Optimizing tacrolimus treatment in kidney transplant recipients

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# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ................................................................................................. iii

LIST OF PAPERS .............................................................................................................. v

SUMMARY ....................................................................................................................... vi

ABBREVIATIONS ............................................................................................................ vii

1 INTRODUCTION ......................................................................................................... 1
   1.1 Tacrolimus in kidney transplantation ..................................................................... 1
       1.1.1 History and current challenges ........................................................................ 1
       1.1.2 Pharmacology ................................................................................................. 3
       1.1.3 Current dosing strategy ................................................................................... 5
   1.2 Population pharmacokinetic modeling ................................................................... 7
       1.2.1 Fundamental principles ................................................................................... 7
       1.2.2 Previous models for tacrolimus ....................................................................... 8
   1.3 Model-based dosing .............................................................................................. 9
       1.3.1 Covariate-guided dosing ................................................................................. 9
       1.3.2 Bayesian forecasting ..................................................................................... 10

2 OBJECTIVES ............................................................................................................ 13

3 METHODS ................................................................................................................. 14
   3.1 Data and study design ........................................................................................ 14
   3.2 Tacrolimus analytical methods ............................................................................ 16
   3.3 Data analyses ..................................................................................................... 17
       3.3.1 General statistics (Paper I and IV) ................................................................. 17
       3.3.2 Population pharmacokinetic modeling (Paper II and III) ................................ 17
       3.3.3 External evaluation of models (Paper III) ....................................................... 23
       3.3.4 Evaluation of dosing strategies (Paper III) ..................................................... 24
       3.3.5 Clinical dose predictions (Paper IV) .............................................................. 24
   3.4 Software .............................................................................................................. 27
   3.5 Ethics .................................................................................................................. 27

4 SUMMARY OF RESULTS ......................................................................................... 28

5 DISCUSSION ............................................................................................................ 32
   5.1 Population pharmacokinetics of tacrolimus ......................................................... 32
   5.2 Bayesian forecasting of tacrolimus doses ............................................................ 34
   5.3 Optimal tacrolimus concentration ........................................................................ 36
   5.4 Methodological considerations and limitations .................................................... 38
       5.4.1 Internal and external validity ......................................................................... 38
       5.4.2 Influence of missing data ............................................................................. 40
   5.5 Clinical implications ............................................................................................. 42

6 CONCLUSIONS ........................................................................................................ 45

7 FUTURE DIRECTIONS ............................................................................................ 46

8 REFERENCES .......................................................................................................... 47
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Oslo, august 2016

Elisabet
LIST OF PAPERS

This thesis is based on the following papers. They are referred to by their Roman numerals in the text. Reprints were made with permissions from the publishers.

**Paper I**
*Nephrology* 2016 Feb; Online publication ahead of print.

**Paper II**

**Paper III**

**Paper IV**
After kidney transplantation, lifelong treatment with immunosuppressive drugs is needed. Tacrolimus is an essential part of most immunosuppressive regimens that are used today. However, dosing of tacrolimus is a major challenge because of considerable variability in dose requirement between patients. Low concentrations must be avoided, because this may increase the risk of organ rejection. Too high blood concentrations, on the other hand, increase the risk of serious adverse effects, including cardiovascular disease, cancer and infections. In this thesis, the overall goal was to evaluate and develop strategies to optimize tacrolimus treatment early after kidney transplantation.

First, the clinical outcomes of standard risk kidney transplant recipients at Oslo University Hospital (January 2009 - March 2013) who received low-target tacrolimus (trough concentrations of 3–7 µg/L), mycophenolate mofetil, glucocorticoids and basiliximab induction are summarized. This immunosuppressive regimen is used at our center based on the results of a large multicenter randomized controlled trial called “ELiTE-Symphony”. In our clinical cohort, one-year renal function, acute rejection rate and patient and graft survival were excellent and comparable to the results of the ELiTE-Symphony study. The results suggest that this regimen is safe and effective also in the “real world” clinical setting.

Then, two population pharmacokinetic models of tacrolimus were developed based on data from kidney-transplanted adults. These are mathematical models that describe the relationship between tacrolimus doses, patient characteristics and tacrolimus blood concentrations over time. A variety of patient characteristics were identified to influence the dose requirement of tacrolimus, including patient fat-free mass, cytochrome P450 3A5 genotype, time after transplantation and prednisolone dose. Furthermore, hematocrit was found to influence the whole blood concentrations, theoretically without influencing the therapeutic unbound concentrations, and was therefore suggested to be a key factor in the interpretation of tacrolimus whole blood measurements. The models predicted tacrolimus concentrations well in an independent patient cohort.

Finally, a randomized controlled trial was conducted in newly transplanted kidney recipients, comparing tacrolimus dosing determined by a population pharmacokinetic model with dosing determined by experienced transplant physicians. This study demonstrated for the first time that model-based dosing for tacrolimus is feasible in the clinical setting and significantly improves target concentration achievement.

In conclusion, the work presented in this thesis contributes with novel knowledge about strategies to optimize tacrolimus treatment early after kidney transplantation. Further research is needed to demonstrate potential impact on long-term outcomes.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>Area under the concentration-time curve</td>
</tr>
<tr>
<td>BOV</td>
<td>Between occasion variability</td>
</tr>
<tr>
<td>BPAR</td>
<td>Biopsy-proven acute rejection</td>
</tr>
<tr>
<td>BQL</td>
<td>Below quantification limit</td>
</tr>
<tr>
<td>BSV</td>
<td>Between subject variability</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CL</td>
<td>Clearance</td>
</tr>
<tr>
<td>CL/F</td>
<td>Apparent clearance</td>
</tr>
<tr>
<td>CMIA</td>
<td>Chemiluminescent microparticle immunoassay</td>
</tr>
<tr>
<td>Cov</td>
<td>Covariate</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
<tr>
<td>Cb</td>
<td>Bound concentration</td>
</tr>
<tr>
<td>Cp</td>
<td>Plasma concentration</td>
</tr>
<tr>
<td>C_{ss,ave}</td>
<td>Average concentration at steady state</td>
</tr>
<tr>
<td>C_{tot}</td>
<td>Total concentration</td>
</tr>
<tr>
<td>C_u</td>
<td>Unbound concentration</td>
</tr>
<tr>
<td>C_wb</td>
<td>Whole blood concentration</td>
</tr>
<tr>
<td>C_0</td>
<td>Trough concentration / Predose concentration</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Cytochrome P450 3A4</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>Cytochrome P450 3A5</td>
</tr>
<tr>
<td>DGF</td>
<td>Delayed graft function</td>
</tr>
<tr>
<td>dnDSA</td>
<td>De novo donor specific antibody</td>
</tr>
<tr>
<td>eGFR</td>
<td>Estimated glomerular filtration rate</td>
</tr>
<tr>
<td>F</td>
<td>Bioavailability</td>
</tr>
<tr>
<td>f</td>
<td>Fraction</td>
</tr>
<tr>
<td>FOCE-I</td>
<td>First order conditional estimation method with interaction</td>
</tr>
<tr>
<td>FFM</td>
<td>Fat-free mass</td>
</tr>
<tr>
<td>FKBP12</td>
<td>FK506-binding protein 12</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>GRP</td>
<td>Index for group parameter value</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>Hct</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>i</td>
<td>Index for individual</td>
</tr>
<tr>
<td>j</td>
<td>Index for observation</td>
</tr>
<tr>
<td>k</td>
<td>Index for occasion</td>
</tr>
<tr>
<td>k_a</td>
<td>Absorption rate constant</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography tandem mass spectrometry</td>
</tr>
<tr>
<td>LLOQ</td>
<td>Lower limit of quantification</td>
</tr>
<tr>
<td>MEIA</td>
<td>Microparticle enzyme immunoassay</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>MMF</td>
<td>Mycophenolate mofetil</td>
</tr>
<tr>
<td>MPE%</td>
<td>Median percentage prediction error</td>
</tr>
<tr>
<td>NFAT</td>
<td>Nuclear factor of activated T-cells</td>
</tr>
<tr>
<td>OFV</td>
<td>Objective function value</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>P</td>
<td>Parameter</td>
</tr>
<tr>
<td>p</td>
<td>Index for plasma based parameter</td>
</tr>
<tr>
<td>pcVPC</td>
<td>Prediction-corrected visual predictive check</td>
</tr>
<tr>
<td>PRA</td>
<td>Panel reactive antibody</td>
</tr>
<tr>
<td>PTDM</td>
<td>Post-transplant diabetes mellitus</td>
</tr>
<tr>
<td>Q</td>
<td>Intercompartmental clearance</td>
</tr>
<tr>
<td>Q/F</td>
<td>Apparent intercompartmental clearance</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>std</td>
<td>Index for a standard value</td>
</tr>
<tr>
<td>Tac</td>
<td>Tacrolimus</td>
</tr>
<tr>
<td>TDM</td>
<td>Therapeutic drug monitoring</td>
</tr>
<tr>
<td>Txt</td>
<td>Time after transplantation</td>
</tr>
<tr>
<td>tlag</td>
<td>Absorption lag time</td>
</tr>
<tr>
<td>V1</td>
<td>Central volume of distribution</td>
</tr>
<tr>
<td>V1/F</td>
<td>Apparent central volume of distribution</td>
</tr>
<tr>
<td>V2</td>
<td>Peripheral volume of distribution</td>
</tr>
<tr>
<td>V2/F</td>
<td>Apparent peripheral volume of distribution</td>
</tr>
<tr>
<td>VPC</td>
<td>Visual predictive check</td>
</tr>
<tr>
<td>wb</td>
<td>Index for whole blood based parameter</td>
</tr>
<tr>
<td>ΔOFV</td>
<td>Difference in objective function value</td>
</tr>
<tr>
<td>ε</td>
<td>Residual error</td>
</tr>
<tr>
<td>η</td>
<td>Random effect parameter</td>
</tr>
<tr>
<td>θ</td>
<td>Fixed effect parameter</td>
</tr>
<tr>
<td>σ</td>
<td>Standard deviation of ε</td>
</tr>
<tr>
<td>ω</td>
<td>Standard deviation of η</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

1.1 Tacrolimus in kidney transplantation

1.1.1 History and current challenges

Historical overview

The first successful human kidney transplantation was performed between twins in 1954.\textsuperscript{1} Because the twins were genetically identical, immunosuppression was not needed.\textsuperscript{2} Transplanting between nonidentical siblings and later unrelated individuals, however, made it clear that the kidney would be rejected in most patients without suppressing the immune system. Initial attempts to suppress the immune system using total body irradiation were unsuccessful, and the focus shifted to the potential value of pharmacological treatment.\textsuperscript{2} In the 1960s, azathioprine was introduced, which together with glucocorticoids produced sufficient immunosuppression to perform successful kidney transplantations between unrelated individuals.\textsuperscript{3} From 1962 and twenty years onwards, combined therapy with azathioprine and glucocorticoids constituted the standard immunosuppression after renal transplantation. Yet, acute rejections were a major obstacle, and only about half of the kidneys survived the first year.\textsuperscript{4}

In 1982, the use of the calcineurin inhibitor cyclosporine was initiated, strikingly improving one-year graft survival to 70-80%.\textsuperscript{4,5} Then, in 1994, another calcineurin inhibitor became available, called FK506 and later tacrolimus. Tacrolimus reduced the acute rejection rate and displayed a generally more beneficial adverse effect profile than cyclosporine.\textsuperscript{6} Gradually, tacrolimus became the preferred calcineurin inhibitor. Tacrolimus is now a key agent in immunosuppressive strategies, usually combined with mycophenolate mofetil, glucocorticoids and induction therapy with anti-interleukin-2 receptor antibodies or a T-lymphocyte-depleting agent.\textsuperscript{7,8} With today’s drugs, one-year graft survival approaches 95%.\textsuperscript{9,10} Although more than 20 years has passed since the introduction of tacrolimus, no new drugs have replaced its role.\textsuperscript{10}
Current challenges

The improvements in short-term outcomes with evolving immunosuppressive drugs have not been translated into major improvements in long-term outcomes.\textsuperscript{11} Ten years after transplantation, about 50\% of the patients will have died or lost their graft.\textsuperscript{12} The main causes of death in renal transplant recipients are cardiovascular disease, cancer and infection.\textsuperscript{13} Tacrolimus-specific adverse effects (hypertension, hyperlipidemia, post-transplant diabetes mellitus) contribute to the cardiovascular disease risk,\textsuperscript{14} whereas the general use of immunosuppressants increases the susceptibility to cancer\textsuperscript{15} and infections.\textsuperscript{16} To reduce the risk of these events, it is desired to keep tacrolimus exposure low. Graft loss after the first year is mainly caused by “chronic allograft dysfunction”, a poorly understood condition characterized by declining renal function due to progressive damage to the allograft.\textsuperscript{17} The factors that contribute to this condition are both of non-immunological and immunological nature. Among the non-immunological factors, calcineurin inhibitor-induced nephrotoxicity is thought to play a central role.\textsuperscript{18} This is another reason to keep tacrolimus exposure low. Immunological damage due to acute rejection episodes and \textit{de novo} donor-specific antibodies (dnDSA), however, are also important contributors to chronic allograft dysfunction.\textsuperscript{17,19} To prevent immunological damage, it is critical that tacrolimus exposure is kept sufficiently high. The therapeutic goal is to dose tacrolimus in a manner that leads to an appropriate balance between too low and too high tacrolimus exposure (Figure 1).

\textit{Figure 1.} Overview of the contribution of sub-optimal tacrolimus exposure to the two main reasons for late graft loss: death with a functioning graft and chronic allograft dysfunction. High tacrolimus exposure increases cardiovascular disease (CVD) risk and increases the susceptibility to cancer and infections. Low tacrolimus exposure increases risk of acute rejection episodes and the development of donor-specific antibodies (DSA), which together with tacrolimus-induced nephrotoxicity are important contributors to the progression of chronic allograft dysfunction.
Prevention of the main causes of death and chronic allograft dysfunction is considered the most important strategy to improve long-term outcomes after kidney transplantation.\textsuperscript{17} Whereas several factors in addition to the sub-optimal dosing of tacrolimus increase the risk of these incidents, many are non-modifiable, such as donor and recipient characteristics. Importantly, the immunosuppressive strategy is a modifiable factor that can be improved.\textsuperscript{20}

### 1.1.2 Pharmacology

**Mechanism of action**

Unbound tacrolimus molecules enter the cytoplasm of T-cells. Here, they form complexes with intracellular proteins (FKBP12) and inhibit the enzyme calcineurin.\textsuperscript{21} Calcineurin inhibition prevents activation of \textit{nuclear factor of activated T-cells} (NFAT), a transcription factor involved in the production of a number of cytokines, including interleukin-2. These cytokines lead to T-cell activation and proliferation, important processes in the allograft rejection process. By suppressing their formation, tacrolimus prevents cell-mediated acute rejection effectively (Figure 2).\textsuperscript{21} Tacrolimus also inhibits T-cell dependent B-cell activation and thereby prevents antibody-mediated rejection.\textsuperscript{22}

\textit{Figure 2.} The mechanism of action of tacrolimus. After entering the T-cell, tacrolimus binds to FK506-binding protein 12 (FKBP12) and forms a complex that inhibits calcineurin. This in turn leads to reduced activation of \textit{nuclear factor of activated T-cells} (NFAT) and thereby reduced production of interleukin-2 and other cytokines that are important in the allograft rejection process.
Pharmacokinetic properties

After oral administration, the immediate-release formulation of tacrolimus is generally rapidly absorbed into the bloodstream. An absorption lag-time of ~1/2 hour has been reported. Tacrolimus is a substrate of P-glycoprotein and the cytochrome P450 isoenzymes (CYP) 3A4 and 3A5. The oral bioavailability is poor (average 25%) and highly variable (4-89%) due to its lipophilic structure and extensive first-pass metabolism mainly in the intestinal wall. Elimination is primarily hepatic, with negligible renal contribution. Based on whole blood, tacrolimus is a low-extraction-ratio drug (3% of liver blood flow).

In blood, tacrolimus distributes into and binds extensively to proteins within blood cells, mainly erythrocytes. The blood:plasma ratio is high and variable between patients (13:1-114:1), and depends on the proportion of erythrocytes in blood (hematocrit), tacrolimus concentration and temperature. In plasma, tacrolimus is ~99% bound to plasma proteins (α-acid glycoprotein, lipoproteins, globulins and albumin). Thus, the unbound concentration available to enter T-cells and cause a pharmacological effect is only about 0.01-0.1% of the whole blood concentration.

Some of the proteins involved in the pharmacokinetic processes of tacrolimus are expressed differently in different genetic sub-populations. The most influential and consistently identified genetic covariate for tacrolimus is a single nucleotide polymorphism in the gene encoding CYP3A5 (6986A>G). Patients with the CYP3A5*1 allele normally express functional CYP3A5 protein in their intestine and liver, and are referred to as CYP3A5 expressers. The CYP3A5*3 allele, on the other hand, is associated with a splicing defect, and homozygous CYP3A5*3 carriers normally express CYP3A5 protein with virtually no enzyme activity (CYP3A5 non-expressers). About 10-30% of the Caucasian population are CYP3A5 expressers. These patients require approximately two times higher doses of tacrolimus compared with non-expressers due to higher clearance and/or lower bioavailability.

The pharmacokinetics of tacrolimus are influenced by several drugs, as well as by food and herbal product constituents. Examples of clinically relevant interacting components include inducers and inhibitors of CYP3A enzymes or P-glycoprotein, such as antifungals, antibiotics, calcium channel blockers, glucocorticoids, St. John’s wort and grapefruit juice. The absorption rate and bioavailability of tacrolimus are decreased in the presence of food, with a more pronounced effect if the fat content is high.
Tacrolimus pharmacokinetics change systematically with time after transplantation, as indicated by a continuous decrease in dose requirement for the maintenance of stable whole blood concentration levels throughout the first year after transplantation.\textsuperscript{37-39} The explanation to this phenomenon is not clear. The most commonly suggested causes include time-dependent increases in hematocrit and serum albumin (increasing the bound tacrolimus concentration in blood) and decreasing CYP3A activity due to tapering of the glucocorticoid dose over time.\textsuperscript{25,37,39}

1.1.3 Current dosing strategy

Therapeutic drug monitoring

Because of the substantial variability in tacrolimus pharmacokinetics, the dose needed to achieve a specific concentration in blood varies considerably between patients. The dose alone is therefore not a good marker of drug effect. To account for the pharmacokinetic variability, drug concentrations are measured in body fluids, followed by dose adjustments to achieve concentrations within a narrow target range that produces an acceptable balance between desired and adverse effects. This procedure is called therapeutic drug monitoring (TDM).\textsuperscript{40} Tacrolimus concentrations are traditionally measured in whole blood rather than in plasma, mainly because of the very low plasma fraction and the more convenient sample handling since the blood:plasma ratio is sensitive to temperature differences.\textsuperscript{41}

Generally, the area under the concentration-time curve (AUC) is considered the best measure of systemic drug exposure. However, AUC monitoring has not been widely accepted due to costs and practical difficulties.\textsuperscript{42} The most common strategy for tacrolimus TDM is to measure the concentration immediately before a dose (trough concentration, Figure 3). This strategy has been used since the first trials,\textsuperscript{43,44} because of the clinical convenience of collecting blood at the morning visit and the fact that some studies have demonstrated a satisfying correlation between tacrolimus trough concentrations and AUC\textsubscript{0-12h} ($r > 0.90$\textsuperscript{24,45}). This correlation, however, has not been reproduced in other studies ($r \leq 0.60$\textsuperscript{46-48}). A single trough concentration level can undoubtedly be associated with numerous and highly variable pharmacokinetic profiles and AUC\textsubscript{0-12h} (Figure 3).
**Introduction**

Figure 3. Simulated concentration-time profile for three virtual patients with different pharmacokinetic characteristics. Doses are adjusted to achieve the same trough concentration, whereas the area under the concentration-time curve varies between the patients.

Tacrolimus treatment is usually initiated with a dose proportional to the total body weight of the patient. Drug concentrations are measured frequently in the early period after transplantation, and multiple dose adjustments are typically performed in attempt to achieve the target concentration. Normally, the clinician evaluates the concentration’s deviation from the target and adjusts the dose based on intuition and experience. However, this strategy is complicated in the case of non-steady state conditions, missed doses, or concentrations measured at non-standard time points within the dose interval. For tacrolimus, intuitive dosing is further complicated by time-varying pharmacokinetics. Achieving and maintaining tacrolimus target concentrations is therefore a challenging task for clinicians, and concentrations below or above the target range has been reported in >40% of patients during the first month using this strategy.49,50

**Target whole blood trough concentration**

A general prerequisite for the use of TDM is that a target concentration can be derived from a well-defined relationship between drug concentrations and drug effects.40 However, this relationship is not fully clear for tacrolimus, although it has been in use for more than 20 years.42 Yet, TDM has been considered a useful strategy to aim for empirically defined target whole blood trough concentration ranges that have been titrated down over the years due to increased clinical experience and more effective concomitant immunosuppressive drugs. The early clinical trials targeted tacrolimus trough concentrations of 10-20 or 10-25 µg/L during the first three months after transplantation, with subsequent reduction to 5-15 µg/L.44,51,52 Combination with anti-interleukin-2 receptor antibody induction (daclizumab or
basiliximab) suggested that further reduction to 5-10 µg/L was safe. In 2007, a large, multicenter, randomized controlled trial called “ELiTE-Symphony” (hereafter referred to as “Symphony”) demonstrated that a “low-target” tacrolimus trough range of 3–7 µg/L from the day of transplantation led to excellent results in terms of one-year renal function without increase in acute rejection rate when combined with mycophenolate mofetil, glucocorticoids and daclizumab induction. Based on these results, a similar low-target tacrolimus-based regimen was implemented at Oslo University Hospital Rikshospitalet in 2009 for standard immunological risk kidney transplant recipients. However, there are very few published experiences from using this strategy in the clinical setting. Many centers still report to be using relatively high tacrolimus target trough concentrations, particularly during the first weeks and months after transplantation (e.g. 10/12-15 µg/L). There is little consensus between transplant centers regarding the appropriate tacrolimus concentration and whether higher concentrations during the early phase are necessary, mainly due to a lack of randomized controlled trials addressing these questions. The applied target trough ranges therefore vary considerably between transplant centers, with time passed since transplantation, concomitant immunosuppression and presumed immunological risk.

1.2 Population pharmacokinetic modeling

1.2.1 Fundamental principles

Population pharmacokinetic models are mathematical models that describe the dynamic relationship between drug doses and drug concentrations in the body over time. The models typically describe this relationship in terms of the absorption, distribution and elimination of drug into, between and out of theoretical body compartments (i.e. compartmental models). They also describe the biological variability associated with the pharmacokinetic parameters for these processes, as well as the functional relationships with patient characteristics (called covariates) that appear to explain parts of the total variability. In addition, they describe variability in concentration measurements originating from measurement error, imprecise logging of time of sampling and time of dosing, and other uncontrollable sources of error. Normally, a goal of population pharmacokinetic modeling is to describe the pharmacokinetics in patients who are representative of the population who are using the drug.
Introduction

(e.g. the transplant population in case of tacrolimus) rather than in healthy individuals. Population pharmacokinetic models are useful to understand and predict the behavior of drugs in the body and to design individual dosing strategies.

1.2.2 Previous models for tacrolimus

Prior to the models presented in this thesis, 14 population pharmacokinetic models had been published for tacrolimus (immediate-release formulation) in kidney-transplanted adults (Table 1). In these models, tacrolimus disposition was most commonly described using two-compartment models. One-compartment models were used exclusively by those with only trough concentrations available. Absorption has usually been described by a first-order process, occasionally with a lag-time.

All the above-mentioned studies used an empirical, data-driven approach to covariate identification. Only a few demographic covariates were identified: Two studies found a relationship with age, one a relationship with total body weight, one with ethnicity, and none with sex. Alternative body size metrics to total weight, such as fat-free mass had not been explored as covariates. In contrast, all the eight models that investigated the influence of CYP3A5 genotype consistently identified a statistically significant effect on apparent clearance (CL/F) ranging from +50% to +150%. CYP3A5 enzymes are expressed both in the intestine and liver, and theoretically influence presystemic and systemic metabolism of tacrolimus independently. Still, none of the models have attempted to model CYP3A5 genotype as an independent covariate on oral bioavailability (F) separated from the effect on clearance (CL). Pharmacokinetic changes with time after transplantation have been modeled as a continuous decrease in CL/F during the first three weeks, three months, and six months, or attributed to time-varying covariates: Hematocrit or prednisolone dose. Those who have investigated the effect of hematocrit have modeled it as a covariate on single pharmacokinetic disposition parameters (e.g. on CL/F separately), rather than as a covariate on the whole blood concentrations. Less consistently identified covariates include aspartate aminotransferase, ABCB1 genotype, MRP2 haplotype, CYP3A4 genotype, drug intake at night, and the concomitant use of calcium channel blockers.
Table 1. Published population pharmacokinetic models for tacrolimus in kidney transplanted adults.

<table>
<thead>
<tr>
<th>Author, reference</th>
<th>No. of subjects</th>
<th>Structural model</th>
<th>Identified covariate relationships</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staatz et al.63</td>
<td>70</td>
<td>1 FO, 70</td>
<td>CL/F ~ ASAT, time post-transplant</td>
</tr>
<tr>
<td>Scholten et al.64</td>
<td>17</td>
<td>2 FO, lag</td>
<td>None</td>
</tr>
<tr>
<td>Antignac et al.65</td>
<td>83</td>
<td>1 FO, 17</td>
<td>CL/F ~ Prednisolone dose &gt;25 mg, time post-transplant</td>
</tr>
<tr>
<td>Press et al.66</td>
<td>31</td>
<td>2 FO, 31</td>
<td>CL/F ~ CYP3A5, Prednisolone dose &gt;10mg</td>
</tr>
<tr>
<td>Grover et al.67</td>
<td>24</td>
<td>2 FO, lag, 24</td>
<td>CL/F ~ Native American origin</td>
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<tr>
<td>Benkali et al.68</td>
<td>32</td>
<td>2 Transit, 32</td>
<td>CL/F ~ Hematocrit</td>
</tr>
<tr>
<td>Musuamba et al.69</td>
<td>19</td>
<td>2 FO, 19</td>
<td>ka ~ CL/F ~ Night time CYP3A5, ABCB1</td>
</tr>
<tr>
<td>Velickovic et al.70</td>
<td>63</td>
<td>1 FO</td>
<td>CL/F ~ Prednisolone dose &gt;25 mg, tacrolimus dose</td>
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<tr>
<td>Woillard et al.71</td>
<td>73</td>
<td>2 Transit, 73</td>
<td>CL/F ~ CYP3A5, hematocrit</td>
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<tr>
<td>Passey et al.72</td>
<td>681</td>
<td>Modeled CL/F only</td>
<td>CL/F ~ CYP3A5, prednisolone, time post-transplant, age, calcium channel blocker</td>
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<tr>
<td>Musuamba et al.73</td>
<td>65</td>
<td>2 FO, lag, 65</td>
<td>CL/F ~ CYP3A5, hematocrit</td>
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<tr>
<td>Ogasawara et al.74</td>
<td>102</td>
<td>2 FO, lag, 102</td>
<td>CL/F ~ CYP3A5, MRP2, age</td>
</tr>
<tr>
<td>Zuo et al.75</td>
<td>161</td>
<td>1 FO</td>
<td>CL/F ~ CYP3A5, CYP3A4, hematocrit</td>
</tr>
<tr>
<td>Han et al.76</td>
<td>80</td>
<td>1 FO</td>
<td>CL/F ~ CYP3A5, hematocrit, time post-transplant Total body weight</td>
</tr>
</tbody>
</table>

ABCB1, adenosine triphosphate-binding cassette B1 genotype; ASAT, aspartate aminotransferase; CL, clearance; CL/F, apparent clearance; Cmp, compartments; CYP3A4, cytochrome P450 3A4 genotype; CYP3A5, cytochrome P450 3A5 genotype; F, bioavailability; FO, first-order; ka, absorption rate constant; lag, lag time; MRP2, multi-resistance-associated protein 2 haplotype; V/F, apparent volume of distribution

1.3 Model-based dosing

One of the major applications of population pharmacokinetic models is to aid in individualization of drug dosing.61,62 Covariates may be used to guide selection of the initial dose, whereas the full model with its description of variability can be used for dose adjustment guidance after individual concentration measurements become available.77 These processes are described in more details in the following section.

1.3.1 Covariate-guided dosing

Prior to the availability of drug concentration measurements, the optimal individual dose can be estimated from the population pharmacokinetic typical parameters and individual
covariate values. All patients with the same covariate values will then receive the same dose. For drugs with a large proportion of pharmacokinetic variability explained by covariates, this method may be adequate to achieve the target concentration in most patients. However, for drugs with large unexplained pharmacokinetic variability, concentrations will still vary considerably between patients after accounting for covariates, and this strategy alone will not be sufficient to reliably achieve the target.

### 1.3.2 Bayesian forecasting

Bayesian forecasting is a method for estimating individual pharmacokinetic parameters, using a combination of prior information and individual information. Prior information is represented by a population pharmacokinetic model for the drug, while individual information is the measured drug concentrations and covariate values for the patient of interest. Estimation of individual pharmacokinetic parameters is performed by balancing these sources of information, taking into account expected variability in the population parameters and drug concentration measurements (Figure 4). As more drug concentrations from the individual become available, the individual information is given more weight, and the prior population information is given less weight.

**Figure 4.** Principle behind Bayesian forecasting. Prior information from a population model is balanced against individual information to obtain estimates of the most likely individual pharmacokinetic parameters.
After individual pharmacokinetic parameter estimates are obtained, they can be used to simulate the individual pharmacokinetic profile into the future and devise a dose regimen to achieve a specific target concentration for the individual (Figure 5).

![Figure 5. Estimated time vs. concentration profile for an individual, showing both past drug exposure (white area) and the future expected exposure with three alternative doses (gray area) based on Bayesian forecasted individual pharmacokinetic parameters.](image)

An advantage of Bayesian forecasting over traditional TDM based on subjective judgment by the clinicians is that it is not dependent on steady-state conditions and absence of missed doses, and that it can take time-varying pharmacokinetics into account (provided that the underlying model includes time or an explanatory covariate for the time-related trend). Furthermore, concentrations measured at any time after dose can be used. The major disadvantage is that it requires a previously developed, appropriate population pharmacokinetic model and specialized software that might be challenging to learn. Several software packages have been developed for the purpose of Bayesian forecasting with varying degrees of user-friendliness.

Bayesian forecasting has been shown superior to standard dosing for several critical dose drugs, including digoxin, aminoglycosides, busulfan, vancomycin, cyclosporine
in kidney transplant recipients and tacrolimus in liver transplant recipients. Despite the fact that numerous models have been developed for tacrolimus in kidney transplant recipients (Table 1), no studies have been published on the clinical use of Bayesian forecasting for tacrolimus after kidney transplantation.
2 OBJECTIVES

The overall objective of the thesis was to evaluate and develop strategies to optimize tacrolimus treatment early after kidney transplantation.

Specific objectives of each paper

I) To evaluate the “real-world” clinical outcomes in standard risk kidney transplant recipients using low-target tacrolimus-based immunosuppression from the day of transplantation (tacrolimus trough concentrations of 3–7 µg/L, mycophenolate mofetil, glucocorticoids and anti-interleukin-2 receptor antibodies) and to compare the results with the results of the Symphony study.

II) To develop a population pharmacokinetic model of tacrolimus in kidney transplant recipients and identify patient characteristics that influence tacrolimus dose requirement.

III) To develop a theory-based population pharmacokinetic model of tacrolimus in kidney transplant recipients and to externally evaluate the model and two alternative empirical models.

IV) To prospectively evaluate the target concentration achievement of tacrolimus early after transplantation using dosing based on Bayesian forecasting compared with conventional dosing performed by experienced transplant physicians.
3 METHODS

3.1 Data and study design

Paper I

Study I was a single-center observational study including standard risk adult patients who received a single kidney transplant at Oslo University Hospital Rikshospitalet between January 1, 2009 and March 31, 2013, and who received low-target tacrolimus twice daily (target trough concentration range 3–7 µg/L), mycophenolate mofetil (0.75 g twice daily), glucocorticoids (gradually tapered from 20 mg to 5 mg) and basiliximab induction. Standard risk patients were defined by the absence of pre-transplant donor specific antibodies, panel reactive antibodies <20%, ABO-compatibility and non-HLA-identical donor. The main clinical outcome parameters included estimated glomerular filtration rate (Cockcroft-Gault formula\(^8\)) and rate of biopsy-proven acute rejections one year after transplantation, as well as one- and three-year graft- and patient survival. These outcome parameters were compared with the one- and three-year results of the low-target tacrolimus arm in the Symphony study.\(^9\)\(^,\)\(^8\)\(^9\) Data were collected from patient medical records and the Norwegian Renal Registry. The final data set included data from 406 patients.

Paper II

Study II was a single-center population pharmacokinetic analysis. Intensively sampled pharmacokinetic data for tacrolimus were obtained from three previous analyses that included patients transplanted between 2006 and 2011\(^9\)\(^0\)\(^-\)\(^9\)\(^2\) (Figure 6A). These data were combined with additional routine TDM data (trough concentrations) from 44 patients transplanted in 2012 (Figure 6B). Four patients contributed data to both data sets. The final data set included 1546 tacrolimus concentrations from 69 patients. Data were collected from patient medical records. Patients who received multiple organ transplants or who used drugs known to interact with tacrolimus pharmacokinetics (except drugs used by >5% of the patients; the drug was then evaluated as a covariate) were excluded from the study.
Methods

Figure 6. Overview of tacrolimus concentrations used in paper II. A Intensively sampled concentration measurements (n=514), B Trough concentrations from routine therapeutic drug monitoring (n=1032).

Paper III

Study III was a population pharmacokinetic analysis performed in collaboration with the Pharmacometrics Group at the School of Pharmacy, University of Queensland, Australia. The dataset used in study II (n=69) was combined with a data set available from this group, comprising 173 patients who received a kidney transplant at the Princess Alexandra Hospital in Brisbane, Australia93 (Figure 7). Patients who used drugs known to interact with tacrolimus pharmacokinetics, including grapefruit juice, or who received multiple organ transplants were excluded from the study. The final data set for model development included 3100 tacrolimus concentrations from 242 patients. In addition, 837 concentrations from 72 independent patients were collected to externally evaluate the models.

Figure 7. Overview of tacrolimus concentrations (n=1554) measured in the 173 patients who were included in the study by Bergmann et al.93 In study III, these data were combined with the data shown in Figure 6. Dots are not connected with lines in this figure due to the large number of patients.
Methods

Paper IV

Study IV was a single-center, prospective, randomized study planned to include 80 consecutive kidney transplant recipients at Oslo University Hospital Rikshospitalet from January 2014. Patients were eligible for the study if they received a kidney as the only transplanted organ, were 18 years or older, were mentally able to comply with study procedures and used tacrolimus-based immunosuppression without concomitant drugs known to interact with tacrolimus pharmacokinetics. Eligible patients were randomized (allocation ratio 1:1 based on a computer-derived, unstratified randomization list with block size = 8) to receive tacrolimus doses determined by a population pharmacokinetic model implemented in a dose prediction software (computer group) or conventional doses determined by transplant physicians (control group) during the first eight weeks after transplantation. Main outcome descriptions for comparison included the median proportion of tacrolimus concentrations within the target range, the incidence of biopsy-proven acute rejections and infections during follow-up, and renal function (glomerular filtration rate, measured by iohexol clearance) and glucose tolerance (oral glucose tolerance test: fasting and 2-hour plasma glucose) eight weeks after transplantation. Both standard risk patients (as defined for paper I, but including those with HLA-identical donor) and high-risk patients (defined by pre-transplant presence of donor specific antibodies, panel reactive antibodies ≥20% or ABO-incompatible donor) were included, but were analyzed separately.

3.2 Tacrolimus analytical methods

In study I and IV, all tacrolimus concentrations were measured using chemiluminescent microparticle immunoassay (CMIA). In study II and III, concentrations measured using CMIA, microparticle enzyme immunoassay (MEIA) and liquid chromatography tandem mass spectrometry (LC-MS/MS) were combined for analysis. LC-MS/MS is considered the reference method, whereas immunoassays overestimate concentrations by cross-reacting with tacrolimus metabolites. This was accounted for by inter-converting tacrolimus concentrations measured by LC-MS/MS ($C_{\text{LC-MS/MS}}$) and immunoassays ($C_{\text{MEIA or CMIA}}$) using a locally established conversion algorithm (Equation 1).

$$C_{\text{LC-MS/MS}} (\mu g/L) = 0.80 \times C_{\text{MEIA or CMIA}} (\mu g/L) + 0.19 (\mu g/L)$$ (1)
Differences in assay precision were accounted for by estimating substudy-specific residual errors during population pharmacokinetic modeling (see section 3.3.2).

3.3 Data analyses

3.3.1 General statistics (Paper I and IV)

In study I and IV, continuous variables were assessed for normality using histograms and the Shapiro-Wilk test. The Shapiro-Wilk test tests the null hypothesis that the population is truly normally distributed and provides a p-value for whether this hypothesis should be rejected. Normally distributed variables were compared between groups using the two-tailed Student’s $t$-test. Non-normally distributed variables were compared using the Mann-Whitney U test, which is the nonparametric equivalent to the Student’s $t$-test. Differences in proportions were analyzed using Fisher’s exact test (paper I) or the $\chi^2$-test (paper IV). These tests lead to approximately the same results with large samples ($>40$ observations), and both tests could theoretically have been used in both analyses. Three-year survival rates (paper I) were estimated using the Kaplan-Meier method to allow inclusion of patients with incomplete follow-up. P-values less than 0.05 were assumed to represent statistical significance.

3.3.2 Population pharmacokinetic modeling (Paper II and III)

Population pharmacokinetic modeling was performed using non-linear mixed effects modeling. The first-order conditional method with interaction (FOCE-I) in NONMEM® v. 7.2 was used as estimation method.

Missing data imputation

Tacrolimus concentrations reported below the lower limit of quantification were discarded because they constituted a negligible proportion of the total data set ($n=2$, <0.2%). Missing time-constant covariate values were imputed using the median population value. Missing hematocrit values were imputed using a linear regression model developed from hematocrit
values and hemoglobin values in all patients with both values available. Remaining missing
time-varying covariates were imputed by carrying forward/backward the last/next known
value.

**Structural model**

One and two-compartment models with first-order or zero-order absorption with or without
a lag time and linear elimination were tested as structural models to describe tacrolimus
pharmacokinetics. The models were parameterized in terms of clearances (CL, Q) and
volumes of distribution (V1, V2) rather than rate constants (Figure 8). Because oral
bioavailability (F) was unknown, these parameters were estimated relative to bioavailability
(e.g. apparent clearance, CL/F).

![Figure 8](image)

*Figure 8. Overview of a two-compartment model. The oral dose is absorbed into the central
compartment (compartment 1) and is distributed into and redistributed from a peripheral
compartment (compartment 2). Drug is finally irreversibly eliminated from the central compartment.
ka, absorption rate constant; k_{12} and k_{21}, rate constants for the transfer between the compartments;
kel, elimination rate constant; CL, clearance; V1, central volume of distribution; V2, peripheral volume
of distribution; Q, intercompartmental clearance. Adapted from Fisher and Shafer.*

Variability between subjects and between dosing occasions was described using a parametric
approach, which means that the variability is assumed to follow a certain shape that can be
described by parameters, such as the mean and the standard deviation. The natural logarithm
of the individual deviation from the typical value and the occasion-specific deviation from
the mean individual value were assumed to be symmetrically distributed around zero. This
exponential model is commonly used because it prevents the parameter value to take on
negative (physiologically impossible) values. The residual error was described using a
combined proportional and additive model. This allows a constant error model to
predominate at low concentrations and a proportional error model to predominate at high concentrations. When multiple studies or tacrolimus concentrations measured using different analytical assays were combined, the residual error was estimated specifically for each substudy or assay type.

Covariates

Theoretically, changes in the erythrocyte-bound concentration of tacrolimus in blood due to changes in hematocrit should equally influence all the pharmacokinetic parameters that are estimated from whole blood concentrations, except absorption parameters. Therefore, hematocrit was included in the best structural model by modeling its effect directly on the whole blood concentrations, rather than as a covariate on single pharmacokinetic parameters.

In paper II, the total concentration of tacrolimus in whole blood \( (C_{\text{tot}}) \) was modeled as a function of the unbound concentration \( (C_u) \), the binding capacity in erythrocytes and the hematocrit percentage \( (\text{Hct}) \), as shown in Equation 2.

\[
C_{\text{tot}} = C_u \times \left( 1 + \frac{C_{\text{bmax}}}{C_u + C_{50}} \right) \times \text{Hct.} 
\]  

(2)

where \( C_{\text{bmax}} \) is the maximum concentration bound to erythrocytes and \( C_{50} \) is the unbound concentration leading to half maximum binding. By assuming that \( C_{\text{tot}} \) mainly reflects the amount in erythrocytes \( (C_b) \), with negligible unbound and plasma bound concentrations \( \text{(total <5\%25,25,28)} \), by assuming that \( C_u \) is very small relative to \( C_{50} \), and by standardizing \( C_{\text{bmax}} \) to a hematocrit of 45\%, Equation 2 was simplified and rewritten as shown in Equation 3.

\[
C_{\text{tot}} \approx C_b = C_{\text{std}} \times R \times \frac{\text{Hct}}{45 \%}, 
\]  

(3)

where \( C_{\text{std}} \) is the standardized concentration and \( R \) is the estimable ratio between \( C_{\text{bmax}} \) and \( C_{50} \) at a hematocrit of 45\%. Equation 3 was included in the model by relating pharmacokinetic parameters and predictions to \( C_{\text{std}} \).

In paper III, a slightly different approach was used: \( C_{\text{tot}} \) was modeled as a function of \( C_p, \text{Hct} \) and values for the binding properties of tacrolimus to erythrocytes obtained from the literature (Equation 4):
\[ C_{\text{tot}} = C_p \times \left(1 + \frac{4.18}{C_p+3.8}\right) \times \text{Hct} \]  

Thus, instead of estimating whole blood based pharmacokinetic parameters, the parameters were estimated with respect to the plasma concentrations \((C_p)\), which were predicted from each pair of \(C_{\text{tot}}\) and \(\text{Hct}\) values.

The effects of the following covariates were evaluated on clearance, volume of distribution and/or bioavailability: weight, fat-free mass, fat mass, age, sex, \(CYP3A5\) genotype, time after transplantation, prednisolone dose, serum creatinine, serum albumin and liver function test values (aspartate aminotransferase, alanine aminotransferase, serum bilirubin and alkaline phosphatase). In paper II, C-reactive protein, methylprednisolone dose, acute rejection episodes and the use of the potentially interacting drugs nifedipine, lansoprazole and cinacalcet were also evaluated. Fat-free mass and fat mass were predicted from total body weight, height and sex using an established algorithm.\(^{98}\) For body size covariates, power models with exponents fixed to \(\frac{2}{3}\) for clearances and 1 for volumes of distributions were used according to allometric scaling theory.\(^{99}\)

Categorical covariates were modeled as the fractional change in the parameter \(P\) for each category compared to a reference category \((P_{\text{std}})\) by using \(\text{if-else}\) statements. This is exemplified in Equation 5, where \(\theta_{\text{cov}}\) represents the estimable fractional change in the parameter for males relative to the female estimate.

\[
P = \begin{cases} 
P_{\text{std}} \\ P_{\text{std}} \times \theta_{\text{cov}} 
\end{cases} \quad \text{if female} 
\begin{cases} 
P_{\text{std}} \\ P_{\text{std}} \times \theta_{\text{cov}} 
\end{cases} \quad \text{if male} \quad (5)
\]

The effect of continuous covariates with little prior information regarding the shape of the potential influence were visually explored by categorization (e.g. age group 20-29 years, 30-39 years and so on) followed by estimation of a mean pharmacokinetic parameter in each category. Then, continuous functions were selected to match the trends. The evaluated function curves included linear (Equation 6 and Figure 9A), power (Equation 7 and Figure 9B) and sigmoidal (Equation 8 and Figure 9C). These functions were expressed relative to a standard value \((\text{Cov}_{\text{std}})\), e.g. a total body weight of 70 kg.
**Methods**

**A Linear model**

\[ P = P_{\text{std}} \times (1 + \theta_{\text{cov}} \times (\text{Cov} - \text{Cov}_{\text{std}})) \]  

**B Power model**

\[ P = P_{\text{std}} \times (\frac{\text{Cov}}{\text{Cov}_{\text{std}}}^{\theta_{\text{cov}}}) \]  

**C Sigmoid model**

\[ P = P_{\min} \times (1 + \frac{\theta_{\text{cov, max}}}{1 + \left(\frac{\text{Cov}}{\theta_{\text{cov, 50}}}\right)^{-\text{Cov, hill}}}) \]  

*Figure 9. Examples of covariate models: A Linear model, B power model, C sigmoid model. P is the pharmacokinetic parameter, P_{\text{std}} is the pharmacokinetic parameter at the standard covariate value called Cov_{\text{std}}, Cov is the covariate (e.g. weight, age), and \theta_{\text{cov}} is the estimable covariate coefficient. In the sigmoid model, there are three estimable covariate coefficients: \theta_{\text{cov, max}} is the asymptotic value of relative increase in P with increasing covariate value, \theta_{\text{cov, 50}} is the covariate value associated with half maximum change in P and \theta_{\text{cov, hill}} describes the steepness of the curve around \theta_{\text{cov, 50}}.*
**Methods**

**Model selection**

In both studies, model selection was guided by statistical comparison of models, biological plausibility, parameter precision and graphical evaluation of the model fit using mainly visual predictive checks. These criteria are explained in more details below. In paper II, statistical criteria were the primary model selection driver (i.e. an empirical approach). In paper III, covariates representing theoretical relationships that were expected from prior knowledge about biological mechanisms were prioritized and included in the model regardless of statistical significance.

**Statistical comparison of models**

In NONMEM, model fitting is based on maximum likelihood estimation. NONMEM describes the model fit by reporting an objective function value (OFV) that is proportional to $-2 \times \log \text{likelihood}$. Due to the negative sign, the OFV is minimized when the likelihood is maximized, and lower values imply improvement in data description. The OFV of two competing models can be statistically compared using the likelihood ratio test if model#2 is reduced to model#1 when the added parameters are fixed to zero (i.e. nested models). The difference in OFV ($\Delta\text{OFV}$) between nested models is approximately $\chi^2$-distributed. Thus, if model#2 includes one additional parameter compared to model#1, a reduction in OFV of $>3.84$ represents statistical significance at $p<0.05$ for the added parameter. Correspondingly, a reduction of $>6.63$ represents $p<0.01$. In paper II, the most statistically significant covariate was included in each round of a forward-inclusion procedure until no more covariates showed statistical significance at $p<0.01$. After completion of covariate inclusion, each covariate was removed separately. If any covariates had lost statistical significance at this stage, the covariate associated with the highest $p$-value was removed. This procedure was repeated until all the remaining covariates were independently statistically significant.

**Biological plausibility**

The criterion of biological plausibility comprises the principle that parameter estimates should take on values that are biologically and physiologically reasonable. Examples of implausible estimates include clearance higher than organ flow or negative relationships between body weight and clearance. The parameter estimates were checked for whether they were biologically plausible at each stage during model building.
Parameter precision

The precision of the parameter estimates was assessed by the bootstrap procedure. Bootstrapping is based on random, repeated resampling of the data set to create multiple new data sets with the same number of subjects, but allowing the same subjects to be present multiple times. The model is then fit to each of these data sets, and the distribution of the estimates for each parameter can be compared with the original model estimates. The 2.5th and 97.5th percentile values of the re-estimated distribution represent the 95% confidence interval. In the present work, 95% confidence intervals were generated by running 100 bootstrap replicates for temporary models (limited by the time-consuming nature of the procedure) and 500-1000 bootstrap replicates for final models.

Graphical evaluation

The most important visual guidance for model development in this thesis was the visual predictive check (VPC). VPCs were generated by using the model to simulate concentration data for 100 virtual patients. Percentiles of the simulated data were plotted against time, together with percentiles of the observed values. The agreement between the median, 5th and 95th percentiles of the model predictions and the observations was assessed to evaluate model fit. Because standard VPC simulations do not take into account the inherent relationship between drug concentrations and subsequent dose adaptations that are generated in clinical TDM data, prediction-corrected VPCs (pcVPCs) were used. In pcVPCs, observations and simulations are normalized to the median population prediction, thus removing covariates and dose differences as sources to variability and overcoming this problem. In addition to standard pcVPCs with “time after dose” on the x-axis, pcVPCs were investigated by continuous covariates on the x-axis and by stratifying by categorical covariates. Standard goodness-of-fit plots were also assessed for key models (agreement between observed and predicted values and residual distribution).

3.3.3 External evaluation of models (Paper III)

For models intended for dosing in the clinic, it is essential that they predict well also for independent patients not used for model development. External evaluation is performed to assess the predictive performance of the model in an external data set, and is considered the most stringent method for model evaluation. Both models (study II and III) were externally evaluated in study III by using Bayesian forecasting to predict the $n + 1^{th}$
tacrolimus concentration based on the population model and information from the first to $n$th concentrations. Predictive performance was assessed by calculating the median percentage prediction error with 90% prediction intervals.\(^\text{106}\)

### 3.3.4 Evaluation of dosing strategies (Paper III)

In paper III, three alternative dosing strategies were evaluated:

- weight-based dosing (0.04 mg/kg/12 h)
- covariate-based dosing
- Bayesian forecasting

The concentration-time profile of 1000 subjects were simulated using the final model and covariate values randomly sampled from the original data set. Each dosing strategy was evaluated by the percentage of the 1000 simulated subjects achieving a steady state average concentration ($C_{ss,\text{ave}}$) within 80-125% of a target $C_{ss,\text{ave}}$ that was derived from a previous study.\(^\text{38}\)

### 3.3.5 Clinical dose predictions (Paper IV)

The dose predictions in study IV was performed using BestDose® (Laboratory of Applied Pharmacokinetics, LA, US).\(^\text{107}\) BestDose is a package that is installed and run within the statistical software R\(^\text{®}\), and is based on non-parametric modeling. The models developed in paper II and III, however, were based on a parametric approach, and could not be implemented in BestDose. A non-parametric model developed using the same data set as described for paper II was therefore implemented in BestDose and used for dose predictions in study IV\(^\text{108}\) (model not presented in this thesis). Non-parametric models assume no particular shape of the between-subject variability distribution, but rather estimate a discrete distribution of pharmacokinetic parameter values (called support points) with associated probabilities\(^\text{109}\) (Figure 10). The collection of support points represents multiple possible models.
Figure 10. Example of parametric (left) and non-parametric (right) distribution of clearance. The parametric approach describes clearance or log-clearance in terms of parameters: the mean ($\mu$) and standard deviation ($\omega$). The non-parametric approach describes clearance as a discrete distribution of support points and associated probabilities.

In BestDose, the support points with associated probabilities are used as prior information for individual pharmacokinetic parameter estimation. When drug concentrations become available for a patient, the software updates the probability estimates specifically for the individual, without changing the original support point values. Then, each of the possible models is weighted by its individual-specific probability to obtain the overall mean individual pharmacokinetic profile. Figure 11 illustrates this by a screenshot from the BestDose software.
Methods

Figure 11. Example of Bayesian dose predictions using BestDose. Each colored line represents a pharmacokinetic profile generated from a set of support points in the nonparametric model, and each profile has its distinct probability (top right). The black solid line represents the weighted (Wtd) mean profile for the individual.

During dose estimation, the software searches for the optimal individual dose by minimizing the squared deviation between the target concentration and the predicted concentration for each of the models, weighted by their individual-specific probability. This is an iterative process, starting at an initial dose estimate (selected by the user; typically the current dose), followed by modifications of the dose until the weighted squared deviation is minimized. The dose estimation process in practice can be described in four steps that are repeated every time a new drug concentration becomes available (Box I).

Box I. The dose estimation process in BestDose.

(i) The user updates the dosing history, covariate values and any available drug concentrations to an Excel file in a specific format.

(ii) The individual pharmacokinetic parameters are estimated.

(iii) The user sets the target (e.g. a target concentration 12 hours after dose in the middle of the target trough range).

(iv) The software estimates the optimal individual dose.
3.4 Software

Modeling and simulation in study II and III was performed using NONMEM® v. 7.2.\textsuperscript{95} Wings for NONMEM®,\textsuperscript{112} was used for running models, bootstrapping and managing results. Graphical investigations and statistical analyses for all studies were carried out using R v.2.15 or later versions.\textsuperscript{113} Dose predictions were performed using BestDose®.\textsuperscript{107}

3.5 Ethics

All studies were approved by the Regional Committee for Medical Research Ethics and were performed in accordance with the Declaration of Helsinki. Study III was also approved by the Princess Alexandra Hospital and University of Queensland Ethics Committees. Study IV is registered at www.ClinicalTrials.gov (NCT02010320). All participants gave written informed consent.
4 SUMMARY OF RESULTS

Paper I

Low-target tacrolimus in de novo standard risk renal transplant recipients - a single-center experience

A total of 15,772 tacrolimus concentrations were recorded and compared with the data observed in the Symphony study (Figure 12). The median number of tacrolimus measurements per patient (n=406) during follow-up was 38 (range 3-86), and 68% of the measurements were between 3 and 7 µg/L. The median [interquartile range] tacrolimus concentration eight weeks and one year after transplantation was 6.1 [5.2-7.1] and 6.0 [5.0-7.1] µg/L, respectively. One year after transplantation, the mean (standard deviation) estimated glomerular filtration rate was 76.8 (28.3) mL/min (Symphony: 65.4 (27.0) mL/min, p<0.001). Biopsy-proven acute rejections were seen in 14.5% of the patients (Symphony 12.3%, p=0.35). One-year death-censored graft survival was 99.0% (Symphony 96.4%) and one-year patient survival was 97.8% (Symphony 97.2%). Corresponding Kaplan-Meier estimates [95% confidence interval (CI)] of three-year values were 96.6% [94.2-99.0%] (Symphony: 93%) and 95.0% [92.6-97.3] (Symphony: 95 %), respectively.

Figure 12. Mean trough concentrations of tacrolimus during the first post-transplant year. Dashed lines represent the target range limits; error bars represent standard deviations. A Norwegian data, B Symphony study, from Ekberg et al.9 Copyright © 2007 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.
Paper II

Importance of hematocrit for a tacrolimus target concentration strategy

The best structural model was a two-compartment model with first order absorption and a lag time. The base model fit was biased over the range of hematocrit values (24-45%, Figure 13a) when not accounting for hematocrit (Figure 13b). Standardization of tacrolimus whole blood concentrations to a hematocrit value of 45% improved the model fit considerably (\(\Delta\text{OFV} -78.3, p<0.001\), Figure 13c). Fat-free mass was the superior body size metric to predict tacrolimus clearance and volume of distributions. Bioavailability was estimated to be 49% [95% CI 38%-58%] lower in CYP3A5 expressers than in CYP3A5 non-expressers. Bioavailability increased with age for both males and females towards a common value at age >55 years. The minimum value at age <40 years was 57% [40-77%] lower in females and 34% [11-64%] lower in males than the maximum value. Finally, bioavailability changed with time after transplantation, best described using two distinct sigmoid functions. The value was highest immediately after transplantation (+104% [60-290%]), with a steep decrease towards a nadir at day 5. Then, it increased during the next 55 days towards an asymptotic value 28% [14%-49%] higher than on day 5. Prediction-corrected VPCs indicated that the model predicted well over time and over the range of covariate values.

Figure 13. a Prediction-corrected tacrolimus whole blood concentrations over the range of hematocrit, b prediction-corrected visual predictive check (pcVPC) over the range of hematocrit using the base model without covariates, c pcVPC over the range of hematocrit using the base model with hematocrit standardized concentrations. Solid red line represents median tacrolimus concentration, dashed red lines represent 90% observation interval (5th to 95th percentile), solid black line represents median model-predicted concentrations, dashed black lines represent 90% prediction interval. Gray-shaded areas represent 95% confidence intervals for each model-predicted percentile.
Results

Paper III

Improved prediction of tacrolimus concentrations early after kidney transplantation using theory-based pharmacokinetic modelling

A two-compartment model with first-order absorption and a lag time was used to describe the data. The pharmacokinetic parameters were estimated based on tacrolimus plasma concentrations, predicted from whole blood concentrations, hematocrit and literature values for the binding of tacrolimus to red blood cells. All pharmacokinetic parameters were allometrically related to fat-free mass (p<0.001). Tacrolimus clearance was 30% higher [95% CI 13-46%] and bioavailability 18% lower [2-29%] in patients expressing at least one CYP3A5 *1 allele compared with CYP3A5 non-expressers. Tacrolimus bioavailability was modeled as a sigmoid $E_{\text{max}}$ function of prednisolone dose (p<0.001), based on prior knowledge of a pharmacokinetic interaction between prednisolone and tacrolimus. Age showed a statistically significant effect on bioavailability, but was not included in the model due to lack of known biological basis. The final model predicted the observations well (Figure 14).

In the external evaluation, the theory-based model was superior, with a median prediction error of $-1.2\%$ [95% CI $-3.01-0.1\%$] and 90% prediction error interval of $-38\%$ [$-32-42\%$] to $+47\%$ [42 - 53%]. Finally, it was found by simulations that Bayesian forecasting is expected to lead to greater target achievement (65%) than weight-based (32%) and covariate-based dosing (37%). Thus, even with Bayesian forecasting, a considerable fraction of concentrations will be outside the suggested target range due to large unexplained between-occasion variability in bioavailability.

Figure 14. Prediction-corrected (PC) visual predictive checks using the final theory-based model. Red solid line represents median observed concentration, red dashed lines represent 5th and 95th percentiles of the observed concentrations, black solid line represents median predicted concentration in 100 simulated subsets of total dataset, black dashed lines represent 5th and 95th percentiles of the predicted concentrations. Grey-shaded areas represent 95% confidence intervals of the prediction percentiles.
Paper IV

Improved tacrolimus target concentration achievement using computerized dosing in renal transplant recipients – a prospective, randomized study

Between January 13, 2014 and June 9, 2014, 102 patients were assessed for eligibility. Of these, 22 were excluded, and 80 were randomized. Two patients were excluded after randomization due to co-treatment with the CYP3A4-inhibitor verapamil (computer group) and cancelled transplantation because of a positive cross-match (control group). Thus, 39 patients received Bayesian-based dosing (32 standard risk, 7 high-risk) and 39 patients received conventional dosing (35 standard risk, 4 high-risk). Seventy-four patients completed the full eight-week study period. Table 2 shows the main outcome variables. The proportion of tacrolimus concentrations within the target range was significantly higher in the computer group than in the control group for both standard risk (Figure 15) and high-risk patients. Eight weeks after transplantation, the patients in the computer group had significantly higher glomerular filtration rate and significantly lower 2-hour plasma glucose. There were no significant differences in the number of rejections or infections.

Table 2. Tacrolimus concentration achievement and clinical outcomes eight weeks after transplant.

<table>
<thead>
<tr>
<th>Model</th>
<th>Computer Group</th>
<th>Control Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number / Median (95% CI)</td>
<td>Number / Median (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Concentrations within target</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard risk</td>
<td>90% (84-95%)</td>
<td>78% (76-82%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High-risk</td>
<td>77% (71-80%)</td>
<td>59% (40-74%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Biopsy-proven acute rejections</td>
<td>3</td>
<td>5</td>
<td>0.45</td>
</tr>
<tr>
<td>Recorded infections</td>
<td>6</td>
<td>3</td>
<td>0.29</td>
</tr>
<tr>
<td>Glomerular filtration rate (mL/min/1.73 m²), mean</td>
<td>59 (55-64)</td>
<td>53 (48-57)</td>
<td>0.046</td>
</tr>
<tr>
<td>Fasting plasma glucose</td>
<td>5.3 (5.1-5.5)</td>
<td>5.5 (5.4-5.7)</td>
<td>0.058</td>
</tr>
<tr>
<td>2-hour plasma glucose</td>
<td>5.9 (5.6-6.6)</td>
<td>6.8 (6.1-8.1)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

**Figure 15.** Observed tacrolimus trough concentrations in the computer group (green) and control group (orange) over time after transplantation in standard risk patients. Solid lines show the median concentration. The shaded area represents the target range. Solid gray line represents the middle of the target range.
5 DISCUSSION

Due to large and time-dependent variability in tacrolimus pharmacokinetics, it is challenging to achieve and maintain the target concentration after kidney transplantation. Additionally, the optimal target concentration of tacrolimus has not been fully defined. The overall goal of this thesis was to evaluate and develop strategies to optimize tacrolimus treatment early after kidney transplantation. Paper I demonstrated that targeting tacrolimus trough concentrations of 3–7 µg/L in standard risk patients from the day of transplantation is safe and effective in the clinical setting. In paper II and III, population pharmacokinetic models of tacrolimus were developed, and a range of patient characteristics that partly explain the pharmacokinetic variability of tacrolimus were identified. The models predicted well in external patients and were found suitable for Bayesian forecasting. Finally, paper IV presented a randomized controlled trial comparing tacrolimus dosing based on Bayesian forecasting with conventional dosing performed by experienced transplant physicians. The target concentration achievement was improved using Bayesian forecasting. With these novel findings, this thesis has contributed to knowledge on strategies to improve tacrolimus treatment early after kidney transplantation.

5.1 Population pharmacokinetics of tacrolimus

Paper II and paper III describe the development of population pharmacokinetic models of tacrolimus using different sample sizes and different covariate selection strategies. Nevertheless, the population pharmacokinetic models share some common features. Both are two-compartment models with first-order absorption, which is in agreement with most published models of tacrolimus (Table 1, page 9). Both models were fundamentally based on accounting for hematocrit differences before traditional covariate investigation. Although different strategies were used (hematocrit standardization in paper II, prediction of plasma concentrations in paper III), the strategies are principally the same and aim to reduce variability in whole blood concentrations caused by differences in hematocrit. This approach
is novel compared with previous models that have evaluated hematocrit only as a covariate on either clearance or volume of distribution. Furthermore, both the presented models identified fat-free mass as the superior body size metric, which is also a novel finding. Previous models have generally not identified any relationship between tacrolimus pharmacokinetics and body size, but have not evaluated fat-free mass.

The presented models identified higher CL/F in patients expressing functional CYP3A5 enzymes. This is in agreement with all the population pharmacokinetic studies for tacrolimus with this covariate available. In paper II, the data did not support the estimation of separate effects on clearance and bioavailability, probably because most CYP3A5 expressers only contributed one data point per dose interval. Paper III, on the other hand, is the first published model that estimated distinct effects of CYP3A5 genotype on tacrolimus clearance and bioavailability. This was reasonable because CYP3A5 enzymes are present both in the liver and the intestine. Of note, the actual bioavailability cannot be estimated without intravenous data, and the estimations only refer to the difference in bioavailability in one group compared to another group (i.e. relative bioavailability).

Some differences in the covariate selection between the presented models are worth noticing. First, bioavailability was found to be lower in females than in males in paper II (p=0.002). This finding was supported by a few studies that reported higher tacrolimus dose requirement in females. However, sex has not been included as a covariate in previously published population pharmacokinetic models for tacrolimus, and the modeling in paper III, which was based on a larger dataset than paper II, did not support the inclusion of sex. This indicates that sex was a falsely detected covariate in paper II. When multiple covariate relationships are tested, the risk of including a false covariate increases. When exploring multiple models, using a more stringent significance criterion has been recommended.

Another difference was that patient age was included as a covariate on bioavailability in paper II and not in paper III. In paper II, this effect was uncertain because of a small number of older patients in the dataset. In paper III the sample size was larger, and there was still a statistically significant, modest, linear increase in bioavailability above 45 years of age. However, age was not included in the model due to the lack of a clear biological mechanism for this observation. Most studies have not described declining intestinal CYP3A or P-glycoprotein expression with ageing, and the extent of drug absorption is generally known to decrease rather than increase in the elderly. Furthermore, with some exceptions, population modeling analyses and other studies have generally not
Discussion

identified a relationship between age and tacrolimus pharmacokinetics in adults.\textsuperscript{118,120} In one large study with considerably more elderly (>65 years) kidney transplant recipients included, however, elderly displayed significantly higher tacrolimus trough concentrations normalized for dose and weight.\textsuperscript{119} Ageing also leads to reduced immunologic responsiveness, indicating that lower tacrolimus exposure may be adequate in older patients.\textsuperscript{120} More studies are needed to characterize tacrolimus pharmacology across the full age range.

A third difference between the models was that time after transplantation was used as an empirical covariate in paper II, whereas in paper III, a prednisolone induction function was used as a mechanistic covariate on tacrolimus bioavailability based on previous knowledge of glucocorticoid-induced CYP3A/P-glycoprotein expression in the small intestine and liver.\textsuperscript{121,122} This difference was due to different covariate modeling strategies in the two studies (empirical vs. mechanistic). Because time after transplantation and prednisolone dose are highly correlating as a consequence of clinical practice, both brought approximately the same information into the models. The prednisolone induction function estimates are somewhat uncertain, do not account for individual differences, and may also reflect underlying time-related changes that are not related to the use of prednisolone.

Both the presented models and one previously published empirical model\textsuperscript{93} were externally evaluated in paper III. The theory-based model was superior to the empirical models, possibly due to the mechanistic approach to covariate identification, which is known to extrapolate better to data not used for model building.\textsuperscript{123,124} The larger patient sample in paper III is also likely to have contributed to this finding. For future applications, the model described in paper III is likely to represent the best selection of the two presented models.

5.2 Bayesian forecasting of tacrolimus doses

Although more than 15 population models have been published for tacrolimus in kidney transplanted adults, paper IV presents the first prospective, clinical evaluation of Bayesian forecasting of tacrolimus doses after kidney transplantation. Compared with conventional dosing performed by transplant physicians, the proportion of tacrolimus trough concentrations within the target concentration range was significantly higher using this strategy, and the median trough concentration was considerably closer to the middle of the target range. These results are not surprising, considering the number of studies that have
demonstrated the superiority of Bayesian forecasting in other therapeutic areas as well as transplanted patients. In addition to better target achievement, renal function and glucose tolerance were better eight weeks after transplantation in the group receiving Bayesian-based doses. These results suggest that generally lower and more consistent tacrolimus exposure translates into improvements in the extent of nephrotoxic and diabetogenic side effects. However, larger and long-term studies are needed to confirm this.

As discussed in paper III, the size of unexplained between-occasion variability (BOV) is important because it ultimately limits the maximum potential for target achievement using TDM or Bayesian forecasting. Although BOV was partly explained by hematocrit and prednisolone dose changes, unexplained BOV remains large for tacrolimus (estimated to CV 23% for bioavailability in paper III). This is illustrated also by the considerable scattering of concentrations around the median in the patients receiving Bayesian-based doses in study IV (Figure 15, page 30). Potential sources of unexplained BOV for tacrolimus include non-adherence, episodes of diarrhea and concomitant intake of drugs or food that influence tacrolimus absorption or CYP3A/P-glycoprotein activity. These factors are challenging to measure and probably not easily included in a population pharmacokinetic model. Thus, reducing the sources of BOV may be a better strategy to make tacrolimus exposure between dosing occasions more consistent. This may be accomplished by actions to reduce non-adherence, prevention of drug-drug interactions and encouraging patients to consistent concomitant food intake. Another approach is to develop new drug formulations with enhanced and less variable bioavailability. The latter has been demonstrated for a new tacrolimus once-daily formulation with “MeltDose” technology. Reducing sources of BOV is probably also important from a clinical point of view because within-patient variation in tacrolimus trough concentrations repeatedly has been associated with poor outcomes after kidney transplantation.

The available dosing software (BestDose) for the conduction of study IV is based on non-parametric modeling. Therefore, a non-parametric model was used rather than the parametric models presented in paper II and III. The non-parametric model was developed using the same data set as the model described in paper II, and the models share the same basic characteristics (two-compartment models with linear elimination and first-order absorption with a lag-time) and covariates (fat-free mass, hematocrit and time after transplantation; CYP3A5 genotype was excluded in study IV because the participants had not been genotyped prior to transplantation). The external predictive performances of the models have been compared and found to be very similar (data not published). Thus, this
choice is not expected to have markedly influenced the conclusions of paper IV. It may, however, have improved identification of outlying patients or patients who belong to pharmacokinetic sub-populations.109

5.3 Optimal tacrolimus concentration

The optimal tacrolimus concentration is the concentration that minimizes adverse effects without compromising protection against acute rejection and the development of de novo donor specific antibodies (dnDSA). In the Symphony study, a tacrolimus target trough concentration of 3–7 µg/L was superior to alternative regimens based on cyclosporine or sirolimus when combined with mycophenolate mofetil, glucocorticoids and anti-interleukin-2 receptor antibody induction.9 Paper I provides supplementary evidence from routine clinical data supporting that this regimen can be safely applied in standard risk kidney transplant recipients with good short-term outcomes (14.5% acute rejection and 5.2% dnDSA within one year). However, neither of these studies was designed to answer whether this tacrolimus concentration range is optimal, as the results were not compared with those of patients treated with alternative tacrolimus strategies. To date, the optimal tacrolimus concentration remains unknown, and targeted concentration ranges vary considerably between transplant centers.42,57

In study I, we performed post-hoc analyses to investigate potential relationships between tacrolimus whole blood trough concentrations and acute rejection or dnDSA formation. However, the concentrations were not significantly different neither in rejecters vs. non-rejecters or in patients with vs. without detectable dnDSA after one year. This is in agreement with previous studies that could not demonstrate a relationship between tacrolimus trough concentration and acute rejection rate131-133 or dnDSA formation.134,135 On the contrary, some studies have suggested various trough concentration thresholds for increased acute rejection risk: <9.3 µg/L136 and <8 µg/L137 during the first five days, <10 µg/L during the first month,138 and <4 µg/L during the first year.139 The inconsistent and sometimes undetectable relationship is probably a result of relatively narrow concentration ranges under evaluation, differences in individual immunological risks and concomitant therapies, concentrations measured at different time periods after transplantation, as well as limited ability of tacrolimus trough concentrations to reflect overall drug exposure (see
In addition, as discussed in paper II, the underlying relationship may also have been diluted by large hematocrit variability and therefore varying erythrocyte accumulation. This confines the ability of the whole blood concentration to reflect the unbound concentration that is available to enter the T-cell and reach the target site. Of note, the direct measurement of tacrolimus within T-cells represents a compelling alternative strategy. In liver transplant recipients, intracellular concentrations of tacrolimus in peripheral blood mononuclear cells (where T-cells constitute the main component) have been found to correlate strongly with acute rejection \((p<0.05)\).\(^{140}\) The same tendency has been observed in kidney transplant recipients \((p=0.09)\).\(^{141}\) However, this strategy is still in its infancy and will probably not become clinically available for a while.\(^{142}\) In the meantime, it may be valuable to interpret tacrolimus whole blood concentrations in the light of the accompanying hematocrit values, which are routinely measured, to get a more accurate perception of the pharmacologically active concentration.

In order to define the optimal tacrolimus concentration, there is a need for concentration-controlled clinical trials that prospectively evaluate alternative targets. In such trials, it would be beneficial to standardize the definition of immunological risk status to facilitate comparison of results between studies. The Bayesian forecasting technique presented in paper IV may be a useful tool to ensure tacrolimus target achievement in future concentration-controlled trials.

5.4 Methodological considerations and limitations

5.4.1 Internal and external validity

Paper I

The internal validity of study I is limited by the lack of an appropriate control group. Ideally, clinical outcomes should be compared with those of comparable patients receiving tacrolimus doses to achieve a different target, such as from patients transplanted during the period prior to implementation of the Symphony regimen in 2009. This, however, was not possible because there were additional differences in immunosuppressive regimen between the periods, such as a considerably higher proportion of patients receiving cyclosporine and higher glucocorticoid doses. The comparison with historical data from the Symphony study
Discussion

should be interpreted with caution due to uneven distribution of patient variables between the studies that may have influenced clinical outcomes.

The external validity is limited by selection bias, which occurs when specific patients are systematically excluded from the study. During the first interval of the study period (2009-2011), standard risk patients received either cyclosporine or tacrolimus. The protocol for this period stated that cyclosporine should be the preferred calcineurin inhibitor for patients >50 years, body mass index >28 kg/m² or with known impaired glucose tolerance. Data from patients receiving cyclosporine were not collected for this study, but due to these criteria it is likely that these patients were systematically different from those who received tacrolimus. The studied sample may therefore not be fully representative for the typical kidney transplant population in Norway. Furthermore, the results were based predominantly on Caucasian patients with standard immunological risk as defined by our local protocol, limiting the relevance of the results to centers with different ethnical compositions and/or different risk definition criteria.

Paper II and III

Paper II and III describe the development of population pharmacokinetic models of tacrolimus using non-linear mixed effects modeling. An advantage of this type of modeling is its ability to appropriately handle sparse and imbalanced data from different sources. Nevertheless, the sampling design is important for the ability to estimate reliable parameters. Paper II was limited by a relatively small study sample (n=69) with a large proportion of trough concentrations (67%, Figure 6, page 15). Trough concentrations represent the least informative pharmacokinetic sample type and return no information about the absorption process. Furthermore, during modeling it was assumed that 12 hours had passed since last dose for these samples, although the exact time after dose was unknown. This uncertainty was probably reflected by wide confidence intervals (paper II, Table 3) of the pharmacokinetic parameters, except clearance. Study III included a considerably larger patient sample (n=242), and the majority of patients contributed multiple tacrolimus concentrations from within the dosing interval. This allowed more reliable estimation of the absorption process, as reflected by the narrower confidence intervals (Paper III, Table 2).

The hematocrit-related components of the pharmacokinetic models relied on pharmacological theory and information about erythrocyte binding from previous studies. The fact that unbound or plasma concentrations were not actually measured in our studies is
clearly a limitation. Furthermore, the models assign the average blood distribution parameters to all patients, whereas in reality the erythrocyte affinity and capacity vary substantially between patients, also at the same hematocrit level.\textsuperscript{24,145} Thus, these models provide a best guess of a tacrolimus hematocrit-standardized (paper II) or plasma (paper III) concentration based on available data, but these estimates cannot replace the value of actually measuring unbound or plasma concentrations.

The external evaluation dataset covered similar covariate ranges as the original datasets. The ability of the models to predict outside these ranges is not known and extrapolation should be done with caution. Moreover, in both studies, patients using concomitant drugs with a known pharmacokinetic interaction with tacrolimus (except those that were used by >5\% of the patients in paper II) or who received multiple organ transplants were excluded. The exclusion of these sub-populations may have led to underprediction of the true variability across the target population as a whole.

Another factor limiting the external validity of the results is that tacrolimus concentrations were measured using three different types of analytical assays. We attempted to account for the systematic difference between assays by using a linear regression equation developed as part of routine procedures at the local laboratory. Still, this inter-conversion may have brought additional uncertainty to the results. Moreover, the conversion equation may be of limited validity at other laboratories. Clearly, assay type needs to be considered when applying the presented results. For example, the pharmacokinetic parameters in paper III are estimated with respect to LC-MS/MS equivalent tacrolimus concentrations. If used for prediction of immunoassay measurements without conversion, the model is expected to underpredict concentrations. Before clinical application at other centers, the model should preferably be retrospectively evaluated on a local patient sample.

**Paper IV**

The randomized, controlled design in study IV protects against potential confounding variables.\textsuperscript{143} However, uneven distribution of group characteristics may still randomly occur, particularly with a limited number of patients. All the measured patient characteristics in study IV were balanced, except a higher number of living donor recipients and ABO mismatches in the computer group. It cannot be excluded that the difference in clinical outcomes between the groups may have been due to uneven distributions of measured or unmeasured patient characteristics that influence outcomes.
The use of blinding was practically impossible, and bias may have been introduced if the physicians in the control group performed differently during their dose decisions for study patients than they do in regular practice. The fact that the median proportion within the target range of 78% [95% CI 76-82%] was higher than the proportion within the same range in paper I (68%) supports this theory. Thus, the true difference between the Bayesian forecasting and conventional dosing may be even larger than observed. Bias may also have been introduced by any specific patient behavior due to study participation. For example, patients may have taken tacrolimus more regularly because they were asked to write down the exact times of drug intake and because the study information underscored the importance of consistent drug intake. The contribution of non-adherence to the total pharmacokinetic variability was therefore probably reduced in this study.

5.4.2 Influence of missing data

Paper I

The data set collected for study I was nearly complete. One-year graft- and patient survival data were available for all patients, and the remaining one-year outcome variables were available for 400 out of 406 patients (98.5%). However, a large proportion of the patients (24% of those without pre-transplant diabetes mellitus) did not perform an oral glucose tolerance test. This was due to a period in 2011 with limited laboratory resources. These data are therefore missing completely at random (i.e. no particular type of patient over-represented in the group with missing data) and should theoretically not have introduced bias to the results.144

Paper II and III

Tacrolimus whole blood concentrations reported below the lower quantification limit (BQL) of the analytical assay were discarded. However, BQL data are not missing at random, and omitting such data may lead to biased parameter estimates.146 Other methods more appropriate than simply discarding the data exist.147 However, simulation studies have shown that the method for handling BQL data when <10% of the observations are BQL is irrelevant.146 In our analysis, <0.2% were BQL, and the effect of discarding these should be negligible.
Of the independent variables, time-constant covariates were complete in the dataset, while some time-varying covariates were missing. The most frequently missing covariates were liver function test values (about 1/3 of the data points in paper II), which were collected approximately once weekly in contrast to multiple times weekly for the dependent variable. The last-value-carried-forward approach for covariate imputation was used because of its simplicity. An alternative and probably better method would be linear interpolation or creating a model to predict the variable from other variables in the dataset. Yet, it is unlikely that this would have revealed a significant effect of liver function test values, considering that there was no trend in the available data. The remaining time-varying covariates were mostly complete, and none were missing at >10% of the data points.

Paper IV

Two patients were excluded from study IV after randomization (one started on the CYP3A4 inhibitor verapamil, one had transplantation cancelled), and four patients did not complete the eight-week study period (three were moved to local hospitals and were unable to complete visits, one had the graft removed after two weeks due to unsuccessful transplantation). The causes of these losses are most likely not related to the study participation, and the data are therefore missing completely at random with respect to study group. These missing data should not have biased the outcome comparison between the groups.

The data describing tacrolimus doses and concentrations were complete. Among the clinical outcome variables, glomerular filtration rate was not measured in eight of the 74 patients who completed the study (three in computer group, five in control group) and three patients did not perform an oral glucose tolerance test. The causes for missing data in these patients were not recorded and may not have been at random. The descriptive variables within the groups may therefore have become biased. Still, the reason for missing renal function and glucose tolerance assessment is not likely to have been related to the participation in this study, and should therefore not have influenced the comparison between the groups.
5.5 Clinical implications

The results presented in paper I provide some reassurance that the immunosuppressive regimen suggested in the Symphony study can be safely applied to standard risk Caucasian kidney transplant recipients in the clinical setting. This may encourage transplant centers that are using more conservative immunosuppressive regimens to reconsider adopting a low-target tacrolimus-based strategy. Of note, similar to the Symphony study, a high percentage of the tacrolimus concentrations (30%) were above 7 µg/L, and only 25% of concentrations were below 5 µg/L. This is likely a result of the physicians being more satisfied with concentrations towards the upper end of the range rather than the lower, as most are aware of the actual tacrolimus concentrations in the Symphony study. Thus, study I provides little new information about the safety of tacrolimus trough concentrations below 5 µg/L.

The population pharmacokinetic models presented in paper II and III include a range of patient characteristics that may be relevant for the clinical dosing of tacrolimus. For example, patient fat-free mass was identified as a superior body size descriptor for tacrolimus pharmacokinetics. Tacrolimus is traditionally dosed in proportion to body weight (mg/kg), as recommended by the manufacturer, despite that very few studies have characterized a relationship between body size and tacrolimus pharmacokinetics. Figure 16 illustrates how tacrolimus clearance is expected to increase with body weight using the mg/kg approach vs. the model-predicted allometric relationship with fat-free mass. From 40 to 120 kg body weight, proportional dosing assumes a three-fold increase in clearance (and dose), whereas the presented models predict only a 60% increase. Fat-free mass is typically not measured early after kidney transplantation, but can be estimated from weight, height and sex using relatively complex equations. It is uncertain whether the potential benefits of using allometric scaling to fat-free mass for the initial tacrolimus dose selection justifies the increased calculation complexity compared with simple linear scaling to total body weight (mg/kg). The dosing strategy simulation presented in paper III suggested that the target concentration achievement would be only modestly improved with covariate-based dosing (including fat-free mass) compared with standard weight-based dosing (37% vs. 32%, respectively). Still, precaution should be taken when dosing tacrolimus by mg/kg to patients with low and high weights to prevent initial underexposure and overexposure, respectively.
The results of paper II and III support previous studies in that patients expressing functional CYP3A5 enzymes require approximately doubled doses to achieve the same tacrolimus trough concentration as CYP3A5 non-expressers.\textsuperscript{32,152} From 2015, patients awaiting kidney transplantation in Norway have been genotyped, and the initial dose for CYP3A5 expressers have been recommended to be doubled (0.08 mg/kg).\textsuperscript{7} This illustrates how results of population pharmacokinetic modeling may translate into clinical use. However, in recent randomized controlled trials, clinical outcomes were not different in patients receiving CYP3A5-based initial doses compared with standard initial doses, and considerable proportions (>50\%) of concentrations were outside the target range (trough concentration 10-15 µg/L during the first two weeks) in both dosing groups.\textsuperscript{55,153} This questions the clinical relevance of pre-transplant \textit{CYP3A5} genotyping, provided the intensive TDM dose adjustments a few days after the initial dose. It also demonstrates that for drugs with large unexplained pharmacokinetic variability, such as tacrolimus, covariate-based dosing alone
is insufficient to reliably achieve the target concentration and highlights the need for proper
dose adaptation strategies in addition to initial dose algorithms.

The usefulness of Bayesian forecasting to predict tacrolimus doses in the clinical
management of kidney transplant recipients was demonstrated in paper IV. However, the
method has not been routinely implemented yet due to the complex methodology and the
need for trained personnel to use it safely. A user-friendlier version of BestDose is currently
under development. Improved target achievement of tacrolimus is likely to translate into
improved long-term graft and patient survival by reducing acute rejection risk, the formation
of de novo donor-specific antibodies and tacrolimus-related adverse effects (Figure 1, page
2).\textsuperscript{20} Long-term data are needed to confirm this anticipation.
6 CONCLUSIONS

The overall objective of the present thesis was to evaluate and develop strategies to optimize tacrolimus treatment after kidney transplantation. The following conclusions have been made:

I) Low-target tacrolimus-based immunosuppression from the day of transplantation is safe and effective in standard risk kidney transplant recipients, also in the “real-world” clinical setting. Clinical outcomes were similar to, or better than, those observed in the Symphony study.

II) Fat-free mass, CYP3A5 genotype, sex, age and time after transplantation influence the dose requirement of tacrolimus after kidney transplantation. Hematocrit predicts variability in whole blood concentrations, but is not expected to influence the unbound, therapeutically active concentration.

III) A theory-based population pharmacokinetic model was developed for tacrolimus and included fat-free mass, CYP3A5 genotype and prednisolone dose as mechanistic covariates. The model predicted better into an external cohort of kidney transplant recipients than two alternative empirical models.

IV) Bayesian forecasting of tacrolimus doses improves target achievement early after kidney transplantation compared with dosing determined by experienced transplant physicians.
7 FUTURE DIRECTIONS

- The theoretical importance of using hematocrit for tacrolimus whole blood concentration interpretation was discussed in paper II and III. Future studies should evaluate whether this pharmacological theory applies to the clinical setting by investigating whether hematocrit-standardized (or unbound) tacrolimus concentrations may predict therapeutic effects or immune function biomarker levels better than whole blood concentrations.

- The current standard is to monitor and target tacrolimus trough concentrations, despite that the AUC is generally considered a better descriptor of drug exposure. Paper IV demonstrated that Bayesian forecasting is clinically feasible and can be used to improve trough concentration achievement. A compelling feature of this technique is that it also offers AUC-guided dosing with great flexibility in sampling times. With the aid of Bayesian forecasting, future studies may be designed to prospectively compare clinical outcomes when targeting trough concentrations vs. AUCs.

- The present thesis focused on tacrolimus pharmacokinetics and the achievement of specific blood concentrations. Due to considerable pharmacodynamic variability for tacrolimus, undesired effects will occur even with perfect concentration control. With the recent progresses in measuring immune function biomarkers, future work should focus on developing joint population pharmacokinetic-pharmacodynamic models. Such models may be used to target specific drug effects in addition to drug concentrations. This represents a promising next step towards individualized dosing of tacrolimus.
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