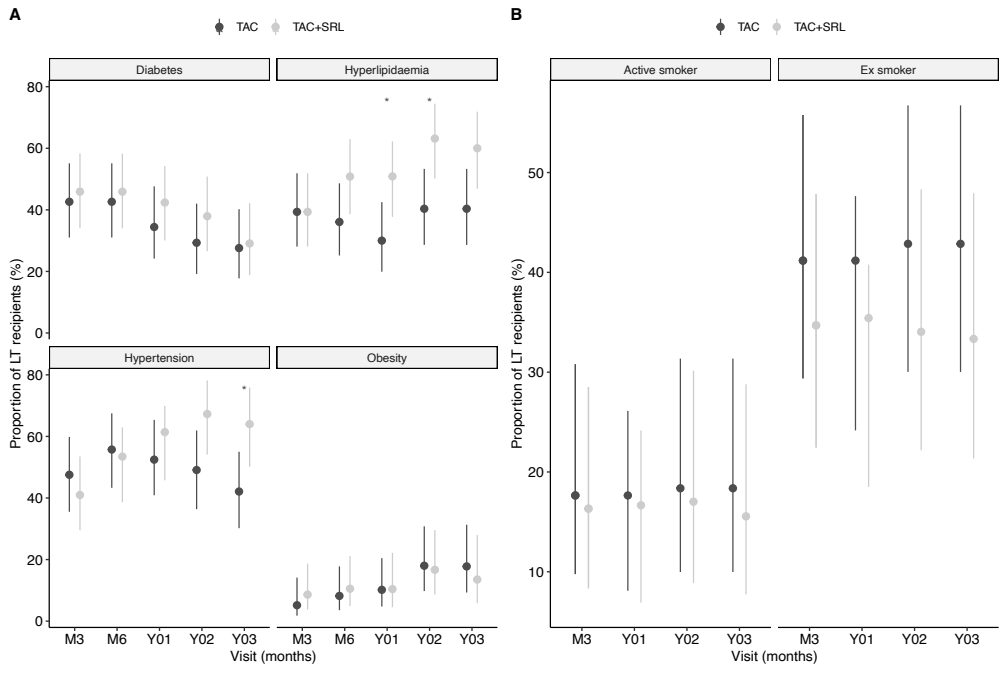

Figure 2. Development of cardiovascular risk factors after LT.

Panels A and B show the proportion of LT recipients with 95%-CI experiencing CV risk factors as indicated by the panel headers. Hypertension significantly differed between the study groups at year 3 (42.1% [24/57], 95%CI 30.2-55.0% versus 64% [32/50], 95%CI 50.1-75,.9%; risk difference: -0.219; 95%CI -0.399– -0.016 p=0.033). Hyperlipidemia significantly differed between the study groups at year 1 (30% [18/60], 95%CI 19.9-42.5% versus 50.9% [30/59], 95%CI 37.7-62.3%; risk difference: -0.208; 95%CI -0.378– -0.021; p=0.025) and year 2 (40.3% [23/57], 95%CI 28.6-53.3% versus 63.2% [36/57], 95%CI 50.2-74.5%; risk difference: -0.228; 95%CI -0.402– -0.032; p=0.024).
*, p-value <0.05

Discussion

In this 36-month trial, low-dose SRL combined with low-dose extended-release TAC compared to normal-dose extended-release TAC resulted in comparable rates of CV morbidity and mortality among LT recipients. Most CV events occurred within the first year after transplantation. After the first year post-LT, the use of low-dose SRL combined with low-dose extended-release TAC resulted in significantly more hyperlipidemia and hypertension compared to normal-dose extended-release TAC.

Our results are in line with the findings of McKenna *et al.*, who compared CV events in a SRL-based regimen and a regimen without SRL. They did not show a difference in the total number of CV events between the SRL cohort and the non-SRL cohort (8.9% vs. 8.1%).(24) Furthermore, another study investigated the prevalence of in-hospital CV events early after transplantation and showed a comparable rate of CV events (11%).(25) Half of the CV events in our study were venous thromboembolisms. We did not find differences in the number of hospitalization days and the usage of anticoagulants in these LT recipients as a possible explanation for these events.

Our finding contradicts the study by Fussner *et al.*, who found a total of 15.3% (70/455) of the LT recipients in their cohort suffering from a CV event during a 3-year follow-up period.(26) A possible explanation for this could be the fact that a higher percentage of the LT recipients was actively smoking in this study compared to our study (51% versus 13.9%).

An important finding in our study is that after the first year a SRL-based regimen resulted in a significantly higher prevalence of hypertension and hyperlipidemia. SRL is strongly associated with hypertension and dyslipidemia as stated in the summary of product characteristics of SRL.(27) Like our results, Di Stefano *et al.* showed that the development of sustained hypertension after LT was related to treatment with a mTOR inhibitor and not to treatment with TAC only.(28) Furthermore, our findings confirm the results by Nguyen *et al.*, who showed a higher incidence of hyperlipidemia associated with mTOR inhibitors compared to TAC.(29) Thereby, they concluded that the higher prevalence of hyperlipidemia with SRL may predispose these LT recipients to more CV events. Conversely, we did not find evidence for a difference in the frequency of CV events in LT recipients treated with SRL during the three-year follow-up. However, higher lipid concentrations and hypertension might influence the prevalence of CV events on the long term, e.g., > 10 years after transplantation rather than already within three years.

The management of CV risk factors is crucial pre-LT and on the long-term post-LT. The COMMIT guideline provides practical recommendations for identification and management of modifiable risk factors after liver and kidney transplant. It states that intervention strategies which target modifiable risk factors (e.g. obesity, diabetes, hypertension, dyslipidemia, smoking and renal dysfunction) will be vital for improving long-term outcomes in LT recipients.(30) Interestingly, we found a decline in the prevalence of diabetes mellitus in the LT recipients in both groups during the study period. For both study groups, at the moment of randomization (i.e. 3 months after transplantation) approximately 45% of the LT recipients had diabetes mellitus compared to approximately 30% of the LT recipients after 3 years. This might be explained by the fact that corticosteroids are tapered and then stopped in the majority of our LT recipients within the first year post-LT in our program and lowering of the TAC trough levels post-LT. The percentage of obese LT recipients could be underestimated in our study due to missing

data. Nowadays, the obesity rate in the world is increasing and nonalcoholic steatohepatitis (NASH) is becoming a growing indication for LT.(31) LT recipients transplanted for NASH are prone to develop a metabolic syndrome post-LT resulting in CV morbidity and mortality. Therefore, lifestyle changes, like frequent exercising and cessation of smoking, individualizing immunosuppressive therapy with regards to side effects and the appropriate management of comorbidities are essential to minimize the risk of CV complications after LT.

An important limitation of this study is the fact that we retrospectively collected additional data with regards to the CV mortality, morbidity and risk factors. As a consequence, some information with regards to the smoking history and obesity is missing. However, we believe that this did not influence the findings for CV events. Every LT recipient is at least annually seen in our outpatient clinic and major CV events are consequently registered in the patients' electronic medical records. Another limitation is that the observation period of three years might be too short to observe every major CV event of interest. However, as shown in other studies, most CV events post-LT occur within the first years after transplantation.

In conclusion, low-dose SRL combined with low-dose extended-release TAC compared to normal-dosed extended-release TAC resulted in comparable rates of CV morbidity and mortality among LT recipients at 36 months. However, after the first year post-LT significantly more hypertension and hyperlipidemia occurred in the SRL-based regimen. Based on this study we do not advocate low-dosed SRL combined with low-dosed extended-release TAC on the long-term.

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Chapter 5

Modifying tacrolimus-related toxicity after liver transplantation comparing life cycle pharma (LCP)-tacrolimus versus extended-released tacrolimus: a multicenter randomized, controlled trial (MOTTO).

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Abstract

Background

The aim of this open-label, multicenter, randomized controlled study was to investigate whether the life cycle pharma (LCP)-tacrolimus compared to the extended-release (ER)-tacrolimus formulation results in a difference in the prevalence of post-transplant diabetes mellitus (PTDM), hypertension and chronic kidney disease at 12 months after liver transplantation.

Methods

Patients were 1:1 randomized to either of the two tacrolimus formulations. The primary endpoint was defined as a composite endpoint of any of three events: sustained (>3 months post randomization) PTDM, new onset hypertension, and/or chronic kidney disease, defined as eGFR<60 mL/min/1.73m^2 for >3 months during the follow-up.

Results

In total, 105 patients were included. In the intention-to-treat analysis, a statistically significant lower proportion of liver transplant recipients in the LCP-tacrolimus group reached the composite primary endpoint at 12 months compared to the extended-release tacrolimus group (50.9% [27/53], 95% confidence interval (CI) 37.9-63.9% versus 71.2% [37/52], 95%CI 57.7-81.7%; risk difference: 0.202; 95%CI 0.002–0.382; p = 0.046). No significant difference was found in the per protocol analysis. In the intention-to-treat and per protocol population, fewer liver transplant recipients in the LCP-tacrolimus group developed CKD and new-onset hypertension compared to the ER-tacrolimus group. No differences in rejection rate, graft and patient survival were found.

Conclusions

A statistically significant and clinically relevant reduction in the prevalence of the composite primary endpoint was found in the LCP-tacrolimus group compared to the ER-tacrolimus group in the first year after liver transplantation with comparable efficacy.

Introduction

Tacrolimus is the cornerstone of the immunosuppressive regimen after liver transplantation (LT). The use of tacrolimus has substantially decreased the risk of acute rejection and has improved short-term outcomes, but these short-term gains are not matched by similar gains in long-term outcomes.(1-3) Tacrolimus was approved in 1994 by the EMA and FDA as twice-daily capsules (Prograf®, Astellas Pharma). In 2007, the first once-daily extended-release (ER)-tacrolimus formulation (Advagraf®, Astellas Pharma) received approval, and in 2014 a second prolonged-release once-daily tacrolimus formulation, life cycle pharma (LCP)-tacrolimus, (Envarsus®; Chiesi Farmaceutici S.p.A.) was approved. The introduction of once-daily tacrolimus formulations improved the medication adherence in liver transplant recipients.(4, 5) Around the world, the choice for a tacrolimus formulation varies among transplant centers and no preference is pronounced.

Tacrolimus is associated with a wide range of side effects with potential negative impact on long-term outcome in liver transplant recipients. Cumulative exposure and peak blood concentration

of tacrolimus are two factors associated with side-effects which are potentially modifiable.(6-8) Nephrotoxicity, post-transplant diabetes mellitus (PTDM), hypertension, and neurotoxicity are the most common side effects specific to CNI's, aside from the risk of infection and the development of de novo malignancy, which are shared by most immunosuppressive agents.(9) Several studies show that in the first years after LT the incidence of PTDM ranges from 10 – 30% and the incidence of hypertension ranges from 40 – 60%.(10) Furthermore, up to 50% of LT recipients will develop chronic kidney disease (CKD) defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73m^2.(11, 12) Apart from the direct nephrotoxic effects of tacrolimus, diabetes mellitus, hypertension and, in the past, recurrent hepatitis C infection have an additive effect on the development of CKD.(13) A number of strategies has been developed to minimize the risk on tacrolimus toxicity including different dosing regimens and combinations with multiple immunosuppressive agents allowing for lower (cumulative) tacrolimus exposure.(12)

LCP-tacrolimus is a prolonged-release tacrolimus formulation utilizing a new drug delivery technology (MeltDose).(14, 15) This formulation has lower peak-through blood level fluctuations and a higher bioavailability compared to the other tacrolimus formulations, resulting in a lower dose requirement to reach a certain tacrolimus exposure.(14, 16, 17) Rayar *et al.* showed that a high intra-patient variability of tacrolimus exposure in LT recipients was associated with poorer outcomes.(18) Furthermore, the tacrolimus immediate-release (IR) formulation (Prograf®) and ER-tacrolimus formulation are associated with a characteristic high peak concentration (Cmax) following dosing, which may be associated with increased neurotoxicity.(19) Whether the high peak concentration (Cmax) is also associated with the increased cardiovascular risk profile of tacrolimus is unknown.

So far, no head-to-head comparison between the two once-daily tacrolimus formulations has been performed to evaluate differences in clinically relevant outcomes. Therefore, the aim of this randomized, controlled study was to investigate whether LCP-tacrolimus compared to ER-tacrolimus results in a difference in the prevalence of PTDM, new onset hypertension and CKD at 12 months after transplantation.

Materials and Methods

Study design and participants

This study was an open-label, multicenter, randomized, controlled trial. Patients were enrolled between April 2019 - October 2021 and prospectively followed for 12 months or until death. Patients were randomized at discharge or within 4 weeks (whichever came first) after liver transplantation from IR-tacrolimus to LCP-tacrolimus or ER-tacrolimus.

Included were adult patients, between 18 and 75 years, after a primary LT. All participants gave written informed consent before any study-related activity. Main exclusion criteria were: multi-organ transplantation, estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2 at the moment of randomization, hepatic artery thrombosis, known hypersensitivity to tacrolimus and the use of a mTOR-inhibitor or the need for an IR-tacrolimus formulation.

The study was performed at two centers in the Netherlands: The Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands and Leiden University Medical Center, Leiden, The Netherlands. The study was approved by the institutional Ethical Committees of these institutions, registered in the EudraCT database (EudraCT: 2018-002856-34) and conducted in accordance with the latest version of the declaration of Helsinki.

Study Endpoints

The primary endpoint was defined as a composite endpoint of any of three events: sustained (>3 months post randomization) PTDM, new onset hypertension and/or CKD.

PTDM was defined according to the definition of diabetes mellitus by the World Health Organization (i.e., fasting plasma glucose value of 7.0 mmol/L or random venous plasma glucose concentration ≥11.1 mmol/l measured at least on two different occasions or HbA1C >48 mmol/mol) and excludes the diagnosis of diabetes mellitus prior to liver transplantation.(20, 21) Since the majority of patients receive high dose prednisolone in the immediate post-transplant period, PTDM was defined by a sustained hyperglycemia after the first 3 months post-LT.

New onset hypertension was defined as a systolic blood pressure of >140 mm Hg or diastolic blood pressure of >90 mm Hg measured during ≥2 office blood pressure measurements. This definition excluded the presence of hypertension prior to liver transplantation.

CKD was defined as grade ≥3 (eGFR <60 mL/min/1.73m^2) for >3 months during the follow-up according to the KDIGO classification.(22) The renal function was measured by serum creatinine and the estimated glomerular filtration rate was calculated using the CKD Epidemiology Collaboration (CKD-EPI) creatinine equation.(23)

Secondary endpoints included: the individual components of the composite endpoint, prevalence of LT recipients having an eGFR <60 or <30 ml/min/1.73m^2 at 3, 6 and 12 post-LT, graft survival, recipient survival, number of episodes and severity of rejections and safety. Furthermore, the cumulative exposure to tacrolimus was calculated by the area under curve of trough concentrations based on work by Rodríguez-Perálvarez *et al.*.(7, 8) and for the patient treated according to the protocol the intra-patient variability (IPV) in tacrolimus in the first 6 and 12 months was quantified as the coefficient of variation as described by van der Veer *et al.*.(24) Serious Adverse Events (SAEs) were described according to the Medical Dictionary for Regulatory Activities (MedDRA). Infections included every viral or bacterial infection that occurred during the study period excluding cholangitis.

Randomization and masking

Participants were randomly assigned (1:1) to either LCP-tacrolimus or ER-tacrolimus according to a computer-generated randomization list by CastorEDC.(25) Stratification was done by center, to ensure an equal distribution of both arms in the two participating centers. Blinding of participants and physicians was not applied.

Procedures

After the transplantation participants received after basiliximab induction, corticosteroids and mycophenolic acid (MPA). From day 5 after transplantation IR-tacrolimus was started. The tacrolimus trough level at the time of randomization had to be at least 6 ng/ml and mycophenolic acid (MPA) had to be discontinued. During the study follow-up, the dose of both tacrolimus

formulations was adapted according to trough levels, aiming for a trough level between 8 and 10 µg/l in the first 3 months and a trough level between 6 and 8 µg/l thereafter. Dose adjustments of both formulations resulting in lower or higher trough levels were allowed in case of severe side-effects or rejection. In case of deterioration of the kidney function, tacrolimus monotherapy could be switched to mycophenolic acid (MPA) or a mTOR-inhibitor in combination with low-dose tacrolimus. Subjects switching tacrolimus therapy will not be replaced, according to the intention-to-treat principle. Corticosteroids were lowered or discontinued within 180 days after randomization at the discretion of the treating physician.

Data collection

Variables collected included recipient socio-demographic, clinical and transplantation parameters, serious adverse events and trough levels of tacrolimus.

Statistical analysis

The percentage of LT recipients reaching the primary composite endpoint in the ER-tacrolimus arm was estimated at 68% (based on historical data, not published, at the Erasmus University Medical Center). The percentage of LT recipients reaching the primary composite endpoint in the LCP-tacrolimus arm was expected to be 30% percentage points lower compared to the control group. To have 80% power to detect a significant difference at the 95% confidence level using Pearson's Chi-square test with continuity correction 96 patients are required. However, to compensate for any unexpected loss 10 additional patients were included resulting in a total of 106 patients deemed to be required.

Variables were described using counts (%) for nominal and ordinal variables and mean (standard deviation, SD) or median (inter-quartile range, IQR) for the continuous variables, depending on the shape of the distribution.

The risk differences for the primary and secondary outcomes between the two treatment arms were compared using the Pearson's chi-squared test (with continuity correction). The corresponding p-values were obtained via Monte Carlo simulation with 1 million simulations. Secondary endpoints were analyzed using the Pearson's Chi-square test, Mann-Whitney U Test or student's t-test. For all statistical tests, a (two-sided) p-value of <0.05 was considered to indicate statistical significance.

A generalized mixed effect model was fitted to examine the kidney function over the course of the study. Besides the treatment arm, visit number and their interaction, the model included covariates shown to be relevant in previous studies: tacrolimus trough levels, recipient age and sex, pre- and post-transplantation hypertension and diabetes mellitus. Participant-specific random intercepts were included to account for correlation among repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. Missing data were considered as missing completely at random. To visualize the estimated associations, the expected kidney function across the course of the study was calculated while fixing the values of all other covariates to the median or reference category. The analysis was performed as an intention-to-treat (ITT) and per protocol (PP). Patients with protocol violations in immunosuppressive therapy, a re-transplantation or death were excluded in the per-protocol analysis. All data were collected in CastorEDC and analyses were conducted with R software (version 4.2.1).(25, 26)

Results

Table 1 presents the baseline characteristics. A total of 105 patients was included, of whom 52 randomized to the ER-tacrolimus and 53 to the LCP-tacrolimus arm (figure 1). Most of the patients was transplanted because of HCC (31/105, 29.5%), primary sclerosing cholangitis (18/105, 17.1%) or (non)alcoholic steatohepatitis (17/105, 16.2%). The mean eGFR at randomization in the ER-tacrolimus and LCP-tacrolimus group was 82 ± 17.8 and 79 ± 20.4 ml/min/1.73m^2. More patients with pre-transplant hypertension were included in the ER-tacrolimus compared to the LCP-tacrolimus group (32.7% versus 20.8%).

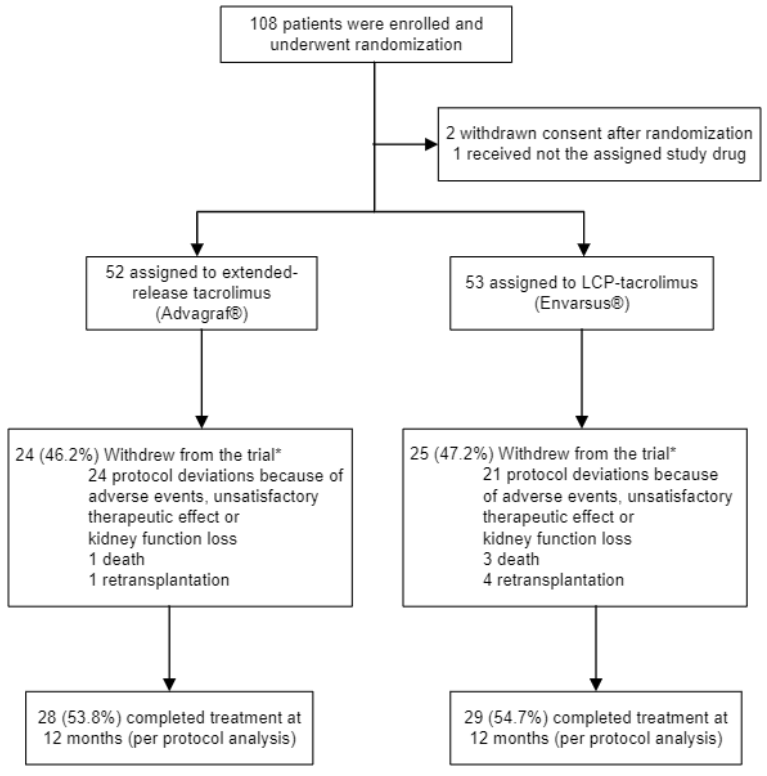

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Figure 1. Enrollment, Randomization, and Follow-up.

* Some LT recipients experiencing protocol deviations died or had a retransplantation.

▶

Table 1. Baseline characteristics

	ER-tacrolimus (n = 52)	LCP-tacrolimus (n= 53)
Recipient demographics at randomization		
Age, year (median, IQR)	58.50 (46.75 – 65.25)	56.50 (46.25 - 63)
Gender, male (n, %)	41 (78.8%)	35 (66%)
Body mass index, kg/m^2 (mean ±SD)	26.13 ± 5.28	25.82 ± 4.56
Ethnicity (n, %)		
Caucasian	46 (88.5%)	49 (92.5%)
Other§	4 (7.6%)	4 (7.5%)
Unknown	2 (3.8%)	1 (1.9%)
Primary Disease (n, %)		
Hepatocellular carcinoma	19 (36.5%)	12 (22.6%)
(Non)alcoholic steatohepatitis	7 (13.5%)	10 (18.9%)
Primary sclerosing cholangitis	10 (19.2%)	8 (15.1%)
Acute liver failure	3 (5.8%)	3 (5.7%)
Cryptogenic cirrhosis	3 (5.8%)	3 (5.7%)
Metabolic diseases	-	4 (7.5%)
Viral Hepatitis	3 (5.8%)	3 (5.7%)
Other‡	7 (13.5%)	11 (20.8%)
Hematology lab		
Hemoglobin, mmol/L (mean ± SD)	6.3 ± 0.9	6.1 ± 0.8
Leucocytes, 10^9/L (mean ± SD)	9.5 ± 4.8	9.5 ± 4.7
Platelets, 10^9/L (mean ±SD)	263 ± 125	249 ± 122
INR (mean ±SD)	1.6 ± 0.5	1.2 ± 0.3
Factor V (median, IQR)	1.71 (1.39 – 1.71)	1.47 (1.16 – 1.66)
Chemistry lab		
Albumin, g/L (mean ± SD)	32.9 ± 4.7	33.7 ± 4.2
Bilirubin, µmol/L (median, IQR)	19 (12.8 – 31.8)	19 (12.3 – 27.5)
Creatinine, µmol/L (mean ± SD)	77 ± 26	82 ± 36
eGFR, ml/min/1.73m^2 (mean ± SD)	82 ± 18	79 ± 20
Cholesterol total, mmol/L (mean ± SD)	3.65 ± 1.06	3.48 ± 0.95
LD lipoproteïn, mmol/L (mean ± SD)	1.96 ± 0.86	1.90 ± 0.86
Triglyceride, mmol/L (mean ± SD)	2.08 ± 0.85	1.87 ± 0.74
Glucose, mmol/L (median, IQR)	6.65 (5.55 – 8.45)	7.15 (5.70 – 9.28)
HbA1c, mmol/mol (mean ± SD)	35.3 ± 10.7	33.8 ± 6.4
Blood pressure		
Diastolic, mmHgG (mean ± SD)	77 ± 10	75 ± 11
Systolic, mmHG (mean ± SD)	130 ± 15	127 ± 17
Heart rate, beats per minute (mean ± SD)	82 ± 14	84 ± 13
Tacrolimus trough blood level, µg/L (mean ± SD)	6.94 ± 3.05	7.51 ± 3.29
Pharmacogenetics (n, %)		
Normal CYP3A4 metabolism	33 (63.5%)	36 (67.9%)
Intermediar CYP3A4 metabolism	4 (7.7%)	2 (3.8%)
Unknown CYP3A4 metabolism	15 (28.8%)	16 (30.2%)
CYP3A5 expressor	9 (17.3%)	7 (13.2%)
CYP3A5 non-expressor	28 (53.8%)	31 (58.5%)
Unknown CYP3A5 status	15 (28.8%)	16 (30.2%)
Recipient demographics pre-transplantation		
Pre-existing Diabetes, Yes (n, %)	11 (21.2%)	13 (24.5%)
Pre-existing Hypertension, Yes (n, %)	17 (32.7%)	11 (20.8%)

Abbreviations: eGFR, estimated glomerular filtration rate based on the CKD-EPI formula; INR, International Normalized Ratio; SD, standard deviation; IQR, interquartile range

§Other includes: Asian and Afro-American

‡Other includes: primary biliary cirrhosis, secondary biliary cirrhosis, autoimmune cirrhosis, cholangiocarcinoma, Caroli disease, polycystic liver disease, neuroendocrine tumor liver metastases

Composite primary endpoint and separate components

Figure 2 shows the proportion of LT recipients reaching the composite primary endpoint and the separate components of the composite primary endpoint in the ITT and PP population. In the ITT population, a statistically significant lower proportion of LT recipients in the LCP-tacrolimus group reached the composite primary endpoint at 12 months compared to the ER-tacrolimus group (50.9% [27/53], 95% confidence interval (CI) 37.9% - 63.9% versus 71.2% [37/52], 95%CI 57.7% - 81.7%; risk difference: 0.202; 95%CI 0.002 – 0.382; p = 0.046). In the PP population the observed difference was not statistically significant (41.4% [12/29], 95%CI 25.5% - 59.3% in the LCP-tacrolimus group versus 64.3% [18/29], 95%CI 45.8% - 79.3% in the ER-tacrolimus group; risk difference: 0.229; 95%CI -0.051 – 0.467; p = 0.11).

In the ITT population, fewer LT recipients in the LCP-tacrolimus group developed CKD, new-onset hypertension and PTDM compared to the ER-tacrolimus group: CKD 26.4% [14/53], 95%CI 16.4% - 39.6% versus 42.3% [22/52], 95%CI 29.9% - 55.8%; risk difference: 0.159; 95%CI -0.035 – 0.339; p=0.10 and new-onset hypertension 38.1% [16/42], 95%CI 24.9% - 53.2% versus 54.3% [19/35] 95%CI 38.2% - 69.5%; risk difference: 0.162; 95%CI -0.076 – 0.379, p=0.18 and PTDM 20% [8/40], 95%CI 10.5% - 34.8% versus 26.8% [11/41] 95%CI 15.7% - 41.9%; risk difference: 0.068; 95%CI -0.133 – 0.262, p=0.60.

In the PP population, less LT recipients in the LCP-tacrolimus group developed CKD and new-onset hypertension compared to the ER-tacrolimus group: CKD 10.3% [3/29], 95%CI 3.6% - 26.4% versus 28.6% [8/28], 95%CI 15.3% - 47.1%; risk difference: 0.182; 95%CI -0.051 – 0.399; p=0.10 and new-onset hypertension 38.1% [8/21], 95%CI 20.8% - 59.1% versus 52.2% [12/23] 95%CI 32.9% - 70.7%; risk difference: 0.141; 95%CI -0.173 – 0.421, p=0.38. No evidence was found for a difference in the development of PTDM between both groups: 13.0% [3/23], 95%CI 4.5% - 32.1% versus 13.0% [3.23], 95%CI 4.5% - 32.1%; risk difference: 0; 95%CI -0.237 – 0.237; p=1.

Sensitivity analyses for new-onset hypertension and PTDM showed similar event rates in both groups when LT recipients with pre-transplant hypertension or diabetes mellitus were included in the analysis (and considered not to have new onset disease) as well as when every LT recipients with hypertension or diabetes mellitus was considered new-onset hypertension or PTDM.

Figure 3A and 3B visualize the individual kidney function measurements, the observed means per group, and the estimated group trajectories across the study period based on the linear mixed-effect model. The results of the models are shown in supplementary table 1. In the ITT and PP population, after the transplantation the mean eGFR gradually declined during the study period. No evidence for differences in the mean eGFR was found between the LCP-tacrolimus group compared to the ER-tacrolimus group over the study period. The linear mixed-effect models confirmed this.

In the PP population, the percentage of LT recipients having an eGFR <60 ml/min/1.73m^2 at 3 months post-LT was 15.4% [4/29] in the LCP-tacrolimus group and 25% [7/28] in the ER-tacrolimus group (p=0.13), at 6 months post-LT 17.2% [5/29] in the LCP-tacrolimus group and 25% [7/28] in the ER-tacrolimus group (p=0.24) and at 12 months post-LT 25% [7/29] in the LCP-tacrolimus group and 28.6% [8/28] in the ER-tacrolimus group (p=0.68). At 3 months, no

LT recipients had an eGFR < 30ml/min/1.73m^2 and at 6 and 12 months one LT recipients in the LCP-tacrolimus group and zero LT recipients in the ER-tacrolimus group had an eGFR < 30ml/min/1.73m^2.

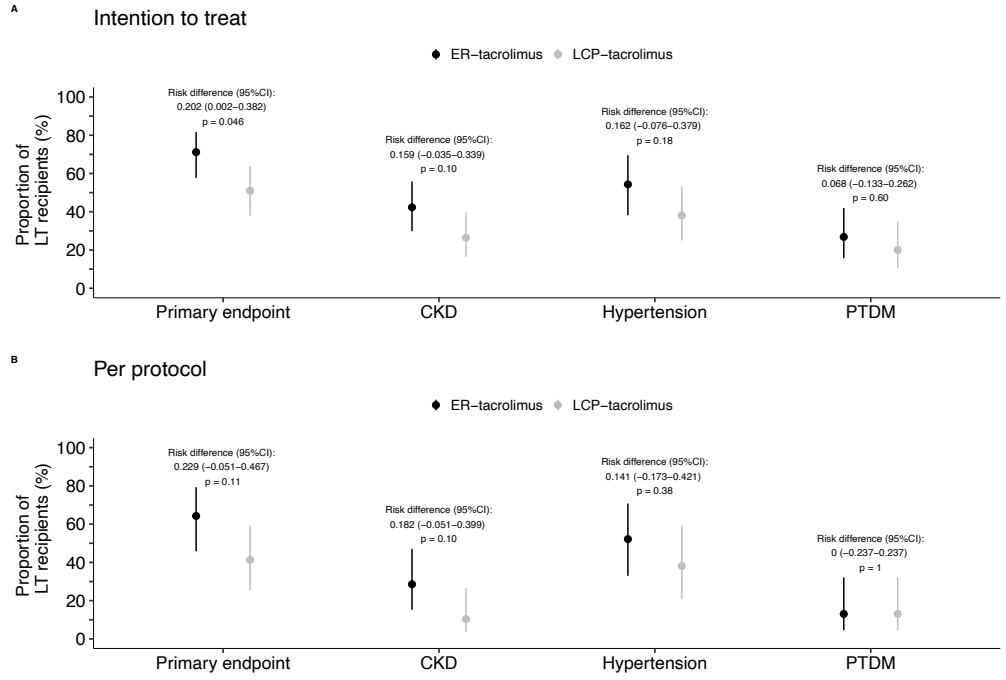

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Figure 2. LT recipients reaching the composite primary endpoint and developing chronic kidney disease, new-onset hypertension and new-onset diabetes after transplantation.

Panel A (intention-to-treat) and B (per protocol) show the proportion of LT recipients with 95%-CI reaching the composite primary endpoint and developing the separate components of the composite primary endpoint in the intention to treat population and per protocol population: CKD defined as grade ≥3 (eGFR <60 mL/min/1.73m^2) for >3 months during the follow-up, new-onset hypertension and PTDM. In the ITT population the composite primary endpoint at 12 months was reached in 50.9% [27/53], 95% confidence interval (CI) 37.9% - 63.9% of the LT recipients in the LCP-tacrolimus group versus 71.2% [37/52], 95%CI 57.7% - 81.7% of the LT recipients in the ER-tacrolimus group; risk difference: 0.202; 95%CI 0.002 – 0.382; p = 0.046. In the PP population the composite primary endpoint at 12 months was reached in 41.4% [12/29], 95%CI 25.5% - 59.3% of the LT recipients in the LCP-tacrolimus group versus 64.3% [18/29], 95%CI 45.8% - 79.3% of the LT recipients in the ER-tacrolimus group; risk difference: 0.229; 95%CI -0.051 – 0.467; p = 0.114.

Abbreviations: CKD, chronic kidney disease; LT, liver transplant; ns, non-significant; PTDM, post-transplant diabetes mellitus.

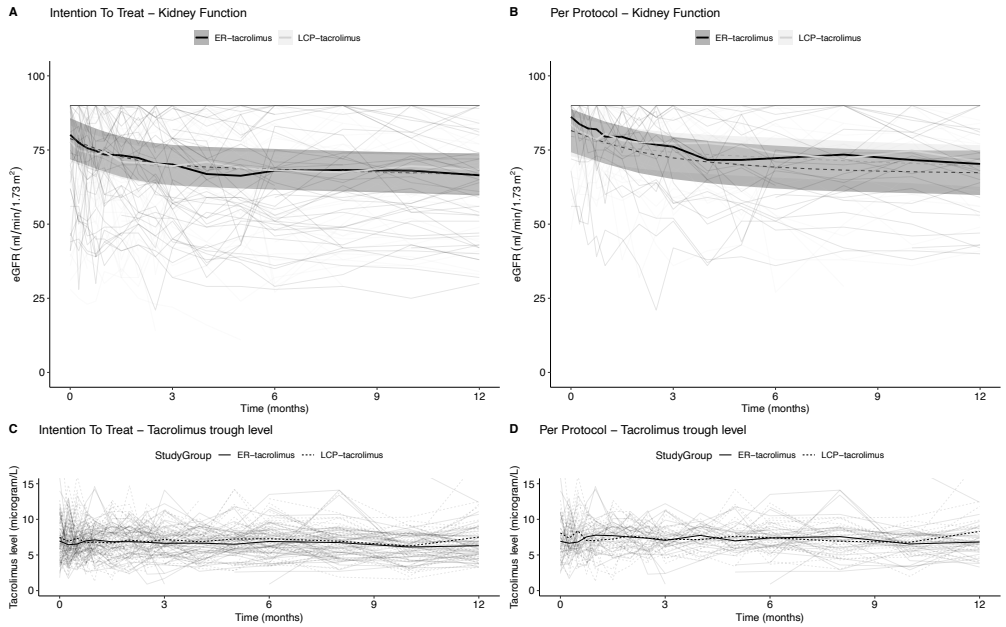

Figure 3. Kidney function and tacrolimus levels in the intention to treat and per protocol population.

A. Individual eGFR trajectories (CKD-EPI formula) and group-wise mean with 95%-confidence interval (CI) during the course of the study of the intention to treat (ITT) population represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, recipient age and sex, hypertension, diabetes mellitus were set to the population median or reference category). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. Missing data were considered as missing completely at random.

B. Individual eGFR trajectories (CKD-EPI formula) and group-wise mean with 95%-CI during the course of the study of the per protocol (PP) population represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough levels, recipient age and sex, hypertension, diabetes mellitus were set to the population median or reference category). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. Missing data were considered as missing completely at random.

C. Mean tacrolimus trough level (µg/L) during the study of the ITT population.

D. Mean tacrolimus trough level (µg/L) during the study of the PP population.

Secondary endpoints: Rejection, graft and patient survival

During the follow-up, no differences in the number of rejection episodes between the study groups were found (table 2). In the LCP-tacrolimus group 6 LT recipients developed 7 episodes of rejection and in the ER-tacrolimus group 5 LT recipients developed 5 episodes of rejection. Rejections were treated according to local protocols with corticosteroids and no anti-thymocyte globulin was used.

In the LCP-tacrolimus group more LT recipients died or had a re-transplantation compared to the ER-tacrolimus group: death 5.6% [3/54] versus 1.9% [1/52] and re-transplantation 7.4% [4/54] versus 1.9% [1/52]. No death or re-transplantation was considered study drug-related: 3 LT recipients died because of multi organ failure, 1 died because of a traumatic intracranial hemorrhage, 4 LT recipients were re-transplanted because of ischemic-type biliary lesions, 1 LT recipient was re-transplanted because of hepatic artery thrombosis.

Immunosuppression

During the study the mean trough levels for tacrolimus were within the target range for both groups for the ITT and PP population (figure 3C and 3D). At the end of the study, in the ITT and PP population, the mean tacrolimus trough levels in the LCP-tacrolimus group was statistically significant higher compared to the ER-tacrolimus group: ITT population 7.6 ± 3.1 µg/L versus 6.3 ± 2.2 µg/L, p = 0.026 and PP population 8.3 ± 3.1 µg/L versus 6.7 ± 2.1 µg/L, p = 0.033.

The median cumulative exposure to tacrolimus based on the area under the curve of trough concentrations was higher at month 12 for the LCP-tacrolimus group compared to the ER-tacrolimus group: ITT population 2697 µg·day/L [IQR 2316–2949] versus 2357 µg·day/L [IQR 1946–2806]; p = 0.018 and PP population 2707 µg·day/L [IQR 2383–2975] versus 2612 µg·day/L [IQR 2219–2976]; p = 0.39. No differences were found in the cumulative exposure to tacrolimus at month 3.

At 6 and 12 months, the intra-patient variability calculated with the coefficient of variation was not different between the groups (supplementary figure 1).

Every LT recipient received 500 mg of methylprednisolone intraoperatively. The median number of days of prednisolone after transplantation, the median cumulative dose and the median dose/day prednisolone during the study in both groups were not different. In the LCP-tacrolimus group, the number of days prednisolone was 146 days [IQR 114 – 180 days], the median cumulative dose prednisolone was 1030 mg [IQR 830 – 1260] and the median dose/day prednisolone was 7.1 mg/day [IQR 6.5 – 8]. In the ER-tacrolimus group, the number of days prednisolone was 151 days [IQR 117 – 175 days], the median cumulative dose prednisolone was 1095 mg [IQR 865 – 1320] and the median dose/day prednisolone was 7.2 mg/day [IQR 6.8 – 8]. During the study, in the ER-tacrolimus group 46.2% [24/52] of the LT recipients switched therapy due to toxicity (renal insufficiency or tremors) or rejection: 22 LT recipients to the combination of ER-tacrolimus and mycophenolic acid, 1 LT recipient to the combination of IR-tacrolimus and everolimus and 1 LT recipients from ER-tacrolimus to LCP-tacrolimus. In the LCP-tacrolimus group 40.4% [21/54] of the LT recipients switched therapy during the study due to toxicity (renal insufficiency or tremors), rejection or the recurrence of hepatocellular carcinoma: 19 LT recipients to the combination of LCP-tacrolimus and mycophenolic acid, 2 LT recipients to LCP-tacrolimus and sirolimus. None of these patients was switched back during the study period.

Safety

Table 2 shows the SAEs and the outcomes of the SAEs during the study period. In total, 160 SAEs were reported: 47.5% [76/160] in the ER-tacrolimus group and 52.5% [84/160] in the LCP-tacrolimus group. SAEs most frequently reported were fever 23.1% [37/160], cholangitis and bile duct obstruction 10% [16/160] and infections 10% [16/160].

	ER-tacrolimus		LCP-tacrolimus	
	No. of patients with event (n=51)	*No. of events (n=76)*	*No. of patients with event (n=53)*	*No. of events (n=84)*
Serious adverse events				
*Fever**	11 (22.0%)	23 (30.3%)	8 (15.1%)	14 (16.7%)
Cholangitis and bile duct obstruction	4 (7.8%)	6 (7.9%)	9 (17.0%)	10 (11.9%)
Infections§	6 (11.7%)	9 (11.8%)	6 (11.3%)	7 (8.3%)
Liver transplant rejection	5 (9.8%)	5 (6.6%)	6 (11.3%)	7 (8.3%)
Kidney injury / failure	3 (5.9%)	4 (5.3%)	4 (7.5%)	7 (8.3%)
Hepatic artery thrombosis	-	-	1 (1.9%)	1 (1.2%)
Other	22 (43.1%)	29 (38.2%)	19 (35.8%)	38 (45.2%)
Outcome				
Death	1 (2.0%)	-	3 (5.7%)	-
Resolved – no sequelae	24 (47.1%)	62 (81.6%)	26.0 (49.1%)	63 (75.0%)
Resolved – with sequelae	26 (51.0%)	14 (18.4%)	24 (45.3%)	21 (25.0%)

▲

Table 2. Serious Adverse Events according to the Medical Dictionary for Regulatory Activities (MedDRA).

*Fever with an unspecified cause and no overlap with the SAEs for cholangitis or infections.

§Infections include every viral or bacterial infection occurred during the study period excluding cholangitis.

Discussion

In this randomized controlled study, it was observed that significantly less LT recipients in the LCP-tacrolimus group reached the composite primary endpoint at 12 months compared to the ER-tacrolimus group at no increased costs in terms of efficacy or safety.

An important recommendation in the COMMIT guideline is the frequent monitoring for unwanted side effects of immunosuppression, such as renal impairment, PTDM, obesity, arterial hypertension and hyperlipidemia.(6) Therefore, we focused in this study on differences in clinically relevant outcomes for both long-acting tacrolimus formulations currently available.

In this study, the use of LCP-tacrolimus had a major positive impact on CNI related nephrotoxicity. The use of LCP-tacrolimus resulted in a 15.9 – 18.2% reduction in the prevalence of CKD grade ≥3 (eGFR <60 mL/min/1.73m^2) for >3 months post-LT. Furthermore, the prevalence of CKD grade ≥3 at 3 and 6 months post-LT was 15 – 17% in the LCP-tacrolimus group, whereas in the ER-tacrolimus group in this study, as in previous studies, the prevalence ranged from 30 - 50%. (11, 12) Interestingly, the mean eGFR over the whole study period was not different between both study groups. This is caused by the fact that when the eGFR of liver transplant recipients deteriorated most transplant physicians reduced the tacrolimus dose or switched the recipient to combination therapy of immunosuppressive drugs. This resulted in an increase of the eGFR over time and by calculating the mean eGFR, information of liver transplant recipients with an eGFR below average is not shown anymore. Whereas by calculating the percentage of liver transplant recipients with CKD a more appropriate view on the development of CKD during the study period is available.

Another interesting finding is the fact that we found less new-onset hypertension in the LCP-tacrolimus group. After solid organ transplantation, immunosuppressive agents play a major role in the development of new-onset hypertension. Both, tacrolimus and corticosteroids are associated with blood pressure elevation. Tacrolimus-induced hypertension has been related to increased sympathetic nervous system activity and increased peripheral vascular resistance, whereas corticosteroid-induced hypertension is related to sodium and water retention.(27) In this study corticosteroids have less contributed to the development of new-onset hypertension since the corticosteroids were lowered or discontinued within a median of 150 days. Furthermore, no difference in the median number of days of prednisolone after transplantation, cumulative dose and median dose/day prednisolone were found. Finally, based on the difference in prevalence of pre-existing hypertension in both groups we performed a sensitivity analysis. This analysis showed similar results when LT recipients with pre-transplant hypertension were included in the analysis and when every LT recipients with hypertension in this study was analyzed as new-onset hypertension. Therefore, the significantly reduced prevalence of new-onset hypertension in the LCP-tacrolimus group is suggested to be a result of the new drug delivery technology with a lower peak concentrations (C_{max}) of this tacrolimus formulation.

Over the last decennia, the exposure to tacrolimus in LT recipients decreased with target trough levels declining from >10 μg/L to the current target range of 6 - 8 μg/L for 3 – 12 months post-LT. Previous studies by Rodriguez-Perálvarez have shown that an increased cumulative exposure to tacrolimus over the years results in increased toxicity (e.g. nephrotoxicity and the incidence of cancer).(7, 8) In this study, the mean tacrolimus trough level and the cumulative exposure to tacrolimus at month 12 was statistically significant higher in the LCP-tacrolimus group compared to the ER-tacrolimus group. In line with a study by Den Bello *et al.*(28), we found that the IPV in the LCP-tacrolimus group was not different compared to the ER-tacrolimus group. Conflicting results regarding the impact of a high IPV in tacrolimus exposure on the long-term outcomes are available.(24, 29) Even though patients in the LCP-tacrolimus group had a higher cumulative exposure to tacrolimus, we found a statistically significant and clinically relevant reduction in the prevalence of the composite primary endpoint.

The development of LCP-tacrolimus was driven based on the large fluctuations in plasma concentration with the other tacrolimus formulations. It has been suggested that high tacrolimus C_{max} following dosing may be associated with increased neurotoxicity.(19) Pre-clinical studies investigating the mechanism behind the development of tacrolimus-related toxicity (e.g. CKD, hypertension, diabetes and neurotoxicity) in relation to peak concentrations are lacking. In this study, we did not evaluate the tacrolimus peak concentrations or actual exposure by measuring the area under the curve after ingestion. However, since the IPV in tacrolimus was comparable between the groups and the calculated cumulative exposure was higher for LCP-tacrolimus, we believe that the lower LCP-tacrolimus peak concentration is the factor that explains the more favorable cardiovascular risk profile. Overall, the tacrolimus peak concentration and the cumulative exposure to tacrolimus over time are the factors associated with the development of tacrolimus-related toxicity. However, the exact mechanism behind the development of tacrolimus-related toxicity needs to be determined.

This is the first head-to-head comparison of the two long-acting tacrolimus formulations available for the prevention of rejection after transplantation evaluating CNI related nephrotoxicity or metabolic side effects. No other study showed a significant improvement in the cardiovascular risk profile with the use of LCP-tacrolimus. Most studies that have been performed focused either on the conversion of immediate-release formulation to LCP-tacrolimus, or investigated only pharmacokinetics, had a retrospective and short-term design or analyzed other primary endpoints (e.g. death, graft failure of biopsy-proven acute rejection)(15, 30-32)

This study has a major limitation, namely the fact that almost half of the LT recipients in both groups switched immunosuppressive therapy due to toxicity (renal insufficiency or tremors), rejection or the recurrence of HCC. While a larger number of LT recipients was switched to another immunosuppressive regimen, mostly combination therapy, this could have introduced selection bias, complicating the interpretation of the results. This type of selection bias has been addressed in several other studies investigating immunosuppressive drugs in transplant recipients. (12, 33, 34) Overall, the results in our ITT analysis might be underestimating the actual effect of tacrolimus on the composite primary endpoint. Although the ITT and PP analysis needs to be cautiously interpreted, our results are consistent in the ITT and PP analysis. Since we studied two formulations of tacrolimus and not two different immunosuppressive regimens, the result is still relevant and reflects the daily clinical practice in transplant care.

Further research evaluating the long-term clinical side-effects in a larger population and the effect on the quality of life is necessary to determine whether LCP-tacrolimus should be the preferred tacrolimus formulation after LT. Currently, the EnGraft-trial enrolling 268 patients is running to evaluate the bioavailability, efficacy and safety of LCP-tacrolimus compared to ER-tacrolimus over a 3-year period. The results are awaited in the following years.(35)

In conclusion, a statistically significant and clinically relevant reduction in the prevalence of the composite primary endpoint was found in the LCP-tacrolimus group compared to the ER-tacrolimus group in the first year after liver transplantation with comparable efficacy. Furthermore, less LT recipients using LCP-tacrolimus develop chronic kidney disease and new-onset hypertension compared to the ER-tacrolimus group in the first year after liver transplantation.

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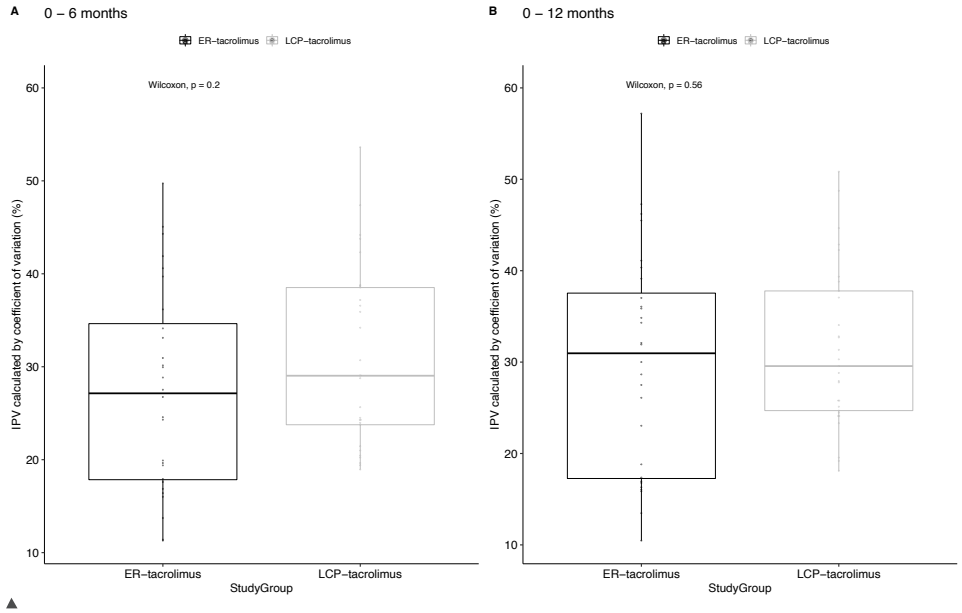
	Model for ITT population (n=1375)		Model for PP-population (n=812)	
Fixed effects	**Estimate**	**95%-CI**	**Estimate**	**95%-CI**
Intercept	109	97.5 - 120	108	97.3 - 121
ns (Visit, df=3)1	-7.42	-10.6 - -4.2	-10.6	-14.7 – -6.43
ns (Visit, df=3)2	-18.5	-22.5 – -14.5	-20.8	-25.8 – -15.7
ns (Visit, df=3)3	-7.59	-9.99 – -5.19	-10.1	-13.0 – -7.15
Study group	-1.81	-7.19 – 3.57	-2.19	-7.98 – 3.59
Tacrolimus trough level	-0.49	-0.61 – -0.36	-0.47	-0.66 – -0.27
Recipient age	-0.49	-0.70 – -0.28	-0.45	-0.65 – -0.24
Recipient sex	6.72	1.20 – 12.2	5.62	-0.72 – 11.9
Hypertension	-0.11	-4.85 – 4.59	-1.22	-6.74 – 4.30
Diabetes Mellitus	-12.3	-17.6 – 7.08	-2.67	-8.58 – 3.24
Interaction between Visit and study group (df =3)1	1.81	-2.88 – 6.50	5.63	-0.14 – 11.4
Interaction between Visit and study group (df =3)2	1.94	-3.78 – 7.66	8.11	0.97 – 15.2
Interaction between Visit and study group (df =3)3	1.06	-2.41 – 4.53	2.62	-1.55 – 6.76
Random effects	**Variance**	**SD**	**Variance**	**SD**
Subject intercept	161.16	12.69	98.98	9.95
Residual	82.22	9.06	76.31	8.74

Supplementary table 1. Results of the generalized mixed effect models.

Abbreviations: CI, confidence interval; ITT, intention to treat; PP, per protocol; SD, standard deviation

Two generalized mixed effect models were fitted, investigating the association between the kidney function during the course of the study (values for the covariates: tacrolimus trough levels, recipient age and sex, hypertension, diabetes mellitus were set to the population median or reference category). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the kidney function was investigated using natural cubic splines. A total of 1375 kidney function measurements of LT recipients in the intention to treat analysis and a total of 812 kidney function measurements of LT recipients in the per protocol analysis were included in the models. To take into account that the kidney function may not only be independently associated with the visit and the study group, the model included the product (=interaction) of the visit and study group as independent variable



Supplementary Figure 1. Tacrolimus intra-patient variability in the PP population

A) Distribution of the intra-patient variability calculated by the coefficient of variation in the PP population during month 0 - 6
B) Distribution of the intra-patient variability calculated by the coefficient of variation in the PP population during month 0 - 12

Abbreviations: IPV, intra-patient variability

Chapter 6

Tremors and health-related quality of life in liver transplant recipients comparing life cycle pharma-tacrolimus and extended-release tacrolimus: a multicenter randomized, controlled trial.

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Abstract

Background

The impact of the two once-daily tacrolimus formulations on health-related quality of life (HRQoL) and the severity of tremors in liver transplant recipients is unknown. We investigated whether life cycle pharma (LCP)-tacrolimus compared to extended-release (ER)-tacrolimus results in a difference in HRQoL and severity of tremors.

Methods

In this multi-center, open-label, randomized, controlled trial, 108 patients were randomized in a 1:1 ratio to either LCP-tacrolimus or ER-tacrolimus after transplantation. HRQoL was assessed with the EQ-5D-5L and SF-36 questionnaire (two generic HRQoL instruments) and the quality of life in essential tremor (QUEST) questionnaire (a domain specific HRQoL instrument). The EQ-5D-5L scores were translated to the societal values. We examined the HRQoL over the course of the study by fitting generalized mixed effect models.

Results

In total, 105 patients were included, 53 in the LCP- and 52 in the ER-tacrolimus arm. Baseline questionnaires were available for every LT recipient. No statistically significant differences were found at 3, 6 and 12 months in the frequency and severity of tremors in LT recipients in the intention-to-treat (ITT) and per protocol population. In the ITT population, at 12 months 25% [10/40], 95% confidence interval (CI) 14.2% - 40.2% of the LT recipients in the LCP-tacrolimus group experienced tremors compared to 30.4% [14/46], 95%-CI 19.1% - 44.8% of the LT recipients in the ER-tacrolimus group; risk difference: 0.054; 95%-CI -0.151 – 0.249; p=0.63. No statistically significant differences in HRQoL were seen between the two groups. During follow-up, the societal values of the EQ-5D-5L health states were lower than those of the general Dutch population in both study arms.

Conclusions

The once-daily LCP-tacrolimus formulation is not associated with an improvement in the HRQoL or a reduction in the occurrence of tremors compared to ER-tacrolimus. Further evaluation of the best tacrolimus regimen resulting in the least neurotoxicity is necessary in order to alleviate this troublesome tacrolimus side effect.

Introduction

Liver transplantation (LT) is the preferred treatment for patients with end-stage liver disease and unresectable hepatocellular carcinoma (HCC). After LT, health-related quality of life (HRQoL) generally reaches a level similar to the general population, except for the aspect of physical functioning.(1,2) In general, transplant recipients need to take lifelong immunosuppressive agents. These agents can cause multiple side effects that might negatively affect the daily life of LT recipients.(3) Therefore, the choice of immunosuppressive agents may impact the HRQoL of LT recipients.

Tacrolimus is the cornerstone of the immunosuppressive regimen after LT and belongs to the class of calcineurin inhibitors (CNIs).(4) CNIs are associated with neurotoxicity and affect the

central and peripheral nervous systems.(5,6) Peripheral tremors are the most frequently occurring neurological side effect and affect 30% - 55% of solid organ transplant recipients.(7) Tacrolimus exposure (whole blood trough concentrations) are associated with the severity of tremors.(7)

Life cycle pharma (LCP)-tacrolimus, (Envarsus®; Chiesi Farmaceutici S.p.A.) is a prolonged-release tacrolimus formulation utilizing a new drug delivery technology (MeltDose).(8,9) This formulation has lower peak-through blood level fluctuations and a higher bioavailability compared to the other tacrolimus formulations, resulting in a lower dose requirement to reach the intended tacrolimus exposure.(8,10) Therefore, it is hypothesized that LCP-tacrolimus could reduce the frequency and severity of peripheral tremors.

A study by Langone *et al.* investigated the change in tremor severity after switching from tacrolimus twice-daily capsules (Prograf®, Astellas Pharma) to LCP-tacrolimus once-daily tablets in kidney transplant recipients.(11) They found that patients on LCP-tacrolimus experienced significantly less tremors. However, a major limitation of this study was the fact that it included only patients that had already experienced a clinically significant tremor observed by a health care provider or by patient complaint and that this study was uncontrolled. Up until now no head-to-head comparison between the two once-daily tacrolimus formulations has been performed.

The aim of this randomized, controlled study was to investigate whether LCP-tacrolimus compared to extended-release (ER)-tacrolimus (Advagraf®, Astellas Pharma) results in a difference in the HRQoL and severity of tremors.

Materials and Methods

Study design and participants

An extensive description of the MOTTO study design has been published previously.(12) In brief, from day 5 after LT patients received twice-daily, immediate-release (IR) tacrolimus. After achieving stable tacrolimus trough levels between 8 – 10 μg/L, patients were randomized in a 1:1 ratio to either LCP-tacrolimus or ER-tacrolimus. During a one-year follow-up period, in the LCP-tacrolimus group 40.4% [21/54] of the LT recipients switched therapy due to toxicity (renal insufficiency or tremors), rejection or to prevent recurrence of hepatocellular carcinoma to the combination of LCP-tacrolimus and mycophenolic acid or LCP-tacrolimus and sirolimus. In the ER-tacrolimus group 46.2% [24/52] of the LT recipients switched therapy due to toxicity (renal insufficiency or tremors) or rejection to the combination of ER-tacrolimus and mycophenolic acid, immediate-release tacrolimus and everolimus or to LCP-tacrolimus.

The study was performed at two centers in the Netherlands: The Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands and Leiden University Medical Center, Leiden, The Netherlands. The study was approved by the institutional Ethical Committees of these institutions, registered in the EudraCT database (EudraCT: 2018-002856-34) and conducted in accordance with the latest version of the declaration of Helsinki. The inclusion period ran from April 2019 until October 2021.

Patient-reported outcomes
The evaluation of the HRQoL and the severity of tremors comprised a pre-defined secondary objective of the MOTTO study. The MOTTO study was initially designed to investigate whether LCP-tacrolimus compared to ER-tacrolimus results in a difference in the prevalence of post-transplant diabetes mellitus, new onset hypertension and chronic kidney disease at 12 months after transplantation.

HRQoL and Severity of Tremor assessments
HRQoL was assessed with the validated Dutch version of the EQ-5D-5L questionnaire and the SF-36 questionnaire (two generic HRQoL instruments) and the quality of life in essential tremor (QUEST) questionnaire (a domain specific HRQoL instrument). The questionnaires were distributed at the day of randomization, month 3, 6 and 12.

The EQ-5D-5L questionnaire is based on a descriptive system that defines health in terms of 5 states: Mobility, Self-Care, Usual Activities, Pain/Discomfort, and Anxiety/Depression.(13) Each dimension has 5 response categories corresponding to no problems, slight problems, moderate problems, severe problems, and extreme problems. EQ-5D-5L scores were transformed to societal values based on the Dutch tariff for the EQ-5D-5L established by Versteegh *et al*..(14)

In the EQ-5D-5L questionnaire, the respondents' overall health on the day of the interview (patient's self-rated HRQoL scores) was rated on a 0–100 hash-marked, vertical visual analogue scale (EQ-VAS). The threshold for the minimally important difference (MID), indicating a clinical meaningful improvement, in the EQ-VAS score was defined as ≥ 7 points.(15)

The SF-36 questionnaire contains 36 items grouped in eight domains: physical functioning, role limitation-physical, pain, general health, energy/fatigue, social functioning, emotional well-being, role limitation-emotional. Each domain is scored between 0 and 100 points, with higher scores indicating better HRQoL.

The QUEST questionnaire is a self-administered questionnaire with 30 items on a five-point scale (0 – 4), corresponding to the frequency (never, rarely, sometimes, frequently, always) with which tremor is perceived to currently impact five domains: physical, psychosocial, communication, hobbies/leisure and work/finance.(16,17) The score on each domain is expressed as a percentage of the total score possible on that domain, with a higher score indicating greater dissatisfaction with that domain of QoL. A total score was computed by calculating the mean of the five domain scores.

Given that the QUEST is 'domain specific' for 'patients with essential tremors', this questionnaire is most likely more sensitive than the generic EQ-5D-5L and SF-36. The value of those two questionnaires is the ability to formulate 'values' of quality of life for cost effectiveness analysis, and these generic questionnaires can measure side effects outside the measuring domain of the QUEST.

Data collection
Variables collected included recipient socio-demographic, clinical and transplantation parameters, the HRQoL and tremor severity and trough levels tacrolimus.

Statistical analysis
The HRQoL analysis included all patients within the MOTTO study who responded to at least one questionnaire, according to the intention-to-treat principle. The EQ-5D-5L, SF-36 and QUEST questionnaire included in the analysis missed <5% based on the total number of measurements across all patients and questions. The missing data were considered as missing completely at random.

Two generalized linear mixed effect models were fitted to examine the HRQoL (EQ-VAS and the societal values of the EQ-5D-5L) over the course of the study. The models included covariates shown or suggested to be relevant: time since transplantation, study group, tacrolimus trough concentrations, kidney function, hemoglobin, recipient age and sex, primary disease, diabetes mellitus and hypertension pre-transplantation as well as the interaction between visit and the study group. Participant specific random intercepts were included to account for correlation among repeated measurement nested within each participant. Natural cubic splines were used to model the potentially nonlinear trajectories of the EQ-VAS and societal values of the EQ-5D-5L over time. The need for these splines was evaluated using likelihood-ratio tests. Splines provide a convenient non-parametric way to flexibly model (potentially) non-linear associations in regression models. Instead of using one polynomial (e.g., a quadratic or cubic function) that spreads over the whole range of the covariate, splines use a set of several polynomial functions that are defined over smaller intervals. This allows the resulting fit to be more flexible and less influenced by outliers than when using a single polynomial. To visualize the estimated associations, the expected HRQoL across the course of the study was calculated while fixing the values of all other covariates to the median or reference category.

Secondary endpoints were analyzed using the Pearson's Chi-square test or Mann-Whitney U Test. Confidence intervals for binomial proportions were calculated using the binconf package for R software. For all statistical tests, a (two-sided) p-value of <0.05 was considered to indicate statistical significance.

Data were approached in an intention-to-treat (ITT) and per protocol (PP) analysis. Patients with protocol violations in immunosuppressive therapy, a re-transplantation or death were excluded in the per protocol analysis. All data were collected in CastorEDC and analysis was conducted with R software (version 4.2.1).(18,19)

Results

Patient and treatment characteristics
A total of 108 LT recipients was included and randomized in the MOTTO study. No LT recipients included in the MOTTO study were diagnosed with a neurological movement disorder pre-transplantation. At baseline, 100% of the LT recipients responded to the EQ-5D-5L, SF-36 and QUEST questionnaires. The response rate decreased during follow up to a minimum of 75.5% at the end of the study (Figure 1).

Table 1 shows the baseline characteristics of the ITT population. No relevant differences in the baseline characteristics for the EQ-5D-5L questionnaire existed between the two groups. More LT recipients in the LCP-tacrolimus group experienced tremors compared to the ER-tacrolimus

group (30.2% [16/53], 95%-confidence interval (CI) 19.9% – 44.3% versus 19.2% [10/52], 95%-CI 10.8% – 31.9%). LT recipients in the LCP-tacrolimus group registered more hours of tremor per day compared to the ER-tacrolimus group (median 4 hours, IQR: 1 – 7 versus median 1 hour, IQR: 1 – 3.5). The mean tacrolimus trough level at the day of randomization in the LCP-tacrolimus group was 7.5 ± 3.3 μg/L and in the ER-tacrolimus group 6.9 ± 3.1 μg/L, p=0.38. LT recipients in the LCP-tacrolimus group were converted to that formulation after 11 days (IQR: 9.25 - 15.25 days) and in the ER-tacrolimus group LT recipients were converted to that formulation after 13.5 days (IQR: 9 - 15.75 days).

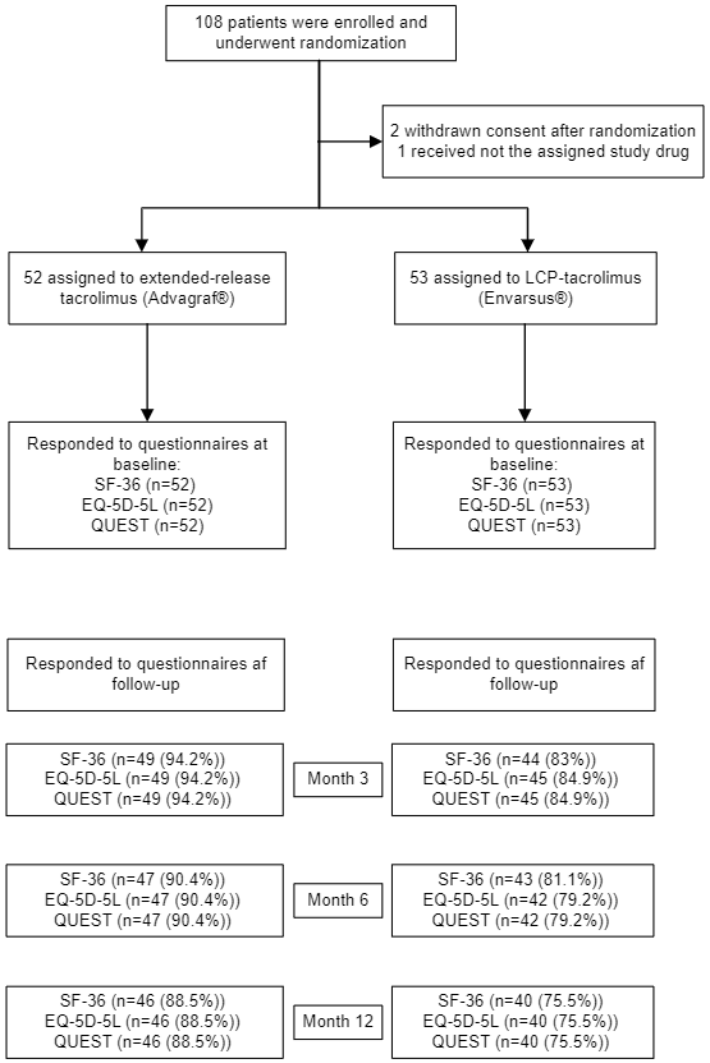

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Figure 1. Enrollment, Randomization, and Follow-up.

	Extended-release tacrolimus (n = 52)	LCP-tacrolimus (n= 53)
Recipient demographics at randomization		
Age, year (median, IQR)	58.50 (46.75 – 65.25)	56.50 (46.25 - 63)
Gender, male (n, %)	41 (78.8%)	35 (66%)
Primary Disease (n, %)		
Hepatocellular carcinoma	19 (36.5%)	12 (22.6%)
(Non)alcoholic steatohepatitis	7 (13.5%)	10 (18.9%)
Primary sclerosing cholangitis	10 (19.2%)	8 (15.1%)
Acute liver failure	3 (5.8%)	3 (5.7%)
Cryptogenic cirrhosis	3 (5.8%)	3 (5.7%)
Metabolic diseases	-	4 (7.5%)
Viral Hepatitis	3 (5.8%)	3 (5.7%)
Other‡	7 (13.5%)	11 (20.8%)
Lab		
Hemoglobin, mmol/L (mean ± SD)	6.25 ± 0.90	6.13 ± 0.84
eGFR, ml/min/1.73m^2 (mean ± SD)	82.08 ± 17.83	79.44 ± 20.43
Tacrolimus trough blood level, μg/L (mean ± SD)	6.94 ± 3.05	7.46 ± 3.28
Smoking (n, %)	11 (21.2%)	8 (14.8%)
Recipient demographics pre-transplantation		
Pre-existing Diabetes, Yes (n, %)	11 (21.2%)	13 (24.5%)
Pre-existing Hypertension, Yes (n, %)	17 (32.7%)	11 (20.8%)
EQ-5D-5L questionnaire		
VAS (mean ± SD) [ref: 0 – 100]	65 ± 15	58 ± 17
Societal values of the EQ-5D-5L based on the Dutch tariff for the EQ-5D-5L (median, IQR) [ref: -0.466 - 1]	0.53 (0.35 – 0.62)	0.56 (0.37 – 0.67)
QUEST questionnaire		
LT recipients and tremors, Yes (n, %)	10 (19.2%)	16 (30.2%)
Hours of tremors per day (median, IQR)	1.0 (1.0 – 3.5)	4.0 (1.0 – 7.0)
Total score QUEST (median, IQR)	1.15 (0.28 – 3.33)	12.29 (1.25 – 23.96)

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Table 1. Baseline characteristics

Abbreviations: eGFR, estimated glomerular filtration rate based on the CKD-EPI formula; INR, International Normalized Ratio; SD, standard deviation; IQR, interquartile range, QUEST, Quality of Life in Essential Tremor

§Other includes: Asian and Afro-American

‡Other includes: primary biliary cirrhosis, secondary biliary cirrhosis, autoimmune cirrhosis, cholangiocarcinoma, Caroli disease, polycystic liver disease, neuroendocrine tumor liver metastases

Tremors

Figure 2 shows the proportion of LT recipients experiencing tremors during study follow up. Table 2 shows the QUEST questionnaire results and the tacrolimus levels of the LT recipients during the study. No statistically significant differences were found at 3, 6 and 12 months in the frequency and severity of tremors in the ITT and PP population. In the ITT population, at 12 months 25% [10/40], 95%-CI 14.2% - 40.2% of the LT recipients in the LCP-tacrolimus group versus 30.4% [14/46], 95%-CI 19.1% - 44.8% of the LT recipients in the ER-tacrolimus group experienced tremors; risk difference: 0.054; 95%CI -0.151 – 0.249; p=0.63. The mean tacrolimus trough level at 12 months in the LCP-tacrolimus group was statistically significantly higher compared to the ER-tacrolimus group: 7.6 ± 3.1 μg/L versus 6.3 ± 2.2 μg/L, p = 0.026.
In the PP population, at 12 months 25% [7/28], 95%-CI 12.7% - 43.4% of the LT recipients in the LCP-tacrolimus group versus 25.9% [7/27], 95%-CI 13.2% - 44.7% of the LT recipients in the ER-tacrolimus group experienced tremors; risk difference: 0.009; 95%-CI -0.237 – 0.257; p = 1.00. The mean tacrolimus trough level at 12 months in the LCP-tacrolimus group was statistically significantly higher compared to the ER-tacrolimus group: 8.3 ± 3.1 μg/L versus 6.7 ± 2.1 μg/L, p = 0.033. Overall, no statistically significant differences were observed in any of the five domains and the total score of the QUEST in the ITT and PP analysis (supplementary Figure 1).

Interestingly we did see effects of switching and dose reduction of tacrolimus in specific cases. During the study, one patient switched from ER-tacrolimus to LCP-tacrolimus, two patients switched from monotherapy LCP-tacrolimus to combination therapy of low-exposure LCP-tacrolimus with mycophenolic acid and one patient switched from monotherapy ER-tacrolimus to combination therapy of low-exposure ER-tacrolimus with mycophenolic acid. In all four LT recipients a reduction in the severity of tremors and an improved QUEST score after this switch was observed.

Health-related Quality of Life outcomes

Supplementary Figure 2 shows the proportion of responses by level of severity for the EQ-5D-5L dimensions during the study period in the ITT population. Overall, patients reported the least issues in the states of Self-Care and Anxiety/Depression and the most problems in the states of Usual Activities and Pain/Discomfort. No evidence for differences between the study groups in any of the five domains was found.

The likelihood-ratio tests indicated non-linear patient specific trajectories of HRQoL scores and the societal values of the EQ-5D-5L. No evidence was found for between-group differences over the course of the study based on the mixed effect models. The hemoglobin level was statistically significantly associated with a higher EQ-VAS and EQ-5D-5L score, whereas tacrolimus trough levels were statistically significantly associated with a lower EQ-VAS and EQ-5D-5L score (supplementary table 1). Figures 3 visualize the expected HRQoL scores and societal values of the EQ-5D-5L together with the corresponding observed values per time point and study group for the ITT and PP population.

At the end of the study, the patient's self-rated HRQoL scores as expressed with the EQ-VAS approximate the mean self-reported EQ-VAS score by the general Dutch population. The per protocol analysis showed comparable results (figure 3A and 3B). For both arms in the ITT and PP population, the societal values of the EQ-5D-5L were below those of the general Dutch population (figure 3C and 3D).

In the ITT population, LT recipients in both groups achieved a clinically meaningful improvement (>7 points) in the EQ-VAS score at 12 months (LCP-tacrolimus: 20.8 points and ER-tacrolimus: 14.3 points difference with baseline). This result persisted in the PP population.

Supplementary figure 3 shows the results from the SF-36 questionnaire. In the ITT and PP population, every domain of the SF-36 questionnaire improved during the follow-up. Most improvement was shown in the domains: physical functioning, social functioning and pain. No statistically significant differences were found between both study groups on any of the eight domains.
An analysis of the EQ-VAS score and the SF-36 questionnaire in relation to tremors did not show statistically significant differences between LT recipients with and without tremor as indicated by the QUEST questionnaire (supplementary figure 4 and 5).

Intention to treat population	**Baseline**		**Month 3**		**Month 6**		**Month 12**	
	ER-tacrolimus (n=52)	LCP-tacrolimus (n=53)	ER-tacrolimus (n=49)	LCP-tacrolimus (n=45)	ER-tacrolimus (n=47)	LCP-tacrolimus (n=42)	ER-tacrolimus (n=46)	LCP-tacrolimus (n=40)
Experienced tremors, Yes (n, %)	10 (19.2)	16 (30.2)	16 (32.7)	15 (33.3)	12 (25.5)	12 (28.6)	14 (30.4)	10 (25)
Severity tremors, hours/day (median, IQR)	1 (1 – 3.5)	4 (1 – 7)	2.5 (1 – 4)	2 (1 – 8.5)	5 (1 – 9)	1.5 (1 – 2.25)	2 (1 – 8)	1 (1 – 2)
Total QUEST score (median, IQR)	1.15 (0.28 – 3.33)	12.29 (1.25 – 23.96)	5.56 (3.19 – 13.44)	7.78 (2.22 – 17.5)	5.78 (1.67 – 15.83)	7.5 (1.87 – 16.56)	6.32 (1.67 – 10)	6.94 (1.46 – 18.75)
Tacrolimus trough level, μg/L (mean (SD))	6.94 (3.05)	7.51 (3.29)	6.59 (2.59)	7.15 (1.99)	6.8 (2.46)	7.03 (2.23)	6.27 (2.15)	7.63 (3.14)
Number of recipients in target range tacrolimus, i.e. month 0-3 between 8-10 μg/L and after month 3 between 6-8 μg/L (n, %)	10 (19.2)	12 (22.2)	7 (14.3)	12 (26.7)	20 (42.6)	14 (33.3)	17 (36.9)	14 (35)
Number of recipients above target range tacrolimus, i.e. month 0-3 > 10 μg/L and after month 3 > 8 μg/L (n, %)	8 (16.7)	9 (18)	4 (8.3)	4 (9.8)	12 (26.1)	15 (36.6)	7 (16.7)	14 (36.8)
Per Protocol population	**Baseline**		**Month 3**		**Month 6**		**Month 12**	
	ER-tacrolimus (n=28)	LCP-tacrolimus (n=29)	ER-tacrolimus (n=26)	LCP-tacrolimus (n=28)	ER-tacrolimus (n=28)	LCP-tacrolimus (n=29)	ER-tacrolimus (n=27)	LCP-tacrolimus (n=28)
Experienced tremors, Yes (n, %)	3 (10.7)	7 (24.1)	7 (26.9)	7 (25)	4 (14.3)	7 (24.1)	7 (25.9)	7 (25)
Severity tremors, hours/day (median, IQR)	2 (1.5 – 3)	1 (1 – 4.5)	1 (1 – 1.5)	2 (1 – 2.5)	4 (1 – 8.25)	2 (1 – 2.5)	1 (1 – 1.75)	1 (1 – 2.5)
Total QUEST score (median, IQR)	1.11 (1.11 – 6.39)	3.33 (2.22 – 19.65)	4.44 (1.53 – 10)	13.33 (6.81 – 15.83)	10.83 (5 – 15.83)	12.08 (4.17 - 17.57)	8.89 (1.11 – 10.83)	5 (1.39 – 13.89)
Tacrolimus trough level, μg/L (mean (SD))	6.91 (3.31)	8.09 (3.55)	6.98 (2.61)	7.15 (1.84)	7.38 (2.64)	7.42 (2.05)	6.73 (2.13)	8.30 (3.05)
Number of recipients in target range tacrolimus, i.e. month 0-3 between 8-10 μg/L and after month 3 between 6-8 μg/L (n, %)	4 (14.3)	3 (10.3)	4 (15.4)	8 (28.6)	15 (53.6)	12 (41.4)	13 (48.1)	11 (39.3)
Number of recipients above target range tacrolimus, i.e. month 0-3 > 10 μg/L and after month 3 > 8 μg/L (n, %)	5 (17.9)	7 (24.1)	3 (11.5)	2 (8)	8 (29.6)	11 (39.2)	5 (19.2)	12 (42.9)

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Table 2. QUEST questionnaire outcomes

Abbreviations: ER-tacrolimus, extended-release tacrolimus; SD, standard deviation, QUEST, Quality of Life in Essential Tremor

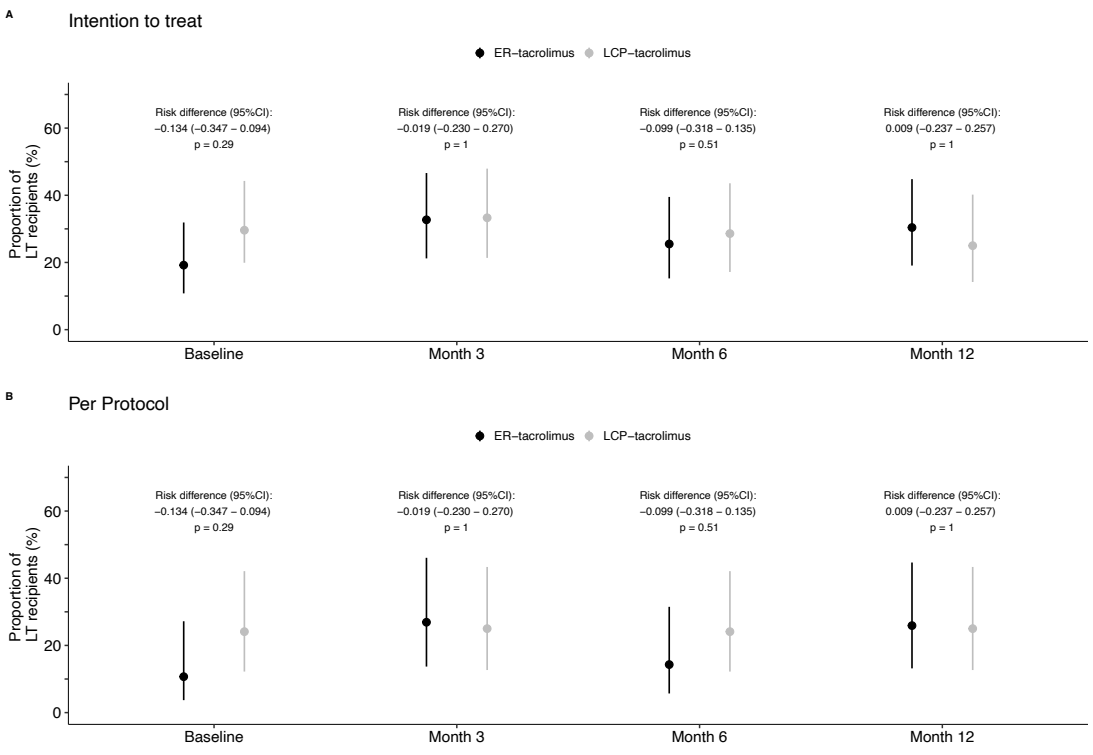

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Figure 2. Proportion of liver transplant recipients experiencing tremors during follow-up

Panel A (intention-to-treat) and B (per protocol) show the proportion of LT recipients with 95%-CI experiencing tremors during follow-up in the intention to treat population and per protocol population. In the ITT population at 12 months 25% [10/40], 95% confidence interval (CI) 14.2% - 40.2% of the LT recipients in the LCP-tacrolimus group versus 30.4% [14/46], 95%CI 19.1% - 44.8% of the LT recipients in the ER-tacrolimus group experienced tremors; risk difference: 0.054; 95%CI -0.151 – 0.249; p=0.63. In the PP population at 12 months 25% [7/28], 95%CI 12.7% - 43.4% of the LT recipients in the LCP-tacrolimus group versus 25.9% [7/27], 13.2% - 44.7% of the LT recipients in the ER-tacrolimus group experienced tremors; risk difference: 0.009; 95%CI -0.237 – 0.257; p = 1.

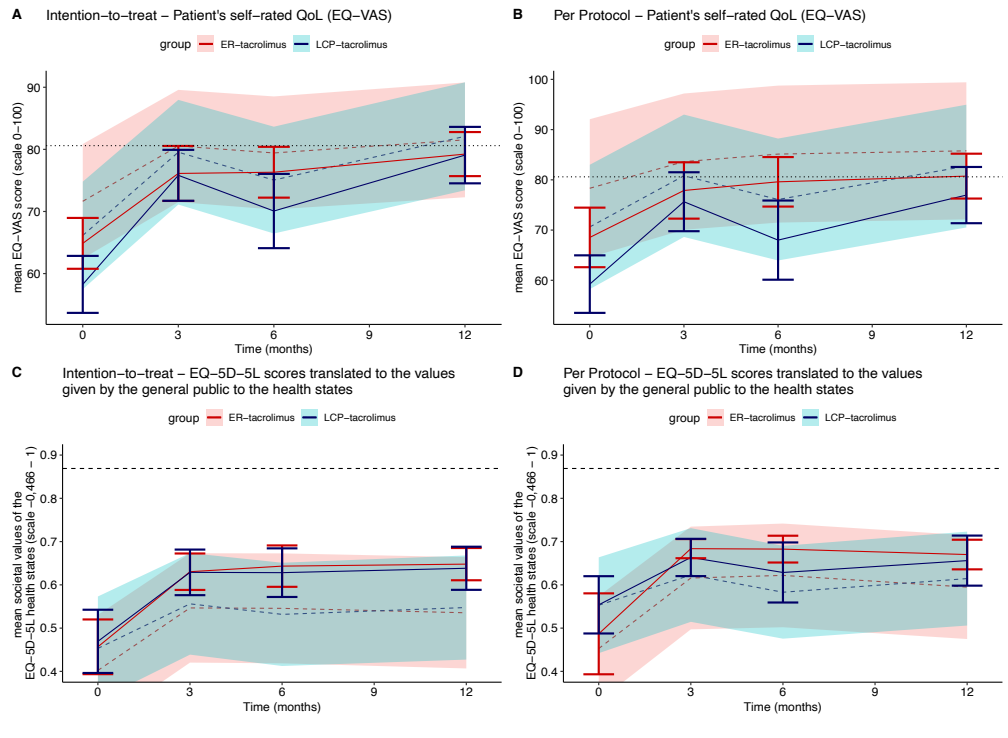

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Figure 3. EQ-VAS score and EQ-5D-5L scores on the dimensions translated to the societal values for the intention-to-treat population

A + B) Patient's self-rated QoL (EQ-VAS)

Group-wise mean EQ-VAS with 95%-confidence interval (CI) during the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough concentrations, kidney function, hemoglobin, recipient age and sex, primary disease, diabetes mellitus and hypertension pretransplantation as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the EQ-VAS was investigated using natural cubic splines. Splines provide a convenient non-parametric way to flexibly model (potentially) non-linear associations in regression models. Instead of using one polynomial (e.g., a quadratic or cubic function) that spreads over the whole range of the covariate, splines use a set of several polynomial functions that are defined over smaller intervals. This allows the resulting fit to be more flexible than when using a single polynomial. Missing data were considered as missing completely at random. Dotted black line indicates the mean self-reported EQ-VAS score by the general Dutch population.(12)

C + D) EQ-5D-5L scores translated to the values given by the general public to the health states

Group-wise mean of the societal values of the EQ-5D-5L health states with 95%-confidence interval (CI) during the course of the study represented as solid lines. The dashed lines and shaded areas indicate the expected values and corresponding 95%-CI from the generalized mixed effect model (values for the covariates: tacrolimus trough concentrations, kidney function, hemoglobin, recipient age and sex, primary disease, diabetes mellitus and hypertension pretransplantation as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the EQ-VAS was investigated using natural cubic splines. Splines provide a convenient non-parametric way to flexibly model (potentially) non-linear associations in regression models. Instead of using one polynomial (e.g., a quadratic or cubic function) that spreads over the whole range of the covariate, splines use a set of several polynomial functions that are defined over smaller intervals. This allows the resulting fit to be more flexible than when using a single polynomial. Missing data were considered as missing completely at random. Dotted black line indicates the mean EQ-5D-5L score given by the general Dutch population to the health states.(12)

Abbreviations: QoL, quality of life; VAS, visual analogue scale

Discussion

This is the first head-to-head comparison of two once-daily tacrolimus formulations, LCP- and ER-tacrolimus, evaluating HRQoL and tremor in the first year after liver transplantation. In this randomized controlled study, we found no significant differences in terms of both HRQoL and the frequency and severity of tremors in LT recipients using LCP-tacrolimus compared to ER-tacrolimus.

The findings of our study are in line with several other studies showing that the HRQoL of LT recipients rapidly improves after LT.(1,20) However, conflicting results regarding the use of different immunosuppressive agents and their impact on the HRQoL of LT recipients are reported.(2,21) We did not find evidence for differences in the HRQoL between both once-daily formulations of tacrolimus, despite a different pharmacokinetic profile and assumed lower peak levels.(8) In addition, in a previous study by our research group we also did not find a difference in the HRQoL between two regimens with different immunosuppressive agents, namely normal dose tacrolimus versus a combination of low dose tacrolimus and sirolimus.(2) During the current study follow-up, the EQ-VAS approximated the mean self-reported EQ-VAS score by the general Dutch population, whereas the societal values of the EQ-5D-5L were below those of the general Dutch population. Based on the limited available evidence, it remains to be determined whether different immunosuppressive agents and different formulations of immunosuppressive agents have a clinically relevant impact on the HRQoL of LT recipients.

We did not find a difference in frequency and severity of tremors between both once daily tacrolimus formulations. This in contrast with a clinical study in kidney transplant recipients with pre-existing tremor. This study demonstrated that kidney transplant recipients after switching to LCP-tacrolimus experienced significantly less tremors.(11) In our study, four patients switched therapy because of tremors. However, only one LT recipient switched between the once daily tacrolimus formulations and the other three LT recipients switched to low-dose combination therapy.

Based on our results and the limited number of patients switching therapy because of tremors, we cannot conclude what the best treatment option is to reduce tremors. In daily clinical practice, when using tacrolimus, up to 50% of the solid organ transplant recipients experience tremors.(6,11) In this study up to 34% of the LT recipients experienced tremors while using tacrolimus. A recent study showed that high tacrolimus trough concentrations were the main determinant of tremor.(7) Interestingly, in our study, the mean tacrolimus trough levels in the LCP-tacrolimus group were statistically significantly higher at the end of the study follow-up, while no differences in frequency and severity of tremor were found. This finding suggests that higher trough levels and a more stable pharmacokinetic profile of LCP-tacrolimus seems not to be related to the occurrence of tremors. Hypothetically more equal tacrolimus trough levels in both study groups might have resulted in less tremors in the LCP-tacrolimus group. Furthermore, previously we showed that the use of LCP-tacrolimus was associated with significantly lower rates of kidney dysfunction and hypertension.(12)

Multiple factors have an influence on the appearance and severity of tremors such as the height of the tacrolimus trough levels, smoking, medical conditions (e.g. hypothyroidism and hypoglycemia) or the use of certain medications (e.g. beta-blockers, bronchodilators, anticonvulsants,

antidepressants).(7,22) The number of LT recipients smoking was equally divided over both study groups. Unfortunately, adequate information regarding the use of concomitant drugs influencing tremors was not available. Since beta-blockers are occasionally prescribed to treat post-transplant hypertension the frequency and severity of tremors in this study might be underestimated.
Another study showed that severe tremor in solid organ transplant recipients was strongly and independently associated with lower physical and mental HRQoL.(7) We could not find lower HRQoL scores for LT recipients experiencing tremors compared to LT recipients without tremors.

A strength of this study is the high response rate and the longitudinal assessment of the HRQoL and severity of tremors. A major limitation is that in both study groups almost half of the LT recipients switched to another immunosuppressive regimen, mostly combination therapy, due to toxicity (renal insufficiency or tremors), rejection or to prevent the development of HCC. This could have caused an under- or overestimation of the HRQoL and severity of tremors. Since the PP analysis showed comparable results to the ITT analysis, we believe the switch in immunosuppressive regimen does not affect the interpretation of the results. Another limitation is the fact that the tremors reported by LT recipients were not evaluated by a physician using the Fahn-Tolosa-Marin tremor reporting scale. This tremor reporting scale was developed to quantify essential tremor severity and has been used in large trials for essential tremor. The QUEST questionnaire is a self-assessment and therefore the results regarding the severity of the tremor are not objectified.

Future research should continue investigating whether switching from one tacrolimus formulation to another, reducing the dose of tacrolimus or switching to combination therapy of tacrolimus with mycophenolic acid is the most effective therapy to reduce neurotoxicity in LT recipients.

In conclusion, based on this clinical study, the once-daily LCP-tacrolimus formulation is not associated with an improvement in the HRQoL or a reduction in the occurrence of tremors compared to ER-tacrolimus. Aiming for lower tacrolimus trough levels seems a better strategy to reduce the severity and frequency of tremors. Further evaluation of the best tacrolimus regimen resulting in the least neurotoxicity is necessary to alleviate this troublesome tacrolimus side effect.

Acknowledgements

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	Intention-to-treat population				Per protocol population			
	Model for EQ-VAS (n=354)		*Model for EQ-5D-5L (n=354)*		*Model for EQ-VAS (n=217)*		*Model for EQ-5D-5L (n=217)*	
Fixed effects	**Estimate**	**95%-CI**	**Estimate**	**95%-CI**	**Estimate**	**95%-CI**	**Estimate**	**95%-CI**
Intercept	57.0	36.6 – 76.7	0.18	-0.089 – 0.45	59.1	29.1 – 84.8	0.33	0.039 – 0.59
ns (Visit, df=3)1	1.44	-6.42 – 9.31	0.061	-0.034 – 0.16	4.91	-5.60 – 15.3	0.084	-0.054 – 0.20
ns (Visit, df=3)2	18.0	8.90 – 27.2	0.28	0.17 – 0.39	13.6	0.55 – 24.7	0.32	0.17 – 0.46
ns (Visit, df=3)3	3.93	-0.59 – 8.51	0.039	-0.015 – 0.095	4.25	-1.57 – 10.3	0.038	-0.038 – 0.11
Study group	-5.49	-11.3 – 0.18	0.051	-0.026 – 0.13	-7.70	-15.4 – 0.04	0.10*	0.023 – 0.18
Tacrolimus trough level	-0.78*	-1.29 – -0.25	-0.0067*	-0.013 – -0.00043	-0.89*	-1.49 – -0.22	-0.0081*	-0.016 – -0.00075
Kidney function	0.05	-0.06 – 0.16	0.00026	-0.0012 – 0.0016	0.01	-0.14 – 0.18	-0.000098	-00019 – 0.046
Hemoglobin level	2.40*	0.81 – 4.06	0.021*	0.00041 – 0.041	2.99*	0.98 – 5.28	0.019	-0.00097 – 0.046
Recipient age	-0.02	-0.22 – 0.19	0.0019	-0.00095 – 0.0048	0.05	-0.20 – 0.32	0.00094	-0.0013 – 0.0031
Recipient sex, male	-1.01	-6.25 – 4.18	0.033	-0.041 – 0.11	1.25	-6.35 – 8.86	0.042	-0.022 – 0.10
Primary disease AIH	-2.52	-13.8 – 8.86	0.088	-0.073 – 0.25	-2.16	-16.3 – 12.4	0.061	-0.057 – 0.18
Primary disease ALF	-3.46	-14.5 – 7.89	0.064	-0.091 – 0.22	-5.76	-28.9 – 17.4	0.041	-0.15 – 0.23
Primary disease Cholestatic liver disease†	-7.16	-14.8 – 0.53	0.099	-0.0083 – 0.21	-8.14	-18.8 – 2.67	0.073	-0.015 – 0.16
Primary disease Crytogenic cirrhosis	-8.83	-19.3 – 1.61	-0.024	-0.17 – 0.12	-15.9	-33.4 – 1.41	0.030	-0.11 – 0.18
Primary disease HCC	-2.93	-9.55 – 3.84	0.049	-0.044 – 0.14	-8.90	-19.2 – 1.63	-0.012	-0.095 – 0.077
Primary disease Metabolic disease	-12.8	-25.9 – 0.27	0.043	-0.14 – 0.23	-14.3	-28.9 – 0.27	-0.043	-0.17 – 0.079
Primary disease other‡	5.19	-4.87 – 15.2	0.061	-0.080 – 0.20	-3.94	-19.0 – 11.2	0.039	-0.085 – 0.16
Primary disease Viral hepatitis	-4.19	-14.3 – 5.99	0.097	-0.047 – 0.24	-10.4	-23.6 – 3.02	0.053	-0.056 – 0.16
Pre-transplant Diabetes Mellitus	2.59	-3.16 – 8.44	-0.013	-0.094 – 0.068	1.27	-6.80 – 9.47	-0.087*	-0.15 – -0.015
Pre-transplant Hypertension	-2.77	-7.92 – 2.30	-0.019	-0.092 – 0.052	-3.34	-10.9 – 4.00	0.0041	-0.060 – 0.064
Interaction between Visit and study group (df =3)1	-4.89	-16.2 – 6.5	-0.064	-0.20 – 0.075	-10.1	-24.3 – 4.13	-0.13	-0.31 – 0.048
Interaction between Visit and study group (df =3)2	8.42	-2.24 – 19.2	-0.093	-0.22 – 0.039	6.88	-7.01 – 20.0	-0.20	-0.37 – -0.039
Interaction between Visit and study group (df =3)3	2.58	-3.76 – 8.97	-0.016	-0.092 – 0.062	0.47	-7.47 – 8.22	-0.028	-0.13 – 0.068
Random effects	**Variance**	**SD**	**Variance**	**SD**	**Variance**	**SD**	**Variance**	**SD**
Subject intercept	97.07	9.85	0.021	0.15	113.1	10.6	0.0054	0.073
Residual	121.9	11.0	0.017	0.13	120.1	10.9	0.018	0.14

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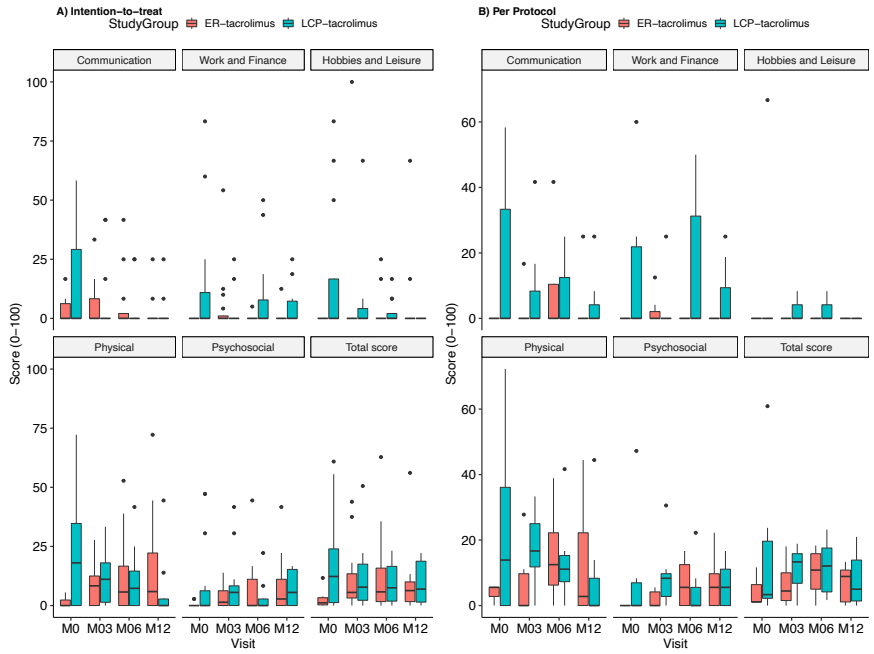
Supplementary table 1. Results of the generalized mixed effect models for EQ-VAS and EQ-5D-5L scores on the dimensions translated to the societal values

Abbreviations: AIH, autoimmune hepatitis; ALF, acute liver failure; CI, confidence interval; HCC, Hepatocellular carcinoma; ITT, intention to treat; PP, per protocol; TAC, tacrolimus; SD, standard deviation; VAS, visual analogue scale; *indicates statistical significance

†Cholestatic liver disease includes: Primary biliary cholangitis, Primary sclerosing cholangitis, Caroli disease; biliary cirrhosis

‡Other includes: Cholangiocarcinoma; Neuroendocrine tumour; Polycystic liver disease

Two generalized mixed effect models were fitted, investigating the association between the EQ-VAS and the societal values of the EQ-5D-5L health states during the course of the study (values for the covariates: tacrolimus trough levels, kidney function, hemoglobin level, recipient age and sex, primary disease, pretransplant diabetes mellitus, pretransplant hypertension as well as the interaction between visit and the study group). Random participant effects were included to account for repeated measurement nested within each participant. The shape of the association with the EQ-VAS was investigated using natural cubic splines. The coefficients of the spline do not have a direct interpretation (see figures for interpretation). Missing data were considered as missing completely at random.



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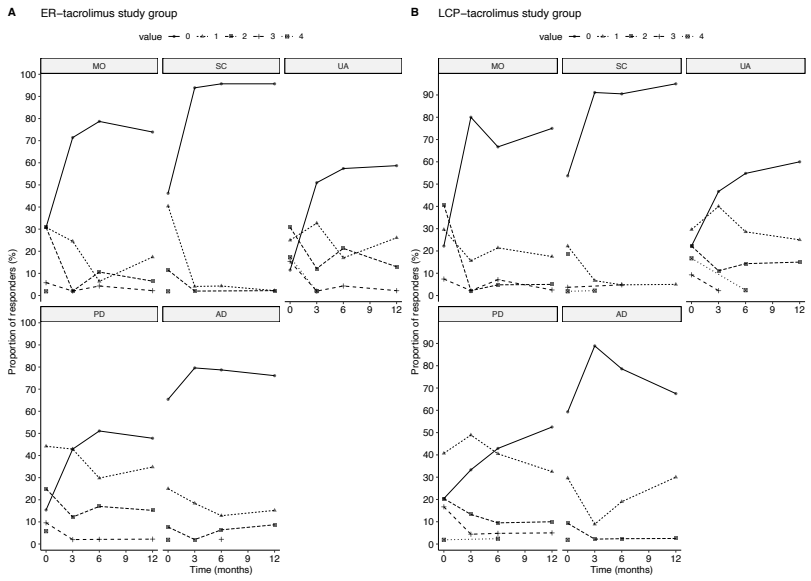
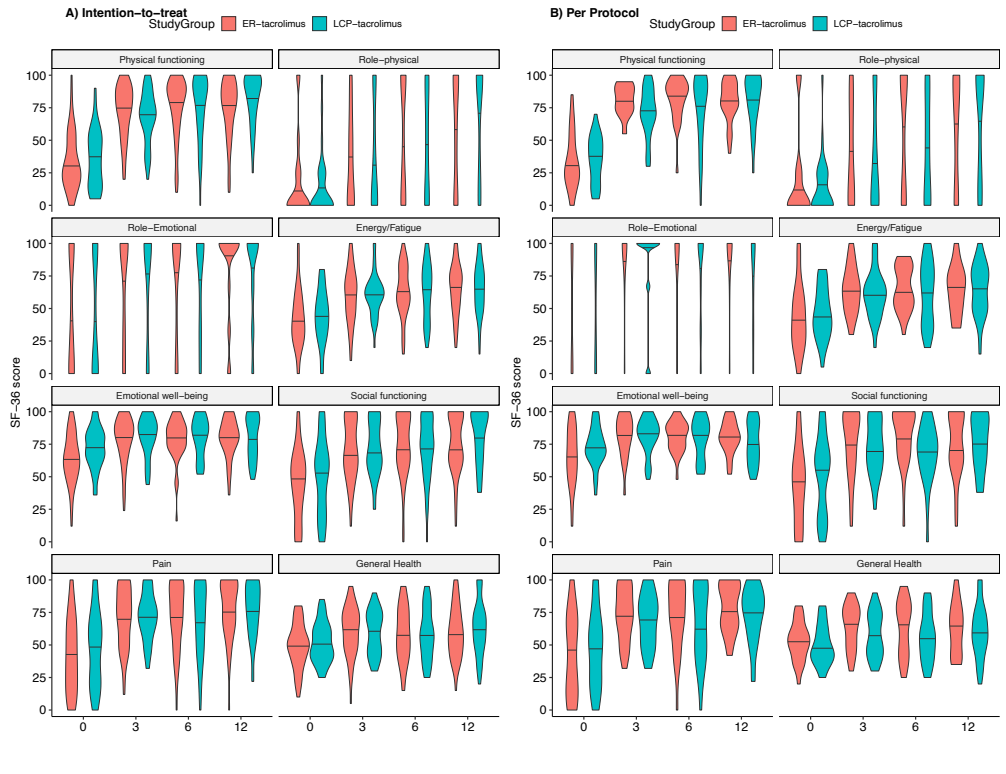
Supplementary figure 1. QUEST questionnaire outcomes for every domain

The boxplots present the distribution of the QUEST questionnaire outcomes. The panels on top indicate the subdomain of the QUEST questionnaire. Medians are indicated by the straight black line. Figure 1A presents the intention-to-treat analysis and figure 1B presents the per protocol analysis. No statistical differences were found.

Abbreviations: QUEST, Quality of Life in Essential Tremor





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Supplementary figure 3. SF-36 questionnaire outcomes for every domain

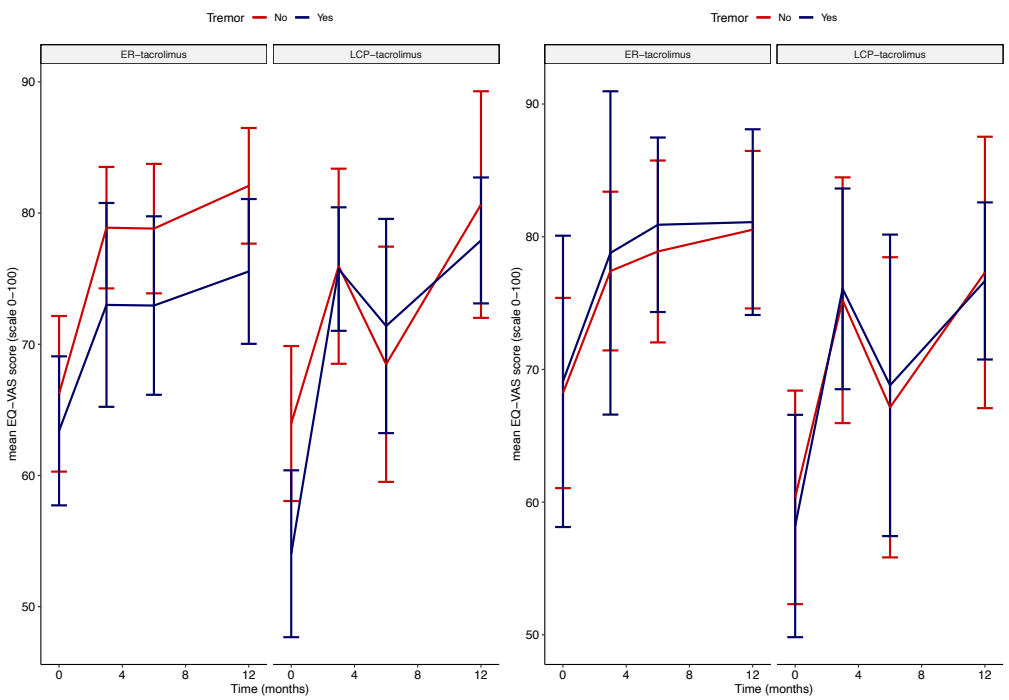
The violin charts present the distribution of the SF-36 questionnaire outcomes. The panels on top indicate the subdomain of the SF-36 questionnaire. Medians are indicated by the straight black line. Figure 3A presents the intention-to-treat analysis and figure 3B presents the per protocol analysis. No statistical differences were found.

◀

Supplementary figure 2. Proportion of responses by level of severity for EQ-5D-5L dimensions for the intention-to-treat population

2A) ER-tacrolimus group: 1, no problems; 2, slight problems; 3, moderate problems; 4, severe problems; 5, Extreme problems
2B) LCP-tacrolimus group : 1, no problems; 2, slight problems; 3, moderate problems; 4, severe problems; 5, Extreme problems

Abbreviations: MO, mobility; SC, self-care; UA, usual activities; PD, pain / discomfort; AD, anxiety / depression.



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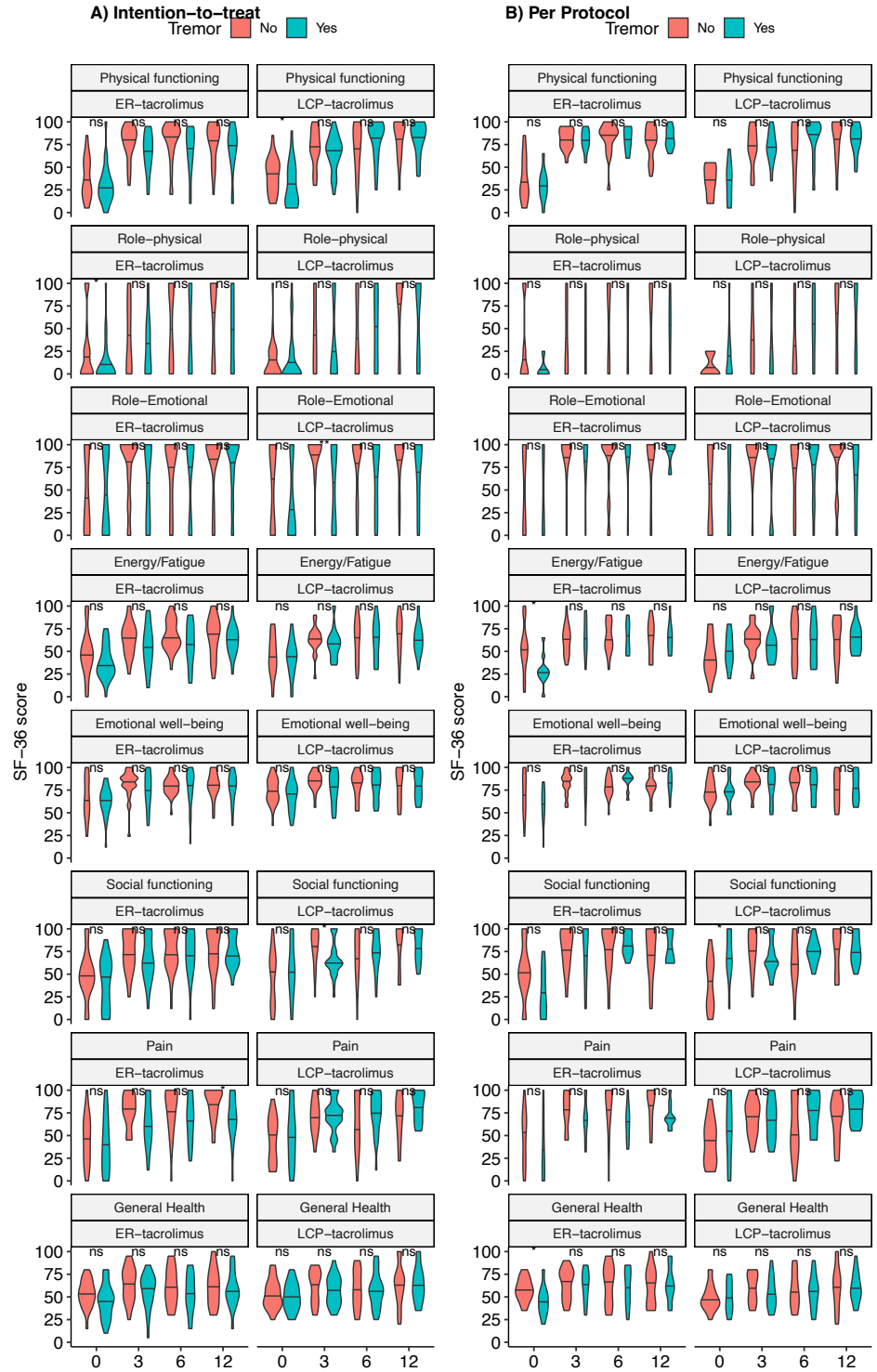
Supplementary figure 4. Subgroup analysis of the EQ-VAS in relation to tremors

Group-wise mean EQ-VAS with 95%-confidence interval (CI) during the course of the study represented as solid lines in relation to tremors. Figure 3A presents the intention-to-treat analysis and figure 3B presents the per protocol analysis. No statistical differences were found in the EQ-VAS score in LT recipients with or without tremors.

▶

Supplementary figure 5. Subgroup analysis of the SF-36 outcomes for every domain in relation to tremors

The violin charts present the distribution of the SF-36 questionnaire outcomes in relation to tremors. The panels on top indicate the subdomain of the SF-36 questionnaire and the study group. Medians are indicated by the straight black line. Figure 4A presents the intention-to-treat analysis and figure 4B presents the per protocol analysis. No statistical differences were found.



Part III

Optimizing therapy for viral complications after transplantation

Chapter 7

Determining the therapeutic range of ribavirin in transplant recipients with chronic hepatitis E virus infection.

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Abstract

The aim of this study was to define the therapeutic range of ribavirin (RBV) in transplant recipients with chronic hepatitis E virus (HEV) infection. In this retrospective, multicenter, cohort study, data of adult transplant recipients with chronic HEV infection, who had been treated with RBV monotherapy between 01-3-2008 and 01-08-2018 were included. ROC-curve analyses were performed and the half-maximal effective RBV concentration was calculated to determine a representative therapeutic range. In 96 patients, RBV monotherapy for a median of three months resulted in a sustained virologic response in 63.5% of the patients, while 88.5% of the patients developed anemia. RBV plasma concentrations at steady-state were significantly higher in clinical responders compared to clinical non-responders: median 1.96 (IQR 1.81–2.70) versus 0.49 (IQR 0.45–0.73) mg/L, p=0.0004. RBV caused a dose-dependent hemoglobin reduction with higher RBV plasma concentrations resulting in more hemoglobin reduction. The therapeutic range of RBV for chronic HEV infection in transplant recipients ranges between 1.8 and 2.3

Introduction

Ribavirin (RBV) for chronic hepatitis E virus (HEV) infection in immunocompromised patients is associated with a sustained virologic response (SVR) of around 80%.(1) The use of RBV is, however, limited by its side effects, which include hemolytic anemia and a decrease in glomerular filtration rate (GFR).(2)

RBV is mainly excreted by the kidneys and has a long half-life (approximately 300 hours). Therefore, RBV steady-state plasma concentrations are not reached until week 8. In patients infected with HCV, a relationship has been described between RBV plasma concentrations, SVR and anemia.(3) The aim of this study was to investigate the association between RBV plasma concentrations and virologic response and anemia in transplant recipients with a chronic HEV infection.

Materials and methods

Study design and setting

This was a retrospective, multicenter study in which four hospitals participated. Data of adult solid organ transplant (SOT) and hematopoietic stem cell transplant (HSCT) patients diagnosed with HEV infection, who had been treated with RBV monotherapy between 01-03-2008 and 01-08-2018 were collected. For all patients, socio-demographic, clinical parameters and laboratory results were collected.

The decision to treat HEV with RBV, the starting and maintenance dose of RBV and the timing of RBV plasma concentrations measurements were determined by the treating physician. No formal therapeutic drug monitoring (TDM) protocol for RBV was implemented in any of the participating hospitals.

A waiver was given for this this retrospective study by the Medical Ethics Committee of the Erasmus MC (MEC-2018-1326).

Response assessment

A SVR was defined as an undetectable level of HEV RNA in serum at least 6 months after completion of RBV therapy.(4) Since 6 months after the completion of therapy, RBV is washed out, we examined the relationship between viral kinetics during RBV treatment and RBV exposure to assess clinical response. Clinical response was defined as a decrease of the HEV RNA load between two measurements with at least a factor 2. Further referred to as “clinical response” in this manuscript. A rise in the HEV RNA load was defined as “clinical non-response”. Declines in HEV RNA load without at least a factor 2 (n=5) were not included. Only the first RBV plasma concentration at steady-state was included in the analysis. For the determination of the lower limit of the therapeutic range, plasma concentrations of patients with at least 90 days between the diagnosis of HEV infection and initiation of RBV therapy were included.

Toxicity assessment

Toxicity of RBV was determined based on the percentage reduction of the hemoglobin (Hb) concentration during a RBV plasma concentration measurement compared to the Hb concentrations at the initiation of RBV therapy (baseline) for each patient. Anemia was defined as a hemoglobin concentration <8.5 mmol/L (men) and <7.5 mmol/L (women). For the toxicity analysis and determination of the upper limit of the therapeutic range, every plasma concentration was included.

Statistical analysis

Variables are described with descriptive statistics and differences in characteristics are described with the Mann-Whitney U test for quantitative data. Receiver operating characteristic (ROC) curve analyses were performed to determine a representative cut-off value for RBV pre-dose concentrations between responders and non-responders. In the analysis of a ROC-curve, an area under the concentration versus time curve (AUC) of > 0.7 is considered to be acceptable to determine a representative cut-off value. The half-maximal effective concentration (EC50) was calculated with nonlinear regression of log concentration versus Hb reduction to determine the maximum cut-off value for the therapeutic range. The EC50 refers to the concentration of RBV which induces a response halfway between the baseline and maximum Hb reduction in percent. All statistical analyses were performed using SPSS for Windows, version 24 and GraphPad Prism version 7.02.

Results

Patients and ribavirin therapy

A total of 92 HEV-infected SOT and 4 HSCT recipients were included. The characteristics of the 96 patients are depicted in table 1. RBV monotherapy for a median of 3 (range 1 – 44) months resulted in a SVR in 63.5% of the patients. In total, 324 RBV plasma samples were included, of which 68 samples of 40 patients were RBV steady-state plasma concentrations.

	Overall (n = 96)
Age, years	56 (22–84)
Gender	
Male	63 (65.6%)
Female	33 (34.4%)
Ethnicity	
Caucasian	91 (94.8%)
African	5 (5.2%)
Body weight, kilograms	74 (43.5–140)
Serum creatinine during RBV therapy, µmol/L	124 (100 – 165)
Kidney function during RBV therapy, ml/min/1.73 m^2	50 (37 – 68)
Type of organ transplant	
Kidney	42 (43.8%)
Liver	19 (19.8%)
Heart	14 (14.6%)
Lung	10 (10.4%)
Pancreas	1 (1.0%)
Kidney and pancreas	3 (3.1%)
Kidney and heart	3 (3.1%)
Stem cell	4 (4.2%)
Immunosuppressive therapy at the start of RBV	
MPA	51 (53.1%)
Glucocorticoid	61 (63.5%)
Calcineurin inhibitors	
Tacrolimus	76 (79.2%)
Cyclosporine A	3 (3.1%)
mTOR inhibitor	
Everolimus	14 (14.6%)
Sirolimus	6 (6.3%)
Tacrolimus pre-dose concentration at initiation of RBV therapy, mcg/L	5.7 (4.5 – 7.7)
Hemoglobin concentration at treatment initiation, mmol/L	8.1 (5.3–10.8)
Positive anti-HEV IgG at the start of RBV	70 (72.9%)
Positive anti-HEV IgM at the start of RBV	74 (77.1%)
Positive serum HEV RNA at the start of RBV	96 (100%)
Interval between diagnosis of HEV infection and start of RBV, days	120 (2–1380)
Duration RBV therapy, days	90 (26–1333)
Sustained Virologic Response	
Yes	61 (63.5%)
No	29 (30.2%)
Unknown	6 (6.3%)

▲

Table 1. Characteristics of patients with HEV infection

Continous variables are displayed as medians and ranges. Categorical variables as counts and percentages. HEV, Hepatitis E Virus; IgG, Immunoglobulin G; IgM, Immunoglobulin M; MPA, mycophenolic acid; mTOR, mammalian target of rapamycin; RBV, ribavirin; RNA, Ribonucleic acid

Therapeutic effect of ribavirin

The RBV plasma concentrations at steady-state were not different between patients with or without SVR (Supplementary figure 1). Whereas RBV plasma concentrations at steady-state were significantly higher in the clinical response group compared to the clinical non-response group: median 1.96 (IQR 1.81-2.70) versus 0.49 (IQR 0.45-0.73) mg/L, p=0.0004. The RBV dose at steady-state was not significantly higher in the clinical response group compared to the clinical non-response group: median 8.44 (IQR 4.92-13.03) versus 8.16 (IQR 4.88-10.51) mg/kg/day, p=0.61 and, total daily dose, median 600 (IQR 400-800) versus 600 (IQR 350 – 800) mg/day, p=0.88 (Supplementary figure 2). No correlation was found between the RBV dose and RBV concentrations (r2=0.040) at steady-state. A worse renal function was not associated with treatment failure (defined as no decline in HEV RNA load).

The ROC-curve established a cut-off point of 1.80 mg/L to achieve a clinical response (sensitivity 66%, specificity 68%, AUC=0.75 (95% CI 0.628 to 0.871, p<0.0001), figure 1A). This decreased to 1.10 mg/L with a sensitivity of 89% and specificity 65%. The ROC-curve analysis revealed no differences when HSCT recipients were excluded.

Ribavirin and toxicity

Eighty-five (88.5%) patients developed anemia during RBV therapy. Twelve (12.5%) patients needed a blood transfusion because Hb concentrations dropped below 5.0 mmol/L. During RBV treatment, 24 (25%) patients had an increasing Hb concentration due to the use of erythropoiesis-stimulating agents or a blood transfusion. RBV caused Hb reduction regardless of the dose. Figure 1B demonstrates a stronger Hb reduction with increasing RBV plasma concentrations. Based on the EC50-cruve, an upper limit of the therapeutic range of 2.3 mg/L was established. A common side effect of MPA is anemia. When assessing the upper limit of the therapeutic range according to the concomitant use of MPA, the upper limit decreased to 1.5 mg/L in patients using MPA and increased to 9.8 mg/L in patients not using MPA. Furthermore, the upper limit of the therapeutic range increased to 3.3 mg/L when this limit was assessed after excluding the HSCT recipients.

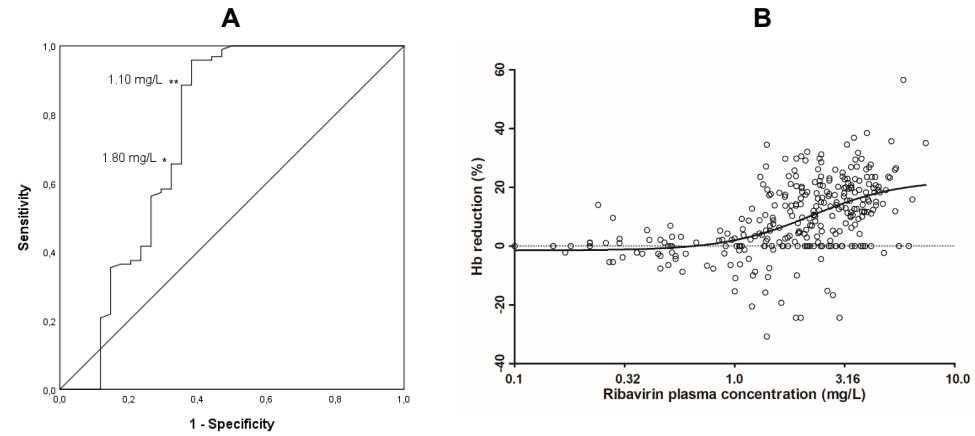

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Figure 1. Determination of the therapeutic range of ribavirin in transplant recipients with chronic HEV infection.

A) ROC-curve for RBV plasma concentration as predictor of effect in chronic HEV patients treated with monotherapy ribavirin. Cut-off point * = 1.8 mg/L; Cut-off point ** = 1.1 mg/L.

B) Toxicity and RBV plasma concentration. EC50 curve: Hemoglobin reduction (%) versus log ribavirin plasma concentration (mg/L). EC50, half-maximal effective concentration; calculated Emax value of 22.5% Hb reduction; Hb, hemoglobin

Discussion

Here, we show that a steady-state RBV therapeutic range of 1.8 – 2.3 mg/L is the optimal range for treating a chronic HEV infection in transplant recipients. Our findings are in line with those of Kamar *et al.* who observed no association between RBV plasma levels and SVR.(5) In our study a SVR (around 60%) was observed which was lower compared to other studies.(1,6,7) An explanation might be that at initiation of RBV therapy, the immunosuppressive therapy was not reduced sufficiently with 44.8% of the patients on triple immunosuppressive therapy. Furthermore, in our cohort MPA was used as immunosuppressive agent in almost 75% of the kidney transplant recipients, whereas MPA was used in 33% of the other transplant recipients. Debing *et al.* showed that MPA has strong antiviral activity *in vitro*.(8) Differences in the use of immunosuppressive agents may have contributed to the lower SVR in our cohort.

As many centers in the world are not able to measure ribavirin concentrations, TDM is not common practice, the more so because RBV exposure appears not to be associated with SVR. However, because we observed no correlation between the RBV dose and RBV plasma concentrations at steady-state, TDM could provide important information on RBV under- or overexposure. A more practical way of dosing ribavirin is to start with 10 mg/kg. Next, we recommend measuring HEV RNA quantitatively in order to identify patients with an insufficient viral response in an early phase after initiation of RBV therapy. Depending on the renal function of a patient, we propose to reduce the dose to 75% (eGFR between 30 and 50 ml/min per 1.73m2) or 50% (eGFR between 10 and 30 ml/min per 1.73m2). Based on our toxicity analysis, regular monitoring of Hb and adjusting the dose accordingly, is sufficient to prevent RBV-related toxicity. A reduction of the dose is desirable in case the Hb concentration drops >15%. RBV should than be stopped for 2 weeks and restarted at half the initial dose.

In SOT recipients or when MPA is not used as an immunosuppressive agent one might aim for higher RBV concentrations. Furthermore, in case of severe toxicity of RBV, a lower limit of the therapeutic range of 1.1 mg/L might be targeted. We recommend aiming for the lower limit of 1.80 mg/L when treatment-naive patients start treatment with RBV for chronic HEV.

A limitation of the present study is its retrospective design. RBV dosing was clinically driven and not every plasma concentration was measured during steady-state.

In conclusion, RBV monotherapy for a median of 3 months resulted in a SVR in 63.5% of the patients, with 88.5% developing anemia. RBV plasma concentrations at steady-state were significantly higher in clinical responders compared to clinical non-responders, defined as a ≥2-fold decrease in HEV RNA load. The therapeutic range of RBV for treating a chronic HEV infection in transplant recipients ranges between 1.8 and 2.3 mg/L.

Acknowledgments

The authors thank the laboratory personnel of the four university hospitals for sample analysis.

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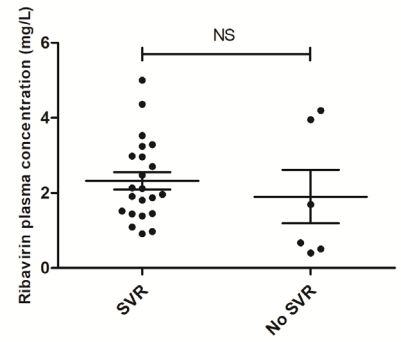
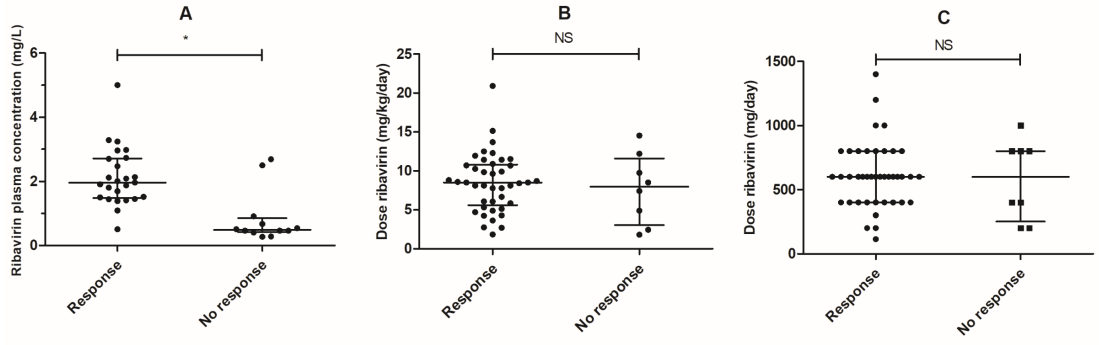


Supplementary figure 1. Ribavirin steady-state plasma concentration versus SVR in patients with a chronic HEV infection.

NS, not significant; SVR, sustained virological response



Supplementary figure 2. Ribavirin steady-state plasma concentration and dose versus response and no response in patients with a chronic HEV infection.

(A) Ribavirin steady-state plasma concentration (mg/L) versus response and no response. (B) Ribavirin weight-based dose (mg/kg/day) versus response and no response. (C) Ribavirin dose (mg/day) versus response and no response. *, significant; NS, Not Significant

Chapter 8

Development of a ribavirin dosing regimen in solid organ transplant recipients with chronic hepatitis E virus infection based on a population pharmacokinetic and pharmacodynamic model.

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Manuscript submitted

Abstract

Background

Ribavirin therapy for the treatment of chronic hepatitis E virus (HEV) infection in solid organ transplant (SOT) recipients is based on case reports and series, but the optimal ribavirin dosing regimen is unknown. We modelled ribavirin plasma concentrations versus virologic response and hemoglobin concentrations. The model was used to select a suitable RBV dosing regimen considering efficacy (decrease in viral load) and safety (hemoglobin).

Methods

Data were collected in a retrospective, multicenter study of adult SOT recipients with chronic HEV infection treated with ribavirin between 09-2009 and 11-2019. Population pharmacokinetic and pharmacodynamic analyses were conducted using nonlinear mixed-effects modeling. Simulations were performed to select the most suitable dosing regimen.

Results

In total, 107 chronically HEV-infected SOT recipients with 305 ribavirin plasma levels, 592 viral load concentrations and 443 hemoglobin concentrations were included. Sustained virologic response was achieved in 68.2% of the subjects. Due to a low IC50, the decline in viral load was independent of ribavirin concentration and dose, whereas hemoglobin decreased with increasing ribavirin concentration and dose. A model-supported ribavirin dose for 180 days of 600 mg/day and kidney function (eGFR) ≥60 ml/min/1.73m^2, 400 mg/day and eGFR 30-59 ml/min/1.73m^2 and 200 mg/day and eGFR ≤30 ml/min/1.73m^2 showed good efficacy and low toxicity.

Conclusions

This study constitutes a first step in determining the optimal ribavirin treatment regimen for chronic HEV infections in SOT recipients. Based on our model, we suggest to perform a non-inferiority trial evaluating the effect of low dose ribavirin on HEV clearance in SOT recipients.

Introduction

Hepatitis E virus (HEV) infection is one of the most common causes of acute viral hepatitis worldwide, and several studies have shown that the number of reported HEV infections has increased over the past decade.(1-4) In immunocompetent individuals, HEV is normally self-limiting.(5) However, in solid organ transplant (SOT) recipients, HEV can cause chronic hepatitis and cirrhosis if undiagnosed or left untreated.

The current clinical practice guidelines on HEV of the European Association for the Study of the Liver (EASL) recommend to lower immunosuppressive drug therapy in SOT recipients with a chronic HEV infection.(5) This results in a sustained virologic response (SVR) in approximately one-third of the SOT recipients.(6) If this is not possible or unsuccessful, a 3-month course of (off-label) ribavirin (RBV) is recommended.(7-10) RBV inhibits HEV replication *in vitro*.(11) *In vivo*, first-line RBV therapy was associated with a SVR in 81.2% of 255 patients.(12) However, the use of RBV is limited by its side effects: mood disturbances, sleeping disorders, neuropathy and (severe) hemolytic anemia. The latter is dose-dependent and often necessitates RBV dose reduction or discontinuation.(13-15)

RBV, a guanosine analogue, is rapidly absorbed and widely distributed in all tissue compartments after oral administration. RBV has a half-life (t1/2) of approximately 300 hours, and consequently RBV steady-state plasma concentrations are not reached until week 8 after the initiation of therapy.(16) Based on previous research, the therapeutic range of RBV for treating a chronic HEV infection in transplant recipients ranges between 1.8 – 2.3 mg/L.(17) For the treatment of a chronic HEV infection, RBV doses range between 29 – 1200 mg daily and treatment duration varies between 0.25 – 18 months.(12)

To investigate the population pharmacokinetics and pharmacodynamics of RBV, cases of chronically HEV-infected SOT recipients treated with RBV were collected retrospectively. The associations between RBV plasma concentrations versus HEV virologic response and hemoglobin concentrations were modeled and dosing regimens simulated to optimize RBV treatment in SOT recipients, considering efficacy (viral load) and safety (hemoglobin).

Methods

Study design and patients

This was a retrospective, multicenter study in which five hospitals participated. Data were collected from adult SOT recipients diagnosed with a chronic HEV infection, who had been treated with RBV between 09-2009 and 11-2019. For all patients, demographic and clinical parameters and laboratory results were collected. Data of 92 of these cases were published previously, but the associations between RBV plasma concentrations and virologic response and hemoglobin concentrations were not analyzed using nonlinear mixed-effects modeling, and no simulation-based evaluation of dosing regimens was performed.(17) The decision and timing to treat HEV with RBV, the starting and maintenance dose of RBV, hemoglobin and viral load plasma concentration measurements were determined by the treating physician. Excluded from the pharmacodynamics analysis were hemoglobin measurements after erythrocyte transfusions, and the viral load was not quantified for five SOT recipients. Viral load measurements showing a relapse of HEV in plasma after one or more preceding negative results were excluded, since insufficient data were available to develop a satisfactory relapse model.

Ethics

A waiver was given for this retrospective study by the Medical Ethics Committee of the Erasmus University Medical Center (MEC-2018-1326).

Software and modelling techniques

Population pharmacokinetic (PK) and pharmacodynamic (PD) analysis was conducted using nonlinear mixed-effects modeling with NONMEM® [version 7.5.0, ICON, Development Solutions, MD, USA]. Pirana [version 2.9.9, Certara, NJ, USA] was used as modeling interface, and results were further analyzed and visualized in R [version 3.6.1, R Foundation for statistical computing, Vienna, Austria].(18) Models were compared using the objective function value (OFV). In general, the simplest model that described the data adequately and was suitable for the intended use was preferred. A model was accepted only if its goodness-of-fit (GoF) and visual predictive check (VPC) or normalized prediction distribution errors (NPDE) were adequate upon visual inspection, its parameter values were deemed to be realistic, and shrinkage values (and RSE's) were sufficiently low (shrinkage preferably below 30%, RSE preferably below 50%). A

bootstrap analysis was performed on the final models with 1000 data sets. There were no PK or hemoglobin observations below the lower limit of quantification (BLQ); viral load observations reported as BLQ were included using the likelihood estimation method (M3).(19)

Pharmacokinetic, hemoglobin and viral load analysis

A schematic representation of the fully integrated model is provided in Figure 1. Model equations are available in the supplementary material. A two-compartment population PK model with first-order absorption previously developed for RBV in patients with chronic hepatitis C virus infection was used as a starting point.(20) As RBV plasma samples per patient were limited, the absorption rate constant (ka), central compartment volume of distribution (Vc), inter-compartmental clearance (Q) and peripheral compartment volume of distribution (Vp) were fixed to the estimates published in the starting model.[20] Due to observed deviations in visual predictive checks, clearance was re-estimated.

Hemoglobin concentrations were modeled using an indirect response model, with a linear inhibitory effect of RBV on the degradation rate (kout).(20) The production rate (kin) was chosen such that baseline hemoglobin concentrations equaled their observed values. In case baseline hemoglobin concentrations were not available (one subject), the typical (median) baseline hemoglobin concentration was used. The parameters kout and the slope of the RBV effect were estimated.

The time course of viral load was modeled using a target-cell-limited model with three compartments representing healthy hepatocytes, infected hepatocytes, and virions. The inhibitory effect (IC50) of RBV on viral replication was described using a sigmoidal Emax model. The viral load was initialized to its observed baseline value, and the fraction of infected hepatocytes was fixed to a low value (rho = 0.001) to prevent unrealistic growth of the liver with clearance of the virus. As the number of hepatocytes was unknown, it was arbitrarily set to one at baseline. To stabilize the model, the elimination rates of healthy hepatocytes (kdeg) and infected hepatocytes (kloss) were fixed to literature estimates(21), and the maximum inhibition of RBV was set to 99.9%. The viral elimination rate (elim) was estimated.

Covariates were selected based on known or theoretical interactions with the pharmacokinetics and pharmacodynamics of RBV. The following covariates were evaluated on CL, kout in the hemoglobin model and the elimination rate of virions: sex, weight, age, estimated GFR based on the MDRD, use of mycophenolic acid, liver enzymes (ALT, AST, GGT), bilirubin and albumin. Covariates were tested through a forward inclusion process at significance level p=0.05, followed by a backward elimination with p=0.01. Continuous covariates were centered on the median, and modelled using a power function. Categorical variables were described as multiplicative effects compared to the neutral category (which was either the natural neutral category or the largest category).

Receiver operating characteristic (ROC) curve analyses were performed to determine a cut-off value for the HEV load between SOT recipients with and without SVR. The cut-off value was subsequently used to estimate the minimum treatment duration of RBV achieving viral clearance. The HEV elimination rate at the end of RBV therapy was evaluated for SOT recipients with and without SVR. This might have the potential to distinguish RBV responders from non-responders. Differences in characteristics were described with the Mann-Whitney U or Kruskal Wallis test for quantitative data.

Simulations

Hemoglobin and viral load Monte Carlo simulations employed the final RBV PK and PD models and established RBV dosing strategies in SOT recipients with chronic HEV infection(10) (200 subjects per dosing regimen and sex) in order to assess the optimum dosage and treatment duration for RBV to achieve viral clearance (viral load <100 IU/ml) and prevent severe anemia (hemoglobin ≤5 mmol/L). The remaining covariates, weight and eGFR based on MDRD, were set to their median values, namely 70 (male) and 75 (female) kg and 57 ml/min/1.73m^2. Baseline concentrations of hemoglobin per sex and viral load were set to their median values. Different RBV dosing regimens for males and females were assessed.

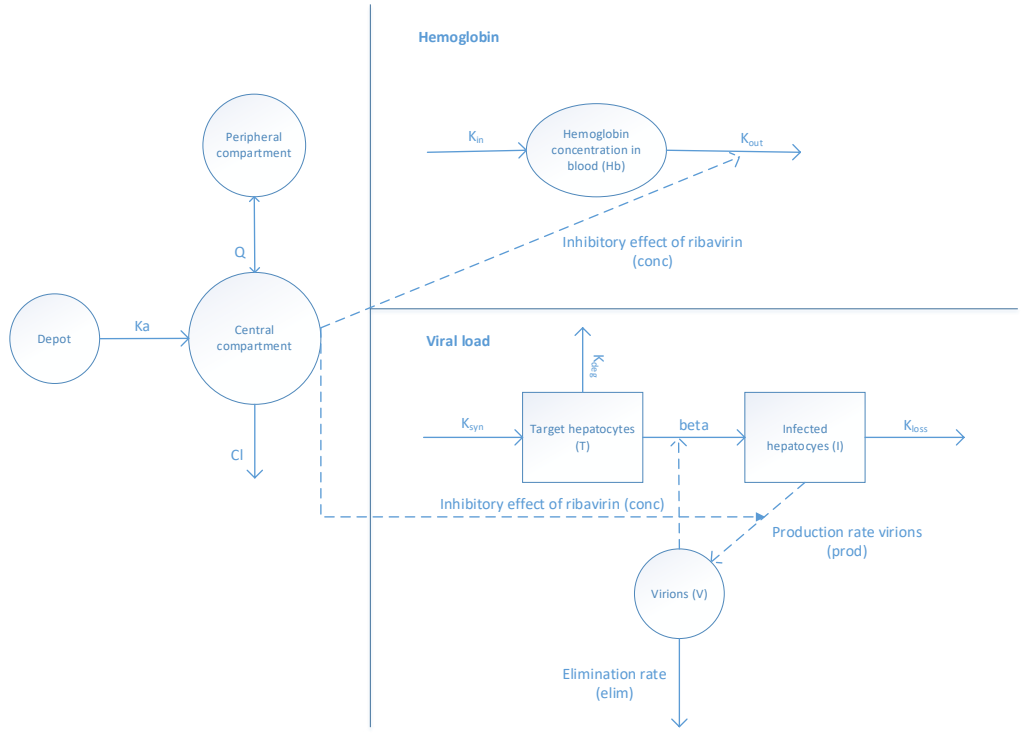

Figure 1. Schematic overview model

Solid arrows indicate mass flow and dashed arrows indicate influence. Ka, absorption constant; Cl, clearance; Q, Distribution clearance; kin, production of hemoglobin; kout, Loss of hemoglobin; Ksyn, production constant of healthy hepatocytes; Kdeg, degradation constant of healthy hepatocytes; Kloss, degradation constant of infected hepatocytes.

Results

Study population

A total of 107 chronically HEV-infected SOT recipients were included, with 305 RBV plasma concentrations (range 0.1 – 6.2 mg/L), 443 hemoglobin concentrations and 592 viral loads; 38% (225/592) of the viral load observations were reported as BLQ (Table 1). RBV with a median dose of 600 mg/day (range 100 – 2400 mg/day) for a median of 3 months (range 1 – 50 months) resulted in SVR in 68.2% of these patients. A clinician-diagnosed relapse occurred in 8.5% (9/106) of the SOT recipients.

	Overall (n = 107)
Age, years	56.9 (22 – 84)
Gender (n, %)	
Male	72 (67.3)
Female	35 (32.7)
Body weight, kilograms	74 (43.5 – 140)
Kidney function during RBV therapy, ml/min/1.73m^2	50 (6 – 117)
Tacrolimus pre-dose concentration at initiation of RBV therapy, mcg/L	6.2 (2.5 – 14.3)
Hemoglobin concentration at treatment initiation, mmol/L	8.3 (5.3 – 11.9)
Viral load at treatment initiation, IU/ml	1886058 (527 – 168000000)
Interval between diagnosis of HEV infection and start of RBV, days	128 (1–1507)
Duration RBV therapy, days	90 (21–1333)
Dose RBV, mg	600 (100 – 2400)
Type of organ transplant (n, %)	
Kidney	47 (43.9)
Liver	19 (17.8)
Heart	16 (14.9)
Lung	15 (14)
Kidney and Pancreas	4 (3.7)
Kidney and Heart	3 (2.8)
Pancreas	1 (0.9)
Lung and Liver	1 (0.9)
Lung and Heart	1 (0.9)
Immunosuppressive therapy at the start of RBV (n, %)	
Tacrolimus	90 (84.9)
Glucocorticoids	76 (71.7)
MPA	62 (58.5)
Everolimus	14 (13.2)
Sirolimus	7 (6.6)
Sustained Virologic Response (n, %)	
Yes	73 (68.2)
No	30 (28)
Unknown	4 (3.7)

▲

Table 1. Characteristics of patients with chronic hepatitis E virus infection

Continuous variables are displayed as medians and ranges. Categorical variables as counts and percentages.

Abbreviations: HEV, Hepatitis E Virus; MPA, mycophenolic acid; RBV, ribavirin.

Population pharmacokinetics

Covariates from the starting model (body weight on central and peripheral volume, and sex on peripheral volume) were maintained. Inter-individual variability (IIV) and eGFR were included on clearance. The eGFR effect above an estimated cut-off value of 57 mL/min/1.73m^2 was capped at maximum (table 2). A proportional residual error was estimated. Shrinkage was below 30% for all random effect parameters.

Hemoglobin concentrations

IIV could be estimated on kout and the slope of the RBV effect. The residual error model was best described with an additive function (table 2). Shrinkage was 31% for IIV on kout and 36% for IIV on the slope of the RBV effect. No covariate appeared to be relevant on the hemoglobin concentrations.

Viral load

The IC50 parameter of the effect was estimated at a value lower than almost all observed concentrations, which destabilized the model. After a sensitivity analysis (results not shown), this parameter was fixed (1000 ng/L). IIV could be estimated on the elimination rate of virions, with shrinkage below 30%. The residual error model was best described with an additive function on the log scale (table 2). Visual analysis demonstrated no covariate effect on the elimination of HEV virions.

The ROC-curve established a theoretical cut-off point for the viral load of 0.00000372 IU/ml at the end of RBV therapy to indicate whether a SOT recipient will reach SVR (sensitivity 62%, specificity 70%, AUC=0.677 (95%-CI 0.557 – 0.797, p=0.005), supplementary figure 1). This value is too low for quantification but illustrates that RBV therapy should be continued when the HEV viral load in blood is negative (<100 IU/ml).

No difference in the elimination rate between SOT recipients with versus without SVR and a short treatment duration (<180 days) was found (0.012h-1 and 0.011h-1, p = 0.49). The elimination rate for SOT recipients with SVR was higher compared to the elimination rate for SOT recipients without SVR who had taken at least 180 days of RBV therapy (0.012h-1 and 0.002h-1, p=0.053) (figure 2).

Model diagnostics

Bootstrap analyses were in good agreement with parameter estimates (table 2). Visual diagnostics showed that RBV concentrations, hemoglobin concentrations and viral load concentrations including BLQ values were predicted by the model with no systematic biases (supplementary figures 2-7).

Model parameter	Description	Population estimate	RSE (%)	Bootstrap of the final model	
Population pharmacokinetic model				**Median**	**95% CI**
Ka (h^{-1})	Absorption constant	2.91 (fixed)	-	-	-
CL (L/h)	Clearance	26.4	15	24.3	15.8 – 36.1
V2 (L)	Volume of central compartment	769 (fixed)	-	-	-
Q1 (L/h)	Distribution clearance to peripheral compartment	104 (fixed)	-	-	-
V3 (L)	Volume of peripheral compartment	3570 (fixed)	-	-	-
eGFR on Cl	Kidney function on clearance	1.32	14	1.22	0.89 – 1.7
WGT on V2	Weight on volume of central compartment	1.29 (fixed)	-	-	-
WGT on V3	Weight on volume of peripheral compartment	0.725 (fixed)	-	-	-
Sex on V3	Sex on volume of peripheral compartment	0.732 (fixed)	-	-	-
Cut-off value on kidney function (mL/min)	-	57	0.1	57.4	52 – 180
IIV CL (%CV)	-	50.5	12	47.2	32.9 – 59.6
Standard deviation proportional error	-	0.377	11	0.375	0.308 – 0.456
Hemoglobin population model					
k_{out} (h^{-1})	Loss of hemoglobin	0.556	26	0.565	0.308 – 0.911
Slope	The slope of the RBV effect	0.102	11	0.102	0.078 – 0.129
IIV on k_{out} (%CV)	-	463	28.2	443.1	211.7 – 781.2
IIV on slope (%CV)	-	52.1	28.8	50.6	23.2 – 92.3
Standard deviation additive error (mmol/L)	-	0.406	8	0.405	0.341 – 0.473
Viral load population model					
TDEG (h)	Half-life of healthy hepatocytes	6398 (fixed)	-	-	-
Factor	Factor for half-life of infected hepatocytes	100 (fixed)	-	-	-
Elimination rate of virions (h^{-1})	-	0.0136	7	0.0126	0.0090 – 0.0171
IC50 (ng/L)	Half maximal inhibitory concentration	1000 (fixed)	-	-	-
Imax	Maximum inhibition concentration	0.999 (fixed)	-	-	-
Rho	Fraction of infect hepatocytes at baseline	0.001 (fixed)	-	-	-
IIV on elimination rate (%CV)	-	71.7	26.1	70.5	24.6 – 130
Standard deviation additive error	-	2.01	16.8	1.99	1.33 – 2.61

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Table 2. Estimated population pharmacokinetic and pharmacodynamic parameters for the final model and bootstrap analysis

Abbreviations: CV, coefficient of variation; IIV, interpatient variability; eGFR, estimated glomerular filtration rate; RSE, relative standard error

For IIV parameters, the estimate is the %CV value, calculated as 100*sqrt(exp(omega^2)-1), where omega^2 is the estimated variance, and the RSE is calculated as 100*SE/(2 omega^2), where SE is the estimated standard error (SE) for the variance omega^2.

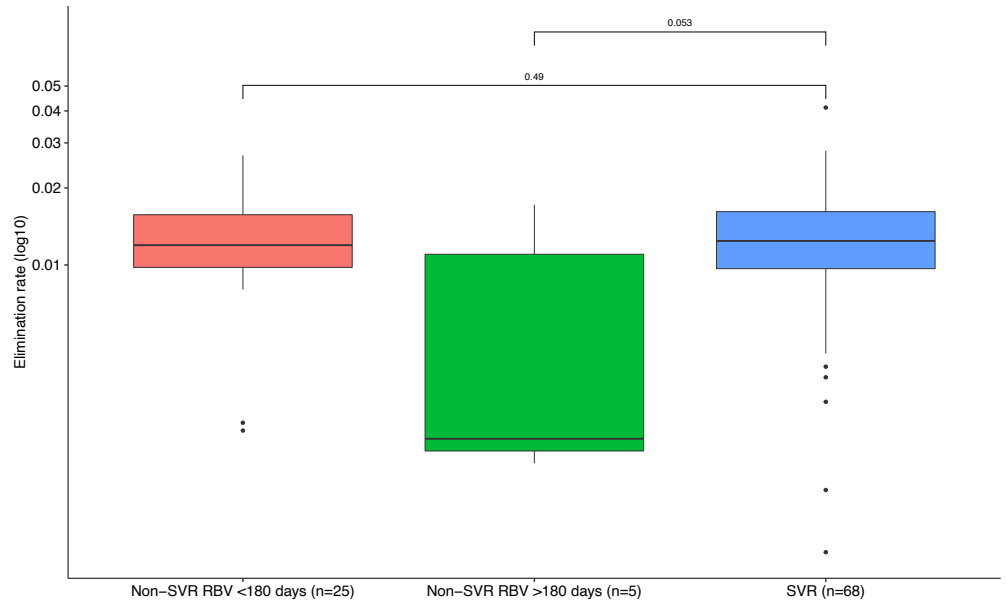

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Figure 2. Evaluation of the HEV elimination rate for solid organ transplant recipients with and without sustained virologic response.

HEV elimination rate for solid organ transplant recipients with and without SVR (non-SVR) and different treatment durations (< or > 180 days).

Abbreviations: RBV, ribavirin; SVR, sustained virologic response

Optimal dosing simulations

Figure 3 shows the hemoglobin concentrations and viral loads over time for several dosing regimens. The decline in viral load did not depend on the RBV dose, whereas the decline in hemoglobin was dependent on the RBV dose and the baseline hemoglobin concentration.

The simulated viral load (figure 3B and 3D) showed a biphasic profile at the 5th percentile, and a monophasic profile at the 95th percentile. At the upper end of the prediction interval, the rate-limiting elimination rate of the virus drove the viral load decay. A slow viral elimination rate for the virus masked the secondary phase of viral load decay, driven by a decrease in the number of infected cells. For high rates (near the 5th percentile), the RBV-induced reduction in viral production translated into a rapid drop in viral load due to the fast turnover. In a second phase, the slower reduction in the number of infected cells reduced viral load further. As there was no IIV on the elimination rate of infected hepatocytes, all high-rate profiles (including the median) heaped up in the second phase.

After 180 days of RBV therapy, the 95% prediction intervals of the viral load for every RBV dosing strategy were at or below the defined cut-off (<100 IU/ml) where HEV viral load is considered negative (figure 3D), but 21 – 24% of the simulated viral loads were not below the (lower) cut-off point established with the ROC-curve for SVR (figure 3D).

Figure 3A and 3C show that with RBV dosing strategies of ≥400 mg per day 11.5 – 26.8% of the simulated hemoglobin levels were severely decreased to ≤5 mmol/L versus 0.5 – 3.8% for dosing regimens of 200 mg per day or every 2 days. Figure 4 and supplementary table 1 show the distribution of hemoglobin reduction, by sex, kidney function, treatment duration and dose. The reduction percentages were independent of the hemoglobin baseline and were similar for men and women.

A model-suggested optimized RBV dose of 600 mg/day with a kidney function ≥60 ml/min/1.73m^2, 400 mg/day with a kidney function 30-59 ml/min/1.73m^2 and 200 mg/day with a kidney function ≤30 ml/min/1.73m^2 for 180 days showed good efficacy and low risk of anemia. Figure 5 shows predicted hemoglobin concentrations for 180 days in SOT recipients with different renal functions using 600 mg/day, 400 mg/day and 200 mg/day RBV. The fraction of SOT recipients reaching hemoglobin ≤5 mmol/L increased with decreasing renal function. This fraction was higher in women than in men, because of their lower baseline hemoglobin concentrations.

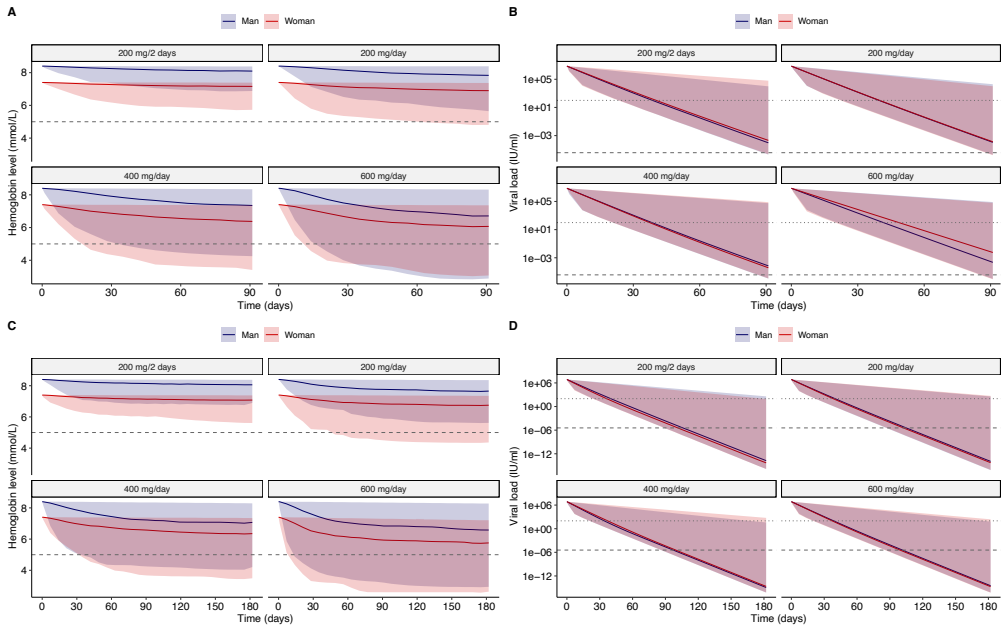

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Figure 3. Dose simulation of ribavirin

A), C) Simulation of hemoglobin (toxicity) for 90 and 180 days RBV therapy as indicated by the panel header. Shaded area represents the 95%-prediction interval. Dashed lines represent the cut-off value of 5 mmol/L below which a blood transfusion is recommended; B), D) Simulation of viral load (efficacy) for 90 and 180 days RBV therapy as indicated by the panel header. Shaded area represents the 95%-prediction interval. Dotted lines represent the cut-off value of 100 IU/ml below which the HEV viral load is considered negative. Dashed lines represent the cut-off value established with the ROC-curve for the viral load of 0.00000372 IU/ml to indicate whether a solid organ transpant recipient will reach SVR. Male and female are overlapping and hard to distinguish.

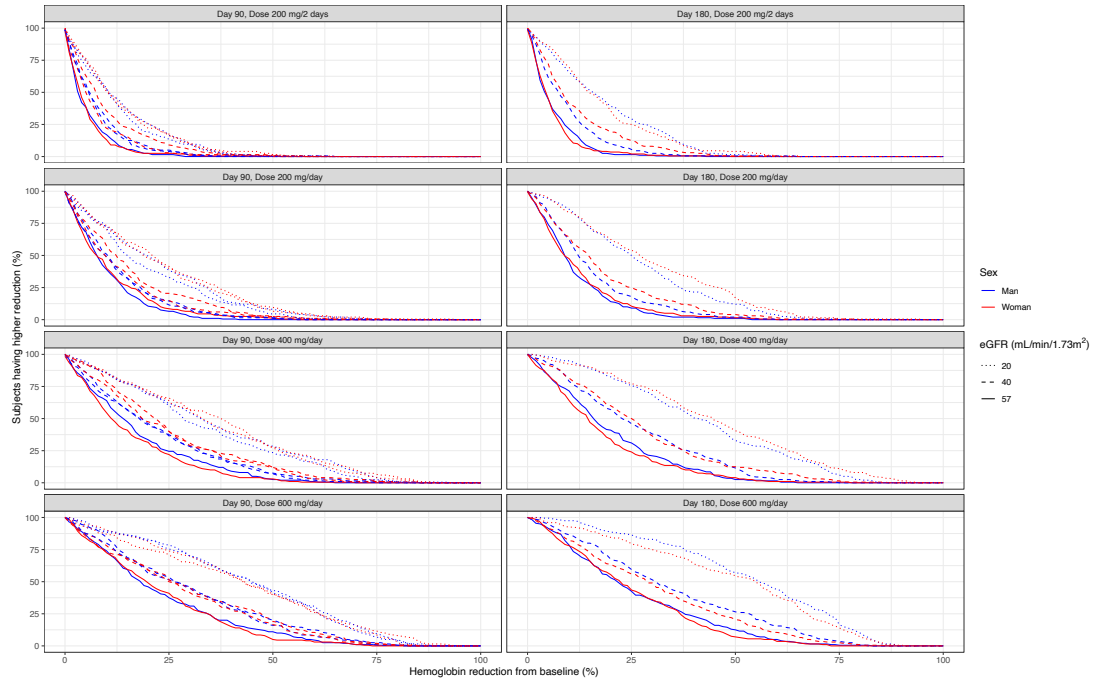

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Figure 4. Subjects having more than the indicated reduction in hemoglobin

Percentage of subjects having more that the indicated reduction in hemoglobin, by kidney function (eGFR) and sex, for 90 and 180 days RBV therapy and different dosing regimens as indicated by the panel header; i.e. 25% of the subjects with an eGFR 57 ml/min using 400 mg/day RBV for 90 days have >25% hemoglobin reduction from baseline.

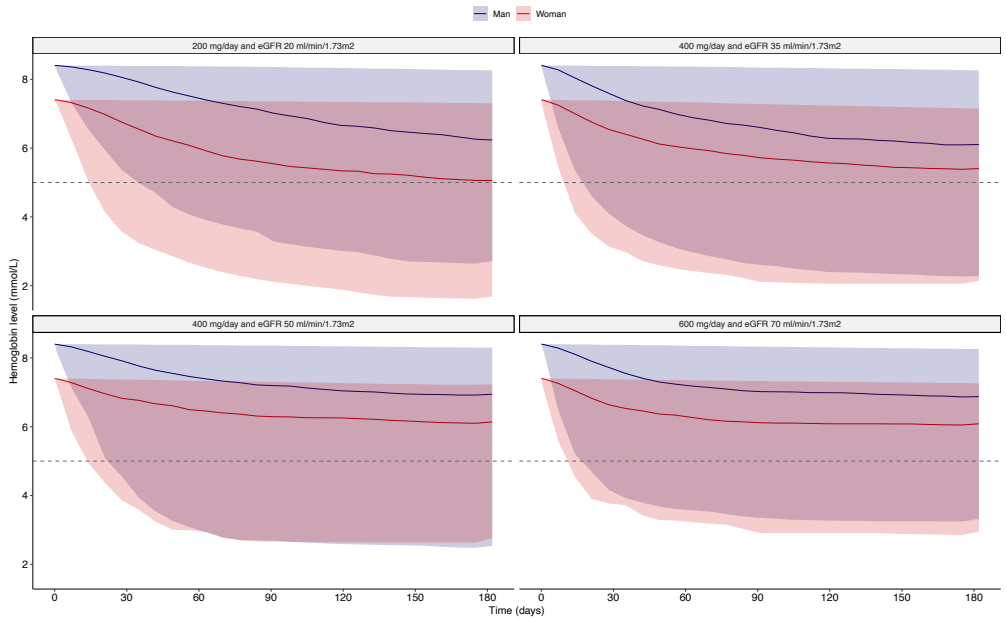

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Figure 5. Simulations of hemoglobin for 180 days in solid organ transplant recipients with different renal function using 200 mg/day and 200 mg/2 days ribavirin

Simulation of hemoglobin (toxicity) for 180 days in solid organ transplant recipients with different renal function (as indicated by the panel header) using 200 mg/day, 400mg/day or 600 mg/day ribavirin. Shaded area represents the 95%-prediction interval. Dashed line represents the cut-off value of 5 mmol/L below which a blood transfusion is recommended.

Discussion

To our knowledge, this is the first population PK/PD model describing the effect of RBV on hemoglobin and viral load in SOT recipients with chronic HEV infection. Currently, RBV therapy for the treatment of chronic HEV infection in SOT recipients is based on case reports and case series, and the optimum RBV dose and treatment duration are unknown.(7, 9, 10) Therefore, treatment may benefit from model-based selection of dosing regimens, considering viral load and hemoglobin concentrations. The model predicts viral clearance and acceptable toxicity (hemoglobin >5 mmol/L) for this population at 600 mg/day with a kidney function ≥60 ml/min/1.73m^2, 400 mg/day with a kidney function 30-59 ml/min/1.73m^2 and 200 mg/day with a kidney function ≤30 ml/min/1.73m^2. Although RBV doses up to 1200 mg/day are commonly used in clinical practice, in our simulations RBV doses ≥400 mg/day resulted in hematological toxicity without improvement in the viral clearance over the model-suggested dosing regimens. RBV therapy should not be stopped too early as this is illustrated by the theoretical low value for the viral load in the ROC analysis. Furthermore, a lower RBV dose will result in less side effects resulting in better treatment compliance.

RBV therapy is associated with dose-dependent anemia and therefore hemoglobin and eGFR dependent dosing is recommended.(22) In our model, the hemoglobin concentration decreases with increasing RBV concentrations. RBV-induced anemia has been studied in patients with chronic hepatitis C virus infection.(23, 24) In one study, bodyweight and hemoglobin concentrations were shown to be relevant covariates in an indirect response model that did not include RBV plasma concentrations.(23) In another model, plasma and intracellular RBV phosphorylation kinetics were linked to the effect of RBV triphosphate accumulation on red blood cell homeostasis.(24) Relevant covariates in this model were sex, weight and inosine triphosphatase (ITPA) genotype. In clinical practice intracellular RBV concentrations and ITPA genotypes are not regularly measured. So far, it remains unclear what impact an ITPA variant phenotype has on ribavirin-induced anemia. Therefore, we developed a model including covariates that will be always available at the start of RBV therapy.

The immunosuppressant mycophenolic acid was shown to inhibit HEV replication *in vitro*, but not *in vivo*.(25) In our cohort, this covariate did not appear to be a significant covariate influencing the elimination of HEV virions. This is possibly due to the high efficacy of RBV.

After the start of RBV therapy, it takes several weeks before steady-state plasma concentrations are reached. Therefore, some centers start with loading doses of RBV for the first couple of days. In patients with HCV, RBV is shown to be a weak inhibitor of HCV viral replication with an IC50 between 2.93 – 9.76 mg/L.(26, 27) In contrast, RBV was a strong inhibitor of HEV viral replication in our cohort, and we fixed the IC50 at 1000 ng/L after performing a sensitivity analysis evaluating different values for IC50. Based on these results and the suggested low IC50 of RBV for HEV, including a loading dose will not result in faster viral clearance. On the contrary, a loading dose might cause more toxicity (hemoglobin drop) in the first days after the start of RBV therapy. Therefore, the added value of a loading dose in the treatment of chronic HEV is disputable.

Currently, still 20 – 30% of the chronically-infected SOT recipients do not reach SVR despite RBV therapy. This might be due to insufficient clearance of HEV at the end of RBV therapy as shown by the persistence of HEV RNA in the stool in patients with undetectable HEV RNA in the serum.[5] We found a six-fold difference in the HEV elimination rate between SOT recipients with and without SVR, including those with a sufficient treatment duration (>180 days). Further research could investigate whether estimating the HEV elimination rate from two HEV RNA measurements in blood within six weeks after the start of RBV could differentiate responders and non-responders to RBV. For non-responders (i.e., patients with a low elimination rate, projected not to reach the clearance threshold within an acceptable time frame), a further reduction in the immunosuppressive therapy might be considered. For responders, RBV should be continued for at least 180 days and thereafter HEV RNA in the stool should be tested negative twice before considering stopping RBV therapy.

This study has several limitations. Firstly, we did not include SOT recipients experiencing a relapse after ribavirin withdrawal, due to insufficient data. Secondly, the insensitivity of the viral load model to the value of IC50 suggests that the given RBV doses tended to result in maximum suppression of HEV for all or most observations. Thirdly, the use of recombinant

erythropoietin and blood transfusions was not included in the model, which could have resulted in an underprediction of the effect of ribavirin on hemoglobin. By correcting for these factors, the model-based dose suggestion would potentially be even lower as proposed now. Finally, as the data was collected retrospectively, we had no control over the dosing regimen, and therefore initial dosing and dose adaptations depended on time varying patient status. This could have affected the results, i.e., an over-estimation of efficacy. SOT recipients who did not achieve viral load reduction would likely be up-titrated during the RBV therapy. A prospective study with controlled dosing of ribavirin and controlled reduction of immunosuppressive therapy before the start of ribavirin would be able to address this.

In conclusion, this study provides a valuable first step in determining the optimal RBV treatment regimen for chronic HEV infections in SOT recipients. Given the model predictions and the data limitations, it seems prudent and feasible to start a non-inferiority, prospective trial evaluating the effect of low dose RBV on HEV clearance in SOT recipients in the near future.

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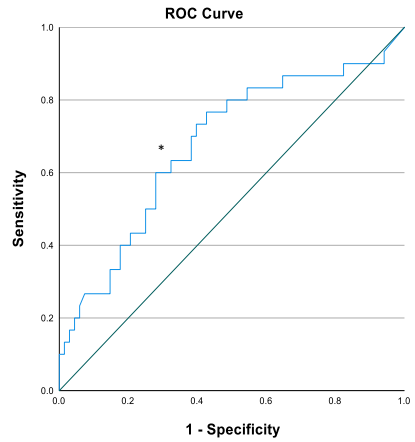
	Dose	Hb reduction 0%	Hb reduction 20%	Hb reduction 40%	Hb reduction 80%	Hb reduction 100%
Male and kidney function 20 ml/min/1.73m²	200mg/2days for 90 days	98.5	25.5	2	0	0
	200mg/2days for 180 days	99	39	7	0	0
	200mg/day for 90 days	100	39	11.5	0	0
	200mg/day for 180 days	100	60	21.5	0	0
	400mg/day for 90 days	100	69.5	34.5	0.5	0
	400mg/day for 180 days	100	82.5	50.5	2.5	0
	600mg/day for 90 days	100	82	56	4.5	0
	600mg/day for 180 days	100	88.5	72	10	0
Female and kidney function 20 ml/min/1.73m²	200mg/2days for 90 days	100	22	4	0	0
	200mg/2days for 180 days	100	35.5	6	0	0
	200mg/day for 90 days	99.5	48	18.5	0.5	0
	200mg/day for 180 days	100	62.5	33	0.5	0
	400mg/day for 90 days	100	69.5	45	4.5	0
	400mg/day for 180 days	100	85.5	55	8	0
	600mg/day for 90 days	100	75	55.5	3	0
	600mg/day for 180 days	100	84.5	64	7	0
Male and kidney function 40 ml/min/1.73m²	200mg/2days for 90 days	98.5	5.5	0.5	0	0
	200mg/2days for 180 days	99.5	10	0.5	0	0
	200mg/day for 90 days	100	19.5	3	0	0
	200mg/day for 180 days	100	25	5	0	0
	400mg/day for 90 days	100	46	16	0	0
	400mg/day for 180 days	100	57	23.5	0	0
	600mg/day for 90 days	100	61	31	0.5	0
	600mg/day for 180 days	100	67.5	36	0.5	0
Female and kidney function 40 ml/min/1.73m²	200mg/2days for 90 days	99.5	15.5	1	0	0
	200mg/2days for 180 days	100	19	3	0	0
	200mg/day for 90 days	100	26.5	6	0	0
	200mg/day for 180 days	100	31	11	0	0
	400mg/day for 90 days	100	47.5	17.5	0.5	0
	400mg/day for 180 days	100	60	20.5	0.5	0
	600mg/day for 90 days	100	59	28	0.5	0
	600mg/day for 180 days	100	63.5	31.5	1	0
Male and kidney function ≥57 ml/min/1.73m²	200mg/2days for 90 days	98.5	2.5	0	0	0
	200mg/2days for 180 days	99.5	2	0.5	0	0
	200mg/day for 90 days	100	10.5	0.5	0	0
	200mg/day for 180 days	100	14	2	0	0
	400mg/day for 90 days	99.5	33.5	9	0	0
	400mg/day for 180 days	99.5	41	10.5	0	0
	600mg/day for 90 days	100	46	18	0.5	0
	600mg/day for 180 days	100	53	23	0.5	0
Female and kidney function ≥57 ml/min/1.73m²	200mg/2days for 90 days	97.5	2.5	0.5	0	0
	200mg/2days for 180 days	98.5	3.5	0.5	0	0
	200mg/day for 90 days	99.5	15.5	2.5	0	0
	200mg/day for 180 days	99.5	18	3	0	0
	400mg/day for 90 days	98.5	31.5	5.5	0	0
	400mg/day for 180 days	100	34	8.5	0	0
	600mg/day for 90 days	100	49	15	0	0
	600mg/day for 180 days	100	55.5	19	0	0

▲

Supplementary table 1. Percentage of subjects with more than the indicated reduction in hemoglobin, by sex, kidney function, ribavirin dose regimens and treatment durations.

For example, 33.5% of the male SOT recipients with an eGFR ≥57 ml/min/1.73m² using 400 mg/day RBV for 90 days experience >20% hemoglobin reduction from baseline (i.e., baseline hemoglobin of 8 mmol/L will drop below 5.6 mmol/L).



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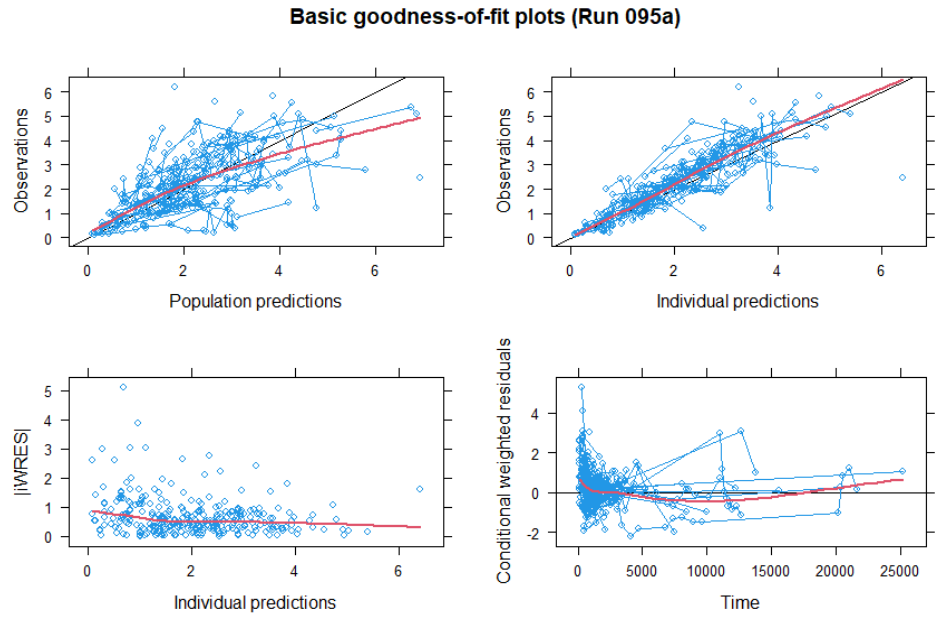
Supplementary figure 1. ROC-curve for HEV load as predictor of effect (SVR) in chronic HEV patients treated with RBV

ROC-curve for RBV plasma concentration as predictor of effect in chronic HEV patients treated with RBV. Cut-off point * = 0.00000372 IU/ml, AUC=0.677 (95%-CI 0.557 to 0.797, p=0.005. At this point, sensitivity was 62% and specificity was 70%.



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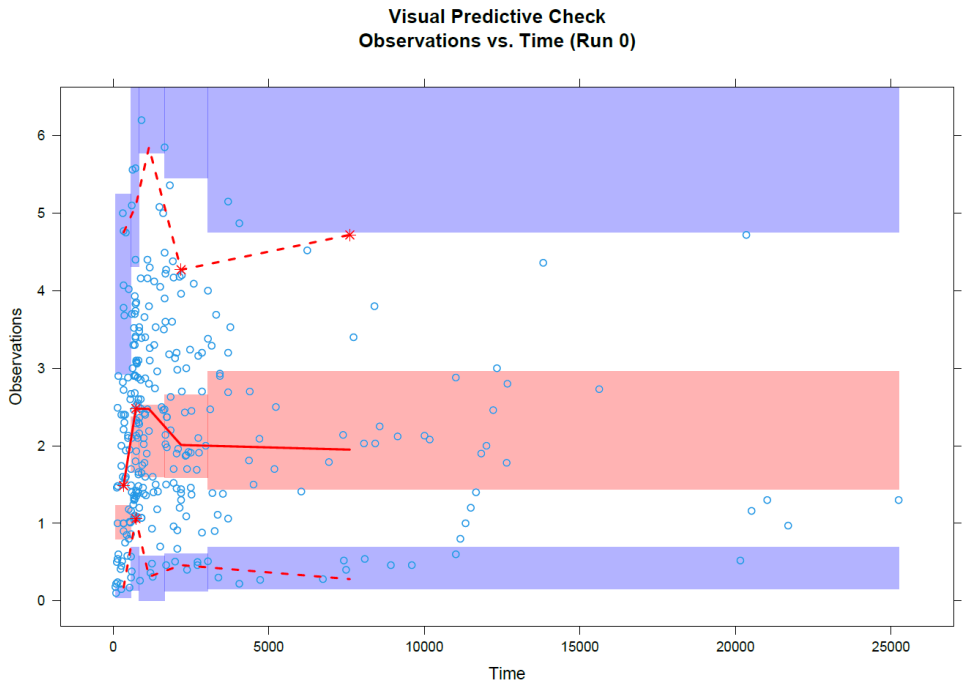
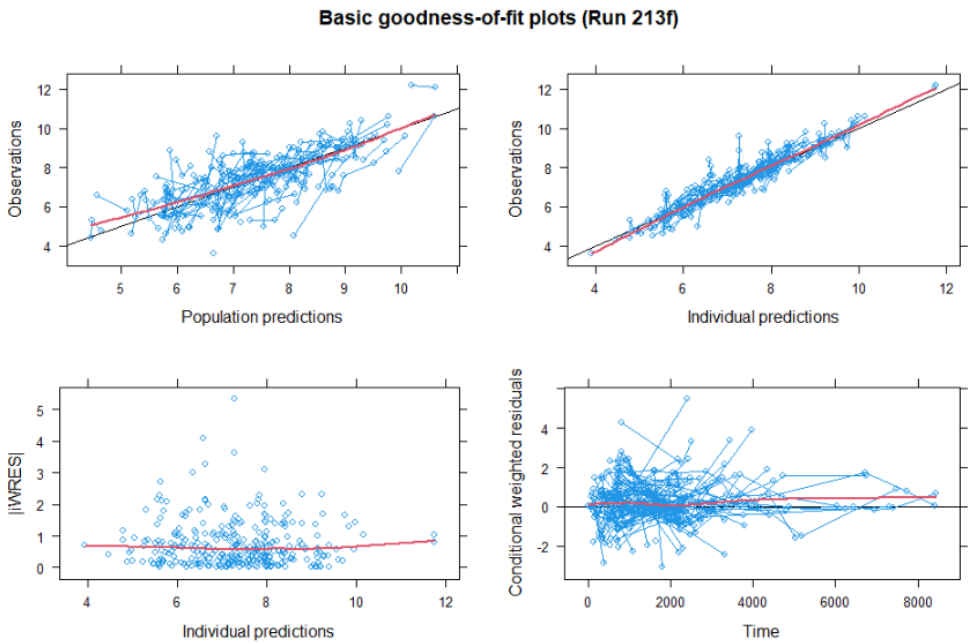
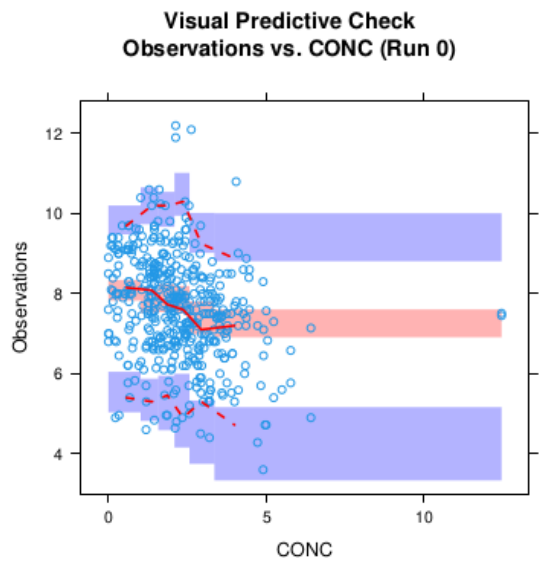
Supplementary figure 2. Visual diagnostics of population pharmacokinetic model



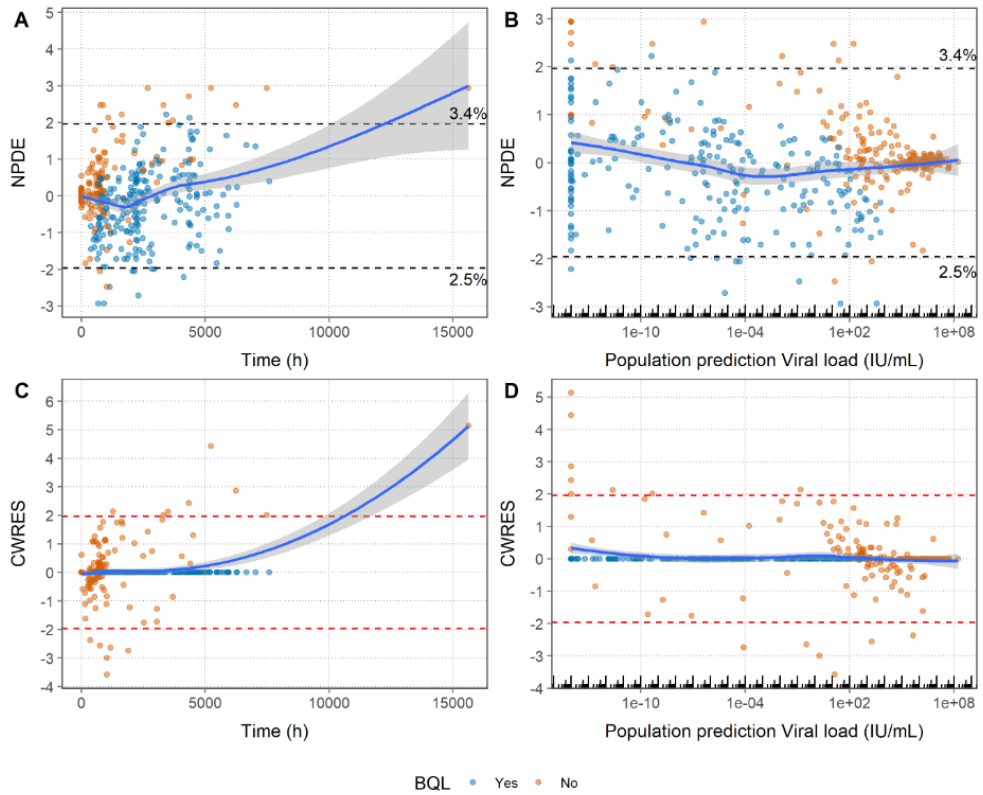
Supplementary figure 3. Visual predictive check of population pharmacokinetic model



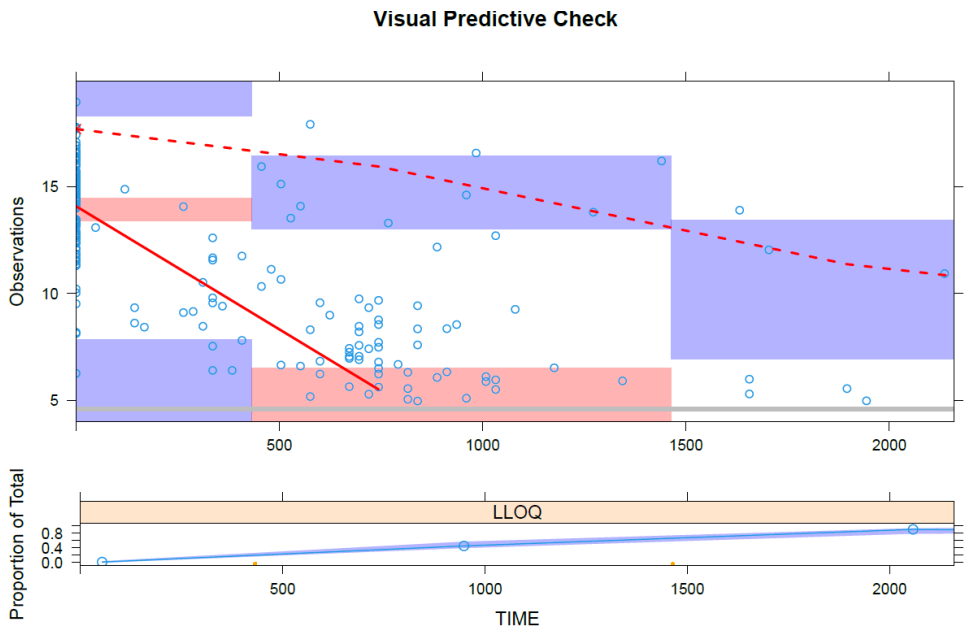
Supplementary figure 4. Visual diagnostics of the hemoglobin population model



Supplementary figure 5. Visual predictive check of the hemoglobin population model



Supplementary figure 6. Visual diagnostics of the viral load population model



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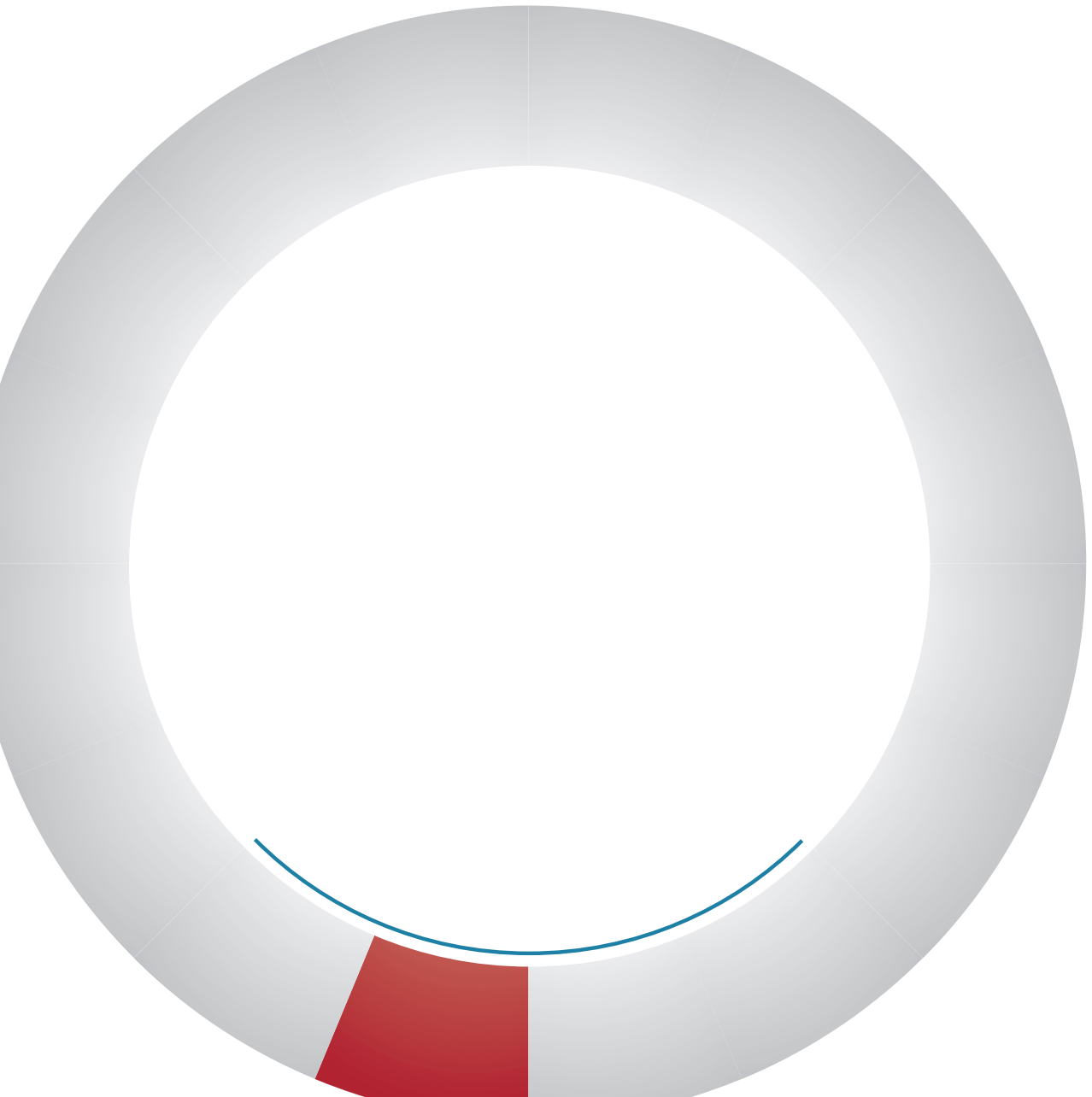
Supplementary figure 7. Visual predictive check of the viral load population model



Chapter 9

The high antibody response in relation to immunosuppressive blood levels in liver transplant recipients after SARS-CoV-2 vaccination: an observational, cohort study.

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Abstract

Objective

Several studies showed that the immunogenicity to SARS-CoV-2 vaccines in solid organ transplant recipients is reduced, with positive serology ranging from 30% - 65%. Until now, no study evaluated the effect of immunosuppressive blood levels on the IgG SARS-CoV-2 anti-spike antibody response after SARS-CoV-2 vaccination.

Design

In this observational, cohort study, we determined the immunogenicity to SARS-CoV-2 vaccination in liver transplant (LT) recipients in relation to the immunosuppressive blood levels after the 2nd dose of mRNA vaccines or the vector vaccine ChAdOx1 nCoV19.

Results

A total of 476 LT recipients were included: 430 received mRNA-1273 vaccine, 25 received BNT162b2 mRNA vaccine and 21 received ChAdOx1 nCoV19 vector vaccine. Seroconversion occurred in 79.0% (376/476) of the LT recipients. LT recipients vaccinated with the mRNA-1273 vaccine had significantly higher IgG SARS-CoV-2 anti-spike antibody levels compared to the other two vaccines, $p<0.001$. The use of mycophenolate mofetil (MMF), regardless the blood level, suppressed the IgG SARS-CoV-2 anti-spike antibody response and resulted in suboptimal responders to the SARS-CoV-2 vaccines, whereas the other immunosuppressive agents did not have that effect.

Conclusion

SARS-CoV-2 vaccination was highly effective in our LT recipient cohort. The mRNA-1273 vaccine results in a superior IgG SARS-CoV-2 anti-spike antibody response. MMF suppressed the IgG SARS-CoV-2 anti-spike antibody response, regardless the blood levels of MMF and the type of vaccination. Consequently, lowering the dose of MMF has no effect on the immunogenicity to SARS-CoV-2 vaccines. Discontinuation of MMF around vaccination for every patient on MMF therapy is suggested to achieve an optimal antibody response.

Introduction

Currently, several mRNA vaccines and adenovirus-based vector vaccines are available showing a strong efficacy in clinical trials.(1-3) SOT recipients are at an increased risk for a complicated course of severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2).(4-6) Vaccination is therefore strongly recommended in all SOT recipients with no preference for either mRNA or vector-based vaccines.(7, 8)

Several studies have shown that the immunogenicity to SARS-CoV-2 vaccines in SOT recipients is reduced, with detectable antibodies ranging from 30% - 65% for all types of solid organ transplantation.(9-13) Additional evidence is appearing regarding changes in cellular and humoral immunity after mRNA or vector-based SARS-CoV-2 vaccines.(13-17) A study by Schmidt *et al.* showed lower antibody and T cell levels in transplant recipients compared to healthy controls. (15) Two other studies in kidney and liver transplant recipients have shown significantly lower IgG anti-spike levels compared to healthy controls after full vaccination.(18)

Variables associated with a reduced immunogenicity are older age, regimens that includes mycophenolate mofetil (MMF), renal insufficiency and time after transplantation.(18, 19)

Recently, the optimal vaccination strategy for the general population and specific target populations such as SOT recipients was discussed. Evidence demonstrate that administering a booster vaccination in healthy adult individuals was well-tolerated and immunogenic. The strongest responses were detected after a booster with mRNA-based vaccines.(20, 21)

Until now, studies on the vaccine efficacy and immunogenicity in LT recipients have been limited. Furthermore, specific guidance with regards to immunosuppressive blood levels in relation to the immunogenicity of SARS-CoV-2 vaccines in SOT recipients is lacking and desperately needed with regards to the current discussion on booster vaccination. The aim of this study was to investigate the effect of immunosuppressive blood levels on the SARS-CoV-2–specific immunogenicity of SARS-CoV-2 vaccination in LT recipients. In addition, the influence of sex, recipients age, kidney function and time between vaccination and transplantation on the immunogenicity of SARS-CoV-2 vaccination was investigated.

Materials and Methods

Study design and patients

This study was an observational, cohort study conducted between March 2021 and July 2021 at the Erasmus MC University Medical Center Rotterdam, the Netherlands and compromised of LT recipients, fully vaccinated and routinely followed at the outpatient clinic. Included were adult LT recipients (>18 years) vaccinated with 2 doses of the mRNA vaccines BNT162b2 or mRNA-1273 or the vector vaccine ChAdOx1 nCoV19. Blood samples were routinely collected and measured. Excluded were patients with a history of a SARS-CoV-2 infection.

This study was approved by the Medical Ethics Committee of the Erasmus MC University Medical Center Rotterdam, the Netherlands (MEC-2021-0810). Patients were not involved in the design, conduct, reporting and dissemination plans of this research.

Immunosuppressive protocol

In our program the induction immunosuppressive therapy consists of basiliximab and methylprednisolone intravenously. On day zero, twice daily 1000 mg mycophenolate mofetil (MMF), the prodrug of MPA, and once daily 20 mg prednisolone is started. From day five twice daily 0.05 mg/kg tacrolimus is introduced and MMF is discontinued after achieving two therapeutic trough levels of tacrolimus; and patients are weaned of the prednisolone. Next, according to the development of renal insufficiency or other side effects the maintenance regimen is adapted, intensified or reduced.

Laboratory tests

SARS-CoV-2 serology

Humoral immune responses to vaccination were measured by using a quantitative assay directed against the SARS CoV-2 Spike (S) antigen (Liaison SARS CoV-2 TrimericS IgG assay, DiaSorin, Italy), with a lower limit of detection of 4.81 BAU/ml.(22) The assay was performed following the manufacturer's instructions in which values ≥ 33,8 BAU/ml were considered reactive.

As is shown in other studies, an arbitrary cut-off for adequate responders based on virus neutralization was set at >68.3 BAU/ml for the vector vaccines and >300 BAU/mL for the mRNA vaccines.(21, 23, 24)

Immunosuppressive drugs

Plasma concentrations of the immunosuppressive drugs were analyzed using validated ultra-high-performance liquid chromatography-tandem mass spectrometry (U-HPLC-MS/MS) methods. Trough levels of the immunosuppressive drugs were included in the analysis. Trough levels below the lower limit of quantification were included with the lower limit of quantification.

Data collection

Socio-demographic, clinical and transplant parameters were extracted from patients' electronic medical records in the hospital information system. The following information was collected from patients' electronic medical records: gender, age, ethnicity, reason for and date of the transplantation, history of SARS-CoV-2 infection, total Ig SARS-CoV-2, IgM SARS-CoV-2, IgG anti-spike SARS-CoV-2 levels, interval between vaccination and lab, interval between transplantation and vaccination, interval between vaccinations, renal function, immunosuppressive drugs and immunosuppressive trough levels.

The renal function was measured by serum creatinine and the estimated glomerular filtration rate (eGFR) was calculated with the CKD-EPI equation.(25)

Statistical analysis

Patient characteristics are summarized using counts (%) for nominal and ordinal variables and mean (SD) or median (inter-quartile range, IQR) for the continuous variables, depending on the shape of the distribution. Categorical variables were compared between groups using the Chi-square test or Fisher's exact test. Continuous variables were compared between groups using a t-test if approximately normally distributed or by Mann-Whitney-U/Kruskall-Wallis test if non-normally distributed.

Differences in the antibody response on SARS-CoV-2 vaccination between the LT recipients were compared based on the Chi-square test. Differences in the IgG anti-spike SARS-CoV-2 levels were tested using the Mann-Whitney-U or Kruskall-Wallis test.

Two multiple linear regression models were fitted, investigating the association between the antibody response on SARS-CoV-2 vaccination and the immunosuppressive trough levels of MPA or tacrolimus in the subset of patients who received the specific immunosuppressive agent. Both models additionally included covariates shown to be relevant in previous studies: the recipients age, kidney function, type of vaccination and time between vaccination and transplantation as well as the interaction between kidney function and age. To visualize the estimated associations, the expected antibody response across the range of trough levels was calculated while fixing the values of all other covariates to the median or reference category.

For all statistical tests, a p-value of <0.05 was considered to indicate statistical significance. All data were collected in a data extraction file and analysis were performed using R software (version 3.6.2).(26)

Results

Study population

A total of 512 LT recipients (512/795 = 64.4% of all alive recipients) were eligible for analysis at the time of reporting. Among these 512 LT recipients, 462 received the mRNA-1273 vaccine, 29 received the BNT162b2 mRNA vaccine and 21 received the ChAdOx1 nCoV19 vector vaccine. In total, 32 LT recipients vaccinated with the mRNA-1273 vaccine and 4 LT recipients with the BNT162b2 mRNA vaccine had a history of a SARS-CoV-2 infection confirmed by PCR before vaccination and were excluded from this analysis. A total of 128 (16.1%, 128/795) LT recipients were not vaccinated and 155 (19.5%, 155/795) LT recipients were not routinely seen at the outpatient clinic during the study period.

Table 1 presents the demographical and clinical characteristics of the study population. The median age was significantly higher for the LT recipients receiving the ChAdOx1 nCoV19 vector vaccine (63 year, IQR: 60-64) and the BNT162b2 mRNA vaccine (71 year, IQR: 59–79) compared to the mRNA-1273 vaccine (59 year, IQR: 49-66), p < 0.001. The three most frequent indications for transplantation were primary sclerosing cholangitis, hepatocellular carcinoma and acute liver failure. The interval between the vaccinations was standardized according to the advice of the Dutch National Institute for Public Health and the Environment (RIVM). LT recipients receiving the mRNA-1273 vaccine had a significantly longer interval between vaccination and quantification of the IgG SARS-CoV-2 anti-spike antibodies, p<0.001 (43 days, IQR 33-56.25 for the mRNA-1273 vaccine versus 31 days, IQR 26-38 for the ChAdOx1 nCoV19 vector vaccine and 31 days, IQR 29-40 for the BNT162b2 mRNA vaccine). Tacrolimus was used in 88.2% of the LT recipients as main immunosuppressive agent and the majority of the LT recipients (51.1%) used monotherapy of tacrolimus. MMF was used in 34% of the LT recipients, mainly in combination therapy with tacrolimus.

SARS-CoV-2 specific antibody response upon SARS-CoV-2 vaccination in LT recipients

Seroconversion occurred in 79.0% (376/476) of our LT recipients. Table 2 presents the SASR-CoV-2 specific antibody response of SARS-CoV-2 vaccination in LT recipients. LT recipients vaccinated with the mRNA-1273 vaccine, the BNT162b2 mRNA vaccine and the ChAdOx1 nCoV19 vector vaccine showed a seroconversion rate of 80.2% (345/430, 95%-CI 76.2% – 83.7%), 72.0% (18/25, 95%-CI 52.4% – 85.7%) and 61.9% (13/21, 95%-CI 40.9% – 79.2%). Most of the LT recipients (76.9%) had negative IgM SARS-CoV-2 serology after vaccination.

Figure 1 shows the IgG SARS-CoV-2 anti-spike antibody response of the seroconverted LT recipients. LT recipients vaccinated with the mRNA-1273 vaccine had significantly higher IgG SARS-CoV-2 anti-spike antibody levels compared to the other two vaccines, p<0.001 (1070 BAU/mL, IQR 242 - 2320 BAU/mL for the mRNA-1273 vaccine versus 231.50 BAU/mL, IQR 77.30 – 633.75 BAU/mL for the BNT162b2 mRNA vaccine and 124 BAU/mL, IQR 31.40 – 203.00 BAU/mL for the ChAdOx1 nCoV19 vector vaccine).

	ChAdOx1 nCoV19 (n=21)	mRNA-1273 (n=430)	BNT162b2 (n=25)	p-value
General characteristics				
Male (n, %)	11 (52.4)	264 (61.4)	11 (44)	0.172⁺
Age at 1st vaccination, years (median [IQR])	63.00 [60.00 - 64.00]	59.00 [49.00 - 66.00]	71.00 [59.00 - 79.00]	<0.001‡
Ethnicity (n, %)				0.673§
Caucasian	21 (100.0)	388 (90.2)	22 (88.0)	
Afro-American	-	22 (5.1)	1 (4.0)	
Asian	-	20 (4.7)	2 (8.0)	
Primary disease for transplantation (n, %)				0.109§
PSC	-	98 (22.8)	3 (12.0)	
HCC	3 (14.3)	93 (21.6)	7 (28.0)	
ALF	3 (14.3)	41 (9.5)	2 (8.0)	
Other cholestatic disease†	5 (23.8)	31 (7.2)	1 (4.0)	
(N)ASH	3 (14.3)	35 (8.1)	4 (16.0)	
Cryptogenic	1 (4.8)	20 (4.7)	1 (4.0)	
Viral hepatitis	2 (9.5)	21 (4.9)	1 (4.0)	
Metabolic disease	-	20 (4.7)	-	
Other*	1 (4.8)	44 (10.2)	5 (20.0)	
Retransplantation	3 (14.3)	27 (6.3)	1 (4.0)	
Interval between vaccinations, days (median [IQR])	77.00 [73.00 - 82.00]	28.00 [28.00 - 28.00]	35.00 [29.75 - 35.00]	<0.001‡
Interval between vaccination and lab, days (median [IQR])	31.00 [26.00 - 38.00]	43.00 [33.00 – 56.25]	31.00 [29.00 - 40.00]	<0.001‡
Interval between transplantation and vaccination, years (median [IQR])	7.00 [2.00 - 14.00]	5.50 [2.00 - 12.00]	14.00 [6.00 - 18.00]	0.020‡
eGFR, ml/min/1.73m2 (mean (SD))	61.24 (20.62)	64.53 (18.31)	56.76 (18.44)	0.097‡
Immunosupressive drug therapy				
Tacrolimus use (n, %)	18 (85.7)	383 (89.1)	19 (76.0)	0.134⁺
Tacrolimus trough level, mcg/L (median [IQR])	5.00 [3.50 - 6.20]	4.70 [3.60 - 6.00]	4.80 [3.95 - 5.50]	0.943‡
Mycophenolate mofetil use (n, %)	7 (33.3)	144 (33.5)	11 (44.0)	0.559⁺
Mycophenolic acid trough level, mg/L (median [IQR])	0.56 [0.53 - 2.00]	1.75 [1.10 - 2.84]	2.25 [1.66 - 2.65]	0.164‡
Ciclosporin use (n, %)	-	10 (2.3)	2 (8.0)	0.211§
Ciclosporin trough level, mcg/L (median [IQR])	-	62.50 [29.25 - 124.75]	26.50 [21.75 - 31.25]	0.197‡
Everolimus use (n, %)	-	9 (2.1)	-	-
Everolimus trough level, mg/L (median [IQR])	-	3.30 [3.20 - 3.50]	-	-
Sirolimus use (n, %)	1 (4.8)	20 (4.7)	-	0.708§
Sirolimus trough level, mcg/L (median [IQR])	7.40 [7.40 -7.40]	4.30 [3.50 - 4.90]	-	0.143‡
Mono therapy (n, %)				0.257§
TAC	11 (52.4)	221 (51.5)	11 (44.0)	
MMF	2 (9.5)	21 (4.9)	4 (16.0)	
No IS drug	1 (4.8)	4 (0.9)	-	
CICLO	-	4 (0.9)	1 (4.0)	
OTHER‡	-	4 (0.9)	-	
Duo therapy (n, %)				0.976§
TAC + MMF	5 (23.8)	103 (24.0)	5 (20.0)	
TAC + CORT	1 (4.8)	24 (5.6)	2 (8.0)	
TAC + SRL	1 (4.8)	15 (3.5)	-	
CICLO + EVR	-	5 (1.2)	1 (4.0)	
OTHER¶	-	20 (4.6)	-	
Triple therapy (n, %)				0.603§
TAC + MMF + CORT	-	7 (1.6)	1 (4.0)	
TAC + SRL + CORT	-	1 (0.2)	-	
Corticosteroid use (n, %)	1 (4.8)	35 (8.1)	3 (12.0)	0.667⁺

▲

Table 1. Demographic and clinical characteristics

Abbreviations: PSC, primary sclerosing cholangitis; HCC, hepatocellular carcinoma; ALF, acute liver failure; (N)ASH, (non)alcoholic steatohepatitis; TAC, tacrolimus; MMF, mycophenolate mofetil; CORT, corticosteroids; SRL, sirolimus; Ciclo, cyclosporine; EVR, everolimus; AZA, azathioprine

†other cholestatic diseases: secondary biliary cirrhosis, primary biliary cirrhosis, congenital biliary diseases;
*other: hepatopulmonary syndrome, polycystic liver disease, autoimmune hepatitis, vascular liver diseases (budd chiari, veno-occlusive disease);
‡other: SRL (n=2), EVR (n=1), AZA (n=1);
¶Other: TAC + AZA (n=7), TAC + EVR (n=5), MMF + EVR (n=3), MMF + SRL (n=2), AZA + CORT (n=1), CICLO + AZA (n=1), MMF + CORT (n=1);
‡p-value based on non-parametric test (Kruskal-Wallis) test; ⁺p-value based on Chi-square test; §p-value based on Fisher's Exact test

	ChAdOx1 nCoV19 (n=21)	mRNA-1273 (n=430)	BNT162b2 (n=25)	p-value
Total Ig SARS-CoV-2 (n, %)				0.089⁺
Positive	13 (61.9)	345 (80.2)	18 (72.0)	
IgM SARS-CoV-2 (n, %)				0.108§
Negative	17 (81.0)	329 (76.5)	20 (80.0)	
Positive	1 (4.8)	62 (14.4)	1 (4.0)	
Unknown	1 (4.8)	30 (7.0)	4 (16.0)	
Borderline	2 (9.5)	9 (2.1)	-	
IgG anti-spike SARS-COV-2 levels (median [IQR])	14.30 [0.00 - 136.00]	650.50 [71.30 – 1787.50]	99.80 [20.40 - 304.00]	<0.001‡

▲

Table 2. Presence of S-specific antibodies after SARS-CoV-2 vaccination in LT recipients

‡p-value based on non-parametric test (Kruskal-Wallis) test; ⁺p-value based on Chi-square test; §p-value based on Fisher's Exact test

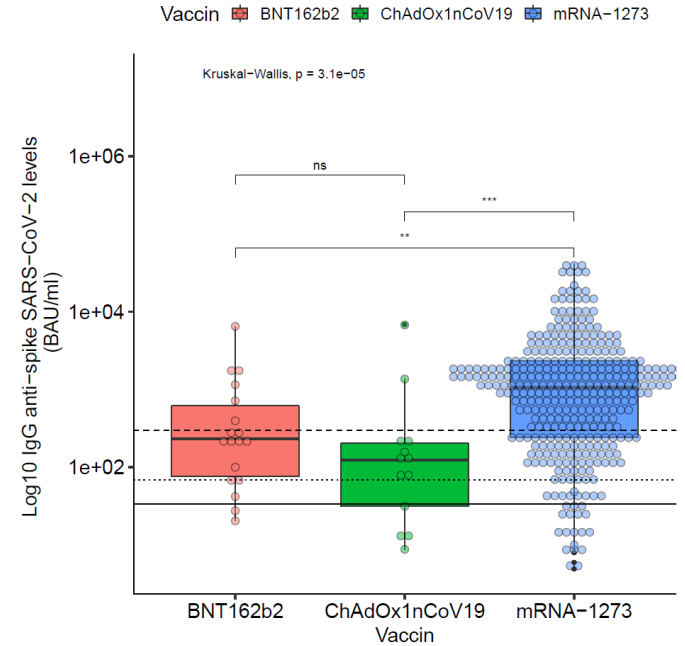

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Figure 1. SARS-CoV-2 specific antibody response in seroconverted LT recipients

Log-transformed IgG SARS-CoV-2 anti-spike antibody levels in seroconverted LT recipients. Data is presented as box plots with individual values. The cut-off of 33.8 BAU/ml is considered reactive following the manufacturer's instructions (solid black line), levels >68.3 BAU/ml indicated a cut-off for adequate response based on other studies for the vector based vaccine (dotted black line) and levels >300 BAU/ml indicated a cut-off for adequate response based on other studies for the mRNA vaccines (dashed black line). Differences tested with Kruskal-wallis and wilcoxon signed rank test.

Immunosuppressive blood levels and antibody response to SARS-CoV-2 vaccination

Figure 2A shows the IgG SARS-CoV-2 anti-spike antibody response according to the type of vaccine and stratified by the use of MMF. LT recipients receiving the mRNA-1273 vaccine and using MMF had significantly lower IgG SARS-CoV-2 anti-spike antibody levels, p <0.001. The use of MMF reduced the median level of IgG SARS-CoV-2 anti-spike antibodies for all vaccines to below the manufacturer's cut-off for considering reactive. We did not find any significant associations between the other used immunosuppressive agents and immunogenicity.

Figure 2B and 2C show the IgG SARS-CoV-2 anti-spike antibodies versus TAC and MPA trough levels including expected values from multivariable linear regression models (see Supplementary Table 1). The median IgG SARS-CoV-2 antibody levels over the TAC trough concentration range were 1090 BAU/ml and TAC trough levels were not associated with an effect on the immunogenicity. The median IgG SARS-CoV-2 antibody levels over the MPA trough concentration range were below the cut-off considered reactive by the manufacturer. MPA trough levels were significantly associated with lower immunogenicity of SARS-CoV-2 vaccination. Overall, IgG SARS-CoV-2 antibody levels were low for recipients using MMF and even for MPA trough levels of ≤1 mg/L.

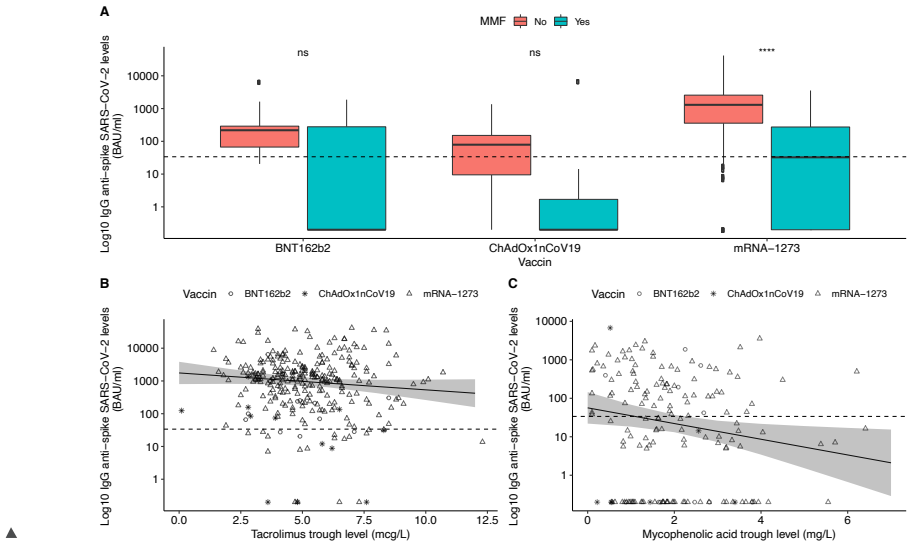

Figure 2. Immunosuppressive blood levels and antibody response to SARS-CoV-2 vaccination

A) Log10-transformed IgG SARS-CoV-2 anti-spike antibody according to the type of vaccine and stratified by the use of MMF (mycophenolate mofetil). The cut-off of 33.8 BAU/ml is considered reactive following the manufacturer's instructions (dashed black line). Differences tested with the Mann-Whitney-U test.

B) Log10-transformed IgG SARS-CoV-2 anti-spike antibodies versus tacrolimus trough levels (mcg/L) in LT recipients vaccinated with a mRNA vaccine. The cut-off of 33.8 BAU/ml is considered reactive following the manufacturer's instructions (dashed black line). The solid black line and shaded areas indicate the expected values and corresponding 95% confidence intervals from a multiple linear regression model including recipient age, kidney function, years between transplantation, type of vaccination as well as the interaction between kidney function and recipient age. The multiple linear regression model was fitted on a total of 275 trough levels of LT recipients receiving solely tacrolimus. The expected values were calculated while fixing the values of all other covariates to the median or reference category.
C) Log10-transformed IgG SARS-CoV-2 anti-spike antibodies versus mycophenolic acid trough levels (mg/L) in LT recipients vaccinated with a mRNA vaccine. The cut-off of 33.8 BAU/ml is considered reactive following the manufacturer's instructions (dashed black line). The solid black line and shaded areas indicate the expected values and corresponding 95% confidence intervals from a multiple linear regression model including recipient age, kidney function, years between transplantation, type of vaccination as well as the interaction between kidney function and recipient age. The multiple linear regression model was fitted on a total of 162 trough levels of LT recipients receiving mycophenolic acid. The expected values were calculated while fixing the values of all other covariates to the median or reference category.

Discussion

This is the first study describing the effect of immunosuppressive trough levels on the IgG SARS-CoV-2 anti-spike antibody response after SARS-CoV-2 vaccination in SOT recipients. The overall efficacy of SARS-CoV-2 vaccination is 79% in our LT recipient cohort. We show a significantly superior IgG SARS-CoV-2 anti-spike antibody response on the mRNA-1273 vaccine compared to the BNT162b2 mRNA vaccine and the ChAdOx1 nCoV19 vector vaccine. The use of MMF, regardless the trough level, is associated with a very poor IgG SARS-CoV-2 anti-spike antibodies response to SARS-CoV-2 vaccination, whereas the other immunosuppressive agents did not have that effect.

Our results are in line with the findings of two studies.(11, 16) Prendecki *et al.* showed a significantly higher IgG SARS-CoV-2 anti-spike antibody response on the BNT162b2 mRNA vaccine compared to the ChAdOx1 nCoV19 vector vaccine in kidney transplant recipients.(11) Thuluvath *et al.* showed that LT recipients and patient with a chronic liver diseases had a poor IgG SARS-CoV-2 anti-spike antibody response on a vector vaccine compared to mRNA vaccines.(16) A possible explanation for the difference in IgG SARS-CoV-2 anti-spike antibody response on mRNA vaccines is the fact that the BNT162b2 mRNA vaccine contains 30 mcg of SARS-CoV-2 mRNA in one dose of 0.3 mL (100 mcg/ml) and the mRNA-1273 vaccine contains 100 mcg SARS-CoV-2 mRNA in one dose of 0.5 mL (200 mcg/ml). Richards *et al.* compared the antibody response in healthy volunteers and showed that the BNT162b2 mRNA vaccine elicited relatively lower antibody levels compared to mRNA-1273.(14)

We found a high antibody response on the SARS-CoV-2 vaccination in our LT recipients, with 80.2% of the LT recipients vaccinated with the mRNA-1273 vaccine having a positive serology test. Our results are superior to many other studies with regards to the immunogenicity of SARS-CoV-2 vaccinations in SOT recipients.(9-13, 15, 16, 18, 27) Until now, all these studies show a low antibody response of 30 – 65% and have in common that > 50% of the transplant recipients used MMF.

MMF is one of the main immunosuppressive agents in SOT recipients and mostly used in combination with tacrolimus. Furthermore, MMF is also being used in other autoimmune disorders. MMF is the prodrug of MPA, an inhibitor of inosine-50-monophosphate dehydrogenase. MPA depletes guanosine nucleotides preferentially in T and B lymphocytes and inhibits their proliferation, thereby suppressing cell-mediated immune responses and antibody formation.[28] Tacrolimus, corticosteroids and mTOR-inhibitors deplete only the T lymphocytes and indirectly the B lymphocytes.(29-31)

Focusing on the mechanism of action and drug levels of the immunosuppressive agents is essential in the ongoing debate to select the right target population for additional vaccinations and to define the right moment for a booster vaccination. Several studies in patients with inflammatory bowel disease, rheumatoid arthritis or other immune-mediated inflammatory disease showed altered antibody responses to SARS-CoV-2 vaccinations in relation to the immunosuppressive drugs used.(32-35) TNFα-inhibitors and IL-receptor antagonists showing no effect on the B lymphocytes, whereas JAK inhibitors, rituximab and the antimetabolite methotrexate showing a significant effect on the differentiation of human B lymphocytes resulting in lower response rates to the SARS-CoV-2 vaccinations.(36-38) It has been shown that the antimetabolite azathioprine

has a weak and non-significant effect on human B lymphocytes and thereby does not affect the response rate to SARS-CoV-2 vaccinations.(24, 39)

As is demonstrated by our results, MMF suppressed the IgG SARS-CoV-2 anti-spike antibody response to below or near the defined cut-off for an adequate response, regardless the trough levels of MMF. Consequently, the use of MMF results in the low immunogenicity in SOT studies evaluating the SARS-CoV-2 vaccines so far. Moreover, the use of MMF is also related to a more severe SARS-CoV-2 infection in unvaccinated LT recipients.(40) Furthermore, it has been shown that the antibody response to the influenza vaccination is modulated by MMF. High doses of MMF alter the T-Helper 2 and B-Cell responses and causing lower seroconversion rates to influenza vaccination.(41) It should be kept in mind that the duration of inhibitory effects of immunosuppressive agents on the T and B lymphocytes differ per agent with for example a single dose of rituximab resulting in a long-lasting B-cell depletion of over 6 to 12 months. Since the effect of MMF on the T and B lymphocytes is reversible, we suggest to discontinue MMF in SOT recipients for at least 6 weeks prior to and after vaccination based on the pharmacodynamical effect of MMF on B lymphocytes as shown by Ganschow *et al.*.(42)

An important limitation of this study is the fact that this is not a randomized trial. Furthermore, we did not evaluate the effect on the T-cell response. However, based on several other studies performed in SOT recipients we expect this to be lower in LT recipients.(11, 24) Lastly, the LT recipients receiving the BNT162b2 mRNA vaccine and the ChAdOx1 nCoV19 vector vaccine have a higher median age compared to the mRNA-1273 vaccine. This is because in the Netherlands, at the beginning of the SARS-CoV-2 vaccination period people > 60 year were the first to be invited due to the availability of the vaccines. Several studies show that a higher age results in a weaker antibody response. However, we believe that the difference in age did just marginally affect our results on the IgG SARS-CoV-2 anti-spike antibody response and is counteracted by the fact that our LT recipients in the mRNA-1273 vaccine group had a shorter period between the transplantation and the vaccination.

In conclusion, SARS-CoV-2 vaccination was highly effective in our cohort with 79% of the LT recipients seroconverted after two vaccinations. The mRNA-1273 vaccine produces a significantly superior IgG SARS-CoV-2 anti-spike antibody response compared to the BNT162b2 mRNA vaccine and the ChAdOx1 nCoV19 vector vaccine. Focusing on the mechanism of action and drug levels of the immunosuppressive agents is essential in the ongoing debate to select the right target population for additional vaccinations and to define the right moment for a booster vaccination. The use of MMF, regardless the trough level, is associated with a very poor IgG SARS-CoV-2 anti-spike antibodies response to SARS-CoV-2 vaccination. As a consequence, lowering the dose of MMF has no beneficial effect. We suggest discontinuing and restarting MPA for at least 6 weeks prior to and after vaccination for every patient on MMF therapy and avoid other agents affecting the B lymphocytes in immunocompromised patients.

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	Model TAC (n=275)	95%-CI	p-value	Model MPA (n=162)	95%-CI	p-value
Constant	2.87	0.57 – 5.18	0.015*	3.85	-0.17 – 7.86	0.061
MPA trough level, mg/L	-	-	-	-0.20	-0.37 – -0.035	0.019*
TAC trough level, mcg/L	-0.051	-0.11 – 0.0095	0.097	-	-	-
Recipient age, years	-0.021	-0.056 – 0.014	0.23	-0.066	-0.13 – -0.0045	0.035*
Kidney function, ml/min/1.73m^2	-0.0024	-0.029 – 0.025	0.86	-0.044	-0.098 – 0.011	0.12
Time between vaccination and transplantation, years	0.019	0.0039 – 0.035	0.014*	0.051	0.023 – 0.079	<0.001*
Type of vaccination						
ChAdOx1nCoV19	-1.06	-1.74 – -0.38	0.0025*	-0.75	-2.04 – 0.54	0.25
mRNA-1273	0.67	0.17 – 1.17	0.0088*	0.34	-0.49 – 1.19	0.42
Interaction between kidney function and age	0.00023	-0.00022 – 0.00069	0.32	0.0011	0.00016 – 0.0019	0.021*

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Supplementary table 1. Multivariable linear regression results

Abbreviations: CI, confidence interval; TAC, tacrolimus; MPA, mycophenolic acid; *indicates statistical significance

Two multivariable linear regression models were fitted, investigating the association between the antibody response on SARS-CoV-2 vaccination and the immunosuppressive trough levels of MPA or tacrolimus in the subset of patients who received the specific immunosuppressive agent. A total of 275 trough levels of LT recipients receiving solely tacrolimus and a total of 162 trough levels of LT recipients receiving mycophenolic acid were included in the models. Variables included in the model were independently associated with an effect on the IgG SARS-CoV-2 anti-spike antibody response. To take into account that the IgG SARS-CoV-2 anti-spike antibody response may not only be independently associated with kidney function and the recipient age, but that the effect of kidney function may change across ages, the model included the product (=interaction) of the kidney function and recipient age as independent variable.

Chapter 10

Positive antibody response in liver transplant recipients on mycophenolate mofetil after the third, fourth and fifth SARS-CoV-2 vaccination; an observational cohort study.

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**Authors contributed equally*

To be sumbitted

Abstract

Background

The negative influence of mycophenolate mofetil (MMF) on the immunogenicity in LT recipients raised the question whether the dosage of the drug should be altered during following SARS-CoV-2 vaccination. This study aimed to investigate the immunogenicity in LT recipients in relation to mycophenolic acid (MPA; the active substance of MMF) blood levels after a third, fourth or fifth mRNA vaccination.

Methods

In this observational, cohort study, we determined the immunogenicity to SARS-CoV-2 vaccination in liver transplant (LT) recipients in relation to the MPA blood levels after the 3th, 4th and 5th dose of mRNA vaccines. Multiple linear regression models were fitted, investigating the association between the antibody response on SARS-CoV-2 and the MPA trough levels for the vaccinations.

Results

In total, 86 LT recipients were included with 92 IgG anti-spike SARS-CoV-2 titers; six patients had titers available after multiple vaccinations. Significantly more LT recipients had positive IgG SARS-CoV-2 serology after the third vaccination (41/48, 85.4%) compared to the second vaccination (20/48, 41.7%), p<0.001. This increased to 90% after the fourth and fifth vaccination. MPA trough levels were not significantly associated with an effect on the IgG SARS-CoV-2 anti-spike antibodies response after a third, fourth or fifth vaccination.

Conclusion

Additional SARS-CoV-2 vaccination was highly effective in our cohort with seroconversion in 85.4% of the LT recipients using MMF after three vaccinations. Regardless the MPA trough levels, LT recipients using MMF show positive IgG anti-spike SARS-CoV-2 levels after additional vaccination. MMF could be continued during additional vaccination.

Introduction

Solid organ transplant (SOT) recipients have an increased risk of a complicated course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection due to comorbidities and the use of immunosuppressive drugs.(1, 2) Over the last year, most SOT recipients in the Netherlands received multiple doses of a SARS-CoV-2 vaccine (BNT162b2 mRNA vaccine, mRNA-1273 vaccine or the ChAdOx1 nCoV19 vector vaccine) to induce an immunogenic response.

Various studies have revealed an attenuated humoral immune response in SOT recipients after two-doses mRNA vaccination in comparison to healthy controls.(3-5) Factors associated with this lower immunogenicity include older age, impaired renal function and a short time between transplantation and vaccination.(4, 6-8) An additional predictor for a negative antibody response is a drug regimen that includes mycophenolate mofetil (MMF). A recent publication by Alejo *et al.* introduced a machine learning algorithm that designated MMF use in SOT recipients as a strong predictor for a negative antibody response.(9) Clinical data confirmed these algorithm results for liver transplant (LT) recipients. Patients that used MMF showed lower seroconversion rates after

two or even three mRNA vaccinations compared to patients who did not use MMF.(6, 7, 10)

At the beginning of 2022, we showed that MMF influences the immunogenicity of LT recipients after two vaccinations, regardless of the mycophenolic acid (MPA; the active substance of MMF) trough levels.(11) Therefore, the negative influence of MMF on the immunogenicity in LT recipients raised the question whether the dosage of the drug should be altered or even temporarily halted during following SARS-CoV-2 vaccination. So far, the effects of MPA trough levels on the immunogenicity after following vaccinations remains unknown. This study aimed to investigate the immunogenicity in LT recipients in relation to MPA blood levels after a third, fourth or fifth mRNA vaccination.

Materials and methods

Study design and patients

This observational study was conducted between October 2021 and July 2022 in a cohort of liver transplant recipients using MMF, treated and monitored at our center. Included was every adult LT recipient using MMF with available IgG SARS-CoV-2 anti-spike antibodies measurements, MPA trough levels, history of SARS-CoV-2 infection and vaccination data (date and type of vaccine). Excluded were patients that died and participated in a SARS-CoV-2 antibody-related trial.

This study was approved by the Medical Ethics Committee of the Erasmus MC University Medical Center Rotterdam, the Netherlands (MEC-2021-0810). Patients were not involved in the design, conduct and dissemination plans of this research. The data collected in the database were anonymized. Subject identification codes were used for included patients for the storage and handling of the data.

Laboratory tests

To measure the humoral immune responses to vaccination, a quantitative assay directed against the SARS-CoV-2 Spike (S) antigen (Liaison SARS CoV-2 TrimericS IgG assay, DiaSorin, Italy) was used. The lower limit of detection for this test was 4.81 BAU/mL.(12) The tests were performed according to the manufacturer's recommendation and values >33.8 BAU/mL were considered reactive. To analyze the MPA plasma concentrations, validated ultra-high-performance liquid chromatography-tandem mass spectrometry (U-HPLC-MS/MS) methods were used. Trough levels below the lower limit of quantification were included in the lower limit of quantification.

Data collection

Socio-demographic, clinical and transplant parameters were extracted from patient's electronic medical records in the hospital information system. The following parameters were collected from the patients' electronic medical records: gender, age, ethnicity, renal function (the estimated glomerular filtration rate, eGFR, was calculated using the CKD-EPI equation), the reason for and the date of the liver transplantation, immunosuppressive drug regimens, immunosuppressive trough levels and IgG anti-spike SARS-CoV-2 levels. The history of SARS-CoV-2 infection, the date of vaccination and the type of vaccination were collected by interviewing the LT recipients.

Statistical analysis

Patient characteristics are summarized using numbers (%) for nominal and ordinal variables. Depending on the shape of the distribution, the continuous variables are summarized by mean (SD) or median (inter-quartile range, IQR). Differences in patient characteristics were tested using the Chi-square test for categorical variables and the Mann-Whitney-U or Kruskall-Wallis test for continuous variables. The difference in LT recipients with positive IgG SARS-CoV-2 serology after the second and third vaccination was tested using the Chi-square test.

A total of three multiple linear regression models were fitted, investigating the association between the antibody response on SARS-CoV-2 and the MPA trough levels for the third, fourth and fifth vaccination. All three models included covariates shown to be relevant in previous studies: age at first vaccination, renal function, the time between the transplantation and the first vaccination as well as the interaction between renal function and age. To visualize the estimated associations, the expected antibody response across the range of trough levels was calculated while fixing the values of all other covariates to the median or reference category.

For all statistical tests, a p-value of <0.05 was considered to indicate statistical significance. All data were collected in a data extraction file and analyses were performed using R software (version 3.6.2).(13)

Results

Study population

A total 86 LT recipients were included having 92 IgG anti-spike SARS-CoV-2 titers available. In total, six LT recipients had IgG anti-spike SARS-CoV-2 titers available for analysis after multiple vaccinations; two LT recipients had titers available after the third and the fourth vaccination, three LT recipients had titers available after the third and the fifth vaccination and one LT recipient had titers available after the fourth and the fifth vaccination. Out of the 86 LT recipients, 14 LT recipients had developed a SARS-CoV-2 infection prior to IgG anti-spike SARS-CoV-2 levels measurement (figure 1).

The demographical and clinical characteristics of the patient population are presented in table 1. The majority of the LT recipients used MMF in combination with tacrolimus. The three most frequent indications for transplantation were hepatocellular carcinoma, primary sclerosing cholangitis and (non)-alcoholic steatohepatitis. Median MPA trough levels after third, fourth or fifth vaccination were between 1.1 – 1.8 mg/L.

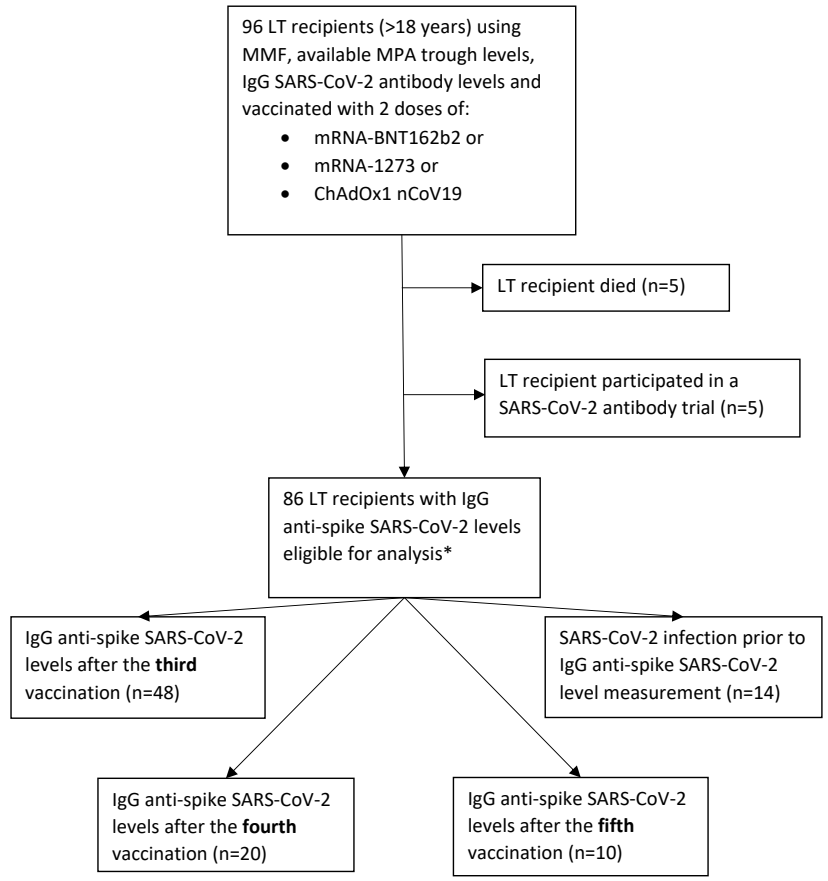

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Figure 1. Flow chart of study inclusion and exclusion criteria.

*six LT recipients had IgG anti-spike SARS-CoV-2 titers available for analysis after multiple vaccinations; two LT recipients had titers available after the third and the fourth vaccination, three LT recipients had titers available after the third and the fifth vaccination and one LT recipient had titers available after the fourth and the fifth vaccination.

	SARS-CoV-2 infection naïve (n=72)	SARS-CoV-2 infection recovered (n=14)	p-value
R-sex = Male (n,%)	40 (55.6)	8 (57.1)	1¶
Age at first vaccination (median [IQR])	64.00 [57.00 - 69.00]	57.50 [56.00 - 67.50]	0.242§
eGFR, ml/min/1.73m^2 (mean (SD))	56.26 (16.03)	61.93 (11.45)	0.212§
Ethnicity (n,%)			0.095¶
Afro-American	2 (2.8)	2 (14.3)	
Asian	7 (9.7)	0 (0.0)	
Caucasian	63 (87.5)	12 (85.7)	
Primary disease for transplantation (n,%)			0.439¶
(N)ASH	11 (15.3)	1 (7.1)	
ALF	6 (8.3)	-	
Cryptogenic	4 (5.6)	1 (7.1)	
HCC	13 (18.1)	5 (35.7)	
Metabolic disease	3 (4.2)	-	
Other cholestatic disease†	4 (5.6)	-	
PSC	12 (16.7)	3 (21.4)	
Retransplantation	6 (8.3)	-	
Viral hepatitis	4 (5.6)	-	
Other‡	9 (12.5)	4 (28.6)	
Immunosuppressive drug therapy (n,%)			
Monotherapy MPA	16 (22.2)	1 (7.1)	0.378¶
Duo therapy:			0.239¶
MPA + CICLO	3 (4.2)	-	
MPA + EVR	2 (2.8)	-	
MPA + CORT	-	1 (7.1)	
MPA + SRL	2 (2.8)	-	
MPA + TAC	47 (65.2)	12 (85.7)	
Triple therapy TAC + MPA + CORT	2 (2.8)	-	1¶
Intervals (median [IQR])			
Transplantation and 1st vaccination, years	6.00 [3.00 - 14.00]	4.50 [3.25 - 7.00]	0.253§
Time between vaccination and antibody titer measurement, days			
After third vaccination	60.00 [32.25 - 85.00]	105.00 [77.00 - 135.00]	0.032§*
After fourth vaccination	33.00 [11.50 - 90.75]	47.00 [45.00 - 48.00]	0.11§
After fifth vaccination	18.00 [10.00 - 28.75]	17.50 [13.25 - 21.75]	0.667§
Time between vaccinations, days			
Second and third vaccination	189.50 [183.25 - 206.75]	188.00 [184.25 - 209.50]	0.710§
Third and fourth vaccination	95.00 [93.00 - 107.00]	94.00 [93.00 - 98.00]	0.949§
Fourth and fifth vaccination	100.00 [94.25, 114.50]	133.00 [123.00, 139.00]	0.004§*
Mycophenolic acid trough levels, mg/L (median [IQR])			
After third vaccination	1.50 [0.97, 2.25]	1.25 [0.79, 1.52]	0.29§
After fourth vaccination	1.75 [1.06, 3.25]	1.44 [1.28, 1.77]	0.497§
After fifth vaccination	1.40 [0.68, 2.00]	1.17 [1.00, 1.33]	1§
SARS-CoV-2 antibody titers, BAU/mL (median [IQR)			
After third vaccination	1270.00 [161.25 - 2335.00]	7220.00 [2850.00 - 17100.00]	0.002§*
After fourth vaccination	2640.00 [585.00 - 6812.50]	5790.00 [34.30 - 16400.00]	0.838§
After fifth vaccination	1439.00 [201.65 - 8877.50]	52410.00 [29815.00 - 75005.00]	0.133§

▲

Table 1. Demographic and clinical characteristics.

Abbreviations: PSC, primary sclerosing cholangitis; HCC, hepatocellular carcinoma; ALF, acute liver failure; (N)ASH, (non)alcoholic steatohepatitis; TAC, tacrolimus; MPA, mycophenolic acid; CORT, corticosteroids; SRL, sirolimus; Ciclo, cyclosporine; EVR, everolimus.

†other cholestatic diseases: secondary biliary cirrhosis, primary biliary cirrhosis, congenital biliary diseases;
‡other: hepatopulmonary syndrome, polycystic liver disease, autoimmune hepatitis, vascular liver diseases (budd chiari, veno-occlusive disease);
§p-value based on non-parametric test (Kruskal-Wallis) test;
¶p-value based on Chi-square test
*indicates p-value <0.05

After the third SARS-CoV-2 vaccination the median IgG anti-spike SARS-CoV-2 levels were significantly higher in the group that experienced a SARS-COV-2 infection compared to the SARS-CoV-2 naïve LT recipients. The interval between the third vaccination and IgG anti-spike SARS-CoV-2 levels measurement was significantly longer in the group that experienced a SARS-COV-2 infection compared to the SARS-CoV-2 naïve LT recipients.

SARS-CoV-2 specific antibody response upon SARS-CoV-2 vaccination

Table 2 presents the SARS-CoV-2 specific antibody response upon SARS-CoV-2 vaccination in LT recipients. The majority (>75%) of the LT recipients were vaccinated with the BNT162b2 mRNA vaccine for their third, fourth or fifth vaccination. After the third SARS-CoV-2 vaccination, 85.4% (41/48) of the LT recipients showed a positive IgG SARS-CoV-2 serology. This increased to 90% after the fourth (18/20) and fifth (9/10) vaccination.

Figure 2A shows the increase of IgG anti-spike SARS-CoV-2 levels after the second and third vaccination. Figure 2B shows the percentage of LT recipients with positive IgG SARS-CoV-2 serology after the second and third vaccination. Significantly more LT recipients had positive IgG SARS-CoV-2 serology after the third vaccination (41/48, 85.4%) compared to the second vaccination (20/48, 41.7%), p<0.001.

	Total number of IgG SARS-CoV-2 titers positive†	IgG anti-spike SARS-CoV-2 levels (median [IQR])
After third vaccination (n=48) (n, %)	41 (85.4)	1270.00 [161.25, 2335.00]
After fourth vaccination (n=20) (n, %)	18 (90.0)	2640.00 [585.00, 6812.50]
After fifth vaccination (n=10) (n, %)	9 (90.0)	1439.00 [201.65, 8877.50]

▲

Table 2. Presence of S-specific antibodies after a third, fourth or fifth SARS-CoV-2 vaccination in LT recipients.

†The cut-off of 33.8 BAU/mL is considered reactive following the manufacturer's instructions.

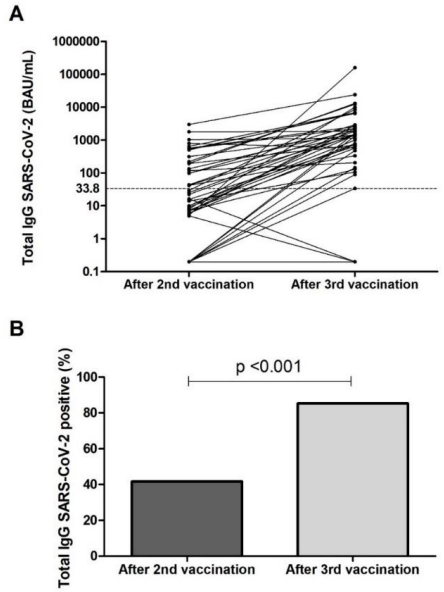

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Figure 2. Difference in the presence of S-specific antibodies after second and third SARS-CoV-2 vaccination in LT recipients.

(A) Total IgG SARS-CoV-2 antibody measurement after the second and third SARS-CoV-2 vaccination in the same patient cohort (n=48). The cut-off of 33.8 BAU/mL is considered reactive following the manufacturer's instructions. (B) Percentage of total IgG SARS-CoV-2 positive patients after the second (20/48, 41.7%) and third (41/48, 85.4%) SARS-CoV-2 vaccination. The difference was tested using the Chi-square test.

Mycophenolic acid blood levels and SARS-CoV-2 antibody response upon vaccination

Figure 3 illustrates the IgG anti-spike SARS-CoV-2 levels after the third, fourth or fifth vaccination versus the MPA trough levels stratified for the type of vaccine. The multiple linear regression models were fitted on a total of 48 MPA trough levels of LT recipients after the third vaccination, 20 MPA trough levels of LT recipients after the fourth vaccination and 10 MPA trough levels of LT recipients after the fifth vaccination. The results from these models are shown in supplementary table 1. The expected values from the multiple linear regression models were added to the plot (figure 3). For every model, MPA trough levels were not significantly associated with an effect on the IgG SARS-CoV-2 anti-spike antibodies response with a median IgG SARS-CoV-2 anti-spike antibody response of 1270, 2640 and 1439 BAU/ml after the third, fourth and fifth vaccination over the MPA trough concentration range.

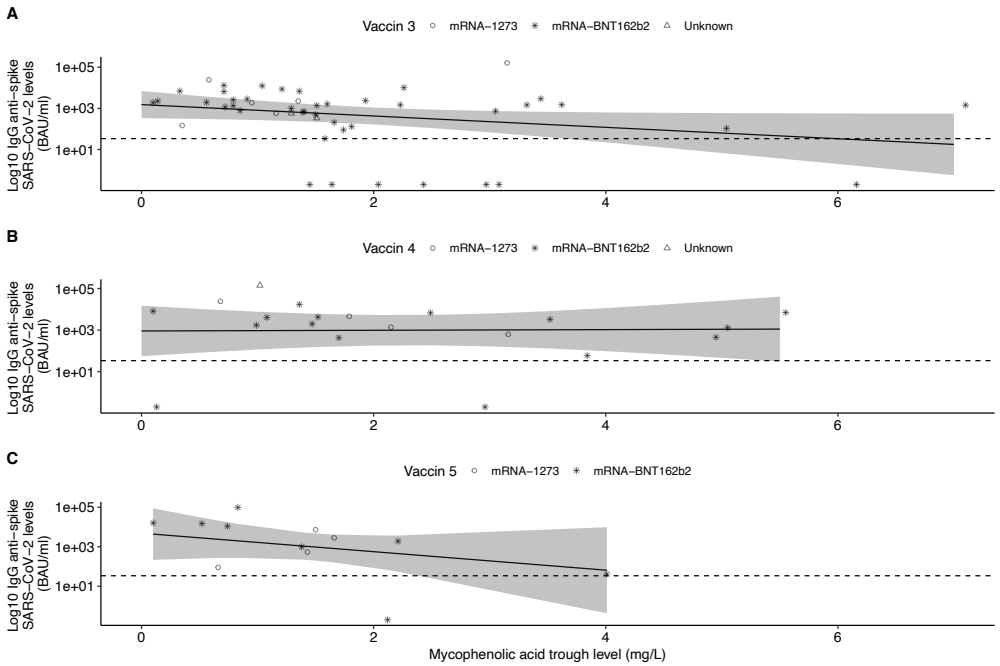

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Figure 3. Immunosuppressive blood levels and antibody response to SARS-CoV-2 vaccination.

Log-transformed IgG SARS-CoV-2 anti-spike antibodies versus mycophenolic acid trough levels after (A) third vaccination (n=48), (B) fourth vaccination (n=20) or (C) fifth vaccination (n=10). The cut-off of 33.8 BAU/mL is considered reactive following the manufacturer's instructions (dotted black line). The solid black line and shaded areas indicate the expected values and corresponding 95% confidence intervals from a multiple linear regression model including recipient age, renal function and years between transplantation and the first SARS-CoV-2 vaccination. The expected values were calculated while fixing the values of all other covariates to the median or reference category.

Discussion

The previously demonstrated association between the use of MMF and low immunogenicity to SARS-CoV-2 vaccination in SOT recipients raised the question whether the MMF dose should be adapted during additional SARS-CoV-2 vaccination.(6, 10, 14) In our cohort of LT recipients we demonstrate that additional SARS-CoV-2 vaccination was highly effective, with seroconversion of 85.4% of the LT recipients using MMF after three vaccinations. Significantly more LT recipients using MMF had positive SARS-CoV-2 serology after the third vaccination compared to the second vaccination.

Our results are in line with several other recent publications on IgG anti-spike SARS-CoV-2 levels after the third SARS-CoV-2 vaccination. Enhanced immunogenicity after the third vaccination compared to the second vaccination has recently been shown in LT recipients.(15, 16) However in both studies, the use of MMF was shown to be a predictor for a negative antibody response. In another recent study in kidney transplant recipients who did not demonstrate seroconversion after their second or third vaccination, increased IgG anti-spike SARS-CoV-2 antibodies were measured after additional vaccination.(17) Interestingly, in this study no difference in the IgG anti-spike SARS-CoV-2 antibodies response was shown between a group that discontinued MMF for 2 weeks around vaccination and a group that continued the use of MMF. So far, up to our knowledge, no study correlated the MPA blood levels in relation to the IgG anti-spike SARS-CoV-2 levels after three or more vaccinations.

The results of our current study are not completely in line with previous findings which we reported.(11) Previously, we showed that the use of MMF, regardless the MPA trough level, was associated with a very poor IgG anti-spike SARS-CoV-2 antibodies response after two SARS-CoV-2 vaccinations. Another study as well showed in a multivariate analysis, that a higher daily dose of MMF was associated with a reduced IgG anti-spike SARS-CoV-2 antibodies response. (6) MPA inhibits both T and B lymphocytes proliferation, thereby suppressing cell-mediated immune responses and antibody formation.(18) However, our current data show the majority of the LT recipients using MMF did become seropositive after additional vaccinations suggesting that the immune response in LT recipients using MMF is delayed and not unresponsive.

The production of IgG anti-spike SARS-CoV-2 antibodies over time in LT recipients using MMF was evaluated by Toniutto *et al*..(6) They measured the IgG anti-spike SARS-CoV-2 antibodies in LT recipients using MMF for a period of up to six months after the second vaccination. More LT recipients became seropositive four and six months after the second vaccination compared to one month after vaccination suggesting a postponed immune response. In this study, the median time between additional vaccination and the IgG anti-spike SARS-CoV-2 antibodies measurement varied between a half and two months. Therefore, the optimal immune response might not have been reached in our population at the time of measuring.

Immunogenicity studies in other SOT recipients showed an enhanced antibody response after the third vaccination.(19-21) Our findings could be extrapolated to other SOT recipients using MMF, since MMF dosage and MPA blood levels do not differ among the SOT recipients.(22) Based on the fact that the use of MMF, regardless the MPA trough level, was not associated with a poor IgG anti-spike SARS-CoV-2 antibody response after additional vaccination we do not recommend stopping or lowering the dose of MMF during additional vaccination. Repeated

vaccinations eventually result in seroconversion in the majority of LT recipients. However, it remains to be determined how often and how many additional vaccinations SOT recipients will need to preserve an adequate humoral response against SARS-CoV-2.

An important limitation of this study is that the incidence of SARS-CoV-2 infections could be underestimated. Asymptomatic infections could have been unnoticed by LT recipients and therefore not reported. However, LT recipients at the Erasmus Medical Center are intensively monitored in the post-transplant care and encouraged to test for SARS-CoV-2. We believe that unreported asymptomatic infections did marginally affect our results. Furthermore, we measured the IgG anti-spike SARS-CoV-2 antibodies at standard scheduled visits resulting in variation in the time between vaccination and antibody measurements.

In conclusion, additional SARS-CoV-2 vaccination was highly effective in our cohort with seroconversion of 85.4% of the LT recipients using MMF after three vaccinations. Regardless the MPA trough levels, we show a positive IgG anti-spike SARS-CoV-2 levels in LT recipients using MMF after additional vaccination. MMF could be continued during additional vaccination.

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Part IV

Addition of a clinical pharmacist in the liver transplant care

Chapter 11

Medication-Related Problems in liver transplant recipients in the outpatient setting: a Dutch cohort study.

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Abstract

Background

After liver transplantation (LTx), adherence to immunosuppressive medication and avoidance of contra-indicated drugs is essential for long-term survival. This study aimed to investigate the prevalence, types and severity of medication-related problems (MRPs) and interventions initiated by a clinical pharmacist (CP) in a cohort of LTx recipients in the outpatient setting.

Method

This study was a retrospective, observational study in LTx recipients that visited the outpatient clinic for an annual check-up. A 20-minutes consultation with a CP consisted of medication reconciliation and consultation about medication, adherence, and adverse drug reactions (ADRs). Discrepancies between actual and intended drug use, and MRPs were identified and the severity of MRPs was assessed. Potential interventions were discussed with the patient and the treating physician and evaluated after one year.

Results

The CP counseled 64 LTx recipients and found 96 discrepancies in 37 patients. Most discrepancies (60.4%, n=58) concerned missing medications.
In total, 98 MRPs were identified in 53 patients (median 2; range 1-5 per patient), with a total of 113 interventions. Most frequent MRPs were: ADRs (22.4%, n=22), nonadherence (19.3%, n=19), unnecessary drugs (16.3%, n=16) and undertreatment (12.2%, n=12). Interventions most frequently proposed included optimization of dosage regimen (21.2%, n=24), individualized recommendation regarding compliance (16.8%, n=19) and drug discontinuation (12.4%, n=14). After one year, 15 of the 19 patients (79%) experienced no longer compliance issues and 27 of the 29 patients (93%) used no drugs with indication issues anymore.

Conclusion

The CP in an outpatient monitoring program for LTx recipients can signal relevant discrepancies and MRPs. This leads to interventions that are accepted by both the patients and the physicians, with a positive effect after one year.

Introduction

Liver transplantation (LTx) is the preferred treatment in patients with end-stage liver disease and hepatocellular carcinoma with 1-year patient survival exceeding 80%. However, survival rates gradually decline over time with 5-year and 10-year patient survival rates of respectively 71 % and 61 %.(1) Adherence to immunosuppressive medication and avoidance of contra-indicated drugs are two potential modifiable risk factors to improve long-term outcome.(2) In addition, due to the development of comorbidities, LTx recipients will usually end up with multiple drugs over the years. Over 30 years of experience, we learned that medication errors contribute to a substantial number of unplanned hospitalizations.(3, 4) In the Netherlands, the Hospital Admissions Related to Medication (HARM) study showed that 5.6% of all unplanned hospitalizations are drug related and that 46% of these were potentially preventable.(5) Therefore, identification and management of medication-related problems (MRPs) opens opportunities to improve medication safety. Several studies have shown that a medication review might contribute to the detection, prevention

and management of MRPs in all sorts of settings.(6, 7)

MRPs are defined as events or circumstances involving drug therapy that actually or potentially interferes with desired health outcomes.(8) Examples of MRPs are adverse drug reactions, drug interactions, nonadherence, unnecessary drug use and untreated indications.
In North-America clinical pharmacists (CP) have been involved in the direct patient care in transplantation since the early 1970s.(9) In the Netherlands, pharmacists working in the hospital as CPs are more recently starting to be involved in the direct care for hospitalized patients.(10) Only a few CPs are involved in the out-patient care as well. As far as we know, no CP has been structurally involved in the out-patient care of liver transplant recipients in the Netherlands.

Taber *et al.* showed that MRPs and adverse drug events commonly occur in kidney transplant recipients resulting in higher rates of acute rejection and lower graft survival rates.(11) Despite the fact that LTx recipients take comparable drugs as kidney transplant recipients, so far no study describes the prevalence and types of MRPs in LTx recipients and the impact of interventions initiated by a CP in this population. By investigating MRPs in LTx recipients more information about MRPs in the transplantation population becomes available resulting in more awareness, possibly earlier detection of MRPs and better prevention strategies.

This study aimed to investigate the prevalence and types of MRPs in a cohort of liver transplant recipients in the outpatient setting in one of the three liver transplant centers of the Netherlands. The secondary objectives were to investigate the severity of the MRPs and the type and impact of interventions initiated by a CP to improve medication use.

Method

Ethics approval

This study was a retrospective, observational study conducted between September – December 2018 at the Erasmus MC University Medical Center Rotterdam, the Netherlands and was approved by the Medical Ethics Committee of the Erasmus University Medical Center (MEC-2019-0784).

Study design

Since 1986, 1271 liver transplantations have been performed in 1116 adult patients at the Erasmus University Medical Center, Rotterdam, the Netherlands. Currently, 713 liver transplantation (LTx) recipients are still alive and 671 are seen at least annually at the outpatient clinic. The other 42 recipients are lost to follow-up. Adult LTx recipients were eligible if they were scheduled for an annual, multidisciplinary medical check-up at the outpatient clinic. During this annual medical check-up, the recipient is seen by a hepatologist or specialized nurse practitioner and a social worker. Since hospital pharmacists have knowledge and experience regarding the pharmacotherapy and comorbidities of LTx recipients, a CP was added to the LTx program of the Erasmus University Medical Center in September 2018 as part of integrated patient care. A newly established 20-minute face-to-face consultation with the CP was added to the annual check-up. Patients were asked to bring their own medication and a list of prescriptions as registered by their community pharmacy. The consultation consisted of medication reconciliation and a conversation about medication, adherence, adverse drug reactions (ADRs) and drug use. Potential

interventions were discussed with the patient and the hepatologist and after consensus initiated by the CP. In total, the CP spent approximately 45 minutes per patient for the preparation of the consultation, the consultation with the patient and the evaluation afterwards with the LTx team. All findings were registered in the patients' electronic medical records for further follow-up by the hepatologist or the CP. The findings of the annual check-up were reported to the primary care physician by the hepatology department. The consulting pharmacist, MBM, completed a special training on the treatment of LTx patients through courses and a mentorship with a transplant hepatologist. One year after the first consultation, all MRPs and proposed interventions per patient were evaluated by the CP in the annual check-up to evaluate the clinical impact of the outpatient monitoring program.

Patients

Adult LTx recipients scheduled for an annual, multidisciplinary medical check-up at the outpatient clinic between September – December 2018 were included in this analysis (figure 1).

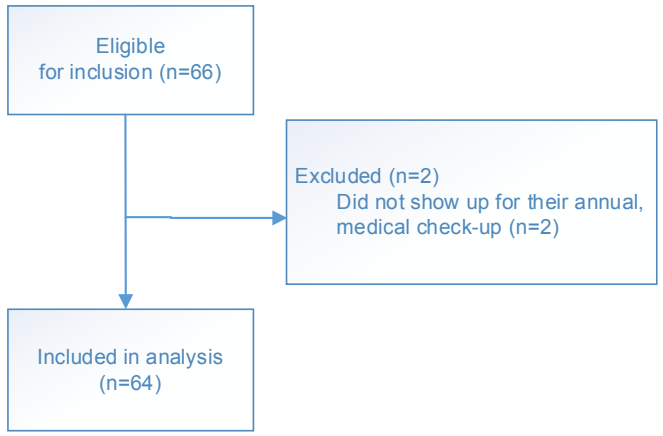

Figure 1. Flowchart of study

Data collection

Socio-demographic and clinical parameters were extracted from patients' electronic medical records in the hospital information system. The following information was collected from patients' electronic medical records: gender, age, presence of comorbidities, reason for and date of the transplantation, information about re-transplantation, medication according to the patients' electronic medical records in the hospital and according to the list of prescriptions distributed by their community pharmacy.

During the face-to-face consultation, the CP retrieved information about drug use reported by the patient, adherence, adverse drug reactions, untreated conditions, problems with medication use and proposed interventions. All information was registered in a data extraction file. Next, MRPs were identified by reviewing all information documented by the CP. No additional information from the patients' electronic medical record was necessary for the assessment of the MRPs.

Assessment of MRPs

The registered information was categorized into predefined categories of MRPs. These categories were based on the classification of the Pharmaceutical Care Network Europe (PCNE) Classification V 9.0. and the classification used by Hayward *et al.* that was applied in patients with cirrhosis (8, 12).

Each identified MRP was categorized and for one MRP, several interventions could have been proposed. All MRPs and interventions were independently categorized by two pharmacists (MBM and SDB). Next, they compared their classifications and when dissensus existed, the panel members reviewed their own classifications and discussed these until consensus was reached.

Assessment of the severity of the MRPs

The severity of the MRPs was assessed with the National Coordinating Council for Medication Error Reporting and Prevention (NCC MERP) index(13). This classification is widely used and categorizes medication errors (for example MRPs) into nine categories (A – I) based on the severity of the related patient outcomes. The first categories (A – D) are associated with errors that caused no or potential harm to the patient. Categories (E – I) are associated with errors that caused harm or even death to the patient.

Each identified MRP was categorized according to the NCC MERP index. The rating was based on the potential impact of the detected MRP on the patient's health status. Several common MRPs were rated in a standardized way: (1) nonadherence, (2) experience complexity in dosage regimen and (3) ADRs in category D (error caused potential harm to patients); (4) use of anticoagulants without indication and (5) use of contraindicated drugs in category E (error caused temporary harm and required intervention). All MRPs were independently categorized by two pharmacists (MBM and NH). Next, they compared their classifications and when dissensus existed, the panel members reviewed their own classifications and discussed these until consensus was reached.

Statistical analysis

No formal sample size calculation was performed. We included all patients in the analysis who received a consultation with the CP during their annual, multidisciplinary medical check-up at the outpatient clinic.

Variables were described with descriptive statistics: n (%) for nominal and ordinal variables and median (inter-quartile range, IQR) for the continuous variables. Statistical software, SPSS for Windows, version 24 (SPSS, Chicago, IL), was used for the analysis. No statistical tests were performed.

Results

The CP counseled 64 patients with a median age of 59.5 years (IQR: 47–66) and a median of seven medications (IQR: 5–8). The most prevalent indication for LTx was cirrhosis. Frequent comorbidities were chronic kidney disease (n=26), cardiovascular disease (n=26), and diabetes mellitus (n=19); 11 patients had no comorbidities. Table 1 presents the clinical and demographical characteristics of the cohort.

	Patients (n = 64)
Age, years (median, IQR)	59.5 (47–66)
Gender	
Male (n, %)	37 (57.8%)
Indication liver transplantation	
Cirrhosis[a]	30 (46.9%)
Hepatitis B Virus	11 (17.2%)
Hepatocellular carcinoma	9 (14.1%)
Acute Liver Failure	7 (10.9%)
Hepatitis C Virus	7 (10.9%)
Other[b]	11 (17.2%)
Time after transplantation, years (median, IQR)	8 (3.5–12.5)
Re-transplantation	
No	59 (92.2%)
Yes	5 (7.8%)
Presence of a comorbidity[c]	
Cardiovascular Disease	26 (40.6%)
Chronic Kidney Disease[d]	26 (40.6%)
Diabetes Mellitus	19 (29.7%)
None	11 (17.2%)
Gastrointestinal	8 (12.5%)
Other[e]	17 (26.6%)
Number of drugs on medication list during consultation (median, IQR)	7 (5–8)

▲

Table 1. Clinical and demographical characteristics

IQR, inter-quartile range

[a] Cirrhosis was caused by Primary Sclerosing Cholangitis (n=17), alcohol abuse (n=3), nonalcoholic steatohepatitis (n=2), Primary Biliary Cholangitis (n=1) and cryptogenic cirrhosis (n=7).
[b] Other includes: Autosomal Dominant Polycystic Kidney Disease (n=3), Alpha-1 Antitrypsin Deficiency (n=2), Hemochromatosis (n=2), Hepatitis D Virus, Budd Chiari, hepatopulmonary syndrome, Wilson's disease
[c] Comorbidity: Every comorbidity is counted separately
[d] Chronic Kidney Disease is defined according to the KDIGO guidelines(18)
[e] Other includes: neurological (n=5), haematological (n=3), dermatological (n=2), thyroid disorders (n=2), psychiatric (n=2), immunological (n=1), pulmonary (n=1) and rheumatological comorbidities (n=1).

Medication discrepancies during consultation

Table 2 presents an overview of the medication discrepancies during consultation. In 37 patients (57.8%), one or more discrepancies were found in the medications registered in the hospital information system and the ones actually used by the patient. Most discrepancies (60.4%) involved missing medications (i.e. medications used by the patient but not registered in the chart). For example, medicines prescribed by the general practitioner as inhaled medication, antihypertensive agents or oral anti-diabetics. All discrepancies in the patients' electronic medical records in the hospital were subsequently corrected by the hepatologist treating the patient. In 27 patients (42.2%) no discrepancy was found.

Type of discrepancy	Number of discrepancies (n = 96)	Number of patients with ≥1 discrepancy (n = 64)	Example of discrepancies
Missing medication in patients' electronic medical records (n, %)	58 (60.4 %)	27 (42.2 %)	Tiotropium inhaler 18 ug was initiated by the general practitioner and not registered in the patients' electronic medical record
Unnecessary medication in patients' electronic medical records (n, %)	23 (24.0 %)	14 (21.9 %)	Hydrochlorothiazide tablets or iron tablets were registered as active medication in the patients' electronic medical record whereas another physician advised the patient to stop the tablets.
Incorrect dose or dose frequency in patients' electronic medical records (n, %)	14 (14.6 %)	9 (14.1 %)	Metoprolol (extended-release) once a year or tacrolimus (extended-release) once daily 5 mg instead of 8 mg was registered in the patients' electronic medical record
Other type of drug within same class in patients' electronic medical records (n, %)	1 (1.0 %)	1 (1.6 %)	Atorvastatin was taken by a patient whereas pravastatin was registered in the patients' electronic medical record

▲

Table 2. Discrepancies between medication recorded in the patients' electronic medical records and actual medication used by patients

Prevalence and examples of MRPs and interventions proposed for MRPs

In total, 98 MRPs were identified in 53 patients, with a median of 2 (range 1 – 5) MRPs per patient. In 34 patients (53.1%) more than one MRP was identified during the consultation. Most frequent MRPs were: ADRs (22.4%), nonadherence (19.3%), unnecessary drugs (16.3%) and undertreatment of known comorbidities (12.2%).

In total, 113 interventions were proposed for the identified MRPs. In some cases, more interventions were proposed for one MRP. Interventions most frequently proposed were dosage optimization (21.2%), individualized recommendation regarding drug compliance (16.8%) and drug discontinuation (12.4%). Most interventions proposed by the CP (93.6%) were followed by both the patients and the hepatologists. Interventions proposed and not accepted by the hepatologist or the patient were interventions in which the hepatologist or the patient had to stop or change the time of administration of a drug that was started by the primary care physician. Interventions were not accepted due to uncertainties about the medication (e.g. indication or no causal relation with side-effects). One year after the consultation with the CP, 79% (15/19) of the patients experienced no compliance issues and 93% (27/29) of patients used no drugs with indication issues anymore. No patient experienced an unplanned hospital admission related to medication during the year after the consultation. Table 3 and 4 present the prevalence and some examples of MRPs and the interventions proposed for MRPs.

Severity of the MRPs

The majority of the MRPs (57.1%, 56/98) was rated in category D (error caused potential harm to patient). In total, 10 MRPs (10.2%) were rated in category E (error caused temporary harm and required intervention) and 1 (1%) MRP was rated in category F (error caused temporary harm and required hospitalization). MRPs rated in category E and F were: use of anticoagulants without indication, use of contra-indicated drugs, dose not adapted in patient with worse renal function, no use of prophylactic antibiotics with major dental surgery and wrong dose frequency of immunosuppressive agents. The other MRPs were rated in category A (22.4%, 22/98; events that have the capacity to cause error) and category C (9.2%, 9/98; error occurred without posing harm to patients)

Medication related problems		N (%) instances of medication related problems (n = 98)	Example of medication related problems
Nonadherence	Intentional	7 (7.1 %)	Mycophenolic acid is taken once daily instead of twice daily
	Unintentional	12 (12.2 %)	Medication during daytime or before bed is forgotten
ADR		22 (22.4 %)	Hypotension and dizziness by blood pressure lowering medication
Drug interaction	Drug - disease	3 (3.1 %)	Use of NSAIDs
Indication	Wrong drug	1 (1.0 %)	Xylometazolin nasal spray used for allergies
	Unneccessary drug	16 (16.3 %)	Use of PPI without indication
	Untreated indication	12 (12.2 %)	Patient with frequent migraine and untreated neuropathic pain
Suboptimal dose	Dose too high	2 (2.0 %)	Normal dose used despite kidney insufficiency
	Dose too low	2 (2.0 %)	Inadequate dose of PPI for prophylaxis of a gastro-intestinal bleeding
Monitoring issues		1 (1.0 %)	New drug started by other specialism which requires monitoring of the liver enzymes regularly
Experienced complexity in dosage regimen		5 (5.1 %)	Too many administration times for medicines e.g. 5 or 6 times daily.
Logistic problems		5 (5.1 %)	Shortage of medicines in community pharmacy
Drug use problems		7 (7.1 %)	Problems with the taste of medicines
Other		3 (3.1 %)	Questions of patients about e.g. interactions, drug use and pregnancy and storage.

▲

Table 3. Prevalence and examples of MRPs

ADR, Adverse Drug Reaction; e.g., for example; MRPs, Medication-Related problems; NSAID; nonsteroidal anti-inflammatory drug; PPI, proton pump inhibitor

Interventions proposed for medication related problems		N (%) interventions proposed for medication related problems (n = 113)	Examples of interventions
Optimizations	Dosage regimen	24 (21.2 %)	Simplification of complex medication schedules from 6 moments of intake to 3.
	Stop	14 (12.4 %)	No indication for PPI or acetylsalicylic acid
	Start	8 (7.1 %)	Laxative for constipation due to opioid usage and sildenafil for erectile dysfunction
	Switch	1 (0.9 %)	Tacrolimus twice-daily formulation to once-daily formulation
Patient handling interventions	Education about medication	11 (9.7 %)	Explanation of the indication for xylometazoline nasal spray; not to be used to treat allergies and to be used for a maximum of 7 days.
	Medication compliance advise	19 (16.8 %)	Information about how to organize medication intake properly, e.g. with the help of an application on your phone or an alarm
	Advise for practical problems with medicines use	8 (7.1 %)	Improving the intake of medication by giving advise how to mask the taste
	Advise on how to reduce ADRs	14 (12.4 %)	Changing the intake of blood pressure medication to the evening to prevent for dizziness
	Advise how to stop medication	2 (1.8 %)	Advise with regards to stop PPI use
	Refer to other health care professional	9 (8.0 %)	Patients with unclear indications for a medicines referred to specialist or general practitioner Patient experiences pain for a long time referred to pain consultant
Dispensing		3 (2.7 %)	Wrong tablets in multidose drug dispensing bags

▲

Table 4. Interventions proposed for MRPs

ADRs, Adverse Drug Reactions; e.g., for example; MRPs, Medication-Related problems; PPI, proton pump inhibitor

Discussion

In this cohort, LTx recipients experience a median of 2 MRPs with the majority of the errors causing potential harm to patients (68.3%). ADRs, nonadherence and the use of unnecessary drugs were the most frequently reported MRPs in this cohort. Interventions most frequently proposed by the CP were dosage optimization, individualized recommendation regarding drug compliance and drug discontinuation. The clinical relevance of this program by the CP is shown by a reduction in patients experiencing compliance issues and patients using drugs with indication issues.

Our results are in line with Taber *et al.*, who evaluated MRPs in kidney transplant recipients.(11) They showed that MRPs commonly occur in kidney transplant recipients with nonadherence and ADRs as most frequently reported MRPs in their cohort. Recently, another study by Hayward *et al.* found results comparable to ours with nonadherence and indication issues as most frequently reported MRPs in a cohort of ambulatory patients with cirrhosis.(12) Interestingly, they found more drug interactions, dose issues and monitoring issues in comparison with our study. An explanation for this difference is the fact that in the Netherlands a nationwide electronical medication monitoring system is implemented with clinical decision support and clinical rules. (14, 15) As a consequence, physicians and pharmacists receive drug safety alerts directly during prescribing preventing for suboptimal doses, drug interactions and monitoring issues (e.g. the measurement of through levels for certain drugs).

Most frequently proposed interventions were dosage optimization, individualized recommendation regarding drug compliance and drug discontinuation. Interestingly, most ADRs and nonadherence issues in this cohort were due to complex medication schedules. Furthermore, the use of unnecessary drugs was approximately one fifth of the MRPs, which shows that a comprehensive review of medication is not regularly performed by the treating physician during the outpatient visit.

MRPs and especially nonadherence have been found to be correlated to multiple factors such as socioeconomic, therapy-related and healthcare organizational. (16) Methods used to improve nonadherence are automated prescription refill assistance, patients' self-reports or eHealth applications. However, MRPs in LTx recipients can only be solved by multi-faceted interventions targeting behavioral, educational and emotional factors and providing multidisciplinary care including a consultation with a CP.

Interventions proposed by the CP were in 93.6% followed by both the patient and the hepatologist. Other international studies show comparable acceptance rates of approximately 95%, whereas studies in the Netherlands show an acceptance rate of approximately 80%.(10, 17) Probably, at the Erasmus University Medical Center an acceptance rate in accordance with international studies is achieved by a recent change in the workflow. CPs are dedicated to specific wards causing intensive collaborations with all health care providers. During some consultations the number of interventions initiated for the MRPs were greater than the total number of MRPs. This is caused by the fact that for some MRPs multiple interventions are possible. For example, nonadherence could be improved by medication optimizations and medication compliance advises. Also, ADRs could be solved by a change in the dosage regimen and an advice on how to reduce or handle adverse drug reactions. Moreover, potential medication related complications were prevented in 68.3% of the patients.

This newly established consultation with the CP is performed during the annual check-up. Possibly a consultation more frequently over the year might be more beneficial, even for recently transplanted patients. A potential hurdle for this kind of involvement of the pharmacist is current absence of financial reimbursement for the CP in the Netherlands. With a reduction in patients experiencing compliance issues and patients using drugs with indication issues we show the clinical relevance of this program. The results of this study implicate that an intensive collaboration between liver transplant healthcare professionals and pharmacists is needed and should be evolved in the near future.

Strengths of our study are the real-life clinical setting, the fact that the MRPs were independently categorized and the good collaboration between the Department of Hepatology and the Department of Hospital Pharmacy. Furthermore, we did assess the severity of the MRPs and evaluated the MRPs and proposed interventions one year after the consultation. As far as we know, this is the first study that describes MRPs in the outpatient setting focusing on liver transplant recipients. Our study has some limitations. Due to the fact that the consultation with the CP was planned one afternoon per week, not every LTx recipient monitored at the outpatient clinic of the Erasmus University Medical Center has been consulted by the CP. As a consequence, we might under- or overestimate the actual prevalence of MRPs in our cohort. However, patients of all hepatologists working at the Erasmus University Medical Center are seen by the CP. Therefore, we assume that this cohort is a good reflection of all LTx recipients monitored at the outpatient clinic. Furthermore, patient satisfaction about the consultation of the CP was not monitored. Further research could focus on this topic, together with the prevention of unplanned hospital admissions of LTx recipients. In the future, a randomized controlled trial could be performed evaluating the effect of a consultation provided by a CP that combines several strategies to reduce MRPs in LTx recipients.

In summary, LTx recipients in this cohort experience a median of 2 MRPs of which ADRs, nonadherence and unnecessary drugs are most frequently reported. The clinical relevance of this program is shown by a reduction in patients experiencing compliance issues and patients using drugs with indication issues. An outpatient monitoring program of a CP for LTx recipients can signal MRPs and lead to interventions that are accepted by both the patients and the hepatologists and hence result in optimization of medication safety in LTx recipients.

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Chapter 12

Evaluation of medication-related problems in liver transplant recipients with and without an outpatient medication consultation by a clinical pharmacist: a cohort study.

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Abstract

Background
Transplant recipients undergo significant changes in their medication regimen during follow-up and are at an increased risk for medication-related problems (MRPs).

Aim
This study aimed to compare the prevalence and types of MRPs and interventions in liver transplant recipients with and without an outpatient medication consultation by a clinical pharmacist as well as the satisfaction with information about medicines and medication adherence.

Method
We performed a single-center, observational cohort study. A retro- and prospective cohort were used and subdivided in a group that did and did not receive a medication consultation. The prevalence and types of MRPs and interventions were identified and categorized. The satisfaction parameters were evaluated using validated questionnaires.

Results
Included were 291 patients. In total, 368 MRPs were identified in 197 patients in the non-medication consultation cohort (median 1; range 1–3 per patient) and 248 MRPs in 94 patients in the medication consultation cohort (median 2; range 1–4 per patient). In the medication consultation cohort, significantly fewer MRPs as unnecessary drugs (17.3% versus 58.7%, p<0.001), suboptimal therapy (2.4% versus 9.5%, p<0.001), untreated indication (2.8% versus 6.8%, p=0.040) and underdosed drugs (0.4% versus 6.3%, p<0.001) were identified. In the non-medication consultation cohort significantly more patients used unnecessary drugs (72.1% versus 39.4%, p<0.001) compared to the medication consultation cohort. Patients in both cohorts are satisfied with the information about medicines and reported a high medication adherence.

Conclusion
Patients in the medication consultation cohort had significantly fewer MRPs and used significantly less unnecessary drugs. Including a clinical pharmacist to the post-transplant care has an added value.

Introduction

Liver transplantation (LT) has become a lifesaving treatment option for patients with end-stage liver disease, hepatocellular carcinoma and acute liver failure [1]. Over the past decades significant developments have been made in the field of LT, which steadily led to improved outcomes and long-term survival.(1,2) However, long-term care after LT remains complex. LT recipients undergo significant changes in their medication regimen during follow-up with an increased risk for medication-related problems (MRPs).

LT recipients need to adhere to difficult and complex therapeutic regimens.(2,3-5) In addition, LT recipients will usually receive more medication over the years due to the development of new-onset diabetes mellitus, hypertension, and hyperlipidemia.(6,7) The addition of more medication could cause MRPs and possibly result in preventable drug-related hospital admissions.(8,9)

A study by Repp *et al.* reported that 40% of the hospital admissions following cardiac transplantation were drug-related of which 58% was preventable.(10)

Hepatologists focus mainly on the liver-related problems and transplant-specific complications. Clinical pharmacists involved in the transplant care provide a broad range of different services to prevent MRPs such as therapeutic drug monitoring, educational activities, management of adverse drug reactions (ADRs), dosing issues and therapy optimizations.(11)

Recently, we showed the added value of an outpatient monitoring program for LT recipients by a clinical pharmacist through signaling relevant discrepancies and MRPs.(12) However, no studies have been done to compare the prevalence of MRPs and interventions in patients with and without an outpatient medication consultation (MC) by a clinical pharmacist.

Aim
This study aimed to compare the prevalence and types of MRPs and interventions in LT recipients with and without an outpatient pharmacy consultation by a clinical pharmacist as well as the satisfaction with information about medicines and medication adherence.

Ethics approval
A waiver was given by the Medical Ethics Committee of the Erasmus University Medical Center (MEC-2019-0784). Patient data were sampled and stored in accordance with privacy regulations.

Method

Study design and setting
We performed an observational cohort study at the Erasmus MC Transplant Institute, University Medical Center Rotterdam, the Netherlands. For our primary objective, we retrospectively collected data between July-December 2020. For the secondary objectives, we prospectively included a second cohort of LT recipients to participate in a questionnaire study in the period March-May 2021. Both cohorts consisted of adult LT recipients >1 year after LT who were scheduled for an annual, multidisciplinary medical check-up at the outpatient clinic. A retro- and prospective cohort were used due to practical and feasibility reasons. The use of questionnaires to evaluate the satisfaction with information about medicines and medication adherence was not regularly done in our clinic. Therefore, the prospective part of this research was performed during a research internship. No differences in the treatment protocol occurred during both periods.

At the start of this study in July 2020, 746 LT recipients were in active follow up after transplantation at the Erasmus MC. Since 2018, a clinical pharmacist has an active role in the annual, multidisciplinary medical check-up of LT recipients by conducting MCs. Detailed information about the content of the MCs by the clinical pharmacist has been previously reported [12]. All check-ups are performed on two weekdays, with the clinical pharmacist only participating on one of these days. This has resulted in two cohorts: a cohort that did not receive a MC (non-MC cohort) and a cohort that did receive a MC (MC cohort). In the non-MC cohort more LT recipients are being seen since more hepatologists have outpatient visits on that day of the week. All findings during the check-ups were registered in the patients' medical records for further follow-up.

Data collection

MRPs and interventions

For the analysis of the primary objective the following baseline characteristics were obtained from the patient medical record: age, gender, indication for liver transplantation, time after transplantation, information about re-transplantation, comorbidities, and number and type of drugs. MRPs and interventions in the non-MC cohort were identified by reviewing all information. This information included the patients' medical history and laboratory results, such as electrolytes, renal function, and blood glucose levels. Medication reconciliation was performed in the MC cohort. MRPs and interventions identified by the clinical pharmacist as well as MRPs solved by the clinical pharmacist in the MC cohort were documented in the patients' medical records.

Assessment of MRPs and interventions

For the non-MC cohort, MRPs were assessed by a pharmacological review based on all available information in the patients' medical record after the LT recipients were seen by a hepatologist. The follow-up and corrections of the detected MRPs in the non-MC cohort was beyond the scope of this research. Two researchers independently identified these MRPs and interventions proposed by the hepatologist and categorized them into predefined categories using the Pharmaceutical Care Network Europe (PCNE) Classification V9.0 (full classification in Supplementary table 1) [8]. Next, the classifications were compared and when dissensus occurred, both researchers reviewed their classifications and discussed these until consensus was reached.

For the MC cohort, the two researchers independently categorized the identified MRP and proposed interventions by the clinical pharmacist as registered in the patients' medical record into the predefined categories using the PCNE Classification V9.0.(8) Only one intervention could have been proposed for each identified MRP.

Satisfaction with Information about Medicines and the Medication Adherence

LT recipients in the second cohort were asked to fill out two questionnaires (translated and validated into Dutch) after their annual medical check-up: the Satisfaction with Information about Medicines (SIMS) and the Medication Adherence Reporting Scale (MARS-5) surveys. (13,14) Besides, patients were asked to report their age, gender and highest reached educational level. The International Standard Classification of Education 2011 (ISCED 2011) was used to convert the Dutch educational system into an international one.(15)

Assessment of the Satisfaction with Information about Medicines and the Medication Adherence

The SIMS assesses patients' satisfaction with 17 items of information considered essential for safe and accurate self-management of medicines according to the recommendations of the Association of the British Pharmaceutical Industry (full survey in Section S1 in the Supplementary Appendix). (13,14) For each item, patients indicate if the information they have received is "too much," "about right," "too little," "none received," or "none needed." Reports of "about right" and "none needed" are classified as satisfied and receive a score of 1. The remaining options are classified as dissatisfied and are scored as 0. The scores are summed up to obtain a satisfaction rating for the total scale ranging from 0 to 17. Higher summary scores indicate a higher degree of satisfaction with information received.(16) A score of ≥13 was interpreted as a satisfied patient and a number

<13 was interpreted as a dissatisfied patient. No threshold for satisfied versus dissatisfied was described in the literature. Therefore, an arbitrary threshold in which >75% of the items of the questionnaire received a score of 1 was chosen by the researchers.

The MARS-5 compromises five short adherence statements (full survey is provided in Section S2 in the Supplementary Appendix) [14]. The MARS-5 survey was scored on a Likert-type scale ranging from "always", "often", "sometimes", "rarely" to "never". The point spread from 1 point for "always" to 5 points for "never". A total adherence rate was obtained for each patient. A total of 25 could be achieved with higher scores indicating higher reported adherence.

Statistical analysis

No formal sample size calculation was performed. We included every patient seen for their annual check-up in the study period at the outpatient clinic in the analysis. Variables were described using counts (%) for nominal and ordinal variables and mean (standard deviation, SD) or median (inter-quartile range, IQR) for the continuous variables, depending on the shape of the distribution.
The primary and secondary endpoints were analyzed using Pearson's Chi-square test or Fisher's exact tests. The latter was used in the case of a low observed count (<10) in at least one of the cohorts. For all statistical tests, a two-sided p-value of <0.05 was considered to indicate statistical significance. Missing values <5% were considered as missing completely at random.
Statistical analysis was performed using SPSS for Windows, version 25.0 (SPSS, Chicago, IL).

Results

Primary endpoint: prevalence of and interventions proposed for MRPs

Between 30/06/2020 – 31/12/2020 a total of 291 LT recipients had their annual, medical check-up; 197 in the non-MC cohort and 94 in the MC cohort. Two LT recipients in the MC cohort did not show up at their annual, medical check-up.

Table 1 shows the baseline clinical and demographical characteristics of the participants. LT recipients in the non-MC cohort had a significantly higher occurrence of renal disorder as comorbidity (p<0.001). No significant differences were found in the number of drugs on the medication list during consultation during the annual check-up (p=0.276).

	non-MC cohort (n = 197)	MC cohort (n = 94)	p-value
Age (year) (median [IQR])	60.0 (49.0-68.0)	60.0 (51.0-68.0)	0.455Ω
Gender			
Male (n, %)	120 (60.9%)	50 (53.2%)	0.211
Indication liver transplantation¥			
Viral hepatitis	43 (21.8%)	29 (30.9%)	0.077
Primary sclerosing cholangitis	45 (22.8%)	21 (22.3%)	0.924
Hepatocellular carcinoma	46 (23.4%)	9 (9.6%)	0.006ʃ*
Alcohol-related liver disease	32 (16.2%)	9 (9.6%)	0.151ʃ
Acute liver failure	18 (9.1%)	5 (5.3%)	0.354ʃ
Biliary cirrhosis	14 (7.1%)	4 (4.3%)	0.441ʃ
Metabolic liver disease	13 (6.6%)	4 (4.3%)	0.595ʃ
Polycystic liver disease	9 (4.6%)	4 (4.3%)	1.000ʃ
Nonalcoholic Steatohepatitis	9 (4.6%)	4 (4.3%)	1.000ʃ
Cryptogenic cirrhosis	10 (5.1%)	6 (6.4%)	0.574ʃ
Other[a]	12 (6.1%)	13 (13.8%)	0.050
Time after transplantation (year) (median [IQR])	7.0 (4.0 - 12.0)	8.0 (4.0-15.0)	0.536Ω
Retransplantation			
Yes	21 (10.7%)	4 (4.3%)	0.076ʃ
Comorbidities¥			
Cardiovascular disease	128 (65.0%)	50 (53.2%)	0.054
Diabetes mellitus	57 (28.9%)	22 (23.4%)	0.321
Renal disorder	36 (18.3%)	4 (4.3%)	<0.001ʃ*
Inflammatory bowel disease	35 (17.8%)	17 (18.1%)	0.947
Bone disease	31 (15.7%)	8 (8.5%)	0.100ʃ
Other[b]	83 (42.1%)	44 (46.8%)	0.452
None	23 (11.7%)	14 (14.9%)	0.441
Number of drugs on medication list during consultation (median [IQR])	6.0 (5.0-9.0)	7.0 (5.0-10.0)	0.276Ω

▲

Table 1. Baseline clinical and demographical characteristics of LT recipients with and without an MC.

Data presented are counts (%) and differences between groups were analyzed using the Pearson's chi-squared test unless otherwise noted.

ΩMann-Whitney U test; ʃFisher's exact test; ¥patients may have had more than one indication for liver transplantation and/or comorbidities; *indicates a statistically significant difference of p<0.05.
[a]other includes biliary atresia (n=5), hemochromatosis (n=4), Budd–Chiari syndrome (n=3), Caroli disease (n=2), Rendu-Osler-Weber (n=2), cystic fibrosis (n=2), Alagille syndrome (n=1), hemangioendothelioma (n=1), Abernethy Syndrome (n=1), echinococcosis (n=1), acute fatty liver of pregnancy (n=1), Crigler–Najjar syndrome (n=1), cholangiocarcinoma (n=1);
[b]other includes haematological, immunological, metabolic and psychological morbidities.

Abbreviations: IQR, inter-quartile range; non-MC cohort, no medication consultation cohort; MC cohort, medication consultation cohort

Medication related problems

In total, 616 MRPs were identified: 368 in the non-MC cohort (median per patient, 1.0; IQR, 1.0 – 3.0) and 248 in the MC cohort (median per patient, 2.0; IQR, 1.0 – 4.0). Most LT recipients had at least one MRP, 173 (87.8%) in the non-MC cohort and 89 (94.7%) in the MC cohort.

Table 2 shows the prevalence and examples of the identified MRPs in both cohorts. In the MC cohort, significantly fewer MRPs as unnecessary drugs (17.3% versus 58.7%, p<0.001), wrong drugs/suboptimal therapy (2.4% versus 9.5%, p<0.001), untreated indication (2.8% versus 6.8%, p=0.040), and too low dosed drugs (0.4% versus 6.3%, p<0.001) were detected compared to the non-MC cohort. In the MC cohort significantly more MRPs as unintentional nonadherence (9.0% versus 0.0%, p<0.001), problems in drug use (24.2% versus 1.6%, p<0.001), questions regarding the drugs (4.0% versus 0.0%, p<0,001), and other problems (28.2% versus 3.0%, p<0.001) were detected compared to the non-MC cohort.

In the non-MC cohort significantly more patients used unnecessary drugs (72.1% versus 39.4%, p<0.001) compared to the MC cohort and 16.5% of the patients in the non-MC cohort used suboptimal doses. The most prevalent unnecessary drugs used were proton pump inhibitors, opioids, benzodiazepines, vitamin D and calcium supplementation. Suboptimal doses were mostly found in patients with poorly controlled diabetes.

A total of 35 LT recipients in the MC cohort had their first MC with the clinical pharmacist, 55 had their second MC and 4 had their third MC. Patients having their first MC with the clinical pharmacist had the most MRPs. The number of MRPs for ADRs in patients having their second MC with the clinical pharmacist was reduced compared to patients having their first MC (16.2% versus 9.7%). The number of MRPs for unnecessary drugs, usage issues and discrepancies in the medication list in patients having their second MC did not differ compared to patients having their first MC.

Interventions proposed for MRPs

Figure 1 shows the interventions proposed for the MRPs. A total of 74 interventions were proposed by the hepatologist in the non-MC cohort (median, 0.0; IQR, 0.0 – 1.0; maximum 2) and 251 interventions were proposed by the clinical pharmacist in the MC cohort (median, 2.0; IQR, 1.0 – 4.0; maximum 10). Interventions in the non-MC cohort and MC cohort were carried out by a hepatologist. The most prevalent interventions by a hepatologist were starting a drug (28/74, 37.8%), changing a dose (24/74, 32.4%), and pausing or stopping a drug (15/74, 20.3%). The most prevalent interventions by the clinical pharmacist were adjusting the patient file (71/251, 28.3%), changing the instructions for use (42/251, 16.7%) and pausing or stopping a drug (36/251, 14.3%).

The clinical pharmacist resolved 251 MRPs of which 155 (61.8%) were accepted by the hepatologist and 46 (18.3%) were not accepted by the hepatologist. Examples of accepted interventions were: lowering the dose of magnesium hydroxide and stopping proton pump inhibitors due to the absence of an indication for the high dose and optimizing the dosing regimen. Examples of interventions not accepted were: stopping drugs prescribed by another physician due to the absence of an indication, optimizing the antihypertensive therapy according to the guidelines and changing to another class of laxatives because of taste complaints by the patient. Due to the need for follow-up, it is unknown if 11 (4.4%) interventions were accepted by the LT recipient and due to the nature of the MRP 39 (15.5%) interventions could not be followed up.

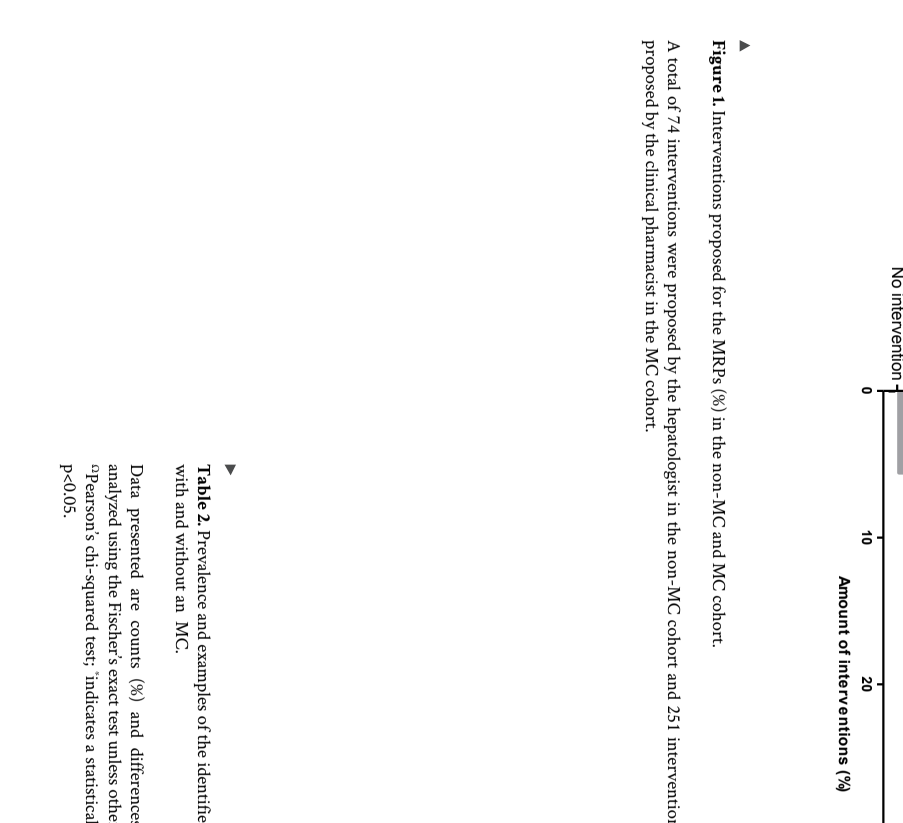

Figure 1. Interventions proposed for the MRPs (%) in the non-MC and MC cohort.

A total of 74 interventions were proposed by the hepatologist in the non-MC cohort and 251 interventions were proposed by the clinical pharmacist in the MC cohort.

Table 2. Prevalence and examples of the identified MRPs in LT recipients with and without an MC.

Medication related problems	N (%) instances of mediation related problems in non-MC cohort (n=368)	N (%) instances of medication related problems in MC cohort (n=248)	p-value	Example of medication related problems
Nonadherence				
Intentional	0 (0.0%)	3 (1.2%)	0.065	Nonadherence with magnesium gluconate due to diarrhea as an adverse drug reaction.
Unintentional	0 (0.0%)	9 (3.6%)	<0.001*	Nonadherence with ursodeoxycholic acid due to forgetting to take the drug during the holiday.
Adverse drug reaction	34 (9.2%)	31 (12.5%)	0.196$^{\Omega}$	Hypomagnesaemia when using a proton pump inhibitor.
Drug interaction	0 (0.0%)	2 (0.8%)	0.162	Use of clindamycin cure during a holiday, which causes an interaction with cyclosporine.
Indication				
Wrong drug/suboptimal therapy	35 (9.5%)	6 (2.4%)	<0.001*	The use of naproxen for headache, which is contraindicated in LT recipients.
Unnecessary drug	216 (58.7%)	43 (17.3%)	0.001$^{\Omega}$*	The use of 3 low dosed antihypertensive drugs by a patient with a well-regulated blood pressure.
Untreated indication	25 (6.8%)	7 (2.8%)	0.040*	No anticoagulant prophylaxis, statin and/or ACE inhibitor in a patient with a history of acute coronary syndrome.
Suboptimal dose				
Dose too high	10 (2.7%)	1 (0.4%)	0.057	Long-term use (> 6 months) of apixaban at a dose of 5 mg twice daily instead of 2.5 mg twice daily because of a worse renal function.
Dose too low	23 (6.3%)	1 (0.4%)	<0.001*	Increase the dose of metformin because of a poorly controlled diabetes mellitus and sufficient renal function.
Dosage regime				
Too frequent	6 (1.6%)	5 (2.0%)	0.763	Magnesium hydroxide usage of 4 times daily while not needed.
Not frequent enough	2 (0.5%)	0 (0.0%)	0.518	Mycophenolate mofetil prescribed once daily instead of twice daily.
Use	6 (1.6%)	60 (24.2%)	<0.001*	Simplification of complex medication schedules
Question	0 (0.0%)	10 (4.0%)	<0.001*	Answering a question regarding the use of tacrolimus during pregnancy.
Other	11 (3.0%)	70 (28.2%)	<0.001$^{\Omega}$*	Discrepancies between medication recorded in the patients' medical records and actual medication used by patient.

Data presented are counts (%) and differences between groups were analyzed using the Fischer's exact test unless otherwise noted.
$^{\Omega}$Pearson's chi-squared test; *indicates a statistical significant difference of p<0.05.

Secondary endpoints: Satisfaction with Information about Medicines and the Medication Adherence

A total of 132 LT recipients participated in the surveys: 84 in the non-MC cohort and 48 in the MC cohort. The completion rate was 80.5%.

Baseline characteristics of participants for the SIMS and MARS-5 surveys are shown in table 3. The median age differed significantly between the two groups (54.0 years (IQR: 43.0-65.0) in the non-MC cohort versus 63.5 years (IQR: 54.0-68.0) in the MC cohort (p = 0.027)). The majority in both groups were men: 60.7% in the non-MC cohort and 64.4% in the MC cohort.

	non-MC cohort (n = 84)	MC cohort (n = 48)	p-value
Age, year (median [IQR])	54.0 (43.0-65.0)	63.5 (54.0-68.0)	0.027Ω*
Gender			
Male (n,%)	51 (60.7%)	31 (64.6%)	0.552
Education¥			
None	2 (2.4%)	2 (4.2%)	0.620ʃ
ISCED 1	3 (3.6%)	4 (8.3%)	0.254ʃ
ISCED 2	14 (16.7%)	7 (14.6%)	0.810ʃ
ISCED 3	33 (39.3%)	16 (33.3%)	0.507
ISCED 6	23 (27.4%)	13 (27.1%)	0.987
ISCED 7	6 (7.1%)	1 (2.1%)	0.421ʃ
ISCED 8	0 (0.0%)	3 (6.3%)	0.046ʃ*
Missing information	3 (3.6%)	1 (2.1%)	-

▲

Table 3. Baseline characteristics of the LT recipients for the SIMS and MARS-5 questionnaire.

Data presented are counts (%) and differences between groups were analyzed using the Pearson's chi-squared test unless otherwise noted.

ΩMann-Whitney U-Test; ʃFisher's Exact Test; *indicates a statistically significant difference of p<0.05; ¥The International Standard Classification of Education 2011 (ISCED 2011) was used to convert the Dutch educational system into an international one. ISCED 4 and 5 does not exist in the Dutch educational system.

Satisfaction with Information about Medicines

Table 4 shows the overall satisfaction and factors associated with the satisfaction of LT recipients with the information about medicines in both cohorts. LT recipients in both cohorts are satisfied with the information about medicines with lower educated LT recipients less satisfied and higher educated LT recipients more satisfied. LT recipients aged < 55 and >65 years appeared to be more satisfied in the MC cohort compared to the non-MC cohort. LT recipients in the MC cohort were more satisfied compared to the non-MC cohort (72,9% versus 64,3%, p=0.309).

LT recipients in both cohorts were less satisfied with the information about the mechanism of action, ADRs and whether the medicine interferes with other drugs (supplementary table 2).

	non-MC satisfied (n = 84)	MC cohort satisfied (n = 48)	p-value
Overall satisfaction	54/84 (64.3%)	35/48 (72.9%)	0.309
Gender			
Male	33/51 (64.7%)	22/31 (71.0%)	0.558
Female	21/33 (63.6%)	12/16 (75.0%)	0.426
Missing information	-	1/1 (100.0%)	-
Age			
< 55 years	26/41 (63.4%)	11/12 (91.7%)	0.055
55 – 65 years	15/20 (75.0%)	9/17 (52.9%)	0.044
>65 years	9/18 (50.0%)	14/17 (82.4%)	0.161
Missing information	4/5 (80.0%)	1/2 (50.0%)	-
Education*			
Low	32/52 (61.5%)	20/29 (69.0%)	0.585
High	20/29 (69.0%)	13/17 (76.5%)	0.504
Missing information	2/3 (66.7%)	2/2 (100.0%)	-

▲

Table 4. Overall satisfaction and factors associated with the satisfaction of LT recipients with the information about medicines in the non-MC and MC cohort.

*Patients are considered highly educated for ISCED classifications of ≥ 6 and low educated for ISCED classifications of < 6.

Medication Adherence

LT recipients in both cohorts had a median MARS-5 score of 24.0 (IQR: 24.0-25.0), which indicates a high medication adherence in both cohorts.

Discussion

To our knowledge, this is the first study describing the impact of a clinical pharmacist by investigating the differences in MRPs in LT patients with and without an MC during an outpatient annual check-up. LT recipients in the MC cohort had significantly fewer MRPs as using of unnecessary drugs, wrong drugs/suboptimal therapy, and having dosing issues. In the MC cohort significantly more MRPs were identified as unintentional nonadherence, problems in drug use, questions regarding the drugs, and other problems. We demonstrated a high prevalence of MPRs in LT recipients in the outpatient setting with a median of 1 MRP in the non-MC cohort and 2 MRPs in the MC cohort. The most frequently reported MRPs in both cohorts were unnecessary drug use, problems in drug use and ADRs. Most LT recipients in both cohorts were satisfied with the information about medicines.

Our findings are in line with the results of Flamme-Obry *et al.*. They showed that an interview with the clinical pharmacist at discharge could help to reduce MRPs in kidney transplant recipient. (17) Flamme-Orby found that their intervention resulted in fewer MRPs as interactions between drugs, ADRs and wrong usage of medicines. By introducing a medication consultation by a clinical pharmacist, we resolved a substantial number of MRPs as the wrong usage of medicines. Furthermore, several studies showed comparable results to ours with regards to the most

prevalent MRPs.(10,18) The most prevalent MRPs in these studies were ADRs, nonadherence issues, and the use of unnecessary drugs. By detecting and preventing the use of unnecessary drugs the clinical pharmacist can contribute to relevant social issues as preventing for unnecessary healthcare costs and the sustainable use of medicines.

Most frequently proposed interventions by the clinical pharmacist in the MC cohort were instructions for use changed, drug paused or stopped and patient file adjusted. Cohen *et al.* showed that a pharmacist-driven medication reconciliation significantly reduced medication discrepancies.(19) Furthermore, another study by Ho *et al.*, showed that pharmacists working as part of the multidisciplinary transplant team identified and resolved medication discrepancies and thereby improved the medication safety at a transplant clinic.(20) Interestingly, in both studies the average number of discrepancies per patient was higher compared to our study. This might be explained by the fact that in the Netherlands medication reconciliation is mandatory for every patient admitted to a hospital. The most frequently proposed interventions by the hepatologist in the non-MC cohort were the start/stop of a drug or the change of the dosage of a drug. This is a consequence of the organization of the Dutch healthcare system in which only doctors are allowed to initiate those interventions. Interventions not accepted by the hepatologist were mostly due to the fact that these drugs were prescribed by other prescribers or patients were not willing to change/stop their medication.

Most LT recipients in both cohorts were satisfied with the information about medicines. In the non-MC cohort, patients indicated that they were less satisfied with the information regarding the mechanism of action, ADRs and possible interactions. This might be explained by the fact that hepatologists focus less on educating patients about their medicines and ADRs. These findings are in line with Klewitz *et al.* who found a high prevalence of dissatisfaction with information about medication, specifically ADRs, in kidney transplant recipients.(16) A study evaluating the SIMS in patients using anticancer agents also showed comparable results to our non-MC cohort with patients dissatisfied with the information concerning the mechanism of action of drugs, the risk of ADRs, and the interference with other drugs.(21) Based on the results in this study, educating patients about their medication, ADRs and dosing regimens is not commonly done but is important to prevent MRPs.

Strengths of our study are the real-life clinical setting and the fact that we included a representative part, almost 50%, of the LT recipients being seen at the outpatient clinic. A limitation of our study is the fact that in the non-MC cohort, MRPs and interventions were detected using the patients' medical records, including the patients' medication list, laboratory results and medical history. The clinical pharmacist did not attend the consultations by the hepatologist. Whereas patients in the MC cohort had an actual MC with the clinical pharmacist. As a consequence, other types of and less MRPs were detected in the non-MC cohort since less information was available. Another limitation is the fact that we did not investigate the impact of the MRPs in relation to potential harm and clinical outcomes. Further research is needed to study the cost-effectiveness of the MCs carried out by a clinical pharmacist. In addition, evaluating the responsibilities and mandate of a clinical pharmacist to resolve MRPs caused by drugs prescribed by different physicians is needed to optimize the medication safety in patients with multiple comorbidities.

Conclusion

LT recipients in the MC cohort had significantly fewer MRPs as the usage of unnecessary drugs, wrong drugs/suboptimal therapy, and having dosing issues. Over 70% of the patients in the non-MC cohort were using unnecessary drugs. LT recipients in both cohorts are satisfied with the information about medicines. Since clinical pharmacists bring different perspectives to post-transplant care, including a clinical pharmacist in the multidisciplinary transplant team has an added value for improving the pharmaceutical care and optimizing medication safety in these patients.

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Category	Subclassification	Definition
Nonadherence	Intentional	The patient uses/takes intentionally less drug than prescribed or does not take the drug at all.
	Unintentional	The patient uses/takes unintentionally less drug than prescribed or does not take the drug at all.
Adverse drug reactions		The patient has a medical problem that is the result of an adverse drug reaction.
Drug interaction		The patient has a medical problem that is the result of a drug-drug, drug-food, or drug-laboratory interaction.
Indication	Wrong drug	The patient has a drug indication but is taking the wrong drug.
	Unnecessary drug	The patient is taking a drug for no medically valid indication.
	Untreated indication	The patient has a medical problem that requires drug therapy but is not receiving a drug for that indication.
Suboptimal dose	Dose too high	The patient has a medical problem that is being treated with too much of the correct drug.
	Dose too low	The patient has a medical problem that is being treated with too little of the correct drug.
Dosage regime	Too frequent	The patient has a medical problem that is being treated with the correct drug of which the dosage regime is too frequent.
	Not frequent enough	The patient has a medical problem that is being treated with the correct drug of which the dosage regime is not frequent enough.
Use		The patient administers/uses the drug in a wrong way.
Question		The patient has questions concerning the medication.
Other		The patients' medication list is incomplete or has discrepancies.

▲ **Supplementary table 1.** MRP classification according to the classification of the Pharmaceutical Care Network Europe version 9.0. (5)

	non-MC cohort (n=84)					MC cohort (n=48)					p-value
	Satisfied (%)		Not satisfied (%)			Satisfied (%)		Not satisfied (%)			
	About right	None needed	Too much	Too little	None received	About right	None needed	Too much	Too little	None received	
What your medicine is called.	90.5	2.4	2.4	3.6	1.2	79.2	12.5	4.2	2.1	2.1	0.177
What your medicine is for.	89.3	2.4	2.4	3.6	2.4	87.5	8.3	2.1	2.1	0	0.438
What it does.	86.9	1.2	2.4	7.1	1.2	89.6	4.2	2.1	4.2	0	0.589
How it works.	78.6	1.2	2.4	10.7	7.1	87.5	2.1	2.1	6.3	2.1	0.624
How long it will take to act.	69.0	4.8	2.4	14.3	9.5	77.1	12.5	0	8.3	2.1	0.125
How you can tell if it is working.	61.9	6.0	1.2	14.3	16.7	68.8	12.5	2.1	10.4	6.3	0.300
How long you will need to be on your medicine.	83.3	9.5	4.8	1.2	1.2	83.3	10.4	2.1	2.1	2.1	0.921
How to use your medicine.	94.0	3.6	2.4	0	0	85.4	8.3	2.1	2.1	2.1	0.280
How to get a further supply.	88.1	4.8	0	3.6	3.6	70.8	16.7	4.2	6.3	2.1	0.039
Whether the medicine has any unwanted effects (side effects).	77.4	2.4	0	16.7	3.6	77.1	8.3	0	14.6	0	0.243
What are the risks of you getting side effects.	67.9	2.4	0	15.5	14.3	75.0	8.3	0	12.5	4.2	0.125
What you should do if you experience unwanted side effects.	67.9	4.8	2.4	14.3	10.7	60.4	16.7	2.1	14.6	6.3	0.222
Whether you can drink alcohol whilst taking this medicine.	71.4	15.5	2.4	3.6	7.1	56.3	31.3	0	6.3	6.3	0.177
Whether the medicine interferes with other medicines.	64.3	6.0	1.2	19.0	9.5	56.3	18.8	2.1	10.4	12.5	0.142
Whether the medication will make you feel drowsy.	60.7	10.7	0	13.1	15.5	64.6	14.6	0	12.5	8.3	0.644
Whether the medication will affect your sex life.	36.9	15.5	0	15.5	32.1	43.8	22.9	4.2	14.6	14.6	0.076
What you should do if you forget to take a dose.	71.4	7.1	1.2	8.3	11.9	72.9	10.4	0	10.4	6.3	0.715

▲ **Supplementary table 2.** Detailed information regarding the degree of satisfaction of the LT recipients with the various aspects of information about medicines.

Chapter 13

Differences in CYP3A genotypes of a liver transplant recipient and the donor liver graft and adjustment of tacrolimus dose.

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Abstract

Tacrolimus (TAC) is well established as main immunosuppressant in most immunosuppressive regimens in solid organ transplantation. Due to the narrow therapeutic window, pre dose Tac levels (C0) are monitored in all patients receiving Tac to reach optimal therapeutic levels. Tac is metabolized in the liver and intestine by the cytochrome P450 3A (CYP3A) isoforms CYP3A4 and CYP3A5. We present a case of an African American woman who underwent a liver transplantation in which adequate Tac levels were difficult to accomplish due to differences in cytochrome P450 3A4/5 (CYP3A4/5) polymorphisms of the transplant recipient and the donor liver graft.

This case report highlights that genotyping the liver transplant recipient and the donor liver graft might provide data which could be used to predict the tacrolimus metabolism post transplantation.

Case Report

After solid organ transplantation, tacrolimus is used to prevent allograft rejection in the long term. TAC is known for its narrow therapeutic window with large interpatient pharmacokinetic variability where underexposure poses a risk to allograft rejection and overexposure might increase the incidence of infections and toxicity.(1) TAC is metabolized in the liver and intestine by the cytochrome P450 3A (CYP3A) isoforms CYP3A4 and CYP3A5. Patients carrying at least one CYP3A5*1 variant allele are considered to be CYP3A5 expressers; these patients have low TAC exposure due to rapid metabolism of Tac. Patients carrying a CYP3A5*3, CYP3A5*6 or CYP3A5*7 variant allele have nonfunctional CYP3A5 protein and are considered to be CYP3A5 non-expressers. Approximately 55% of African Americans are carriers of the CYP3A5*1 variant allele.(2) CYP3A5 expressers require a TAC dose that is approximately 1.5 – 2-fold higher than non-expressers to reach equivalent TAC exposure.(3) Also, the effect of the drug-drug interactions between TAC and CYP3A4/CYP3A5 inducers/inhibitors will be enhanced in CYP3A5 expressers. Monostory *et al.* found an association between TAC blood levels in liver transplant recipients and donors' CYP3A5 genotype as well as CYP3A4 expression.

We present a case in which the genotype of the donor liver graft had a significantly less important effect on TAC pharmacokinetics than the genotype of the liver transplant recipient during the first month post transplantation. A 33-year-old African American woman, known to have sickle cell disease, G6PD deficiency, osteoporosis and colitis ulcerosa, received an uncomplicated donation after brain death (DBD) liver transplantation (LT) because of a cirrhosis and recurrent cholangitis due to primary sclerosing cholangitis (PSC). TAC was initially started at day 5 at a lower dose (2 mg twice daily; 62 kg) because of a postoperative pulmonary infection.

Target TAC whole blood levels were set at 6 – 10 µg/L in the first month after LT followed by target TAC whole blood levels of 4 – 8 µg/L from the second month onwards.(4) TaTACc levels were measured by ultra-high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UHPL-MS/MS Xevo TQ, Water Chromatography, BV, USA). After several dose adjustments shown in Figure 1, the TAC trough level was still inadequate (4.5 µg/L) at day 16, which resulted in an additional dose increase to 24 mg twice daily. A daily dose of 48 mg correlates with a dose of 0.8 mg/kg/day in our patient, which may potentially lead to toxic peak

levels. To prevent potentially toxic peak levels, the dosing interval of TAC was shortened to 16 mg three times daily at day 17. Because of the lower doses per administration, lower peak levels will be reached. Subsequently, TAC trough levels will be higher due to a shorter elimination time of TAC.

The AUC is the best marker for total drug exposure and could be calculated based on a limited number of blood samples strategy using Bayesian estimation. At day 18, blood samples were drawn 30 minutes before the next dose and 1, 2 and 4 hours after TAC dosing; the measured concentrations were 12.3, 11.6, 12.8 and 28.9 µg/L respectively. Note that at day 18, a single dose of fluconazole 400mg was administered because of its ability to inhibit CYP3A enzymes. The AUC_{0-8} was 240 µg*u/L, calculated with MW/Pharm and the trough level was 12.3 µg/L. It should be taken into account that our patient was on a three times daily dosing regimen, which reflects an AUC_{0-8}. Our calculated AUC_{0-24} (720 µg*u/L) was higher than the target AUC_{0-24} (400 – 420 µg*u/L).(5) Guy-Viterbo *et al.* (6) showed that fluconazole significantly increased TAC trough levels from day 2 to 30 post transplantation, especially in CYP3A5 expresser recipients. The combination of single-dose fluconazole administration and shortening of the dosing interval may have positively influenced the TAC exposure. However, our patient did not have a fungal infection, so multiple daily dosing of fluconazole to efficiently balance inhibition of CYP3A5 would not be appropriate. As biopsies of the liver graft were already taken, we genotyped both our patient and the donor liver graft after informed consent was obtained. Genomic DNA was extracted from whole blood of the patient and from the donor liver biopsy using the Total Nucleic Acid DNA isolation kit on a MagnaPure Compac (Roche Diagnostics, Mannheim, Germany). Genotyping of the CYP3A4*22 and CYP3A5*3, *6 and *7 SNPs were performed using the TaqMan® (ThermoFisher Scientific, CA, USA) genotyping assays according to manufacturer instructions. The results suggests that our patient is a CYP3A5 expresser (CYP3A5 *1/*1) with a normal CYP3A4 enzyme activity (CYP3A4 *1/*1B) explaining low TAC exposure. However, the results of the donor liver graft showed that the donor liver has a reduced CYP3A4 activity (CYP3A4 *1/*22) and nonfunctional CYP3A5 enzymes (CYP3A5 *3/*3). In theory, this genotype would cause higher TAC exposures in patients.(6)

Several studies showed that, in adult liver transplant patients, CYP3A5 expression in liver donor grafts and in transplant recipients resulted in higher TAC daily doses to achieve adequate TAC exposure. Initially, the recipient CYP3A activity seems to have the greatest influence on TAC pharmacokinetics, but this changes over time when the donor CYP3A activity becomes more important.(7-10) In the case of our patient, the metabolism of TAC in the intestine also had a more important effect on TAC pharmacokinetics than the metabolism of TAC in the donor liver in the first month after transplantation. However, these aforementioned studies mostly describe the influence of the transplant recipients' and donor liver grafts' CYP3A5 status on TAC metabolism. In this case, the donor liver graft was a CYP3A5 non-expresser, but had a reduced CYP3A4 activity (CYP3A4 *1/*22), which have not yet been studied in combination with a transplant recipient CYP3A5 expresser. Therefore, we could hypothesize that the clearance of TAC by the donor liver is reduced because of its decrease in CYP3A4 expression and therefore more TAC is metabolized in the intestine, resulting in a substantially increased clearance because of its CYP3A5 expression. At day 31, our patient achieved adequate TAC levels (6.0 µg/L) with a dosing regimen of 10mg TAC three times a day. If we had genotyped this patient before

transplantation, we would have started with a dose of 0.3 mg/kg/day. This would have resulted in higher pre dose concentrations early after transplantation, however not as high as needed to reach the therapeutic window.

In conclusion, this case shows the difficulties of adjusting dosing regimens to obtain adequate TAC levels in patients with CYP3A genetic polymorphisms.

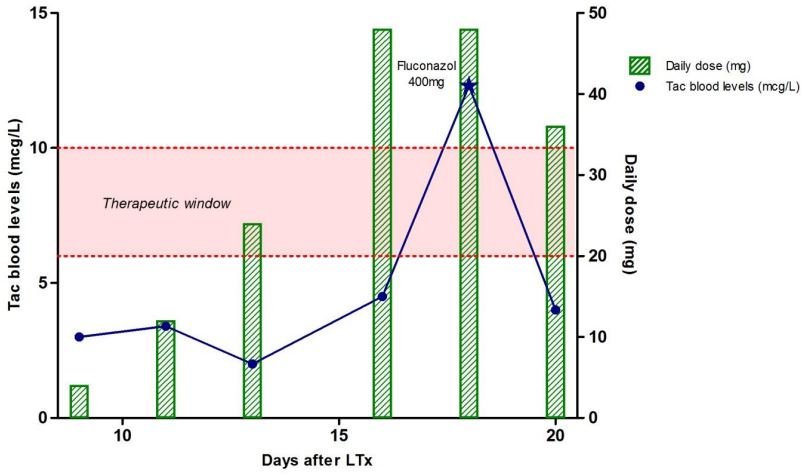

Figure 1. TAC trough levels in µg/L (blue line) and daily doses in mg (green bar) versus days after LTx.

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Chapter 14

Oral antibiotics lower mycophenolate mofetil drug exposure, possibly by interfering with the enterohepatic recirculation: a case series.

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Abstract

Mycophenolate mofetil has an important role as immunosuppressive agent in solid organ transplant recipients. Exposure to the active mycophenolic acid (MPA) can be monitored using therapeutic drug monitoring. We present three cases in which MPA exposure severely decreased after oral antibiotic co-administration. By diminishing gut bacteria β-glucuronidase activity, oral antibiotics can prevent deglucuronidation of the inactive MPA-7-O-glucuronide metabolite to MPA and thereby possibly prevent its enterohepatic recirculation. This pharmacokinetic interaction could result in rejection, which makes it clinically relevant in solid organ transplant recipients, especially when therapeutic drug monitoring frequency is low. Routine screening for this interaction, preferably supported by clinical decision support systems, and pragmatic close monitoring of the MPA exposure in cases is advised.

Introduction

Mycophenolate mofetil (MMF) is the backbone of immunosuppression in solid organ transplantation patients to reduce the risk of rejection.(1-3) MMF interferes with de novo synthesis of purine nucleotides in B- and T-lymphocytes by reversibly inhibiting inosine monophosphate dehydrogenase. This results in decreased lymphocyte proliferation and decreased antibody production.(4,5)

Upon ingestion, the pro-drug MMF is rapidly hydrolyzed to the active metabolite mycophenolic acid (MPA; Figure 1).(6) MPA is metabolized by uridine diphosphate-glucuronosyltransferase (UGT) isoenzymes into the inactive MPA-7-O-glucuronide (MPAG).(7) MPAG is excreted via the bile to the intestines and deglucuronidated into MPA again by β-glucuronidase enzymes present in intestinal bacteria. MPA is then reabsorbed into the circulation.(6) This enterohepatic recirculation of MPA comprises up to 60% of the exposure.(6)

MPA and its metabolites exhibit large interindividual pharmacokinetic variability. A relationship between MPA concentrations and allograft rejection has been documented, which makes therapeutic drug monitoring (TDM) of MPA an important tool to prevent inadequate drug concentrations that increase the risk of organ rejection or MPA toxicity.(8-10) The most adequate measurement of MPA exposure is a 12-hour area under the concentration-time curve (AUC[0-12h], reference: 30 – 60 mg*h/L).(11) The MPA C_{max} is observed 0-6 hours after a single dose of MMF orally. A second peak after 6 hours represents re-absorption of MPA through enterohepatic recirculation.(6) This method is sometimes used in clinical setting to address MPA exposure, however steady state MPA trough concentrations are more frequently assessed as a surrogate parameter for exposure.

Emerging evidence suggests disturbance of the MPA exposure during concomitant use of other drugs, e.g. antibiotics.(12,13) Furthermore, the summary of product characteristics of MMF-originator CellCept® specifically mentions ciprofloxacin, amoxicillin/clavulanic acid, norfloxacin/metronidazole and trimethoprim/sulfamethoxazole for this interaction.(14) Nevertheless, routine screening for this interaction is currently not routine clinical practice. We discuss three patients with decreased MPA concentrations during concomitant oral antibiotics.

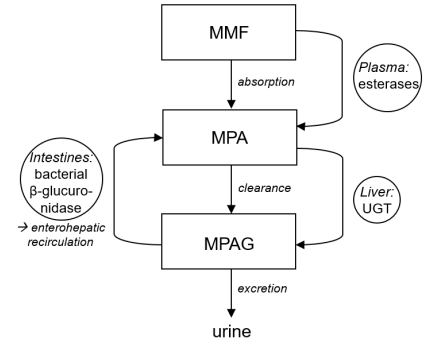


Figure 1. Pharmacokinetics of mycophenolate mofetil (MMF)

MMF mycophenolate mofetil; MPA mycophenolic acid; MPAG MPA-7-O-glucuronide; UGT uridine diphosphate glucuronosyltransferases.

Case #1

A 53-year-old male patient received a liver transplant because of end stage liver disease caused by primary sclerosing cholangitis. He started on tacrolimus, MMF and prednisolone orally. Because of adequate tacrolimus concentrations, MMF was discontinued. For an intra-abdominal infection, intravenous vancomycin and ciprofloxacin were administered. Because of neurological side effects of tacrolimus, the tacrolimus dose was reduced and MMF was reintroduced. The first plasma concentration of MPA (day 4; Figure 2A) was 1.48 mg/L (reference 1-3 mg/L according to local protocol).11 However, on day 8, the MPA concentration was very low and remained low (0.19-0.25 mg/L) after increasing the MMF dose to 1000mg t.i.d. Concomitant albumin concentrations were normal (36-42 g/L, reference 35-50 g/L). As on day 6 ciprofloxacin was switched to oral, an oral ciprofloxacin-induced disturbance of MPA concentrations was suspected. On day 20, all antibiotics were discontinued and on day 22 the MPA concentration was above therapeutic range: 3.47 mg/L. The MMF dose was then reduced to 1000mg b.i.d. The kidney function was mildly decreased in this period of time (eGFR 50-70 ml/min), which was slightly better than the week before (eGFR 40-50 ml/min), but decreased compared to the week after this period of time (eGFR 70-80 ml/min).

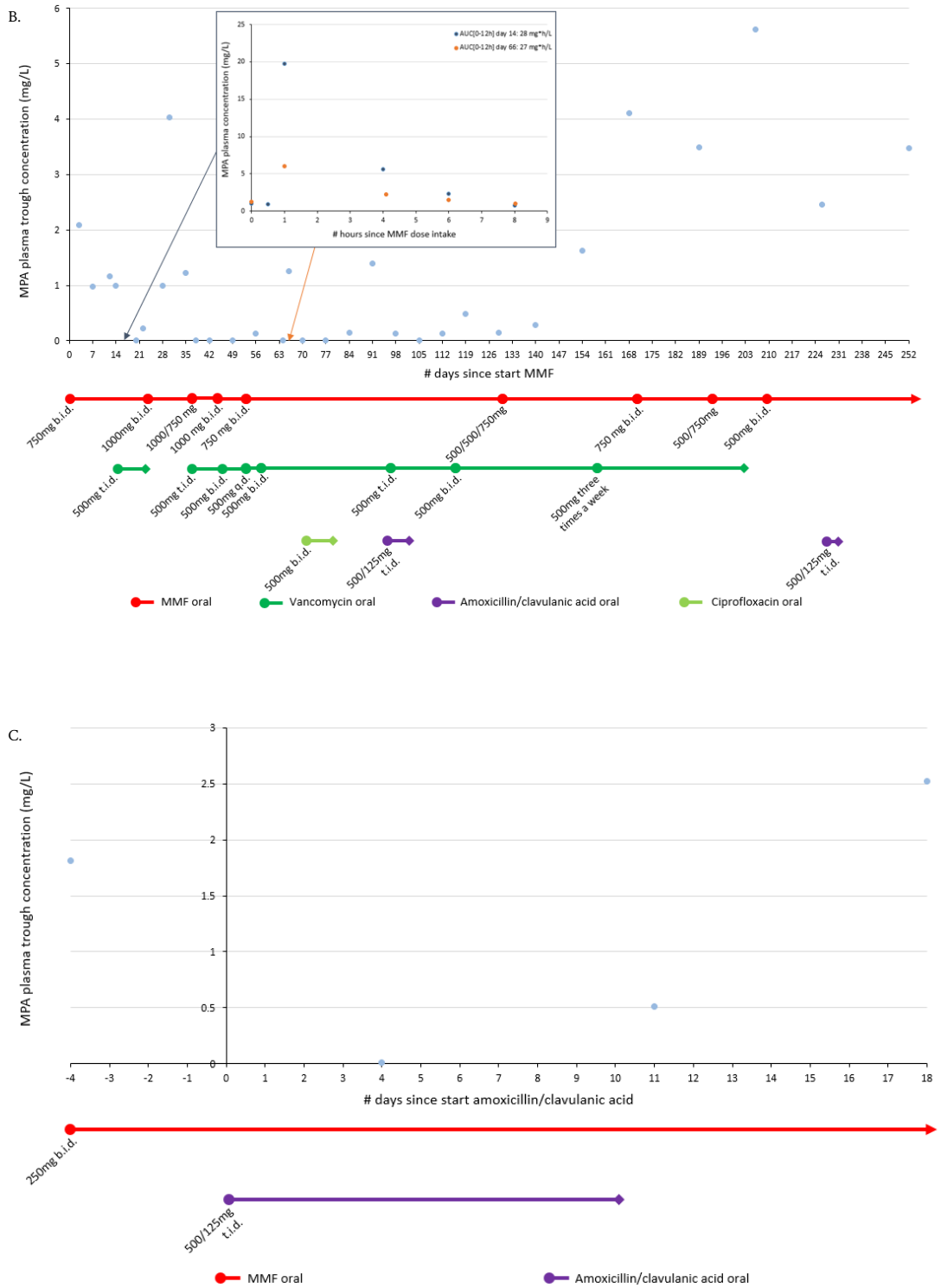
Case #2

A 13-year-old boy was admitted to the hospital for an elective living related kidney transplantation because of congenital uropathy. He received immunosuppression with tacrolimus, MMF and prednisolone orally. When the patient developed diarrhea, proven due to intestinal Clostridium difficile by fecal PCR, oral vancomycin was started for 10 days. Soon thereafter, MPA plasma concentrations decreased to undetectable concentrations (Figure 2B). After increasing the MMF dose to 1000mg b.i.d. and stopping vancomycin, MPA concentrations increased again to above therapeutic range. Unfortunately, due to a persistent Clostridium infection, the patient received prolonged vancomycin therapy and short courses of ciprofloxacin and amoxicillin/clavulanic acid. On several occasions, subtherapeutic MPA concentrations and AUC[0-12h]s were measured for which the MMF dose was adjusted to doses ranging from 1000 to 2500 mg per day, taking into account vancomycin dose changes. Upon lowering the dose and eventually stopping vancomycin as the diarrhea improved, MPA concentrations increased quickly to (above) therapeutic range and MMF was reduced to 500mg b.i.d. The kidney function was stably mildly decreased in this period of time (eGFR 58-74 ml/min), while the albumin concentration was in the normal range (32 – 49 g/L).

Case #3

A 13-year-old girl visited the emergency room with a fever, abdominal pain and a suspected urinary tract infection, 1 year and 9 months since her second renal transplant. She was on immunosuppression with tacrolimus and MMF with adequate exposure (AUC[0-12h] 51 mg*h/L). While awaiting urine cultures, amoxicillin/clavulanic acid 500/125 mg t.i.d. was administered for 10 days (Figure 2C). On day 4, she reported at the outpatient clinic to feel much better although now having diarrhea. The MPA concentration was undetectable. The diarrhea was a suspected side effect of amoxicillin/clavulanic acid, but not likely to be solely responsible for the undetectable MPA plasma concentration. Seven days after completing the antibiotic course, the MPA concentration was in range: 2.52 mg/L (reference >1.9 mg/L according to local protocol).11 The kidney function was stably mildly decreased in this period of time (eGFR 52-62 ml/min). No albumin concentration was measured.

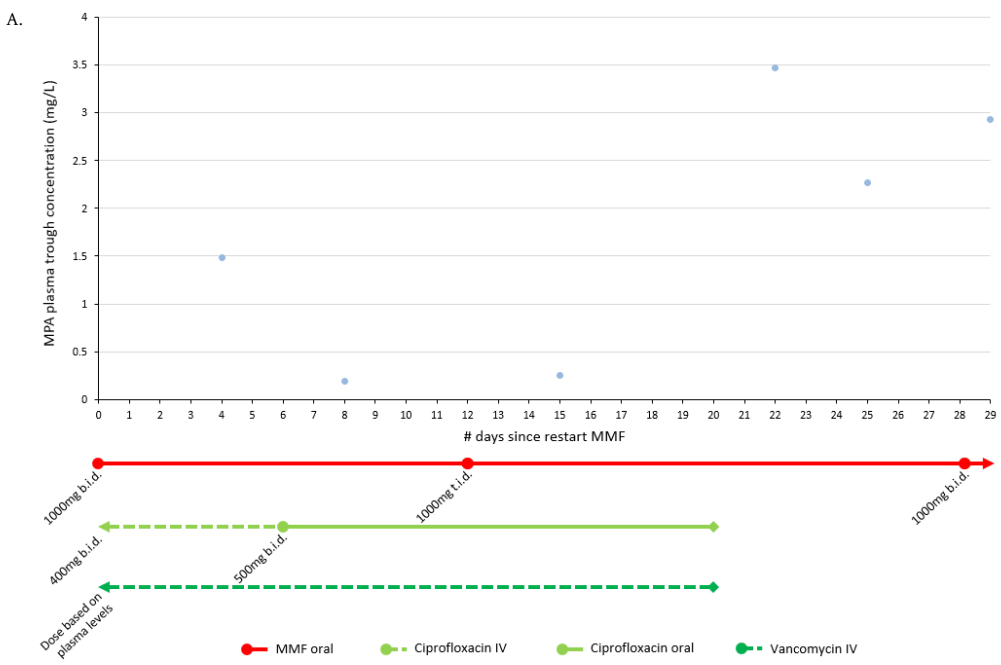
All three patients were Caucasian. These patients were all medication adherent – both anamnestically and proven by administration registration and/or TDM. None of the patients were co-treated with ciclosporin, which is known to inhibit the MPA enterohepatic recirculation, or any other non-antibiotic medication that interacts with MMF or its metabolites.(14)



▼ **Figure 2.** Plasma MPA concentrations and co-medication over time.

Lines connect individual measurements but do not themselves reflect measured values. A. Case 1; B. Case 2; C. Case 3.

AUC[0-12h] reference 30-60 mg*h/l.11 AUC area under the curve; MPA mycophenolic acid; MMF mycophenolate mofetil; b.i.d. twice daily; q.d. once daily; t.i.d. three times a day; IV intravenous.



Discussion and conclusion

We describe three patients with a significant reduction in MPA exposure after starting oral antibiotics, which increased again after lowering the dose or discontinuation of the antibiotics.

Our findings are in line with the few available small cohorts and case series.(12-19) It has been shown that many oral antibiotics can cause an interaction with decreasing MMF concentrations, including rifampicine, norfloxacine/metronidazole, selective bowel decontamination (mycostatin/tobramycin/cefuroxime), ciprofloxacin and amoxicillin/clavulanic acid.(12,13,15-19) However, the summary of product characteristics of MMF-originator CellCept® specifically mentions only ciprofloxacin, amoxicillin/clavulanic acid, norfloxacin/metronidazole and trimethoprim/sulfamethoxazole for such an interaction.(14) The co-administered oral ciprofloxacin and amoxicillin/clavulanic acid in case 2 may thus have had an additive effect on the MPA concentrations, but it seems unlikely that this fully explains the prolonged decreased trough concentrations and AUCs in this case. To the best of our knowledge, we add to the existing literature by being the first to report a similar effect for oral vancomycin.

The exact mechanism underlying the interaction is still unclear. As the AUC[6-12h] and MPA trough concentration are affected predominantly, without a significant effect on AUC[0-6h], Cmax or tmax, the previously suggested mechanism of the interaction is interference with the enterohepatic recirculation.(17) A recent study in hematopoietic cell transplantation patients showed that the MPA trough concentrations, MPA AUC[4-8h], and acyl-glucuronide metabolite (acylMPAG) AUC[4-8h]/AUC[0-8h] ratio and also the abundance of *Bacteroides* species were greater in patients with a higher MPA enterohepatic recirculation compared to patients with low MPA enterohepatic recirculation.(20) This suggests that *Bacteroides* species and the enterohepatic recirculation indeed play an important role in the MPA pharmacokinetics and that antibiotics affecting this system may influence MPA exposure. We observed not only a rapid decline of MPA trough concentrations after start of antibiotic treatment, but also rapid recovery after cessation of antibiotics. This suggests that the deglucuronidating activity of the gut flora and accordingly enterohepatic circulation can be reconstituted.(18) This is corroborated by previous literature, which showed profound and rapid effects of antibiotics, with dysbiosis occurring within three to four days after start of ciprofloxacin in human and recovering – although to an altered state - one week after discontinuation.(21,22) As shown in an *in vitro* experiment, the reduction of MPA exposure might not solely depend on eradication of β-glucuronidase producing bacteria, but also on direct non-competitive inhibition of intestinal β-glucuronidase activity.(23) This is also illustrated by case 1, in which MPA trough concentrations recovered very quickly after withdrawal of ciprofloxacin. A possible enterohepatic circulation interfering effect therefore seems antibiotic-specific rather than a group effect, as inhibition of *in vitro* β-glucuronidase was observed for ciprofloxacin and enoxacin but not for levofloxacin and ofloxacin.(23)

Recently, more evidence is appearing regarding the influence of immunosuppressants on the gut microbiome. Tacrolimus and prednisolone are associated with pro-inflammatory dysbiosis, and alterations in the intestinal barrier and MMF is associated with pro-inflammatory dysbiosis and increased endotoxemia.(24) In mice it is shown that MMF was responsible for an increase in *Clostridia* and *Bacteroides* spp. β-Glucuronidase is expressed by some Bacteroides and as a consequence MMF stimulates the activity of gut β-glucuronidase in the cecum and the colon.(25) Furthermore, in these mice it was shown that addition of vancomycin was responsible for

a decrease in *Bacteroides*, β-glucuronidase activity, and free MPA in mice stool.(25) This is an interesting finding, as *Bacteroides* are a genus of gram-negative bacteria and vancomycin only affects gram-positive bacteria such as enterococci and staphylococci. Nevertheless, antibiotics against gram-negative bacteria might be suspect for having a significant impact on the MPA blood concentrations in transplant recipients, but other antibiotics may have a similar effect through an alternative or indirect mechanism. This warrants further research.

Because the enterohepatic recirculation may account for up to 60% of the MPA AUC[0-12h] and bacterial infections are common in patients using immunosuppressants such as MMF, interference with the enterohepatic recirculation by antibiotics may have a significant impact on MPA exposure and result in potentially ineffective immunosuppression.(6) In a clinical setting, TDM of MPA is performed regularly. However, outpatient prescribers less familiar with transplant patients, may start antibiotics for various indications. Unfortunately, this interaction is not regularly monitored and many physicians and (community) pharmacists' may not be aware of this effect of oral antibiotics on MMF. Furthermore, most of these interactions are not included in clinical decision support systems, which makes routine identification and management of these interactions difficult. Without digital monitoring for the MMF-antibiotics interaction, medication reconciliation is essential for prescribers to be informed about the current (antibiotic) drug use by their patients. Altered exposure to MPA can be detected using TDM. However, trough concentrations as were mostly measured in our cases according to local routine clinical practice, are not strongly associated with the exposure, for which the AUC[0-12h] (full or even with limited sampling strategies) is a better measure.(11) MPA trough concentrations also exhibit more intra-individual variability than the AUC[0-12h].(11) In addition, it is important to take into account that the enterohepatic recirculation predominantly influences the AUC[6-12h]. For this reason, trough concentrations may not adequately represent changes in overall MPA exposure as a result of the interaction. Also, one should bear in mind that the effect on the MPA plasma concentrations may reduce again with continued antibiotic use and usually diminishes within days after antibiotic discontinuation.(14) Although a pre-emptive dose increase is not supported by the literature so far, close monitoring of the MPA exposure, ideally with AUC[0-12h], and graft function during and shortly after antibiotic use is necessary. A pragmatic approach would be to measure the MPA trough concentration 3-4 days after the start of a >1 week course of an interfering antibiotic, adjust the dose accordingly and repeat this about a week after the antibiotic course.

Although more prospective research is needed into which antibiotics are involved in this interaction and through which exact mechanism, we recommend caution in transplant recipients on MMF with co-prescriptions for oral antibiotics to prevent organ rejection. Furthermore, we suggest routine screening for the combination of MMF and oral antibiotics interfering with the enterohepatic recirculation, preferably using clinical decision support systems.

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Part V

General discussion and summary

Chapter 15

General discussion and future perspectives

Midas B. Mulder

In this thesis multiple aspects of pharmaceutical care for liver transplant recipients have been evaluated. The main findings are discussed in this chapter, along with the future perspectives of the pharmaceutical care for liver transplant recipients.

Optimizing immunosuppressive therapy in liver transplant recipients

Liver transplantation (LT) is the preferred treatment in patients with end-stage liver disease and hepatocellular carcinoma (HCC), with 1-year patient survival exceeding 80%.(1) After transplantation, the overall approach to immunosuppression varies widely between transplant centers worldwide. Immunosuppression after LT can be divided into the induction and maintenance phase. During the maintenance phase, the cornerstone of the immunosuppressive regimen after LT are the calcineurin inhibitors (CNIs), specifically tacrolimus.(2,3) Prolonged use of tacrolimus is associated with significant short- and long-term toxicity, such as neurotoxicity, nephrotoxicity, post-transplant diabetes mellitus (PTDM) and hypertension.(4-6) Several studies showed that after transplantation an overwhelming majority (>50%) of LT recipients develop chronic kidney disease defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73m2.(7,8)

The addition of sirolimus to the immunosuppressive regimen

In the last decade, many studies investigated different immunosuppressive strategies in order to prevent the short- and long-term toxicity of immunosuppression.(9) Tacrolimus minimizations studies have been performed in which tacrolimus is reduced or withdrawn completely.(10,11) To prevent chronic kidney disease in our liver transplant patients, tacrolimus is postponed early after transplantation and introduced on day 5 after LT based on the ReSpECT study.(12) Another approach applied to prevent significant tacrolimus toxicity is to start combination therapy of low-dose tacrolimus and mycophenolic acid or low-dose tacrolimus and a mTOR-inhibitor (everolimus or sirolimus).(13-17) In addition, mTOR-inhibitors suppress the mTOR signaling pathway which plays a role in tumor angiogenesis and proliferation in HCC.(18) Results from several studies suggest that sirolimus reduces the HCC recurrence rate after LT, which makes sirolimus in combination with low-dose tacrolimus an interesting immunosuppressive regimen for patients transplanted because of HCC.(19-21)

To date, the combination of tacrolimus and sirolimus, a mTOR inhibitor, has not been extensively studied on the long-term toxicity. We evaluated (**chapter 2**) whether the combination of low-dose sirolimus and low-dose extended-release tacrolimus compared to normal-dose extended-release tacrolimus results in a difference in the renal function and comparable rates of rejection, graft and patient survival at 36 months after transplantation (the LOLIII study). In this study 196 patients were included and we found that the incidence of chronic kidney disease at 36 months was not different between the control (once daily normal-dose extended-release tacrolimus) and interventional group (once daily combination therapy of SRL and low-dose extended-release tacrolimus): 50.8%, 95% confidence interval 39.7% – 59.9% versus 43.7%, 95% confidence interval 32.8% - 52.8%. No differences in the graft and patient survival and the safety were found between the groups.

In a subset study of the LOLIII study (**chapter 4**) we evaluated the cardiovascular morbidity and mortality and the development of cardiovascular risk factors. In this study 122 liver transplant

recipients were included and we found no difference in the cumulative incidence of any major cardiovascular event (i.e. atrial fibrillation, heart failure, stroke, venous thromboembolism, cardiac arrest and coronary artery disease) at 36 months after transplantation (8.3% versus 8.2%). However, significantly more liver transplant recipients in the interventional group (once daily combination therapy of sirolimus and low-dose extended-release tacrolimus) suffered from hyperlipidemia and hypertension after one year. No differences in the occurrence of diabetes mellitus were found.

Based on these two studies we can conclude that the once daily combination therapy of sirolimus and low-dose extended-release tacrolimus does ultimately not provide less toxicity compared to monotherapy with tacrolimus. However, for selected patients, e.g. liver transplant patients with renal insufficiency but without a metabolic syndrome or patients transplanted because of the presence of a hepatocellular carcinoma, this combination could still be a valuable strategy to minimize tacrolimus exposure.

Life Cycle Pharma-tacrolimus versus extended-release tacrolimus

In 2014 the prolonged-release once-daily tacrolimus formulation, life cycle pharma (LCP)-tacrolimus, (Envarsus®; Chiesi Farmaceutici S.p.A.) was approved and entered the market. Until that moment, liver transplant recipients worldwide have been treated with either the twice-daily capsules (Prograf®, Astellas Pharma) or the once-daily extended-release (ER)-tacrolimus formulation (Advagraf®, Astellas Pharma). LCP-tacrolimus is a prolonged-release tacrolimus formulation utilizing a new drug delivery technology (MeltDose).(22,23) This formulation has lower peak concentration (Cmax), lower peak-through blood level fluctuations and a higher bioavailability compared to the other tacrolimus formulations, resulting in a lower dose requirement to reach a certain tacrolimus exposure.(22,24)

Despite all the effort taken until 2018 to optimize immunosuppressive protocols to reduce CNI toxicity, still significant toxicity occurred. After the introduction of LCP-tacrolimus no head-to-head comparison between the two once-daily tacrolimus formulations had been performed until 2018. We hypothesized (**chapter 5**) that the new tacrolimus formulation (LCP-tacrolimus) could reduce CNI-related toxicity (i.e., neurotoxicity, nephrotoxicity, PTDM and hypertension) compared to the other once-daily ER-tacrolimus formulation in liver transplant recipients. Therefore, in April 2019 we started a multicenter, randomized, controlled trial (the MOTTO study) to investigate whether LCP-tacrolimus compared to ER-tacrolimus results in a difference in clinically relevant outcomes. The primary endpoint was defined as a composite endpoint of any of three events: sustained (>3 months post randomization) PTDM, new onset hypertension, and/or chronic kidney disease, defined as eGFR<60 mL/min/1.73m2 for >3 months during the follow-up. In the MOTTO trial, 106 patients were included. In the intention-to-treat analysis, a statistically significant lower proportion of liver transplant recipients in the LCP-tacrolimus group reached the composite primary endpoint at 12 months compared to the extended-release tacrolimus group (50.9% [27/53], 95% confidence interval (CI) 37.9-63.9% versus 71.2% [37/52], 95%CI 57.7-81.7%; risk difference: 0.202; 95%CI 0.002–0.382; p = 0.046). No differences in rejection rate, graft and patient survival were found. We concluded that LCP-tacrolimus resulted in a significant and clinically relevant decrease in the prevalence of the primary endpoint as compared to ER-tacrolimus in the first year after liver transplantation with comparable efficacy.

Health-related quality of life and immunosuppression

Next to the clinically relevant outcomes, nowadays health-related quality of life (HRQoL) is an important and integral part of the evaluation of interventions in modern medicine.(25) Physical, mental and social aspect of health can be assessed by asking patients directly. These domains of health are called patient-reported outcomes and are measured using standard, validated, questionnaires, patient-reported outcome measures (PROMs).(26) By understanding the different aspects of health in which a patient is most affected, health care providers can make better choices in patient care and facilitate better communication with the patients.

After LT, HRQoL generally reaches a similar level as the general population, except for physical functioning.(27,28) Most transplant recipients need to take lifelong immunosuppressive agents. These immunosuppressive agents can cause multiple side effects that might negatively affect the daily life of LT recipients.(29) Therefore, the choice of immunosuppressive agents could have an impact on the HRQoL of LT recipients. Furthermore, fatigue and tremors in liver transplant recipients are a major issue. Peripheral tremors are the most frequent occurring neurological side effect in about 30% - 55% of the solid organ transplant recipients on tacrolimus.(30) Tacrolimus blood trough concentration are associated with the severity of tremors.(30)

The impact of different immunosuppression regimes and the two once-daily tacrolimus formulations on the HRQoL, the severity of fatigue and tremors in LT recipients is largely unknown. Therefore, we evaluated these aspects as a secondary endpoint in the two multicenter, randomized controlled trials (the LOLIII and MOTTO study). In HRQoL study of the LOLIII (**chapter 3**) we found that liver transplant recipients reported the least problems in the states of Self-Care and Anxiety/Depression and the most problems in the states of Usual Activities and Pain/Discomfort. No significant differences in HRQoL and severity of fatigue were seen in the combination of low-dose sirolimus and low-dose extended-release tacrolimus group compared to normal-dose extended-release tacrolimus group. In the HRQoL study of the MOTTO (**chapter 6**) we found no statistically significant differences in the HRQoL and frequency and severity of tremors in LT recipients between the LCP-tacrolimus group compared to the ER-tacrolimus. In the intention-to-treat population, at 12 months 25% [10/40], 95% confidence interval (CI) 14.2% - 40.2% of the LT recipients in the LCP-tacrolimus group experienced tremors compared to 30.4% [14/46], 95%-CI 19.1% - 44.8% of the LT recipients in the ER-tacrolimus group; risk difference: 0.054; 95%-CI -0.151 – 0.249; p=0.63. Based on our studies we cannot recommend one immunosuppression regime or once-daily tacrolimus formulation over the other to improve the HRQoL. After LT, multiple aspects contribute to HRQoL and based on comorbidities and the risk of side effects (e.g. renal insufficiency, metabolic syndrome, tremors or other forms of neurotoxicity) of the LT recipient the choice of immunosuppression should be made.

In daily clinical practice, patient-reported outcome measures (PROMs) are currently not systematically used in the transplant care although this might be useful to optimize the LT recipients' well-being in the long-term.(26,31) The most frequent PROMs are instruments evaluating the generic HRQoL such as the SF-36 questionnaire, whereas LT-specific instruments are lacking. Generic instruments have several desirable characteristics, like a wide scope and the possibility to compare HRQoL with populations norms. But given that we found minimal differences in the HRQoL using the generic HRQoL instruments, more sensitive LT-specific instruments may have be a better potential to guide treatment options.

A major limitation of the LOLIII and MOTTO study is the fact that almost half of the patients in the study groups (interventional and control) switched immunosuppressive therapy because of toxicity (deterioration of the kidney function), side effects (tremors), rejection or preference of the treating physician. This is a consequence of performing studies with immunosuppressive drugs that reflect the clinical practice setting in transplant care. However, the significant deviation and the high number of patients switching the immunosuppressive regimen could introduce selection bias and therefore difficulties with interpreting the intention to treat and per protocol results. As a results of the high number of LT recipients switching therapy, the results in the intention to treat analysis might be underestimated. The results in the intention to treat and per protocol analysis are consistent in the LOLIII and MOTTO studies supporting the main conclusions of these studies. In other studies, investigating immunosuppressive agents this type of bias has also been addressed.(32,33)

Future research with immunosuppressive agents

In the future, research with immunosuppressive agents in the transplant field should continue focusing on minimizing CNI toxicity (specifically less chronic kidney disease, hypertension, diabetes mellitus and tremors). In the past, several studies on CNI withdrawal have been performed with disappointing results. These studies showed that in only 20 – 40% of very carefully selected LT recipients CNI withdrawal was feasible.(34,35) Due to the high risk of acute rejection, especially on the short-term after transplantation, the clinical benefit of this strategy is very limited. To increase the clinical benefit of this strategy a research group is investigating biomarker-guided immunosuppression withdrawal. However, recently the investigated biomarker appeared to be not accurate in predicting the success of immunosuppression withdrawal.(36) Based on the limited progression, CNI withdrawal strategies in the current setting of liver transplantation are presumably not the way to go.

A new study with LCP-tacrolimus aiming for CNI minimization and thus lower tacrolimus trough levels (for example tacrolimus trough levels of 4-6 μg/L for monotherapy tacrolimus) and evaluating clinically relevant outcomes such as kidney function, cardiovascular disease, recurrence of disease and development of de novo malignancies after one year could be of added value to the field. Furthermore, the beneficial effect of LCP-tacrolimus should be evaluated in other solid organ transplant recipients. Finally, a one size fits all immunosuppressive approach is not suitable anymore with the increasing number of pre-existing comorbidities in liver transplant patients. The etiologies in cirrhosis and thus the indications for LT are shifting with a decrease in LTs for viral hepatitis C and an increase in LTs for non-alcoholic steatohepatitis (NASH) or the new name "metabolic dysfunction-associated steatohepatitis" (MASH).(37) These patients often have more pre-existing comorbidities before the transplantation such as obesity, diabetes mellitus and hypertension. Therefore, a more personalized way of initiating and adapting the immunosuppressive protocol in liver transplant recipients should be investigated in relation to the development of CNI toxicity and cardiovascular risk factors. In addition, a stricter compliance with the study protocol should be aimed for in future trials to reduce the chance of selection bias, increase the generalizability and clinical impact and to easy the interpretation of the results of the immunosuppressive trial.

Currently, many research groups are investigating machine perfusion technologies to improve the quality of the livers and eventually increase the number of organs available for transplantation.(38)

Machine perfusion technologies could also become a platform for immunological modulation and repair/regeneration of damaged donor livers before implantation.(39) Some research has been performed on safety and efficacy with regulatory T-cell-based cell therapy post-liver transplantation in order to reduce or completely discontinue immunosuppression.(40,41) In the future, Advanced Therapy Medicinal Products (ATMPs) could be developed that interfere with the human leukocyte antigen (HLA) system of the donor organ. These ATMPs could be administered during the machine perfusion in order to reduce the immunogenicity of the organ before transplantation and consequently reduce the need for immunosuppressive agents' post-transplantation. Finally, progression in organ bioengineering and xenotransplantation could also alter the need for immunosuppression following transplantation.

Optimizing therapy for viral complications after transplantation

Viral infections are well recognized complications of immunosuppression and can occur from the donor (donor-derived infections), reactivation of endogenous latent virus, nosocomial sources or from the community.(42) Viral infections occur usually after the first month after LT. In this thesis we focused on the treatment of chronic hepatitis E virus infections with ribavirin and the antibody response of liver transplant recipients after vaccination against severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infections.

Chronic hepatitis E virus and optimal dosing strategy for ribavirin

Hepatitis E virus (HEV) infection is one of the most common causes of acute viral hepatitis worldwide and in solid organ transplant (SOT) recipients, HEV can cause chronic hepatitis and cirrhosis if undiagnosed or left untreated.(43,44) The current clinical practice guidelines on HEV of the European Association for the Study of the Liver (EASL) recommend to lower immunosuppressive drug therapy in SOT recipients with a chronic HEV infection.(45) This results in a sustained virologic response (SVR) in approximately one-third of the SOT recipients.(46) If this is not possible or unsuccessful, a 3-month course of (off-label) ribavirin is recommended.(47-50) Ribavirin inhibits HEV replication *in vitro*.(51) *In vivo*, first-line ribavirin therapy was associated with a SVR of around 80%.(52) However, the use of RBV is associated with some severe side effects such as mood disturbances, sleeping disorders, neuropathy and (severe, dose-dependent) hemolytic anemia.

Due to the low incidence of chronic HEV infections in SOT recipients, no pharmaceutical company is currently investigating anti-viral therapies for this indication. Furthermore, ribavirin has even been withdrawn from the Dutch market. Ribavirin was formally only registered to administer to patient with a chronic hepatitis C virus infection. With the introduction of the new, more effective direct-acting antivirals for chronic hepatitis C virus infection, only a very few patients used ribavirin in the Netherlands yearly and therefore the pharmaceutical company has decided to withdraw ribavirin for the Dutch market.

The current research in the field of ribavirin and a chronic HEV infection is dependent on investigator-initiated case-reports and case series. So far, no study investigated the optimal therapeutic range and dose regimen for ribavirin in SOT recipients suffering from a chronic HEV infection. Currently, ribavirin doses range between 29 and 1200 mg daily and treatment duration varies between 0.25 and 18 months.(52)

In a multicenter cohort study, we collected retrospectively data of adult SOT recipients with chronic HEV infection. First, we performed a study (**chapter 7**) evaluating the ribavirin trough levels, hemoglobin levels and viral load. In this analysis (**chapter 7**) we found that higher ribavirin plasma concentrations resulted in more hemoglobin reduction and that the therapeutic range of ribavirin for chronic HEV infection in SOT recipients ranged between 1.8 and 2.3 mg/L. However, this analysis is a simple approach and not reflecting complex biological processes. Therefore, in the subsequent population PK and pharmacodynamic (PD) study we used non-linear mixed-effect modeling (NONMEM) (**chapter 8**) in which a compartmental approach is applied.(53) Compartmental modeling methods consider the body to consist of a finite number of interconnected compartments (e.g., blood, organs, and other tissues). NONMEM is the golden standard in drug development in order to explore and describe PK and PD variability in different patient groups and an established population PK/PD model has the possibility to be used in simulations and inform about optimal dosing regimens.(54) We found (**chapter 8**) that a model-supported ribavirin dose of 600 mg/day with a kidney function ≥60 ml/min/1.73m^2, 400 mg/day with a kidney function 30-59 ml/min/1.73m^2 and 200 mg/day with a kidney function ≤30 ml/min/1.73m^2 for 180 days showed good efficacy and low toxicity. Interestingly, due to the suggested low IC50 of ribavirin for HEV, potentially even lower dosages of ribavirin might be possible in the treatment of a chronic HEV infection in SOT recipients. However, we could not simulate the effect of lower ribavirin dosages with our model as compared to the recommended dosages in chapter 8 due to safety concerns regarding the efficacy of ribavirin and the fact that not enough SOT recipients in our cohort were treated with lower dosages. In addition, the most important finding from our analysis is that ribavirin should not be stopped to soon, i.e. within 6 months, due the persistence of HEV RNA in the stool in patients with undetectable HEV RNA in the serum.(45) Moreover, SOT recipients with a chronic HEV infection treated with a lower dosage of ribavirin for a longer time period have a higher chance of a successful treatment compared to SOT recipients treated with higher dosages. Since the use of ribavirin is limited by dose-dependent (severe) hemolytic anemia which often necessitates ribavirin dose reduction or discontinuation. Based on our study it seems prudent and feasible to start a non-inferiority, prospective trial evaluating the effect of low dose ribavirin on HEV clearance in SOT recipients in the near future.

Immunosuppressive drugs and the immunogenicity to SARS-CoV-2 vaccines

The severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) originated as a human virus in December 2019.(55) Since then, the virus has been travelling all over the world. In the beginning of 2021 four vaccines became available in the European Union to prevent coronavirus disease 2019 (Covid-19), which is caused by SARS-CoV-2.(56) These mRNA vaccines and adenovirus-based vector vaccines showed a strong efficacy in clinical trials.(57-59) However, several studies showed that the immunogenicity to SARS-CoV-2 vaccines in SOT recipients was reduced after the first vaccinations, with detectable antibodies ranging from 30% - 65% for all types of solid organ transplantation.(60-64) Variables associated with a reduced immunogenicity are older age, regimens that includes mycophenolate mofetil (MMF), renal insufficiency and time after transplantation.(65,66)

As described in the introduction of this thesis, many different immunosuppressive agents with different blood levels and mechanisms of action are used in the transplant field. Specific guidance with regards to immunosuppressive blood levels in relation to the immunogenicity of

SARS-CoV-2 vaccines in SOT recipients was lacking. Therefore, we investigate the effect of immunosuppressive blood levels on the SARS-CoV-2–specific immunogenicity of SARS-CoV-2 vaccination in LT recipients.

First, we showed (**chapter 9**) that two doses of mRNA vaccines (BNT162b2 or mRNA-1273) or the vector vaccine (ChAdOx1 nCoV19) was highly effective in our LT recipient cohort with seroconversion occurring in 79.0% (376/476) of the LT recipients. Focusing on the mechanism of action and drug levels of the immunosuppressive agents is essential to select the right target population for additional vaccinations and to define the right moment for a booster vaccination. MMF is the prodrug of mycophenolic acid (MPA), an inhibitor of inosine-50-monophosphate dehydrogenase. MPA depletes guanosine nucleotides preferentially in T and B lymphocytes and inhibits their proliferation, thereby suppressing cell-mediated immune responses and antibody formation.(67) Tacrolimus, corticosteroids and mTOR-inhibitors deplete only the T lymphocytes and indirectly the B lymphocytes.(68-70) In our population the majority of LT recipients is using monotherapy of tacrolimus which could explain the high seroconversion rate.

Previously, it has been shown that the antibody response to the influenza vaccination is modulated by MMF. High doses of MMF alter the T-Helper 2 and B-Cell responses and causing lower seroconversion rates to influenza vaccination.(71) Therefore, LT recipients do receive two influenza vaccinations whereas in the population without immunosuppression one influenza vaccination is sufficient. With the ongoing pandemic of SARS-CoV2-2 in 2021 and 2022 additional vaccinations for the population including SOT recipients became available. As a result, we could investigate whether the immunogenicity in LT recipients using MMF after a third, fourth or fifth SARS-CoV-2 vaccination was altered.

We found (**chapter 10**) that LT recipients using MMF had a high seroconversion rate (41/48, 85.4%) after three SARS-CoV-2 vaccinations. This was a significantly higher percentage compared to the seroconversion rate after two SARS-CoV-2 vaccinations in LT recipients using MMF (20/48, 41.7%). Furthermore, regardless the MPA trough levels, LT recipients using MMF showed positive IgG anti-spike SARS-CoV-2 levels after the 3rd, 4th and 5th vaccination. We concluded that LT recipients can continue MMF during additional vaccinations and that LT recipients using MMF do need at least one additional vaccination to reach a comparable seroconversion rate as LT recipients not using MMF.

Lessons learned for future research in optimizing therapy for viral complications after transplantation

A limitation of the work that we performed on ribavirin and chronic hepatitis E virus is the fact that the data were collected retrospectively and that we had no control over the dosing regimen of ribavirin. Therefore, initial dosing and dose adaptations depended on time varying patient status which could have resulted in confounding by indication. This could have affected the results, i.e., an over-estimation of efficacy. SOT recipients who did not achieve viral load reduction would likely be up-titrated during the ribavirin therapy. In the future a prospective study with controlled dosing would be able to address this and to evaluate the efficacy and safety of the proposed dosing regimen. Ideally, this prospective study will be performed with a control arm to compare the new dosing regimen with the current practice. However, since yearly only a very limited number of SOT recipients with a chronic HEV infection in the Netherlands start with ribavirin therapy, a

controlled study would be hardly unfeasible to perform solely in the Netherlands. A European multicenter study under the umbrella of either the European Centre for Disease Control or the European Society of Organ Transplantation is advocated to study new dosing regimen of ribavirin in solid organ transplant recipients with HEV infection. Furthermore, to increase the generalizability of the results a protocol with fixed steps describing when and to what dosage the immunosuppression should be lowered before the start of ribavirin is essential.

The most important lesson learned for the future in immunosuppressive drugs and the immunogenicity to SARS-CoV-2 vaccines based on our two studies (**chapter 9 and 10**) is the fact that considering and focusing on the mechanism of action of the immunosuppressive agents is crucial to achieve good immunogenicity to vaccinations. In case a new pandemic occurs, health care providers should consider whether their patients use drugs that deplete the T lymphocytes or the B lymphocytes or both and individualize their immunosuppressive medication accordingly to optimize the immune response. Especially, in patients using immunosuppressive agents affecting the B lymphocytes (e.g. MMF, JAK inhibitors and rituximab) one should be aware, monitor the immune response and even stop these agents temporarily or administer additional vaccinations.

Addition of a clinical pharmacist in the liver transplant care

Outpatient clinical pharmacy service

After transplantation, LT recipients will usually end up with multiple drugs over the years due to the development of comorbidities such as renal insufficiency, hypertension and post-transplant diabetes mellitus. Furthermore, adherence to immunosuppressive medication and avoidance of contra-indicated drugs are two potential modifiable risk factors to improve long-term outcomes in LT recipients.(72) Over the years, we learned that medication errors contribute to a substantial number of unplanned hospitalizations.(73,74) Therefore, identification and management of medication-related problems (MRPs) is essential to improve medication safety.

In the Anglo-Saxon countries, clinical pharmacists have been involved in the direct patient care in transplantation since the early 1970s. In the Netherlands, in the last decades the role of community pharmacists has been evolved from a product to a service and patient focus. However, pharmacists working in the hospital as clinical pharmacists are more recently starting to be involved in the direct care for hospitalized patients. In addition, only a few clinical pharmacists are involved in the outpatient care as well.

So far, no clinical pharmacist has been structurally involved in the outpatient care of liver transplant recipients in the Netherlands. At the Erasmus University Medical Center, we have added a clinical pharmacist to the LT program in September 2018 as part of the integrated patient care. A newly established 20-minute face-to-face consultation with the clinical pharmacist was added to the annual check-up of the LT recipients. We evaluated the addition of the clinical pharmacist in the outpatient setting. In a first cohort study (**chapter 11**) we evaluated 64 LT recipients who had a 20-minutes consultation with the clinical pharmacist and found that most frequent MRPs were ADRs, nonadherence, unnecessary drugs and undertreatment. Next, we compared a group of LT recipients with and without an outpatient medication consultation by a clinical pharmacist (**chapter 12**) to evaluate the prevalence and types of MRPs. Again, we found

that the group LT recipients with an outpatient medication consultation by a clinical pharmacist experienced significantly fewer MRPs as unnecessary drugs, suboptimal therapy, untreated indications and underdosed drugs.

In the current global situation with the aging of the population and increasing healthcare costs adding a clinical pharmacist to the outpatient care of transplant recipients can contribute to sustainable use of medicines and prevents for unnecessary drug use. However, currently adequate financial reimbursement for outpatient activities of the clinical pharmacists in the Netherlands is still lacking and preventing institutionalization of this outpatient service.

In Germany, recently five clinical pharmacy services are now reimbursed by all health insurance companies.(75) One of the five services is a medication review including follow-up for patients taking immunosuppressants post-transplantation. In the Netherlands, medication reviews are reimbursed for community pharmacists and not for clinical pharmacists. The amount of compensation for community pharmacists is depending on the health insurance company of their patient. Since most community pharmacists have no or very little experience with the transplant care, they will not perform a high-quality medication review for transplant recipients. Therefore, in the future an adequate reimbursement and a sustainable provision of transplant services by a clinical pharmacist in every transplant hospital in the Netherlands are necessary to improve the clinical care for these patients.

Therapeutic drug monitoring of immunosuppressive agents

In **chapter 13 and 14** we highlighted in a case-report and case series the importance of adding a clinical pharmacist to the transplant care. First, in **chapter 13** we presented a case of an African American woman who underwent a liver transplantation in which adequate tacrolimus levels were difficult to accomplish due to differences in cytochrome P450 3A4/5 (CYP3A4/5) polymorphisms of the transplant recipient and the donor liver graft. This case-report showed that genotyping the liver transplant recipient and the donor liver graft might provide relevant data which could be used in population pharmacokinetic and pharmacodynamic models to predict the tacrolimus metabolism post-transplantation and to increase efficacy and reduce toxicity. In the near future, personalized medicine in the transplant care will be of greater interest and model-informed dosing tools will become available in the hospital information systems to optimize the dosing of immunosuppressants. In **chapter 14** we presented a case-series of one adult liver transplant recipient and two teenager kidney transplant recipients in which mycophenolic acid exposure severely decreased after oral antibiotic co-administration. This pharmacokinetic interaction could result in rejection, which makes it clinically relevant in solid organ transplant recipients, especially when the therapeutic drug monitoring frequency is low. The pharmacological background of this interaction and the potential impact marks the importance of an intensive collaboration between clinical pharmacists and transplant physicians.

Future research for clinical pharmacists in the liver transplant care

Future research of added value is a pharmaco-economical study to show the cost-effectiveness of the addition of the clinical pharmacist in the outpatient setting. An analysis of the finding in our two studies (**chapter 11 and 12**) in relation to quality-of-life health-care outcomes would be of interest to calculate the quality-adjusted life year of the new intervention. Furthermore, implementing our outpatient service in other transplant hospitals in the Netherlands and evaluate

this service in those settings could be of added value to design a nationwide framework of clinical pharmacy services for transplant recipients. This framework and a pharmaco-economical study could be helpful to convince the politics and health insurance companies that reimbursing patient-focused pharmacy services by a clinical pharmacist are worth it. Finally, investigating the possibilities of artificial intelligence and e-Health platforms to optimize the pharmaceutical care for transplant recipients is of great interest. Recently, a study protocol of a randomized controlled trial investigating the addition of information technology to increase medication adherence in kidney transplant recipients has been published.(76) Other possibilities for clinical pharmacy services in e-Health platforms in the transplant field are guidance and monitoring of side effects, medication reconciliation and education of transplant recipients.

Conclusion

In the next decades, due to technological developments, healthcare providers will have to deal with an increase in the diagnostic possibilities, surgical techniques and new innovative drugs. Furthermore, the care for liver transplant recipients will become more complex due to an increase in comorbidities. Therefore, as shown in this thesis adapting immunosuppressive agents based on patients' comorbidities and side effects is essential in order to modify and minimize immunosuppressive related toxicity. We showed that LCP-tacrolimus provides better results compared to ER-tacrolimus. Secondly, based on findings in this thesis we suggested that low-dose ribavirin for at least 180 days has a positive effect on hepatitis E virus clearance in solid organ transplant recipients. Next, immunosuppressive agents affecting the B lymphocytes reduce the immunogenicity of vaccination in LT recipients. This should be considered when vaccinating LT recipients. Finally, liver transplantation is only possible with a multidisciplinary team and interprofessional collaborations. Until the start of this thesis, in the Netherlands clinical pharmacists were solely involved in therapeutic drug monitoring of immunosuppressive agents and computerized medication monitoring. In this thesis, we showed that clinical pharmacists can have an added value in the clinical and outpatient transplant care and increase the medication safety and efficacy for these patients. Overall, we added new insights to the field of optimizing drug therapy for LT recipients to improve patient outcomes.

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Appendices

Summary

Liver transplantation (LT) is the preferred treatment in patients with end-stage liver disease and hepatocellular carcinoma. An effective immunosuppressive regimen is essential to reduce graft loss due to acute or chronic rejection. However, too much immunosuppression could cause complications and several causes of death in transplant recipients such as infections, renal failure, malignancies and cardiovascular events. In this thesis, we aimed to optimize drug therapy for liver transplant recipients to improve patient outcomes.

Part II – Optimizing immunosuppressive therapy in liver transplant recipients.
In **chapter 2** we investigated whether the combination of low-dose sirolimus and low-dose extended-release tacrolimus (interventional group) compared to normal-dose extended-release tacrolimus (control group) resulted in a difference in the renal function and comparable rates of rejection, graft and patient survival at 36 months after transplantation (LOLIII study). This study was an open-label, multicenter randomized, controlled trial. The primary endpoint was the cumulative incidence of chronic kidney disease (CKD) defined as grade ≥3 (estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m^2) at 36 months after transplantation. In total, 196 patients were included. CKD at 36 months was not different between the control and interventional group (50.8%, 95% confidence interval (CI) 39.7% – 59.9%) versus 43.7%, 95% CI: 32.8% - 52.8%). Only at six months after transplantation, the eGFR was higher in the interventional group compared to the control group (mean eGFR 73.1±15 versus 67.6±16 mL/min/1.73m^2, p=0.02) in the intention-to-treat population. No differences in the secondary endpoints and the number of serious adverse events were found between the groups. These findings show that once-daily low-dose SRL combined with low-dose extended-release tacrolimus does ultimately not provide less CKD grade ≥3 at 36 months compared to normal-dose extended-release tacrolimus.

In **chapter 3**, we investigated the impact of the immunosuppressive regimens in the LOLIII study on the health-related quality of life (HRQoL) and the severity of fatigue. HRQoL was measured with the EQ-5D-5L questionnaire, the EQ-VAS and the severity of fatigue questionnaire (FSS). The EQ-5D-5L scores were translated to the societal values. We examined the HRQoL and the FSS over the course of the study by fitting generalized mixed effect models. In the multi-center open-label, randomized, controlled LOLIII study, 196 patients were randomized 90-days after transplantation to 1) once daily normal-dose tacrolimus or 2) once daily combination therapy of low-dose sirolimus and tacrolimus. Baseline questionnaires were available for 87.7% (172/196) of the patients. Overall, patients reported the least problems in the states of Self-Care and Anxiety/Depression and the most problems in the states of Usual Activities and Pain/Discomfort. No significant differences in HRQol and FSS were seen between the two groups. During follow-up, the societal values of the EQ-5D-5L health states and the patient's self-rated EQ-VAS score were a little lower than those of the general Dutch population in both study arms. In conclusion, the HRQoL and FSS was comparable in the 36 months after liver transplantation in both study groups. The HRQoL of all transplanted patients approximated that of the general Dutch population, suggesting little to no residual symptoms in the long-term after transplantation.

Chapter 4 of this thesis aimed to evaluate the cardiovascular morbidity and mortality in a subset of the LOLIII study. The primary endpoint was the cumulative incidence of any major cardiovascular event at 36 months after transplantation. The secondary endpoint was to assess the development of cardiovascular risk factors. In total, 122 LT recipients were included.
No difference in the cumulative incidence of any major cardiovascular event at 36 months after transplantation was found. Significantly less LT recipients in the control group suffered from hyperlipidemia compared to the interventional group; year 1 30% [18/60], versus 50.9% [30/59]; risk difference: -0.208; 95%CI -0.378– -0.021; p=0.025) and year 2 (40.3% [23/57] versus 63.2% [36/57]; risk difference: -0.228; 95%CI -0.402– -0.032; p=0.024. The prevalence of hypertension was significantly lower in the control group compared to the interventional group at year 3: 42.1% [24/57] versus 64% [32/50]; risk difference: -0.219; 95%CI -0.399– -0.016 p=0.033). These findings show that a sirolimus-based regimen resulted in comparable cardiovascular morbidity and mortality among liver transplant recipients at 36 months compared to monotherapy tacrolimus. However, after the first year post-LT significantly more hypertension and hyperlipidemia occurred in the sirolimus-based regimen.

In **chapter 5** we investigated whether the Life Cycle Pharma (LCP)-tacrolimus formulation compared to the extended-release (ER) tacrolimus formulation resulted in a difference in the prevalence of post-transplant diabetes mellitus, hypertension and chronic kidney disease (CKD) at 12 months after liver transplantation in an open-label, multicenter, randomized controlled study (MOTTO study). Patients were 1:1 randomized to either of the two tacrolimus formulations. The primary endpoint was defined as a composite endpoint of any of three events: sustained (>3 months post randomization) post-transplant diabetes mellitus, new onset hypertension, and/or CKD, defined as eGFR<60 mL/min/1.73m^2 for >3 months during the follow-up. In total, 105 patients were included. In the intention-to-treat analysis, significantly less liver transplant recipients in the LCP-tacrolimus group reached the composite primary endpoint at 12 months compared to the extended-release tacrolimus group (50.9% [27/53], 95% confidence interval (CI) 37.9-63.9% versus 71.2% [37/52], 95%CI 57.7-81.7%; risk difference: 0.202; 95%CI 0.002–0.382; p=0.046). No significant difference in the composite primary endpoint was found in the per protocol analysis. In the intention-to-treat population, fewer LT recipients in the LCP-tacrolimus group developed CKD and new-onset hypertension compared to the ER-tacrolimus group: CKD 26.4% [14/53], 95%CI 16.4-39.6% versus 42.3% [22/52], 95%CI 29.9-55.8%; risk difference: 0.159; 95%CI -0.035–0.339; p=0.102 and new-onset hypertension 38.1% [16/42], 95%CI 24.9-53.2% versus 54.3% [19/35] 95%CI 38.2-69.5%; risk difference: 0.162; 95%CI -0.076–0.379, p=0.175. No differences in rejection rate, graft and patient survival were found. In conclusion, in the LCP-tacrolimus group significantly less liver transplant recipients reached the composite primary endpoint compared to the ER-tacrolimus group in the first year after liver transplantation with comparable efficacy.

Next, in **chapter 6** we evaluated the health-related quality of life and severity of tremors in the MOTTO study. HRQoL was assessed with the EQ-5D-5L and SF-36 questionnaire (two generic HRQoL instruments) and the quality of life in essential tremor (QUEST) questionnaire (a domain specific HRQoL instrument). The EQ-5D-5L scores were translated to the societal values. We examined the HRQoL over the course of the study by fitting generalized mixed effect models. In total, 105 patients were included, 53 in the LCP- and 52 in the ER-tacrolimus arm. Baseline questionnaires were available for every LT recipient. No statistically significant differences were found at 3, 6 and 12 months in the frequency and severity of tremors in LT recipients in the intention-to-treat (ITT) and per protocol population. In the ITT population, at 12 months 25% [10/40], 95% confidence interval (CI) 14.2% - 40.2% of the LT recipients in the LCP-tacrolimus group experienced tremors compared to 30.4% [14/46], 95%-CI 19.1% - 44.8% of the LT recipients in the ER-tacrolimus group; risk difference: 0.054; 95%-CI -0.151 – 0.249; p=0.63.
No statistically significant differences in HRQoL were seen between the two groups. During follow-up, the societal values of the EQ-5D-5L health states were lower than those of the general Dutch population in both study arms. These findings suggest that the once-daily LCP-tacrolimus formulation is not associated with an improvement in the HRQoL or a reduction in the occurrence of tremors compared to ER-tacrolimus.

Part III – Optimizing therapy for viral complications after transplantation.

Chapter 7 aimed to define the therapeutic range of ribavirin in transplant recipients with chronic hepatitis E virus (HEV) infection in a retrospective, multicenter, cohort study. Data of adult transplant recipients with chronic HEV infection, who had been treated with ribavirin monotherapy between 01-3-2008 and 01-08-2018 were included. ROC-curve analyses were performed and the half-maximal effective ribavirin concentration was calculated to determine a representative therapeutic range. In 96 patients, ribavirin monotherapy for a median of three months resulted in a sustained virologic response in 63.5% of the patients, while 88.5% of the patients developed anemia. Ribavirin plasma concentrations at steady-state were significantly higher in clinical responders compared to clinical non-responders: median 1.96 (IQR 1.81–2.70) versus 0.49 (IQR 0.45–0.73) mg/L, p=0.0004. Ribavirin caused a dose-dependent hemoglobin reduction with higher ribavirin plasma concentrations resulting in more hemoglobin reduction. The therapeutic range of ribavirin for chronic HEV infection in transplant recipients ranges between 1.8 and 2.3 mg/L.

In **chapter 8** we modelled ribavirin plasma concentrations versus virologic response and hemoglobin concentrations using nonlinear mixed-effects modeling. The model was used to select a suitable ribavirin dosing regimen considering efficacy (decrease in viral load) and safety (hemoglobin). Data were collected in a retrospective, multicenter study of adult solid organ transplant recipients with chronic HEV infection treated with ribavirin between 09-2009 and 11-2019. In total, 107 chronically HEV-infected solid organ transplant recipients with 305 ribavrin plasma levels, 592 viral load concentrations and 443 hemoglobin concentrations were included. Sustained virologic response was achieved in 68.2% of the subjects. Due to a low IC50, the decline in viral load was independent of RBV concentration and dose, whereas hemoglobin decreased with increasing RBV concentration and dose. A model-supported ribavirin dose of 600 mg/day with a kidney function ≥60 ml/min/1.73m^2, 400 mg/day with a kidney function 30-59 ml/min/1.73m^2 and 200 mg/day with a kidney function ≤30 ml/min/1.73m^2 for 180 days showed good efficacy and low toxicity. This study constitutes a first step in determining the optimal RBV treatment regimen for chronic HEV infections in SOT recipients. Based on our model, it seems feasible to start a non-inferiority, prospective trial evaluating the effect of low dose ribavirin on HEV clearance in solid organ transplant recipients.

In **chapter 9** we evaluated the effect of immunosuppressive blood levels on the IgG SARS-CoV-2 anti-spike antibody response after SARS-CoV-2 vaccination. In this observational, cohort study, we determined the immunogenicity to SARS-CoV-2 vaccination in liver transplant (LT) recipients in relation to the immunosuppressive blood levels after the 2nd dose of mRNA vaccines or the vector vaccine ChAdOx1 nCoV19. A total of 476 LT recipients were included: 430 received mRNA-1273 vaccine, 25 received BNT162b2 mRNA vaccine and 21 received ChAdOx1 nCoV19 vector vaccine. Seroconversion occurred in 79.0% (376/476) of the LT recipients. LT recipients vaccinated with the mRNA-1273 vaccine had significantly higher IgG SARS-CoV-2 anti-spike antibody levels compared to the other two vaccines, p<0.001.
The use of mycophenolate mofetil (MMF), regardless the blood level, suppressed the IgG SARS-CoV-2 anti-spike antibody response and resulted in suboptimal responders to the SARS-CoV-2 vaccines, whereas the other immunosuppressive agents did not have that effect. SARS-CoV-2 vaccination was highly effective in our LT recipient cohort. The mRNA-1273 vaccine results in a superior IgG SARS-CoV-2 anti-spike antibody response. MMF suppressed the IgG SARS-CoV-2 anti-spike antibody response, regardless the blood levels of MMF and the type of vaccination. Consequently, lowering the dose of MMF has no effect on the immunogenicity to SARS-CoV-2 vaccines.

Chapter 10 of this thesis aimed to investigate the immunogenicity in liver transplant recipients in relation to mycophenolic acid (the active substance of mycophenolate mofetil) blood levels

after a third, fourth or fifth mRNA SARS-CoV-2 vaccination. In this observational, cohort study, we determined the immunogenicity to SARS-CoV-2 vaccination in liver transplant recipients in relation to the mycophenolic acid blood levels after the 3rd, 4th and 5th dose of mRNA vaccines or the vector vaccine ChAdOx1 nCoV19. Multiple linear regression models were fitted, investigating the association between the antibody response on SARS-CoV-2 and the MPA trough levels for the vaccinations. In total, 86 liver transplant recipients were included with 92 IgG anti-spike SARS-CoV-2 titers; six patients had titers available after multiple vaccinations. Significantly more liver transplant recipients had positive IgG SARS-CoV-2 serology after the third vaccination (41/48, 85.4%) compared to the second vaccination (20/48, 41.7%), p<0.001. This increased to 90% after the fourth and fifth vaccination. Mycophenolic acid trough levels were not significantly associated with an effect on the IgG SARS-CoV-2 anti-spike antibodies response after a third, fourth or fifth vaccination. These findings showed that additional SARS-CoV-2 vaccination was highly effective in our cohort. Regardless the mycophenolic acid trough levels, liver transplant recipients using mycophenolate mofetil showed positive IgG anti-spike SARS-CoV-2 levels after additional vaccination. Mycophenolate mofetil could be continued during additional vaccination.

Part IV – Addition of a clinical pharmacist in the liver transplant care.

In 2018 a newly established 20-minute face-to-face consultation for liver transplant recipients with the clinical pharmacist was added to the annual check-up of these patients. The consultation consisted of medication reconciliation and a conversation about medication, adherence, adverse drug reactions and drug use.

Chapter 11 aimed to investigate the prevalence, types and severity of medication-related problems and interventions initiated by a clinical pharmacist in a cohort of liver transplant recipients in the outpatient setting. Discrepancies between actual and intended drug use, and medication-related problems were identified and the severity of medication-related problems was assessed. Potential interventions were discussed with the patient and the treating physician and evaluated after one year. The clinical pharmacist counseled 64 liver transplant recipients and found 96 discrepancies in 37 patients. Most discrepancies (60.4%, n=58) concerned missing medications. In total, 98 medication-related problems were identified in 53 patients (median 2; range 1-5 per patient), with a total of 113 interventions. Most frequent medication related problems were: adverse drug reactions (22.4%, n=22), nonadherence (19.3%, n=19), unnecessary drugs (16.3%, n=16) and undertreatment (12.2%, n=12).

Interventions most frequently proposed included optimization of dosage regimen (21.2%, n=24), individualized recommendation regarding compliance (16.8%, n=19) and drug discontinuation (12.4%, n=14). After one year, 15 of the 19 patients (79%) experienced no longer compliance issues and 27 of the 29 patients (93%) used no drugs with indication issues anymore. In conclusion, the clinical pharmacist in an outpatient monitoring program for liver transplant recipients can signal relevant discrepancies and medication related problems. This leads to interventions that are accepted by both the patients and the physicians, with a positive effect after one year.

Chapter 12 aimed to compare the prevalence and types of medication-related problems and interventions in liver transplant recipients with and without an outpatient medication consultation by a clinical pharmacist as well as the satisfaction with information about medicines and medication adherence. A retro- and prospective cohort were used and subdivided in a group that did and did not receive a medication consultation. The prevalence and types of MRPs and interventions were identified and categorized. The satisfaction parameters were evaluated using validated questionnaires. Included were 291 patients.

In total, 368 MRPs were identified in 197 patients in the non-medication consultation cohort (median 1; range 1–3 per patient) and 248 MRPs in 94 patients in the medication consultation cohort (median 2; range 1–4 per patient). In the medication consultation cohort, significantly fewer MRPs as unnecessary drugs (17.3% versus 58.7%, p<0.001), suboptimal therapy (2.4% versus 9.5%, p<0.001), untreated indication (2.8% versus 6.8%, p=0.040) and underdosed drugs (0.4% versus 6.3%, p<0.001) were identified. In the non-medication consultation cohort significantly more patients used unnecessary drugs (72.1% versus 39.4%, p<0.001) compared to the medication consultation cohort. Patients in both cohorts are satisfied with the information about medicines and reported a high medication adherence. These findings showed that patients in the medication consultation cohort had significantly fewer MRPs and used significantly less unnecessary drugs. Including a clinical pharmacist to the post-transplant care has an added value.

Chapter 13 presented a case of an African American woman who underwent a liver transplantation in which adequate tacrolimus levels were difficult to accomplish due to polymorphisms in the cytochrome P450 3A4/5 enzymes of the transplant recipient and the donor liver graft. This case report highlights that genotyping the liver transplant recipient and the donor liver graft might provide data which could be used to predict the tacrolimus metabolism post transplantation.

In **chapter 14** we presented three cases in which mycophenolic acid exposure severely decreased after oral antibiotic co-administration. By diminishing gut bacteria β-glucuronidase activity, oral antibiotics can prevent deglucuronidation of the inactive mycophenolic acid-7-O-glucuronide metabolite to mycophenolic acid and thereby possibly prevent its enterohepatic recirculation. This pharmacokinetic interaction could result in rejection, which makes it clinically relevant in solid organ transplant recipients, especially when therapeutic drug monitoring frequency is low. Routine screening for this interaction, preferably supported by clinical decision support systems, and pragmatic close monitoring of the MPA exposure in cases is advised.

In the next decades, due to technological developments, healthcare providers will have to deal with an increase in the diagnostic possibilities, surgical techniques and new innovative drugs. Furthermore, the care for liver transplant recipients will become more complex due to an increase in comorbidities. Therefore, as shown in this thesis adapting immunosuppressive agents based on patients' comorbidities and side effects is essential in order to modify and minimize immunosuppressive related toxicity.
We showed that LCP-tacrolimus provides better results compared to ER-tacrolimus. Secondly, based on findings in this thesis we suggested that low-dose ribavirin for at least 180 days has a positive effect on hepatitis E virus clearance in solid organ transplant recipients. Next, immunosuppressive agents affecting the B lymphocytes reduce the immunogenicity of vaccination in LT recipients. This should be considered when vaccinating LT recipients. Finally, liver transplantation is only possible with a multidisciplinary team and interprofessional collaborations. Until the start of this thesis, in the Netherlands clinical pharmacists were solely involved in therapeutic drug monitoring of immunosuppressive agents and computerized medication monitoring. In this thesis, we showed that clinical pharmacists can have an added value in the clinical and outpatient transplant care and increase the medication safety and efficacy for these patients. Overall, we added new insights to the field of optimizing drug therapy for LT recipients to improve patient outcomes.

Nederlandse samenvatting (Dutch summary)

Voor patiënten met eindstadium leverziekte is levertransplantatie de voorkeursbehandeling. Sinds de eerste levertransplantatie in 1967 zijn er significante ontwikkelingen geweest binnen dit veld onder andere op het gebied van chirurgische technieken, postoperatieve zorg, het voorkomen van afstoting, de terugkeer van de oorspronkelijke leverziekte en het voorkomen van complicaties door het noodzakelijke en langdurig gebruik van immunosuppressiva. Door ontwikkelingen op het gebied van immunosuppressieve medicatie is het optreden van acute en chronische rejectie inmiddels relatief zeldzaam geworden. Echter, langdurig gebruik van (te veel) immuunsuppressie kan voor complicaties zorgen en leiden tot verschillende oorzaken van overlijden bij transplantatie patiënten. Oorzaken van overlijden kunnen zijn: de ontwikkeling van ernstige infecties, nierfalen, maligniteiten of cardiovasculaire events. In dit proefschrift beschrijven we hoe we het geneesmiddelengebruik bij levertransplantatie patiënten kunnen optimaliseren, zodat de patiënt uitkomsten verbeteren.

Part II – Optimaliseren van immunosuppressiva gebruik bij levertransplantatie patiënten.

In **hoofdstuk 2** hebben we onderzocht of de combinatie van laag-gedWoseerd sirolimus en laag-gedoseerd tacrolimus met vertraagde afgifte (interventie groep) vergeleken met normaal-gedoseerd tacrolimus met vertraagde afgifte (controlegroep) leidt tot een verschil in de nierfunctie en daarbij vergelijkbare aantallen rejectie, transplantaat- en patiëntoverleving op 36 maanden na levertransplantatie (LOLIII studie). Het betrof een open-label, multicenter gerandomiseerd, gecontroleerd onderzoek. Het primaire eindpunt was de cumulatieve incidentie van chronische nierziekte (CKD) gedefinieerd als graad ≥3 (geschatte nierfunctie (eGFR) <60 mL/min/1.73m^2) op 36 maanden na levertransplantatie. In totaal zijn 196 patiënten geïncludeerd. CKD op 36 maanden was niet verschillend tussen de controle en interventie groep (50.8%, 95% betrouwbaarheidsinterval (BI) 39.7% – 59.9%) versus 43.7%, 95% BI: 32.8% - 52.8%). De eGFR was alleen op 6 maanden na levertransplantatie hoger in de interventie groep vergeleken met de controlegroep (gemiddelde eGFR 73.1±15 versus 67.6±16 mL/min/1.73m^2, p=0.02) in de intention-to-treat populatie. Er werden geen verschillen in secondaire eindpunten en aantal ernstige ongewenste voorvallen gevonden. Dit onderzoek bewijst dat laag-gedoseerd sirolimus en laag-gedoseerd tacrolimus met vertraagde afgifte niet leidt tot minder CKD-graad ≥3 leidt op 36 maanden na levertransplantatie vergeleken met normaal-gedoseerd tacrolimus met vertraagde afgifte.

In **hoofdstuk 3** hebben we onderzocht wat de invloed van de immunosuppressieve behandelingen in de zogeheten LOLIII-studie zijn op de gezondheid gerelateerde kwaliteit van leven (HRQoL) en de ernst van vermoeidheid. HRQoL is gemeten middels de EQ-5D-5L vragenlijst, de EQ-VAS en de ernst van vermoeidheid vragenlijst (FSS). De EQ-5D-5L scores zijn vertaald naar de maatschappelijke waarden. De HRQoL en FSS gedurende de studieduur is geanalyseerd middels een gegeneraliseerd mixed effect model. In het multicenter, open-label, gerandomiseerde, gecontroleerde onderzoek zijn 196 patiënten 90 dagen na levertransplantatie gerandomiseerd naar 1) eenmaal daags normaal-gedoseerd tacrolimus met vertraagde afgifte of 2) eenmaal daags laag-gedoseerd sirolimus en laag-gedoseerd tacrolimus met vertraagde afgifte. Op baseline waren 87.7% (172/1967) vragenlijsten beschikbaar voor analyse. In het algemeen rapporteerden patiënten de minste problemen op de gebieden van zelfzorg en angst/somberheid en de meeste problemen op de gebieden van dagelijkse activiteiten en pijn/ongemak. Er werden geen significante verschillen in de HRQoL en FSS tussen beide groepen gevonden. Tijdens de studieperiode zijn de maatschappelijke waarden van de EQ-5D-5L gezondheidsstaten en de door patiënten zelf gescoorde EQ-VAS scores lager vergeleken met de waarden gegeven door de algemene Nederlandse populatie in beide studiegroepen. Samenvattend kunnen we concluderen dat de HRQoL en FSS vergelijkbaar is in beide studiegroepen op 36 maanden na levertransplantatie. De HRQoL van alle getransplanteerde patiënten benadert het gemiddelde voor de HRQoL gegeven door de Nederlandse populatie.

In **hoofdstuk 4** van dit proefschrift hebben we de cardiovasculaire morbiditeit en mortaliteit in levertransplantatie patiënten onderzocht als subonderdeel van de LOLIII-studie. Het primaire eindpunt in dit onderzoek was de cumulatieve incidentie van een groot cardiovasculair event op 36 maanden na de levertransplantatie. Het secundaire eindpunt betrof het vaststellen van de ontwikkeling van cardiovasculaire risicofactoren na een transplantatie. In totaal hebben we in deze sub studie 122 levertransplantatie patiënten geïncludeerd. Geen significante verschillen in de cumulatieve incidentie van een groot cardiovasculair event op 36 maanden na de levertransplantatie werden gevonden. Echter, significant minder levertransplantatie patiënten in de controle groep (eenmaal daags normaal-gedoseerd tacrolimus met vertraagde afgifte) ontwikkelden hyperlipidemie vergeleken met de interventie groep (eenmaal daags laag-gedoseerd sirolimus en laag-gedoseerd tacrolimus met vertraagde afgifte): jaar 1 30% [18/60], versus 50.9% [30/59]; risicoverschil: -0.208; 95%BI -0.378– -0.021; p=0.025) en jaar 2 (40.3% [23/57] versus 63.2% [36/57]; risicoverschil: -0.228; 95%BI -0.402– -0.032; p=0.024. Ook het voorkomen van hypertensie was significant lager in de controlegroep vergeleken met de interventie groep op jaar 3 na levertransplantatie: 42.1% [24/57] versus 64% [32/50]; risicoverschil: -0.219; 95%BI -0.399– -0.016 p=0.033). Een sirolimus-gebaseerd immunosuppressief regime bij levertransplantatie patiënten resulteert in een vergelijkbare cardiovasculaire morbiditeit en mortaliteit vergeleken met monotherapie tacrolimus. Wel ontwikkelden, één jaar na levertransplantatie, significant meer patiënten hypertensie en hyperlipidemie bij gebruik van een sirolimus-gebaseerd immunosuppressief regime.

In **hoofdstuk 5** hebben we onderzocht of de Life Cycle Pharma (LCP)-tacrolimus formulering met vertraagde afgifte (interventie groep) vergeleken met de andere geregistreerde tacrolimus formulering met vertraagde afgifte (controlegroep) resulteerde in een verschil in de prevalentie van diabetes mellitus, hypertensie en chronische nierziekte op 12 maanden na levertransplantatie. Dit hebben wij onderzocht in een open-label, multicenter, gerandomiseerd gecontroleerde studie (MOTTO studie). Patiënten zijn 1:1 gerandomiseerd naar een van beide tacrolimus formuleringen. Het primaire eindpunt betrof een samengesteld eindpunt van een van de volgende 3 gebeurtenissen: diabetes mellitus na transplantatie >3 maanden na randomisatie, nieuw ontstaande hypertensie en/ of chronische nierziekte, gedefinieerd als een nierfunctie (eGFR) <60 mL/min/1.73m^2 gedurende > 3 maanden tijdens de follow-up. In totaal zijn er 105 patiënten geïncludeerd. Uit de intention-to-treat analyse bleek dat significant meer levertransplantatie patiënten in de interventie groep vergeleken met de controlegroep het samengestelde primaire eindpunt bereikten: 50.9% [27/53], 95%BI 37.9-63.9% versus 71.2% [37/52], 95%BI 57.7-81.7%; risicoverschil: 0.202; 95%BI 0.002–0.382; p=0.046. Dit significant verschil werd niet gevonden in de per protocol analyse. Verder bleek uit de intention-to-treat analyse dat numeriek minder levertransplantatie patiënten in de interventie groep chronische nierziekte en nieuw ontstaande hypertensie ontwikkelden vergeleken met de controle groep: chronische nierziekte 26.4% [14/53], 95%BI 16.4-39.6% versus 42.3% [22/52], 95%BI 29.9-55.8%; risicoverschil: 0.159; 95%BI -0.035–0.339; p=0.102 en nieuw ontstaande hypertensie 38.1% [16/42], 95%BI 24.9-53.2% versus 54.3% [19/35] 95%BI 38.2-69.5%; risicoverschil: 0.162; 95%BI -0.076–0.379, p=0.175. De verschillen waren statistisch niet significant. Geen verschillen in rejectie, orgaan- en patiëntoverleving werden gevonden. Op basis van dit onderzoek kunnen we concluderen dat levertransplantatie patiënten in de LCP-tacrolimus groep significant minder vaak het samengestelde primaire eindpunt bereikten vergeleken met de andere geregistreerde tacrolimus formulering met vertraagde afgifte tijdens het eerste jaar na levertransplantatie waarbij vergelijkbare effectiviteit behaald werd.

Vervolgens hebben we in **hoofdstuk 6** de gezondheid gerelateerde kwaliteit van leven (HRQoL) en de ernst van tremoren in de MOTTO studie onderzocht. HRQoL is gemeten middels de EQ-5D-5L en SF-36 vragenlijst (twee generieke HRQoL instrumenten) en de ernst van tremoren met de quality of life in essential tremor (QUEST) vragenlijst (een domein specifiek HRQoL instrument).

De EQ-5D-5L scores zijn vertaald naar de maatschappelijke waarden. De HRQoL gedurende de studieduur is geanalyseerd middels gegeneraliseerde mixed effect models. In totaal zijn er 105 patiënten geïncludeerd, waarvan 53 in de LCP-tacrolimus groep (interventie groep) en 52 in de groep met de andere geregistreerde tacrolimus formulering met vertraagde afgifte (controlegroep). Voor iedere geïncludeerd levertransplantatie patiënt waren de vragenlijsten op baseline beschikbaar. In de intention-to-treat en per protocol analyse werden geen statistisch significante verschillen gevonden in de frequentie en ernst van tremoren in levertransplantatie patiënten op maand 3, 6 en 12 na transplantatie. In de intention-to-treat populatie ervaarden in de interventie groep op 12 maanden na transplantatie 25% [10/40], 95%BI 14.2% - 40.2% van de levertransplantatie patiënten tremoren vergeleken met 30.4% [14/46], 95%BI 19.1% - 44.8% van de levertransplantatie patiënten in de controlegroep; risicoverschil: 0.054; 95%BI -0.151 – 0.249; p=0.63. Geen statistisch significante verschillen in HRQoL werden gevonden tussen de 2 groepen. De bevindingen in dit onderzoek suggereren dat de LCP-tacrolimus formulering niet geassocieerd is met een verbetering van de HRQoL of een daling in het voorkomen van tremoren vergeleken met de andere geregistreerde tacrolimus formulering met vertraagde afgifte.

Part III – Optimaliseren van de behandelingen voor virale complicaties na een transplantatie.

In **hoofdstuk 7 en 8** hebben we onderzoek gedaan naar het optimale therapeutische gebied en behandelregime van ribavirine bij orgaantransplantatie patiënten met een chronische hepatitis E virus (HEV) infectie. Allereerst hebben we in **hoofdstuk 7** het optimale therapeutische gebied voor ribavirine in kaart gebracht op basis van een retrospectief, multicenter, cohortonderzoek met ROC-curve analyses en het berekenen van de concentratie waarbij 50% van het maximale effect (EC50) wordt bereikt. In totaal zijn er in dit cohort 96 orgaantransplantatie patiënten geïncludeerd die tussen 1-3-2008 en 1-8-2028 behandeld zijn met monotherapie ribavirine voor een mediaan van 3 maanden. Dit resulteerde bij 63.5% van de patiënten in een “sustained virologic response” (SVR) en 88.5% van de patiënten ontwikkelden anemie. Ribavirine plasmaconcentraties in steady-state waren statistisch significant hoger in patiënten met een klinisch relevante respons vergeleken met de patiënten zonder klinisch relevante respons: mediaan 1.96 (IQR 1.81–2.70) versus 0.49 (IQR 0.45–0.73) mg/L, p=0.0004. Ribavirine veroorzaakte een dosis-afhankelijke hemoglobine daling bij hogere ribavirine plasmaconcentraties. Het optimale therapeutische gebied voor ribavirine bij de behandeling van transplantatie patiënten met een chronische HEV infectie ligt tussen de 1.8 en 2.3 mg/L.

In **hoofdstuk 8** hebben we vervolgens een model gemaakt op basis van de ribavirine plasmaconcentraties en de virologische respons (effectiviteit) en hemoglobine concentraties (toxiciteit) gebruik makend van niet-lineaire mixed-effect models. Het model was gebruikt om het meest geschikte doseerregime voor ribavirine te bepalen aan de hand van effectiviteit en toxiciteit. Het betrof een retrospectieve, multicenter onderzoek onder orgaantransplantatie patiënten met een chronische HEV-infectie die tussen 09-2009 en 11-2019 behandeld zijn met monotherapie ribavirine. In totaal zijn er 107 patiënten geïncludeerd waarbij 305 ribavirine plasmaconcentraties, 592 virale concentraties en 443 hemoglobine concentraties beschikbaar waren. SVR was bereikt in 68.2% van de patiënten. De daling in de hoeveelheid virussen in het bloed bleek onafhankelijk te zijn van de ribavirine concentratie en dosering vanwege een lage concentratie waarbij 50% van het minimale effect wordt bereikt (IC50). Daarentegen daalde de hemoglobine waarde met een stijgende ribavirine concentratie en dosering. Op basis van het model werd een optimale ribavirine dosering gevonden met goede effectiviteit en lage toxiciteit van 600 mg/dag bij patiënten met een nierfunctie ≥60 ml/min/1.73m^2, 400 mg/dag bij patiënten met een nierfunctie tussen 30 - 59 ml/min/1.73m^2 en 200 mg/dag bij patiënten met een nierfunctie ≤30 ml/min/1.73m^2 gedurende 180 dagen. Dit onderzoek is de eerste stap in het bepalen van het optimale ribavirine behandelregime in orgaantransplantatie patiënten met een chronische HEV-infectie.

In **hoofdstuk 9** hebben we in een observationeel, cohortonderzoek bepaald wat de immunogeniciteit op SARS-CoV-2 vaccinatie in levertransplantatie patiënten was in relatie tot de concentraties van immunosuppressiva in het bloed na de 2e dosering van een van beide mRNA vaccins of het vector vaccin ChAdOx1 nCoV19. In totaal hebben we 476 levertransplantatie patiënten geïncludeerd: 430 ontvingen het mRNA-1273 vaccin, 25 ontvingen het BNT162b2 mRNA vaccin en 21 ontvingen het ChAdOx1 nCoV19 vector vaccin. Seroconversie gebeurde in 79.0% (376/476) van de levertransplantatie patiënten. Levertransplantatie patiënten gevaccineerd met het mRNA-1273 vaccin hadden een significant hogere IgG SARS-CoV-2 anti-spike antilichaam hoogte in het bloed vergeleken met de andere 2 vaccins, p<0.001. Het gebruik van mycofenolaat mofetil (MMF), ongeacht de bloedspiegel, onderdrukte de IgG SARS-CoV-2 anti-spike antilichaam reactie en resulteerde in suboptimale reacties op de SARS-CoV-2 vaccins, terwijl de andere immunosuppressieve middelen niet dit effect hadden. Vaccinatie tegen SARS-CoV-2 was zeer effectief in ons cohort. Het mRNA-1273 vaccin resulteert in een superieure IgG SARS-CoV-2 anti-spike antilichaam reactie. MMF onderdrukte de SARS-CoV-2 anti-spike antilichaam reactie, ongeacht de bloedspiegel en het soort SARS-CoV-2 vaccin. Het verlagen van de dosering MMF heeft geen effect op het vergroten van de immunogeniciteit van SARS-CoV-2 vaccins.

In **hoofdstuk 10** van dit proefschrift hebben we vervolgens in een observationeel, cohortonderzoek bepaald wat de immunogeniciteit op SARS-CoV-2 vaccinatie in levertransplantatie patiënten was in relatie tot de concentraties van mycofenolzuur in het bloed na de derde, vierde of vijfde dosering van een van beide mRNA vaccins. In totaal zijn er 86 levertransplantatie patiënten geïncludeerd met 92 IgG anti-spike SARS-CoV-2 antilichaam bepalingen; zes patiënten hadden IgG anti-spike SARS-CoV-2 antilichaam bepalingen beschikbaar na meerdere vaccinaties. Significant meer levertransplantatie patiënten hadden positieve IgG SARS-CoV-2 serologie na de 3e vaccinatie (41/48, 85.4%) vergeleken met de 2e vaccinatie (20/48, 41.7%), p<0.001. Dit verhoogde tot 90% na de vierde en vijfde vaccinatie. Mycofenolzuur dalspiegels bleken niet significant geassocieerd met een effect op de IgG SARS-CoV-2 anti-spike lichaam reactie na de derde, vierde of vijfde vaccinatie. Deze resultaten tonen dat additionele SARS-CoV-2 vaccinatie zeer effectief was in ons cohort. Ongeacht de mycofenolzuur dalspiegel hebben levertransplantatie patiënten die mycofenolaat mofetil gebruikten in dit cohort een positieve IgG anti-spike SARS-CoV-2 respons na additionele vaccinaties. Mycofenolaat mofetil kan worden blijven gebruikt tijdens additionele SARS-CoV-2 vaccinaties.

Part IV – Toevoeging van een ziekenhuisapotheker in de zorg voor de levertransplantatie patiënt.

In 2018 hebben we een 20-minuten durend poliklinisch bezoek voor levertransplantatie patiënten bij een ziekenhuisapotheker toegevoegd aan de jaarlijkse controle van deze patiënten. In de poliklinische bespreking wordt ingegaan op het gebruik van de juiste medicatie in relatie tot de indicatie, tijdstippen van inname, therapietrouw, bijwerkingen en praktische zaken waar patiënten tegen aanlopen bij het gebruik van hun medicatie.

In **hoofdstuk 11** hebben we in een cohort levertransplatnatie patiënten in de poliklinische setting onderzocht wat het voorkomen was van, de soorten en ernst van medicatie-gerelateerde problemen. Daarnaast hebben we de interventies geïnitieerd door een ziekenhuisapotheker in kaart gebracht. Potentiele interventies zijn besproken met patiënt en behandelend specialist en geëvalueerd na één jaar. In totaal zijn er 64 levertransplantatie patiënten geconsulteerd door de ziekenhuisapotheker en werden daarbij 96 discrepanties gevonden in 37 patiënten in het actuele medicatiegebruik versus het medicatiegebruik volgens het ziekenhuisinformatiesysteem. De meeste discrepanties (58/96, 60.4%) waren het ontbreken van medicijnen.

Er werden 98 medicatie-gerelateerde problemen gevonden in 53 patiënten (mediaan 2; range 1-5 per patiënt), met een totaal van 113 interventies. Meest frequente medicatie-gerelateerde problemen waren: bijwerkingen (22/98, 22.4%), therapietrouw (19/98, 19.3%), onnodig geneesmiddel gebruik (16/98, 16.3%) en onderhandeling (12/98, 12.2%). De meest voorkomende interventies door de ziekenhuisapotheker waren optimaliseren van het doseerregime (24/113, 21.2%), geïndividualiseerde aanbevelingen voor verbeteren therapietrouw (19/113, 16.8%) en stoppen van medicatie (14/113, 12.4%). Na 1 jaar ervaarden 15 van de 19 patiënten (79%) geen therapietrouwproblemen meer en 27 van de 29 patiënten (93%) hadden geen geneesmiddelen met een onjuiste indicaties meer in gebruik. Op basis van dit onderzoek kunnen we concluderen dat door de toevoeging van een ziekenhuisapotheker aan een poliklinisch programma voor levertransplantatie patiënten relevante discrepanties en medicatie-gerelateerde problemen kunnen worden gedetecteerd. De gepleegde interventies worden geaccepteerd worden door zowel patiënt als specialist.

In **hoofdstuk 12** hebben we onderzocht of er verschil is in de prevalentie en soorten medicatie-gerelateerde problemen en interventies tussen groepen levertransplantatie patiënten met en zonder een poliklinisch consult door een ziekenhuisapotheker. Tevens hebben we onderzocht wat de tevredenheid met de informatie over medicijnen was en hoe de therapietrouw van de patiënten was. Hiervoor is een retro- en prospectief cohort onderzocht en onderverdeeld in een groep dat wel en geen medicatie consult bij een ziekenhuisapotheker ontvangen had. Er zijn 291 patiënten geïncludeerd. In totaal zijn er in de groep zonder een consult bij een ziekenhuisapotheker 368 medicatie-gerelateerde problemen ontdekt in 197 patiënten (mediaan 1; range 1 – 4 per patiënt) en in de groep met een consult bij een ziekenhuisapotheker 248 medicatie-gerelateerde problemen in 94 patiënten (mediaan 2; range 1 – 4 per patiënt). In het cohort met een consult bij een ziekenhuisapotheker werden significant minder medicatie-gerelateerde problemen gevonden zoals onnodig geneesmiddel gebruik (17.3% versus 58.7%, $p<0.001$), suboptimale behandeling met geneesmiddelen (2.4% versus 9.5%, $p<0.001$), onbehandelde indicaties (2.8% versus 6.8%, $p=0.040$) en te laag gedoseerde geneesmiddelen (0.4% versus 6.3%, $p<0.001$). In het cohort zonder een consult bij een ziekenhuisapotheker significant meer patiënten gebruikten onnodige geneesmiddelen (72.1% versus 39.4%, $p<0.001$) vergeleken met het cohort dat wel een consult bij een ziekenhuisapotheker had ontvangen. Levertransplantatie patiënten in beide cohorten waren tevreden over de informatie die ze ontvingen over hun geneesmiddelen en gaven zelf een hoge mate van therapietrouw aan. Deze bevindingen tonen aan dat levertransplantatie patiënten in het cohort dat wel een consult bij een ziekenhuisapotheker ontvingen significant minder medicatie-gerelateerde problemen ervaarden en significant minder onnodige geneesmiddelen gebruikten. Het includeren van een ziekenhuisapotheker in de post-transplantatie zorg is van toegevoegde waarde.

In **hoofdstuk 13** presenteren we een case-report van een afro-Amerikaanse vrouw die een levertransplantatie onderging en waarbij het lastig was om adequate tacrolimus bloedspiegel te bereiken vanwege een polymorfisme in cytochroom P450 3A4/5 enzymen bij de ontvanger en donor. Dit case-report laat zien dat genotypering van de levertransplantatie ontvanger en donor van de lever informatie oplevert dat bruikbaar is bij het voorspellen van het tacrolimus metabolisme post-transplantatie.

Tot slot presenteren we in **hoofdstuk 14** een drietal casussen waarbij de blootstelling aan mycofenolzuur zeer verlaagd is na de start en toediening van orale antibiotica. Orale antibiotica kunnen de deglucuronidatie van het inactieve mycofenolzuur-7-O-glucoronide voorkomen en zodoende mogelijk de enterohepatische kringloop doorbreken vanwege het reduceren van de β-glucuronidase activiteit door darmbacteriën. Deze farmacokinetische interactie kan resulteren in rejectie waardoor het een klinisch relevante interactie is in solide orgaantransplantatie patiënten, vooral bij de casussen waarbij minder frequent therapeutic drug monitoring uitgevoerd wordt.

Wij adviseren routinematige screening op deze interactie, bij voorkeur ondersteund door klinische beslisondersteuning, en pragmatische, nauwlettende monitoring van de mycofenolzuur concentratie in patiënten at-risk.

Vanwege de vele technologische ontwikkelingen zullen zorgmedewerkers in de komende jaren moeten leren omgaan met een toename aan diagnostische mogelijkheden, nieuwe chirurgische technieken en nieuwe innovatieve geneesmiddelen. Daarnaast zal de zorg voor levertransplantatie patiënten complexer worden door een toename van comorbiditeiten in deze patiënten. Het is daarom noodzakelijk, zoals in dit proefschrift beschreven, dat de immunosuppressieve medicatie van een patiënt moet worden aangepast op basis van de comorbiditeiten en bijwerkingen om zodoende het risico op immunosuppressiva gerelateerde toxiciteit te verminderen. In dit proefschrift tonen wij aan dat LCP-tacrolimus de voorkeur verdient boven ER-tacrolimus. Tevens raden wij, op basis van de bevindingen in dit proefschrift, een lagere dosering ribavirine gedurende minimaal 180 dagen aan voor de behandeling van een chronische hepatitis E infectie in solide orgaantransplantatie patiënten aan. Daarnaast laten wij zien dat immunosuppressieve middelen, die werken op de B lymfocyten, de immunogeniciteit van SARS-CoV-2 vaccinaties in levertransplantatie patiënten beïnvloeden, maar dat herhaalde vaccinatie leidt tot een goede immunogeniciteit van de vaccins. Tot slot, levertransplantatie is alleen mogelijk met een multidisciplinair team en interprofessionele samenwerkingen. Tot het moment van starten met het onderzoek in dit proefschrift waren ziekenhuisapothekers in Nederland alleen betrokken op het gebied van therapeutic drug monitoring van de immunosuppressieve middelen en het uitvoeren van elektronische medicatiebewaking. Dit proefschrift heeft duidelijk gemaakt dat ziekenhuisapothekers ook andere en positieve bijdragen kunnen leveren bij de dagelijkse klinische en poliklinische levertransplantatiezorg en deze zorg effectiever en veiliger maken. Dit proefschrift geeft nieuwe inzichten in de optimalisatie van geneesmiddeltherapie voor de levertransplantatie patiënt, maar deze is zeker nog niet optimaal.

List of abbreviations

ADR	Adverse drug reaction
AUC	Area under the concentration-time curve
CAD	Coronary artery disease
CKD	Chronic kidney disease
Cmax	Maximum concentration
CNIs	Calcineurin inhibitors
CP	Clinical pharmacist
CV	Cardiovascular
CYP	Cytochrome P450
DBD	Donation after brain death
EASL	European Association for the Study of the Liver
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
ER	Extended-release
FDA	Food and Drug Administration
HARM	Hospital Admissions Related to Medication
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HEV	Hepatitis E virus
HRQoL	Health-related Quality of Life
HSCT	Hematopoietic stem cell transplant
IQR	Interquartile range
ISCED	International Standard Classification of Education
ITT	Intention to treat
KDIGO	Kidney Disease Improving Global Outcomes
LCP	Life cycle pharma
LT	Liver transplantation
MARS	Medication Adherence Reporting Scale
MC	Medication consultation
MedDRA	Medical Dictionary for Regulatory Activities
MMF	Mycophenolate mofetil
MPA	Mycophenolic acid
MPAG	MPA-7-O-glucuronide
MRP	Medication-related problem
mTOR	Mammalian target of rapamycin
NASH	Nonalcoholic steatohepatitis
NCC MERP	National Coordinating Council for Medication Error Reporting and Prevention
NODAT	New onset diabetes after transplantation
NOTR	Dutch Organ Transplantation Registry
PCNE	Pharmaceutical Care Network Europe
PD	Pharmacodynamics
PK	Pharmacokinetics
PP	Per protocol
PSC	Primary sclerosing cholangitis
PTDM	Post-transplant diabetes mellitus
QUEST	Quality of life in essential tremor
RBV	Ribavirin
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus type 2
SD	Standard deviation
SIMS	Satisfaction with Information about Medicines
SIR	Sirolimus
SOT	Solid Organ Transplant
SRL	Sirolimus
SVR	Sustained virologic response
TAC	Tacrolimus
TDM	Therapeutic drug monitoring
RCT	Randomized controlled trial
ROC	Receiver operating characteristic
tBPAR	Treated biopsy proven acute rejection
UGT	Uridine diphosphate-glucuronosyltransferase
U-HPLC-MS/MS	Ultra-high-performance liquid chromatography-tandem mass spectrometry
VAS	Visual analogue scale
VTE	Venous thromboembolism
WHO	World Health Organization

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List of publications

Related to this thesis

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Erasmus MC Department: Gastroenterology and Hepatology	Promotor:	Prof. Dr. H.J. Metselaar
	Supervisor:	Dr. B.C.M. de Winter

	Year
PhD training	
Courses	
HESPERIS course – *ESOT*, V*ienna*	2018
Teach the Teacher III - *Erasmus MC, Rotterdam*	2019
Introduction course to population PK/PD modeling with NONMEM® - *UMCG, Groningen*	2019
Scientific Integrity - *Erasmus MC, Rotterdam*	2019
EndNote workshop - *Erasmus MC, Rotterdam*	2019
Logistic Regression - *Erasmus Summer Program, Rotterdam*	2020
English writing course - *Erasmus MC, Rotterdam*	2020
BROK® (Basic Course Rules and Organisation for Clinical researchers) - *NFU*	2020
Motivational Interviewing Course – *NVZA, Utrecht*	2021
(Inter)national conferences	
Dutch Hospital Pharmacy Congres (oral) - *Hilversum*	2019
NTV Bootcongres (2x oral) - *Roermond*	2020
55th EASL International Liver Congress (poster) - *digital*	2020
ILTS congress (poster and oral) - *digital*	2021
NTV Bootcongres (oral) - *digital*	2021
56th EASL International Liver Congress - *digital*	2021
36th Erasmus Liver day (oral) - *digital*	2021
20th ESOT Congress (2x oral) - *Milan*	2021
Dutch Hospital Pharmacy Congres (oral) - *Utrecht*	2021
NTV Bootcongres (oral) - *Leiden*	2022
NTS Lustrumevent (oral) - *Leiden*	2022
Digestive Disease Days (2x oral) - *Veldhoven*	2022
20th IATDMCT Congress (oral) - *Prague*	2022
ILTS congress (poster) - *digital*	2022
Dutch Hospital Pharmacy Congres (2x oral) – *'s-Hertogenbosch*	2022
NTV Bootcongres (poster and oral) - *Groningen*	2023
ILTS congress (oral) - *Rotterdam*	2023
21th ESOT Congress (oral) - *Athens*	2023
Dutch Hospital Pharmacy Congres (oral) – *Papendal*	2023
Grants / Awards	
Best registration presentation - *NVZA*	2019
Supervisor of the year - *Hospital Pharmacy Erasmus MC, Rotterdam*	2020
Best idea in transplantation – *NTV Bootcongres, Roermond*	2021

Teaching	
Lecturing	
Teaching pharmacology to medical students – *Erasmus MC, Rotterdam*	2019 - 2023
Teaching pharmacology to pharmacy students – *University Utrecht, Utrecht*	2019 - 2023
Teaching Dutch hospital pharmacy residents – *NVZA, Utrecht*	2019 - 2023
U.P.S.V. "Unitas Pharmaceuticorum" Dies Natalis symposium - *digital*	2021
VJA voorjaarsdag "Goed Verteerbaar" - *Oegstgeest*	2022
Supervising Master's theses	
G. Durmaz (6 month research master)	2018
B. Dogba (6 month research master)	2021
M. van Dinter (6 month research master)	2021
T. Hooijman (6 month research master)	2021/2022

Memberships	
Koninklijke Nederlandse Maatschappij ter bevordering der Pharmacie (KNMP)	2007 – 2024
Nederlandse Vereniging van ZiekenhuisApothekers (NVZA)	2014 – 2024
Nederlandse Transplantatie Vereniging (NTV)	2018 – 2024
Nederlandse Vereniging voor Hepatologie (NVH)	2019 – 2024
European Society for Organ Transplantation (ESOT)	2020 - 2024

Extracurricular	
Board member of the Dutch pharmacy Post Academic Educational Organisation – *PAOfarmacie, Bilthoven*	2021 - 2023
Chair of the Educational Committee of the Dutch Hospital Pharmacy Association – *NVZA, Utrecht*	2020 - 2023
Development of an educational movie for liver transplant recipients – *Erasmus MC, Rotterdam*	2021
Workshop "Klinisch Farmaceutisch Redeneren" organized during Dutch Hospital Pharmacy Congres - *Papendal*	2023

Curriculum vitae

Midas Berend Mulder was born on the 11th of August 1989 in Enschede, the Netherlands. He grew up in Hengelo and studied Pharmacy at the University of Utrecht from 2009 – 2014. As part of his Master's degree, he performed a six-month research project at the University of Florida in Gainesville (United States of America) under the supervision of Prof. Dr. H. Derendorf. In this project he focused on the pharmacokinetics and pharmacodynamics of antibiotics which resulted in two publications.

After his graduation in 2014, Midas started his career as a pharmacist in the clinical pharmacy at the Franciscus Gasthuis & Vlietland hospital in Rotterdam, the Netherlands. In 2015 Midas got the opportunity to start his residency in the same hospital under the supervision of Dr. E.M. Westerman. During his 4 years of residency Midas spent one year of training at the Erasmus University Medical Center under the supervision of Prof. Dr. P.M.L.A. van den Bemt. In this hospital he got involved in the care for liver patients. His research project during the residency focused on a testosterone-based dosing regimen of androgen deprivation therapy in patients with locally advanced or metastatic prostate cancer (MIDAS-trial). For this research he received the "best registration presentation" award of the Dutch Hospital Pharmacy Association (NVZA) in 2019.

Midas finished his residency in hospital pharmacy by the end of 2018. Afterwards he got the opportunity to start working as a clinical pharmacist at the Erasmus University Medical Center. In this hospital he got focused and specialized in the pharmaceutical care for complex gastrointestinal and Hepato Pancreato Biliary patients including liver transplant recipients. Simultaneously he started as a PhD-student at the Department of Gastroenterology and Hepatology under the supervision of Prof. Dr. H.J. Metselaar focusing on the optimization of drug therapy for liver transplant recipients.

As one of the first clinical pharmacists in the Netherlands, Midas started an outpatient monitoring program specifically for liver transplant recipients. In this outpatient monitoring program he optimized the medication of liver transplant recipients transplanted in the Erasmus University Medical Center. During his conversations he focused on on the actual medication used, the adherence, adverse drug reactions (ADRs) and the way liver transplant recipients used their drugs. Furthermore, Midas is involved in educational activities and boards, e.g. chair of the educational committee of the Dutch Hospital Pharmacy Association (NVZA) and board member of the Dutch pharmacy Post Academic Educational Organisation (PAOfarmacie). In addition, in 2021 Midas developed together with Caroline den Hoed (hepatologist) an educational movie for liver transplant recipients named "Grip op je pillen".

Currently, Midas is working as a clinical pharmacist and deputy trainer of clinical pharmacy residents at the Haaglanden Medical Center in The Hague. In this hospital he is responsible for the medication safety, medication reconciliation, educational activities and research. Clinically he is still involved in the care for the gastroenterology and hepatology patients.

Midas is married to Miriam Stuart-Fox and they live with their daughter Maxime in The Hague, The Netherlands.

Dankwoord

Begin 2019 mocht ik starten met het uitvoeren van mijn promotieonderzoek en het schrijven van dit mooie proefschrift. Terwijl ik als farmaciestudent geen moment aan het uitvoeren van een promotieonderzoek had gedacht, werd ik in het ziekenhuis gegrepen door klinisch onderzoek dat direct iets kan bijdragen aan de patiëntenzorg. De afgelopen jaren heb ik veel mogen leren over één onderwerp, maar heb ik ook kansen gehad om mij in de breedte te ontwikkelen binnen het onderzoeksveld en op onderwijsgebied. De afgelopen jaren hebben veel mensen mij adviezen en ondersteuning geven. Een aantal personen wil ik in het bijzonder benoemen.

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