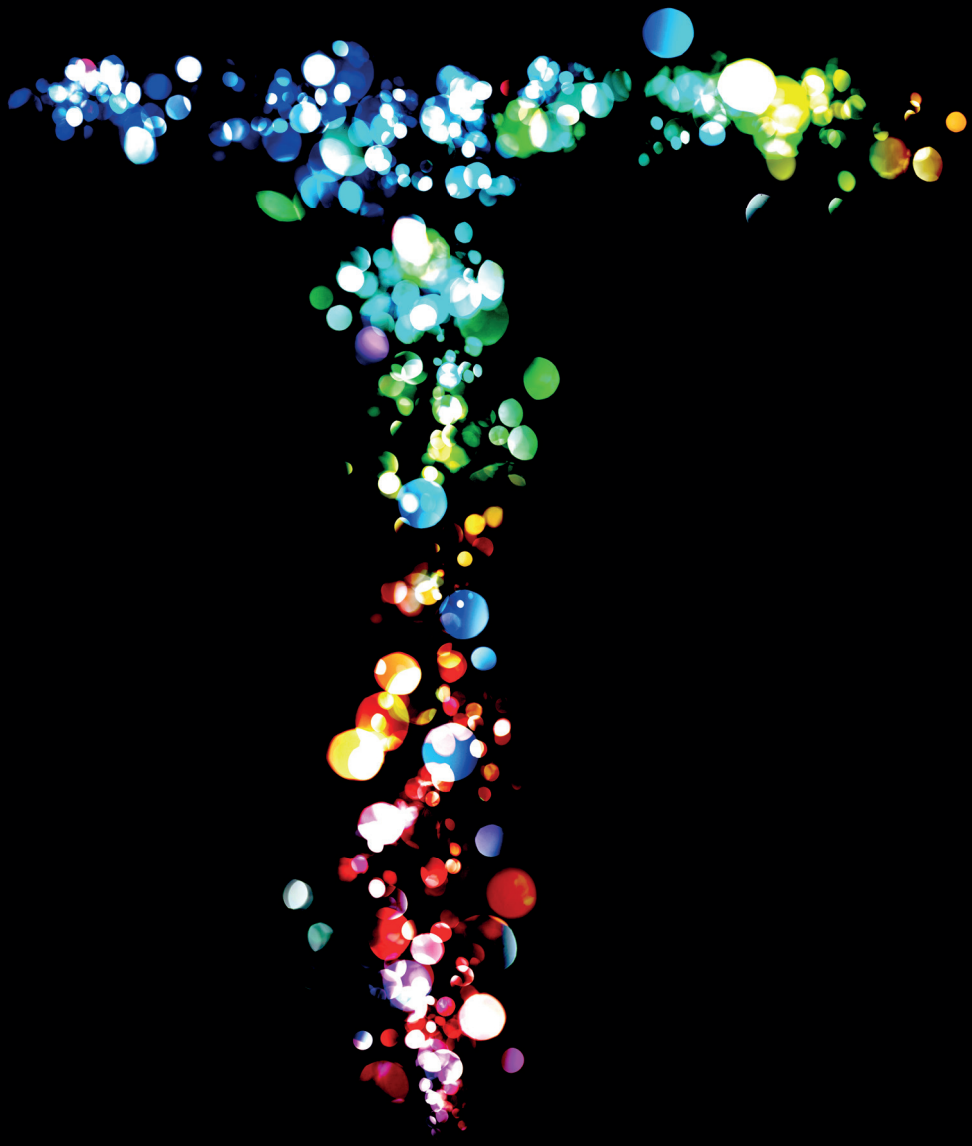
Tacrolimus pharmacokinetics and toxicity in the first days after heart and lung transplantation



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PhD thesis, Utrecht University, the Netherlands

The work presented in this thesis was performed at the Dutch Poisons Information Center and the department of Intensive Care in close collaboration with the pharmacy lab and the departments of heart and lung transplantation of the University Medical Center Utrecht, the Netherlands and the department of lung transplantation of the Antonius Hospital, the Netherlands.

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Tacrolimus pharmacokinetics and toxicity in the first days after heart and lung transplantation

Tacrolimus farmacokinetiek en toxiciteit in de eerste dagen na hart- en longtransplantatie
(met een samenvatting in het Nederlands)

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PROLOGUE

I will never forget Anna. She was admitted to our intensive care when I was still a resident. Anna, 45 years of age and suffering from end-stage idiopathic pulmonary fibrosis was on the transplantation list for 2 years. Mechanical ventilation was started because of respiratory insufficiency due to pneumonia. The antibiotics were effective, yet due to the end-stage fibrosis mechanical ventilation continued to be necessary. When she received the good news, we were all delighted. Anna would get new lungs!

The postoperative course differed from our expectations. Recurrent intra-thoracic bleeding with long periods of shock (low blood pressure and insufficient tissue perfusion) occurred. Although the team was determined to drag Anna through this difficult period, acute kidney injury developed as a result of the shock and the use of nephrotoxic drugs. Tacrolimus concentrations were frequently far out of the therapeutic range jeopardizing her kidney function. Renal replacement therapy was needed for a long time. Pleural effusions with atelectasis and consolidations of both lungs together with bronchomalacia and ICU acquired weakness accounted for failure to wean from the ventilator. Re-transplantation was not an option because of the concomitant kidney injury. Anna, the strongest woman I've ever met, decided to stop her treatment after six months of fighting. The team was devastated. Could the renal complications have been prevented? How does tacrolimus affect renal function early after lung transplantation?

This thesis is dedicated to and designated for patients like Anna.[1]

[1] The case is anonymized in accordance with privacy policy

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OBJECTIVES AND OUTLINE OF THIS THESIS

Objectives

The objectives of this thesis are to gain insight into how pharmacokinetics early after heart and lung transplantation affect the risk of tacrolimus (nephro-)toxicity. We used a population pharmacokinetic model of tacrolimus whole-blood, and total and unbound plasma to examine the pharmacokinetics in the first week after thoracic organ transplantation. The ultimate goal was to guide the transplant physician to improve tacrolimus dosing in the clinically unstable thoracic organ transplant patient and diminish tacrolimus (nephro-)toxicity.

Outline of this thesis

In **Part one** prevailing knowledge of tacrolimus pharmacokinetics is reviewed. We outline the changes in physiological conditions in the clinically unstable thoracic organ transplant patients affecting the variability in tacrolimus pharmacokinetics.

Part two describes the frequency of acute kidney injury in the first two weeks after lung transplantation, **Chapter 2a**, and heart transplantation, **Chapter 2b**, and the development to chronic kidney injury. The relationship of AKI with increased whole-blood tacrolimus concentrations is depicted.

In **Part three** pharmacokinetics of whole-blood, **Chapter 3**, and of total and unbound plasma concentrations, **Chapter 4b**, are described. The inter-individual and inter-occasion variability in pharmacokinetic parameters are shown. A guidance to improve tacrolimus dosing is disposed. In addition, in **Chapter 4a**, the development of a feasible analysis of tacrolimus plasma concentrations with HPLC-MS/MS is described.

Part four, Chapter 5, discusses the variability in tacrolimus pharmacokinetics in clinically unstable thoracic organ transplant recipients and the effects on tacrolimus blood concentrations. Future perspectives on improving tacrolimus dosing in clinically unstable patients are outlined. **Chapter 6a** and **Chapter 6b** summarize this thesis.

CHAPTER 1

Pharmacokinetics and toxicity of tacrolimus early after heart and lung transplantation

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ABSTRACT

Annually, about 8.000 heart and lung transplantations are successfully performed worldwide. However, morbidity and mortality pose a major concern. Renal failure in heart and lung transplant recipients is an essential adverse cause of morbidity and mortality, often originating in the early postoperative phase. At this time of clinical instability, the kidneys are exposed to numerous nephrotoxic stimuli. Among these, tacrolimus toxicity plays an important role, and its pharmacokinetics may be significantly altered in this critical phase by fluctuating drug absorption, changed protein metabolism, anemia and (multi-)organ failure. Limited understanding of tacrolimus pharmacokinetics in these circumstances is hampering daily practice. Tacrolimus dose adjustments are generally based on whole-blood trough levels, which widely vary early after transplantation. Moreover, whole-blood trough levels are difficult to predict and are poorly related to the area under the concentration-time curve. Even within the therapeutic range, toxicity may occur. These shortcomings of tacrolimus monitoring may not hold for the unbound tacrolimus plasma concentrations, which may better reflect tacrolimus toxicity. This review focuses on post-transplant tacrolimus pharmacokinetics, discusses relevant factors influencing the unbound tacrolimus plasma concentrations and tacrolimus (nephro-)toxicity in heart and lung transplantation patients.

INTRODUCTION

Heart and lung transplants are among the most successful solid organ transplantations in the world.[1] Still, long-term morbidity and mortality are significantly jeopardized by chronic kidney disease.[2,3] It has been shown that chronic kidney disease often originates from kidney injury acquired early after transplantation.[2,3] The underlying mechanisms of acute kidney injury are incompletely unraveled, but shock, systemic inflammation and tacrolimus nephrotoxicity are considered the most important factors. Serious clinical instability is frequently found in both heart and lung transplant recipients early after transplantation.[4,5] These unfavorable clinical conditions set the stage for highly fluctuating pharmacokinetics of tacrolimus with increased unbound plasma concentrations, which potentiate the risk of kidney injury. Here, we summarize current knowledge regarding tacrolimus pharmacokinetics as derived from healthy persons and patients undergoing solid organ transplantation. Insight is provided into how altered pharmacokinetics early after heart and lung transplantation affect the risk of tacrolimus (nephro-)toxicity.

Tacrolimus and its efficacy in heart and lung transplantation

The immunosuppressant tacrolimus has been of paramount importance since the 1990s in the modern era of heart and lung transplantation. Tacrolimus acts as a potent calcineurin inhibitor and has significantly contributed to contemporary 5-year-survival rates of roughly 85% for heart and 60% for lung transplantation.[6,7] In most studies, tacrolimus exhibits higher patient and organ survival rates than the calcineurin inhibitor cyclosporine. Moreover, tacrolimus leads to lower rejection rates and longer freedom from rejection.[8-10] Sirolimus, an immunosuppressant of the mTOR inhibitor group, is discouraged in the early phase after transplantation owing to wound-healing complications, especially bronchial dehiscence in lung recipients.[11] At present, when prioritizing efficacy, tacrolimus is the first choice immunosuppressive drug for heart and lung transplant recipients in the early phase post-transplantation. Consequently, improving tacrolimus management in heart and lung transplant recipients is of utmost importance.

Pharmacokinetics of tacrolimus in healthy persons

The pharmacokinetics of tacrolimus are best described by a 2-compartment model with first-order absorption and first-order elimination from the central compartment.[12] The mean disposition half-life of tacrolimus is about 12 hours.[13] Therefore, steady state concentrations are expected in two to three days. The therapeutic levels of whole-blood tacrolimus trough concentrations range from 5-20 µg/L, but to prevent toxicity the usual range is 5-15 µg/L.[14,15] In daily practice, whole-blood tacrolimus trough concentrations 12 hours after administration are generally used for therapeutic drug monitoring, even though it has been demonstrated that 6 hours post-administration concentrations better

correlate with the 12-hour area under the concentration-time curve (AUC) in stable transplantation patients.[12,16-18]

Bioavailability of tacrolimus

Tacrolimus administered orally is rapidly absorbed with a mean time to maximal concentration (Tmax) of 1-2 hours, while the composition of food may highly influence its absorption.[19] High fat as well as high carbohydrate meals may substantially decrease the maximal concentration (Cmax) and increase Tmax.[20] The highly lipophilic character of tacrolimus largely explains this phenomenon.

Another factor regulating tacrolimus bioavailability is P-glycoprotein (Pgp), which is an adenosine triphosphate (ATP)-driven efflux pump (Figure 1). Pgp is predominantly situated in the apical membrane of the mature epithelial cells but also in hepatocytes, renal proximal tubular cells, the blood-brain barrier and leucocytes.[21,22] There is a pharmacokinetic linkage between Pgp and cytochrome P-450 enzyme 3A (CYP3A) (Figure 1). When tacrolimus passes Pgp and enters the enterocyte, it is metabolized by CYP3A. Hereafter, Pgp pumps tacrolimus and its metabolites into the gut lumen where it is transported into more distal segments of the bowel containing lower amounts of both enzymes.[23-26]

The expression of Pgp and CYP3A is influenced by genetics. P-glycoprotein is encoded by the ABCB1 gene in humans. The single nucleotide polymorphisms (SNPs) 1199G>A and 2677G>T/A, 3435C>T and 1236C>T, whether present individually or in linkage, significantly minimize Pgp activity (0–28%) and result in a higher bioavailability of tacrolimus.[27,28] The expression of ABCB1 is influenced by ethnicity. The combined haplotype (2677G>T/A, 3435C>T, 1236C>T) is present in approximately 35% of Mexican Americans, 32% of Caucasians, 27% of Asian Americans and 5% of African Americans.[29-31] Another regulator of the ABCB1 genes is the pregnane X receptor (encoded by NR1I2). SNPs in the NR1I2 gene have been associated with reduced Pgp expression in the gut. Consequently, the pregnane X receptor 7635G>A and 8055T variant alleles may result in higher bioavailability of tacrolimus as well.[32,33]

Yet, another transporter of tacrolimus influencing oral bioavailability is the organic anion transporting polypeptide-C (OATP-C) (encoded by SLCO1B1), which is specifically expressed in the liver and takes part in the biliary excretion of tacrolimus. The SNP in the SLCO1B1 gene 521T>C significantly increases tacrolimus blood concentrations and the SNP 388A>G significantly decreases tacrolimus blood concentrations.[28]

The bioavailability of tacrolimus has been found to be roughly 15%, though may widely vary in healthy persons due to the aforementioned phenomena.[34] In the first days after transplantation, the bioavailability may be even more variable (Figure 2).

Blood distribution of tacrolimus

The binding of tacrolimus to blood components is an important factor in its pharmacokinetics.[35] Tacrolimus is mainly found within erythrocytes (85%-95%), only a small part being localized in lymphocytes (roughly 0.5%). In plasma, approximately 60% of tacrolimus is bound to the proteins albumin and α1-acid glycoprotein (AGP), 30% to high-density lipoprotein (HDL), 8% to low-density lipoprotein (LDL) and 1% to very low-density lipoprotein (VLDL). Only 0.3-2% of plasma tacrolimus is unbound.[36]

In more detail, tacrolimus is strongly bound to the cytosolic proteins cyclophilin and FK506 binding protein within the red blood cells.[35,37] Due to the extensive distribution of tacrolimus into the erythrocytes, its apparent volume of distribution based on whole-blood concentrations is much lower (1.0 to 1.5 L/kg) than based on plasma concentrations (about 30 L/kg).[38] Additionally, influx and efflux of tacrolimus from plasma into red blood cells and vice versa is rapid with clearance rates of 0.276 ml/min and 1.70 ml/min, whereby equilibrium is established within 2 minutes.[39] Because of this fast repartitioning, many authors prefer whole-blood tacrolimus concentrations instead of tacrolimus plasma concentrations to monitor patients' treatment, which seems adequate when erythrocytes and proteins are within normal limits.[38]

Metabolism of tacrolimus

Tacrolimus is mainly metabolized in the liver, but also in the gut and kidney. This process is mediated by, so called, phase I and II metabolism. Phase I metabolism occurs through the mixed-function oxidase system primarily by CYP3A4/5.[40,41] Phase II metabolism takes place in the liver by demethylation, glucuronidation, sulfation, acetylation, and conjugation. The resulting metabolites are only present in low concentrations in the blood and have minor pharmacological activity when compared to tacrolimus itself. Except for neurotoxicity, metabolites of tacrolimus are thought to be of minor clinical relevance.[42]

Significant inter-patient variation is present in the expression and function of CYP3A4 and CYP3A5, which is caused by the SNPs of genes encoding for these enzymes.

The frequency of the CYP3A4-392A>G SNP, also known as CYP3A4*1B, is predominantly found in Africans (in approximately 50%).[43] The CYP3A4*1B variant allele increases CYP3A4 expression and decreases tacrolimus concentrations.[44] Another SNP, CYP 3A4*18B: 82266G>A, is only expressed in Asians and also results in higher CYP3A4 expression.[44] The CYP 3A4*22 SNP is only expressed in 5% of Caucasians and causes low CYP3A4 expression. The CYP3A4*22 SNP in combination with CYP3A5 non-expression can easily result in supra-therapeutic tacrolimus levels and hence in increased toxicity of tacrolimus.[45]

The expression levels of CYP3A5*1 or *3 may influence metabolism of tacrolimus extensively and may be more important than CYP3A4 polymorphisms.[26,44,46] The CYP3A5*1/*1 and CYP3A5*1/*3 genotype (CYP3A5 expressers) is associated with significantly lower whole-blood tacrolimus concentrations when compared with the CYP3A5*3/*3 genotype (CYP3A5 non-expressers).[32,47] The CYP3A5*3 allele also shows distinctive ethnic diversity with allelic frequencies of about 35% in African-Americans, 70% in Asians and 95% in Caucasians.[48,49] Furthermore, the expression of CYP3A5 enzymes may differ between and within organs. For instance, CYP3A5 may be better expressed in the kidney than in the liver and within the kidney, CYP3A5 is predominantly expressed in the tubules metabolizing tacrolimus and decreasing nephrotoxicity.[41] The metabolisation of tacrolimus in the gut may be affected by CYP3A5-expression affecting bioavailability, which may be around 50% lower in CYP3A5-expressers in comparison to CYP3A5 non-expressers.[46]

Due to these large differences in CYP3A expression between individuals, it may be beneficial to identify CYP3A expression before transplantation to better predict tacrolimus blood concentrations and reduce (nephro-)toxicity directly after transplantation.[50]

Clearance of tacrolimus

Tacrolimus is mainly excreted via the bile, while the renal clearance rate amounts to less than 1% of the total body clearance.[51] Approximately 80-95% of the total tacrolimus dose is excreted via feces and more than 99% is excreted as metabolite.[51]

The systemic plasma clearance of tacrolimus is high (0.6-5.4 L/kg/hr), whereas whole body clearance, based on whole-blood concentrations, is much lower (0.03-0.09 L/kg/hr). Thus, the binding to blood components such as erythrocytes or proteins plays a major role in tacrolimus pharmacokinetics.[38]

Pharmacokinetics of tacrolimus early after heart and lung transplantation

The complexity of tacrolimus pharmacokinetics is markedly increased by a diversity of influences occurring in the peri-operative phase of heart and lung transplantation. The cardiopulmonary bypass itself alters pharmacokinetics by hemodilution, hypo-albuminemia and hypothermia as well as adsorption and sequestration in the bypass circuit.[52-54] Furthermore, the surgical procedure itself, its duration and potential complications, the blood transfusions, as well as ischemia-reperfusion injury of the transplanted organ(s) may all contribute to subsequent systemic inflammation. This, in turn, may alter organ function as well as blood cell and protein concentrations influencing tacrolimus pharmacokinetics.

The early post-operative period is mainly characterized by hemodynamic instability, the need for blood transfusions and the occurrence of systemic inflammation, which all

contribute to fluctuating tacrolimus pharmacokinetics and the increased risk of kidney injury. A subset of patients requires extended periods of extracorporeal support, i.e., veno-arterial or veno-venous extracorporeal membrane oxygenation, which has an additional impact on tacrolimus pharmacokinetics in the postoperative phase. In unstable patients especially, it is challenging to determine appropriate tacrolimus dosages as steady state concentration may not be reached given the prolonged mean disposition half-life time of up to 50 hours.[13,35]

As a result of these dosing difficulties in the first days after heart and lung transplantation, tacrolimus nephrotoxicity, which originates from vasoconstriction of afferent and efferent glomerular arterioles, often ensues.[55] When whole-blood and especially unbound tacrolimus plasma concentrations are increased, a stronger vaso-constrictive effect is suspected leading to acute kidney injury. The acute kidney injury is further aggravated by cardiac dysfunction, hypoxia, hypovolemia, large volume shifts and use of vasopressors (Table 1).[56] Pre-transplant risk factors such as impaired renal function, hypertension, diabetes, renal hypoperfusion, poor nutritional status, low muscle mass, weight loss and edema increase the risk for postoperative kidney injury.[57-59] Importantly, renal injury observed early after transplantation indicates an increased risk of developing chronic renal failure, which has been found in up to 50% after one year and 70% after five years.[3,60] This underscores the need to address the unresolved clinical problem of maintaining whole-blood tacrolimus trough concentrations within the therapeutic range to prevent nephrotoxicity.

Unfortunately, the relationship between whole-blood tacrolimus trough concentrations and the AUC is highly variable, especially peri-operatively, making interpretation of the former very challenging.[12,16-18] Even when tacrolimus concentrations are in the therapeutic range, toxicity may occur because of high-unbound tacrolimus plasma concentrations.[61] The variables influencing the bound and unbound tacrolimus concentrations may considerably change during the early postoperative phase.

Bioavailability of tacrolimus early after heart and lung transplantation

In hemodynamically unstable patients, the motility of the intestinal tract is significantly altered. This has a major impact on tacrolimus bioavailability, since intraluminal transport to the duodenum is limited, being its predominant site of intestinal absorption. On the other hand, a sudden increase in absorption may well occur when gut motility recovers upon hemodynamic improvement.

Furthermore, in situations of inflammation, ischemia-reperfusion injury, diarrhea and shock, Pgp-expression in the gut wall may be reduced leading to decreased Pgp levels

and an increase in whole-blood tacrolimus trough concentrations up to 100%.[17,19,25,62,63] Pgp levels generally normalize within 48 hours after the insult.[17,19,63]

Tacrolimus bioavailability is also importantly influenced by drug-drug interactions encompassing a large number of different drugs administered directly after heart and lung transplantation (See Table 2 and 3). A subset of these drugs significantly affects CYP3A and Pgp activity, e.g., corticosteroids induce the expression of intestinal and hepatic CYP3A and Pgp as does tacrolimus itself.[64] The overall effect of higher Pgp and CYP3A levels is a reduced and delayed absorption of orally administered tacrolimus.[23,25] By inhibiting intestinal Pgp as well as CYP3A activity, the absorption of tacrolimus increases and may result in very high blood concentrations.

Therefore, some authors prefer the sublingual or intravenous route over oral administration to obtain more stable tacrolimus concentrations.[65,66] However, absorption is minimal when tacrolimus is administered sublingually and prolonged intravenous administration is limited by toxic concentrations of the solvent polyoxyl-60-hydrogenated castor oil (HCO-60), causing additional renal injury.[67] At this moment, the preferred route of administration is oral, while sublingual or intravenous application is discouraged. When significant gut motility disturbances are observed, the intravenous route may be considered for a limited period of time.

Blood distribution of tacrolimus early after heart and lung transplantation

Under conditions of clinical instability, the resulting changes in blood composition alter plasma concentrations of unbound tacrolimus, e.g., through differences in erythrocytes concentrations, as mentioned before.[39,68] Anemia, as often encountered in this period, increases the unbound tacrolimus plasma concentrations, whereas red blood cells transfusions reduce it.

Furthermore, blood distribution of tacrolimus is affected by the concentrations of albumin, lipoproteins and AGP, which often change early after heart and lung transplantation. Hypo-albuminemia results from liver failure due to diminished production of proteins and from renal failure due to protein loss by the kidney. Decreased albumin concentrations may also be caused by a shortage of dietary protein, increased capillary permeability and hemodilution. Also, in renal failure, the number of tacrolimus-binding locations on the albumin molecule is reduced as a result of conformational changes and competitive binding of substances to albumin, such as fatty acids or uremic toxins.[69] Additionally, lipoprotein concentrations in general decrease rapidly in the peri-operative phase and may drop as low as 50%, being a result of decreased synthesis and enhanced catabolism. As a consequence, the decrease of the primary tacrolimus-binding lipoprotein HDL results in increased unbound tacrolimus plasma concentrations.[70,71] In contrary, the acute phase

protein AGP is often increased in case of inflammation and also after administration of corticosteroids, macrolide antibiotics and tacrolimus.[72,73] As a result, increased AGP concentrations may result in reduced unbound tacrolimus plasma concentrations.[74]

Thus, early after transplantation, the unbound tacrolimus plasma concentrations may change due to an altered blood composition, while the whole-blood concentrations may remain unchanged (Table 4). These conditions favor the measurement of the unbound plasma concentrations in unstable patients.

Metabolism of tacrolimus early after heart and lung transplantation

The metabolism of tacrolimus depends not only on hepatic intrinsic clearance, but also on hepatic blood flow as reflected by an intermediate extraction ratio.[75] Therefore, under conditions of shock, tacrolimus metabolism is impaired and may substantially increase its concentrations.[76]

Another phenomenon arising during periods of shock is the predominance of tacrolimus metabolisation in the gut as compared to the liver. Intestinal CYP3A levels are usually 10–50% of the concentration found in the liver, but during shock or systemic inflammation intestinal CYP3A, expressed primarily in the duodenum, may equalize or even exceed the hepatic levels.[77]

These high CYP3A concentrations in the proximal intestine increase tacrolimus metabolism and decrease whole-blood concentrations in times of shock.

Clearance of tacrolimus early after heart and lung transplantation

In the unstable clinical phase, whole-body clearance of tacrolimus and its metabolites is influenced by a diversity of factors, among which severe cholestasis, anemia and hypo-albuminemia may all substantially alter the clearance.[76] Cholestasis reflects hepatic dysfunction, which decreases the metabolism and transport of tacrolimus into the bile, resulting in a reduced clearance of tacrolimus. Anemia and hypo-albuminemia increase the unbound concentrations, which could augment the uptake of tacrolimus into the liver resulting in a higher clearance. This may explain the finding that patients with a low hematocrit (<0.35) have a higher whole-body clearance of tacrolimus (up to 46%) than patients with a higher hematocrit.[78,79] Also, in patients with hypo-albuminemia (albumin level <35 mg/L) clearance of tacrolimus is much higher (up to 16%) than in patients with albumin concentrations >35 mg/L.[78] These changes in whole-body clearance support the theory that steady state concentrations are often not reached within the first days after the initial dose of tacrolimus in unstable transplantation patients.[51]

Drug-drug interaction of tacrolimus

Heart and lung transplant recipients often receive a large number of different drugs that interfere with tacrolimus (See Table 2 and 3). A subset of these drugs may influence CYP3A, which metabolizes >90% of tacrolimus. Thus, inhibition or induction of CYP3A will lead to clinically significant changes in tacrolimus metabolism, whereby CYP3A inhibition is almost immediately effective and CYP3A induction is a slow process.[21] Therefore, when a drug interacting with tacrolimus pharmacokinetics is initiated or withdrawn, careful monitoring of the whole-blood tacrolimus concentrations and prompt adjustment of the dose is recommended.

Tacrolimus pharmacokinetics in Cystic Fibrosis

Cystic fibrosis (CF) constitutes a multi-system disorder, which may affect liver, pancreas and intestinal tract potentially causing a large scale of metabolic derangements. Therefore, the pharmacokinetics of tacrolimus in CF patients substantially differ from non-CF patients. Two underlying mechanisms are suggested. First, fat absorption is severely hampered due to pancreatic insufficiency resulting in high-fat containing stools. As a consequence, the absorption of tacrolimus, which is highly lipophilic, may be lowered to as much as 40%, whereas the rate of absorption is slower increasing the Tmax.[12] Next, total body clearance of tacrolimus is increased, likely by an increased phase II metabolism in these patients leading to reduced whole-blood tacrolimus concentrations.[80] Subsequently, in CF patients much higher doses of tacrolimus are generally required to achieve equivalent blood concentrations.[16]

Conclusions and future perspectives

Tacrolimus toxicity is an important determinant of morbidity and mortality after heart and lung transplantation. Clinical instability, especially in the early phase after transplantation, gives rise to fluctuating tacrolimus pharmacokinetics and subsequent nephrotoxicity. Clinicians should be aware of the spectrum of clinical conditions that influences tacrolimus pharmacokinetics, such as systemic inflammation, hemorrhage and shock, all of which result in higher variations of tacrolimus concentrations and therefore complicate adequate dosing.

In clinical practice, it remains cumbersome and unsatisfactory to prescribe well-titrated individualized daily administration of tacrolimus early after transplantation to prevent toxic levels in this phase. Even when the whole-blood tacrolimus concentrations are in the therapeutic range, toxicity may develop because the unbound plasma concentrations can accidentally increase to high levels. The unbound concentration has been shown to be an important factor in cellular uptake, and may increase glomerular vasoconstriction leading to nephrotoxicity in the early days after transplantation.[81]

Thus, from a mechanistic point of view, the plasma concentration of unbound tacrolimus is a more reasonable monitoring parameter for optimal tacrolimus dosing in the unstable patient. This concept of tacrolimus monitoring is novel and will help to avoid toxic tacrolimus concentrations but it necessitates the development of an effective analytical method to determine the unbound plasma concentrations. Unfortunately, at present, current assays used for routine tacrolimus monitoring lack the sensitivity to adequately measure the low unbound plasma concentrations. Until such analyses become available, unbound tacrolimus plasma concentrations can be predicted based on the concentrations of a subset of known bio-variables influencing them. Although pharmacokinetic modeling has been performed, these formulas are not appropriate for the unstable transplantation patient. Creating such a model is of utmost importance to decrease tacrolimus toxicity in the early days after transplantation. The erythrocyte count and the plasma protein concentrations of albumin, AGP and HDL all are pivotal variables, which have to be considered in this complex computation. This review provides initial guidance to clinicians in adjusting tacrolimus dosing regimens on the basis of these bio-variables.

REFERENCES

1. Christie JD, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report-2012. *J Heart Lung Transplant*. 2012;31(10):1073-1086. doi:10.1016/j.healun.2012.08.004.
2. Wehbe E, Duncan AE, Dar G, Budev M, Stephany B. recovery from AKI and short- and long-term outcomes after lung transplatation. *Clinical Journal of the American Society of Nephrology*. 2013;8(1):19-25. doi:10.2215/CJN.04800512.
3. Paradela de la Morena M, La Torre Bravos De M, Prado RF, et al. Chronic Kidney Disease After Lung Transplantation: Incidence, Risk Factors, and Treatment. *TPS*. 2010;42(8):3217-3219. doi:10.1016/j.transproceed.2010.05.064.
4. Taylor DO, Edwards LB, Aurora P, et al. Registry of the International Society for Heart and Lung Transplantation: twenty-fifth official adult heart transplant report-2008. *J Heart Lung Transplant*. 2008;27(9):943-956. doi:10.1016/j.healun.2008.06.017.
5. Vicente R, Morales P, Ramos F, Solé A, Mayo M, Villalain C. Perioperative complications of lung transplantation in patients with emphysema and fibrosis: experience from 1992-2002. *TPS*. 2006;38(8):2560-2562. doi:10.1016/j.transproceed.2006.08.048.
6. Kaczmarek I, Zaruba M-M, Beiras-Fernandez A, et al. Tacrolimus with mycophenolate mofetil or sirolimus compared with calcineurin inhibitor-free immunosuppression (sirolimus/mycophenolate mofetil) after heart transplantation: 5-year results. *HEALUN*. 2013;32(3):277-284. doi:10.1016/j.healun.2012.11.028.
7. Neurohr C, Huppmann P, Zimmermann G, et al. Tacrolimus and mycophenolate mofetil as first line immunosuppression after lung transplantation. *Transpl Int*. 2009;22(6):635-643. doi:10.1111/j.1432-2277.2009.00843.x.
8. Griffith BP, Bando K, Hardesty RL, Armitage JM. A prospective randomized trial of FK506 versus cyclosporine after human pulmonary transplantation. *Transplantation*. 1994;57(6):848-851. doi: 10.1097/00007890-199403270-00013
9. Treede H, Glanville AR, Klepetko W, et al. Tacrolimus and cyclosporine have differential effects on the risk of development of bronchiolitis obliterans syndrome: Results of a prospective, randomized international trial in lung transplantation. *HEALUN*. 2012;31(8):797-804. doi:10.1016/j.healun.2012.03.008.
10. Guethoff S, Meiser BM, Groetzner J, et al. Ten-Year Results of a Randomized Trial Comparing Tacrolimus Versus Cyclosporine A in Combination With Mycophenolate Mofetil After Heart Transplantation. *Transplantation*. 2013;95(4):629-634. doi:10.1097/TP.0b013e318277e378.
11. Groetzner J, Kur F, Spelsberg F, et al. Airway anastomosis complications in de novo lung transplantation with sirolimus-based immunosuppression. *HEALUN*. 2004;23(5):632-638. doi:10.1016/S1053-2498(03)00309-7.
12. Monchaud C, de Winter BC, Knoop C, et al. Population pharmacokinetic modelling and design of a Bayesian estimator for therapeutic drug monitoring of tacrolimus in lung transplantation. *Clinical Pharmacokinetics*. 2012;51(3):175-186. doi:10.2165/11594760-000000000-00000.

13. Phapale PB, Kim S-D, Lee HW, et al. An integrative approach for identifying a metabolic phenotype predictive of individualized pharmacokinetics of tacrolimus. *Clin Pharmacol Ther*. 2010;87(4):426-436. doi:10.1038/clpt.2009.296.
14. Undre NA, Meiser BM, Uberfuhr P, et al. Pharmacokinetics of tacrolimus (FK506) in primary orthoropic heart transplant patients. *Transplantation Proceedings*. 1998;30(4):1112-1115. doi:10.1016/S0041-1345(98)00173-0.
15. Berge M, Chevalier P, Benammar M, et al. Safe Management of Tacrolimus Together With Posaconazole in Lung Transplant Patients With Cystic Fibrosis. *therapeutic drug monitoring*. 2009;31(3):396-399. doi:10.1097/FTD.0b013e31819de6fd.
16. Saint-Marcoux F, Knoop C, Debord J, et al. Pharmacokinetic Study of Tacrolimus in Cystic Fibrosis and Non-Cystic Fibrosis Lung Transplant Patients and Design of Bayesian Estimators Using Limited Sampling Strategies. *Clinical Pharmacokinetics*. 2005;44(12):1317-1328. doi:10.2165/00003088-200544120-00010.
17. Kuypers D. Clinical efficacy and toxicity profile of tacrolimus and mycophenolic acid in relation to combined long-term pharmacokinetics in de novo renal allograft recipients. *Clinical Pharmacology & Therapeutics*. 2004;75(5):434-447. doi:10.1016/j.clpt.2003.12.009.
18. Mendonza AE, Zahir H, Gohh RY, Akhlaghi F. Tacrolimus in diabetic kidney transplant recipients: pharmacokinetics and application of a limited sampling strategy. *therapeutic drug monitoring*. 2007;29(4):391-398. doi:10.1097/FTD.0b013e31811f319b.
19. Maes BD, Lemahieu W, Kuypers D, et al. Differential Effect of Diarrhea on FK506 Versus Cyclosporine A Trough Levels and Resultant Prevention of Allograft Rejection in Renal Transplant Recipients. *Am J Transplant*. 2002;2(10):989-992. doi:10.1034/j.1600-6143.2002.21018.x.
20. Bekersky I, Dressler D, Mekki QA. Effect of low- and high-fat meals on tacrolimus absorption following 5 mg single oral doses to healthy human subjects. *J Clin Pharmacol*. 2001;41(2):176-182. doi: 10.1177/00912700122009999
21. Christians U, Jacobsen W, Benet LZ. Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clinical Pharmacokinetics*. 2002;41(11):813-851. doi: 10.2165/00003088-200241110-00003
22. Shitara Y, Maeda K, Ikejiri K, Yoshida K, Horie T, Sugiyama Y. Clinical significance of organic anion transporting polypeptides (OATPs) in drug disposition: their roles in hepatic clearance and intestinal absorption. *Biopharm Drug Dispos*. 2013;34(1):45-78. doi:10.1002/bdd.1823.
23. Christians U, Jacobsen W, Benet LZ, Lampen A. Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clinical Pharmacokinetics*. 2002;41(11):813-851. doi:10.2165/00003088-200241110-00003.
24. Paine MF, Khalighi M, Fisher JM, Shen DD. Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *journal of pharmacological and experimental therapy*. 1997;283(3):1-11.
25. Masuda S. Tacrolimus therapy according to mucosal MDR1 levels in small-bowel transplant recipients*1. *Clinical Pharmacology & Therapeutics*. 2004;75(4):352-361. doi:10.1016/j.clpt.2003.11.374.

26. Goto M, Masuda S, Kiuchi T, et al. CYP3A5*1-carrying graft liver reduces the concentration/oral dose ratio of tacrolimus in recipients of living-donor liver transplantation. *Pharmacogenetics*. 2004;14(7):471-478. doi:10.1097/01.fpc.0000114747.08559.49.
27. Staatz DCE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 Single Nucleotide Polymorphisms on the Pharmacokinetics and Pharmacodynamics of Calcineurin Inhibitors: Part II. *Clinical Pharmacokinetics*. 2010;49(4):207-221. doi:10.2165/11317550-000000000-00000.
28. Elens L, Capron A, Van Kerckhove VR, et al. 1199G > A and 2677G > T/A polymorphisms of ABCB1 independently affect tacrolimus concentration in hepatic tissue after liver transplantation. *Pharmacogenetics and Genomics*. 2007;17:873-883. doi: 10.1097/FPC.0b013e3282e9a533
29. Kroetz DL, Pauli-Magnus C, Hodges LM, et al. Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. *Pharmacogenetics*. 2003;13(8):481-494. doi:10.1097/01.fpc.0000054113.14659.b9.
30. Cho JH, Yoon YD, Park JY, et al. Impact of Cytochrome P450 3A and ATP-Binding Cassette Subfamily B Member 1 Polymorphisms on Tacrolimus Dose-Adjusted Trough Concentrations Among Korean Renal Transplant Recipients. *TPS*. 2012;44(1):109-114. doi:10.1016/j.transproceed.2011.11.004.
31. Mancinelli L. The pharmacokinetics and metabolic disposition of tacrolimus: A comparison across ethnic groups. *Clinical Pharmacology & Therapeutics*. 2001;69(1):24-31. doi:10.1067/mcp.2001.113183.
32. Press RR, Ploeger BA, Hartigh JD, et al. Explaining Variability in Tacrolimus Pharmacokinetics to Optimize Early Exposure in Adult Kidney Transplant Recipients. *therapeutic drug monitoring*. 2009;31(2):187-197. doi:10.1097/FTD.0b013e31819c3d6d.
33. Barraclough KA, Staatz CE, Johnson DW, et al. Kidney transplant outcomes are related to tacrolimus, mycophenolic acid and prednisolone exposure in the first week. *Transplant International*. 2012;25(11):1182-1193. doi:10.1111/j.1432-2277.2012.01553.x.
34. Karamperis N, Povlsen JV, Højskov C, Poulsen JH, Pedersen AR, Jørgensen KA. Comparison of the pharmacokinetics of tacrolimus and cyclosporine at equivalent molecular doses. *TPS*. 2003;35(4):1314-1318. doi: 10.1016/S0041-1345(03)00481-0
35. Jusko WJ, Piekoszewski W, Klintmalm GB, et al. Pharmacokinetics of tacrolimus in liver transplant patients. *Clinical Pharmacology & Therapeutics*. 1995;57(3):281-290. doi:10.1016/0009-9236(95)90153-1.
36. Zahir H, Nand RA, Brown KF, Tattam BN. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. *journal of pharmacological and toxicological methods*. 2001;46:27-35. doi: 10.1016 S1056-8719(02)00158-2
37. Fung JJ. Tacrolimus and transplantation. *Transplantation*. 2004;77(Supplement):S41-S43. doi:10.1097/01.TP.0000126926.61434.A5.
38. Wallemacq DPE, Verbeeck RK. Comparative Clinical Pharmacokinetics of Tacrolimus in Paediatric and Adult Patients. *Clinical Pharmacokinetics*. 2001;40(4):283-295. doi:10.2165/00003088-200140040-00004.

39. Chow F-S, Piekoszewski W, Jusko WJ. Effect of hematocrit and albumin concentration on hepatic clearance of tacrolimus (FK506) during rabbit liver perfusion. *Drug Metabolism and Disposition*. 1997;25(5):610-616. doi: 10.1016/1043-6618(95)86427-x
40. Masuda S. Effect of intestinal P-glycoprotein on daily tacrolimus trough level in a living-donor small bowel recipient. *Clinical Pharmacology & Therapeutics*. 2000;68(1):98-103. doi:10.1067/mcp.2000.107912.
41. Joy MS, Hogan SL, Thompson BD, Finn WF, Nickeleit V. Cytochrome P450 3A5 expression in the kidneys of patients with calcineurin inhibitor nephrotoxicity. *Nephrology Dialysis Transplantation*. 2007;22(7):1963-1968. doi:10.1093/ndt/gfm133.
42. Yanagimachi M, Naruto T, Tanoshima R, et al. Influence of CYP3A5 and ABCB1 gene polymorphisms on calcineurin inhibitor-related neurotoxicity after hematopoietic stem cell transplantation. *Clinical Transplantation*. 2010;24(6):855-861. doi:10.1111/j.1399-0012.2009.01181.x.
43. Lamba JK, Lin YS, Thummel K, et al. Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. *Pharmacogenetics*. 2002;12(2):121-132. doi:10.1097/00008571-200203000-00006.
44. Hesselink D. Genetic polymorphisms of the CYP3A4, CYP3A5, and MDR-1 genes and pharmacokinetics of the calcineurin inhibitors cyclosporine and tacrolimus. *Clinical Pharmacology & Therapeutics*. 2003;74(3):245-254. doi:10.1016/S0009-9236(03)00168-1.
45. Elens L, Van Schaik RH, Panin N, et al. Effect of a new functional CYP3A4 polymorphism on calcineurin inhibitors' dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenomics*. 2011;12(10):1383-1396. doi:10.2217/pgs.11.90.
46. Størset E, Holford N, Hennig S, et al. Improved prediction of tacrolimus concentrations early after kidney transplantation using theory-based pharmacokinetic modelling. *British Journal of Clinical Pharmacology*. 2014;78(3):509-523. doi: 10.1111/bcp.12361
47. Gervasini G, Garcia M, Macias RM, Cubero JJ, Caravaca F, Benitez J. Impact of genetic polymorphisms on tacrolimus pharmacokinetics and the clinical outcome of renal transplantation. *Transplant International*. 2012;25(4):471-480. doi:10.1111/j.1432-2277.2012.01446.x.
48. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nature genetics*. 2001;27:383-391. doi: 10.1038/86882
49. Hustert E, Haberl M, Burk O, et al. The genetic determinants of the CYP3A5 polymorphism. *Pharmacogenetics*. 2001;11:773-779. doi: 10.1097/00008571-200112000-00005
50. Sy SKB, Heuberger J, Shilbayeh S, Conrado DJ, Derendorf H. A Markov chain model to evaluate the effect of CYP3A5 and ABCB1 polymorphisms on adverse events associated with tacrolimus in pediatric renal transplantation. *AAPS J*. 2013;15(4):1189-1199. doi:10.1208/s12248-013-9528-9.
51. Moller A, Iwasaki K, Kawamura A, Teramura Y. The disposition of 14C-labeled tacrolimus after intravenous and oral administration in healthy human subjects. *Drug Metabolism and Disposition*. 1999;27(6):633-636.

52. Shekar K, Roberts JA, Welch S, et al. ASAP ECMO: Antibiotic, Sedative and Analgesic Pharmacokinetics during Extracorporeal Membrane Oxygenation: a multi-centre study to optimise drug therapy during ECMO. *BMC Anesthesiol*. 2012;12(1):29. doi:10.1186/1471-2253-12-29.
53. Van Saet A, de Wildt SN, Knibbe CAJ, Bogers ADJJC, Stolker RJ, Tibboel D. The effect of adult and pediatric cardiopulmonary bypass on pharmacokinetic and pharmacodynamic parameters. *Curr Clin Pharmacol*. 2013;8(4):297-318. doi: 10.2174/15748847113089990067
54. Pea F, Pavan F, Furlanut M. Clinical relevance of pharmacokinetics and pharmacodynamics in cardiac critical care patients. *Clinical Pharmacokinetics*. 2008;47(7):449-462. doi:10.2165/00003088-200847070-00002.
55. Textor SC, Wiesner R, Wilson DJ, et al. Systemic and renal hemodynamic differences between FK506 and cyclosporine in liver transplant recipients. *Transplantation*. 1993;55(6):1332-1339. doi: 10.1097/00007890-199306000-00023
56. De Lima JJ, Xue H, Coburn L, et al. Effects of FK506 in rat and human resistance arteries. *Kidney International*. 1999;55(4):1518-1527. doi:10.1046/j.1523-1755.1999.00366.x.
57. Bloom RD, Reese PP. Chronic Kidney Disease after Nonrenal Solid-Organ Transplantation. *Journal of the American Society of Nephrology*. 2007;18(12):3031-3041. doi:10.1681/ASN.2007040394.
58. Bloom RD, Doyle AM. Kidney disease after heart and lung transplantation. *Am J Transplant*. 2006;6(4):671-679. doi:10.1111/j.1600-6143.2006.01248.x.
59. Ishani A, Erturk S, Hertz MI, Matas AJ, Savik K, Rosenberg ME. Predictors of renal function following lung or heart-lung transplantation. *Kidney International*. 2002;61(6):2228-2234. doi:10.1046/j.1523-1755.2002.00361.x.
60. Stehlik J, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report--2012. *J Heart Lung Transplant*. 2012;31(10):1052-1064. doi:10.1016/j.healun.2012.08.002.
61. Lefaucheur C, Nochy D, Amrein C, et al. Renal Histopathological Lesions After Lung Transplantation in Patients with Cystic Fibrosis. *Am J Transplant*. 2008;8(9):1901-1910. doi:10.1111/j.1600-6143.2008.02342.x.
62. Omae T, Goto M, Shimomura M, et al. Transient up-regulation of P-glycoprotein reduces tacrolimus absorption after ischemia–reperfusion injury in rat ileum. *Biochemical Pharmacology*. 2005;69(4):561-568. doi:10.1016/j.bcp.2004.10.016.
63. Lemahieu W, Maes B, Verbeke K, Rutgeerts P, Geboes K, Vanrenterghem Y. Cytochrome P450 3A4 and P-glycoprotein Activity and Assimilation of Tacrolimus in Transplant Patients with Persistent Diarrhea. *Am J Transplant*. 2005;5(6):1383-1391. doi:10.1111/j.1600-6143.2005.00844.x.
64. Kuypers DR. Influence of interactions between immunosuppressive drugs on therapeutic drug monitoring. *Ann Transplant*. 2008;13(3):11-18.
65. Reams BD, Palmer SM. Sublingual tacrolimus for immunosuppresion in lung transplantation. *American Journal of Respiratory Medicine*. 2002;1(2):91-98. doi: 10.1007/bf03256598

66. Knoop C, Thiry P, Saint-Marcoux F, Rousseau A, Marquet P, Estenne M. Tacrolimus Pharmacokinetics and Dose Monitoring After Lung Transplantation for Cystic Fibrosis and Other Conditions. *Am J Transplant*. 2005;5(6):1477-1482. doi:10.1111/j.1600-6143.2005.00870.x.
67. Jiang T, Acosta D. An in vitro model of cyclosporine-induced nephrotoxicity. *Fundam Appl Toxicol*. 1993;20(4):486-495. doi: 10.1093/toxsci/20.4.486
68. Sharina H, Kek TL, Kwong SJS, et al. Pharmacogenotyping of CYP3A5 in predicting dose-adjusted trough levels of tacrolimus among Malaysian kidney-transplant patients. *http://dxdoiorg/101139/cjpp-2013-0128*. 2013;26(1):506-515. doi:10.1139/cjpp-2013-0128.
69. Otagiri M. A Molecular Functional Study on the Interactions of Drugs with Plasma Proteins. *DMPK*. 2005;20(5):309-323. doi:10.2133/dmpk.20.309.
70. Lüthold S, Berneis K, Bady P, Müller B. Effects of infectious disease on plasma lipids and their diagnostic significance in critical illness. *Eur J Clin Invest*. 2007;37(7):573-579. doi:10.1111/j.1365-2362.2007.01826.x.
71. Van Leeuwen HJ, Heezius ECJM, Dallinga GM, van Strijp JAG, Verhoef J, van Kessel KPM. Lipoprotein metabolism in patients with severe sepsis. *Critical Care Medicine*. 2003;31(5):1359-1366. doi:10.1097/01.CCM.0000059724.08290.51.
72. Piekoszewski W, Jusko WJ. Plasma protein binding of tacrolimus in humans. *J Pharm Sci*. 1993;82(3):340-341. doi: 10.1002/jps.2600820325
73. Zsila F. Overlapping Ligand Specificity of P-Glycoprotein and Serum 1-Acid Glycoprotein: Evidences and Potential Implications. *Curr Drug Metab*. 2007;8:563-593. doi: 10.2174/138920007781368854
74. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Factors affecting variability in distribution of tacrolimus in liver transplant recipients. *British Journal of Clinical Pharmacology*. 2004;57(3):298-309. doi: 10.1046/j.1365-2125.2003.02008.x
75. Sasa H, Hashimoto Y, Shimizu T. Hepatic extraction of tacrolimus in rats with experimental liver diseases. *Biol Pharm Bull*. 1998;21(6):610-614. doi: 10.1248/bpb.21.610
76. Gonschior A-K, Christians U, Winkler M, Linck A, Baumann J, Sewing K-F. Tacrolimus (FK506) metabolite patterns in blood from liver and kidney transplant patients. *Clinical Chemistry*. 1996;42(9):1426-1432. doi: 10.1097/00007691-199508000-00148
77. Capone D, Gentile A, Imperatore P, Palmiero G, Basile V. Effects of itraconazole on tacrolimus blood concentrations in a renal transplant recipient. *Ann Pharmacother*. 1999;33(10):1124-1125. doi: 10.1345/aph.18409
78. HamzahSharina, Kek T, Kwong SS, et al. Pharmacogenotyping of CYP3A5 in predicting dose-adjusted trough levels of tacrolimus among Malaysian kidney-transplant patients. *http://dxdoiorg/101139/cjpp-2013-0128*. 2013;27(1):422-430. doi:10.1139/cjpp-2013-0128.
79. Staatz C. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. *Clinical Pharmacology & Therapeutics*. 2002;72(6):660-669. doi:10.1067/mcp.2002.129304.

80. Rey E, Tréluyer JM, Pons G. Drug disposition in cystic fibrosis. *Clinical Pharmacokinetics*. 1998;35(4):314-329. doi: 10.2165/00003088-199835040-00004
81. Rifai N, Chao F-F, Pham Q, Thiessen J, Soldin SJ. The role of lipoproproteins in the transport and uptake of cyclosporine and dihydro-tacrolimus into HepG2 and JURKAT cell lines. *Clinical Biochemistry*. 2013;29:1-7. doi: doi.org/10.1016/0009-9120(96)00001-x
82. Paterson DL, Singh N. Interactions between tacrolimus and antimicrobial agents. *Clin Infect Dis*. 1997;25(6):1430-1440. doi: 10.1086/516138
83. Boyer A, Gruson D, Bouchet S, et al. Aminoglycosides in Septic Shock. *Drug Saf*. 2013;36(4):217-230. doi:10.1007/s40264-013-0031-0.
84. Nolin TD, Himmelfarb J. Mechanisms of Drug-Induced Nephrotoxicity. In: Uetrecht J, ed. *Adverse Drug Reactions*. Vol 196. Handbook of Experimental Pharmacology. Berlin, Heidelberg: Springer Berlin Heidelberg; 2009:111-130. doi:10.1007/978-3-642-00663-0_5.
85. Sheiner PA, Mor E, Chodoff L, et al. Acute renal failure associated with the use of ibuprofen in two liver transplant recipients on FK506. *Transplantation*. 1994;57(7):1132-1133. doi: 10.1097/00007890-199404000-00026
86. Sands M, Brown RB. Interactions of cyclosporine with antimicrobial agents. *Rev Infect Dis*. 1989;11(5):691-697. doi: 10.1093/clinids/11.5.691
87. Schwarz A, Perez-Canto A. Nephrotoxicity of antiinfective drugs. *Int J Clin Pharmacol Ther*. 1998;36(3):164-167. doi: 10.1002/cpt.462
88. Iwasaki K, Matsuda H, Nagase K, Shiraga T, Tokuma Y, Uchida K. Effects of twenty-three drugs on the metabolism of FK506 by human liver microsomes. *Res Commun Chem Pathol Pharmacol*. 1993;82(2):209-216.
89. Lampen A, Christians U, Guengerich FP, et al. Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions, and interindividual variability. *Drug Metab Dispos*. 1995;23(12):1315-1324.
90. Christians U, Schmidt G, Bader A, et al. Identification of drugs inhibiting the in vitro metabolism of tacrolimus by human liver microsomes. *British Journal of Clinical Pharmacology*. 1996;41(3):187-190. doi: 10.1111/j.1365-2125.1996.tb00181.x
91. Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metabolism Reviews*. 1997;29(1-2):413-580. doi: 10.3109/03602539709037591
92. Wacher VJ, Salphati L, Benet LZ. Active secretion and enterocytic drug metabolism barriers to drug absorption1PII of original article: S0169-409X(96)003304. The article was originally published in Advanced Drug Delivery Reviews 20 (1996) 99–112.1. *Advanced Drug Delivery Reviews*. 2001;46(1-3):89-102. doi:10.1016/S0169-409X(00)00126-5.
93. Lam S, Partovi N, Ting LSL, Ensom MHH. Corticosteroid interactions with cyclosporine, tacrolimus, mycophenolate, and sirolimus: fact or fiction? *Annals of Pharmacotherapy*. 2008;42(7):1037-1047. doi:10.1345/aph.1K628.

94. Zhou S-F, Xue CC, Yu X-Q, Li C, Wang G. Clinically Important Drug Interactions Potentially Involving Mechanism-based Inhibition of Cytochrome P450 3A4 and the Role of Therapeutic Drug Monitoring. *therapeutic drug monitoring*. 2007;29(6):687-710. doi:10.1097/FTD.0b013e31815c16f5.
95. Kunicki PK, Sobieszcza ska-Ma ek MG. Pharmacokinetic Interaction Between Tacrolimus and Clarithromycin in a Heart Transplant Patient. *therapeutic drug monitoring*. 2005;27(1):107-108. doi:10.1097/00007691-200502000-00020.
96. Iwasaki K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. *DMPK*. 2007;22(5):328-335. doi:10.2133/dmpk.22.328.
97. Mori T, Aisa Y, Kato J, Nakamura Y, Ikeda Y, Okamoto S. Drug interaction between voriconazole and calcineurin inhibitors in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant*. 2009;44(6):371-374. doi:10.1038/bmt.2009.38.
98. Berge M, Guillemain R, Boussaud V, et al. Voriconazole pharmacokinetic variability in cystic fibrosis lung transplant patients. *Transplant Infectious Disease*. 2009;11(3):211-219. doi:10.1111/j.1399-3062.2009.00384.x.
99. Floren LC, Bekersky I, Benet LZ, et al. Tacrolimus oral bioavailability doubles with coadministration of ketoconazole. *Clinical Pharmacology & Therapeutics*. 1997;62(1):41-49. doi:10.1016/S0009-9236(97)90150-8.
100. Mihara A, Mori T, Aisa Y, et al. Greater impact of oral fluconazole on drug interaction with intravenous calcineurin inhibitors as compared with intravenous fluconazole. *Eur J Clin Pharmacol*. 2007;64(1):89-91. doi:10.1007/s00228-007-0395-0.
101. Prasad TN, Stiff DD, Subbotina N, et al. FK 506 (Tacrolimus) metabolism by rat liver microsomes and its inhibition by other drugs. *Res Commun Chem Pathol Pharmacol*. 1994;84(1):35-46.
102. Mieles L, Venkataramanan R, Yokoyama I, Warty VJ, Starzl TE. Interaction between FK506 and clotrimazole in a liver transplant recipient. *Transplantation*. 1991;52(6):1086-1087. doi: 10.1097/00007890-199112000-00029
103. Moreno M, Latorre A, Manzanares C, et al. Clinical management of tacrolimus drug interactions in renal transplant patients. *TPS*. 1999;31(6):2252-2253. doi: 10.1016/s0041-1345(99)00325-5
104. Manez R, Martin M, Raman V, Silverman D, Jain A. Fluconazole therapy in transplant recipients receiving FK506. *Transplantation*. 1994;57(10):1521-1523. doi: 10.1097/00007890-199405000-00022
105. Osowski CL, Dix SP, Lin LS, Mullins RE, Geller RB, Wingard JR. Evaluation of the drug interaction between intravenous high-dose fluconazole and cyclosporine or tacrolimus in bone marrow transplant patients. *Transplantation*. 1996;61(8):1268-1272. doi: 10.1097/00007890-199604270-00026
106. Kramer MR, Merin G, Rudis E, et al. Dose adjustment and cost of itraconazole prophylaxis in lung transplant recipients receiving cyclosporine and tacrolimus (FK 506). *Transplantation Proceedings*. 1997;29(6):2657-2659. doi:10.1016/S0041-1345(97)00546-0.

107. Billaud EM, Guillemain R, Tacco F, Chevalier P. Evidence for a pharmacokinetic interaction between itraconazole and tacrolimus in organ transplant patients. *British Journal of Clinical Pharmacology*. 1998;46:271-274.
108. Furlan V, Parquin F, Penaud JF, et al. Interaction Between Tacrolimus and Itraconazole in a Heart–Lung Transplant Recipient. *Transplantation Proceedings*. 1998;30(1):187-188. doi:10.1016/S0041-1345(97)01226-8.
109. Hairhara Y, Makuuchi M, Kawarasaki H, et al. Effect of Fluconazole on Blood Levels of Tacrolimus. *Transplantation Proceedings*. 1999;31(7):2767–1. doi:10.1016/S0041-1345(99)00560-6.
110. Herzig K, Johnson DW. Marked elevation of blood cyclosporin and tacrolimus levels due to concurrent metronidazole therapy. *Nephrol Dial Transplant*. 1999;14(2):521-523. doi: 10.1093/ndt/14.2.521b
111. Macías MO, Salvador P, Hurtado JL, Martín I. Tacrolimus–itraconazole interaction in a kidney transplant patient. *Ann Pharmacother*. 2000;34(4):536-536. doi:10.1345/aph.17461.
112. Cervelli MJ, Russ GR. Itraconazole-Tacrolimus Drug Interaction. *therapeutic drug monitoring*. 2003;25(4):483-484. doi:10.1097/00007691-200308000-00012.
113. Pai MP, Allen S. Voriconazole inhibition of tacrolimus metabolism. *CLIN INFECT DIS*. 2003;36(8):1089-1091. doi:10.1086/374252.
114. Soltero L, Carbajal H, Rodríguez-Montalvo C, Valdés A. Coadministration of tacrolimus and ketoconazole in renal transplant recipients: cost analysis and review of metabolic effects. *Transplantation Proceedings*. 2003;35(4):1319-1321. doi:10.1016/S0041-1345(03)00450-0.
115. Page RL, Klem PM, Rogers C. Potential elevation of tacrolimus trough concentrations with concomitant metronidazole therapy. *Ann Pharmacother*. 2005;39(6):1109-1113. doi:10.1345/aph.1E399.
116. Shitrit D, Ollech JE, Ollech A, Bakal I, Saute M. Itraconazole Prophylaxis in Lung Transplant Recipients Receiving Tacrolimus (FK 506): Efficacy and Drug Interaction. *The Journal of Heart and Lung Transplantation*. 2005;24:2148-2152.
117. Tintillier M, Kirch L, Goffin E, Cuvelier C, Pochet J-M. Interaction between voriconazole and tacrolimus in a kidney-transplanted patient. *Nephrol Dial Transplant*. 2005;20(3):664-665. doi:10.1093/ndt/gfh593.
118. Vasquez EM, Shin GP, Sifontis N, Benedetti E. Concomitant Clotrimazole Therapy More Than Doubles the Relative Oral Bioavailability of Tacrolimus. *therapeutic drug monitoring*. 2005;27:587-591. doi: 10.1097/01.ftd.0000151186.91464.7c
119. El-Dahshan KF, Bakr MA, Donia AF, Badr AE-S, Sobh MA-K. Ketoconazole-Tacrolimus Coadministration in Kidney Transplant Recipients: Two-Year Results of a Prospective Randomized Study. *Am J Nephrol*. 2006;26(3):293-298. doi:10.1159/000094133.
120. Banjeree R, Lyster H, Leaver N, Banner NR. Coadministration of Itraconazole and Tacrolimus After Thoracic Organ Transplantation. *Transplantation Proceedings*. 2001;33:1600-1602. doi: 10.1016/s0041-1345(00)02608-7
121. Mathis AS, Friedman GS. Coadministration of Digoxin With Itraconazole in Renal Transplant Recipients. *American Journal of Kidney Diseases*. 2001;37(2):E18. doi:10.1053/ajkd.2001.21363.

122. Tuteja S, Alloway RR, Johnson JA, Gaber AO. The effect of gut metabolism on tacrolimus bioavailability in renal transplant recipients. *Transplantation*. 2001;71(9):1303-1307. doi: 10.1097/00007890-200105150-00021
123. Vasquez E, Pollak R, Benedetti E. Clotrimazole increases tacrolimus blood levels: a drug interaction in kidney transplant patients. *Clinical Transplantation*. 2001;15(2):95-99.
124. Toda F, Tanabe K, Ito S, et al. Tacrolimus trough level adjustment after administration of fluconazole to kidney recipients. *Transplantation Proceedings*. 2002;34(5):1733-1735. doi:10.1016/S0041-1345(02)03001-4.
125. Venkataramanan R, Zang S, Gayowski T, Singh N. Voriconazole inhibition of the metabolism of tacrolimus in a liver transplant recipient and in human liver microsomes. *Antimicrob Agents Chemother*. 2002;46(9):3091-3093.
126. Kuypers DR, Claes K, Evenepoel P, Vanrenterghem Y. Clinically relevant drug interaction between voriconazole and tacrolimus in a primary renal allograft recipient. *Transplantation*. 2006;81(12):1750-1752. doi:10.1097/01.tp.0000226080.71764.8c.
127. Leather H, Boyette RM, Tian L, Wingard JR. Pharmacokinetic Evaluation of the Drug Interaction between Intravenous Itraconazole and Intravenous Tacrolimus or Intravenous Cyclosporin A in Allogeneic Hematopoietic Stem Cell Transplant Recipients. *Biology of Blood and Marrow Transplantation*. 2006;12(3):325-334. doi:10.1016/j.bbmt.2005.10.022.
128. Lumlertgul D, Noppakun K, Rojanasthien N, et al. Pharmacokinetic study of the combination of tacrolimus and fluconazole in renal transplant patients. *J Med Assoc Thai*. 2006;89 Suppl 2:S73-S78.
129. Saad AH, DePestel DD, Carver PL. Factors Influencing the Magnitude and Clinical Significance of Drug Interactions Between Azole Antifungals and Select Immunosuppressants. *Pharmacotherapy*. 2006;26(12):1730-1744. doi:10.1592/phco.26.12.1730.
130. Sansone Parsons A, Krishna G, Martinho M, Kantesaria B, Gelone S, Mant TG. Effect of oral posaconazole on the pharmacokinetics of cyclosporine and tacrolimus. *Pharmacotherapy*. 2007;27(6):825-834. doi:10.1592/phco.27.6.825.
131. Roedler R, Neuhauser MM, Penzak SR. Does Metronidazole Interact with CYP3A Substrates by Inhibiting Their Metabolism Through This Metabolic Pathway? Or Should Other Mechanisms Be Considered? *Annals of Pharmacotherapy*. 2007;41(4):653-658. doi:10.1345/aph.1H401.
132. Lu C, Hatsis P, Berg C, Lee FW, Balani SK. Prediction of pharmacokinetic drug-drug interactions using human hepatocyte suspension in plasma and cytochrome P450 phenotypic data. II. In vitro-in vivo correlation with ketoconazole. *Drug Metab Dispos*. 2008;36(7):1255-1260. doi:10.1124/dmd.107.018796.
133. Nivoix DY, Levêque D, Herbrecht R, Koffel J-C, Beretz L, Ubeaud-Sequier G. The Enzymatic Basis of Drug-Drug Interactions with Systemic Triazole Antifungals. *Clinical Pharmacokinetics*. 2008;47(12):779-792. doi:10.2165/0003088-200847120-00003.
134. Sun S, Li Y, Guo Q, Shi C, Yu J, Ma L. In vitro interactions between tacrolimus and azoles against Candida albicans determined by different methods. *Antimicrob Agents Chemother*. 2008;52(2):409-417. doi:10.1128/AAC.01070-07.

135. Federico S, Carrano R, Capone D, Gentile A, Palmiero G, Basile V. Pharmacokinetic Interaction between Levofloxacin and Ciclosporin or Tacrolimus in KidneyTransplant Recipients. *Clinical Pharmacokinetics*. 2006;45(2):169-175. doi:10.2165/00003088-200645020-00003.

136. Sifontis NM, Benedetti E, Vasquez EM. Clinically Significant Drug Interaction Between Basiliximab and Tacrolimus in Renal Transplant Recipients. *Transplantation Proceedings*. 2002;34(5):1730-1732. doi:10.1016/S0041-1345(02)03000-2.

137. Seifeldin RA, Marcos-Alvarez A, Gordon FD, Lewis WD, Jenkins RL. Nifedipine interaction with tacrolimus in liver transplant recipients. *Ann Pharmacother*. 1997;31(5):571-575. doi: 10.1177/106002809703100508

138. Hebert MF, Fisher RM, Marsh CL, Dressler D, Bekersky I. Effects of rifampin on tacrolimus pharmacokinetics in healthy volunteers. *J Clin Pharmacol*. 1999;39(1):91-96. doi: 10.1177/00912709922007499

139. Kothari J, Nash M, Zaltzman J, Ramesh Prasad GV. Diltiazem use in tacrolimus-treated renal transplant recipients. *Journal of Clinical Pharmacy and Therapeutics*. 2004;29(5):425-430. doi:10.1111/j.1365-2710.2004.00578.x.

140. Li J, Wang X, Wang C, et al. Rapid and simultaneous determination of tacrolimus (FK506) and diltiazem in human whole blood by liquid chromatography–tandem mass spectrometry: Application to a clinical drug–drug interaction study. 2008;867(1):111-118. doi:10.1016/j.jchromb.2008.03.024.

141. Van de Plas A, Dackus J, Christiaans MHL, Stolk LML, van Hooff JP, Neef C. A pilot study on sublingual administration of tacrolimus. *Transplant International*. 2009;22(3):358-359. doi:10.1111/j.1432-2277.2008.00779.x.

142. Boubenider S, Vincent I, Lambotte O, et al. Interaction between theophylline and tacrolimus in a renal transplant patient. *Nephrol Dial Transplant*. 2000;15(7):1066-1068. doi: 10.1093/ndt/15.7.1066

143. Matsuda H, Iwasaki K, Shiraga T, Tozuka Z, Hata T, Guengerich FP. Interactions of FK506 (tacrolimus) with clinically important drugs. *Res Commun Mol Pathol Pharmacol*. 1996;91(1):57-64.

144. Tjia JF, Colbert J, Back DJ. Theophylline metabolism in human liver microsomes: inhibition studies. *Journal of Pharmacology and Experimental Therapeutics*. 1996;276(3):912-917. doi:10.1002/(ISSN)2052-1707.

145. Bailey DG, Malcolm J, Arnold O, Spence JD. Grapefruit juice-drug interactions. *British Journal of Clinical Pharmacology*. 1998;46(2):101-110. doi: 10.1046/j.1365-2125.1998.00764.x

146. Fukatsu S, Fukudo M, Masuda S, et al. Delayed effect of grapefruit juice on pharmacokinetics and pharmacodynamics of tacrolimus in a living-donor liver transplant recipient. *DMPK*. 2006;21(2):122-125. doi:10.2133/dmpk.21.122.

147. Peynaud D, Charpiat B, Vial T, Gallavardin M, Ducerf C. Tacrolimus severe overdosage after intake of masked grapefruit in orange marmalade. *Eur J Clin Pharmacol*. 2007;63(7):721-722. doi:10.1007/s00228-007-0323-3.

148. Tang JT, Andrews LM, Van Gelder T, et al. Pharmacogenetic aspects of the use of tacrolimus in renal transplantation: recent developments and ethnic considerations. *Expert Opin Drug Metab Toxicol*. 2016;12(5):555-565. doi:10.1517/17425255.2016.1170808.
149. Venkataramanan R, Swaminathan A, Prasad T, et al. Clinical pharmacokinetics of tacrolimus. *Clinical Pharmacokinetics*. 1995;29(6):404-430. doi:10.2165/00003088-199529060-00003.
150. Mourad M, Mourad G, Wallemacq P, et al. Sirolimus and Tacrolimus Trough Concentrations and Dose Requirements after Kidney Transplantation in Relation to CYP3A5 and MDR1 Polymorphisms and Steroids. *Transplantation*. 2005;80(7):977-984. doi:10.1097/01.TP.0000174131.47469.D2.
151. Anglicheau D. Pharmacokinetic interaction between corticosteroids and tacrolimus after renal transplantation. *Nephrology Dialysis Transplantation*. 2003;18(11):2409-2414. doi:10.1093/ndt/gfg381.
152. Undre NA, Schafer A. Factors Affecting the Pharmacokinetics of Tacrolimus in the First Year After Renal Transplantation. *Transplantation Proceedings*. 1998;30(4):1261-1263. doi:10.1016/S0041-1345(98)00234-6.
153. Shimada T, Terada A, Yokogawa K, et al. Lowered blood concentration of tacrolimus and its recovery with changes in expression of CYP3A and P-glycoprotein after high-dose steroid therapy. *Transplantation*. 2002;74(10):1419-1424. doi:10.1097/01.TP.0000038287.39271.8F.
154. Duijnhoven EM, Boots JMM, Christiaans MHL, Stolk LML, Undre NA, Hooff JP. Increase in tacrolimus trough levels after steroid withdrawal. *Transplant International*. 2003;16(10):721-725. doi:10.1111/j.1432-2277.2003.tb00230.x.
155. Park S-I, Felipe CR, Pinheiro-Machado PG, et al. Tacrolimus pharmacokinetic drug interactions: effect of prednisone, mycophenolic acid or sirolimus. *Fundamental & Clinical Pharmacology*. 2009;23(1):137-145. doi:10.1111/j.1472-8206.2008.00644.x.
156. Kiuchi T, Tanaka K, Inomata Y, et al. Experience of tacrolimus-based immunosuppression in living-related liver transplantation complicated with graft tuberculosis: interaction with rifampicin and side effects. *TPS*. 1996;28(6):3171-3172.
157. Prasad, Tata NV, Subbotina N, et al. Metabolism of tacrolimus (FK 506) in rat liver microsomes. Effect of rifampin and dexamethasone. *Res Commun Mol Pathol Pharmacol*. 1997;96(1):107-110.
158. Bhaloo S, Prasad GVR. Severe reduction in tacrolimus levels with rifampin despite multiple cytochrome P450 inhibitors: a case report. *Transplantation Proceedings*. 2003;35(7):2449-2451. doi:10.1016/j.transproceed.2003.08.019.
159. Chenhsu RY, Loong CC, Chou MH, Lin MF, Yang WC. Renal allograft dysfunction associated with rifampin-tacrolimus interaction. *Ann Pharmacother*. 2000;34(1):27-31. doi: doi.org/10.1345/aph.19069
160. Finch CK, Chrisman CR, Baciewicz AM, Self TH. Rifampin and rifabutin drug interactions: an update. *Arch Intern Med*. 2002;162(9):985-992. doi: 10.1001/archinte.162.9.985
161. Roby C. St John's Wort: Effect on CYP3A4 activity. *Clinical Pharmacology & Therapeutics*. 2000;67(5):451-457. doi:10.1067/mcp.2000.106793. doi: 10.1067/mcp.2000.106793

162. Hebert MF, Park JM, Chen Y-L, Akhtar S, Larson AM. Effects of St. John's Wort (Hypericum perforatum) on Tacrolimus Pharmacokinetics in Healthy Volunteers. *The Journal of Clinical Pharmacology*. 2013;44(1):89-94. doi:10.1177/0091270003261078.
163. Dürr D. St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. *Clinical Pharmacology & Therapeutics*. 2000;68(6):598-604. doi:10.1067/mcp.2000.112240.
164. Wang Z, Gorski JC, Hamman MA, Huang SM, Lesko LJ, Hall SD. The effects of St John's wort (Hypericum perforatum) on human cytochrome P450 activity. *Clinical Pharmacology & Therapeutics*. 2001;70(4):317-326. doi: 10.1016/s0009-9236(01)17221-8
165. Mai I, Stormer E, Bauer S, Kruger H, Budde K, Roots I. Impact of St John's wort treatment on the pharmacokinetics of tacrolimus and mycophenolic acid in renal transplant patients. *Nephrology Dialysis Transplantation*. 2003;18(4):819-822. doi:10.1093/ndt/gfg002.
166. Borrows R, Chusney G, Loucaidou M, et al. Analysis of Factors Influencing Tacrolimus Levels and Immunoassay Bias in Renal Transplantation. *The Journal of Clinical Pharmacology*. 2013;47(8):1035-1042. doi:10.1177/0091270007303765.
167. Warty V, Venkataramanan R, Zendehrouh P, et al. Distribution of FK506 in plasma lipoproteins in transplant patients. *Transplantation Proceedings*. 1991;23(1):954-955.
168. Hochleitner BW, Bösmüller C, Nehoda H, et al. Increased tacrolimus levels during diarrhea. *Transpl Int*. 2001;14(4):230-233. doi:10.1007/s0014710140230.
169. Venkataramanan R, Jain A, Cadoff E. Pharmacokinetics of FK 506: preclinical and clinical studies. *Transplantation*. 1990.

TABLES

Table 1. Nephrotoxic drugs with mechanism of action in combination with tacrolimus

Drug	Hypothetical mechanisms of action	References
Aminoglycosides (gentamycin, neomycin, tobramycin)	Additive or synergistic: tubular apoptosis and/or necrosis	21,82-84
Amphotericin B	Synergistic: afferent vasoconstriction	21,82,84
Non-steroidal anti-inflammatory drugs (ibuprofen, diclofenac, aspirin)	Synergistic: afferent vasoconstriction and/or interstitial nephritis and/or papillary necrosis	21,82,84,85
ACE inhibitors (captopril)	Synergistic: efferent vasodilatation	21,82
Co-trimoxazole (sulfamethoxazole)	Additive: interstitial nephritis	86,87
(Val)gancyclovir/ acyclovir	Additive: intra-tubular obstruction	84

Table 2. Interactions resulting in increased tacrolimus concentrations

Drug	Study	Route of drug administration	Effect on tacrolimus	Proposed mechanism of interaction	References
Glucocorticoids	In vitro: human liver and intestinal microsomes	Tacrolimus oral, glucocorticoids oral or IV	Short term (first hours): inhibition of tacrolimus metabolism, Long term (after hours to days): inducing of tacrolimus metabolism	Cortisol, dexamethasone, prednisone, prednisolone, methylprednisolone: CYP3A4 inhibitor, substrate and inducer, Pgp substrate and inducer Hydrocortisone: CYP3A4 substrate and inducer, Pgp substrate and inhibitor	21,88-94
Macrolide (Erythromycin, Clarithromycin)	In vivo: human, case-report and In vitro: human liver and intestinal microsomes and rat liver microsomes	Tacrolimus oral, erythromycin oral or IV	Inhibition of tacrolimus metabolism, 2 to 6-fold increase in trough concentration	CYP3A4/5 substrate and inhibitor, Pgp substrate and inhibitor	88-90,92,94,95
Azoles (Ketoconazole, Fluconazole, Itraconazole, Voriconazole, Posaconazole, Clotrimazole, Metronidazole)	In vivo; human, prospective studies (healthy volunteers, patients),	Tacrolimus oral or IV and ketaconazole oral or IV, fluconazole oral or iv itraconazole oral or IV, voriconazole oral or IV, posaconazole oral, clotrimazole oral, metronidazole oral	Dose reduction 54 – 78% for oral and 42% for iv or 2 to17.5-fold reduction in dose to maintain therapeutic trough concentrations or 2 to 9-fold increase in trough concentration, 2-fold increase in maximal plasma concentration, 2 to 5-fold increase in AUC and oral clearance decreased, no effects after IV administration	CYP3A substrate and inhibitor, Pgp substrate and inhibitor, CYP3A5 expresser is associated with a reduced susceptibility for the inhibitory effects of fluconazole on tacrolimus metabolism	15,64,77,88-90,92,94,96-134

Table 2. Interactions resulting in increased tacrolimus concentrations

Drug	Study	Route of drug administration	Effect on tacrolimus	Proposed mechanism of interaction	References
Levofloxacin	In vivo: human, prospective study (5 patients)	Tacrolimus oral and levofloxacin oral	25% increase in AUC	CYP3A substrate; only 5% hepatic metabolism, Pgp substrate	94,135
Basiliximab	In vivo; human, retrospective data analysis (12 patients)	Tacrolimus oral and basiliximab IV	63% increase in trough concentration on day 3, 30% dose reduction in first week to maintain therapeutic trough concentrations	IL-2R induced alteration of tacrolimus metabolism by down regulating the hepatic cytochrome P450 system	136
Calcium antagonists: Phenylalkamine: Verapamil, and Benzothiazepine: Diltiazem, and Dihydropiridines: Nifedipine, Amlodipine, and Nicardipine	In vivo: human, prospective study (6 patients) and human, retrospective data analysis (150 patients), In vitro; human liver, intestinal microsomes and rat liver microsomes	Tacrolimus oral and verapamil oral and diltiazem oral, nifedipine oral, nicardipine IV	No change to 4-fold increase or 55% increase in trough concentration, 21-38% decreased in daily dose to maintain therapeutic trough concentrations 25-82% increase in AUC and Cmax	CYP3A4/5 inhibitor, CYP3A4 substrate, Pgp substrate and inhibitor	88-90,92,94,96, 101,137-140

Table 2. Interactions resulting in increased tacrolimus concentrations

Drug	Study	Route of drug administration	Effect on tacrolimus	Proposed mechanism of interaction	References
Omeprazole	In vivo: human, prospective study (51 patients) and case-report (2 patients), In vitro; human liver or intestinal microsomes	Tacrolimus oral and omeprazole oral or IV	No clinical effect or 2 to 3-fold increase in trough concentration	CYP3A4 substrate and inhibitor, Pgp substrate and inhibitor	56
Anti-retroviral drugs (HIV protease inhibitors)	In vivo: human, prospective study (73 patients)	Tacrolimus oral and protease inhibitor oral (nelfinavir, ritonavir)	75-99% or 30-140 –fold lower tacrolimus dose and 7-fold longer dosing interval to maintain therapeutic trough concentrations, 34% to 99% decrease in oral clearance	Amprenavir: CYP3A4 substrate and inhibitor, Pgp substrate, Atazanavir and indinavir: CYP3A4 substrate and inhibitor, Pgp substrate, inhibitor and inducer, Lopinavir and ritonavir: CYP3A4 substrate, inhibitor and inducer, Pgp substrate, inhibitor and inducer, Nelfinavir and saquinavir: CYP3A4 substrate and inhibitor, Pgp substrate and inhibitor	65,66
Amiodarone	In vivo; human, case-report	Tacrolimus oral and amiodarone oral or IV	75% dose reduction to maintain therapeutic trough concentrations	CYP3A4 substrate and inhibitor, Pgp inhibitor	141

Table 2. Interactions resulting in increased tacrolimus concentrations

Drug	Study	Route of drug administration	Effect on tacrolimus	Proposed mechanism of interaction	References
Theophylline	In vivo; human, case-report,	Tacrolimus oral and theophylline oral	3-fold increase in trough concentration	CYP3A4 substrate and inhibitor	94,142-144
Grapefruit	In vivo; human, 2 case reports,	Tacrolimus oral and grape fruit juice oral	2 to10-fold increase in trough concentration	CYP3A4 inhibitor	145-147

Table 3. Interactions resulting in decreased tacrolimus concentrations

Drug	Study	Route of administration	Effect on tacrolimus	Proposed mechanism of interaction	References
Glucocorticoids	In vivo; human, prospective study (778 patients), In vitro; rat liver microsomes	Tacrolimus oral or IV and corticosteroids oral	Short term (first hours): inhibition of tacrolimus metabolism, Long term (after hours to days): inducing of tacrolimus metabolism, a concomitant prednisolone dose of more than 10 mg/d increases the apparent clearance of tacrolimus by 15-36% (and thus a 15% lower bioavailability) at 5 mg/d 12-14% decrease in AUC	Cortisol, dexamethasone, prednisone, prednisolone, methylprednisolone: CYP3A4 inhibitor, substrate and inducer, Pgp substrate and inducer, Hydrocortisone: CYP3A4 substrate and inducer, Pgp inhibitor and substrate	21,37,44,63,91-94,101,148-155
Carbamazepine	In vivo: human, case report	Tacrolimus oral and carbamazepine oral	AUC/dose ratio reduction with 50-70%	CYP3A4 induction	94
Phenytoin	In vivo: human, case report (6 patients)	Tacrolimus oral and phenytoin oral or IV	Decrease in tacrolimus whole-blood levels, 2 to 3-fold increase in dose to maintain therapeutic trough concentrations	CYP3A4 induction	94
Phenobarbital	In vivo: human, case-reports (2 patients)	Tacrolimus oral and phenobarbital IV	Increase clearance	CYP3A4 inducer, Pgp inhibitor	94

Table 3. Interactions resulting in decreased tacrolimus concentrations

Drug	Study	Route of administration	Effect on tacrolimus	Proposed mechanism of interaction	References
Rifampicin	In vivo: human, case report (10 patients), In vitro: rat liver microsomes	Tacrolimus oral and rifampicin oral or IV	5 to >10-fold increase in dose to maintain therapeutic trough concentrations, 50% increased clearance, 50% decrease in oral bio-availability, no effect on rat liver metabolism	CYP3A4 and Pgp induction	103,138,156-160
St John's wort	In vivo: human, prospective study (10 healthy volunteers and 11 patients)	Tacrolimus oral and St John's wort oral	34-50% decrease in AUC, 2 to 5-fold decrease in trough concentration	CYP3A4 and Pgp inducer	161-165

IV = intravenous

Table 4. Influencing factors on tacrolimus blood concentrations early after heart and lung transplantation. The effects are assumptions based on literature and physiological concepts: ⬌ no effect, ↑ and ↓ small effect, ↑↑ and ↓↓ mild effect, ↑↑↑ and ↓↓↓ large effect.

Factor	Effect on tacrolimus whole-blood concentrations	Effect on unbound tacrolimus plasma concentrations	Reference
Bio-variables			
Anemia	↓⬌	↑↑↑	38,39,74,78,149
Blood transfusion	↑⬌	↓↓↓	74
Hypo-albuminemia	⬌	↑↑↑	74,78
High AGP	⬌	↓	74,166
Low HDL	⬌	↑	74,167
Low LDL	⬌	↑	74,167
Low VLDL	⬌	↑	74,167
Organ dysfunction			
Ileus	↓↓↓	⬌	14,54
Restored gut motility	↑↑↑	⬌	14
Diarrhea	↑↑	⬌	17,19,63,168
Low Pgp (shock,inflammation)	↑↑	⬌	40,62,63
ECMO	↓↓	↓	52-54
Liver dysfunction	↑	⬌	76
Cholestasis	↑	⬌	76
Kidney dysfunction	⬌	↑	169

FIGURES

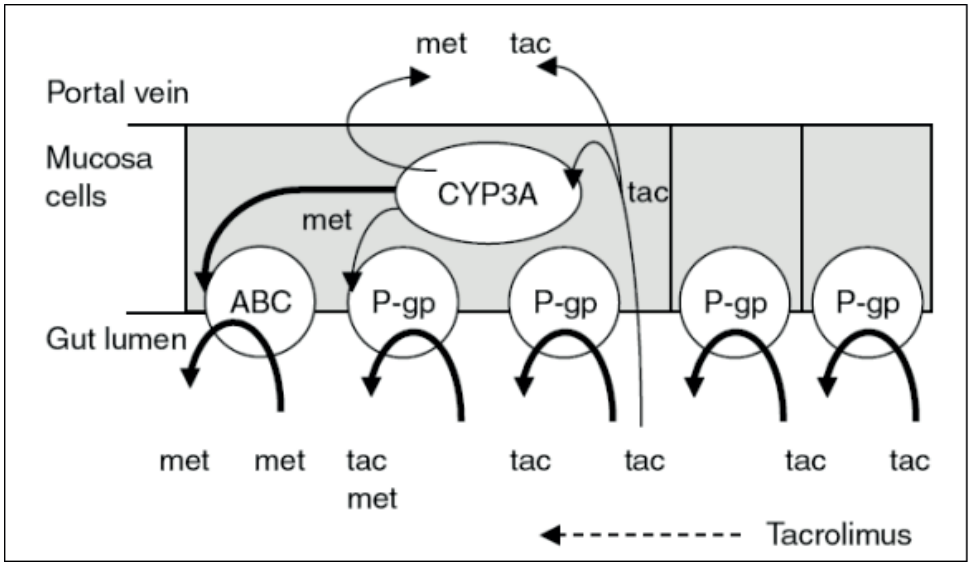

Fig. 1. Proposed interactions between tacrolimus metabolism and active efflux of tacrolimus in the small intestinal mucosa. Two potential cooperative mechanisms between cytochrome P450 enzymes and active efflux transporters have been proposed: I. P-glycoprotein regulates the access of tacrolimus to CYP3A enzymes and prevents CYP3A enzymes from being overwhelmed by the high drug concentrations in the intestine. With tacrolimus being repeatedly transported out of the mucosa cells and being reabsorbed again, leads to a higher exposure of CYP3A to tacrolimus and repeated exposure leads to a more efficient metabolism of tacrolimus in the intestine. II. The metabolites of tacrolimus are better substrates of the active transporter than the parent drug, thus metabolite efflux is facilitated even if the parent drug is present in high concentrations. ABC = ATP-binding cassette transporter other than P-glycoprotein; met = tacrolimus metabolite; P-gp = P-glycoprotein; tac = tacrolimus.[21]

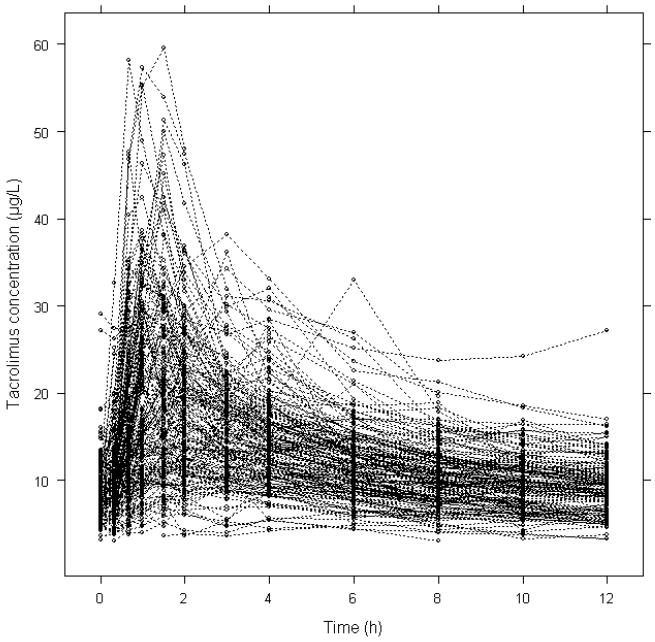

Fig. 2. Whole-blood tacrolimus concentration-time profiles obtained from 78 lung transplant patients during the first year post-transplantation showing a large variability in whole-blood concentrations. The dots represent the observed concentration-time points.[12]

CHAPTER 2a

2a

High tacrolimus blood concentrations early after lung transplantation and the risk of kidney injury

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ABSTRACT

Purpose

Lung-transplant recipients often develop acute kidney injury (AKI) evolving into chronic kidney disease (CKD). The immunosuppressant tacrolimus might be associated with the emergence of AKI. We analyzed the development and recovery of kidney injury after lung transplantation and related AKI to whole-blood tacrolimus trough concentrations and other factors causing kidney injury.

Methods

We retrospectively studied kidney injury in 186 lung-transplantation patients at the UMC Utrecht between 2001 and 2011. Kidney function and whole-blood tacrolimus trough concentrations were determined from day 1 to 14 and at 1, 3, 6 and 12 months postoperative. Systemic inflammatory response syndrome (SIRS), septic shock, and nephrotoxic medications were evaluated as covariate for AKI. We analyzed liver injury and drug-drug interactions.

Results

AKI was present in 85 (46%) patients. Tacrolimus concentrations were supra-therapeutic in 135 of 186 patients (73%). AKI in the first week after transplantation was related to supra-therapeutic tacrolimus concentrations (OR 1.55; 95% CI 1.06-2.27), ≥3 other nephrotoxic drugs (OR 1.96; 95% CI 1.02-3.77), infection (OR 2.48; 95% CI 1.31-4.70) and cystic fibrosis (OR 2.17; 95% CI 1.16-4.06). Recovery rate of AKI was lower than expected (19%) and the cumulative incidence of severe CKD at one year was 15%.

Conclusions

After lung-transplantation, AKI is common and often evolves into severe CKD, which is a known cause of morbidity and mortality. Supra-therapeutic whole-blood tacrolimus trough concentrations are related to the early onset of AKI. Conscientious targeting tacrolimus blood concentrations might be vital in the early phase after lung transplantation.

INTRODUCTION

Each year, approximately 4000 lung transplantations are performed worldwide (ISHLT.org). Many of these lung-transplantation patients develop acute kidney injury (AKI).[1-3] Prevention of AKI in lung transplant recipients is vital because it is associated with the development of chronic kidney disease (CKD) increasing morbidity and mortality.[2] AKI in the first days after transplantation may be due to shock, systemic inflammation and/or nephrotoxic drugs.[4-6] One such nephrotoxic drug is tacrolimus, a very effective immunosuppressant belonging to the calcineurin inhibitor class and ubiquitously used in lung transplant patients.[7-11]

Tacrolimus nephrotoxicity in the early phase after transplantation is associated with several pharmacokinetic factors, which influence tacrolimus blood concentrations profoundly. For instance, the bioavailability of tacrolimus is highly variable due to gut dysmotility, changes in metabolism and altered clearance due to liver injury.[12] Furthermore, tacrolimus metabolism may change with drug-drug interactions by inhibiting or competing with the transporter P-glycoprotein (Pgp) and the enzymes Cytochrome P450 (CYP) 3A4/5.[13,14] These variations in pharmacokinetics in the early phase may result in high fluctuations in the whole-blood tacrolimus concentrations, increasing tacrolimus toxicity and decreasing tacrolimus efficacy.[15,16]

We hypothesized that tacrolimus nephrotoxicity might have a crucial role in the development of AKI after lung transplantation. Therefore, the purpose of this retrospective study was to investigate the development and recovery of kidney injury after lung transplantation and relate AKI to whole-blood tacrolimus trough concentrations.

PATIENTS AND METHODS

All lung transplantation patients hospitalized at the University Medical Center Utrecht (UMCU) from July 2001 to February 2011 were retrospectively studied. Tacrolimus whole-blood trough concentrations were analyzed during the first year post transplantation. The immunosuppressive regimen consisted further of basiliximab, corticosteroids and mycophenolate mofetil. Cofactors influencing tacrolimus blood concentrations and renal function were recorded as well. AKI was defined by the "Kidney Disease: Improving Global Outcomes" (KDIGO) Clinical Practice Guideline criteria and CKD by the "CKD Epidemiology Collaboration equation" (CKD-EPI). Both were solely based on measurement of creatinine. For more detailed information on the variables, co-variates and the used statistical analyses see the supplemental text; "Patients and Methods".

RESULTS

Patients 'characteristics

A total of 186 patients were included. Twenty-nine patients died in the first year, 11 of whom within 14 days. Patients died of bleeding (9), heart failure (1), primary graft failure (1), acute rejection (1), infection (5), hemorrhagic cerebrovascular accident (3), chronic respiratory failure (4), carcinoma (1) or an unknown cause (4). Two peaks in the age distribution were observed (Fig. 1a). The category 18-40 years contained significantly more CF patients than the category >40 years (X^2 88.09, 2 df, p<0.001). Table 1 shows the patients' characteristics. The main differences between the two groups, "AKI" or "no AKI", involved the frequency of CF patients, the frequency of perioperative extracorporeal membrane oxygenation (ECMO), and the frequency of occurrence of infection. Further, systemic inflammatory response syndrome (SIRS) was most frequently observed on day 2 (89%, 159 out of 186) and shock on day 1 (48%, 88 out of 186). On day 6, these percentages were decreased to 34% (61 out of 186) and 5% (8 out of 186), respectively.

Mean baseline creatinine was 71 µmol/L (SD 35 µmol/L). Within the first 14 days after transplantation 85 patients (46%) developed AKI (all 3 AKI stages pooled together) (Fig. S2). Forty-two percent of the patients (78 out of 186) presented at least one episode of AKI between day 1 and day 6. The frequency of AKI was highest on day 3 (24%, N=43 out of 175). The most serious AKI ("AKI stage 3"), was especially frequent within the first two weeks after transplantation and occurred most often on days 6 and 7 (3.5%; 6 out of 173) (Fig. 1b). Fig. 1c shows the early peak in serum creatinine with a partial decrease up to 1 month and hereafter a slow increase in serum creatinine over time. Renal replacement therapy was needed in 9% of patients on the intensive care (17 out of 186). Almost all patients received nephrotoxic drugs other than tacrolimus within the first week after transplantation (96% of patients) (Table 1). On day 2 most patients received nephrotoxic drugs other than tacrolimus (73%; 130 out of 179). The differences in patient numbers arise from transfer to another hospital, death and the discontinuation of tacrolimus (Fig. S2).

Variables influencing whole-blood tacrolimus concentrations

As can be seen in Fig. 1, the median whole-blood tacrolimus trough concentration first increases and then levels off. Between day 1 and day 6, 73% of patients showed supra-therapeutic concentrations (135 out of 186). Supra-therapeutic concentrations were observed most often on days 4 and 5 (50%; 87 out of 174 and 54%; 93 out of 174 of patients). At 6 months, 10% of patients (16 out of 107) showed elevated tacrolimus concentrations. Whole-blood tacrolimus trough concentrations differed significantly over time (day (estimate 1.24: 95% CI 1.02 to 1.46) and day squared (estimate -0.13: 95% CI -0.15 to -0.10)), (Table 2). Cystic fibrosis was significantly related to supra-therapeutic concentrations (estimate -0.16: 95% CI -0.28 to -0.03).

Patients often received at least one drug that could increase tacrolimus concentrations (e.g., 81% of patients on day 1 (149 out of 184) and 36% on day 5 (63 out of 173) and within 2 weeks 97%) (Table 1). At the same time, they also frequently received drugs that could decrease whole-blood tacrolimus concentrations (e.g., 63% on day 2 (112 out of 179), and 37% on day 5 (64 out of 173) and within 2 weeks 84%) (Table 1). Receiving two or more drugs, which potentially increase tacrolimus concentrations, was a significant predictor of a change in concentrations (estimate -0.13:95% CI -0.25 to -0.00) (Table 2). The groups were too small to differentiate the distinctive drugs.

Variables influencing AKI

The variables "supra-therapeutic whole-blood tacrolimus trough concentration" (OR 1.55: 95% CI 1.06-2.27), "infection" (OR 2.48: 95% CI 1.31- 4.70), "CF" (OR 2.17: 95% CI 1.16-4.06) and "≥3 nephrotoxic drugs other than tacrolimus" (OR 1.96: 95% CI 1.02-3.77) were all significantly associated with AKI in Generalized Estimating Equations (GEE) analyses when day 2 to day 6 were concerned (Table 3). When day 2 to day 14 were incorporated, "supra-therapeutic tacrolimus concentration" (OR 1.52: 95% CI 1.04-2.24), "CF" (OR 2.23: 95% CI 1.26-4.33) and "infection" (OR 2.31: 95% CI 1.23-4.34) were significantly associated. These analyses were based on the highest level of tacrolimus obtained, regardless of the duration of the supra-therapeutic concentration.

Relationship between AKI, recovery and CKD

AKI was recovered in 19% of patients (16 out of 85) at 1 month (Fig. S2). At 1 year after lung transplantation, the frequency of patients with severe CKD was 15% (23 out of 149 patients still at risk). The frequency of severe CKD for patients with "no-AKI between day 1 and day 14" was 16 out of 84 (19%), for patients with "AKI between day 1 and day 14 with recovery at one month" was 2 out of 14 (14%), and for patients with "AKI between day 1 and day 14 without recovery at one month" was 5 out of 48 patients (10%). In both groups of patients with AKI between 14 and 25% of patients were either lost to follow-up, discontinued tacrolimus and changed to sirolimus or had missing creatinine levels (AKI and recovery 25% (4 out of 16), AKI without recovery 14% (9 out of 64)). The mortality rate in the group of no-AKI was 1% (1 out of 87), in the group of AKI with recovery was 6% (1 out of 16) and in the group of AKI without recovery was 20% (13 out of 64). Significant differences in cumulative incidence of death were observed between the three categories of patients ($p<0.001$). Significant differences in cumulative incidence of the combined outcome "death and severe CKD" were also observed between the three categories of patients ($p=0.002$). The cumulative incidence of death significantly differed between the groups 1 and 2 on the one hand, and group 3 on the other ($p<0.001$). The cumulative incidence of the combined outcome death and severe CKD also differed between the groups 1 and 2 on the one hand, and group 3 on the other ($p=0.001$). The mortality rate and severe CKD incidence rate did not differ significantly between CF and non-CF patients ($p=0.77$ and 0.17, respectively).

DISCUSSION

We draw three main conclusions from the data with respect to AKI after lung transplantation: (1) it frequently occurs in the first 14 days, (2) it shows low recovery rates and often evolves to severe chronic kidney disease, and (3) it is related to increased whole-blood tacrolimus trough concentrations.

We found high incidence rates of AKI similar to other studies on renal function in lung-transplanted patients treated with tacrolimus.[2,17] The high occurrence rate of AKI in lung transplants might be due to a high occurrence rate of clinical instability. We related AKI to the occurrence of infection and almost all patients exhibited SIRS and shock.[2] CF patients are especially at risk for AKI, because of a high rate of diabetes and exposure to antimicrobials, and they exhibit a high risk for postoperative complications due to a high rate of infections, hemorrhage and perioperative ECMO use.[18,19] We found that CF was related to supra-therapeutic tacrolimus concentrations as additional risk factor for AKI.

Apart from the high incidence rates of AKI, we observed a low rate of convalescence from AKI. Such low improvement rates of AKI have been reported previously. Wehbe et. al. reported that recovery to the pre-transplant renal function occurred in 34% of lung-transplant patients with AKI.[2] This is in contrast to patients with septic shock, in which recovery of renal function often occurs (73% of patients) and is, in a large part, complete (60% of patients).[20] We further observed that the cumulative incidence of death and severe CKD was significantly higher in the group of patients with AKI that had not recovered at one month. This is in accordance with Wehbe et. al.[2] Additionally, we observed, when no AKI occurred, that almost a fifth of patients developed severe CKD after a year. Moreover, slow deterioration of renal function may very well be related to the continuous administration of tacrolimus because it is one of the main constant nephrotoxic factors in lung transplant patients. This is in accordance with previous findings. A gradual increase of tacrolimus toxicity during the first year after transplantation has been shown in renal biopsies.[21] Especially, CYP3A5*3 carriers are associated with increased risk of kidney injury compared to CYP3A5*1 carriers. It is thought that CYP3A5*1 carriers are protected from nephrotoxicity due to a decreased exposure to tacrolimus.[22-24] Expression of CYP3A5 presumably has also a role in nephrotoxicity by directly affecting the tubular cells; reduced presence of CYP3A5 within the tubular cells increases nephrotoxicity possibly due to a diminished metabolisation of tacrolimus.[25]

A high whole-blood tacrolimus trough level was a risk factor for the development of AKI. Tacrolimus was above therapeutic range in more than half of the patients in the first 6 days, which emphasizes the challenges of tacrolimus dosing in the early phase after transplantation. In particular, patients with SIRS or septic shock (the majority of patients)

may have organ failure, which changes the pharmacokinetics. This in turn, may lead to these supra-therapeutic whole-blood tacrolimus levels. Also after recovery from clinical instability, tacrolimus dosage remains challenging. After 6 months, almost one out of ten patients still exhibited supra-therapeutic whole-blood tacrolimus trough levels.

Interestingly, the median whole-blood tacrolimus trough concentrations were not that far above the therapeutic range in the first week after transplantation. This may indicate that the whole-blood tacrolimus trough concentrations were not the only contributing factor to tacrolimus toxicity. We hypothesize that the unbound tacrolimus plasma concentrations are potentially more responsible for the nephrotoxicity than the whole-blood tacrolimus concentrations. Only the unbound plasma concentration of a drug is biologically active and potentially toxic. Tacrolimus is highly bound to erythrocytes, albumin, α1-acid glycoprotein (AGP) and high-density lipoprotein (HDL) in stable clinical conditions, though the capacity of tacrolimus to bind may widely fluctuate in times of systemic inflammation and shock.[26] Erythrocytes and proteins are known to extensively vary during clinical instability.[27,28] Unfortunately, the unbound tacrolimus plasma concentrations cannot be measured by routine analyses. Consequently, knowledge of unbound tacrolimus plasma concentrations is scarce as well as its relation to nephrotoxicity. Our data showed large decreases in the numbers of erythrocytes and protein levels in the majority of patients in the first week after transplantation (See Table S3). These decreases may have influenced unbound plasma concentrations without having an effect on whole-blood concentrations. Therefore, the unbound tacrolimus plasma concentrations may be a more sensitive biomarker of nephrotoxicity.

Remarks regarding this study

There are some limitations to this study due to the retrospective design. Several explanatory variables influencing pharmacokinetics of tacrolimus could not be investigated because of the number of missing values. For instance, the effect of variations in variables like acidosis, changes in fluid balance, gut motility or variations in concentrations and activity of CYP 3A4/5 and P-glycoprotein could not be examined. They all may have an effect on whole-blood tacrolimus concentrations and should be considered as residual confounders.

The whole-blood tacrolimus trough concentrations were measured at approximately 12 h after administration. Tacrolimus elimination half-life time and time to trough level may substantially variate in solid organ recipients.[29] Therefore, the "apparent" trough levels monitored and used for dose adjustments according to the current practice may not always reflect optimal trough levels.[30]

Cystic fibrosis patients showed unexpectedly higher whole-blood tacrolimus concentrations. Cystic fibrosis patients generally have a decreased bioavailability and higher phase II metabolism resulting in a lower area under the concentration-time curve.[31,32] Whether these CF patients received higher doses or had a decreased metabolism due to variations in CYP3A4/5 or P-glycoprotein gene expression, and drug-drug interactions could not be determined.

Different definitions of renal function complicate the comparison of the results with other studies.[1,17,33,34] To allow for a better comparison, we analyzed our data with the criteria used by Wehbe et al., i.e., the KDIGO criteria were used without the urine output.[2] Plasma creatinine levels in lung transplantation patients after surgery may overestimate renal function due to pronounced muscle loss and depressed production of creatinine, which may result in lower creatinine plasma concentrations and an underestimation of the percentage of kidney injury.[35,36]

Investigations like ultrasound, biopsy, and urine analyses are not performed at a regular basis in clinically unstable lung transplant patients. Therefore, other causes of kidney injury are not excluded in this cohort and may have had an effect on kidney function. All AKI was attributed to tacrolimus and this might lead to an overestimation of the nephrotoxicity caused by tacrolimus. Important alternatives are the predisposing factors for AKI, which are shown in Table 1.

CONCLUSIONS

AKI is common after lung transplantation and is associated with both morbidity and mortality. We related supra-therapeutic whole-blood tacrolimus concentrations, next to three or more other nephrotoxic drugs, CF and infection to AKI in the first week after transplantation. There was a high occurrence of hemodynamic instability peri-operatively. Whereas hemodynamic instability is a known cause of AKI, recovery to pre-transplant renal function is expected. We observed an ongoing deterioration of renal function even when patients were considered stable. We related supra-therapeutic whole-blood tacrolimus concentrations early after transplantation to the emergence of AKI. This study underlines the significance of unraveling tacrolimus pharmacokinetics early after transplantation in order to decrease AKI in this vulnerable group of patients.

REFERENCES

1. Paradela de la Morena M, La Torre Bravos De M, Prado RF, et al. Chronic Kidney Disease After Lung Transplantation: Incidence, Risk Factors, and Treatment. *TPS*. 2010;42(8):3217-3219. doi:10.1016/j.transproceed.2010.05.064.
2. Wehbe E, Duncan AE, Dar G, Budev M, Stephany B. recovery from AKI and short- and long-term outcomes after lung transplatation. *Clinical Journal of the American Society of Nephrology*. 2013;8(1):19-25. doi:10.2215/CJN.04800512.
3. Ojo AO, Held PJ, Port FK, et al. Chronic Renal Failure after Transplantation of a nonrenal organ. *N Engl J Med*. 2003;349(10):931-940. doi:10.1056/NEJMoa021744.
4. Sharma P, Welch K, Eikstadt R, Marrero JA, Fontana RJ, Lok AS. Renal outcomes after liver transplantation in the model for end-stage liver disease era. *Liver Transpl*. 2009;15(9):1142-1148. doi:10.1002/lt.21821.
5. De Mendonca A, Vincent JL, Suter PM, et al. Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA score. *Intensive Care Med*. 2000;26(7):915-921. doi:10.1007/s001340051281.
6. Leroy S, Isapof A, Fargue S, et al. Tacrolimus nephrotoxicity: beware of the association of diarrhea, drug interaction and pharmacogenetics. *Pediatr Nephrol*. 2010;25(5):965-969. doi:10.1007/s00467-009-1402-8.
7. Mayer AD, Dmitrewski J, Squifflet JP, et al. Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation*. 1997;64(3):436-443. doi:10.1097/00007890-199708150-00012.
8. Haddad EM, McAlister VC, Renouf E, Malthaner R, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus for liver transplanted patients. McAlister V, ed. *Cochrane Database Syst Rev*. 2006;(4):CD005161. doi:10.1002/14651858.CD005161.pub2.
9. Kur F, Reichenspurner H, Meiser BM, et al. Tacrolimus (FK506) as primary immunosuppressant after lung transplantation. *Thorac Cardiovasc Surg*. 1999;47(3):174-178. doi:10.1055/s-2007-1013136.
10. Penninga L, Penninga EI, Møller CH, Iversen M, Steinbrüchel DA, Gluud C. Tacrolimus versus cyclosporin as primary immunosuppression for lung transplant recipients. *Cochrane Database Syst Rev*. 2013;5:CD008817. doi:10.1002/14651858.CD008817.pub2.
11. Kaczmarek I, Zaruba M-M, Beiras-Fernandez A, et al. Tacrolimus with mycophenolate mofetil or sirolimus compared with calcineurin inhibitor-free immunosuppression (sirolimus/mycophenolate mofetil) after heart transplantation: 5-year results. *HEALUN*. 2013;32(3):277-284. doi:10.1016/j.healun.2012.11.028.
12. Sikma MA, van Maarseveen EM, Donker DW, Meulenbelt J. Letter to the editor: "Immunosuppressive drug therapy - biopharmaceutical challenges and remedies". *Expert Opin Drug Deliv*. 2015;12(12):1955-1957. doi:10.1517/17425247.2015.1106687.

13. Christians U, Jacobsen W, Benet LZ. Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clinical Pharmacokinetics*. 2002;41(11):813-851. doi:10.2165/00003088-200241110-00003.
14. Kuypers DR, De Jonge H, Naesens M, Vanrenterghem Y. Effects of CYP3A5 and MDR1 single nucleotide polymorphisms on drug interactions between tacrolimus and fluconazole in renal allograft recipients. *Pharmacogenetics and Genomics*. 2008;18(10):861-868. doi:10.1097/FPC.0b013e328307c26e.
15. Sikma MA, van Maarseveen EM, van de Graaf EA, et al. Pharmacokinetics and Toxicity of Tacrolimus Early After Heart and Lung Transplantation. *Am J Transplant*. 2015;15(9):2301-2313. doi:10.1111/ajt.13309.
16. Snell GI, Ivulich S, Mitchell L, Westall GP, Levvey BJ. Evolution to twice daily bolus intravenous tacrolimus: optimizing efficacy and safety of calcineurin inhibitor delivery early post lung transplant. *Ann Transplant*. 2013;18:399-407. doi:10.12659/AOT.883993.
17. Lefaucheur C, Nochy D, Amrein C, et al. Renal Histopathological Lesions After Lung Transplantation in Patients with Cystic Fibrosis. *Am J Transplant*. 2008;8(9):1901-1910. doi:10.1111/j.1600-6143.2008.02342.x.
18. Meachery G, De Soyza A, Nicholson A, et al. Outcomes of lung transplantation for cystic fibrosis in a large UK cohort. *Thorax*. 2008;63(8):725-731. doi:10.1136/thx.2007.092056.
19. Wang Y, Kurichi JE, Blumenthal NP, et al. Multiple variables affecting blood usage in lung transplantation. *J Heart Lung Transplant*. 2006;25(5):533-538. doi:10.1016/j.healun.2005.12.004.
20. Garzotto F, Piccinni P, Cruz D, et al. RIFLE-Based Data Collection/Management System Applied to a Prospective Cohort Multicenter Italian Study on the Epidemiology of Acute Kidney Injury in the Intensive Care Unit. *Blood Purif*. 2011;31(1-3):159-171. doi:10.1159/000322161.
21. Nankivell BJ, Borrows RJ, Fung CLS, O'Connell PJ, Allen RDM, Chapman JR. The Natural History of Chronic Allograft Nephropathy. *N Engl J Med*. 2003;349(24):2326-2333. doi:10.1056/NEJMoa020009.
22. Staatz DCE, Goodman LK, Tett SE. Effect of CYP3A and ABCB1 Single Nucleotide Polymorphisms on the Pharmacokinetics and Pharmacodynamics of Calcineurin Inhibitors: Part II. *Clinical Pharmacokinetics*. 2010;49(4):207-221. doi:10.2165/11317550-000000000-00000.
23. Fukudo M, Yano I, Yoshimura A. Impact of MDR1 and CYP3A5 on the oral clearanceof tacrolimus and tacrolimus-related renal dysfunction in adult living-donor liver transplant patients. *Pharmacogenetics and Genomics*. 2008;18:413-423. doi:10.1097/FPC.0b013e3282f9ac01.
24. Shi Y, Li Y, Tang J, et al. Influence of CYP3A4, CYP3A5 and MDR-1 polymorphisms on tacrolimus pharmacokinetics and early renal dysfunction in liver transplant recipients. *Gene*. 2013;512(2):226-231. doi:10.1016/j.gene.2012.10.048.
25. Joy MS, Hogan SL, Thompson BD, Finn WF, Nickeleit V. Cytochrome P450 3A5 expression in the kidneys of patients with calcineurin inhibitor nephrotoxicity. *Nephrology Dialysis Transplantation*. 2007;22(7):1963-1968. doi:10.1093/ndt/gfm133.

26. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Factors affecting variability in distribution of tacrolimus in liver transplant recipients. *British Journal of Clinical Pharmacology*. 2004;57(3):298-309. doi:10.1111/j.1365-2125.2003.02008.x.
27. Zahir H, Nand RA, Brown KF, Tattam BN. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. *journal of pharmacological and toxicological methods*. 2001;46(1):27-35. doi:10.1016 S1056-8719(02)00158-2.
28. Van Leeuwen HJ, Heezius ECJM, Dallinga GM, van Strijp JAG, Verhoef J, van Kessel KPM. Lipoprotein metabolism in patients with severe sepsis. *Critical Care Medicine*. 2003;31(5):1359-1366. doi:10.1097/01.CCM.0000059724.08290.51.
29. Dansirikul C, Staatz CE, Duffull SB, Taylor PJ, Lynch SV, Tett SE. Sampling times for monitoring tacrolimus in stable adult liver transplant recipients. *therapeutic drug monitoring*. 2004;26(6):593-599. doi:10.1159/000445896.
30. Dasari BVM, Hodson J, Nassir A, et al. Variations in Practice to Therapeutic Monitoring of Tacrolimus following Primary Adult Liver Transplantation. *Int J Organ Transplant Med*. 2016;7(1):1-8.
31. Rey E, Tréluyer JM, Pons G. Drug disposition in cystic fibrosis. *Clinical Pharmacokinetics*. 1998;35(4):314-329. doi:10.2165/00003088-199835040-00004.
32. Monchaud C, de Winter BC, Knoop C, et al. Population pharmacokinetic modelling and design of a Bayesian estimator for therapeutic drug monitoring of tacrolimus in lung transplantation. *Clinical Pharmacokinetics*. 2012;51(3):175-186. doi:10.2165/11594760-000000000-00000.
33. Lin YH, Lin CC, Wang CC, et al. The 4-Week Serum Creatinine Level Predicts Long-Term Renal Dysfunction After Adult Living Donor Liver Transplantation. *TPS*. 2012;44(3):772-775. doi:10.1016/j.transproceed.2012.03.034.
34. Neuberger JM, Mamelok RD, Neuhaus P, et al. Delayed Introduction of Reduced-Dose Tacrolimus, and Renal Function in Liver Transplantation: The 'ReSpECT' Study. *Am J Transplant*. 2009;9(2):327-336. doi:10.1111/j.1600-6143.2008.02493.x.
35. Endre ZH, Pickering JW, Walker RJ. Clearance and beyond: the complementary roles of GFR measurement and injury biomarkers in acute kidney injury (AKI). *Am J Physiol Renal Physiol*. 2011;301(4):F697-F707. doi:10.1152/ajprenal.00448.2010.
36. Bragadottir G, Redfors B, Ricksten S-E. Assessing glomerular filtration rate (GFR) in critically ill patients with acute kidney injury - true GFR versus urinary creatinine clearance and estimating equations. *Critical Care*. 2013;17(3):R108. doi:10.1186/cc12777.

TABLES

Table 1. Patients' characteristics

Variables	All patients N=186 (100%)	Follow up ≥ day 14 & no AKI day 2-14 N=87 (47%)	Follow up ≥ day 14 & AKI day 2-14 N=85 (46%)	P value [a]
	N (%)			
Male	91 (49%)	36 (41%)	47 (55%)	0.07
Death day 1-14	11 (6%)	0 (0%)	0 (0%)	--- [b]
Death day 1-1 year	29 (16%)	1 (1%)	17 (20%)	<0.001
Reason transplantation	--	--	--	0.03
CF	57 (31%)	17 (20%)	33 (39%)	0.005
COPD, Emphysema, Alpha-1-antitrypsin deficiency	80 (43%)	48 (55%)	28 (33%)	0.003
Sarcoidosis / ILD / UIP	14 (8%)	7 (8%)	6 (7%)	0.81
Others: PAH, IPF, bronchiectasis, allergic alveolitis, LCH, LAM	35 (19%)	15 (17%)	18 (21%)	0.51
Double transplantation	148 (80%)	64 (74%)	73 (86%)	0.045
Diabetes mellitus	40 (22%)	13 (15%)	21 (25%)	0.11
Preoperative ECMO	1 (0.5%)	1 (1%)	0 (0%)	1.00
Perioperative ECMO	118 (63%)	45 (52%)	62 (73%)	0.004
ICU admission before	19 (10%)	7 (8%)	11 (13%)	0.29
Complications	91 (49%)	35 (40%)	45 (53%)	0.10
Reoperation due to bleeding	43 (23%)	13 (15%)	20 (24%)	0.15
Infection	48 (26%)	15 (17%)	33 (39%)	0.002
Rejection	22 (12%)	7 (8%)	14 (17%)	0.09
Other	27 (15%)	9 (10%)	17 (20%)	0.08
At least once during day 1-6				
Liver injury	53 (29%)	17 (20%)	31 (37%)	0.013
Anemia	182 (98%)	85 (98%)	85 (100%)	0.50
Low protein concentration	129 (69%)	58 (67%)	65 (77%)	0.15

Table 1. Patients' characteristics

Variables	All patients N=186 (100%)	Follow up ≥ day 14 & no AKI day 2-14 N=87 (47%)	Follow up ≥ day 14 & AKI day 2-14 N=85 (46%)	P value [a]
	N (%)			
Supra-therapeutic whole-blood tacrolimus trough concentration	135 (73%)	61 (70%)	71 (84%)	0.04
SIRS	172 (93%)	81 (93%)	81 (95%)	0.7
Shock	115 (62%)	47 (54%)	61 (72%)	0.016
At least one drug increasing tacrolimus concentration	181 (97%)	85 (98%)	85 (100%)	0.50
At least one drug decreasing tacrolimus concentration	157 (84%)	71 (82%)	78 (92%)	0.05
Nephrotoxic drugs other than tacrolimus	178 (96%)	85 (98%)	83 (98%)	1.0
		Mean (SD)		P value [a]
Age (yr)	46 (13.3)	47 (12.8)	45 (13.9)	0.2
BMI	22 (3.7)	23 (3.6)	22 (3.8)	0.8

[a] chi-square test, Fisher's exact test or t-test where appropriate
[b] No statistics are computed because no death occurred

Table 2. Linear mixed model to test the variables influencing whole-blood tacrolimus trough concentrations

Fixed effect	Estimate[1, 2]	95% CI	
CF	-0.16	-0.28	-0.03
Liver injury	0.04	-0.08	0.16
Drugs increasing tacrolimus	-0.02	-0.09	0.05
≥2 drugs increasing tacrolimus	-0.13	-0.25	0.00
1 or 2 drugs decreasing tacrolimus	-0.06	-0.13	0.01
Day	1.24	1.02	1.46
Day squared[3]	-0.13	-0.15	-0.10

[1] Estimate = regression coefficient in linear mixed model, with log(tacrolimus concentration) as outcome variable
[2] Estimate of intercept = -0.19
[3] A quadratic term is included in the model because there was no linear relationship between outcome variable and factors included in the model

Table 3 General estimating equations (GEE) analyses to test the variables influencing AKI

	Day 2-6 [1, 3]			Day 2-14 [2, 4]		
	OR	**95% CI**		**OR**	**95% CI**	
Supra-therapeutic whole-blood tacrolimus trough concentration	1.55	1.06	2.27	1.52	1.04	2.24
SIRS [5]	0.92	0.65	1.28	NA		
Shock [5]	1.56	0.82	2.95	NA		
CF	2.17	1.16	4.06	2.33	1.26	4.33
Nephrotoxic drugs other than tacrolimus [5]				NA		
1 nephrotoxic drug	2.04	0.94	4.41			
2 nephrotoxic drugs	1.40	0.73	2.69			
≥3 nephrotoxic drugs	1.96	1.02	3.77			
Double transplantation	2.07	0.77	5.54	2.15	0.81	5.69
Perioperative ECMO	1.11	0.58	2.10	1.09	0.57	2.06
Infection	2.48	1.31	4.70	2.31	1.23	4.34

[1] d2-d6: data concerning day 2 up to day 6
[2] d2-d14: data concerning day 2 up to day 14
[3] d2-d6: estimate of the intercept: -2.96
[4] d2-d14: estimate of the intercept: -2.82
[5] Data not available between day 7 and day 14

FIGURES

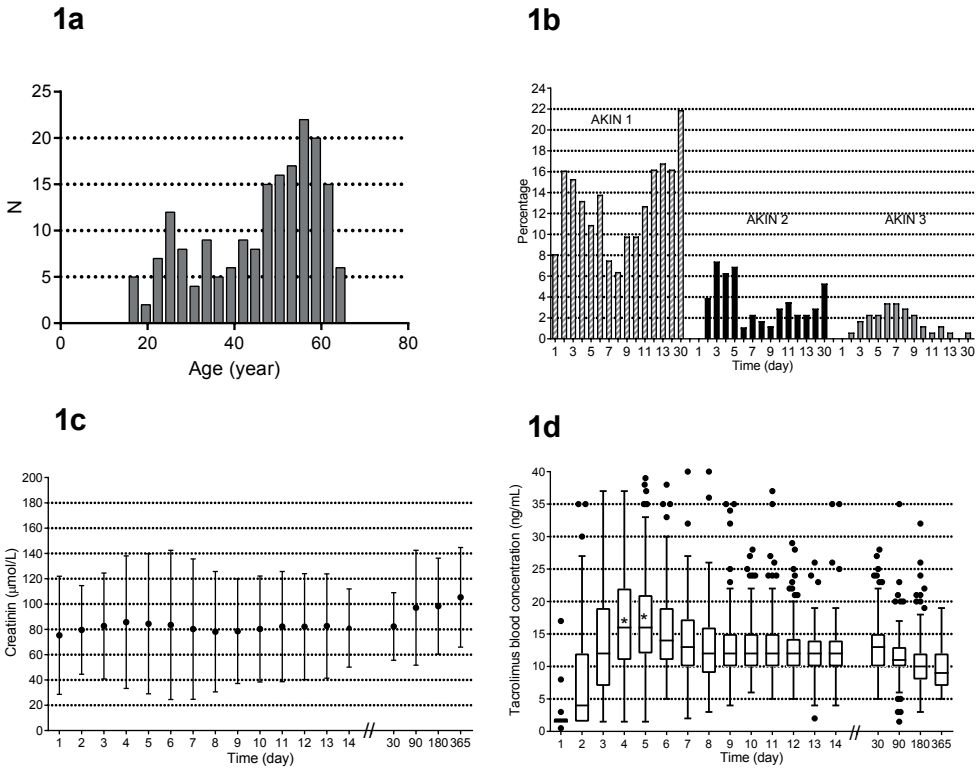

Fig. 1

1a: Histogram of age (years)

1b: Prevalence of AKI per day between day 1 and month 1 by stage (3 stages)

1c: Serum creatinine over time (Mean and SD)

1d: Whole-blood tacrolimus trough concentrations over time (Box: 25th,median and 75th percentiles), the asterisks in the boxplots show that the medians differed significantly from 15 ng/mL on day 4 and 5

SUPPLEMENTARY MATERIAL

High tacrolimus blood concentrations early after lung transplantation and the risk of kidney injury

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PATIENTS AND METHODS

The study was conducted in compliance with the 2008 Declaration of Helsinki and Good Clinical Practice guidelines and with local and national regulatory requirements and laws. The accredited review board for human studies of the UMCU approved the study (IRB protocol number 11-357/G-C).

Patients and therapy

All lung transplantation patients hospitalized at the UMCU from July 2001 to February 2011 were retrospectively studied. Patients were analyzed from the transplantation date until one year after transplantation. Tacrolimus was orally dosed twice daily from the first postoperative day onwards with a starting dose of 0.1 mg/kg. Adjustments on basis of interactions with other drugs, gut dysmotility and liver injury were left to the discretion of the attending physician. Additional dosing was based on daily whole-blood tacrolimus trough concentrations at 6 am (C12h). Dose adjustments were often made on a daily basis and steady state was not necessarily reached at the time of dose adjustments. A whole-blood tacrolimus trough concentration between 9 and 15 ng/mL was considered therapeutic.[1] Although tacrolimus trough concentrations were measured daily we included only the tacrolimus blood concentrations that were taken at day 1 to 14 and at 1, 3, 6 and 12 months after transplantation. The tacrolimus blood concentrations were analyzed using a micro-particle enzyme immunoassay (Abbott IMx™). The immunosuppressive regimen consisted further of basiliximab induction therapy on day 1 and 4 post-operative [20 mg intravenously], corticosteroids [prednisolon 25 mg per day qid intravenously and tapered off to 25 mg od orally after four days] and mycophenolate mofetil [starting dose 1500 mg orally bid, tapered off to 1000 mg bid]. The administration of the following potentially nephrotoxic drugs other than tacrolimus was recorded as a categorical variable with more categories (0, 1, 2 or ≥3): (val)acyclovir, (val)ganciclovir, tobramycin, gentamicin, furosemide, vancomycin and amphotericin B. Other factors potentially related to kidney injury were recorded as well, such as septic shock, systemic inflammatory response syndrome (SIRS), diagnosis of sarcoidosis, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), or alpha-1-antitrypsin deficiency, diabetes, body mass index (BMI), pre-transplant renal function, perioperative extracorporeal membrane oxygenation (ECMO), double lung transplantation, and also postoperative profound bleeding for which reoperation was needed, infection and acute rejection.[2]

Definitions of acute kidney injury (AKI) and chronic kidney disease (CKD) (See Table S1)

AKI was classified according to the "Kidney Disease: Improving Global Outcomes" (KDIGO) Clinical Practice Guideline, which distinguishes 3 stages.[3] These stages were solely based

on serum creatinine concentration because urine data were unavailable. Classification was determined by the most severe stage of AKI. Indications for renal replacement therapy were stage 3 combined with hyperkalemia, untreatable hypervolemia, uncorrectable metabolic acidosis and severe azotemia. A serum creatinine lower or equal to the baseline creatinine + 5% indicated renal recovery after an AKI event. CKD was determined according to the estimated GFR using the "CKD Epidemiology Collaboration equation (CKD EPI)".[4] Albuminuria was not known. Therefore, only serum creatinine was used for categorizing CKD. Severe CKD was defined as having a stage 4 or 5 and was evaluated after 3 and 6 months and 1 year.

Definitions of the covariates (See Table S1)

SIRS has been defined according to the definition of the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine Consensus Conference (SCCM).[5] Patients considered to have septic shock had SIRS in combination with infusion of at least one inotrope/vasopressive agent. Liver injury was defined as a bilirubin >34 µmol/L or an alanine aminotransferase (ALAT) >90 U/L for men and >70 U/L for women.[6] Drugs influencing tacrolimus pharmacokinetics were also recorded: drugs that potentially increase tacrolimus blood concentrations by inhibition or substrate competition of the enzymes CYP3A4/5 and transporter Pgp and drugs that potentially decrease tacrolimus blood concentrations by induction of the enzymes CYP3A4/5 or transporter Pgp.[7]

Statistical analyses (See Fig. S1 and Table S2)

Statistical analyses were executed using SPSS version 15.0 for Windows (SPSS® Inc., Chicago, USA) and SAS version 9.2 for Windows (SAS® Institute Inc., Cary, NC, USA). Variables are presented as mean (standard deviation (SD)), median (25th and 75th percentiles), Odds ratio (OR) or estimate with 95% confidence interval (CI) or number (proportion) where appropriate. Chi-square tests or Fisher's exact tests were used to test differences between groups of patients for categorical data. T-tests or Wilcoxon Rank Tests were used for continuous data.

Variables influencing the tacrolimus whole-blood trough concentrations

Mixed model analyses were applied to investigate the variables influencing the whole-blood tacrolimus trough concentrations. Analyses were performed using the "PROC MIXED option" in SAS (See Fig. S1 and Table S2). The patient's identification number was the subject variable. Different variables (Cystic Fibrosis, liver dysfunction, drugs possibly increasing tacrolimus blood concentrations and drugs possibly decreasing tacrolimus blood concentrations) were tested independently and included as fixed factors. Time was included as both a within-subject and a between-subject variable.

Variables influencing AKI

In longitudinal data with a dichotomous outcome, outcomes within one patient are correlated. To account for this within-patient effect, we applied Generalized Estimation Equation (GEE) analyses.[8] The "PROC GENMOD" in SAS was used to study whether a high whole-blood tacrolimus trough concentration, SIRS, septic shock and the administration of a nephrotoxic drug, not being tacrolimus, were predictive of AKI up to 6 and 14 days after transplantation.

Relationship between AKI, recovery and CKD

Kaplan-Meier analyses were performed to study the probability of surviving without CKD up to one year. We distinguished three groups of patients: group 1 included patients without AKI between day 1 and day 14; group 2 included patients having AKI between day 1 and day 14 with recovery at 1 month; and group 3 included patients with AKI between day 1 and day 14 without recovery at 1 month. Differences between the three groups of patients were tested using the log rank test. Differences between CF and non-CF patients were tested using the same method.

REFERENCES

1. Monchaud C, Marquet P. Pharmacokinetic optimization of immunosuppressive therapy in thoracic transplantation: part I. *Clinical Pharmacokinetics*. 2009;48(7):419-462. doi:10.2165/11317230-000000000-00000.
2. Grimm JC, Lui C, Kilic A, et al. A risk score to predict acute renal failure in adult patients after lung transplantation. *Ann Thorac Surg*. 2015;99(1):251-257. doi:10.1016/j.athoracsur.2014.07.073.
3. Kellum JA, Lameire N, KDIGO AKI Guideline Work Group. Diagnosis, evaluation, and management of acute kidney injury: a KDIGO summary (Part 1). *Crit Care*. 2013;17(1):204. doi:10.1186/cc11454.
4. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612. doi:10.1097/GME.0000000000000416.
5. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. In: Vol 101. 1992:1644-1655. doi:10.1378/chest.101.6.1644.
6. Baran DA, Galin ID, Zucker MJ, et al. Can initial tacrolimus trough levels be predicted from clinical variables? *Transplantation Proceedings*. 2004;36(9):2816-2818. doi:10.1016/j.transproceed.2004.09.037.
7. Sikma MA, van Maarseveen EM, van de Graaf EA, et al. Pharmacokinetics and Toxicity of Tacrolimus Early After Heart and Lung Transplantation. *Am J Transplant*. 2015;15(9):2301-2313. doi:10.1111/ajt.13309.
8. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*. 1986;42(1):121-130. doi:10.2307/2531248.

FIGURES

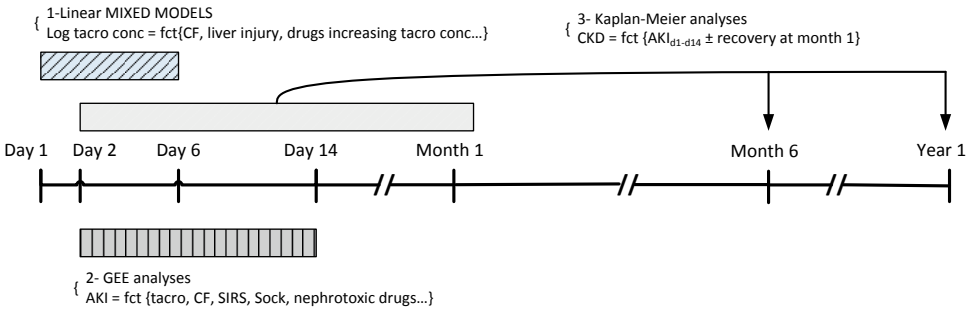

Fig. S1 Performed statistical analyses: 1- Linear mixed model, 2- GEE analyses and 3- Kaplan-Meier analyses

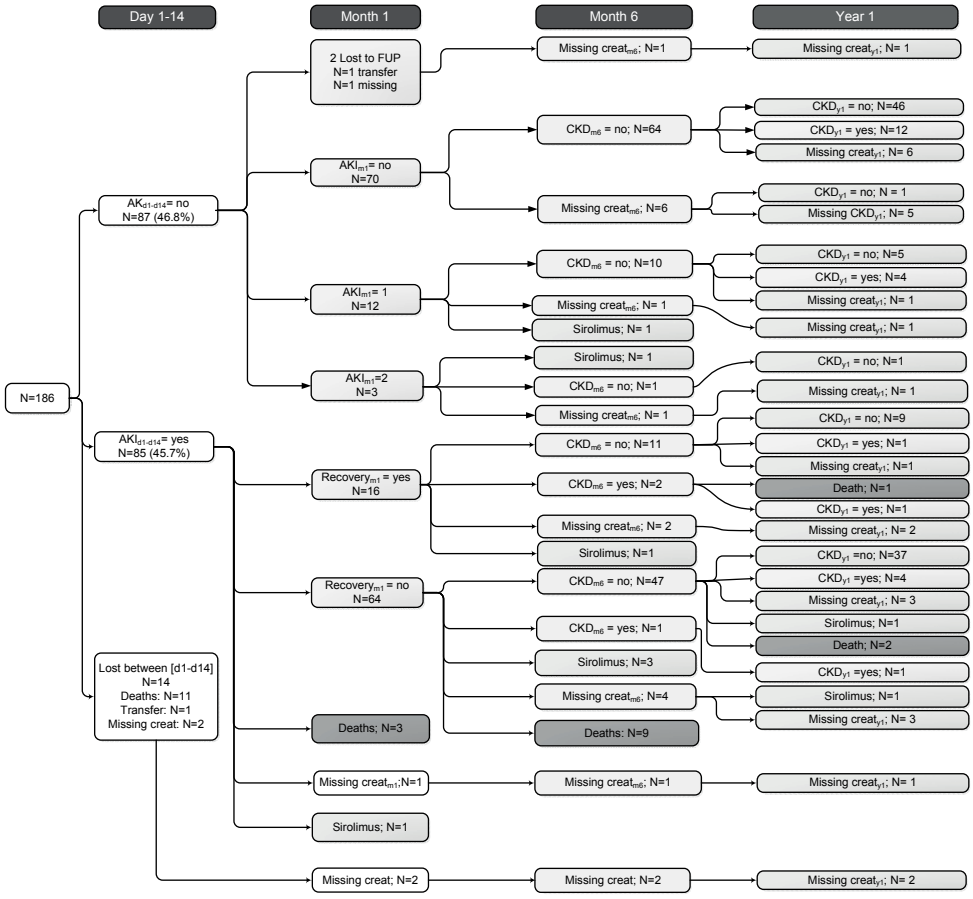

Fig. S2 Flow-chart: Frequency of AKI and severe chronic kidney disease between day 1 and year 1

2a

TABLES

Table S1 Definitions of the covariates

Covariate	Definition
AKI	"No AKI"; No increase in serum creatinine from baseline or serum creatinine <354 µmol/L. Stage 1; Increase in serum creatinine ≥26 µmol/L or 150-200% from baseline. Stage 2; Increase in serum creatinine >200% and ≥300% from baseline. Stage 3; Increase in serum creatinine >300% or ≥354 µmol/L with an acute increase of minimally 44 µmol/L or initiation of renal replacement therapy
CKD	Defined as Glomerular filtration rate (GFR) categories; G1=normal GFR: ≥90 mL/min /1.73m^2, G2=mildly decreased: 60–89 mL/min /1.73m^2, G3a/b=mildly to severely decreased: 30-59 mL/min /1.73m^2, G4= severely decreased: 15-29 mL/min /1.73m^2, G5= Kidney failure: <15 mL/min/1.73m^2. GFR = 141 x [min(Scr/κ),1)α x max(Scr/κ),1)-1.209] x Age-0.993 x 1.018 [if female] x [1.157 if Black] α is 0.329 for females and 0.411 for males; min indicates minimum of Scr/κ or 1, and max indicates maximum of Scr/κ or 1
SIRS	Presenting 2 or more of the following criteria: body temperature <36 °C or >38 °C, heart rate >90/min, respiratory rate >20/min, PaCO2 <32 mmHg, mechanical ventilation and leucocyte count <4 X10^9/L or >12 X10^9/L
Septic shock	SIRS plus at least one inotrope/ vasopressant (norepinephrine, dopamine, dobutamine and milrinone)
Liver injury	Bilirubin >34 µmol/L or an ALAT >90 U/L for men and >70 U/L for women
Nephrotoxic drugs other than tacrolimus	Tobramycin, vancomycin, (val)aciclovir, (val) ganciclovir, furosemide, Amfo B
Drugs increasing tacrolimus blood concentrations by inhibition or substrate competition of the CYP3A4/5 and Pgp enzymes	Basiliximab, erythromycin, fluconazole, voriconazole, (es)omeprazole, amlodipine, nicardipine, diltiazem, haloperidol and amiodarone
Drugs potentially decreasing tacrolimus blood concentrations by induction of CYP3A4/5 or Pgp enzymes	Corticosteroids and rifampicin

Table S2 Type of analysis used for the different outcome variables with the potential confounders

Type of analysis	Outcome variable	Tested variable(s)	Potential confounder(s)
Linear Mixed model	Whole-blood tacrolimus trough concentration[1]	CF Liver injury Other drugs increasing tacrolimus concentration Other drugs decreasing tacrolimus concentration[2]	
GEE analysis[3]	AKI between day 2-6	Supra-therapeutic whole-blood tacrolimus trough concentrations day 2-6	SIRS Shock CF Nephrotoxic drugs other than tacrolimus Double lung transplantation Perioperative ECMO Infection
	AKI between day 2-14	Supra-therapeutic whole-blood tacrolimus trough concentrations day 2-14	CF Double lung transplantation Perioperative ECMO Infection
Kaplan-Meier analyses	Severe CKD up to 1 year	CF AKI between day 2-14 ± recovery at 1 month	

[1] The tacrolimus whole-blood concentration was log transformed for fitting purposes.

[2] Fixed factors were: CF, liver injury, the number of drugs possibly decreasing the tacrolimus concentration and the number of drugs possibly increasing the tacrolimus concentration, included as categorical variables. The effect of drugs on the tacrolimus concentration was tested one day after their initiation. Observations were clustered within individuals (patients' identification number as subject variable) and time (expressed in day) was entered as both a within-subject and a between-subject variable. A quadratic term for day had to be included in the model for fitting purposes as the relationship between whole-blood tacrolimus concentration and day was non-linear.

[3] A binary logistic model was selected, with a logit link and an exchangeable working matrix. The outcome variable was "AKI", with two categories: "normal" corresponding to the no AKI and "abnormal" corresponding to the AKIN stages 1, 2, and 3 together. The significance of the variables was tested by Wald chi-square tests.

Table S3 Concentrations of Hb, Ht, albumin and total protein day 1 to 6

		Hb[1,2] mmol/L	Ht[1,2]	Albumin[1,3] g/L	Total Protein[1,3] g/L
Median		6.1	.29	23.2	47
Percentile	25th	5.3	.25	19.4	41
	75th	6.8	.33	26.5	53

[1] Anemia was defined as Ht <0.35 or Hb <7 mmol/L and low protein as albumin <20 g/L or total protein concentration <45 g/L.
[2] Anemia was observed in 98% of patients (182 out of 186).
[3] A low protein level was found in 69% of patients (129 out of 186).

CHAPTER 2b

2b

Association of whole-blood tacrolimus concentrations with kidney injury in heart transplantation patients

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ABSTRACT

Background and Objectives

Acute kidney injury (AKI) is frequently observed after heart transplantation and is associated with morbidity and mortality. However, many confounding factors also contribute to the development of AKI in heart transplants. We hypothesized that supra-therapeutic whole-blood tacrolimus trough concentrations are associated with AKI.

Methods

In a retrospective observational cohort from April 2005 to December 2012, all adult heart transplantation patients were included. AKI was assessed in the first 2 weeks after transplantation as classified by the Kidney Disease Improving Global Outcomes Network (KDIGO). Whole-blood tacrolimus trough concentrations were determined from day 1 to day 14 and at 1, 3, 6 and 12 months post transplantation. The therapeutic range was 9 to 15 ng/ml the first 2 months and tapered to 5 to 8 ng/ml thereafter. The relationship between supra-therapeutic tacrolimus trough concentrations and AKI was evaluated. The impact of various potentially confounding factors on tacrolimus concentrations and AKI was considered.

Results

We included 110 patients. AKI occurred in 57% of patients in the first week. Recovery from AKI was seen in 24%. The occurrence of chronic kidney disease (CKD) was 19% at 1 year. Whole-blood tacrolimus trough concentrations were often supra-therapeutic and, despite correction for confounding factors, independently associated with AKI (OR 1.66; 95% CI 1.20 -2.31).

Conclusions:

Supra-therapeutic whole-blood tacrolimus trough concentrations are independently associated with the development of AKI in adult heart transplantation patients. More stringent dosing of tacrolimus early after transplantation may be critical in preserving the kidney function. (IRB UMC Utrecht protocol number 12-071)

INTRODUCTION

Over 104,000 heart transplantations have been performed worldwide since 1967.[1] The immunosuppressive regimen has improved considerably since then.[1-3] The introduction of tacrolimus, a very effective immunosuppressive drug, has substantially contributed to increased survival and decreased rejection rates.[1-4] Despite its success, tacrolimus often has serious side effects, such as nephrotoxicity.[3] Tacrolimus-induced nephrotoxicity frequently evolves into chronic kidney disease (CKD).[5] The occurrence of CKD in heart-transplanted patients is reported to be 26% after 1 year, 52% after 5 years and in 68% by 10 years. Of these patients, 81% has been treated with the immunosuppressant tacrolimus as the preferred calcineurin inhibitor.[6] It has been acknowledged that CKD after heart transplantation contributes considerably to increasing mortality rates over time.[7-9] Logically, prevention of acute kidney injury (AKI) might prevent subsequent CKD in heart transplants.[10]

The etiology of AKI in the perioperative phase is often multifactorial. Various factors collectively contribute to the development of AKI, e.g., a high baseline creatinine, a long surgery time, the use of cardiopulmonary bypass, shock, inflammation, the administration of blood products, and nephrotoxic drugs.[10-14] At present, the evidence on tacrolimus trough concentrations being related to AKI after heart transplantation is circumstantial and largely derived from other solid organ transplantations. Therefore, the association between AKI after heart transplantation and tacrolimus is still not fully elucidated.

Our research hypothesis was that supra-therapeutic whole-blood tacrolimus trough concentrations are an independent factor in the development of AKI in adult heart transplant recipients. In addition, we analyzed whether AKI after heart transplantation is associated with subsequent development to CKD.

PATIENTS AND METHODS

Inclusions and exclusions criteria

Data of all heart transplantation patients at the University Medical Center Utrecht between April 2005 and December 2012 were retrospectively examined. No multi-organ transplantations were performed. Patients who died within 24 h were excluded as well as patients with preoperative glomerular filtration rate (GFR) <40ml/min defined by the Modification of Diet in Renal Disease formula (MDRD).[15] In these patients tacrolimus was postponed for several days and basiliximab was used as immunosuppression. Patients who have died on the first day could not be analyzed for kidney injury, because of low exposure to tacrolimus and the delayed increase in plasma creatinine.

Immunosuppressive regimen and dosing

The protocol of the transplantation center demanded that tacrolimus was started at an oral dose of 2 mg twice daily. Further, dosing was based on tacrolimus whole-blood trough concentrations at 6 am (12 h post dose). A whole-blood tacrolimus trough concentration between 9 and 15 ng/ml was considered therapeutic in the first two months and thereafter tapered towards 5 to 8 ng/ml providing no rejection was encountered.[16] Blood samples for tacrolimus concentration were immediately drawn before the administration and, therefore, represent trough levels. Steady state was not needed for dose adjustments. Corrections on basis of trough concentrations, kidney and liver function, gut motility and interactions with other drugs were left to the discretion of the transplantation cardiologist. Accompanying immunosuppression comprised of corticosteroids [prednisolone 50 mg intravenously directly postoperative followed by 25 mg twice daily and tapered off to 20 mg twice daily orally after 6 days] and mycophenolate mofetil [1000 mg orally twice daily]. Basiliximab was not administered in combination with tacrolimus.

Tacrolimus assay

The measurements from days 1 to 14, and at 1, 3, 6 and 12 months after transplantation were used for analysis using a micro-particle enzyme immunoassay in accordance with the required quality standards. The lower limit of quantification was 2 ng/ml and intraday imprecision was ±15% (Abbott IMx™ assay II, Abbott laboratories, Malvern, USA).[17]

Definition of kidney injury

Acute kidney injury was classified according to the Kidney Disease Improving Global Outcomes Network (KDIGO) criteria, which distinguishes three classes (See also Supplementary materials Table S1).[18] Urine data were unavailable; therefore, AKI classification was solely based on serum creatinine concentration: AKI stage 1; Increase in serum creatinine ≥26 μmol/L or 150-200% from baseline, AKI stage 2; Increase in serum creatinine >200% and ≤300% from baseline, AKI stage 3; Increase in serum creatinine

>300% or ≥354 µmol/L with an acute increase of minimally 44 µmol/L or initiation of renal replacement therapy. Baseline creatinine was the last creatinine prior to surgery. Indications for renal replacement therapy were stage 3 combined with one of the following characteristics: hyperkalemia, severe hypervolemia, uncorrectable metabolic acidosis and serious uremia.

Recovery of AKI was defined as a reduction in peak AKI stage within 3 days, which is consistent for 48 h between day 1 to day 14.[19] Persistence of AKI was determined at 1 month by comparing the creatinine at 1 month to the baseline creatinine among patients with AKI between day 1 and day 14. Chronic kidney disease (CKD) was determined as the estimated GFR using the "CKD Epidemiology Collaboration equation".[20] CKD was defined as having a stage 3, 4 or 5 and was assessed after 3 and 6 months and 1 year.

Collection and definitions of other covariates considered in the analyses

Drugs interacting with tacrolimus were also documented. Co-medication increasing tacrolimus blood concentrations encompassed macrolides, azoles, calcium antagonists, haloperidol and amiodarone (Additional information is given in the online resource Table S.1, showing the definitions of the covariates). Corticosteroids are known to decrease tacrolimus blood concentrations.[21] Liver dysfunction increases tacrolimus blood concentrations. Liver injury was defined as bilirubin >34 µmol/L or alanine aminotransferase (ALT) >90 U/L for men and >70 U/L for women.[22]

Systemic inflammatory response syndrome (SIRS) was determined according to the definition of the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine Consensus Conference (SCCM).[23] The only modification to the SIRS-criteria was that the heart frequency, providing one SIRS-point, was established as 100/min instead of 90/min, because in many heart transplant patients the standard pacemaker configuration is set at 100/min. Shock was specified as mean arterial pressure <60 mmHg or use of at least one of the following vasopressors/inotropes: norepinephrine, epinephrine, phenylephrine, vasopressin, dopamine, dobutamine or milrinone. We also collected information on potentially nephrotoxic drugs other than tacrolimus that were used in this cohort of heart-transplantation patients, particularly (val)acyclovir or (val)ganciclovir (CMV prophylaxis), tobramycin, gentamicin, trimethoprim/ sulfamethoxazole (pneumocystis jiroveci prophylaxis), penicillins, furosemide, vancomycin and amphotericin B. The covariates SIRS, shock and nephrotoxic drugs were not available between day 7 and 14.

Statistics

Statistical analyses are outlined in the Supplementary materials Table S.2. Variables are presented as mean (with standard deviation [SD]), median (with interquartile range [IQR]) or number (proportion) where appropriate. A Generalized Estimating Equations (GEE)

procedure was used to test whether AKI was significantly associated with prior supra-therapeutic whole-blood tacrolimus trough concentrations, after correction for possible confounders like shock and baseline characteristics (preoperative ventricular assist device (VAD), ischemic cardiomyopathy (ICM), diabetes mellitus (DM), surgery time >400 minutes and extracorporeal membrane oxygenation postoperative (ECMO)). GEE analysis was chosen, because it accounts for the correlation among the repeated observations for a given patient.[24] The outcome variable "AKI" was "0" when not meeting the KDIGO criteria and "1" when meeting one of the KDIGO classes (1, 2, or 3).

Linear mixed models were used to study the relationships between different variables (liver injury, other administered drugs and time) and supra-therapeutic whole-blood tacrolimus trough concentration. Effects were considered significant when p-values were lower than or equal to 0.005 (instead of 0.05 as usual, to compensate for multiple comparisons). Kaplan-Meier analyses were used to compare two groups of patients: group 1 included patients without AKI between day 1 and day 14; group 2 included patients having AKI between day 1 and day 14. Statistical analyses were carried out using SPSS version 15.0 for Windows and SAS version 9.2 for Windows (SAS Institute Inc., USA).

RESULTS

Demographics

Between April 2005 and December 2012, 114 patients underwent heart transplantation in our hospital. In 4 patients, tacrolimus was postponed and basiliximab was part of the initial immunosuppressive strategy. No patient died within 24 h. We analyzed 110 patients of whom five died within the first 30 days and additionally three within one year. Causes of death were right ventricular failure (2), pulmonary bleeding (1), primary graft failure (2), acute rejection (1), and infection (2). Two patients were transferred to other hospitals and three were lost-to-follow-up. Table 1 shows the patient characteristics. Within 1 year, three patients stopped tacrolimus due to severe side effects as neuro- and nephrotoxicity and switched to sirolimus (N=1) or cyclosporine (N=2). (The frequencies of AKI and CKD are also shown in the Supplementary materials Fig. S.1).

Renal function

Fig. 1 shows the distribution of serum creatinine over time. The median baseline creatinine was 102 µmol/L (IQR 84-126 µmol/L). The highest median creatinine of 117 µmol/L was observed at day 3 and slowly decreased to 91 µmol/L at 1 year. The frequency of patients presenting at least one episode of AKI between day 1 and day 6 was 57% (63 out of 110). AKI was most prevalent on day 3 (44%). There was a decrease in frequency of AKI to 17% at day 14, with an increase at one month to 25%. The most serious AKI stage 3 [based on "KDIGO criteria"] was most often observed on day 4 (6%). Renal replacement therapy was needed in 6% of patients within the first 14 days (7 out of 110). Recovery from AKI was observed in 15 out of 64 patients (24%). Five patients among the 110 (4.5 %) had recurrent AKI. Persistent kidney injury at one month was not associated with the occurrence of CKD (p=0.7). The frequency of patients with CKD was 19% at one year. CKD or death was not significantly related to the occurrence of AKI (p=0.14).

Variables influencing AKI

GEE-analyses showed that AKI was associated with prior supra-therapeutic whole-blood tacrolimus trough concentrations both in the first week (OR 1.66; 95% CI 1.20-2.31) and in the first 2 weeks (OR 2.10; 95% CI 1.35-3.25) after transplantation, even after correction for the variables ICM, VAD, DM, age, contrast administration, surgery time >400 minutes and ECMO post operation. Among the patients who presented a supra-therapeutic level, the median duration was 2 days (minimum 1 day, maximum 5 days). Nephrotoxic drugs as described in the method section were frequently co-administered during the first week after transplantation (95%, 104 out of 110) and most often on day one and two (72% and 75%, respectively). At day 6, 33% of the patients used nephrotoxic drugs. Shock was observed in 96% of patients (105 out of 110) during the first 6 days. The occurrence

of shock was most often seen on day one (84%) and decreased to 50% on day 6. The frequency of SIRS was highest on day 2 (86%) with a decrease to 31% on day 6 (Table 2).

Tacrolimus blood concentrations

We analyzed a total of 473 tacrolimus whole-blood trough concentrations within the first week after transplantation. In 34% of patients (37 out of 110), a supra-therapeutic concentration was measured at least once. Whole-blood tacrolimus trough concentrations ranged from 1.5 to 35 ng/mL with the highest median at day 4, 5, 6 and 7 of 10 ng/mL. At month 3, the median tacrolimus concentration was 9 ng/mL, indicating a supra-therapeutic concentration. Median whole-blood tacrolimus trough concentrations showed to be below therapeutic range during the first 3 days, at day 10 and 12, at month 6 and at 1 year (See Fig. 2). At day 4, 5 and 6 the highest frequencies of supra-therapeutic whole-blood tacrolimus trough concentrations were measured (16% on all days). At day 14 3% had a supra-therapeutic level and at 1 month this frequency increased to 8%. (See Table S.3 in the Supplementary materials, demonstrating the variables influencing whole-blood tacrolimus concentrations). Liver injury was often observed within the first week after transplantation (56%, 61 out of 110). The highest occurrence of liver injury was observed on day 2 post transplantation (28%) with a decrease to 16% on day 6. Supra-therapeutic whole-blood tacrolimus trough concentrations were not significantly associated with liver injury ($p=0.03$) (See Table S.3 in the Supplementary materials, demonstrating the variables influencing whole-blood tacrolimus concentrations). Almost all patients used drugs increasing tacrolimus concentrations within the first week after transplantation (95%, 104 out of 110). Drugs increasing tacrolimus concentrations were most frequently used on day 1 (82%). From day 2 on drugs decreasing tacrolimus concentrations were applied. The prevalence of the administration of these drugs was highest on day 2 (71%). One patient was treated with high dose methylprednisolone for 6 days because of suspected rejection. Drugs, which could affect tacrolimus concentrations, increasing as well as decreasing, were not significantly associated with whole-blood tacrolimus trough concentrations (p values 0.86 and 0.07, respectively, See also in the Supplementary materials Table S.3).

DISCUSSION

We found a high incidence rate of AKI after heart transplantation. AKI was independently associated with supra-therapeutic tacrolimus concentrations, which were most often observed in the first week.

The high incidence rate of AKI (57%) in our cohort of heart transplant patients was in accordance with Tjahjono et.al, in which AKI was seen in 59% of the patients within the first week.[12] These high incidence rates showed to be higher than in a cohort of lung transplants and may be based on the high frequency of shock and on the high median baseline creatinine, reflecting a decreased renal function pre-operatively.[12,25] The high baseline creatinine is in accordance with previous observations of heart transplants.[6] A diminished renal function may further deteriorate in case of shock and inflammation, the use of a pulmonary bypass circuit, increased administration of blood products as well as with the administration of nephrotoxic drugs, such as tacrolimus.[12] It was suggested that a supra-therapeutic tacrolimus concentration at day 3 is a risk factor for the development of AKI. This was observed in a pediatric heart transplantation cohort, though it did not reach significance.[11] We showed that a prior supra-therapeutic whole-blood tacrolimus trough concentration was an independent predictor of AKI in an adult heart transplantation cohort.

The occurrence of AKI is a predictor of CKD. In a group of 300 heart transplantation patients, AKI was related to CKD [10]. The risk of progression to CKD is related to the stages of AKI. Even mild AKI, stage 1, increases the risk of CKD.[26] We could not relate AKI to CKD. This lack of association may have been caused by a too small sample size or too short follow-up period. Nonetheless, we observed a low recovery rate of AKI in our cohort.[27,28] Recovery of AKI in heart transplants is important. AKI persistent at 1 month after transplantation has been shown to significantly decrease survival rates compared to patients with complete renal recovery.[1,10,13,28] Moreover, earlier reports showed a steady increase in the percentage and severity of CKD after heart transplantation in the first 10 years with a median time to progression to CKD stage 4 of 3 years.[1,9] Hence, the importance of AKI in the development of CKD shows to be pivotal and tacrolimus may have an important part in it.

Tacrolimus is an ongoing assault to the kidneys, because it is administered continuously. Nephrotoxicity induced by tacrolimus is assumed to be caused by acute vascular and tubular damage, and chronic irreversible tubule-interstitial fibrosis.[29-37] Renal biopsies show a gradual increase of arteriolar hyalinosis, glomerulosclerosis as well as interstitial fibrosis in patients treated with calcineurin inhibitors.[38] Paradoxically, anatomical abnormalities may go unnoticed and tacrolimus nephrotoxicity might be present without clinical loss of renal function. Nankivell et.al. observed a mean GFR of 60 ml/min in the presence of

grade I nephropathy and of 50 ml/min in the presence of chronic nephropathy of grade II or higher.[38]

To avoid early tacrolimus nephrotoxicity, decreased doses and delayed introduction have been suggested. To preclude early rejection when tacrolimus is postponed, an interleukin-2 receptor inhibitor or mTOR inhibitor could be administered.[39-44] Unfortunately, mTOR inhibitors are poorly tolerated, with almost one third discontinuing the drug within one year after transplantation.[41,44-46] Rejection rates may be higher in tacrolimus free regimens, therefore tacrolimus is still the preferred immunosuppressive drug after heart transplantation.[41]

Tacrolimus was carefully dosed in our cohort, reflected by the low starting dose and the (sub-)therapeutic median whole-blood concentrations. Dosages were substantially lower than in a cohort of lung transplantation patients.[25] Yet, tapering of corticosteroids, increasing tacrolimus blood concentrations, was started in a later stage as in the lung transplantation cohort.[25] Nevertheless, we found a high frequency of supra-therapeutic concentrations during the first 2 weeks. This cohort of heart transplants showed a higher frequency of liver injury and shock compared to the cohort of lung transplantation patients, reflecting the higher frequency of clinical instability.[25] Furthermore, we observed a high prevalence of drugs influencing the blood tacrolimus concentrations. Although none of the aforementioned variables individually influenced the development of subsequent supra-therapeutic tacrolimus concentrations, all combined might still contribute to supra-therapeutic tacrolimus concentrations. Tacrolimus dosing is complex and extremely difficult in the clinically unstable phase after heart transplantation.

To prevent from supra-therapeutic whole-blood trough concentrations, an even more tentative dosing scheme could be used. Targeting at the narrow therapeutic range may have contributed to the supra-therapeutic levels. Starting with a low dose and using only an upper level, may prevent peaks in the concentrations. This qualitative dosing scheme may prevent erratic tacrolimus concentrations and thus supra-therapeutic tacrolimus concentrations.

Limitations of this study

There are some notable limitations to this study due to its retrospective character. Several variables influencing pharmacokinetics of tacrolimus, for instance, the effect of variations in HDL, alpha-1-acid glycoprotein, acidosis, changes in fluid balance, gut motility and variations in concentrations or activity of CYP 3A4/5 and P-glycoprotein, could not be studied.

In our study, only 18 patients were still followed at 1-year post transplantation and developed CKD. A larger sample size would increase the reliability of our results, e.g. the potential influence of tacrolimus on the occurrence of CKD is likely to remain undetected in our study (Kaplan-Meier analysis). Similarly, it is plausible that our study had inadequate power to detect the effect of other drugs and liver injury on the whole-blood tacrolimus trough concentration (mixed model analysis).

Trough concentrations were assumed, though with one sample per 24-hour period the assumption may be insufficient. Especially, clinically unstable patients may lack steady state and large changes in half-life times may occur. This may hamper the interpretation of the blood concentrations.

We could not analyze all the known factors related to AKI after heart transplantation, e.g., the amount of blood products administered, inotrope and ventilation duration. Moreover, ultrasound, biopsy, and urine analyses have not been performed. Therefore, not all causes of kidney injury have been investigated in this study and they may have had an effect on kidney function.

Another limitation is the estimation of AKI itself. At present, the KDIGO criteria are considered to be the best option. However, plasma creatinine provides only an indication of renal function. Plasma creatinine in heart transplantation patients shortly after surgery may overestimate renal function.[47] Moreover, CKD-EPI tends to overestimate GFR.[9] Uniformity in defining kidney injury as well as recovery from AKI may be an important improvement for the comparison of renal outcome in heart transplant recipients.

CONCLUSIONS

This study shows that AKI after heart transplantation is correlated with supra-therapeutic whole-blood tacrolimus trough concentrations. Low recovery rates of AKI might even be a reflection of ongoing tacrolimus toxicity. Therefore, the prevention of tacrolimus nephrotoxicity early after transplantation may be crucial in preserving kidney function. The appropriate tailoring of tacrolimus dosing early after transplantation could be a key factor in improving transplantation outcomes.

REFERENCES

1. Stehlik J, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report--2012. *J Heart Lung Transplant*. 2012;31(10):1052-1064. doi:10.1016/j.healun.2012.08.002.
2. Guethoff S, Meiser BM, Groetzner J, et al. Ten-Year Results of a Randomized Trial Comparing Tacrolimus Versus Cyclosporine A in Combination With Mycophenolate Mofetil After Heart Transplantation. *Transplantation*. 2013;95(4):629-634. doi:10.1097/TP.0b013e318277e378.
3. Penninga L, Møller CH, Gustafsson F, Steinbrüchel DA, Gluud C. Tacrolimus versus cyclosporine as primary immunosuppression after heart transplantation: systematic review with meta-analyses and trial sequential analyses of randomised trials. *Eur J Clin Pharmacol*. 2010;66(12):1177-1187. doi:10.1007/s00228-010-0902-6.
4. Zijlstra LE, Constantinescu AA, Manintveld O, et al. Improved long-term survival in Dutch heart transplant patients despite increasing donor age: the Rotterdam experience. *Transplant International*. 2015;28(8):962-971. doi:10.1111/tri.12503.
5. Nankivell BJ, P'Ng CH, O'Connell PJ, Chapman JR. Calcineurin Inhibitor Nephrotoxicity Through the Lens of Longitudinal Histology: Comparison of Cyclosporine and Tacrolimus Eras. *Transplantation*. 2016;100(8):1723-1731. doi:10.1097/TP.0000000000001243.
6. Lund LH, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Official Adult Heart Transplant Report--2013; focus theme: age. *The Journal of Heart and Lung Transplantation*. 2013;32(10):951-964. doi:10.1016/j.healun.2013.08.006.
7. Healy AH, Stehlik J, Edwards LB, McKellar SH, Drakos SG, Selzman CH. Predictors of 30-day post-transplant mortality in patients bridged to transplantation with continuous-flow left ventricular assist devices-An analysis of the International Society for Heart and Lung Transplantation Transplant Registry. *J Heart Lung Transplant*. 2016;35(1):34-39. doi:10.1016/j.healun.2015.07.007.
8. Lund LH, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-second Official Adult Heart Transplantation Report--2015; Focus Theme: Early Graft Failure. *J Heart Lung Transplant*. 2015;34(10):1244-1254. doi:10.1016/j.healun.2015.08.003.
9. Söderlund C, Löfdahl E, Nilsson J, Reitan Ö, Higgins T, Rådegran G. Chronic kidney disease after heart transplantation: a single-centre retrospective study at Skåne University Hospital in Lund 1988-2010. *Transplant International*. 2016;29(5):529-539. doi:10.1111/tri.12710.
10. De Santo LS, Romano G, Amarelli C, et al. Implications of acute kidney injury after heart transplantation: what a surgeon should know. *Eur J Cardiothorac Surg*. 2011;40(6):1355–61–discussion1361. doi:10.1016/j.ejcts.2011.02.068.
11. MacDonald C, Norris C, Alton GY, et al. Acute kidney injury after heart transplant in young children: risk factors and outcomes. *Pediatr Nephrol*. 2016;31(4):671-678. doi:10.1007/s00467-015-3252-x.
12. Tjahjono R, Connellan M, Granger E. Predictors of Acute Kidney Injury in Cardiac Transplantation. *Transplantation Proceedings*. 2016;48(1):167-172. doi:10.1016/j.transproceed.2015.12.006.

13. Gude E, Andreassen AK, Arora S, et al. Acute renal failure early after heart transplantation: risk factors and clinical consequences. *Clinical Transplantation*. 2010;24(6):E207-E213. doi:10.1111/j.1399-0012.2010.01225.x.
14. Delgado JF, Crespo-Leiro MG, Gómez-Sánchez MA, et al. Risk factors associated with moderate-to-severe renal dysfunction among heart transplant patients: results from the CAPRI study. *Clinical Transplantation*. 2010;24(5):E194-E200. doi:10.1111/j.1399-0012.2010.01249.x.
15. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130(6):461-470. doi: 10.7326/0003-4819-130-6-199903160-00002
16. Aidong W, Zhenjie C, Tong L, et al. Therapeutic drug monitoring of tacrolimus in early stage after heart transplantation. *TPS*. 2004;36(8):2388-2389. doi:10.1016/j.transproceed.2004.06.037.
17. Salm P, Rutherford DM, Taylor PJ, Black MJ, Pillans PI. Evaluation of microparticle enzyme immunoassay against HPLC-mass spectrometry for the determination of whole-blood tacrolimus in heart- and lung-transplant recipients. *Clinical Biochemistry*. 2000;33(7):557-562. doi: 10.1016/s0009-9120(00)00163-6
18. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract*. 2012;120(4):c179-c184. doi:10.1159/000339789.
19. Chawla LS, Bellomo R, Bihorac A, et al. Acute kidney disease and renal recovery: consensus report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup. In: Vol 13. Nature Publishing Group; 2017:241-257. doi:10.1038/nrneph.2017.2.
20. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612. doi:10.1097/GME.0000000000000416.
21. Christians U, Jacobsen W, Benet LZ, Lampen A. Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clinical Pharmacokinetics*. 2002;41(11):813-851. doi:10.2165/00003088-200241110-00003.
22. Kramer L, Jordan B, Druml W, Bauer P, Metnitz PGH. Incidence and prognosis of early hepatic dysfunction in critically ill patients? A prospective multicenter study. *Critical Care Medicine*. 2007;35(4):1099–e7. doi:10.1097/01.CCM.0000259462.97164.A0.
23. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. In: Vol 41. 2013:580-637. doi:10.1097/CCM.0b013e31827e83af.
24. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*. 1986;42(1):121-130. doi: 10.2307/2531248
25. Sikma MA, Hunault CC, van de Graaf EA, et al. High tacrolimus blood concentrations early after lung transplantation and the risk of kidney injury. *Eur J Clin Pharmacol*. 2017;73(5):573-580. doi:10.1007/s00228-017-2204-8.
26. Xu J-R, Zhu J-M, Jiang J, et al. Risk Factors for Long-Term Mortality and Progressive Chronic Kidney Disease Associated With Acute Kidney Injury After Cardiac Surgery. *Medicine (Baltimore)*. 2015;94(45):e2025. doi:10.1097/MD.0000000000002025.

27. Garzotto F, Piccinni P, Cruz D, et al. RIFLE-Based Data Collection/Management System Applied to a Prospective Cohort Multicenter Italian Study on the Epidemiology of Acute Kidney Injury in the Intensive Care Unit. *Blood Purif*. 2011;31(1-3):159-171. doi:10.1159/000322161.
28. Lee JM, Lee S-A, Cho H-J, et al. Impact of perioperative renal dysfunction in heart transplantation: combined heart and kidney transplantation could help to reduce postoperative mortality. *Ann Transplant*. 2013;18:533-549. doi:10.12659/AOT.889103.
29. Hortelano S, Castilla M, Torres AM, Tejedor A, Boscá L. Potentiation by nitric oxide of cyclosporin A and FK506-induced apoptosis in renal proximal tubule cells. *J Am Soc Nephrol*. 2000;11(12):2315-2323.
30. Nankivell BJ, Borrows RJ, Fung CLS, O Connell PJ, Chapman JR, Allen RDM. Delta Analysis of Posttransplantation Tubulointerstitial Damage. *Transplantation*. 2004;78(3):434-441. doi:10.1097/01.TP.0000128613.74683.D9.
31. Esteva-Font C, Ars E, Guillen-Gomez E, et al. Ciclosporin-induced hypertension is associated with increased sodium transporter of the loop of Henle (NKCC2). *Nephrology Dialysis Transplantation*. 2007;22(10):2810-2816. doi:10.1093/ndt/gfm390.
32. Catarsi P. Angiotensin-converting enzyme (ACE) haplotypes and cyclosporine A (CsA) response: a model of the complex relationship between ACE quantitative trait locus and pathological phenotypes. *Human Molecular Genetics*. 2005;14(16):2357-2367. doi:10.1093/hmg/ddi238.
33. Randhawa PS, Shapiro R, Jordan ML, Starzl TE, Demetris AJ. The Histopathological Changes Associated with Allograft Rejection and Drug Toxicity in Renal Transplant Recipients Maintained on FK506. *The American Journal of Surgical Pathology*. 1993;17(1):60-68. doi:10.1097/00000478-199301000-00007.
34. Bai JPF, Lesko LJ, Burckart GJ. Understanding the Genetic Basis for Adverse Drug Effects: The Calcineurin Inhibitors. *Pharmacotherapy*. 2010;30(2):195-209. doi:10.1592/phco.30.2.195.
35. Naesens M, Kuypers DRJ, Sarwal M. Calcineurin Inhibitor Nephrotoxicity. *Clinical Journal of the American Society of Nephrology*. 2009;4:481-508. doi:10.2215/cjn.04800908.
36. Ozdemir BH, Ozdemir FN, Demirhan B, Haberal M. TGF-beta1 Expression in Renal Allograft Rejection and Cyclosporine A Toxicity. *Transplantation*. 2005;80(12):1681-1685. doi:10.1097/01.tp.0000185303.92981.d6.
37. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporine-Associated Chronic Nephropathy. *N Engl J Med*. 1984;311(11):699-705. doi:10.1056/NEJM198409133111103.
38. Nankivell BJ, Borrows RJ, Fung CLS, O'Connell PJ, Allen RDM, Chapman JR. The Natural History of Chronic Allograft Nephropathy. *N Engl J Med*. 2003;349(24):2326-2333. doi:10.1056/NEJMoa020009.
39. Sánchez-Lázaro IJ, Almenar Bonet L, Martínez-Dolz L, et al. Repeated daclizumab administration to delay the introduction of calcineurin inhibitors in heart transplant patients with postoperative renal dysfunction. *Rev Esp Cardiol*. 2011;64(3):237-239. doi:10.1016/j.recesp.2010.05.001.
40. González-Vílchez F, de Prada JAV, Exposito V, et al. Avoidance of calcineurin inhibitors with use of proliferation signal inhibitors in de novo heart transplantation with renal failure. *J Heart Lung Transplant*. 2008;27(10):1135-1141. doi:10.1016/j.healun.2008.07.020.

41. Kaczmarek I, Zaruba M-M, Beiras-Fernandez A, et al. Tacrolimus with mycophenolate mofetil or sirolimus compared with calcineurin inhibitor-free immunosuppression (sirolimus/mycophenolate mofetil) after heart transplantation: 5-year results. *HEALUN*. 2013;32(3):277-284. doi:10.1016/j.healun.2012.11.028.
42. Arora S, Gude E, Sigurdardottir V, et al. Improvement in renal function after everolimus introduction and calcineurin inhibitor reduction in maintenance thoracic transplant recipients: The significance of baseline glomerular filtration rate. *HEALUN*. 2012;31(3):259-265. doi:10.1016/j.healun.2011.12.010.
43. Gullestad L, Mortensen S-A, Eiskjær H, et al. Two-Year Outcomes in Thoracic Transplant Recipients After Conversion to Everolimus With Reduced Calcineurin Inhibitor Within a Multicenter, Open-Label, Randomized Trial. *Transplantation*. 2010;90(12):1581-1589. doi:10.1097/TP.0b013e3181fd01b7.
44. Kaplinsky E, González-Costello J, Manito N, et al. Renal Function Improvement After Conversion to Proliferation Signal Inhibitors During Long-Term Follow-up in Heart Transplant Recipients. *Transplantation Proceedings*. 2012;44(9):2564-2566. doi:10.1016/j.transproceed.2012.09.045.
45. Thibodeau JT, Mishkin JD, Patel PC, et al. Tolerability of sirolimus: a decade of experience at a single cardiac transplant center. *Clinical Transplantation*. 2013;27(6):945-952. doi:10.1111/ctr.12269.
46. Manito N, Delgado JF, Crespo-Leiro MG, et al. Twelve-month efficacy and safety of the conversion to everolimus in maintenance heart transplant recipients. *World J Transplant*. 2015;5(4):310-319. doi:10.5500/wjt.v5.i4.310.
47. Bragadottir G, Redfors B, Ricksten S-E. Assessing glomerular filtration rate (GFR) in critically ill patients with acute kidney injury - true GFR versus urinary creatinine clearance and estimating equations. *Critical Care*. 2013;17(3):R108. doi:10.1186/cc12777.

TABLES

Table 1. Patients' characteristics

	All patients N=110 (100%)	FUP ≥ d14 & no AKI day 2-14 N= 39 (35%)	FUP ≥ d14 & AKI d2-14 N= 63 (25%)	P value [a]
Age (yr)	47 (13)	47 (12)	47 (14)	0.88
Male	74 (67%)	28 (72%)	40 (63%)	0.39
ICM	38 (35%)	19 (49%)	15 (24%)	0.01
DM	6 (5.5%)	1 (2.6%)	5 (7.9%)	0.40
VAD preoperative	55 (50%)	21 (54%)	31 (49%)	0.65
Surgery time >400min	34 (31%)	6 (15%)	25 (40%)	0.01
ECMO postoperative	7 (6.4%)	2 (5.1%)	4 (6.3%)	1.00
Death day 1-14	4 (3.6%)	0 (0%)	0 (0%)	b
Death day 1-1 year	8 (7%)	1 (3%)	3 (5%)	1.00
At least once during day 1-6:				
Liver injury	49 (45%)	13 (33%)	30 (48%)	0.16
Anemia	107 (97%)	38 (97%)	62 (98%)	1.00
Hypo-albuminemia or too low total protein concentration	38 (35%)	12 (31%)	21 (33%)	0.79
Supra-therapeutic whole-blood tacrolimus trough concentration	37 (34%)	13 (33%)	21 (33%)	1.00
SIRS	107 (97%)	37 (95%)	62 (98%)	0.56
Shock	92 (84%)	32 (82%)	55 (87%)	0.47
At least one drug increasing tacrolimus concentration	104 (95%)	36 (92%)	60 (95%)	0.67
At least one drug decreasing tacrolimus concentration	85 (77%)	38 (97%)	52 (83%)	0.32
Nephrotoxic drugs other than tacrolimus	104 (95%)	38 (97%)	58 (92%)	0.40

Values are presented as n (%), except for age, which are mean (SD)

a chi-square test, Fisher's exact test or t-test was used, where appropriate

b No statistics were computed because no death occurred

AKI= acute kidney injury, min=minute, ICM= ischemic cardiomyopathy, DM=diabetes mellitus, VAD= ventricular assist device, ECMO=extracorporeal membrane oxygenation, SIRS= systemic inflammatory response syndrome, SD=standard deviation, yr=year

Table 2. GEE analyses to test the variables influencing AKI

	Day 2-6 [a, c]		Day 2-14 [b, d]	
Variable	**OR**	**95% CI**	**OR**	**95% CI**
Supra-therapeutic whole-blood tacrolimus trough concentration	1.66	1.20 - 2.31	2.10	1.35 - 3.25
SIRS [e]	1.29	0.96 - 1.75	NA	NA
Shock [e]	1.24	0.91 - 1.67	NA	NA
Nephrotoxic drugs [e]			NA	NA
1 nephrotoxic drug	1.28	0.74 - 2.23	NA	NA
2 nephrotoxic drugs	1.07	0.60 - 1.91	NA	NA
≥3 nephrotoxic drugs	1.02	0.57 - 1.83	NA	NA
ICM	2.23	0.97 - 5.11	1.29	0.58 - 2.83
DM	1.72	0.38 - 7.77	1.97	0.57 - 6.83
VAD pre-transplantation	0.36	0.15 - 0.86	0.50	0.24 - 1.04
ECMO post-operation	2.73	0.77 - 9.68	3.02	0.95 - 9.63
Surgery time >400 min	2.56	1.03 - 6.36	2.66	1.27 - 5.58
Age (years)	1.00	0.97 - 1.03	1.00	0.97 - 1.03
Contrast	0.76	0.28 - 2.01	0.75	0.31 - 1.78

[a] d2-d6: data concerning day 2 up to day 6
[b] d2-d14: data concerning day 2 up to day 14
[c] d2-d6: estimate of the intercept: -2.9593
[d] d2-d14: estimate of the intercept: -2.82
[e] Data not available between day 7 and day 14

GEE=generalized estimating equation, AKI= acute kidney injury, OR = odds ratio, CI = confidence interval, SIRS= systemic inflammatory response syndrome, ICM= ischemic cardiomyopathy, DM=diabetes mellitus, VAD= ventricular assist device, ECMO=extracorporeal membrane oxygenation, min=minute

FIGURES

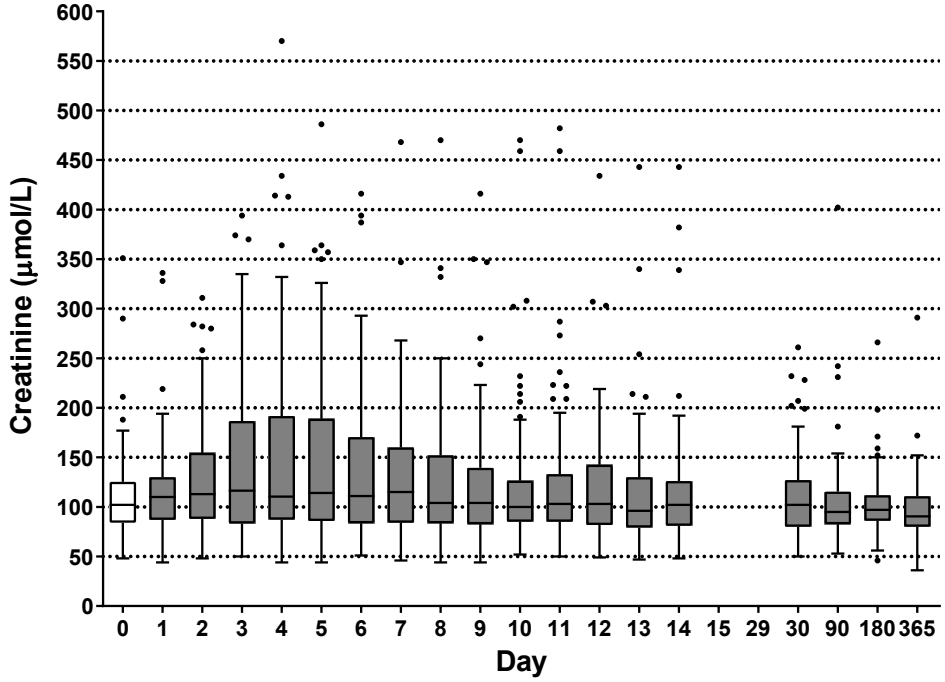

Fig. 1 Boxplot of creatinine (µmol/L) between day 1 and year 1. Day 0 is baseline creatinine. We used Tukey Boxplots.

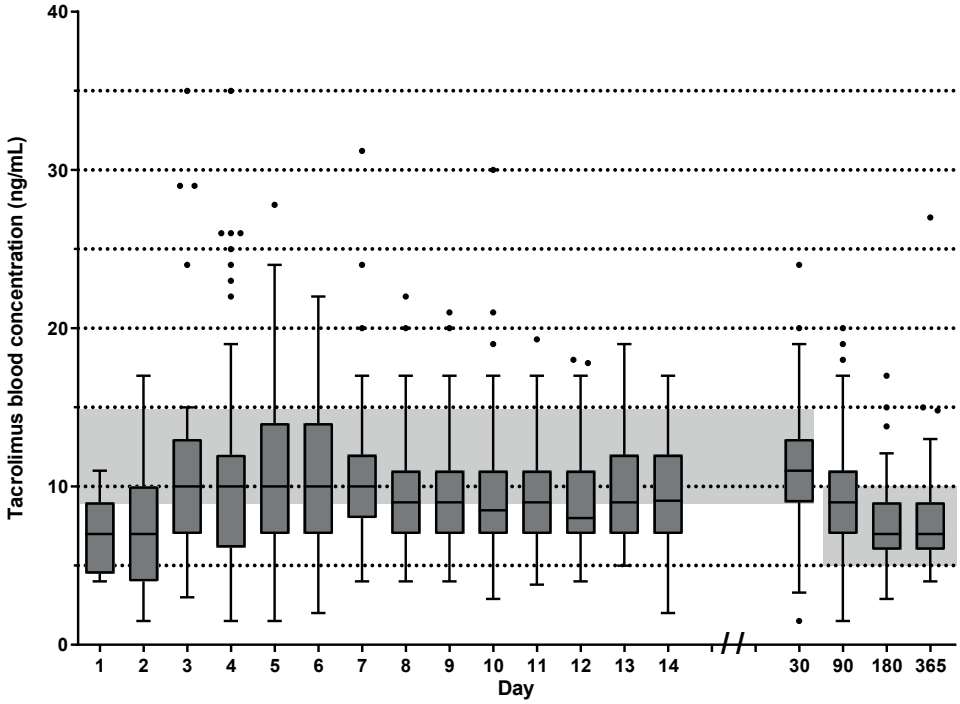

Fig. 2. Boxplot of whole-blood tacrolimus trough concentrations between day 1 and year 1 ("Tukey boxplots"). The therapeutic range of 9-15 ng/ml and 5-8 ng/ml are indicated as grey bars in the Figure.

SUPPLEMENTARY MATERIALS

Association of whole-blood tacrolimus concentrations with kidney injury in heart transplantation patients

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FIGURES

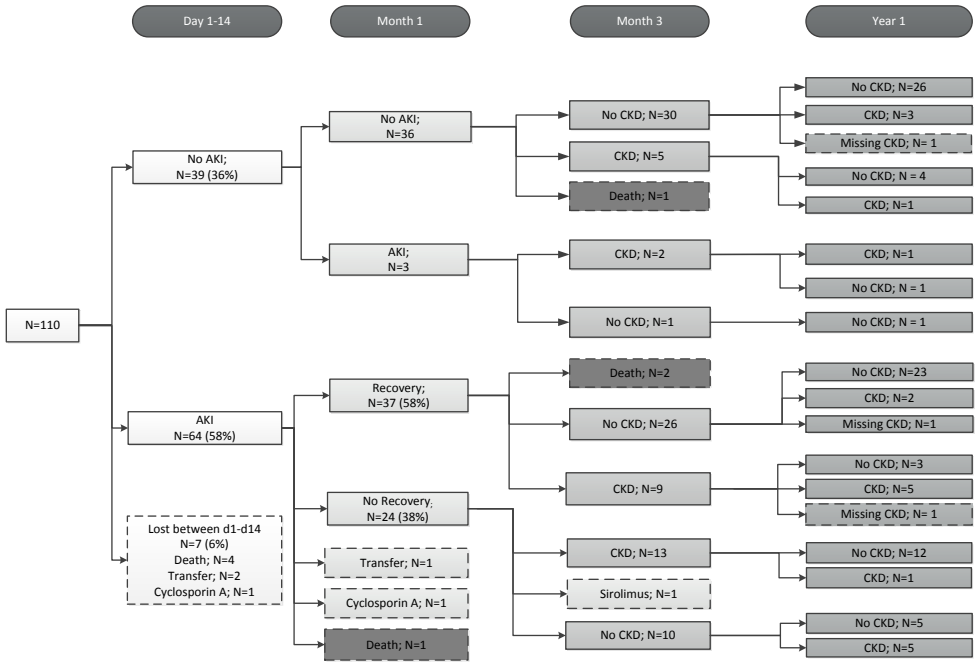

Fig. S.1 Frequency of AKI and chronic kidney disease between day 1 and year 1
AKI=acute kidney injury, CKD=chronic kidney disease

TABLES

Table S.1 Definitions of the covariates

Covariate	Definition
AKI	"No AKI"; No increase in serum creatinine from baseline or serum creatinine <354 µmol/L. AKI stage 1; Increase in serum creatinine ≥26 µmol/L or 150-200% from baseline. AKI stage 2; Increase in serum creatinine >200% and ≥300% from baseline. AKI stage 3; Increase in serum creatinine >300% or ≥354 µmol/L with an acute increase of minimally 44 µmol/L or initiation of renal replacement therapy
Recovery of AKI	A reduction in peak AKI stage within 3 days and consistent for 48 h between day 1 to day 14
Recurrent AKI	Two periods of AKI within 14 days with a minimum of 48 h in between
CKD	GFR categories; G1=normal GFR: ≥90 ml/min/1.73 m^2, G2=mildly decreased: 60–89 ml/min/1.73 m^2, G3a/b=mildly to severely decreased: 30-59 ml/min/1.73 m^2, G4= severely decreased: 15-29 ml/min/1.73 m^2, G5= Kidney failure: <15 mL/min/1.73 m^2. GFR = 141 x [min(Scr/κ),1)α x max(Scr/κ),1)-1.209] x Age-0.993 x 1.018 [if female] x [1.157 if Black] α is 0.329 for females and 0.411 for males; min indicates minimum of Scr/κ or 1, and max indicates maximum of Scr/κ or 1
SIRS	Presenting 2 or more of the following criteria: body temperature <36 °C or >38 °C, heart rate >100 / min, respiratory rate >20/min, PaCO2 <32 mmHg, mechanical ventilation and leucocyte count <4 $X10^9$ /L or >12 $X10^9$ /L
Shock	Mean arterial pressure <60 mmHg or use of norepinephrine, epinephrine, phenylephrine, vasopressin, dopamine, dobutamine or milrinone
Liver injury	Bilirubin >34 µmol/L or an ALT >90 U/L for men and >70 U/L for women
Nephrotoxic drugs	Amoxicillin/clavulanate, flucloxacillin, benzylpenicillin, piperacillin/tazobactam, tobramycin, vancomycin, (val)aciclovir, (val) ganciclovir, trimethoprim/sulfamethoxazole, furosemide

Table S.1 Definitions of the covariates

Covariate	Definition
Drugs increasing tacrolimus blood concentrations by inhibition or substrate competition of the CYP3A4/5 and Pgp enzymes	Tobramycin, erythromycin, neomycin, trimethoprim/sulfamethoxazole, fluconazole, voriconazole, (es) omeprazole, amlodipine, nicardipine, diltiazem, haloperidol, amiodarone
Drugs potentially decreasing tacrolimus blood concentrations by induction of CYP3A4/5 or Pgp enzymes	Corticosteroids and rifampicin
Anemia	Ht <0.35 or Hb <7.0 mmol/L
Low protein concentration	Albumin <20 g/L or total protein concentration <45 g/L

AKI= acute kidney injury, CKD= chronic kidney dysfunction, GFR=glomerular filtration rate, SIRS=systemic inflammatory response syndrome, Scr=serum creatinine, min=minute, CYP=cytochrome P450, Pgp=p-glycoprotein, Ht=hematocrit, Hb=hemoglobin

Table S.2 Type of analysis used for the different outcome variables with the potential confounders

Type of analysis	Outcome variable	Tested variable(s)	Potential confounder(s)
GEE analysis[a]	AKI between day 2-6	Supra-therapeutic whole-blood tacrolimus trough concentrations day 2-6 prior to AKI event	Other nephrotoxic drugs Shock SIRS Surgery time >400 min Preoperative VAD Postoperative ECMO Ischemic or non-ischemic heart failure DM Age Contrast administration
	AKI between day 2-14	Supra-therapeutic whole-blood tacrolimus trough concentrations day 2-14 prior to AKI event	Surgery time >400 min Preoperative VAD Postoperative ECMO Ischemic or non-ischemic heart failure DM Age Contrast administration
Kaplan-Meier analyses	CKD up to 1 year	Logrank test comparing the group of patients with AKI between day 1 and day 14 versus the group of patients without AKI between day 1 and day 14	
Linear Mixed model	Whole-blood tacrolimus trough concentration[b]	Liver injury Other drugs increasing tacrolimus concentration Other drugs decreasing tacrolimus concentration[c]	

[a] A binary logistic model was selected, with a logit link and an exchangeable working matrix. The outcome variable was "AKI", with two categories: "normal" corresponding to no AKI and "abnormal" corresponding to the KDIGO classes 1, 2, and 3 together. The significance of the variables was tested by Wald chi-square tests.

[b] The tacrolimus whole-blood concentration was log transformed for fitting purposes.

[c] Fixed factors were: liver injury, included as categorical variables; day (linear and quadratic terms), the number of drugs possibly decreasing the tacrolimus concentration and the number of drugs possibly increasing the tacrolimus concentration included as continuous variables. The effect of drugs on the tacrolimus concentration was tested one day after their initiation. Observations were clustered within individuals (patients' identification number as subject variable) and the time (expressed in day) was the "repeated" variable, entered as a within-subject variable. A quadratic term for day had to be included in the model for fitting purposes as the relationship between whole-blood tacrolimus concentration and day was non-linear.

GEE=generalized estimating equation, AKI= acute kidney injury, SIRS= systemic inflammatory response syndrome, VAD= ventricular assist device oxygenation, ECMO=extracorporeal membrane, DM=diabetes mellitus, min=minute

Table S.3: Linear mixed model to test the variables influencing whole-blood tacrolimus concentrations

Fixed effect	Estimate [a, b]	P value[c]
Liver injury	-0.15	0.03
Drugs increasing tacrolimus	-0.008	0.86
Drugs decreasing tacrolimus	-0.09	0.07
Day	0.58	<0.0001
Day squared	-0.05	0.0002

[a] Estimate = regression coefficient in linear mixed model, with log(tacrolimus concentration) as outcome variable
[b] Estimate of intercept = 0.70
[c] P value of 0.005 is significant

CHAPTER 3

Extremely high variability of whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation

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ABSTRACT

Purpose

Oral tacrolimus is initiated peri-operatively in heart and lung transplantation patients. Tacrolimus-related toxicity may occur early potentially leading to morbidity and mortality. Few studies on oral tacrolimus pharmacokinetics early post-transplantation have been performed.

Methods

We conducted a pharmacokinetic study in 30 thoracic organ transplants on the intensive care of the University Medical Center Utrecht in the first week post-transplantation (NTR 3912/ EudraCT 2012-001909-24). Whole-blood tacrolimus 12-hour profiles were examined with High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS) and analysed with population pharmacokinetic modelling.

Results

The concentration-time profiles showed extreme variability. Concentrations at 12 hours were outside target range in 69% of the cases. A two-compartment model with first order absorption adequately described tacrolimus concentrations. Median apparent clearance was 19.6 L/hr (95%CI: 16.2 – 22.9) and apparent distribution volumes were, V1 231L (95%CI: 199 – 267) and V2 521L (95%CI: 441 – 634). Inter-occasion (dose-to-dose) variability far exceeded inter-individual variability with an estimated variability in relative bioavailability of 55% (95%CI: 48.5 – 64.4).

Conclusions

The extreme variability of tacrolimus pharmacokinetics early after thoracic organ transplantation is particularly determined by excessive variability in bioavailability making individualized dosing based on measured concentrations impossible. To bypass bioavailability, we suggest administering tacrolimus intravenously and aiming below the upper therapeutic range early post-transplantation.

INTRODUCTION

The immunosuppressant tacrolimus, which is a calcineurin inhibitor, is extensively used in thoracic organ transplantation patients. Tacrolimus is generally administered orally, because of the suspected hepatotoxicity and nephrotoxicity of the solvent polyoxyl-60-hydrogenated castor oil (HCO-60) used in intravenous formulations.[1] Unfortunately, tacrolimus has a narrow therapeutic range, making it difficult to attain therapeutic targets in clinically unstable patients, like patients early after heart and lung transplantation.[2,3] Yet, sufficient therapeutic exposure is important, because variable tacrolimus concentrations increase the risk of transplanted organ dysfunction and death.[4-6] A supra-therapeutic whole-blood tacrolimus trough concentration in the first week after thoracic transplantation has been related to acute kidney injury (AKI), which is, on its own, a risk factor for poor outcome.[2,3,7-9] Therefore, pharmacokinetic (PK) guided dosing is of vital importance and commonplace nowadays. The most important prerequisite for appropriate dosing based on measured drug concentrations is that exposure after dose adaptation can be adequately predicted based on measured exposure. This basically requires a relatively low dose-to-dose variability compared to inter-individual variability. As a consequence, knowledge of the complex tacrolimus pharmacokinetics in clinically unstable thoracic organ recipients is crucial. Few studies on the pharmacokinetics of oral tacrolimus have been performed early after transplantation and the few conducted included clinically stable patients. These studies showed variable pharmacokinetics in the different patient groups, e.g., 40% lower bioavailability in cystic fibrosis (CF) patients, a 40% higher clearance in Cytochrome P450 3A5 (CYP3A5) expressers, and decreased clearance of tacrolimus after several days of immunosuppressive therapy.[10-22]

Unfortunately, there are no studies that focus on the intra-patient (dose-to-dose) variation of oral tacrolimus pharmacokinetics directly post-transplantation. Thoracic organ recipients often experience prolonged surgery time, ischemia of the transplanted organ(s), reperfusion oedema, acute rejection, bleeding and massive blood transfusions or infection, which result in shock, inflammation and organ failure.[23] Due to all the above-mentioned changes, altered drug bioavailability, distribution, metabolism and clearance will influence tacrolimus pharmacokinetics early after transplantation.

We hypothesized that thoracic organ recipients show a large variability of whole-blood tacrolimus 12-hours post dose concentrations (C12h) during the first days after transplantation. We analysed oral tacrolimus pharmacokinetics in 10 heart and 20 lung transplants within the first six days after transplantation.

PATIENTS AND METHODS

Compliance with Ethical Standards

This was a descriptive and prospective study in 10 heart and 20 lung transplantation patients in the first 6 days after transplantation. Informed consent was obtained from all individual participants included in the study. The accredited review board for human studies of the University Medical Center Utrecht (UMC Utrecht) approved the study (NTR 3912/ EudraCT 2012-001909-24).

Patients

All consecutive thoracic organ recipients admitted to the intensive care unit (ICU) of the UMC Utrecht between June 2013 and March 2015 and meeting the inclusion criteria and not the exclusion criteria were studied. Inclusion criteria were patients older than 18 years, treated with tacrolimus and obtained informed consent. Exclusion criteria were patients who died within one day after admission, had known allergies for tacrolimus and macrolides or received total parenteral nutrition.

The immunosuppressive regimen

Tacrolimus, (Prograft® Astellas Pharma Europe) was dosed orally twice daily (bid). The initial dose was 0.1 mg/kg bid for the lung transplantation patients and 2 mg bid for the heart transplantation patients and was started at the day after transplantation. Dose adjustments were based on whole-blood tacrolimus concentrations 12 hours post dose (C12h) taken drug-drug interactions, gut dysmotility and liver injury into account. The therapeutic range was from 9 to 15 ng/ml for all patients. Steady state was not necessarily reached at the time of dose adjustments in accordance with common clinical practice.

The immunosuppressive regimen of heart recipients consisted further of corticosteroids [prednisolone 50 mg intravenously directly postoperative followed by 25 mg bid] and mycophenolate mofetil (MMF) [1000 mg orally bid]. For lung transplantation recipients, the immunosuppressive regimen consisted of basiliximab induction therapy on day 1 and 4 post-operative [20 mg per day intravenously], corticosteroids [prednisolon 25 mg four times per day (qid) intravenously and tapered off to 30 mg once daily orally after four days] and MMF [starting dose 1500 mg orally bid, tapered off to 1000 mg bid].

Tacrolimus analyses

Twelve hours whole-blood tacrolimus concentrations were determined daily from the transplantation date until 6 days after transplantation as long as they were admitted to the intensive care. Blood samples were collected daily during one dose interval. Analysis of tacrolimus was performed with High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS) (Thermo Fisher Scientific) with a lower limit of quantification

of 0.5 ng/ml and intraday imprecision of <5%. The HPLC-MS/MS method was adapted from and validated according to latest European Medicines Agency (EMA) guidelines.[24] The assay has a linear dynamic range of 1-50 ng/mL. Between-run and between-day imprecision (measured by coefficients of variation) were within 10% and bias was under 3%. Low, median and high controls were all within 15%. Furthermore, results over 5 years from an international inter-proficiency testing program for tacrolimus showed that all external quality controls were within 15%.

Covariates

Severity of illness was recorded as a Sequential Organ Failure Assessment (SOFA) score, which was recorded on the intensive care once a day (See also Table S2 in the Supporting Information for the description of the covariates). Shock was determined as mean arterial pressure of ≤60 mmHg or infusion of at least one inotropic or vasopressive agent. Systemic Inflammatory Response Syndrome (SIRS) was defined according to the definition of the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine Consensus Conference (SCCM).[25] Diarrhoea and ileus were documented as well as liver injury. Daily fluid balance and body weight were recorded. Drugs influencing tacrolimus pharmacokinetics were recorded: drugs that potentially increase tacrolimus blood concentrations by inhibition or substrate competition of the CYP3A4/5 enzymes and ATP-binding cassette sub-family B member 1 (ABCB1) transporter and drugs that potentially decrease tacrolimus blood concentrations by induction of CYP3A4/5 enzymes or ABCB1 transporter.[26,27]

Nephrotoxicity

Renal function was evaluated during the first 6 days as long as the patient was admitted to the intensive care and 1 month after transplantation in the out-patient department. Renal clearance was measured by the analyses of creatinine concentration in 24 hours urine. The occurrence of AKI and Augmented Renal Clearance (ARC), as well as the use of Continuous Veno-Venous Hemofiltration (CVVH) were monitored.

Population pharmacokinetic analysis

Non-linear mixed effects modelling (NONMEM) version 7.3.0 was used for modelling tacrolimus PK. The Piraña software program version 2.9.4 was used as an interface for NONMEM and R for Windows version 3.3.1 was used to analyse the results.

Area under the concentration-time curve (AUC) was computed using the following equation:

$$AUC = F * AMT / CL$$

were F is the relative bioavailability, AMT is the amount of drug in µg and CL is the clearance in L/hr. AUC is displayed in µg•hr/L.

Population pharmacokinetic modelling

A two compartment linear model with first order oral absorption was used. On some occasions, the first observation within 30 minutes after dosing was the maximum concentration in the dose interval, indicating extremely rapid absorption on these occasions. To reduce absorption model complexity, dosing on these occasions was treated as zero order oral absorption with duration of absorption equal to the time interval between dosing and the first observation (Figure 1). The structural model included the following parameters: CL/F (apparent clearance), Q/F (inter-compartmental clearance), V1/F (volume of distribution of central compartment), V2/F (volume of distribution of peripheral compartment) and ka (dissociation constant).

Inter-individual variability and inter-occasion variability were described assuming a log normal distribution with the following equation:

$$P_{kjm} = \theta_k * e^{(\eta_{kj} + \kappa_{km})}$$

in which P_{kjm} is the estimate for parameter k for the j^{th} individual at occasion m, θ_k is the population value for the k^{th} PK parameter, η_{kj} represents the inter-individual variability which is assumed to have a normal distribution with mean 0 and standard deviation ω_k and κ_{km} represents the inter-occasion variability (dose-to-dose variability) which is assumed to have a mean 0 and standard deviation of π_k. Although the absolute bioavailability was not identifiable, variability in the relative bioavailability was estimated similarly in which θ_k was fixed to 1.

The residual error was assumed to be proportional to the predicted concentration:

$$C_{ij} = Cpred_{ij} \ (1 + e_{ij})$$

in which C_{ij} is the i^{th} observation for the j^{th} individual, $Cpred_{ij}$ is the tacrolimus concentration predicted by the model, and e_{ij} is the difference between C_{ij} and $Cpred_{ij}$.

The modelling process was performed using the stochastic approximation expectation maximisation (SAEM) estimation method with interaction. The likelihood was subsequently established using the Monte Carlo importance Sampling EM assisted by mode a posteriori estimation method (IMPMAP). The parameter precision was estimated using the SIR procedure (Sampling Importance Resampling).[28] The values of concentrations below the lower limit of quantification (LLOQ) were discarded (3.9%; 46 values out of 1180). Model diagnostics were performed by visual checks of standard diagnostic plots i.e. 'goodness of fit' plots.

Statistical analyses

Variables are presented as median (with the 1st and 3rd quartiles (Q1;Q3)), range or number (proportion) where appropriate.

RESULTS

Patients' characteristics

Thirty patients were included (See Table 1 for the characteristics, Table S1 and S2 in the Supplementary materials for more details and the definitions of covariates). No patient died within the timeframe of the study. No organ rejection was observed in a one year time period. Twenty six patients were supported with cardiopulmonary bypass during surgery. Furthermore, 9 patients (7 lung transplants) were supported with extended extracorporeal membrane oxygenation (ECMO) for a median duration of 4 days (Q1;Q3 2;6). The median SOFA score was 7 (Q1;Q3 4;12). The frequency of shock was 93% (28 out of 30) and of SIRS was 100% (30 out of 30). Gut dysmotility was observed in 97% of the patients (29 out of 30), with ileus in 90% of the patients (27 out of 30) and diarrhoea in 60% of the patients (18 out of 30), respectively. Three of 10 cystic fibrosis patients showed diarrhoea from day 2 and 3 on. All CF-patients had exocrine pancreas insufficiency that was substituted when enteral feeding was started. Potential drug-drug interactions were observed in all patients. The number of drugs potentially increasing tacrolimus concentrations ranged from 0 to 6. The number of drugs potentially decreasing tacrolimus concentrations ranged from 0 to 2. Median baseline creatinine was 66 µmol/L (Q1;Q3 53;98). Median baseline creatinine clearance was 85 ml/(min 1.73m^2) (Q1;Q3 73;116). Augmented renal clearance was seen in 7 out of 30 patients (23%) with a median duration of 1 day (Q1;Q3 1;2). AKI was observed in 47% of patients (14 out of 30). The frequency of patients with AKI showing recovery of creatinine clearance at one month was 64% (9 out of 14).

Pharmacokinetic profiles

The total number of C12h whole-blood profiles was 119 with a median of 5 profiles per patient (range 1-6). Characteristic features of tacrolimus whole-blood concentrations together with dosages are illustrated in Fig. 2.

Observed pharmacokinetics

The median of C12h was 9.5 ng/ml (range 0.5-38.7). The majority of whole-blood concentrations, 69.4%, were out of the target range (9-15 ng/ml). Sub-therapeutic concentrations were observed in 51% and supra-therapeutic concentrations were observed in 19% of the patients. The median of the maximum concentration (Cmax) was 18.5 ng/ml (range 2.1-74.7). The median of the time to maximum concentration (Tmax) was 1.6 hours (range 0.4-8).

Tacrolimus pharmacokinetic model

A 2-compartmental model with first-order absorption best fitted the data (schematic illustration of the pharmacokinetic model; See Fig. 1). The parameter precision was acceptable for all relevant parameters, as represented in the low 95% confidence interval

(CI) of the parameter estimates, indicating that all parameters could be reliably estimated (See Table 2). The goodness-of-fit plots showed no systematic bias (Fig.3). The observed versus individual predicted whole-blood tacrolimus concentrations were well centered around the identity line (upper right panel).

IOV in relative bioavailability was estimated at 55.0% (95% CI 48.5-64.4). The IOV of apparent clearance was 29.5% (95% CI 20.7-38.9) and the IOV of apparent distribution volume was 35.1% (95% CI 27.0-48.0). Only IIV on CL/F could be estimated, estimation of all other IIV elements lead to estimates close to 0 and/or unsuccessful runs, most likely due to the fact that variability proved to be dominated by the corresponding IOV. Given that the IOV exceeded the IIV and the variability of the time-independent covariates, such as sex, age and genotype, as well as time-dependent covariates, such as gut dysmotility, shock and corticosteroid use, were not expected to relevantly explain the large variability in bioavailability, no covariate relationships were tested. The median AUC was 151.2 ug•hr/L (range 31.2-2525). The median terminal T1/2 was 9.4 hours (range 6.0-31.4).

DISCUSSION

In this pharmacokinetic analysis in 30 thoracic organ recipients, we showed that the bioavailability of tacrolimus displays extreme inter-occasion variability in the first week after transplantation. Other PK-parameters also showed a large inter-occasion variability. Such huge variabilities hamper any prediction on next-dose tacrolimus concentrations based upon previous concentrations. In other words, PK-guided dosing is of limited added value in clinically unstable patients. Nevertheless, daily therapeutic drug monitoring remains worthwhile for the prevention of toxicity, since subsequent doses will be omitted in case of high concentrations. In search for optimisation of tacrolimus therapy, it may be useful to circumvent bioavailability by intravenous administration during clinical instability.

In general, tacrolimus is orally dosed after thoracic organ transplantation even when gut dysmotility ensues, because tacrolimus has known nephrotoxicity and intravenous administration may cause additional nephrotoxicity due to the use of the solvent HCO-60.[1,29] When the oral route is not feasible, sublingual administration has been recommended over intravenous administration by some authors, though this is not sufficiently substantiated yet and therefore not common practice.[30]

Currently, only very few studies have analysed oral tacrolimus pharmacokinetics in clinically unstable transplantation patients.[13,20] Unfortunately, none of these studies reported severity of illness, shock or organ failure hampering comparison of the outcomes. Our cohort of thoracic organ transplant patients showed high SOFA scores and many patients had shock in the first week corresponding with major physiological instability. We observed an extremely high inter-occasion, i.e. between doses, variability in bioavailability independent of the dose indicating unpredictable uptake of tacrolimus, which is probably related to this clinical instability. This is in contrast to kidney transplant recipients early after transplantation, in whom bioavailability showed to be dose dependent and with a much smaller inter-occasion variability of only 23-28%.[31,32] Renal transplant patients are often clinically stable and many do not need ICU support in the days after transplantation. Tacrolimus target concentrations are frequently reached in renal recipients within 3 days, which is different from our cohort of heart and lung recipients.[33] Despite the large differences in absorption variability, the observed clearance as well as the variability in clearance was in accordance to earlier reports.[12,13,22,32,34] The distribution volumes in our cohort were only slightly higher with equivalent inter-occasion variability compared to another study conducted one week after lung transplantation.[34]

There are several explanations for the huge variability in tacrolimus pharmacokinetics in our study population, which are all associated with clinical instability. First, we observed

a high incidence of gut dysmotility in all of our patients with and without CF. Ileus delays tacrolimus transport to the duodenum where tacrolimus is mainly absorbed.[35] On the other hand, in some occasions we observed very rapid absorption. This prompt absorption of tacrolimus with a sudden peak in blood concentration may be due to a short transit time to the duodenum, which may be caused by a hyperdynamic state and (e.g. mycophenolate-associated) diarrhoea.[35-37] Increased gut perfusion may increase absorption by augmented enteric drug extraction due to a larger difference in concentration between intracellular and blood locations. Diarrhoea may increase absorption by shortening transit time and maximising luminal degradation and dissolution of tacrolimus.[38] Furthermore, diarrhoea, shock and inflammation may result in decreased ABCB1 and CYP3A4/5 expression.[39-41] These phenomena could all increase tacrolimus transport and also shorten transit time over the gut wall into the blood compartment and give rise to sudden and higher peak concentrations.[35]

Second, the distribution of tacrolimus may fluctuate due to changes in diffusion into erythrocytes and lower binding to (lipo)proteins, because of red blood cells transfusion, bleeding, dilution caused by fluid resuscitation, capillary leakage and the use of an extracorporeal circuit.[42] Anaemia and hypoalbuminaemia were observed in all the patients (See Supplementary materials Table S1), which may lead to higher unbound tacrolimus concentrations with lower whole-blood levels at equivalent doses.[10,13,43] The application of an extracorporeal circuit, such as the cardiopulmonary bypass, ECMO or continuous renal replacement therapy, may significantly alter the disposition of tacrolimus because of adsorption to the bypass equipment, acute haemodilution, hypoalbuminaemia and hypothermia.[44,45] Third, drug-drug interactions influence tacrolimus bioavailability. We observed drug-drug interactions in all patients. For example, corticosteroids induce CYP3A enzymes and ABCB1. Therefore, tapering of corticosteroids decreases the activity of both and may highly increase tacrolimus bioavailability, especially in CYP3A5 non-expressers.[39,46,47] Last, changes in clearance of tacrolimus might contribute to fluctuations in whole-blood concentrations. Shock and inflammation highly influence organ function and subsequently the metabolisation rate of the liver.[26,48,49]

All these variations in the covariates of tacrolimus pharmacokinetics give rise to the high fluctuations in tacrolimus whole-blood concentrations and hence, an extremely high dose-to-dose variability persisting in the first week after thoracic organ transplantation.[27] The dose-to-dose variability in bioavailability far exceeded other sources of variability. No covariate relationships were identified as no covariate, time-dependent and time-independent, were sufficient to explain the large variability in bioavailability. Yet, fluctuations in tacrolimus concentrations are a known significant risk factor for rejection and toxicity, and deteriorating the outcome in heart and lung transplants.[2,3,6-9]

Future perspectives

Circumventing the extreme variability in bioavailability by applying tacrolimus intravenously may improve tacrolimus dosing despite the higher costs and risk of additional nephrotoxicity of the solvent HCO-60.[1,27,50] It will result in a decrease in fluctuations of tacrolimus concentrations with a subsequent decrease in the incidence of supra-therapeutic C12h. Therapeutic drug monitoring of C12h on a daily base is mandatory in clinically unstable patients to improve dosing.[3] We, however, showed that due to the huge inter-occasion variability targeting the therapeutic range is impossible. The therapeutic range of 10 to 15 ng/ml, which is often used, is difficult to attain in clinically unstable patients. Our findings support a more cautious approach. Targeting below the upper level of the therapeutic range (e.g., 15 ng/ml) at day 5 after transplantation and not increasing the dose when low concentrations exist until clinical stability is established, could help to prevent toxicity. Tacrolimus should be temporarily stopped when supra-therapeutic concentrations arise. However, even lower therapeutic ranges might be advocated as concentrations of >10 ng/ml have already been associated with AKI.[51] It has even been suggested to aim at an initial concentration of less than 4 ng/ml, though early rejection may then arise jeopardizing the outcome.[52] To maintain adequate immunosuppression, corticosteroid dosage should not be tapered during clinical instability. In addition, accurate dosing of cell cycle inhibitors (e.g., MMF) and interleukin receptor blockers (e.g., basiliximab) is advocated to diminish the risk of rejection.[52-56] In heart transplants, initiation of tacrolimus may even be postponed for one week when an interleukin receptor blocker is administered.[57] However, for lung transplantation patients postponing tacrolimus may have very negative consequences due to acute rejection.[58]

Limitations and strengths

One of the strong features of this study is the use of full 12-hour pharmacokinetic profiles on a daily base and the use of High Performance Liquid Chromatography with tandem Mass Spectrometry (HPLCMS/MS) for analyses of the blood concentrations. However, some limitations need to be addressed. Overall, the sample size was relatively small albeit that these patients were sampled all days during ICU admission. The aim of this study was to examine tacrolimus pharmacokinetics in clinically unstable patients. Although there is abundant knowledge of tacrolimus pharmacokinetics in other populations, this information is lacking for this extremely vulnerable population. Tacrolimus is initiated in the clinically unstable phase, which makes it necessary to enhance knowledge of tacrolimus pharmacokinetics in the clinically unstable patient. For these patients, it is of vital importance to dose tacrolimus correctly. These data are a good reflection of clinical practice, since large variations in whole-blood concentrations are frequently observed in the early phase after heart and lung transplantation.

CONCLUSIONS

Variability in tacrolimus whole-blood concentrations was excessive in clinically unstable thoracic organ transplantation patients. Tacrolimus pharmacokinetics showed an extreme high inter-occasion and intra-individual variability in pharmacokinetics. Particularly, the inter-occasion variability in relative bioavailability is excessive and is superimposed upon the variability in apparent distribution and apparent clearance. This makes PK-guided dosing to a pre-set narrow therapeutic range an impossible task in these patients. We suggest administering tacrolimus intravenously and aiming below the upper therapeutic range in the first days after transplantation.

REFERENCES

1. Worbs S, Köhler K, Pauly D, et al. Ricinus communis intoxications in human and veterinary medicine-a summary of real cases. *Toxins (Basel)*. 2011;3(10):1332-1372. doi:10.3390/toxins3101332.
2. Sikma MA, Hunault CC, van de Graaf EA, et al. High tacrolimus blood concentrations early after lung transplantation and the risk of kidney injury. *Eur J Clin Pharmacol*. 2017;73(5):573-580. doi:10.1007/s00228-017-2204-8.
3. Sikma MA, Hunault CC, Kirkels JH, Verhaar MC, Kesecioglu J, de Lange DW. Association of Whole Blood Tacrolimus Concentrations with Kidney Injury in Heart Transplantation Patients. *Eur J Drug Metabol Pharmacokinet*. 2018;43(3):311-320. doi:10.1007/s13318-017-0453-7.
4. Gallagher HM, Sarwar G, Tse T, et al. Erratic tacrolimus exposure, assessed using the standard deviation of trough blood levels, predicts chronic lung allograft dysfunction and survival. *J Heart Lung Transplant*. 2015;34(11):1442-1448. doi:10.1016/j.healun.2015.05.028.
5. Rayar M, Tron C, Jézéquel C, et al. High Intrapatient Variability of Tacrolimus Exposure in the Early Period After Liver Transplantation Is Associated With Poorer Outcomes. *Transplantation*. 2018;102(3):e108-e114. doi:10.1097/TP.0000000000002052.
6. Gueta I, Markovits N, Yarden-Bilavsky H, et al. High tacrolimus trough level variability is associated with rejections after heart transplant. *Am J Transplant*. 2018;18(10):2571-2578. doi:10.1111/ajt.15016.
7. Baran DA, Galin ID, Sandler D, et al. Predictors of early renal insufficiency in cardiac transplant recipients initiated on tacrolimus. *TPS*. 2002;34(5):1872-1873. doi: 10.1016/s0041-1345(02)03104-4
8. Wehbe E, Duncan AE, Dar G, Budev M, Stephany B. recovery from AKI and short- and long-term outcomes after lung transplatation. *Clinical Journal of the American Society of Nephrology*. 2013;8(1):19-25. doi:10.2215/CJN.04800512.
9. Tjahjono R, Connellan M, Granger E. Predictors of Acute Kidney Injury in Cardiac Transplantation. *Transplantation Proceedings*. 2016;48(1):167-172. doi:10.1016/j.transproceed.2015.12.006.
10. Størset E, Holford N, Midtvedt K, Bremer S, Bergan S, Åsberg A. Importance of hematocrit for a tacrolimus target concentration strategy. *Eur J Clin Pharmacol*. 2014;70(1):65-77. doi:10.1007/s00228-013-1584-7.
11. Baran DA, Galin ID, Sandler D, et al. A novel tacrolimus dosing strategy in cardiac transplantation: drug levels, renal function, and biopsy results. *TPS*. 2002;34(5):1834-1835. doi: 10.1016/s0041-1345(02)03096-8
12. Han N, Ha S, Yun H-Y, et al. Population pharmacokinetic-pharmacogenetic model of tacrolimus in the early period after kidney transplantation. *Basic Clin Pharmacol Toxicol*. 2014;114(5):400-406. doi:10.1111/bcpt.12176.
13. Staatz C. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. *Clinical Pharmacology & Therapeutics*. 2002;72(6):660-669. doi:10.1067/mcp.2002.129304.

14. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Factors affecting variability in distribution of tacrolimus in liver transplant recipients. *British Journal of Clinical Pharmacology*. 2004;57(3):298-309. doi: 10.1046/j.1365-2125.2003.02008.x
15. Li L, Li C-J, Zheng L, et al. Tacrolimus dosing in Chinese renal transplant recipients: a population-based pharmacogenetics study. *Eur J Clin Pharmacol*. 2011;67(8):787-795. doi:10.1007/s00228-011-1010-y.
16. Antignac M, Barrou B, Farinotti R, Lechat P, Urien S. Population pharmacokinetics and bioavailability of tacrolimus in kidney transplant patients. *British Journal of Clinical Pharmacology*. 2007;64(6):750-757. doi:10.1111/j.1365-2125.2007.02895.x.
17. Wallemacq DPE, Verbeeck RK. Comparative Clinical Pharmacokinetics of Tacrolimus in Paediatric and Adult Patients. *Clinical Pharmacokinetics*. 2001;40(4):283-295. doi:10.2165/00003088-200140040-00004.
18. Aidong W, Zhenjie C, Tong L, et al. Therapeutic drug monitoring of tacrolimus in early stage after heart transplantation. *TPS*. 2004;36(8):2388-2389. doi:10.1016/j.transproceed.2004.06.037.
19. Saint-Marcoux F, Knoop C, Debord J, et al. Pharmacokinetic Study of Tacrolimus in Cystic Fibrosis and Non-Cystic Fibrosis Lung Transplant Patients and Design of Bayesian Estimators Using Limited Sampling Strategies. *Clinical Pharmacokinetics*. 2005;44(12):1317-1328. doi:10.2165/00003088-200544120-00010.
20. Molinaro M, Regazzi MB, Pasquino S, et al. Pharmacokinetics of tacrolimus during the early phase after heart transplantation. *TPS*. 2001;33(3):2386-2389. doi: 10.1016/s0041-1345(01)02032-2
21. Marquet P, Albano L, Woillard J-B, et al. Comparative clinical trial of the variability factors of the exposure indices used for the drug monitoring of two tacrolimus formulations in kidney transplant recipients. *Pharmacol Res*. 2018;129:84-94. doi:10.1016/j.phrs.2017.12.005.
22. Uno T, Wada K, Matsuda S, et al. Impact of the CYP3A5*1 Allele on the Pharmacokinetics of Tacrolimus in Japanese Heart Transplant Patients. *Eur J Drug Metabol Pharmacokinet*. 2018;43(6):665-673. doi:10.1007/s13318-018-0478-6.
23. Ascenzi P, Bocedi A, Notari S, Fanali G, Fesce R, Fasano M. Allosteric modulation of drug binding to human serum albumin. *MRMC*. 2006;6(4):483-489. doi: 10.2174/138955706776361448
24. Marinova M, Artusi C, Brugnolo L, Antonelli G, Zaninotto M, Plebani M. Immunosuppressant therapeutic drug monitoring by LC-MS/MS: workflow optimization through automated processing of whole blood samples. *Clinical Biochemistry*. 2013;46(16-17):1723-1727. doi:10.1016/j.clinbiochem.2013.08.013.
25. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. In: Vol 41. 2013:580-637. doi:10.1097/CCM.0b013e31827e83af.
26. Christians U, Jacobsen W, Benet LZ, Lampen A. Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clinical Pharmacokinetics*. 2002;41(11):813-851. doi:10.2165/00003088-200241110-00003.

27. Sikma MA, van Maarseveen EM, van de Graaf EA, et al. Pharmacokinetics and Toxicity of Tacrolimus Early After Heart and Lung Transplantation. *Am J Transplant*. 2015;15(9):2301-2313. doi:10.1111/ajt.13309.
28. Dosne A-G, Bergstrand M, Harling K, Karlsson MO. Improving the estimation of parameter uncertainty distributions in nonlinear mixed effects models using sampling importance resampling. *J Pharmacokinet Pharmacodyn*. 2016;43(6):583-596. doi:10.1007/s10928-016-9487-8.
29. Collin C, Boussaud V, Lefeuvre S, et al. Sublingual tacrolimus as an alternative to intravenous route in patients with thoracic transplant: a retrospective study. *Transplantation Proceedings*. 2010;42(10):4331-4337. doi:10.1016/j.transproceed.2010.09.126.
30. Doligalski CT, Liu EC, Sammons CM, Silverman A, Logan AT. Sublingual administration of tacrolimus: current trends and available evidence. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*. 2014;34(11):1209-1219. doi:10.1002/phar.1492.
31. Ekberg H, Mamelok RD, Pearson TC, Vincenti F, Tedesco-Silva H, Daloze P. The challenge of achieving target drug concentrations in clinical trials: experience from the Symphony study. *Transplantation*. 2009;87(9):1360-1366. doi:10.1097/TP.0b013e3181a23cb2.
32. Størset E, Holford N, Hennig S, et al. Improved prediction of tacrolimus concentrations early after kidney transplantation using theory-based pharmacokinetic modelling. *British Journal of Clinical Pharmacology*. 2014;78(3):509-523. doi: 10.1111/bcp.12361
33. Størset E, Åsberg A, Skauby M, et al. Improved Tacrolimus Target Concentration Achievement Using Computerized Dosing in Renal Transplant Recipients-A Prospective, Randomized Study. *Transplantation*. April 2015. doi:10.1097/TP.0000000000000708.
34. Monchaud C, de Winter BC, Knoop C, et al. Population pharmacokinetic modelling and design of a Bayesian estimator for therapeutic drug monitoring of tacrolimus in lung transplantation. *Clinical Pharmacokinetics*. 2012;51(3):175-186. doi:10.2165/11594760-000000000-00000.
35. Sikma MA, van Maarseveen EM, Donker DW, Meulenbelt J. Letter to the editor: "Immunosuppressive drug therapy - biopharmaceutical challenges and remedies". *Expert Opin Drug Deliv*. 2015;12(12):1955-1957. doi:10.1517/17425247.2015.1106687.
36. Hesselink DA, Bouamar R, Elens L, Van Schaik RHN, Van Gelder T. The role of pharmacogenetics in the disposition of and response to tacrolimus in solid organ transplantation. *Clinical Pharmacokinetics*. 2014;53(2):123-139. doi:10.1007/s40262-013-0120-3.
37. Tang JT, Andrews LM, Van Gelder T, et al. Pharmacogenetic aspects of the use of tacrolimus in renal transplantation: recent developments and ethnic considerations. *Expert Opin Drug Metab Toxicol*. 2016;12(5):555-565. doi:10.1517/17425255.2016.1170808.
38. Mittal N, Thompson JF, Kato T, Tzakis AG. Tacrolimus and diarrhea: pathogenesis of altered metabolism. *Pediatric Transplantation*. 2001;5(2):75-79. doi: 10.1034/j.1399-3046.2001.005002075.x
39. Kuypers DR, De Jonge H, Naesens M, Vanrenterghem Y. Effects of CYP3A5 and MDR1 single nucleotide polymorphisms on drug interactions between tacrolimus and fluconazole in renal allograft recipients. *Pharmacogenetics and Genomics*. 2008;18(10):861-868. doi:10.1097/FPC.0b013e328307c26e.

40. Lemahieu W, Maes B, Verbeke K, Rutgeerts P, Geboes K, Vanrenterghem Y. Cytochrome P450 3A4 and P-glycoprotein Activity and Assimilation of Tacrolimus in Transplant Patients with Persistent Diarrhea. *Am J Transplant*. 2005;5(6):1383-1391. doi:10.1111/j.1600-6143.2005.00844.x.
41. Nakamura A, Amada N, Haga I, Tokodai K, Kashiwadate T. Effects of elevated tacrolimus trough levels in association with infectious enteritis on graft function in renal transplant recipients. *Transplantation Proceedings*. 2014;46(2):592-594. doi:10.1016/j.transproceed.2013.11.040.
42. Zahir H, Nand RA, Brown KF, Tattam BN. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. *journal of pharmacological and toxicological methods*. 2001;46:27-35. doi: 10.1016/s1056-8719(02)00158-2
43. De Jonge H, Vanhove T, de Loor H, Verbeke K, Kuypers DRJ. Progressive decline in tacrolimus clearance after renal transplantation is partially explained by decreasing CYP3A4 activity and increasing haematocrit. *British Journal of Clinical Pharmacology*. 2015;80(3):548-559. doi:10.1111/bcp.12703.
44. Shekar K, Roberts JA, Welch S, et al. ASAP ECMO: Antibiotic, Sedative and Analgesic Pharmacokinetics during Extracorporeal Membrane Oxygenation: a multi-centre study to optimise drug therapy during ECMO. *BMC Anesthesiol*. 2012;12(1):29. doi:10.1186/1471-2253-12-29.
45. Pea F, Pavan F, Furlanut M. Clinical relevance of pharmacokinetics and pharmacodynamics in cardiac critical care patients. *Clinical Pharmacokinetics*. 2008;47(7):449-462. doi:10.2165/00003088-200847070-00002.
46. Christians U, Jacobsen W, Benet LZ. Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clinical Pharmacokinetics*. 2002;41(11):813-851. doi: 10.2165/00003088-200241110-00003
47. Staatz CE, Størset E, Bergmann TK, Hennig S, Holford N. Tacrolimus pharmacokinetics after kidney transplantation--Influence of changes in haematocrit and steroid dose. *British Journal of Clinical Pharmacology*. 2015;80(6):1475-1476. doi:10.1111/bcp.12729.
48. Tata PNV, Subbotina N, Burckart GJ, et al. Species-dependent hepatic metabolism of immunosuppressive agent tacrolimus (FK-506). *Xenobiotica*. 2009;39(10):757-765. doi:10.1080/00498250903114478.
49. Lunde I, Bremer S, Midtvedt K, et al. The influence of CYP3A, PPARA, and POR genetic variants on the pharmacokinetics of tacrolimus and cyclosporine in renal transplant recipients. *Eur J Clin Pharmacol*. 2014;70(6):685-693. doi:10.1007/s00228-014-1656-3.
50. Snell GI, Ivulich S, Mitchell L, Westall GP, Levvey BJ. Evolution to twice daily bolus intravenous tacrolimus: optimizing efficacy and safety of calcineurin inhibitor delivery early post lung transplant. *Ann Transplant*. 2013;18:399-407. doi:10.12659/AOT.883993.
51. Atalan HK, Gucyetmez B, Aslan S, Yazar S, Polat KY. Postoperative acute kidney injury in living donor liver transplantation recipients. *Int J Artif Organs*. 2017;41(1):37-42. doi:10.5301/ijao.5000638.

52. Knotek M, Mihovilović K, Galešić Ljubanović D, Maksimović B. Tacrolimus or mycophenolate in kidney transplantation-less, or more? *Am J Transplant*. 2014;14(5):1220-1220. doi:10.1111/ajt.12676.
53. Zhang H, Fu Q, Zheng Y, et al. Effect of Early Immunosuppression Therapy on De Novo Anti-Human-Leukocyte-Antigen Antibody After Kidney Transplantation. *Transplantation Proceedings*. 2018;50(8):2382-2387. doi:10.1016/j.transproceed.2018.03.043.
54. Zhang H, Liu L, Li J, et al. The efficacy and safety of intensified enteric-coated mycophenolate sodium with low exposure of calcineurin inhibitors in Chinese de novo kidney transplant recipients: a prospective study. *Int J Clin Pract*. 2016;70 Suppl 185(5 Pt 2):22-30. doi:10.1111/ijcp.12813.
55. Baran DA, Galin ID, Zucker MJ, et al. Can initial tacrolimus trough levels be predicted from clinical variables? *Transplantation Proceedings*. 2004;36(9):2816-2818. doi:10.1016/j.transproceed.2004.09.037.
56. Ekberg H, Bernasconi C, Tedesco-Silva H, et al. Calcineurin inhibitor minimization in the Symphony study: observational results 3 years after transplantation. *Am J Transplant*. 2009;9(8):1876-1885. doi:10.1111/j.1600-6143.2009.02726.x.
57. Kittipibul V, Tantrachoti P, Ongcharit P, et al. Low-dose basiliximab induction therapy in heart transplantation. *Clinical Transplantation*. 2017;31(12):e13132. doi:10.1111/ctr.13132.
58. Yusen RD, Edwards LB, Dipchand AI, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirty-third Adult Lung and Heart-Lung Transplant Report-2016; Focus Theme: Primary Diagnostic Indications for Transplant. *J Heart Lung Transplant*. 2016;35(10):1170-1184. doi:10.1016/j.healun.2016.09.001.

TABLES

Table 1. Patients' characteristics

	N=30 (%)	Median (Q1;Q3)
Male	15 (50%)	--
Age (yr)	--	43 (34;60)
Bodyweight (kg)		73.5 (61;86)
Length (cm)		173.5 (169;176)
Reason for transplantation		
Heart **(N=10)**		
Ischemic CMP	5 (16.7%)	--
Non-ischemic CMP	5 (16.7%)	--
Lung **(N=20)**		
Cystic Fibrosis	10 (33.3%)	--
COPD	4 (13.3%)	--
ILD	6 (20%)	--
Double lung transplantation	18 (90%)	--
Parameters		
SOFA score		7 (4;12)
SIRS at least once between day 1-6	30 (100%)	
SIRS duration (days)		4.5 (3;6)
Shock at least once between day 1-6	28 (93,3%)	
Shock duration (days)		2 (1;3)
Liver dysfunction at least once between day 1-6	14 (47%)	
Gut dysmotility frequency	29 (96.7%)	
Ileus at least once between day 1-6	27 (90%)	
Ileus duration		2 (2; 3)
Diarrhea at least once between day 1-6	18 (60%)	
Diarrhea duration		1 (0;2)
Fluid balance (L/day)		
Day 1		1.5 (0.2;3)

Table 1. Patients' characteristics

	N=30 (%)	Median (Q1;Q3)
Day 2		1.2 (0.4;2.2)
Day 3		0.5 (-0.2;1.5)
Day 4		0.4 (-0.8;0.9)
Day 5		-0.3 (-1.0;1.1)
Day 6		-0.2 (-1.4;0.0)
Change in bodyweight (kg)		
Day 1 - baseline		1.5 (0;6)
Day 2 - baseline		6.5 (0.8;11)
Day 3 - baseline		8 (0;14)
Day 4 - baseline		10 (0.5;14.5)
Day 5 - baseline		7 (-1;14.5)
Day 6 - baseline		5 (-0.8;18.5)
Postoperative ECMO frequency	8 (27%)	
Postoperative ECMO duration (days)		4 (2;6)
Tacrolimus		
Tacrolimus C12h (ng/ml) (min-max)		9.5 (0.5-38.7)
Cmax (ng/ml)		18.5 (2.1-74.7)
Tmax (hr)		1.6 (0.4-8.0)
AUC (ug.hr/L)		151.2 (31.2-2525)
T1/2 (hr)		9.4 (6.0-31.4)
Patients with at least one drug increasing tacrolimus between day 1-6	30 (100%)	
Number of drugs increasing tacrolimus concentration (min-max)		0-6
Patients with at least one drug decreasing tacrolimus between day 1-6	30 (100%)	
Number of drugs decreasing tacrolimus concentration (min-max)		0-2

Table 1. Patients' characteristics

	N=30 (%)	Median (Q1;Q3)
Renal function		
Baseline creatinine (µmol/L)		66 (53;98)
Baseline creatinine clearance (ml/min/1.73 m^2)		85 (73;116)
ARC at least once between day 1-6	7 (23.3%)	
ARC duration (days)(min-max)		1 (1-6)
AKI at least once between day 1-6	14 (47%)	
AKI recovery at 1 month	9[a] (64%)	

[a] of 14 patients

SOFA=Sepsis-related organ failure assessment, SIRS=systemic inflammatory response syndrome, ECMO= extracorporeal membrane oxygenator, C12h=concentration at 12 hours after administration, Cmax=maximum C12h, Tmax= time to maximum concentration, AUC= area-under-the-concentration-time curve,T1/2= terminal half-life time, ARC= Augmented renal clearance, AKI= acute kidney injury

Table 2. Final population pharmacokinetic parameters with 95% confidence interval based on SIR

Pharmacokinetic parameter	Estimate (95% CI)	Inter-patient variability % (95% CI)	Inter-occasion variability % (95%CI)
CL/F (L/h)	19.6 (16.2 – 22.9)	34.6 (24.2 – 48.6)	29.5 (20.7 – 38.9)
V_1/F (L)	231 (199 – 267)	n.e.	35.1 (27.0 – 48.0)
k_a (1/h)	0.579 (0.456 – 0.778)	n.e.	98.3 (81.1 – 121)
Q/F (L/h)	58.2 (49.7 – 69.3)	n.e.	n.e.
V_2/F (L)	521 (441 – 634)	n.e.	n.e.
F	Fixed to 1	n.e.	55.0 (48.5 – 64.4)
Residual unexplained variability (%)	14.0 (13.3 – 14.6)		

SIR=Sampling Importance Resampling, n.e.= not estimated, CI=confidence interval, ka=absorption rate constant

FIGURES

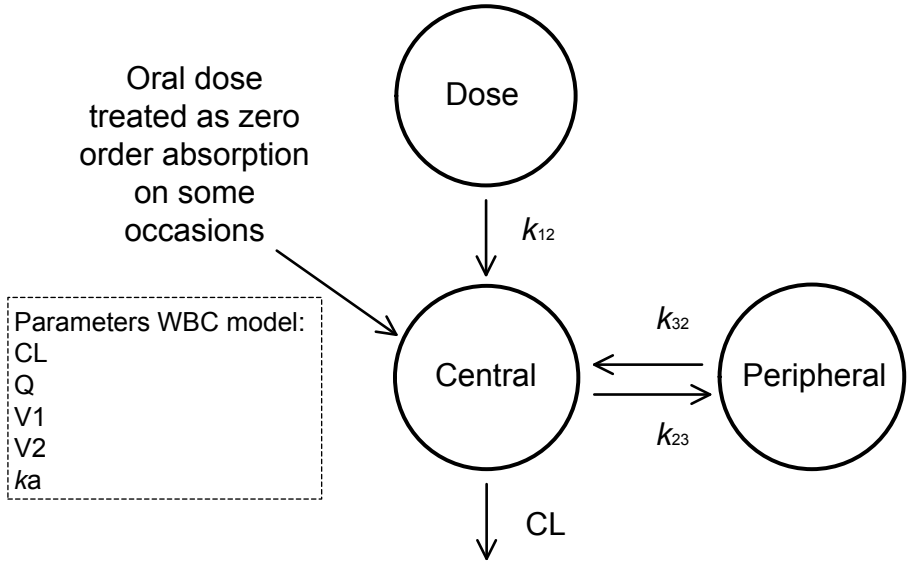

Fig. 1 Schematic representation of the population pharmacokinetic whole-blood concentration (WBC) model for tacrolimus. The absorption phase is described with a rate constant (k12). The oral dose was treated as IV administration on some occasions. The central compartment, with volume V_1, is in rapid equilibrium with the peripheral compartment, which has a volume V_2. Drug transfer between this peripheral compartment and the central compartment is described with the inter-compartmental clearance parameter Q with the following equations: $k23 = Q / V_1$ and $k32 = Q / V_2$. The volumes of distribution for tacrolimus can be estimated from this model. CL is the tacrolimus clearance

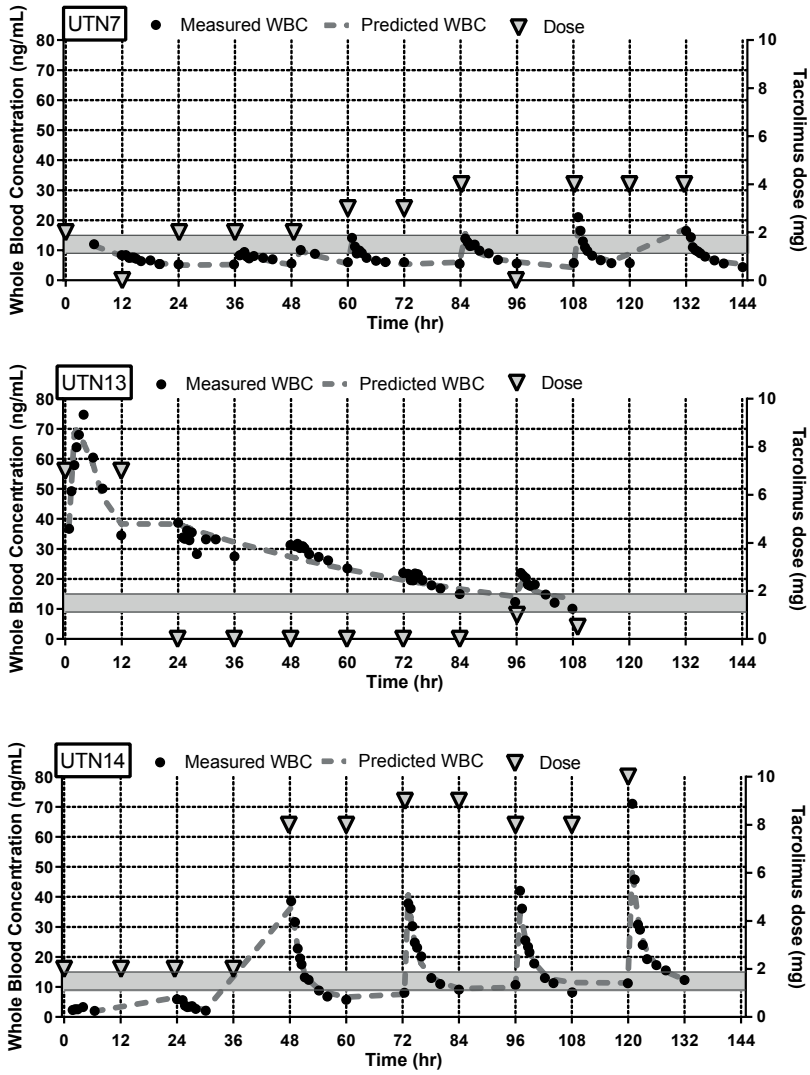

Fig. 2 Three illustrative individual whole-blood concentration (WBC) profiles with the administrated tacrolimus dose and including the individual predicted lines. The therapeutic range for tacrolimus C12h is indicated with the grey bars. UTN7: This patient showed C12h below the therapeutic range despite increasing dosages. The patient was a heart transplant with shock and circulatory support for 4 days. Gut dysmotility was observed for 3 days. UTN13: In this patient absorption was rapid and complete with a short Tmax and a high Cmax. This patient was an uncomplicated non-CF lung transplantation patient. Cardiopulmonary bypass was used during surgery. A hyperdynamic circulation combined with augmented renal clearance existed for 3 days. Day 4 and 5 diarrhoea was observed. UTN14: This patient was a heart transplant patient and had severe bleeding during surgery for which 2 red blood cell units were administered on day 1. For 5 days, he was supported with ECMO, vasopressors and inotropes, because of shock. Fluid balances ranged from 1 to 4 litres. No gut dysmotility occurred. Very low C12h were measured during day 1 and 2, which would fit low absorption or distribution into the red blood cells. From day 3 to day 6 a high clearance was observed. High doses were needed for therapeutic C12h

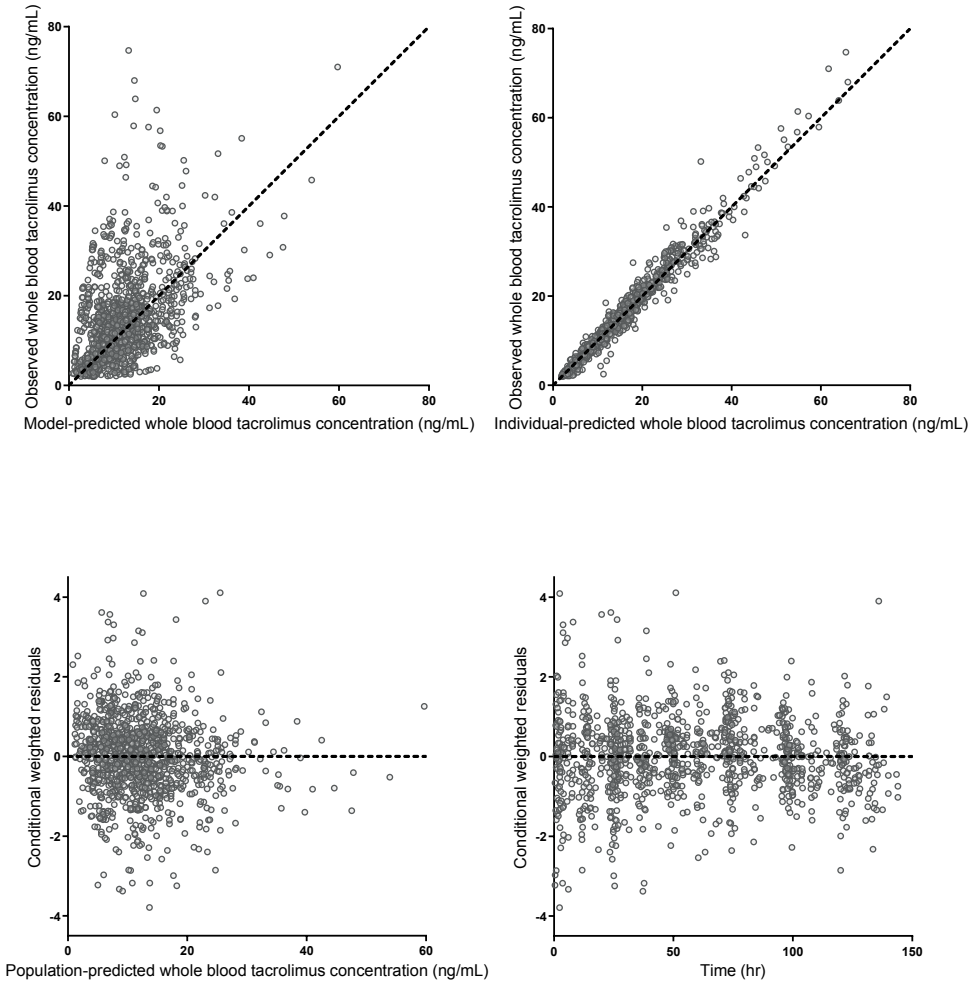

Fig. 3 Goodness-of-fit plots

SUPPLEMENTARY MATERIALS

Extremely high variability of whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation

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FIGURES

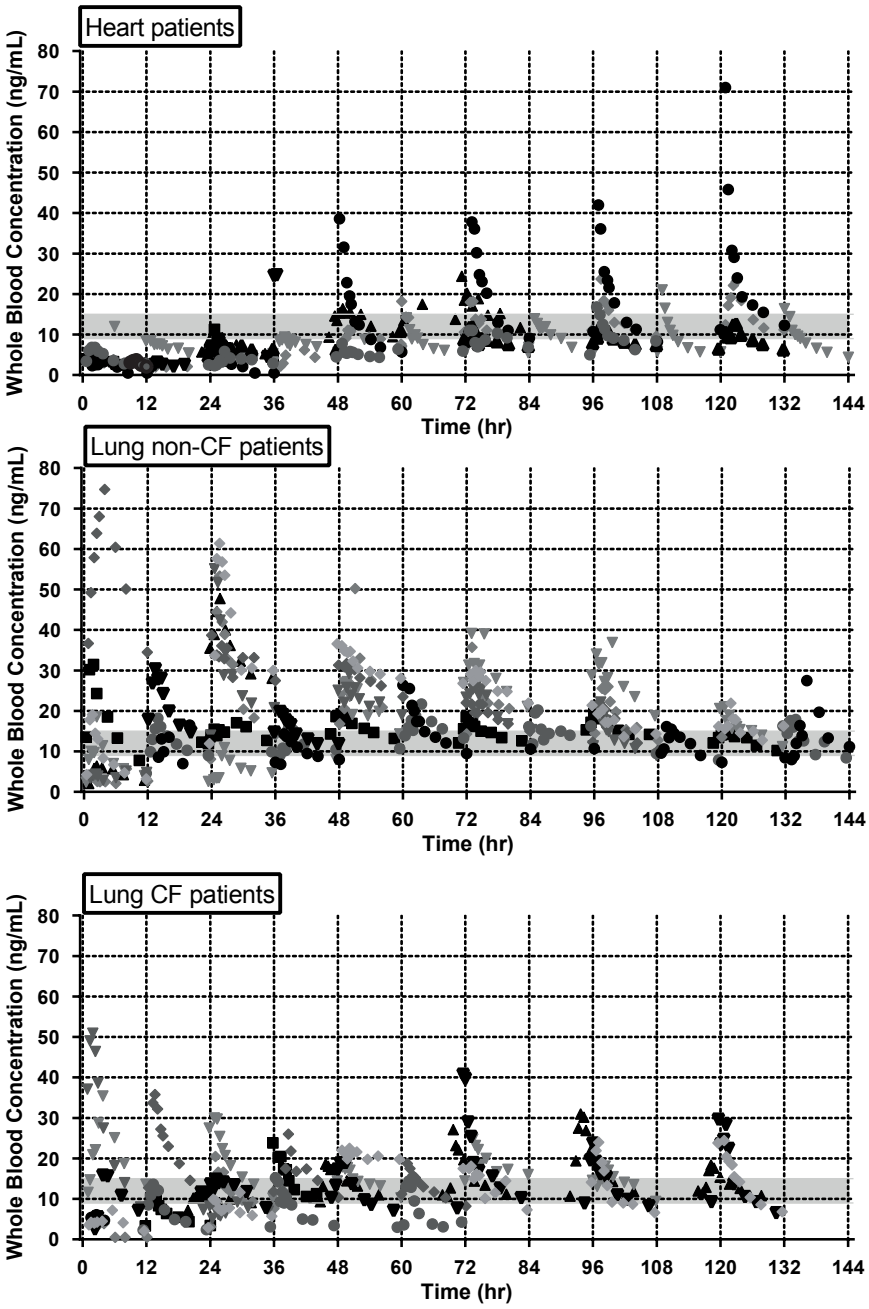

Fig. S1 Whole-blood tacrolimus concentrations over time subdivided into group of patients: 10 heart transplant patients, 10 lung transplant patients without CF, and 10 lung transplant patients with CF. The grey bar corresponds with the therapeutic range (9-15 ng/ml)

TABLES

Table S1. Definitions of the covariates

Covariate	Definition
Baseline creatinine clearance	The baseline creatinine clearance was the last clearance before surgery or, when there was no measured clearance, clearance was calculated with the "CKD Epidemiology Collaboration equation (CKD EPI)"[1]
Augmented renal clearance	Creatinine clearance >130 ml/min[2]
AKI	Decrease of 50% in creatinine clearance or the use of CVVH[3] Creatinine clearance calculation: $C_{cr} = \frac{U_{cr} \times \text{24-hour volume in ml}}{P_{cr} \times 24 \times 60 \text{ min}}$
Renal recovery	Serum creatinine below or equal to 125% of the baseline creatinine[4]
CKD	eGFR categories; G1=normal GFR: ≥90 ml/min/1.73 m^2, G2=mildly decreased: 60–89 ml/min/1.73 m^2, G3a/b=mildly to severely decreased: 30-59 ml/min/1.73 m^2, G4= severely decreased: 15-29 ml/min/1.73 m^2, G5= Kidney failure: <15 mL/min/1.73 m^2. eGFR = 141 x [min(Scr/κ),1) α x max(Scr/κ),1)-1.209] x Age-0.993 x 1.018 [if female] x [1.157 if Black] α is 0.329 for females and 0.411 for males; min indicates minimum of Scr/κ or 1, and max indicates maximum of Scr/κ or 1
SIRS	Presenting 2 or more of the following criteria: body temperature <36 °C or >38 °C, heart rate >90 /min for lung recipients and >100 /min for heart recipients, respiratory rate >20/min, PaCO2 <32 mmHg, mechanical ventilation and leucocyte count <4 $X10^9$ /L or >12 $X10^9$ /L
Shock	Mean arterial pressure <60 mmHg or use of norepinephrine, epinephrine, phenylephrine, vasopressin, dobutamine or milrinone
SOFA score	Sequential Organ Failure Assessment measured per day. The total SOFA was calculated as the sum of all daily SOFA scores during the intensive care stay for each patient.
Diarrhea	Defecation >2 times/day
Ileus	No defecation for ≥3 days or gastric retention of ≥500 mL/day
Liver injury	Bilirubin >34 µmol/L or an ALT >90 U/L for men and >70 U/L for women

Table S1. Definitions of the covariates

Covariate	Definition
Drugs potentially increasing tacrolimus blood concentrations by inhibition or substrate competition of the CYP3A4/5 and ABCB1 enzymes	Tobramycin, erythromycin, neomycin, trimethoprim/ sulfamethoxazole, fluconazole, voriconazole, (es)omeprazole, amlodipine, nicardipine, diltiazem, haloperidol, amiodarone
Drugs potentially decreasing tacrolimus blood concentrations by induction of CYP3A4/5 or ABCB1 enzymes	Corticosteroids and rifampicin

AKI= acute kidney injury, Ccr=creatinine clearance, Ucr=urine creatinine concentration, Pcr= plasma creatinine concentrations, CKD=chronic kidney dysfunction, (e)GFR=(estimated) glomerular filtration rate, SIRS=systemic inflammatory response syndrome, Scr=serum creatinine, min=minute, CYP=Cytochrome P450, ABCB1=p-glycoprotein

Table S2. Patient's characteristics indicated per day

	N=30 (%)	Median (1st and 3rd quartile)
SOFA scores per day		
Day1		9 (7;17)
Day2		8 (5;10)
Day3		5 (3;8)
Day4		5 (3;15)
Day5		4 (3;11)
Day6		4 (2;9)
Frequency of Ileus per day		
Day1	27 (90%)	
Day2	24 (80%)	
Day3	14 (47%)	
Day4	5 (17%)	
Day5	2 (7%)	
Day6	2 (7%)	
Frequency of diarrhea per day		
Day1	0 (0%)	
Day2	2 (7%)	
Day3	8 (27%)	
Day4	9 (30%)	
Day5	11 (37%)	
Day6	10 (33%)	

Table S2. Patient's characteristics indicated per day

	N=30 (%)	Median (1st and 3rd quartile)
Frequency of liver dysfunction per day		
Day1	7/30 (23%)	
Day2	6/30 (20%)	
Day3	3/27 (11%)	
Day4	3/21 (14%)	
Day5	4/17 (24%)	
Day6	3/17 (18%)	
Frequency of ECMO use per day		
Day1	8 (27%)	
Day2	7 (23%)	
Day3	5 (19%)	
Day4	4 (19%)	
Day5	3 (18%)	
Day6	3 (18%)	
Number of drugs increasing tacrolimus concentration (min-max)		
Day1		2 (1-4)
Day2		1 (0-2)
Day3		1 (0-4)
Day4		1 (0-5)
Day5		0 (0-6)
Day6		1 (0-4)
Number of patients with drugs increasing tacrolimus concentration		
Day1	30 (100%)	
Day2	23 (77%)[a]	
Day3	23 (85%)[a]	
Day4	17 (81%)[a]	
Day5	14 82%)[a]	
Day6	16 (94%)[a]	
Number of drugs decreasing tacrolimus concentration (min-max)		
Day1		0 (0-0)
Day2		1 (1-2)
Day3		1 (0-1)
Day4		1 (0-1)
Day5		1 (0-1)
Day6		1 (0-1)
Number of patients with drugs decreasing tacrolimus concentration		
Day1	0 (0%)	
Day2	30 (100%)	
Day3	26 (96%)	
Day4	21 (100%)	
Day5	17 (100%)	
Day6	17 (100%)	

Table S2. Patient's characteristics indicated per day

	N=30 (%)	Median (1st and 3rd quartile)
Renal function		
Baseline Creatinine clearance (ml/min/1.73m2)		85 (73;116)
Pharmacogenetic analyses		
Slow metabolizers of CYP 3A5	21 (70%)	
Slow metabolizers of CYP3A4		
Homozygosity CYP3A4*22	0 (0%)	
Heterozygosity and Homozygosity of POR*28	18 (60%)	
PPARα	16 (53%)	
Decreased ABCB1 activity		
Decreased PXR activity	18 (60%)	
G1199A homozygosity	0 (0%)	
C1236T homozygosity	6 (20%)	
G2677T homozygosity	8 (27%)	
C3435T homozygosity	8 (27%)	
OATP1B1 homozygosity	0 (0%)	

[a] of the patients still at the ICU

REFERENCES

1. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612. doi:10.1097/GME.0000000000000416.
2. Hobbs ALV, Shea KM, Roberts KM, Daley MJ. Implications of Augmented Renal Clearance on Drug Dosing in Critically Ill Patients: A Focus on Antibiotics. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*. 2015;35(11):1063-1075. doi:10.1002/phar.1653.
3. Waikar SS, Bonventre JV. Creatinine kinetics and the definition of acute kidney injury. *Journal of the American Society of Nephrology*. 2009;20(3):672-679. doi:10.1681/ASN.2008070669.
4. Chawla LS, Bellomo R, Bihorac A, et al. Acute kidney disease and renal recovery: consensus report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup. In: Vol 13. Nature Publishing Group; 2017:241-257. doi:10.1038/nrneph.2017.2.

CHAPTER 4a

4a

Development of a simple and rapid method to measure the free fraction of tacrolimus in plasma using ultrafiltration and LC-MS/MS

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ABSTRACT

Background

Tacrolimus is an immunosuppressant mainly used in the prophylaxis of solid organ transplant rejection. Therapeutic drug monitoring of tacrolimus is essential for avoiding toxicity related to overexposure and transplant rejection from underexposure. Previous studies suggest that unbound tacrolimus concentrations in the plasma may serve as a better predictor of tacrolimus-associated nephro- and neurotoxicity compared to tacrolimus concentration in whole-blood. Monitoring the plasma concentrations of unbound tacrolimus might be of interest in preventing tacrolimus-related toxicity. Therefore, the aim was to develop a method for the measurement of total and unbound tacrolimus concentrations in plasma.

Methods

The sample preparation for the determination of the plasma concentrations of unbound tacrolimus consisted of an easy-to-use ultrafiltration method followed by solid phase extraction. To determine the total concentration of tacrolimus in plasma, a simple method based on protein precipitation was developed. The extracts were injected into a Thermo Scientific HyPurity C18 column using gradient elution. The analytes were detected by liquid chromatography-mass spectrometry using a triple quadruple with positive ionization.

Results

The method was validated over a linear range of 1.00–200 ng/L for unbound tacrolimus concentrations in plasma and 100–3200 ng/L for total plasma concentrations. The lower limit of quantification was 1.00 ng/L in ultrafiltrate and 100 ng/L in plasma. The inaccuracy and imprecision for the determination of unbound tacrolimus concentrations in ultrafiltrate and plasma showed a maximum CV of 11.7% and a maximum bias of 3.8%.

Conclusion

A rapid and easy method based on ultrafiltration and LC-MS/MS was established to measure the total and unbound tacrolimus concentrations in plasma. This method can facilitate further investigations on the relationship between plasma concentrations of unbound tacrolimus and clinical outcomes in transplant recipients.

INTRODUCTION

Tacrolimus is the cornerstone of immunosuppressive therapy that can prevent rejection in solid organ transplant patients.[1] Although tacrolimus is effective, its use comes with a risk of toxicity. Neuro- and nephrotoxicity are frequently observed during tacrolimus treatment, increasing the morbidity and mortality of transplant patients.[2,3] Tacrolimus has a narrow therapeutic window. Thus, the high inter- and intra-patient variability in pharmacokinetic parameters are visualized by the high fluctuations in tacrolimus concentrations especially in the early post transplant setting.[4] Therefore, therapeutic drug monitoring (TDM) of whole-blood tacrolimus concentrations is recommended in clinical practice. Although monitoring the whole-blood levels has been proven effective in preventing organ rejection, whole-blood levels show a poor association with tacrolimus-related side effects such as neuro- and nephrotoxicity.[5-11] Blood binding affects the disposition of tacrolimus, and the plasma concentrations of tacrolimus were inversely correlated with the hematocrit value.[12] The average blood to plasma ratio of tacrolimus is 8 in liver and 15 in kidney and heart transplant patients, suggesting that this ratio is dependent on the nature of the organ transplanted.[13,14] The unbound fraction of tacrolimus is low [<3% of the total plasma concentration and <0.5% of the whole-blood concentration], and toxicity could probably be best related to the unbound tacrolimus plasma concentrations.[12,15-22] This may be attributed to the fact that only free or unbound drug in the plasma (Fu) can migrate to tissue compartments. In previous studies on unbound tacrolimus concentrations in plasma, the whole-blood tacrolimus concentrations did not differ between organ-transplant patients who experienced tacrolimus-related toxicity, and those who did not.[18,19] In contrast, the unbound tacrolimus concentrations were observed to be significantly higher in patients experiencing tacrolimus-related toxicity.[18] These findings suggest that the unbound concentrations of tacrolimus correlate better with toxicity than the whole-blood concentrations do. Therefore, the plasma concentrations of unbound tacrolimus might be useful for toxicity monitoring purposes. Nevertheless, only whole-blood concentrations are used for monitoring because the analysis of the unbound tacrolimus concentration in plasma is complex, and is currently not available for everyday practice. Moreover, the assay for unbound concentration may be inaccurate owing to temperature-dependent distribution into the whole-blood and plasma and challenges associated with assay sensitivity.[23] To further investigate the relationship between unbound tacrolimus concentration and clinical outcomes in the early post transplant population, we aimed to develop a bio-analytical method to quantify the unbound tacrolimus concentrations in the plasma.

MATERIALS AND METHODS

Chemicals and reagents

Tacrolimus was purchased from Sigma-Aldrich (Munich, Germany), and the internal standard (IS) tacrolimus [$^{13}C,^{2}H_2$] was purchased from Alsachim (Strasbourg, France). Water with 0.1% ammonium acetate was obtained from Sigma-Aldrich. Acetonitrile, methanol, and water were purchased from Biosolve (Valkenswaard, the Netherlands). Zinc sulfate was obtained from Merck (Darmstadt, Germany). For the isolation of unbound tacrolimus concentration in the plasma, Centrifree® ultrafiltration devices from Merck Millipore (Darmstadt, Germany) were used. The OASIS HLB solid-phase extraction cartridge was obtained from Waters (Milford, USA). Newborn calf serum was obtained from Gibco-Life technologies (Paisley, UK).

Calibrators and quality control samples

For the determination of unbound tacrolimus plasma concentration, a stock solution of tacrolimus at a concentration of 500 µg/L was prepared and diluted with methanol to prepare solutions having concentrations of 0.25 µg/L and 1.25 µg/L. From these calibrators, standards were prepared in phosphate buffer at concentrations of 1.00, 5.00, 20.0, 50.0, 100, and 200 ng/L. The quality control (QC) samples were prepared from a second stock solution of tacrolimus. The lower limit of quantification (LLOQ), LOW, MED, and HIGH controls were prepared in phosphate buffer at concentrations of 1.00, 30.0, 75.0, and 150 ng/L. The calibrators and QC solutions were diluted twice with methanol prior to solid-phase extraction. For the determination of total tacrolimus plasma concentration, the stock solution of tacrolimus was diluted with methanol to a concentration of 10 µg/L. From this solution, calibrators were prepared in newborn calf serum at concentrations of 100, 200, 400, 800, 1600, and 3200 ng/L. The quality control (QC) samples were prepared from a second stock solution of tacrolimus. The LLOQ, LOW, MED, and HIGH controls were prepared in newborn calf serum at concentrations of 100, 500, 1500 and 3000 ng/L. For the preparation of the internal standard (IS) tacrolimus [^{13}C, $^{2}H_2$], a stock solution was diluted with methanol to obtain a concentration of 10 µg/L.

Sample preparation for unbound tacrolimus concentration in plasma

A 1.5 mL aliquot of plasma was distributed over three Centrifree® ultrafiltration devices. The filled ultrafiltration devices were centrifuged (2500 g) for 60 minutes at 25°C. After centrifugation, the filtrates were pooled. A 500 µL aliquot of ultrafiltrate was diluted twice with methanol, and to this dilution, 50 µL IS was added. The solid-phase extraction method described by Annesley and Clayton for immunosuppressant drugs in whole-blood was optimized and used for the determination of plasma concentration of unbound tacrolimus.[24] Solid phase extraction was performed with a 30 mg, 1 mL Waters OASIS HLB cartridge. The cartridge was conditioned with 1 mL of methanol followed by 1 mL of

water. The sample was slowly transferred through the cartridge. The cartridge was washed twice with 1 mL of water and air-dried under reduced pressure. Tacrolimus was eluted into a clean test tube with 1 mL of acetonitrile. Thereafter, acetonitrile was evaporated under nitrogen. The residue was reconstituted in 50 µL methanol/water (50/50 (v/v)). After vortexing, the volumes were inserted into glass vials containing inserts. A 25 µL aliquot of the sample was injected into the LC-MS/MS system.

Sample preparation for total plasma concentration

To a 200 µL aliquot of plasma sample, 200 µL each of 0.1 mol/L zinc sulfate, IS and methanol were added. After vortexing, the samples were centrifuged at 11290 g for 5 min. A 25 µL aliquot of the sample was injected into the LC-MS/MS system.

Instrumentation and conditions

Tacrolimus was quantified using the Thermo Scientific (Waltham, MA) Quantiva LC-MS/MS system with an Ultimate 3000 UHPLC. The Quantiva mass spectrometer was operated in positive electrospray ionization and selected reaction monitoring mode. A method for analyzing tacrolimus in whole-blood, previously described by Koster et al., was optimized for this specific UHPLC system.[25] The analytical column was a HyPurity C18 50 x 2.1 mm column with 3 µm particle size (Thermo Scientific). The auto sampler temperature was set at 10°C, and the column temperature was kept at 60°C. Chromatographic separation was performed by means of a gradient with a flow rate of 500 µL/min and a total runtime of 2.5 min. The gradient was achieved using water with ammonium acetate (mobile phase A), water (mobile phase B), and methanol (mobile phase C). The gradient is represented in Table 1.

Analytes were detected by MS/MS via heated electro spray ionization (HESI)-interface in selected reaction monitoring (SRM)-mode. The parent ions, product ions, collision energy, and radio frequency (RF) lens were optimized in the authors' laboratory. For tacrolimus, the parent and product ions were set at a mass-to-charge ratio (m/z) of 821.5 and 768.5 m/z, respectively. For tacrolimus [^{13}C, $^{2}H_2$], the parent and product ions were set at a mass-to-charge ratio (m/z) of 825.5 and 772.6 m/z, respectively. The collision energies for tacrolimus and tacrolimus [^{13}C, $^{2}H_2$] were 19 and 18 V, respectively. The RF lens 90 V was used for both compounds.

High-purity nitrogen was used as sheath gas and auxiliary gas, and argon was used as the collision gas. The cycle time was set at 0.3 s for both compounds. The optimum ion transfer tube temperature was 325°C, and the vaporizer temperature was maintained at 300°C. The ion spray voltage was set at 3500 V, and the sheath gas, auxiliary gas, and ion sweep gas pressures were set at 40, 25, and 1 Arb, respectively.

Method validation

The validation of the analysis of unbound and total tacrolimus concentration in human plasma included the following parameters according to the FDA guidelines for bio-analytical validation (http://www.fda.gov/downloads/Drugs/Guidances/ucm070107.pdf).

Selectivity

The selectivity of the method was assessed for potential matrix interferences. The chromatograms of six batches of blank human plasma samples and ultrafiltrate samples were evaluated to ensure that there are no interfering peaks at the retention time of tacrolimus and the IS tacrolimus [^{13}C, $^{2}H_2$].

Linearity, inaccuracy, and imprecision

For the unbound tacrolimus concentration in plasma, seven calibration points in the range of 1.00 – 200 ng/L were used to determine linearity on three separate days using linear regression analyses. For the total plasma concentration, seven calibration points in the range of 100–3200 ng/L were used to determine linearity on three separate days using linear regression analyses. The concentrations were calculated by linear regression using the calculated ratios of analyte/internal standard by area. For the determination of inaccuracy and imprecision, the QC samples (LLOQ, LOW, MED, and HIGH) were prepared and analyzed in five-fold in three separate runs on three different days. Within-run, between-run, and overall coefficients of variation (CV) were calculated using one-way ANOVA. The inaccuracy and imprecision were determined at the maximum tolerated bias and CV (20% for LLOQ, 15% for LOW, MED, and HIGH).

Recovery and matrix effects

To evaluate the extraction recovery of the solid-phase extraction, five replicates of blank phosphate buffer spiked with tacrolimus at a concentration of 75.0 ng/L, before and after the sample preparation were compared. Six blank plasma samples spiked with tacrolimus at a concentration of 1500 ng/L, before and after the sample preparation were compared. To determine the extraction recovery, the mean peak area ratio of the samples spiked before preparation was compared to the mean peak area ratio of the samples spiked after preparation. Matrix effect, expressed as matrix factors (MFs), was determined by comparing the mean area ratio response of the five blank plasma samples with the mean area ratio of samples prepared in methanol/water (50/50 (v/v)) end solution spiked at 75.0 ng/L after preparation. The matrix effect in plasma was determined by comparing the mean area ratio response of six blank plasma samples with the mean area ratio of samples prepared in Milli-Q water at 1500 ng/L after preparation.

Stability

The auto sampler stability of tacrolimus in the ultrafiltrate was determined using a sample prepared in methanol/water [50/50 (v/v)] end solution. The determination of auto sampler stability of tacrolimus in plasma was performed using a sample prepared in newborn calf serum. The samples were analyzed every 2 h for a period of 20 h at 10°C. The freeze–thaw stability at −80°C was determined using a sample prepared in newborn calf serum and analyzed in five-fold during three cycles. The solutions were stable if the deviation from nominal value was less than 15%. Long-term stability was tested by storing five patient samples in the freezer at -80°C for 196 days.

RESULTS

Selectivity

No interference peak was detected for tacrolimus and tacrolimus [^{13}C, $^{2}H_2$] in the tested blank human plasma and ultrafiltrate. Representative chromatograms of blank human plasma, a calibrator at LLOQ level, and the internal standard are shown in Figure 1.

Linearity, inaccuracy, and imprecision

A weighting factor of $1/x^2$ was chosen for the determination of linearity in ultrafiltrate, and no weighting factor was chosen for the determination of linearity in plasma. The correlation coefficient (R) for both the calibration curves is shown in Figure 2A and B. The validation results for inaccuracy and imprecision are within the maximum tolerated bias and CV (20% for LLOQ and 15% for LOW, MED, and HIGH; Table 2).

Recovery and matrix effects

The extraction recovery for tacrolimus in the ultrafiltrate and plasma was 105% and 107%, respectively. The matrix effect, expressed as matrix factor, was 1.0 for tacrolimus in both ultrafiltrate and plasma. This indicated that there were no significant matrix effects for tacrolimus in the ultrafiltrate or plasma.

Stability

The results of the stability are shown in Table 3. The unbound tacrolimus concentration in the end solution remained stable in the auto sampler for 22 h at 10°C, with a bias of 10.4% (CV 2.2%). The stability of the analyte was determined after three freeze-thaw cycles and evaluated by calculating the within-run and between-run coefficients of variation (CV). It is not possible to calculate the bias because we used a spiked sample; therefore, the nominal tacrolimus unbound concentration is not known. The within-run and between-run variances were both within the acceptance criteria of 15%. Five patient samples covering a concentration range of 4.75–12.2 ng/L with a median concentration of 9.72 ng/L were analyzed. Although freeze-thaw stability had no effect on the unbound tacrolimus concentration, a median increase of 37.7% with a range of 27.4 to 82.5% in the unbound tacrolimus concentration was observed after the samples were stored for 196 days. The total tacrolimus concentration in the end solution was stable in the auto sampler at 1°C for 22 h, with a bias of 3.2% (CV 1.1%). The freeze-thaw stability was determined in three cycles and a bias of -2.7% was observed. This is within the acceptance criteria of 15%. The total plasma concentration of tacrolimus was stable in plasma stored at -80°C for at least 196 days, and a bias of less than 15% was observed.

DISCUSSION

Several techniques, such as equilibrium dialysis, ultracentrifugation, and ultrafiltration, are available for the determination of the unbound fraction of a drug. The most commonly used method for measuring the unbound fraction of a drug is equilibrium dialysis. However, depending on the properties of the compound, this method can be rather time consuming, and is not suitable for unstable compounds. As tacrolimus is a highly bound compound, it takes more time to reach equilibrium. This can cause bacterial growth and shifts in plasma pH and free fatty acid concentration. Another technique for the determination of unbound drug fraction is ultracentrifugation. The advantage of ultracentrifugation is that there are fewer issues associated with nonspecific binding to centrifugation tubes compared to that associated with binding to dialysis or ultrafiltrate membranes. However, in this method, a large amount of samples cannot be processed at once.[26] In this study, ultrafiltration was performed to separate unbound tacrolimus from the bound tacrolimus. Compared to other techniques such as ultracentrifugation and equilibrium dialysis, ultrafiltration is straightforward and easy-to-use, and therefore has a higher sample throughput. Although the recovery results during ultrafiltration demonstrated no significant adsorption of the analyte to the nonspecific binding (NSB) sites, the awareness of lower and variable recoveries during this process remains warranted since it has been reported earlier.[27] In spite of the risk of adsorption, ultrafiltration has many advantages.

During the first method development, the minimal sample volume required to collect a minimum of 500 µL ultrafiltrate was investigated. The ultrafiltration devices were filled with maximum 1 mL plasma, which resulted in approximately 125 µL ultrafiltrate, after 10 min of centrifugation. However, the unbound fraction of tacrolimus was low [<3% of the total plasma concentration and <0.5% of the whole-blood concentration], and it was therefore necessary to develop a method with a low LLOQ (1 ng/L).[12,15-20,28] A good signal-to-noise ratio for the tacrolimus peak in the chromatogram (Figure 1) of LLOQ could only be achieved with minimum 500 µL of ultrafiltrate. Distributing 1.5 mL of plasma over three ultrafiltration tubes robustly produced at least 500 µL ultrafiltrate per sample for further sample cleanup. Thereafter, owing to the very low solubility of tacrolimus in water, and for preventing the adsorption of tacrolimus to the plastic container of the ultrafiltration device, an aliquot of 500 µL ultrafiltrate was directly diluted with methanol. In addition, newborn calf serum was used for the preparation of calibration curve and quality control samples for the determination of the total tacrolimus concentration, since validation showed no interfering peaks and matrix effects. Finally, linearity in the ultrafiltrate was determined using a $1/x^2$-weighting factor, and no weighting factor was used for the determination of linearity in plasma, because for the unbound tacrolimus concentration, $1/x^2$-weighting factor showed the best fit for this large calibration range (1.00 – 200 ng/L).

Notably, the increase in the unbound tacrolimus concentration observed after long-term storage of the samples can be explained by the occurrence of plasma lipolysis. Lipolysis can increase the free fatty acid levels in plasma resulting in fatty acid-induced protein conformational changes. This may influence the binding of small molecules to proteins.[26] Therefore, long-term storage of plasma cannot be allowed, and the samples should be freshly ultra-filtered. For studies investigating the unbound tacrolimus concentration, "fresh" filtration shortly after the samples are drawn and thereafter long-term storage at -80°C may be recommended.

CONCLUSION

A fast and highly sensitive LC-MS/MS method was developed and validated for the quantitation of total and unbound plasma concentrations of tacrolimus. Compared to previously reported methods, the workflow is straightforward and easy-to-use, facilitating large scale investigations on the relationship between unbound tacrolimus plasma concentrations and clinical outcomes in transplant recipients.

REFERENCES

1. Guethoff S, Meiser BM, Groetzner J, et al. Ten-Year Results of a Randomized Trial Comparing Tacrolimus Versus Cyclosporine A in Combination With Mycophenolate Mofetil After Heart Transplantation. *Transplantation*. 2013;95(4):629-634. doi:10.1097/TP.0b013e318277e378.
2. Ferrara JL, Deeg HJ. Graft-versus-host disease. *N Engl J Med*. 1991;324(10):667-674. doi:10.1056/NEJM199103073241005.
3. Grimm M, Rinaldi M, Yonan NA, et al. Superior prevention of acute rejection by tacrolimus vs. cyclosporine in heart transplant recipients--a large European trial. *Am J Transplant*. 2006;6(6):1387-1397. doi:10.1111/j.1600-6143.2006.01300.x.
4. Sikma MA, van Maarseveen EM, van de Graaf EA, et al. Pharmacokinetics and Toxicity of Tacrolimus Early After Heart and Lung Transplantation. *Am J Transplant*. 2015;15(9):2301-2313. doi:10.1111/ajt.13309.
5. Kershner RP, Fitzsimmons WE. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation*. 1996;62(7):920-926. doi: 10.1097/00007890-199610150-00009
6. Schwartz M, Holst B, Facklam D, Buell D. FK 506 in liver transplantation: correlation of whole blood levels with efficacy and toxicity. The US Multicenter FK 506 Dose Optimization. *TPS*. 1995;27(1):1107.
7. Staatz C, Taylor P, Tett S. Low tacrolimus concentrations and increased risk of early acute rejection in adult renal transplantation. *Nephrol Dial Transplant*. 2001;16(9):1905-1909. doi: 10.1093/ndt/16.9.1905
8. Takahara S, Kokado Y, Kameoka H, et al. Monitoring of FK 506 blood levels in kidney transplant recipients. *TPS*. 1994;26(4):2106-2108.
9. Winkler M, Wonigeit K, Undre N, et al. Comparison of plasma vs whole blood as matrix for FK 506 drug level monitoring. *TPS*. 1995;27(1):822-825.
10. Hebert MF, Zheng S, Hays K, et al. Interpreting tacrolimus concentrations during pregnancy and postpartum. *Transplantation*. 2013;95(7):908-915. doi:10.1097/TP.0b013e318278d367.
11. Yoshida EM, Marotta PJ, Greig PD, et al. Evaluation of renal function in liver transplant recipients receiving daclizumab (Zenapax), mycophenolate mofetil, and a delayed, low-dose tacrolimus regimen vs. a standard-dose tacrolimus and mycophenolate mofetil regimen: a multicenter randomized clinical trial. *Liver Transpl*. 2005;11(9):1064-1072. doi:10.1002/lt.20490.
12. Jusko WJ, Piekoszewski W, Klintmalm GB, et al. Pharmacokinetics of tacrolimus in liver transplant patients. *Clinical Pharmacology & Therapeutics*. 1995;57(3):281-290. doi:10.1016/0009-9236(95)90153-1.
13. Warty V, Zuckerman S, Venkataramanan R, et al. Tacrolimus analysis: a comparison of different methods and matrices. *therapeutic drug monitoring*. 1995;17(2):159-167. doi: 10.1097/00007691-199504000-00010

14. Warty V, Zuckerman S, Venkataramanan R, Lever J, Fung J, Starzl T. FK506 measurement: comparison of different analytical methods. *therapeutic drug monitoring*. 1993;15(3):204-208. doi: 10.1097/00007691-199306000-00005
15. Iwasaki K, Miyazaki Y, Teramura Y. Binding of tacrolimus (FK506) with human plasma proteins re-evaluation and effect of mycophenolic acid. *Res Commun Mol Pathol Pharmacol*. 1996;94(3):251-257.
16. Nagase K, Iwasaki K, Nozaki K, Noda K. Distribution and protein binding of FK506, a potent immunosuppressive macrolide lactone, in human blood and its uptake by erythrocytes. *J Pharm Pharmacol*. 1994;46(2):113-117. doi:10.1111/j.2042-7158.1994.tb03752.x.
17. Weiss HM, Fresneau M, Moenius T, Stuetz A, Billich A. Binding of pimecrolimus and tacrolimus to skin and plasma proteins: implications for systemic exposure after topical application. *Drug Metab Dispos*. 2008;36(9):1812-1818. doi:10.1124/dmd.108.021915.
18. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Changes in tacrolimus distribution in blood and plasma protein binding following liver transplantation. *therapeutic drug monitoring*. 2004;26(5):506-515. doi: 10.1097/00007691-200410000-00008
19. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Factors affecting variability in distribution of tacrolimus in liver transplant recipients. *British Journal of Clinical Pharmacology*. 2004;57(3):298-309. doi: 10.1046/j.1365-2125.2003.02008.x
20. Zahir H, Nand RA, Brown KF, Tattam BN. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. *journal of pharmacological and toxicological methods*. 2001;46:27-35. doi: 10.1016/s1056-8719(02)00158-2
21. Zheng S, Easterling TR, Umans JG, et al. Pharmacokinetics of tacrolimus during pregnancy. *therapeutic drug monitoring*. 2012;34(6):660-670. doi:10.1097/FTD.0b013e3182708edf.
22. Lefaucheur C, Nochy D, Amrein C, et al. Renal Histopathological Lesions After Lung Transplantation in Patients with Cystic Fibrosis. *Am J Transplant*. 2008;8(9):1901-1910. doi:10.1111/j.1600-6143.2008.02342.x.
23. Machida M, Takahara S, Ishibashi M, Hayashi M. Effect of temperature on hematocrit on plasma concentration of FK506. *Transplantation Proceedings*. 1991;23(6):2753-2754. doi: 10.5980/jpnjurol1989.84.1088
24. Annesley TM, Clayton L. Simple extraction protocol for analysis of immunosuppressant drugs in whole blood. *Clinical Chemistry*. 2004;50(10):1845-1848. doi:10.1373/clinchem.2004.037416.
25. Koster RA, Dijkers ECF, Uges DRA. Robust, High-Throughput LC-MS/MS Method for Therapeutic Drug Monitoring of Cyclosporine, Tacrolimus, Everolimus, and Sirolimus in Whole Blood. *therapeutic drug monitoring*. 2009;31(1):116-125. doi:10.1097/FTD.0b013e318192304c.
26. Howard ML, Hill JJ, Galluppi GR, McLean MA. Plasma protein binding in drug discovery and development. *Comb Chem High Throughput Screen*. 2010;13(2):170-187. doi: 10.2174/138620710790596745
27. Lee K-J, Mower R, Hollenbeck T, et al. Modulation of nonspecific binding in ultrafiltration protein binding studies. *Pharm Res*. 2003;20(7):1015-1021. doi: 10.1023/a:1024406221962
28. Zheng S, Davis CL, Hebert MF. Pharmacokinetics of Tacrolimus During Pregnancy. *therapeutic drug monitoring*. 2012;34:660-670. doi: 10.1097/ftd.0b013e3182708edf

TABLES

Table 1. Gradient

Time (min)	A	B	C
0.00	5	65	30
0.36	5	65	30
0.37	5	20	75
1.00	5	12	83
1.10	5	0	95
1.60	5	0	95
1.61	5	65	30
2.50	5	65	30

Table 2. Validation results of unbound and total tacrolimus plasma concentrations

	Correlation coefficient (R) (linear range)	Nominal conc. (ng/L)	Mean (ng/L)[1]	Within-run[2]		Between-run[3]	
				Imprecision CV (%)	Inaccuracy CV (%)	Imprecision CV (%)	Inaccuracy CV (%)
Ultrafiltrate							
Tacrolimus	0.9997 (1-200 ng/L)	1.00	1.04 ± 0.05	9.3	2.0	1.7	3.8
		30.0	29.9 ± 0.51	2.4	-1.8	2.5	-0.3
		75.0	76.0 ± 1.52	1.3	-0.8	3.3	1.4
		150	155 ± 2.21	1.4	1.2	2.4	3.5
Plasma							
Tacrolimus	0.9999 (100-3200 ng/L)	100	101 ± 6.55	3.1	-4.5	11.3	0.7
		500	490 ± 17.2	2.0	2.7	6.0	-2.1
		1500	1461 ± 58.5	2.9	2.2	6.6	-2.6
		3000	2915 ± 120	1.2	3.6	7.3	-2.8

[1] Mean ± standard deviation
[2] within-run (n=5)
[3] between-run (n=3)

Table 3. Stability results of unbound and total tacrolimus plasma concentrations

		Nominal Concentration (ng/L)	Within-run CV (%)	Between-run CV (%)	Overall bias (%)
Ultrafiltrate					
Tacrolimus	F/T stability	n/a [1]	7.7	10.7	n/a
	AS stability	5.0	2.2	n/a	10.4
Plasma					
Tacrolimus	F/T stability	1500	2.7	6.5	-2.7
	AS stability	1500	1,1	n/a	3.2

[1] Nominal concentration not known

Abbreviations: CV=coefficient of variation, F/T=stability of three freeze-thaw cycles, AS=auto sampler stability

FIGURES

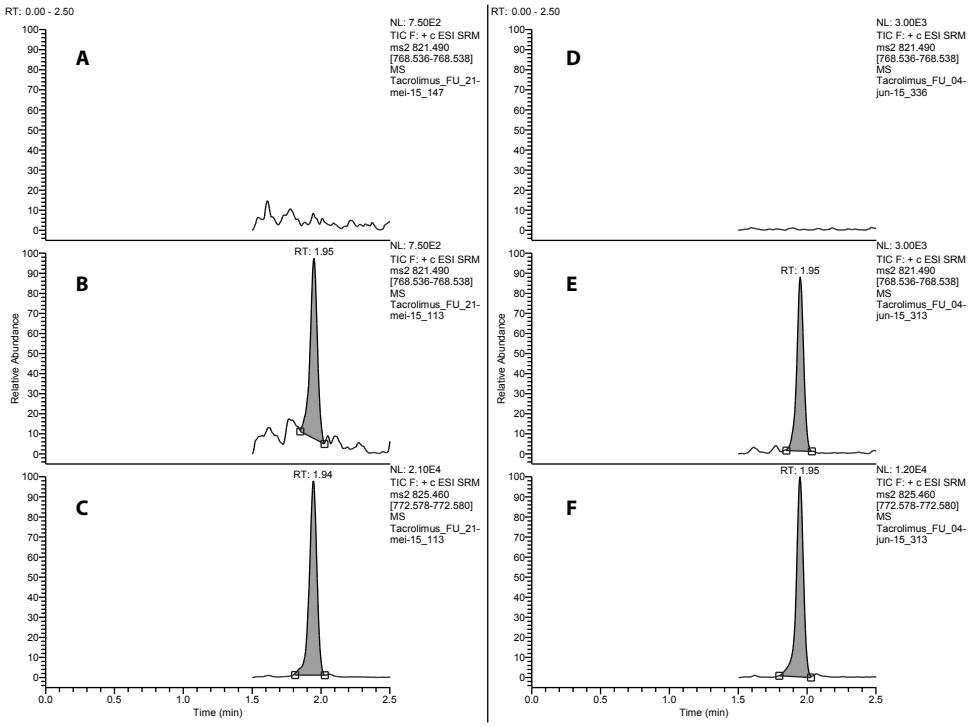

Fig. 1. Representative chromatogram of a blank human plasma (ultrafiltrate) (A), standard at LLOQ level (ultrafiltrate) (B), internal standard (ultrafiltrate) (C), Blank human plasma (D), standard at LLOQ level in plasma (E), and internal standard in plasma (F)

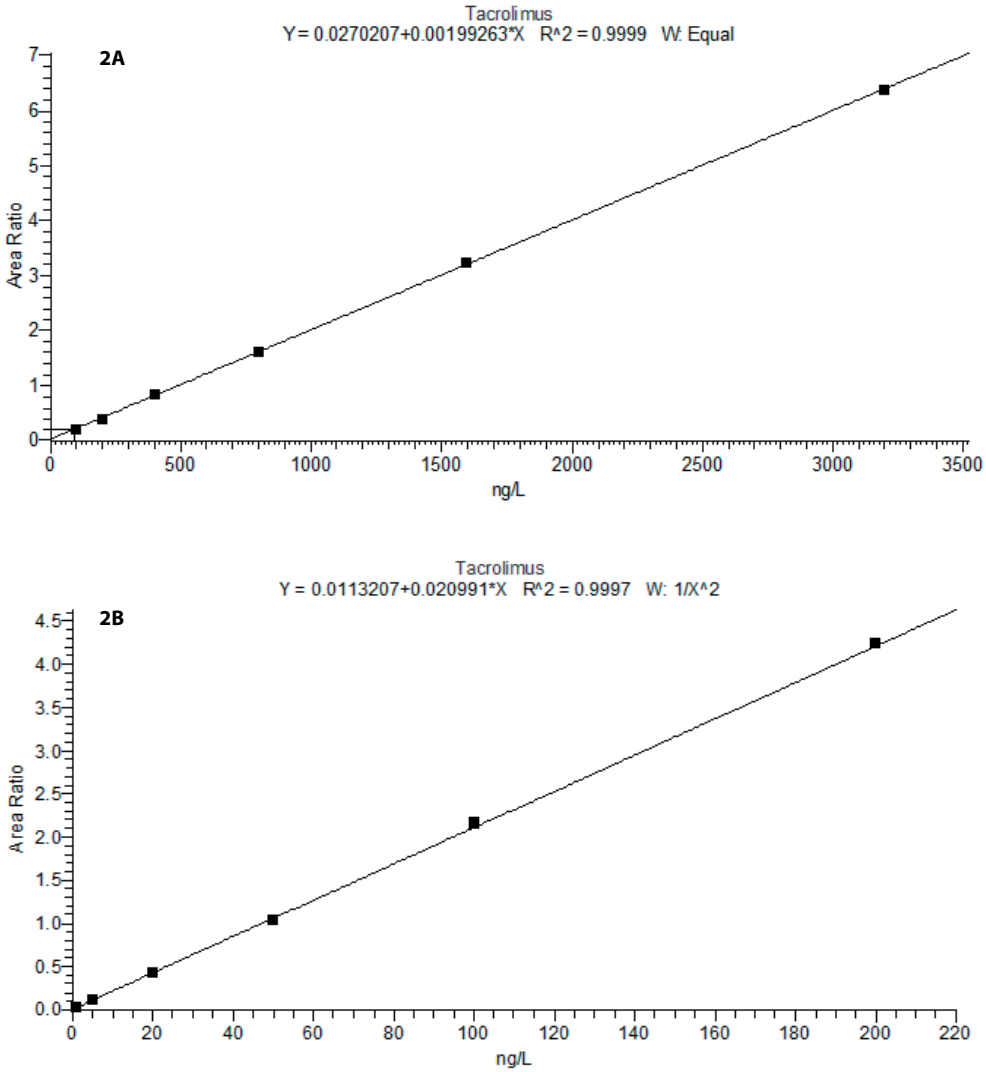

Fig. 2A and B. Representative calibration curves of tacrolimus in human plasma (2A) and ultrafiltrate (2B)

CHAPTER 4b

4b

Unbound plasma, total plasma and whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation

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This manuscript is submitted

ABSTRACT

Background

Therapeutic drug monitoring of tacrolimus whole-blood concentrations is standard care in thoracic organ transplantation. Nevertheless, toxicity may appear with alleged therapeutic concentrations possibly related to variability in unbound concentrations. However, pharmacokinetic data on unbound concentrations are not available.

Methods

Twelve-hours tacrolimus whole-blood, total and unbound plasma concentrations of 30 thoracic organ recipients were analyzed with HPLC-MS/MS directly after transplantation (NTR 3912/ EudraCT 2012-001909-24). Pharmacokinetic modeling was performed using non-linear mixed effects modeling.

Results

Plasma concentration was <1% of whole-blood concentration. Maximum binding capacity (Bmax) of erythrocytes was directly proportional to hematocrit and estimated at 2700 pg/ml (95%CI 1750-3835) with a dissociation constant (Kd) of 0.142 pg/ml (95%CI 0.087-0.195). The inter-individual variability in the binding constants was considerable (27% Bmax, and 29% for the linear binding constant of plasma).

Conclusions

Tacrolimus association with erythrocytes was high and suggested non-linear distribution at high concentrations. Monitoring hematocrit-corrected whole-blood tacrolimus concentrations might improve clinical outcomes in clinically unstable thoracic organ transplants.

INTRODUCTION

Since 1996, tacrolimus is used as immunosuppressant in solid organ transplantation. Ever since, exposure and outcome relationships of tacrolimus have been extensively studied resulting in worldwide consensus on its therapeutic window.[1] Nevertheless, there is room for improvement because patients with alleged therapeutic whole-blood concentrations are still at risk of tacrolimus-related toxicity and rejection.[2-6] Tacrolimus extensively binds to red blood cells and blood proteins. As a consequence, tacrolimus whole-blood distribution is strongly affected by hematocrit and proteins concentrations, e.g., albumin, lipoproteins and α_1-acid glycoprotein (AAG).[7-11] While whole-blood concentrations are commonly used for therapeutic drug monitoring (TDM), the unbound tacrolimus plasma concentrations might be better related to tacrolimus toxicity and efficacy.[8,12,13] Especially early after heart and lung transplantation, the concentrations of red blood cells and (lipo)proteins show high intra- and interpatient variation.[14] This may give rise to extreme variability in unbound tacrolimus concentrations in the clinically unstable phase after thoracic organ transplantation.

Studies investigating the unbound tacrolimus plasma concentrations are scarce, because quantification of unbound tacrolimus concentrations is bio-analytically challenging and time consuming.[7] As such, the relationship between whole-blood and unbound concentrations has not systematically been studied and no pharmacokinetic models are currently available predicting the unbound concentrations based on whole-blood concentrations. Furthermore, a therapeutic range of unbound tacrolimus plasma concentrations is currently lacking for routine therapeutic drug monitoring.[7-9,15]

This study aimed to quantify the pharmacokinetics (PK) of whole-blood, and total and unbound plasma tacrolimus in patients early after heart and lung transplantation. With this model we studied the effect of erythrocyte binding and evaluated whether monitoring based on unbound or total plasma concentrations is feasible as predictor of clinical outcomes.

RESULTS

Patient characteristics

Ten heart and twenty lung transplantation patients were enrolled in the study and completed the study protocol. Half of the patients were women (15 out of 30) and the median age was 43 (range 34 - 60). All heart transplantation patients were diagnosed with dilated cardiomyopathy of which 5 patients had ischemic cardiomyopathy and 1 was diagnosed with giant cell myocarditis. Reasons for lung transplantation were cystic fibrosis, chronic obstructive pulmonary disease, idiopathic pulmonary arterial hypertension, idiopathic pulmonary fibrosis, bronchiectasis, Langerhans cell histiocytosis and sarcoidosis. In Table 1 patient characteristics are summarized. For additional information on clinical characteristics see also Table S1 in the Supplementary materials.

Descriptive pharmacokinetics

The total number of whole-blood tacrolimus profiles 0-12 hours was 119 with a median of 5 profiles per patient (range 1-6). Ninety total and unbound plasma tacrolimus 0-12 hours profiles were obtained with a median of 3 per patient (range 0-6). The whole-blood, and total and unbound plasma pharmacokinetic parameters are shown in Table 2. The majority of tacrolimus was associated with erythrocytes as plasma concentrations were <1% of the whole-blood concentrations. Tacrolimus unbound fraction was <0.0001 in this population. In Figure 1a the observed relationship between unbound plasma and whole-blood concentrations is shown, suggesting non-linear binding of tacrolimus to erythrocytes. Total plasma concentrations of tacrolimus showed a linear relationship with the unbound plasma concentrations (See Figure 1b).

Model development

The previously developed 2-compartmental model for whole-blood concentrations with mixed zero-order and first-order absorption was extended with the total and unbound plasma concentration data (schematic illustration of the pharmacokinetic model; See Figure S1 **Chapter 3**). To reduce model complexity, the parameters related to absorption and the associated variability were fixed to the previously estimated values. Non-linear binding to erythrocytes and linear binding to plasma proteins best described the data. Furthermore, the maximum binding capacity to erythrocytes (Bmax) was directly proportional to hematocrit.

Table 3 shows the parameter estimates and precision of the final model. The parameter precision was acceptable for all relevant parameters, as represented by the small 95% confidence interval (95% CI) of the parameter estimates. The residual unexplained variability was low for the whole-blood concentrations 16.7% (95% CI 15.8 - 17.6) and higher for the unbound 36.3% (95% CI 33.9 - 40.4) and total plasma concentrations 31.6%

(95% CI 28.6 - 34.2). Substantial correlation of residual variability between whole-blood and plasma concentration was found as expected. Goodness-of-fit plots of unbound tacrolimus plasma concentrations exhibited data well centered around the identity line.

Results of PK modeling and simulations

Large inter-occasion variability in bioavailability was found, which dominates inter-individual variability (See Table 3). The dissociation constant (Kd) of distribution to erythrocytes was estimated at 0.142 pg/ml (95% CI 0.087 - 0.195), which is relatively high and indicates slight non-linear distribution relevant only at high tacrolimus concentrations. However, this model was superior to a linear binding model. The non-specific binding constant (Nplasma) for total plasma concentrations was estimated at 137 (95% CI 120 - 152) indicating that total plasma concentrations were typically 137-fold higher than unbound plasma concentrations. Moreover, the inter-individual variability in the binding constants was also considerable (27% for Bmax WBC and 29% for Nplasma).

Simulations of different hematocrit ratios ranging from 0.25 to 0.50 at a fixed whole-blood concentration of 9 ng/ml were conducted. The unbound concentration decreased with increasing hematocrit and ranged from 1.06 pg/ml to 2.14 pg/ml. A non-linear relationship between unbound plasma and hematocrit was observed as shown in Figure 2.

DISCUSSION

This is the first report of a population pharmacokinetic model of whole-blood, total and unbound tacrolimus plasma concentrations. Accumulation of tacrolimus in erythrocytes was high relative to plasma concentrations. The whole-blood to unbound plasma concentration ratios differed with changes in hematocrit and showed saturation in the higher range of whole-blood tacrolimus concentrations. Consequently, the combination of high whole-blood tacrolimus concentrations with low hematocrit concentrations may result in extremely high unbound plasma concentrations and hence, in toxicity. From a theoretical perspective, the unbound tacrolimus plasma concentrations would be a better surrogate for the prediction of clinical outcomes. Yet, the analysis of unbound tacrolimus plasma concentrations is challenging and not easily standardized. As a linear relationship between total and unbound tacrolimus plasma concentrations was found, measurement of total plasma concentrations may be considered as an alternative predictive biomarker of clinical outcomes. However, the accuracy and precision of plasma tacrolimus concentration quantification is vulnerable to hemolysis of whole-blood samples. To circumvent these bio-analytical challenges, hematocrit-corrected whole-blood concentrations may be the most feasible and suitable surrogate for the prediction of clinical outcomes.

The whole-blood concentrations far exceeded total plasma concentrations, indicating that tacrolimus mainly distributes within erythrocytes. This is in line with earlier reports, although the plasma-to-blood ratio we found was considerably lower.[8,15,16] We observed a large variability in unbound tacrolimus plasma concentrations. Moreover, the observed high inter-individual variability in the binding constants (Bmax WBC and Nplasma) indicates highly variable binding of tacrolimus in the central compartment. It has been shown that tacrolimus whole-blood apparent clearance is inversely correlated to hematocrit and erythrocytes count.[17-20] Although comparable with a study in pregnant women, we observed a low median and wide range in hematocrit in this population compared to studies after liver and kidney transplantation.[8,9,12,21] This may be explained by frequent major bleedings peri-operatively, bone marrow depression due to inflammation, blood cell transfusions needed to optimize oxygen demand and hemolysis due to the use of extracorporeal equipment, which are all common events in thoracic transplant patients in the early post-transplant phase. Moreover, we observed a non-linear relationship between whole-blood and unbound tacrolimus plasma concentrations suggesting saturation of erythrocytes. The relatively high dissociation constant indicates that binding to erythrocytes was only slightly non-linear in the observed unbound concentration range, which is substantiated by the relative large confidence interval of this parameter. Although suggested by in vitro observations, saturation has never been observed in vivo before.[8,22,23] A combination of high tacrolimus concentrations with low hematocrit

concentrations may result in excessively high unbound concentrations and consequently lead to tacrolimus-related toxicity.

Interestingly, the maximum binding capacity of erythrocytes (Bmax) showed wide inter-patient variability. Different protein content within erythrocytes may explain this finding. Tacrolimus is bound to the cytoplasmic FK506 binding protein (FKBP) 12 and to a lesser extent to the membrane-associated FKBP13.[16,24-26] Saturation of these proteins within the erythrocyte is the most logical explanation for this effect.[26,27]

The simulation of unbound tacrolimus concentrations with different hematocrit and fixed whole-blood concentration indicated that patients with a low hematocrit tend to have lower whole-blood concentrations, whilst the unbound concentration is around the population mean. In practice, transplant physicians will increase the dose, even though the unbound concentrations as well as the total plasma concentrations will increase as shown by our data. Increasing the dose may lead to higher toxicity by higher unbound concentrations. On the contrary, the risk for rejection is low early after lung transplantation while prevention from cellular and antibody-mediated rejection is also controlled with the addition of induction therapy with an interleukin 2 receptor antagonist, anti-thymocyte globulins or anti-CD52 monoclonal antibodies next to triple therapy with corticosteroids, an anti-proliferative agent and tacrolimus.[28] Notwithstanding a low whole-blood concentration does not imply a low unbound plasma concentration.

Importantly, sample procurement may highly influence unbound tacrolimus plasma concentrations. Swift analysis of the samples is important as well as incubation and centrifugation temperatures. Hemolysis may influence the plasma concentrations whilst no difference was found between analyses at 20°C or 37°C.[15,29,30] In this study, blood samples were immediately centrifuged to minimize erythrocytes damage and tacrolimus distribution from red blood cells to plasma. Importantly, at lower temperatures (4°C) affinity to erythrocytes is higher than at room and body temperature.[29] In this study temperature was kept constant during centrifugation and subsequent filtration at 25°C to diminish a temperature dependent effect on the binding of tacrolimus to erythrocytes.[31] This vulnerability to hemolysis makes analyses of plasma tacrolimus concentrations as a routine practice a major challenge. Some results from previous studies suggest that hematocrit-corrected whole-blood concentrations could be of use for improved target exposure.[11,22,32] The advantage of monitoring hematocrit-corrected whole-blood concentrations over unbound or total plasma concentrations is, that it may be easily implemented in daily transplantation practice.

Strong aspects of this study are the use of full 12-hours profiles, the use of High Performance Liquid Chromatography with tandem Mass Spectrometry (HPLC-MS/MS) for

analyses of tacrolimus concentrations and the use of non-linear mixed-effects modeling (NONMEM) for pharmacokinetic modeling. Earlier studies on unbound concentrations were mostly performed with immuno-assays, which in itself have large variations and may have unreliable results especially in the low range of the unbound concentrations.[7,9,33]

Although a relatively small group of patients was included, the number of tacrolimus profiles was sufficient to perform pharmacokinetic modeling. Nevertheless, the observational character excluded the investigation of causal relationships between unbound plasma concentrations and clinical outcomes. The unbound concentrations have not been related to toxicity before, though have been shown to be related to efficacy.[8] This study did not aim to investigate the associations between tacrolimus plasma concentrations and toxicity and efficacy.

CONCLUSIONS

A two-compartment pharmacokinetic model was designed with mixed zero and first order absorption of tacrolimus whole-blood, total and unbound plasma concentrations in thoracic organ recipients in the first week after transplantation. The unbound concentration was mainly influenced by the variability in erythrocytes count. Erythrocyte binding was saturable. Subsequently, the total or unbound tacrolimus plasma concentrations might be better predictors of clinical outcomes. Nevertheless, robust bio-analysis of the unbound tacrolimus plasma as well as the total plasma concentrations is challenging. Therefore, hematocrit-corrected whole-blood concentrations may serve as the most feasible and suitable predictive exposure measure to improve clinical outcomes and should be further explored in future studies.

METHODS

Data were derived from a previous population pharmacokinetic analysis, involving 30 thoracic organ transplantation patients.(See **Chapter 3**) The accredited review board for human studies of the University Medical Center Utrecht (UMC Utrecht) approved the study (NTR 3912/ EudraCT 2012-001909-24).

Patients

The immunosuppressive regimen contained tacrolimus, Prograft® (Astellas Pharma Europe), a cell cycle blocker, an interleukin 2 inhibitor and corticosteroids. Tacrolimus was dosed orally twice daily (bid) starting with 0.1 mg/kg bid for the lung recipients and 2 mg bid for the heart recipients and was started at the day of transplantation. Dose adjustments were based on whole-blood tacrolimus concentrations at 6 am (C12h). The therapeutic window ranged from 9 to 15 ng/ml for all patients. (See for specific details also **Chapter 3**)

Tacrolimus analyses

Twelve hours profiles of unbound and total tacrolimus plasma concentrations together with whole-blood tacrolimus concentrations were analyzed daily from the transplantation date until 6 days after transplantation as long as they were admitted to the intensive care. Blood samples were collected between 6 pm and 6 am. Blood samples for measurement of unbound and total tacrolimus plasma concentrations were drawn at 0, 2 [or 3 in case of CF], 6 and 12 hours after administration of tacrolimus and collected in vacutainer tubes of 10 ml containing EDTA. Blood samples were immediately centrifuged at the laboratory of the UMC Utrecht. Hereafter, plasma samples were stored at -80°C and were analyzed at the end of the study. Analyses of unbound tacrolimus plasma concentrations was performed as described by Stienstra et.al.[31] The method was validated over a linear range of 1.00–200 pg/ml for unbound tacrolimus concentrations in plasma and 100–3200 pg/ml for total plasma concentrations. The lower limit of quantification was 1.00 pg/ml in ultrafiltrate and 100 pg/ml in plasma. The inaccuracy and imprecision for the determination of unbound tacrolimus concentrations in ultrafiltrate and plasma showed a maximum coefficient of variation (CV) of 11.7% and a maximum bias of 3.8%.

Analyses of whole-blood tacrolimus was conducted using HPLC-MS/MS (Thermo Fisher Scientific) with a lower limit of quantification of 0.5 ng/ml and intraday imprecision of <5%. The HPLC-MS/MS method was adapted from and validated according to the latest European Medicines Agency (EMA) guidelines.[34] The assay has a linear dynamic range of 1-50 ng/ml. Between-run and between-day imprecision (measured by CV) were within 10% and bias was under 3%. Low, median and high controls were all within 15%. Furthermore, results over 5 years from an international inter-proficiency testing program for tacrolimus showed that all external quality controls were within 15%. The unbound

tacrolimus concentrations were quantified using the Thermo Scientific (Waltham, MA) Quantiva LC-MS/MS system with an Ultimate 3000 UHPLC.

Covariates

Clinical and laboratory data were collected for the study period for: sex, age, reason for transplantation, type of transplantation, length and bodyweight, the sequential organ failure assessment score (SOFA), use of extracorporeal membrane oxygenation (ECMO), administration of red blood cells, concentrations of hematocrit, albumin, high density lipoproteins (HDL), AAG and pH (See also Table 1 and S1).

Population pharmacokinetic analysis

NONMEM version 7.3.0 was used for modeling tacrolimus PK. The Piraña software program version 2.9.4 was used as an interface for NONMEM and R for Windows version 3.3.1 was used to analyze the results.

Mixed-effects modeling

A previously developed model for whole-blood tacrolimus PK as developed on the same dataset was used as starting point for model development. In short, this was an open two compartment linear model with first order oral absorption. For some dosing occasions, zero order absorption was used. The structural model included the following parameters: CL, Q, V1, V2 and ka. The rate of binding of tacrolimus within the central compartment to red blood cells and proteins was considered to be much higher than distribution to the peripheral compartment and elimination. Therefore, whole-blood, total plasma and unbound plasma concentrations were assumed to be in equilibrium at all times. To incorporate total and unbound plasma concentrations, models for linear binding kinetics (Equation 1) and models for saturable binding equilibriums (Equation 2) were tested:

$$Equation\ 1:\ WBC = Nplasma * UPC$$

$$Equation\ 2:\ WBC = Bmax * UPC/(Kd + UPC)$$

In which WBC is whole-blood concentration, Nplasma is the non-specific binding constant, UPC is unbound tacrolimus plasma concentration, Bmax corresponds to the maximum binding capacity and Kd is the equilibrium dissociation constant. When the unbound plasma concentration is equal to Kd, the whole-blood concentration is half of the maximum binding capacity.

Total plasma concentrations were related to the unbound plasma concentrations similarly. As tacrolimus is mainly bound to erythrocytes, hematocrit was introduced in the model by

multiplying Nplasma or Bmax with the observed hematocrit with the last observation of hematocrit carried forward.

Inter-individual variability and inter-occasion variability were described assuming a log normal distribution with the following equation:

$$P_{kjm} = \theta_k * e^{(\eta kj + \kappa km)}$$

in which P_{kjm} is the estimate for parameter k for the j^{th} individual at occasion m, θ_k is the population value for the k^{th} PK parameter, η_{kj} represents the inter-individual variability which is assumed to have a normal distribution with mean 0 and standard deviation ω_k and κ_{km} represents the inter-occasion variability which is assumed to have a mean 0 and standard deviation of π_k. The residual error was assumed to be proportional to the predicted concentration:

$$C_{ij} = Cpred_{ij}\,(1 + e_{ij})$$

in which C_{ij} is the i^{th} observation for the j^{th} individual, $Cpred_{ij}$ is the tacrolimus concentration predicted by the model, and e_{ij} is the difference between C_{ij} and $Cpred_{ij}$. All values of eij were assumed to be normally distributed with mean 0 and standard deviation σ. Residual error was separately estimated for whole-blood, total and unbound plasma concentrations. Correlation between these residual error components was estimated using the L2 data option of NONMEM.

The modeling process was performed using the stochastic approximation expectation maximization (SAEM) estimation method with interaction. The likelihood was subsequently established using the Monte Carlo importance Sampling EM assisted by mode a posteriori estimation method (IMPMAP). The parameter precision was estimated using the SIR procedure (Sampling Importance Resampling).[35] The values of concentrations below the lower limit of quantification (LLOQ) were discarded (3.9%; 46 values out of 1180). Model diagnostics were performed by visual checks of standard diagnostic plots i.e. 'goodness of fit' plots (See Figure 3).

Pharmacokinetic simulation

The effect of hematocrit on unbound tacrolimus plasma concentrations was assessed by simulation of unbound plasma concentrations for different hematocrit values at a constant whole-blood concentration (9 ng/ml) using final typical parameter estimates.

Statistical analyses

Variables are presented as, median (with the 1st and 3rd quartiles (Q1; Q3)), range, 95%CI or number (proportion) where appropriate.

REFERENCES

1. Wallemacq P, Armstrong VW, Brunet M, et al. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. In: Vol 31. 2009:139-152. doi:10.1097/FTD.0b013e318198d092.
2. Rayar M, Tron C, Jézéquel C, et al. High Intrapatient Variability of Tacrolimus Exposure in the Early Period After Liver Transplantation Is Associated With Poorer Outcomes. *Transplantation*. 2018;102(3):e108-e114. doi:10.1097/TP.0000000000002052.
3. Gueta I, Markovits N, Yarden-Bilavsky H, et al. High tacrolimus trough level variability is associated with rejections after heart transplant. *Am J Transplant*. 2018;18(10):2571-2578. doi:10.1111/ajt.15016.
4. Bouamar R, Shuker N, Hesselink DA, et al. Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomized-controlled clinical trials(†). *Am J Transplant*. 2013;13(5):1253-1261. doi:10.1111/ajt.12191.
5. Sikma MA, Hunault CC, Kirkels JH, Verhaar MC, Kesecioglu J, de Lange DW. Association of Whole Blood Tacrolimus Concentrations with Kidney Injury in Heart Transplantation Patients. *Eur J Drug Metabol Pharmacokinet*. 2018;43(3):311-320. doi:10.1007/s13318-017-0453-7.
6. Sikma MA, Hunault CC, van de Graaf EA, et al. High tacrolimus blood concentrations early after lung transplantation and the risk of kidney injury. *Eur J Clin Pharmacol*. 2017;73(5):573-580. doi:10.1007/s00228-017-2204-8.
7. Zahir H, Nand RA, Brown KF, Tattam BN. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. *journal of pharmacological and toxicological methods*. 2001;46:27-35. doi: 10.1016/s1056-8719(02)00158-2
8. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Factors affecting variability in distribution of tacrolimus in liver transplant recipients. *British Journal of Clinical Pharmacology*. 2004;57(3):298-309. doi: 10.1046/j.1365-2125.2003.02008.x
9. Zheng S, Davis CL, Hebert MF. Pharmacokinetics of Tacrolimus During Pregnancy. *therapeutic drug monitoring*. 2012;34:660-670. doi: 10.1097/ftd.0b013e3182708edf
10. Brooks E, Tett SE, Isbel NM, Staatz CE. Population Pharmacokinetic Modelling and Bayesian Estimation of Tacrolimus Exposure: Is this Clinically Useful for Dosage Prediction Yet? *Clinical Pharmacokinetics*. 2016;55(11):1295-1335. doi:10.1007/s40262-016-0396-1.
11. Schijvens AM, van Hesteren FHS, Cornelissen EAM, et al. The potential impact of hematocrit correction on evaluation of tacrolimus target exposure in pediatric kidney transplant patients. *Pediatr Nephrol*. October 2018:1-9. doi:10.1007/s00467-018-4117-x.
12. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Changes in tacrolimus distribution in blood and plasma protein binding following liver transplantation. *therapeutic drug monitoring*. 2004;26(5):506-515. doi: 10.1097/00007691-200410000-00008

13. Hebert MF, Zheng S, Hays K, et al. Interpreting tacrolimus concentrations during pregnancy and postpartum. *Transplantation*. 2013;95(7):908-915. doi:10.1097/TP.0b013e318278d367.
14. Sikma MA, van Maarseveen EM, van de Graaf EA, et al. Pharmacokinetics and Toxicity of Tacrolimus Early After Heart and Lung Transplantation. *Am J Transplant*. 2015;15(9):2301-2313. doi:10.1111/ajt.13309.
15. Bittersohl H, Schniedewind B, Christians U, Luppa PB. A simple and highly sensitive on-line column extraction liquid chromatography-tandem mass spectrometry method for the determination of protein-unbound tacrolimus in human plasma samples. *J Chromatogr A*. 2018;1547:45-52. doi:10.1016/j.chroma.2018.03.010.
16. Nagase K, Iwasaki K, Nozaki K, Noda K. Distribution and protein binding of FK506, a potent immunosuppressive macrolide lactone, in human blood and its uptake by erythrocytes. *J Pharm Pharmacol*. 1994;46(2):113-117. doi:10.1111/j.2042-7158.1994.tb03752.x.
17. Benkali K, Prémaud A, Picard N, et al. Tacrolimus population pharmacokinetic-pharmacogenetic analysis and Bayesian estimation in renal transplant recipients. *Clinical Pharmacokinetics*. 2009;48(12):805-816. doi:10.2165/11318080-000000000-00000.
18. Zheng S, Easterling TR, Umans JG, et al. Pharmacokinetics of tacrolimus during pregnancy. *therapeutic drug monitoring*. 2012;34(6):660-670. doi:10.1097/FTD.0b013e3182708edf.
19. De Jonge H, Vanhove T, de Loor H, Verbeke K, Kuypers DRJ. Progressive decline in tacrolimus clearance after renal transplantation is partially explained by decreasing CYP3A4 activity and increasing haematocrit. *British Journal of Clinical Pharmacology*. 2015;80(3):548-559. doi:10.1111/bcp.12703.
20. Andrews LM, Hesselink DA, Van Gelder T, et al. A Population Pharmacokinetic Model to Predict the Individual Starting Dose of Tacrolimus Following Pediatric Renal Transplantation. *Clinical Pharmacokinetics*. 2018;57(4):475-489. doi:10.1007/s40262-017-0567-8.
21. Størset E, Holford N, Hennig S, et al. Improved prediction of tacrolimus concentrations early after kidney transplantation using theory-based pharmacokinetic modelling. *British Journal of Clinical Pharmacology*. 2014;78(3):509-523. doi: 10.1111/bcp.12361
22. Størset E, Holford N, Midtvedt K, Bremer S, Bergan S, Åsberg A. Importance of hematocrit for a tacrolimus target concentration strategy. *Eur J Clin Pharmacol*. 2014;70(1):65-77. doi:10.1007/s00228-013-1584-7.
23. Chow F-S, Piekoszewski W, Jusko WJ. Effect of hematocrit and albumin concentration on hepatic clearance of tacrolimus (FK506) during rabbit liver perfusion. *Drug Metabolism and Disposition*. 1997;25(5):610-616.
24. Biagiotti S, Paoletti MF, Fraternale A, Rossi L, Magnani M. Drug delivery by red blood cells. Muzykantov V, ed. *IUBMB Life*. 2011;63(8):621-631. doi:10.1002/iub.478.
25. Biagiotti S, Rossi L, Bianchi M, et al. Immunophilin-loaded erythrocytes as a new delivery strategy for immunosuppressive drugs. *J Control Release*. 2011;154(3):306-313. doi:10.1016/j.jconrel.2011.05.024.

26. Walensky LD, Gascard P, Fields ME, et al. The 13-kD FK506 binding protein, FKBP13, interacts with a novel homologue of the erythrocyte membrane cytoskeletal protein 4.1. *J Cell Biol*. 1998;141(1):143-153. 10.1083/jcb.141.1.143
27. Van Acker K, Bultynck G, Rossi D, et al. The 12 kDa FK506-binding protein, FKBP12, modulates the Ca(2+)-flux properties of the type-3 ryanodine receptor. *J Cell Sci*. 2004;117(Pt 7):1129-1137. doi:10.1242/jcs.00948.
28. Scheffert JL, Raza K. Immunosuppression in lung transplantation. *J Thorac Dis*. 2014;6(8):1039-1053. doi:10.3978/j.issn.2072-1439.2014.04.23.
29. Machida M, Takahara S, Ishibashi M, Hayashi M. Effect of temperature on hematocrit on plasma concentration of FK506. *Transplantation Proceedings*. 1991;23(6):2753-2754. doi: 10.5980/jpnjurol1989.84.1088
30. J BA, M WRH, H BG, van der J H, G K, van H A. FK 506: monitoring in plasma or in whole blood? *Transplantation Proceedings*. 1991;23(6):325-330. doi:10.1016/j.intimp.2009.12.003.
31. Stienstra NA, Sikma MA, van Dapperen AL, de Lange DW, Van Maarseveen EM. Development of a Simple and Rapid Method to Measure the Free Fraction of Tacrolimus in Plasma Using Ultrafiltration and LC-MS/MS. *therapeutic drug monitoring*. 2016;38(6):722-727. doi:10.1097/FTD.0000000000000351.
32. Gérard C, Stocco J, Hulin A, et al. Determination of the most influential sources of variability in tacrolimus trough blood concentrations in adult liver transplant recipients: a bottom-up approach. *AAPS J*. 2014;16(3):379-391. doi:10.1208/s12248-014-9577-8.
33. Piekoszewski W, Jusko WJ. Plasma protein binding of tacrolimus in humans. *J Pharm Sci*. 1993;82(3):340-341. doi: 10.1002/jps.2600820325
34. Marinova M, Artusi C, Brugnolo L, Antonelli G, Zaninotto M, Plebani M. Immunosuppressant therapeutic drug monitoring by LC-MS/MS: workflow optimization through automated processing of whole blood samples. *Clinical Biochemistry*. 2013;46(16-17):1723-1727. doi:10.1016/j.clinbiochem.2013.08.013.
35. Dosne A-G, Bergstrand M, Harling K, Karlsson MO. Improving the estimation of parameter uncertainty distributions in nonlinear mixed effects models using sampling importance resampling. *J Pharmacokinet Pharmacodyn*. 2016;43(6):583-596. doi:10.1007/s10928-016-9487-8.

TABLES

Table 1. Patient characteristics

Characteristic	N=30 (%)	Median (Q1;Q3)
Male	15 (50%)	--
Age (yr)	--	43 (34;60)
Bodyweight (kg)	--	73.5 (61;86)
Length (cm)	--	173.5 (169;176)
Reason for transplantation		
Heart (N=10)		
Ischemic CMP	5 (17%)	--
Non-ischemic CMP	5 (17%)	--
Lung (N=20)		
Cystic Fibrosis	10 (33%)	--
COPD	3 (10%)	--
IPAH	2 (7%)	--
Bronchiectasis	1 (3%)	--
Sarcoidosis	1 (3%)	--
Langerhans cell histiocytosis	1 (3%)	--
Idiopathic pulmonary fibrosis	2 (7%)	--
Double lung transplantation	18 (90%)	--
Parameters		
Ht[a]		
Day 1		0.31 (0.28;0.35)
Day 2		0.28 (0.25;0.30)
Day 3		0.27 (0.25;0.28)
Day 4		0.27 (0.25;0.29)
Day 5		0.27 (0.24;0.30)
Day 6		0.28 (0.27;0.29)
Alb[b]		
Day 1		26.2 (22.5;29.3)

Table 1. Patient characteristics

Characteristic	N=30 (%)	Median (Q1;Q3)
HDL[c]		
Day 1		0.84 (0.70;1.06)
AAG[d]		
Day 1		0.89 (0.76;1.18)
pH[e]		
Day 1		7.39 (7.33;7.43)
Administration of packed red blood cells (ml/day)		
Day 1		275 (275;550)
Postoperative ECMO frequency	8 (27%)	
Postoperative ECMO duration (days)		4 (2;6)

CMP= cardiomyopathy, COPD= chronic obstructive pulmonary disease, IPAH= idiopatic pulmonary arterial hypertension, Ht= Hematocrit, Alb=Albumin, HDL=High Density Lipoprotein, AAG=α1-acid glycoprotein, ECMO= extracorporeal membrane oxygenator
Normal ranges: [a]Ht; male 0.41-0.50, female 0.36-0.46; [b]Alb; 35-50 g/L, [c]HDL; male 0.90-1.70, female 1.10-2.00 mmol/L; [d]AAG ; 0.5-1.2 g/L; [e]pH; 7.35-7.45

Table 2. Pharmacokinetic parameters

Observed pharmacokinetics	Median (min-max)
Unbound tacrolimus plasma concentrations	
C12h (pg/ml)	1.84 (0.42-11)
Cmax (pg/ml)	2.97 (0.51-12.2)
Tmax (hr)	2.3 (1.2-14.0)
Total Plasma Concentrations	
C12h (pg/ml)	282 (46-1373)
Cmax (pg/ml)	403.5 (61-2640)
Tmax (hr)	2.25 (0.4-14.0)
Whole-blood tacrolimus concentrations	
C12h (ng/ml)	9.5 (0.5-38.7)
Cmax (ng/ml)	18.5 (2.1-74.7)
Tmax (hr)	1.6 (0.4-8.0)

C12h=concentration at 12 hours after administration, Cmax=maximum C12h, Tmax= time to maximum concentration

Table 3. Final population pharmacokinetic parameters with 95% confidence interval based on SIR

Parameter	Estimated value (95% CI)	IIV (%) (95% CI)	IOV (%) (95% CI)
CL (L/h)	20.9 (16.8 - 24.7)	42.1 (30 - 60)	
V1 (L)	220 (187 - 246)	10 Fixed	
Ka (1/h)	0.579 Fixed	10 Fixed	98.3 Fixed
Q (L/h)	72.0 (529 - 767)	10 Fixed	
V2 (L)	469 (399 - 579)	10 Fixed	
Bmax (WBC) (pg/ml)	2700 (1750 - 3835)	27 (19-36)	
Kd (WBC) (pg/ml)	0.142 (0.087 - 0.195)	3 Fixed	
Nplasma	137 (120 - 152)	29 (22 - 41)	
F	1 Fix	10 Fix	65 (58 - 84)
RUV	**SD-PE (95% CI)**		
WBC	16.7% (15.8 - 17.6)		
UPC	36.3% (33,9 - 40.4)		
TPC	31.6% (28.6 - 34.2)		
Correlation RUV (WBC, UPC and TPC)	**R**		
R (WBC, UPC)	0.26		
R (WBC, TPC)	0.51		
R (UPC, TPC)	0.51		
Parameter	**Range (min – max)**		
AUC (WBC) (ug.hr/L)	151.2 (31.2 - 2525)		
AUC (UPC) (ng.hr/L)	266 (10 - 928)		
T1/2 (WBC) (hr)	9.4 (6.0 - 31.4)		

SIR=sampling importance resampling, IIV=inter individual variability, IOV=inter occasional variability, Cl=clearance, V=distribution volume, ka=absorption constant rate, Q=inter-compartmental clearance, Bmax=maximum binding capacity, kd=diffusion constant rate, N2=total plasma to unbound plasma coefficient, F=bioavailability, UPC=unbound plasma concentration, RUV=Residual unexplained variability, SD-PE=Standard Deviation Point Estimate, WBC=whole-blood concentration, TPC=total plasma concentration, R=correlation coefficient

FIGURES

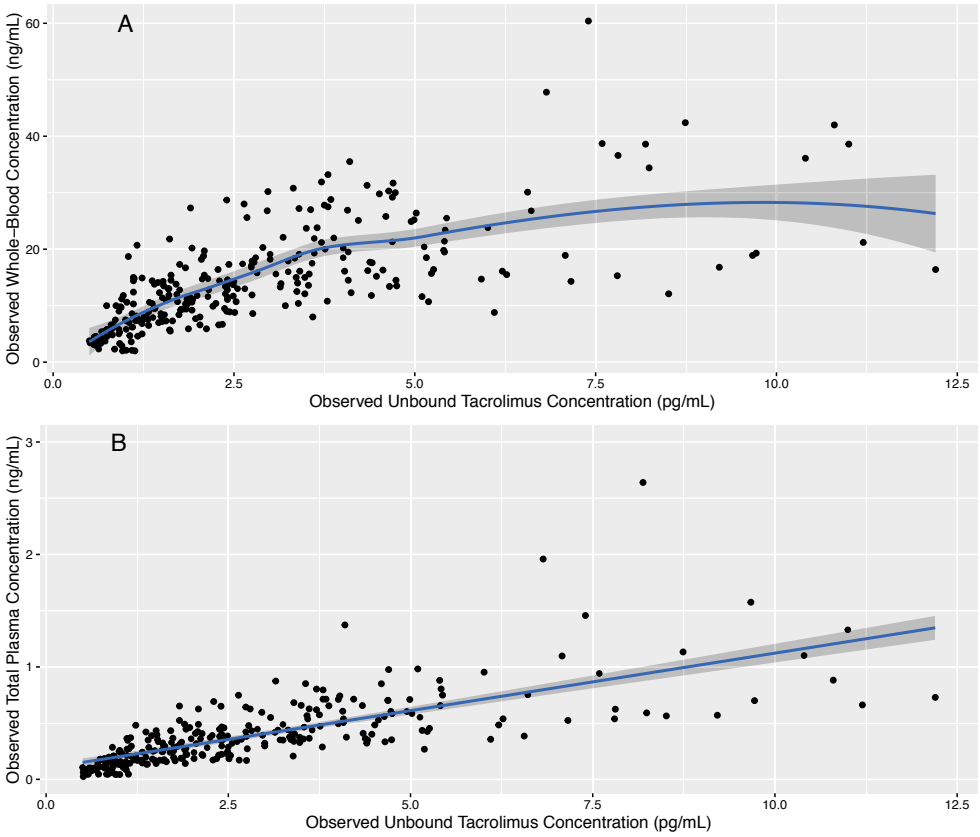

Fig. 1a and 1b. a. Tacrolimus unbound concentrations versus tacrolimus whole-blood concentrations. The figure shows a non-linear relationship between tacrolimus unbound plasma concentrations (UPC) and whole-blood concentrations (WBC). b. Tacrolimus unbound concentrations versus tacrolimus total plasma concentrations (TPC). The figure shows a linear relationship between unbound tacrolimus plasma concentrations and total plasma concentrations.

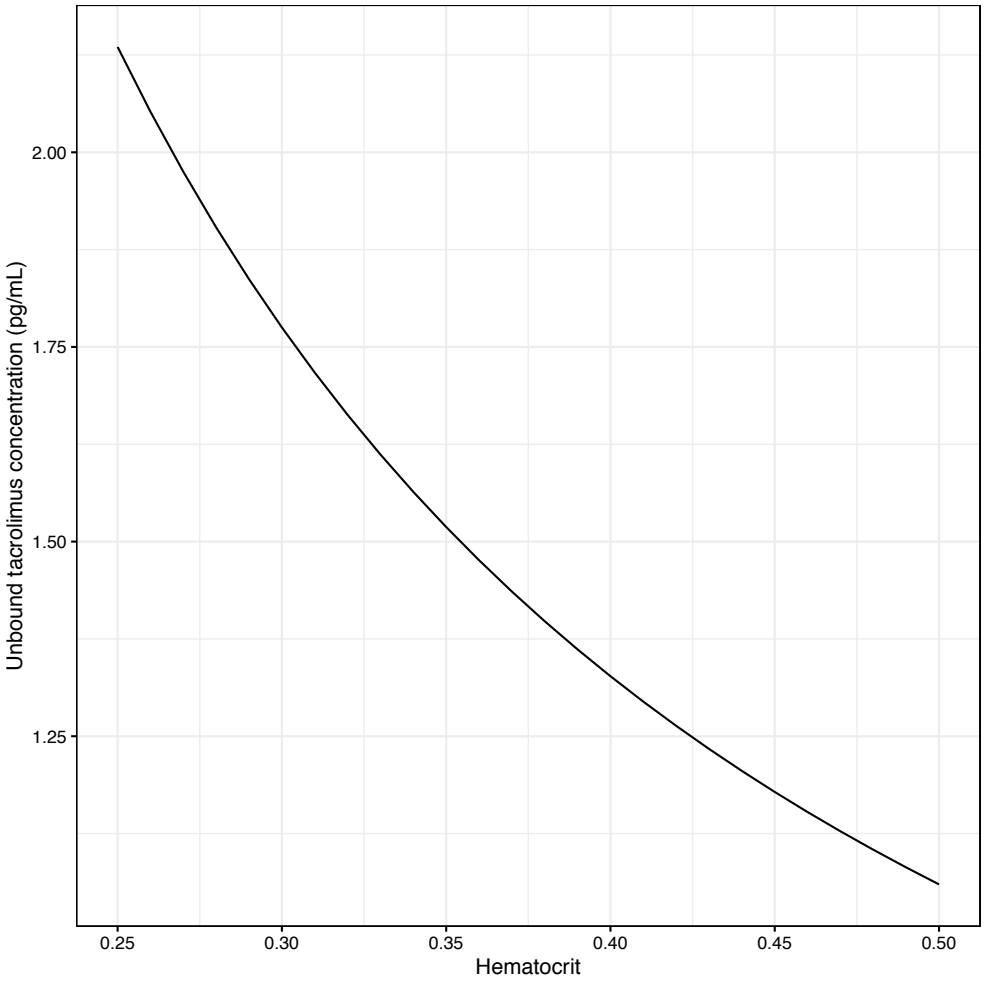

Fig. 2. Simulations of different hematocrit values with fixed whole-blood concentration of 9 ng/ml. On the Y-axis the unbound tacrolimus plasma concentrations are plotted against hematocrit.

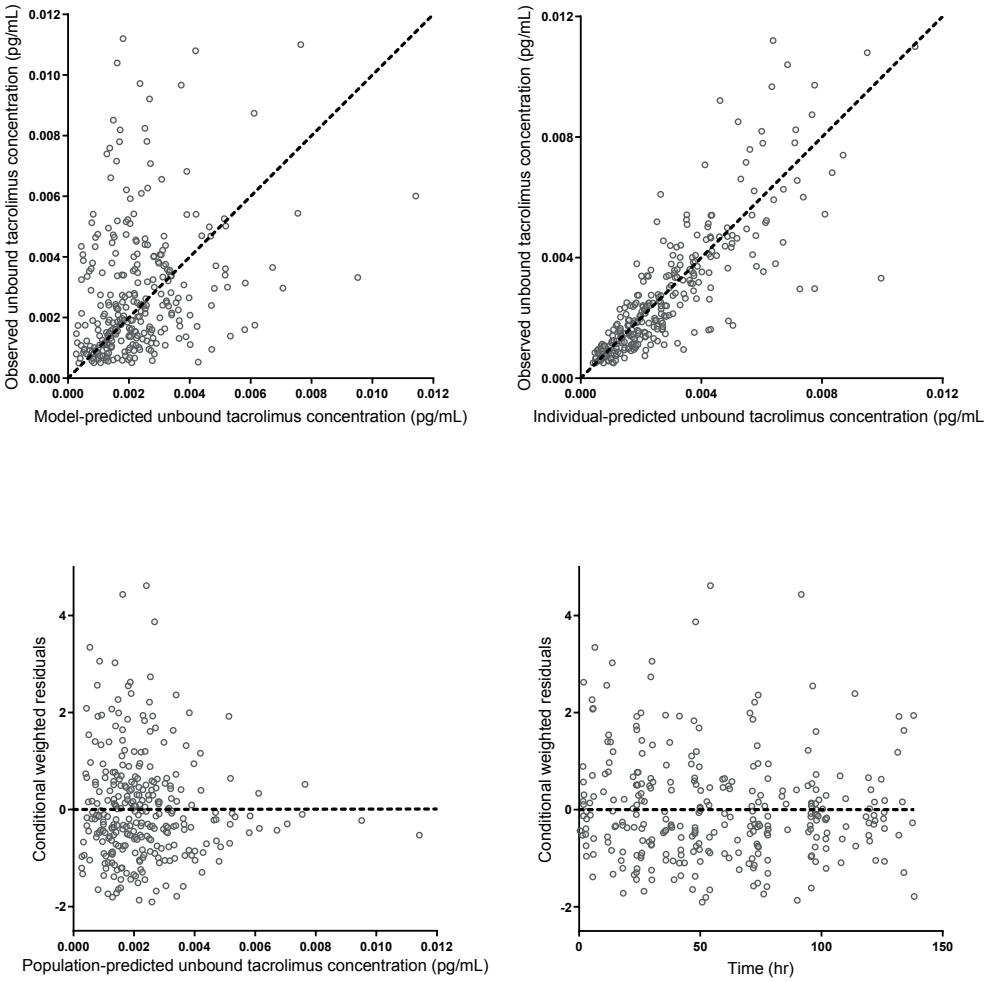

Fig. 3. Goodness-of-fit plots of predicted unbound tacrolimus plasma concentrations

SUPPLEMENTARY MATERIALS

Unbound plasma, total plasma and whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation

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FIGURES

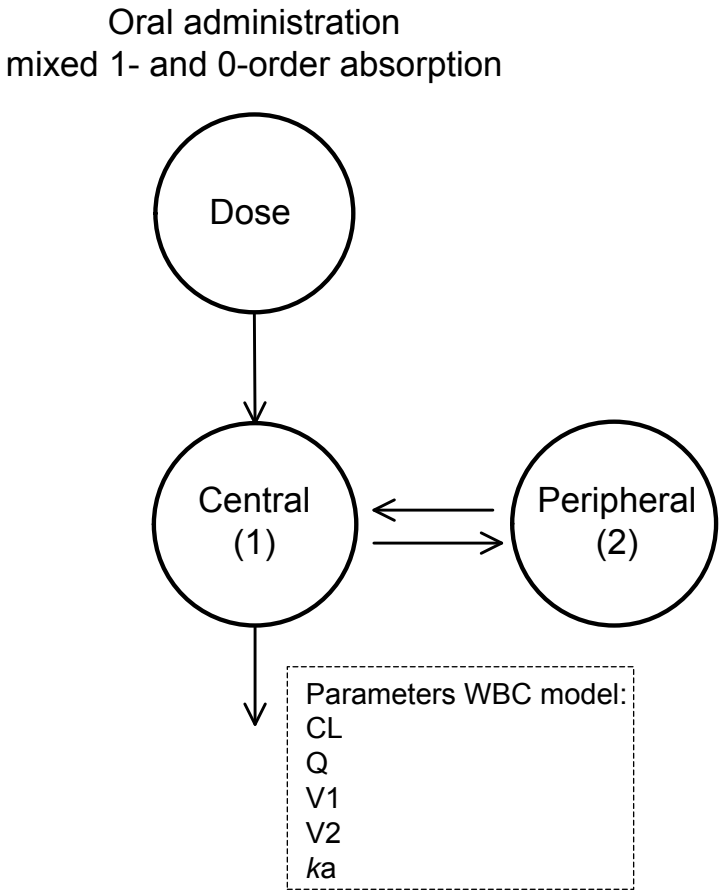

Figure S1. Schematic representation of the population pharmacokinetic whole-blood concentration (WBC) model for tacrolimus. The central compartment, with volume V1, is swiftly in equilibrium with the peripheral compartment represented by volume V2. Drug transfer between this peripheral compartment and the central compartment is described with the inter-compartmental clearance parameter Q. ka is the absorption rate constant and CL is the whole-blood tacrolimus clearance. The unbound plasma concentration of tacrolimus (UPC) was computed using a non-linear model, as follows: UPC = (WBC * kd1) / (Bmax* Ht - WBC) with kd1 = dissociation constant (fitted parameter); Bmax = maximum binding capacity (fitted parameter) and Ht = observed hematocrit (last observation carried forward). The total plasma concentration (TPC) was computed using a linear model, as follows: TPC = Nplasma * UPC with Nplasma = non-specific binding constant for total plasma concentrations (fitted parameter).

TABLES

Table S1. Parameters displayed per day

Parameters	N=30 (%)	Median (Q1;Q3)
Hematocrit		
Day 1		0.31 (0.28;0.35)
Day 2		0.28 (0.25;0.30)
Day 3		0.27 (0.25;0.28)
Day 4		0.27 (0.25;0.29)
Day 5		0.27 (0.24;0.30)
Day 6		0.28 (0.27;0.29)
Albumin (g/L)		
Day 1		26.2 (22.5;29.3)
Day 2		25.7 (22.2;28.2)
Day 3		25.3 (23.0;26.6)
Day 4		24.9 (22.6;27.1)
Day 5		24.3 (22.5;26.9)
Day 6		24.0 (22.8;25.4)
High Density Lipoprotein (mmol/L)		
Day 1		0.84 (0.70;1.06)
Day 2		0.89 (0.73;1.09)
Day 3		0.89 (0.76;1.04)
Day 4		0.93 (0.75;1.05)
Day 5		0.94 (0.80;1.01)
Day 6		0.96 (0.83;1.11)
α1-acid glycoprotein (g/L)		
Day 1		0.89 (0.76;1.18)
Day 2		1.37 (1.07;1.55)
Day 3		1.66 (1.37;2.04)
Day 4		1.79 (1.64;2.27)
Day 5		1.82 (1.67;2.24)
Day 6		1.90 (1.30;2.11)

Table S1. Parameters displayed per day

Parameters	N=30 (%)	Median (Q1;Q3)
pH		
Day 1		7.39 (7.33;7.43)
Day 2		7.40 (7.38;7.43)
Day 3		7.43 (7.39;7.46)
Day 4		7.44 (7.40;7.46)
Day 5		7.46 (7.45;7.48)
Day 6		7.47 (7.44;7.50)
Administration of packed red blood cells (ml/day) among patients receiving transfusion		
Day 1		550 (275;825)
Day 2		275 (275;688)
Day 3		275 (275;275)
Day 4		275 (275;275)
Day 5		413 (275;413)
Day 6		275 (275;1100)
SOFA scores per day		
Day 1		9 (7;17)
Day2		8 (5;10)
Day 3		5 (3;8)
Day 4		5 (3;15)
Day 5		4 (3;11)
Day 6		4 (2;9)
Frequency of Ileus per day		
Day 1	27 (90%)	
Day 2	24 (80%)	
Day 3	14 (47%)	
Day 4	5 (17%)	
Day 5	2 (7%)	
Day 6	2 (7%)	

Table S1. Parameters displayed per day

Parameters	N=30 (%)	Median (Q1;Q3)
Frequency of diarrhea per day		
Day 1	0 (0%)	
Day 2	2 (7%)	
Day 3	8 (27%)	
Day 4	9 (30%)	
Day 5	11 (37%)	
Day 6	10 (33%)	
Frequency of liver dysfunction per day		
Day 1	7 (23%)	
Day 2	6 (20%)	
Day 3	3 (10%)	
Day 4	3 (13%)	
Day 5	7 (23%)	
Day 6	6 (20%)	
Frequency of ECMO use per day		
Day 1	8 (27%)	
Day 2	7 (23%)	
Day 3	5 (17%)	
Day 4	4 (13%)	
Day 5	3 (10%)	
Day 6	3 (10%)	
Number of drugs increasing tacrolimus concentration (min-max)		
Day 1		2 (1-4)
Day 2		1 (0-2)
Day 3		1 (0-4)
Day 4		1 (0-5)
Day 5		0 (0-6)
Day 6		1 (0-4)

Table S1. Parameters displayed per day

Parameters	N=30 (%)	Median (Q1;Q3)
Number of patients with drugs increasing tacrolimus concentration		
Day 1	30 (100%)	
Day 2	23 (77%)	
Day 3	23 (77%)	
Day 4	17 (57%)	
Day 5	14 (47%)	
Day 6	16 (53%)	
Number of drugs decreasing tacrolimus concentration (min-max)		
Day 1		0 (0-0)
Day 2		1 (1-2)
Day 3		1 (0-1)
Day 4		1 (0-1)
Day 5		1 (0-1)
Day 6		1 (0-1)
Number of patients with drugs decreasing tacrolimus concentration		
Day 1	0 (0%)	
Day 2	30 (100%)	
Day 3	26 (87%)	
Day 4	21 (70%)	
Day 5	18 (60%)	
Day 6	18 (60%)	
Renal function		
Baseline Creatinine clearance (ml/min/1.73m2)		85 (73;116)
Pharmacogenetic analyses		
Slow metabolizers of CYP 3A5	21 (70%)	
Slow metabolizers of CYP3A4		
Homozygosity CYP3A4*22	0 (0%)	
Heterozygosity and Homozygosity of POR*28	18 (60%)	

Table S1. Parameters displayed per day

Parameters	N=30 (%)	Median (Q1;Q3)
PPARα	16 (53%)	
Decreased ABCB1 activity		
Decreased PXR activity	18 (60%)	
G1199A homozygosity	0 (0%)	
C1236T homozygosity	6 (20%)	
G2677T homozygosity	8 (27%)	
C3435T homozygosity	8 (27%)	
OATP1B1 homozygosity	0 (0%)	

CHAPTER 5

5

Discussion and future perspectives Optimizing tacrolimus dosing in the early post-transplant phase in thoracic organ recipients

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This manuscript is submitted

DISCUSSION AND FUTURE PERSPECTIVES

Tacrolimus is considered the cornerstone of immunosuppressant regimens of solid organ transplantation since the late 20th century. Early after thoracic organ transplantation, tacrolimus is difficult to dose because of considerable physiological changes due to clinical instability. We will discuss the variability in tacrolimus pharmacokinetics due to these physiological changes and the consequences for therapeutic monitoring and dosing.

Extensive research has demonstrated the efficacy of tacrolimus in solid organ transplantation.[1-3] For instance, acute rejection rates after 6 months (BPAR grade ≥ 3A) have shown to be significantly lower for tacrolimus (28%) than for cyclosporine A (42%).[4] Although tacrolimus is known to be effective, heart and lung transplantation patients often show signs of toxicity and rejection.[5-8] Toxicity and rejection both have major consequences for the outcome of heart and lung transplantation with a higher risk for morbidity and mortality.[5,9-11] Acute kidney injury often evolves into chronic kidney disease, and appears in approximately half of the patients during the first weeks after thoracic organ transplantation.[5,6] The occurrence of acute kidney injury has been associated with supra-therapeutic (>15 ng/ml) whole-blood tacrolimus trough concentrations in the first week after thoracic organ transplantation (See also **Chapters 2a and 2b**).[7,8] Moreover, a higher rejection rate has been associated with a high variability in whole-blood concentrations after heart and lung transplantation.[12,13] Therefore, it is of the utmost importance to prevent supra-therapeutic whole-blood concentrations and to reduce the variability in tacrolimus concentrations.

In the first days after transplantation, heart and lung recipients frequently show a high variability in tacrolimus blood concentrations, due to clinical instability caused by shock and systemic inflammation (See Figure 1 for a schematic overview of tacrolimus pharmacokinetics and Table 4 **Chapter 1** for the effect of physiological changes).[14] The systemic inflammation resulting in organ dysfunction is due to the surgical procedure with the application of (extended) extracorporeal circulation, as well as ischemia-reperfusion injury of the transplanted organ(s) and bleeding with blood transfusions.[14] For instance, gut dysmotility may highly influence absorption of tacrolimus which is already limited in stable patients with an estimated bioavailability of around 25%.[15-17] Furthermore, drug-drug interactions may lead to a large variability in tacrolimus pharmacokinetics.[14] Corticosteroids for instance, are involved in induction of the CYP3A enzymes and the ABCB1-transporter, respectively. Tapering of corticosteroids increases bioavailability of tacrolimus.[18] These unfavorable clinical conditions alter the pharmacokinetics and induce large variations in tacrolimus concentrations. We demonstrated that whole-blood tacrolimus concentrations at 12 hours post-administration (C12h) highly vary after heart and lung transplantation and this extremely high dose-to-dose variability persists in the

first week post-transplantation (See **Chapter 3**). The majority of C12h, almost 70%, were out of the target range (9-15 ng/ml). Half of patients displayed sub-therapeutic concentrations and approximately 20% of patients displayed supra-therapeutic concentrations. Moreover, the inter-occasion (dose-to-dose) variability in pharmacokinetics was extreme and exceeded the inter-patient variability. Personalizing the dose based on C12h showed to be virtually impossible in clinically unstable thoracic organ recipients. Yet, this does not mean that tacrolimus monitoring is redundant. It should still be used to prevent toxicity (See **Chapter 3**).

We showed that the inter-occasion variability was mainly caused due to a substantial variability in relative bioavailability in patients exhibiting clinical instability. Extremely slow (Tmax >8 hours) as well as extremely rapid (Tmax <30 minutes) absorption was observed. The variability in this bioavailability (55%) far exceeded the variability of other pharmacokinetic parameters, such as clearance (35%), indicating highly variable absorption of tacrolimus (**Chapter 3**). The situation totally differs from that of kidney transplant recipients early after transplantation, in whom bioavailability has been shown to be dose dependent and with a much smaller inter-occasion variability of approximately 25%.[16,17] To circumvent this high variability in bioavailability, intravenous administration may be preferred over oral administration early post-transplantation. Applying tacrolimus intravenously may improve tacrolimus dosing despite the higher costs and risk of additional nephrotoxicity of the solvent HCO-60.[14,19,20]

In fact, one may even wonder if the whole-blood tacrolimus concentration is an adequate predictor of clinical outcomes in the early post-transplant phase. Although acute kidney injury seems to be associated with supra-therapeutic whole-blood concentrations, the relation between tacrolimus whole-blood exposure and the development of nephrotoxicity is poor. Even within the therapeutic whole-blood concentration range, tacrolimus-associated nephrotoxicity arises.[2,3] The unbound tacrolimus plasma concentrations might be a better proxy for the prediction of clinical outcomes.[21] Tacrolimus is particularly distributed into erythrocytes, next to being associated with (lipo)proteins (See also Figure 2).[22,23] Within the erythrocytes, tacrolimus is known to be highly associated to the FK-binding protein.[24-27] In the clinically unstable transplant patient, erythrocyte counts may highly fluctuate due to bleeding, red blood cells transfusions, dilution, bone marrow depression and hemolysis due to extracorporeal equipment. This has large consequences with regard to the interpretation of the whole-blood concentrations, e.g., a decrease in red blood cells decreases whole-blood concentrations, though not necessarily the unbound concentrations. To correct for these low whole-blood tacrolimus concentrations, transplant physicians might be enticed to raise the dose. However, raising the dose may lead to higher unbound concentrations posing the patient at a higher risk of toxicity, whilst decreasing the dose or at least not increasing the dose might be more appropriate based on the

unbound concentration (**Chapter 4b**). Yet, a 12-hour whole-blood concentration above 15 ng/ml necessitates lowering or discontinuing the dose until concentrations fall below this level. Aiming for the lower therapeutic range value in the early phase after transplantation may decrease the unbound concentration, hence the risk for toxicity. For instance, 9 ng/ml could be targeted when the therapeutic whole-blood range is 9-15 ng/ml.

Although the unbound concentration is known to be related to hematocrit, studies investigating the unbound tacrolimus plasma concentrations are scarce, because quantification of unbound tacrolimus concentrations is bio-analytically challenging, costly and time consuming.[28] Yet, the accuracy and precision of plasma concentration quantification is vulnerable to hemolysis of the whole-blood sample. As such, the relationship between whole-blood and unbound concentrations has not systematically been studied and no pharmacokinetic models are currently available to predict the unbound concentrations based on whole-blood concentrations. Moreover, a therapeutic range of unbound tacrolimus plasma concentrations is lacking for routine therapeutic drug monitoring.[22,28-30] We studied the variability of plasma concentrations in clinically unstable thoracic organ recipients with a viable analysis of unbound and total tacrolimus plasma concentrations (See also **Chapter 4b**).[31] It was confirmed that the majority of tacrolimus in whole-blood was distributed into erythrocytes. Less than 1% was present in plasma and thus, associated with (lipo)proteins. Interestingly, the whole-blood concentrations presented saturation, which probably originates from saturation of binding to FK-binding protein within the erythrocytes.[27,32] This saturation of erythrocytes may especially occur at low hematocrit concentrations. When hematocrit decreases, there is a non-linear increase in the unbound concentration. Because of the large influence of hematocrit on whole-blood concentrations, hematocrit-corrected whole-blood concentrations may be suitable as substitute for the prediction of clinical outcomes. As an example, a decline in hematocrit from 0.35 to 0.26 may indicate an increase in the unbound tacrolimus plasma concentrations by approximately 25%. Lowering the dose by 25% should be sufficient to control the unbound tacrolimus plasma concentration and subsequently, reduce toxicity. Therapeutic drug monitoring based on hematocrit-corrected whole-blood concentrations may be directly implemented in daily practice and may improve tacrolimus dosing in clinically unstable thoracic organ recipients in order to reduce toxicity and rejection.

How may we improve tacrolimus dosing even further in future? In heart transplantation patients postponing tacrolimus up to two weeks by using an interleukin 2 inhibitor has shown to be equally effective with less toxicity.[33] This may be more efficacious than lowering the dose.[34,35] For lung transplantation patients postponing calcineurin inhibition may be a risky strategy because of the relatively higher risk of rejection in these patients. Increasing the time within therapeutic range by 10% has been associated with a significantly lower likelihood of acute rejection at 1 year.[36] Therefore, improving tacrolimus dosing in the

clinically unstable phase remains particularly necessary in pulmonary transplant patients. Wearable nanotechnology detecting the continuous changes in tacrolimus concentrations may improve precision dosing in the near future.[37] Furthermore, computerized dosing, such as covariate and Bayesian dosing, may help to improve tacrolimus dosing.[38] With covariate-based dosing, the dosing is based on patient-specific variables (like weight, age, genetics and serum creatinine) using population pharmacokinetic parameter values. The Bayesian approach uses the same a priori population pharmacokinetic parameter values as the initial estimate for an individual. However, when one or more patient's drug levels are measured, the Bayesian approach uses them to adjust the next estimate, taking into consideration the variability of both the population parameters and the blood concentrations.[38,42] Covariate-based and Bayesian-based dosing have already been shown to improve the achievement of target concentrations in renal transplant recipients.[17,39,40] When a Bayesian dosing approach based on a two-compartment model with first-order absorption and a lag time was used in renal transplant patients, the median proportion of concentrations within the target range was significantly higher for the computer group (77%) than in the control group (59%).[41] However, tacrolimus pharmacokinetics are highly variable in clinically unstable thoracic organ recipients and do not reach steady state in the first days after transplantation. This makes prediction of the next unbound tacrolimus plasma concentration highly complex and prediction of the next dose a challenge, even with the use of computerized calculations. In future, computational tools such as neuronal networks, directly learning and responding to the physiological and pharmacokinetic changes in the clinically unstable thoracic organ recipients, may help improve tacrolimus personalized dosing.[43-45]

In conclusion, tacrolimus pharmacokinetics differs between clinically stable and clinically unstable patients such as thoracic organ recipients. This results in higher rates of tacrolimus nephrotoxicity in these latter patients. In unstable thoracic organ recipients, we found a large inter-occasion variability in relative bioavailability, which makes PK-guided dosing of orally administered tacrolimus of limited added value. Within the blood compartment, erythrocytes appeared to be an important factor to consider, as tacrolimus is mainly associated with these cells. In clinically unstable thoracic organ transplant patients, erythrocytes concentrations are highly variable, subsequently changing the unbound tacrolimus concentrations. We observed a nonlinear relationship between whole-blood and unbound tacrolimus plasma concentrations with saturation of erythrocytes when hematocrit decreases. To improve tacrolimus personalized dosing in future, we recommend administering tacrolimus intravenously and aiming at the lower therapeutic range value in the first days after transplantation. Monitoring hematocrit-corrected whole-blood concentrations may further improve tacrolimus dosing.

REFERENCES

1. Atalan HK, Gucyetmez B, Aslan S, Yazar S, Polat KY. Postoperative acute kidney injury in living donor liver transplantation recipients. *Int J Artif Organs*. 2017;41(1):37-42. doi:10.5301/ijao.5000638.
2. Rayar M, Tron C, Jézéquel C, et al. High Intrapatient Variability of Tacrolimus Exposure in the Early Period After Liver Transplantation Is Associated With Poorer Outcomes. *Transplantation*. 2018;102(3):e108-e114. doi:10.1097/TP.0000000000002052.
3. Nankivell BJ, P'Ng CH, O'Connell PJ, Chapman JR. Calcineurin Inhibitor Nephrotoxicity Through the Lens of Longitudinal Histology: Comparison of Cyclosporine and Tacrolimus Eras. *Transplantation*. 2016;100(8):1723-1731. doi:10.1097/TP.0000000000001243.
4. Grimm M, Rinaldi M, Yonan NA, et al. Superior prevention of acute rejection by tacrolimus vs. cyclosporine in heart transplant recipients--a large European trial. *Am J Transplant*. 2006;6(6):1387-1397. doi:10.1111/j.1600-6143.2006.01300.x.
5. Wehbe E, Duncan AE, Dar G, Budev M, Stephany B. recovery from AKI and short- and long-term outcomes after lung transplatation. *Clinical Journal of the American Society of Nephrology*. 2013;8(1):19-25. doi:10.2215/CJN.04800512.
6. Tjahjono R, Connellan M, Granger E. Predictors of Acute Kidney Injury in Cardiac Transplantation. *Transplantation Proceedings*. 2016;48(1):167-172. doi:10.1016/j.transproceed.2015.12.006.
7. Sikma MA, Hunault CC, van de Graaf EA, et al. High tacrolimus blood concentrations early after lung transplantation and the risk of kidney injury. *Eur J Clin Pharmacol*. 2017;73(5):573-580. doi:10.1007/s00228-017-2204-8.
8. Sikma MA, Hunault CC, Kirkels JH, Verhaar MC, Kesecioglu J, de Lange DW. Association of Whole Blood Tacrolimus Concentrations with Kidney Injury in Heart Transplantation Patients. *Eur J Drug Metabol Pharmacokinet*. 2018;43(3):311-320. doi:10.1007/s13318-017-0453-7.
9. Paradela de la Morena M, La Torre Bravos De M, Prado RF, et al. Chronic Kidney Disease After Lung Transplantation: Incidence, Risk Factors, and Treatment. *TPS*. 2010;42(8):3217-3219. doi:10.1016/j.transproceed.2010.05.064.
10. Ojo AO, Held PJ, Port FK, et al. Chronic Renal Failure after Transplantation of a nonrenal organ. *N Engl J Med*. 2003;349(10):931-940. doi:10.1056/NEJMoa021744.
11. Mastrobuoni S, Ubilla M, Cordero A, Herreros J, Rabago G. Two-Dose Daclizumab, Tacrolimus, Mycophenolate Mofetil, and Steroid-Free Regimen in De Novo Cardiac Transplant Recipients: Early Experience. *Transplantation Proceedings*. 2007;39(7):2163-2166. doi:10.1016/j.transproceed.2007.06.073.
12. Gueta I, Markovits N, Yarden-Bilavsky H, et al. High tacrolimus trough level variability is associated with rejections after heart transplant. *Am J Transplant*. 2018;18(10):2571-2578. doi:10.1111/ajt.15016.
13. Gallagher HM, Sarwar G, Tse T, et al. Erratic tacrolimus exposure, assessed using the standard deviation of trough blood levels, predicts chronic lung allograft dysfunction and survival. *J Heart Lung Transplant*. 2015;34(11):1442-1448. doi:10.1016/j.healun.2015.05.028.

14. Sikma MA, van Maarseveen EM, van de Graaf EA, et al. Pharmacokinetics and Toxicity of Tacrolimus Early After Heart and Lung Transplantation. *Am J Transplant*. 2015;15(9):2301-2313. doi:10.1111/ajt.13309.
15. Sikma MA, van Maarseveen EM, Donker DW, Meulenbelt J. Letter to the editor: "Immunosuppressive drug therapy - biopharmaceutical challenges and remedies". *Expert Opin Drug Deliv*. 2015;12(12):1955-1957. doi:10.1517/17425247.2015.1106687.
16. Ekberg H, Mamelok RD, Pearson TC, Vincenti F, Tedesco-Silva H, Daloze P. The challenge of achieving target drug concentrations in clinical trials: experience from the Symphony study. *Transplantation*. 2009;87(9):1360-1366. doi:10.1097/TP.0b013e3181a23cb2.
17. Størset E, Holford N, Hennig S, et al. Improved prediction of tacrolimus concentrations early after kidney transplantation using theory-based pharmacokinetic modelling. *British Journal of Clinical Pharmacology*. 2014;78(3):509-523. doi: 10.1111/bcp.12361
18. Lam S, Partovi N, Ting LSL, Ensom MHH. Corticosteroid interactions with cyclosporine, tacrolimus, mycophenolate, and sirolimus: fact or fiction? *Annals of Pharmacotherapy*. 2008;42(7):1037-1047. doi:10.1345/aph.1K628.
19. Snell GI, Ivulich S, Mitchell L, Westall GP, Levvey BJ. Evolution to twice daily bolus intravenous tacrolimus: optimizing efficacy and safety of calcineurin inhibitor delivery early post lung transplant. *Ann Transplant*. 2013;18:399-407. doi:10.12659/AOT.883993.
20. Worbs S, Köhler K, Pauly D, et al. Ricinus communis intoxications in human and veterinary medicine-a summary of real cases. *Toxins (Basel)*. 2011;3(10):1332-1372. doi:10.3390/toxins3101332.
21. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Changes in tacrolimus distribution in blood and plasma protein binding following liver transplantation. *therapeutic drug monitoring*. 2004;26(5):506-515. doi: 10.1097/00007691-200410000-00008
22. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Factors affecting variability in distribution of tacrolimus in liver transplant recipients. *British Journal of Clinical Pharmacology*. 2004;57(3):298-309. doi: 10.1046/j.1365-2125.2003.02008.x
23. Rifai N, Chao F-F, Pham Q, Thiessen J, Soldin SJ. The role of lipoproteins in the transport and uptake of cyclosporine and dihydro-tacrolimus into HepG2 and JURKAT cell lines. *Clinical Biochemistry*. 1996;29(2):149-155. doi: 10.1016/0009-9120(96)00001-x
24. Biagiotti S, Paoletti MF, Fraternale A, Rossi L, Magnani M. Drug delivery by red blood cells. Muzykantov V, ed. *IUBMB Life*. 2011;63(8):621-631. doi:10.1002/iub.478.
25. Biagiotti S, Rossi L, Bianchi M, et al. Immunophilin-loaded erythrocytes as a new delivery strategy for immunosuppressive drugs. *J Control Release*. 2011;154(3):306-313. doi:10.1016/j.jconrel.2011.05.024.
26. Nagase K, Iwasaki K, Nozaki K, Noda K. Distribution and protein binding of FK506, a potent immunosuppressive macrolide lactone, in human blood and its uptake by erythrocytes. *J Pharm Pharmacol*. 1994;46(2):113-117. doi:10.1111/j.2042-7158.1994.tb03752.x.
27. Walensky LD, Gascard P, Fields ME, et al. The 13-kD FK506 binding protein, FKBP13, interacts with a novel homologue of the erythrocyte membrane cytoskeletal protein 4.1. *J Cell Biol*. 1998;141(1):143-153. doi: 10.1083/jcb.141.1.143

28. Zahir H, Nand RA, Brown KF, Tattam BN. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. *journal of pharmacological and toxicological methods*. 2001;46:27-35. doi: 10.1016/s1056-8719(02)00158-2
29. Zheng S, Davis CL, Hebert MF. Pharmacokinetics of Tacrolimus During Pregnancy. *therapeutic drug monitoring*. 2012;34:660-670. doi: 10.1097/ftd.0b013e3182708edf
30. Bittersohl H, Schniedewind B, Christians U, Luppa PB. A simple and highly sensitive on-line column extraction liquid chromatography-tandem mass spectrometry method for the determination of protein-unbound tacrolimus in human plasma samples. *J Chromatogr A*. 2018;1547:45-52. doi:10.1016/j.chroma.2018.03.010.
31. Stienstra NA, Sikma MA, van Dapperen AL, de Lange DW, Van Maarseveen EM. Development of a Simple and Rapid Method to Measure the Free Fraction of Tacrolimus in Plasma Using Ultrafiltration and LC-MS/MS. *therapeutic drug monitoring*. 2016;38(6):722-727. doi:10.1097/FTD.0000000000000351.
32. Van Acker K, Bultynck G, Rossi D, et al. The 12 kDa FK506-binding protein, FKBP12, modulates the Ca(2+)-flux properties of the type-3 ryanodine receptor. *J Cell Sci*. 2004;117(Pt 7):1129-1137. doi:10.1242/jcs.00948.
33. Kittipibul V, Tantrachoti P, Ongcharit P, et al. Low-dose basiliximab induction therapy in heart transplantation. *Clinical Transplantation*. 2017;31(12):e13132. doi:10.1111/ctr.13132.
34. Podesser BK, Rinaldi M, Yona NA, et al. Comparison of low and high initial tacrolimus dosing in primary heart transplant recipients: a prospective European multicenter study. *Transplantation*. 2005;79(1):65-71. doi: 10.1097/01.tp.0000140965.83682.d6
35. Guethoff S, Stroeh K, Grinninger C, et al. De novo sirolimus with low-dose tacrolimus versus full-dose tacrolimus with mycophenolate mofetil after heart transplantation--8-year results. *J Heart Lung Transplant*. 2015;34(5):634-642. doi:10.1016/j.healun.2014.11.025.
36. Ensor CR, Iasella CJ, Harrigan KM, et al. Increasing tacrolimus time-in-therapeutic range is associated with superior one-year outcomes in lung transplant recipients. *Am J Transplant*. 2018;18(6):1527-1533. doi:10.1111/ajt.14723.
37. Yang Y, Gao W. Wearable and flexible electronics for continuous molecular monitoring. *Chem Soc Rev*. 2018;363:301. doi:10.1039/c7cs00730b.
38. Mould DR, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development-part 2: introduction to pharmacokinetic modeling methods. *CPT Pharmacometrics Syst Pharmacol*. 2013;2(4):e38–14. doi:10.1038/psp.2013.14.
39. Marquet P, Albano L, Woillard J-B, et al. Comparative clinical trial of the variability factors of the exposure indices used for the drug monitoring of two tacrolimus formulations in kidney transplant recipients. *Pharmacol Res*. 2018;129:84-94. doi:10.1016/j.phrs.2017.12.005.
40. Andrews LM, Hesselink DA, van Schaik RHN, et al. A population pharmacokinetic model to predict the individual starting dose of tacrolimus in adult renal transplant recipients. *British Journal of Clinical Pharmacology*. 2019;85(3):601-615. doi:10.1111/bcp.13838.

41. Størset E, Åsberg A, Skauby M, et al. Improved Tacrolimus Target Concentration Achievement Using Computerized Dosing in Renal Transplant Recipients-A Prospective, Randomized Study. *Transplantation*. April 2015. doi:10.1097/TP.0000000000000708.
42. Donagher J, Martin JH, Barras MA. Individualised medicine: why we need Bayesian dosing. *Intern Med J*. 2017;47(5):593-600. doi:10.1111/imj.13412.
43. Agatonovic-Kustrin S, Beresford R. Basic concepts of artificial neural network (ANN) modeling and its application in pharmaceutical research. *J Pharm Biomed Anal*. 2000;22(5):717-727. doi: 10.1016/s0731-7085(99)00272-1
44. Yamamura S. Clinical application of artificial neural network (ANN) modeling to predict pharmacokinetic parameters of severely ill patients. *Advanced Drug Delivery Reviews*. 2003;55(9):1233-1251. doi: 10.1016/s0169-409x(03)00121-2
45. Komorowski M, Celi LA, Badawi O, Gordon AC, Faisal AA. The Artificial Intelligence Clinician learns optimal treatment strategies for sepsis in intensive care. *Nat Med*. 2018;24(11):1716-1720. doi:10.1038/s41591-018-0213-5.
46. Mittal N, Thompson JF, Kato T, Tzakis AG. Tacrolimus and diarrhea: pathogenesis of altered metabolism. *Pediatric Transplantation*. 2001;5(2):75-79. doi: 10.1034/j.1399-3046.2001.005002075.x
47. Christians U, Schmitz V, Haschke M. Functional interactions between P-glycoprotein and CYP3A in drug metabolism. *Expert Opin Drug Metab Toxicol*. 2005;1(4):641-654. doi:10.1517/17425255.1.4.641.
48. Renton KW. Regulation of drug metabolism and disposition during inflammation and infection. *Expert Opin Drug Metab Toxicol*. 2005;1(4):629-640. doi:10.1517/17425255.1.4.629.
49. Christians U, Jacobsen W, Benet LZ, Lampen A. Mechanisms of clinically relevant drug interactions associated with tacrolimus. *Clinical Pharmacokinetics*. 2002;41(11):813-851. doi:10.2165/00003088-200241110-00003.

FIGURES

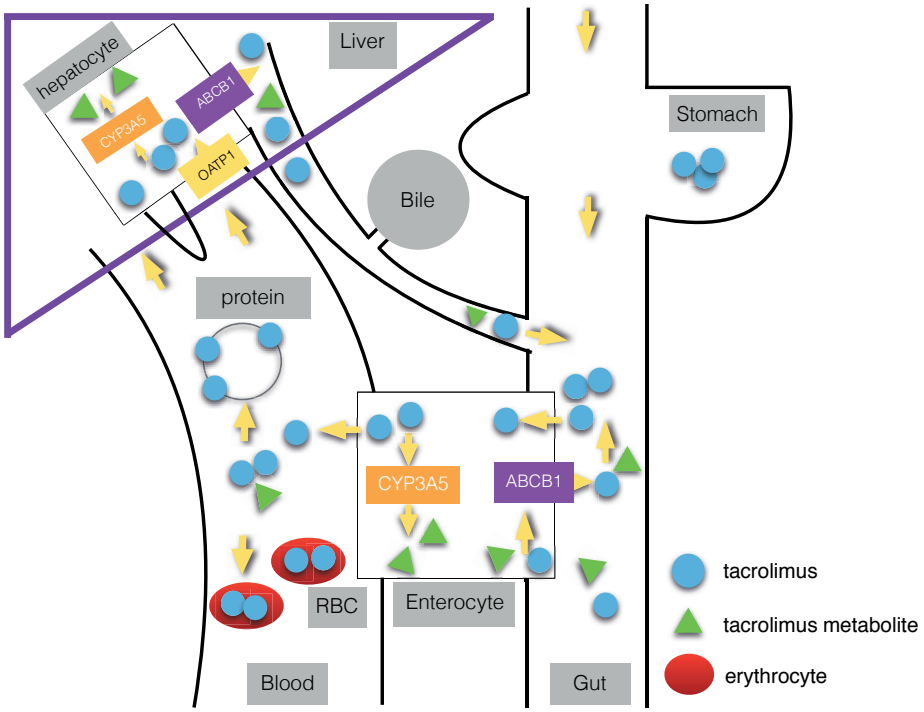

Fig. 1 Schematic overview of tacrolimus pharmacokinetics: gut transport, absorption, blood distribution, hepatic metabolism and excretion of tacrolimus. Clinical instability causes a cascade of processes influencing all these aspects of tacrolimus pharmacokinetics. Tacrolimus is generally administered orally. Inflammation may result in reduced blood flow and ileus reducing bioavailability by delaying transport, minimizing luminal degradation and dissolution, and decreasing contact with the gut wall.[46] On the opposite, increased blood flow increases gut motility shortening transit time and increased degradation and dissolution of tacrolimus. A sudden peak in the blood concentrations may occur. In the enterocyte, Cytochrome P450/3A5 (CYP3A5) is the main enzyme metabolizing tacrolimus. Tacrolimus is repeatedly taken up and pumped out of the enterocytes into the gut lumen by the transporter ATP-binding cassette B1 (ABCB1) increasing the probability of tacrolimus being metabolized.[47] Shock and inflammation decrease the activity of the CYP3A5 enzymes and the ABCB1 transporter.[48] Saturation of the CYP3A5 enzymes may occur facilitating tacrolimus transport into the blood resulting in higher uptake into the blood compartment.[48] Drug-drug interactions may also have large effects on tacrolimus metabolism. For instance, corticosteroids induce CYP3A-enzymes and the ABCB1-transporter.[49] Corticosteroids are administered in high doses in the first days after transplantation and tapered thereafter increasing tacrolimus absorption. In the blood, tacrolimus distributes mainly into erythrocytes and to a lesser extent to (lipo)proteins (albumin, high density lipoprotein, and α1-acid glycoprotein).[21] Inflammation, blood loss and blood transfusions may increase unbound concentrations by anemia and hypoalbuminemia. Also, inflammation occurs upon initiation of extracorporeal life support. Further, extracorporeal life support may increase blood volume, decrease protein concentrations, cause hemolysis and sequestration of tacrolimus into the equipment. CYP3A4/5: cytochrome P450 3A4/5, OATP1: organic anion–transporting peptide, ABCB1: efflux pump of the ABCB1 cassette, RBC: red blood cells.

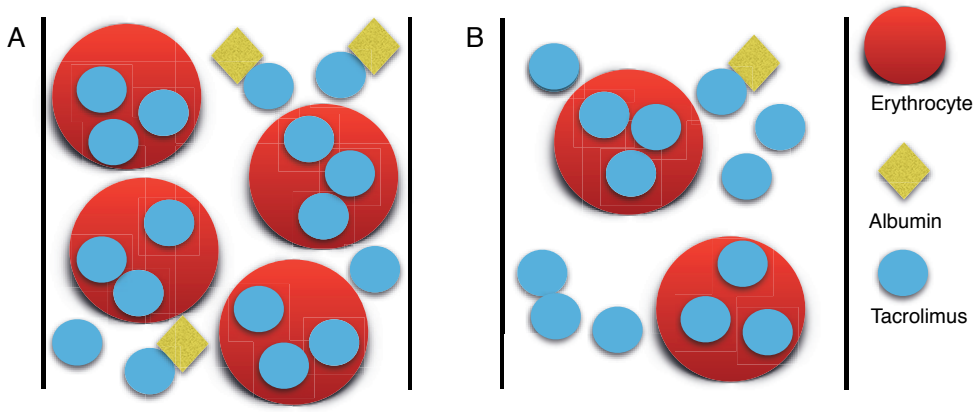

Fig. 2 A theoretical representation of a decrease in erythrocyte and albumin concentrations resulting in changes in whole-blood, total plasma and unbound tacrolimus concentrations. Figure A shows a situation with hematocrit and albumin in normal ranges. Figure B shows a decreased erythrocytes count with a decrease in whole-blood concentration and an increase in unbound concentration. A decrease in albumin concentration may increase the unbound plasma concentration to a lesser extent.

CHAPTER 6a

6a

Summary

SUMMARY

The aim of this thesis was to increase the knowledge of tacrolimus pharmacokinetics early after heart and lung transplantation. Tacrolimus has been on the market since the 1990s and is a very effective immunosuppressant of the calcineurin inhibitor class. However, its use carries a risk of nephrotoxicity. Acute kidney injury after heart and lung transplantation frequently occurs and often evolves into chronic kidney disease jeopardizing transplantation outcomes.[1-6] Causes of acute kidney injury are multiple, though tacrolimus nephrotoxicity may play a prominent role. Nephrotoxicity may occur at supra-therapeutic concentrations and even within the narrow therapeutic range, possibly due to changes in the unbound tacrolimus plasma concentrations.[7] A high variability in tacrolimus blood concentrations is frequently observed early after heart and lung transplantation. This may be due to the complex tacrolimus pharmacokinetics combined with large physiological changes in these patients. Inflammation and shock may cause clinical instability with multiple organ dysfunction, altered protein metabolism, acid-base imbalance, fluid overload and diminished hematopoiesis. Most of these alterations may influence pharmacokinetics of tacrolimus separately. This thesis shows the extreme variability in tacrolimus pharmacokinetics in the clinically unstable phase of thoracic organ transplantation and serves as guidance for transplant physicians to improve tacrolimus dosing in these patients.

In **Chapter 1** a comprehensive review is conducted. Tacrolimus pharmacokinetics in clinically unstable thoracic organ transplantation patients are outlined versus tacrolimus pharmacokinetics in clinically stable patients. During clinical instability, a cascade of processes occurs influencing all aspects of tacrolimus pharmacokinetics: bioavailability, distribution volume, metabolism and clearance. The transplantation itself causes an inflammatory response. Other causes of inflammation may be primary graft dysfunction, ischemia-reperfusion injury, acute rejection and the use of extracorporeal equipment. Inflammation may result in variable pharmacokinetics.[8] We describe the influence of the physiological changes early after heart and lung transplantation as well as drug-drug interactions on tacrolimus pharmacokinetics (See also **Table 2, 3 and 4 in Chapter 1**).

In **Chapter 2 (2a and 2b)**, we retrospectively studied tacrolimus nephrotoxicity in two cohorts: one cohort early after lung transplantation, 186 patients, and one cohort early after heart transplantation, 110 patients. We observed an incidence of AKI of 46% and 57% in lung and heart recipients, respectively. We found that supra-therapeutic whole-blood tacrolimus concentrations (>15 ng/ml) were independently related to the emergence of AKI. AKI typically developed one or two days after supra-therapeutic concentrations. Concentrations were supra-therapeutic in 73% of lung transplant patients and in 34% of heart transplant patients. This may correspond to a more prudent dosing regimen

implemented by the cardiac transplant physicians. Recovery rates of AKI were low (19% for lung transplants and 24% for heart transplants, respectively). Chronic kidney disease developed in 15% of lung recipients and 19% of heart recipients. These studies showed that supra-therapeutic tacrolimus concentrations directly after thoracic organ transplantation are important predictors for the emergence and further development of kidney disease. It is therefore important to improve understanding of tacrolimus pharmacokinetics in the early stage after thoracic organ transplantation.

In **Chapter 3**, whole-blood tacrolimus pharmacokinetics in 20 lung and 10 heart transplant patients were studied. Concentrations twelve-hours after administration (C12h) were outside target range in 69% of the cases, with supra-therapeutic concentrations in 19% and sub-therapeutic concentrations in 51% of the patients. High SOFA scores were found, corresponding with considerable clinical instability. Inflammation and shock were found in the vast majority of patients, 100% and 93% respectively. Gut dysmotility was observed in 97% of patients independent of the presence of cystic fibrosis. Ileus occurred in 90% of the patients and diarrhea in 60% of the patients. A two-compartment model with mixed first-order and zero-order absorption was developed. We showed an extreme variability in tacrolimus whole-blood pharmacokinetics. Interestingly, the inter-occasion variability far exceeded inter-individual variability. Both heart and lung, CF and non-CF, transplant recipients all showed large inter-occasion variability. The extreme variability of tacrolimus pharmacokinetics was particularly determined by an excessive variability in relative bioavailability of 55%. For clinically unstable patients, this means that it is nearly impossible to predict the next tacrolimus dose. To bypass this extreme variability in bioavailability, we suggest administering tacrolimus intravenously early post-transplantation. Furthermore, we suggest aiming for the lower therapeutic level in the early phase after transplantation to minimize tacrolimus toxicity, which means 9 ng/ml with a therapeutic range of 9-15 ng/ml.

In **Chapter 4a,** a fast and highly sensitive liquid chromatography-mass spectrometry method was developed and validated for the quantitation of total and unbound tacrolimus plasma concentrations. The unbound concentration of a drug is the part influencing efficacy and toxicity. Tacrolimus unbound plasma concentrations have been analyzed before, though with complex, time-consuming, and indirect methods.[9-11] Therefore, studies on tacrolimus plasma concentrations are scarce. The sample preparation for the determination of the plasma concentrations of unbound tacrolimus consisted of ultrafiltration followed by solid phase extraction. To determine the total tacrolimus plasma concentration, a simple method based on protein precipitation was developed. The extracts were injected into a Thermo Scientific HyPurity C18 column using gradient elution. The analytes were detected by liquid chromatography-mass spectrometry using a triple quadruple with positive ionization (LC-MS/MS). The method was validated over a

linear range of 1–200 ng/L for unbound tacrolimus plasma concentrations and 100–3200 ng/L for total plasma concentrations. Total and unbound tacrolimus plasma concentrations could be accurately measured.

In **Chapter 4b**, we studied the total and unbound tacrolimus plasma concentrations in the cohort of 20 lung and 10 heart transplantation patients. The two-compartment pharmacokinetic (PK) model with mixed first and zero-order absorption was extended with the total and unbound tacrolimus plasma concentrations. Tacrolimus was for more than 99% associated with erythrocytes. The maximum binding constant to whole-blood concentrations (Bmax WBC) was directly proportional to hematocrit. Hematocrit therefore, mainly influences tacrolimus content within whole-blood. Considering the wide variation in hematocrit values we observed during the first weeks after heart and lung transplantation (hematocrit range 0.24-0.35), this may have highly affected the dose-to-dose variation in whole-blood concentrations. When hematocrit decreases, tacrolimus blood content decreases. The variation in hematocrit affected not only whole-blood, but also unbound concentrations. Yet, we observed extremely low total plasma concentrations in our thoracic organ cohort; <1% of whole-blood concentrations. Moreover, total plasma concentrations were typically 137-fold higher than unbound plasma concentrations. The range of the unbound plasma concentration largely differed between occasions (0.42-11 pg/ml) corresponding to the large variability predominantly in hematocrit. We observed, a non-linear relationship between unbound plasma concentrations and hematocrit especially in the higher range of whole-blood concentrations. This may be due to a saturation of erythrocytes when hematocrit decreases. Interestingly, the maximum binding capacity of erythrocytes showed a wide inter-patient variability of 27%. FK-binding protein (FKBP) is the cytosolic protein binding tacrolimus within the cell. The nonlinear relationship may have been caused by different content of FKBP12 and FKBP13 within the erythrocytes.[12,13] The combination of high whole-blood tacrolimus concentrations with low hematocrit concentrations may result in extremely high unbound plasma concentrations and hence, in toxicity. Theoretically, the unbound tacrolimus plasma concentrations would be a better surrogate for the prediction of clinical outcomes. Although we developed a viable analysis for total and unbound tacrolimus plasma concentrations, hemolysis might influence plasma concentrations in daily practice. Samples need to be immediately sent to the laboratory and directly prepared. Regarding the extremely low concentrations as well, this makes the measurements difficult to interpret. Therefore, our suggestion is to implement hematocrit corrected whole-blood concentrations in daily transplantation practices. Hematocrit-corrected tacrolimus dosing supports targeting therapeutic tacrolimus unbound plasma concentrations and is feasible in current transplant practices.

REFERENCES

1. Wehbe E, Duncan AE, Dar G, Budev M, Stephany B. recovery from AKI and short- and long-term outcomes after lung transplatation. *Clinical Journal of the American Society of Nephrology*. 2013;8(1):19-25. doi:10.2215/CJN.04800512.
2. Paradela de la Morena M, La Torre Bravos De M, Prado RF, et al. Chronic Kidney Disease After Lung Transplantation: Incidence, Risk Factors, and Treatment. *TPS*. 2010;42(8):3217-3219. doi:10.1016/j.transproceed.2010.05.064.
3. Ojo AO, Held PJ, Port FK, et al. Chronic Renal Failure after Transplantation of a nonrenal organ. *N Engl J Med*. 2003;349(10):931-940. doi:10.1056/NEJMoa021744.
4. Lund LH, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Official Adult Heart Transplant Report--2013; focus theme: age. *The Journal of Heart and Lung Transplantation*. 2013;32(10):951-964. doi:10.1016/j.healun.2013.08.006.
5. Healy AH, Stehlik J, Edwards LB, McKellar SH, Drakos SG, Selzman CH. Predictors of 30-day post-transplant mortality in patients bridged to transplantation with continuous-flow left ventricular assist devices-An analysis of the International Society for Heart and Lung Transplantation Transplant Registry. *J Heart Lung Transplant*. 2016;35(1):34-39. doi:10.1016/j.healun.2015.07.007.
6. Söderlund C, Löfdahl E, Nilsson J, Reitan Ö, Higgins T, Rådegran G. Chronic kidney disease after heart transplantation: a single-centre retrospective study at Skåne University Hospital in Lund 1988-2010. *Transplant International*. 2016;29(5):529-539. doi:10.1111/tri.12710.
7. Hebert MF, Zheng S, Hays K, et al. Interpreting tacrolimus concentrations during pregnancy and postpartum. *Transplantation*. 2013;95(7):908-915. doi:10.1097/TP.0b013e318278d367.
8. Udy AA, Roberts JA, Lipman J. Clinical implications of antibiotic pharmacokinetic principles in the critically ill. *Intensive Care Med*. 2013;39(12):2070-2082. doi:10.1007/s00134-013-3088-4.
9. Piekoszewski W, Jusko WJ. Plasma protein binding of tacrolimus in humans. *J Pharm Sci*. 1993;82(3):340-341. doi: 10.1002/jps.2600820325
10. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Changes in tacrolimus distribution in blood and plasma protein binding following liver transplantation. *therapeutic drug monitoring*. 2004;26(5):506-515. doi: 10.1097/00007691-200410000-00008
11. Zheng S, Davis CL, Hebert MF. Pharmacokinetics of Tacrolimus During Pregnancy. *therapeutic drug monitoring*. 2012;34:660-670.doi: 10.1097/ftd.0b013e3182708edf
12. Walensky LD, Gascard P, Fields ME, et al. The 13-kD FK506 binding protein, FKBP13, interacts with a novel homologue of the erythrocyte membrane cytoskeletal protein 4.1. *J Cell Biol*. 1998;141(1):143-153. doi: 10.1083/jcb.141.1.143
13. Van Acker K, Bultynck G, Rossi D, et al. The 12 kDa FK506-binding protein, FKBP12, modulates the Ca(2+)-flux properties of the type-3 ryanodine receptor. *J Cell Sci*. 2004;117(Pt 7):1129-1137. doi:10.1242/jcs.00948.

CHAPTER 6b

6b

Samenvatting

SAMENVATTING

Het doel van dit proefschrift was de kennis omtrent tacrolimus farmacokinetiek direct na hart- en longtransplantatie te vergroten. Tacrolimus is op de markt sinds de jaren '90 en is een zeer effectief immunosuppressivum in de klasse calcineurine-remmers, maar is ook nefrotoxisch. Acute nierschade na hart- en longtransplantatie ontstaat frequent en ontwikkelt zich vaak tot chronische nierziekte. Dit beïnvloedt de transplantatie uitkomsten negatief.[1-6] De oorzaken van acute nierschade kort na transplantatie zijn divers, maar tacrolimus nefrotoxiciteit zou een belangrijke rol kunnen spelen. Nefrotoxiciteit kan ontstaan bij supra-therapeutische concentraties, maar ook binnen de nauwe therapeutische breedte, waarschijnlijk veroorzaakt door de ongebonden tacrolimus concentraties.[7] Een hoge variabiliteit in de tacrolimus bloedconcentraties wordt vaak gezien in de eerste dagen na hart- en longtransplantatie. Dit kan komen door de complexe tacrolimus farmacokinetiek gecombineerd met grote fysiologische veranderingen in deze patiënten. Inflammatie en shock kunnen klinische instabiliteit veroorzaken met multipele orgaandysfunctie, veranderd eiwit metabolisme, zuur-base dysbalans, vloeistof overbelasting en verminderde hematopoëse. Veel van deze veranderingen beïnvloeden de farmacokinetiek van tacrolimus afzonderlijk. Dit proefschrift toont extreme variabiliteit van tacrolimus farmacokinetiek in klinisch instabiele hart- en longtransplantatie patiënten en helpt transplantatie-artsen om het doseren van tacrolimus in deze patiënten te verbeteren.

In **Hoofdstuk 1** is een uitgebreid overzicht gegeven van tacrolimus farmacokinetiek in klinisch instabiele hart- en longtransplantatie patiënten versus tacrolimus farmacokinetiek in klinisch stabiele patiënten. Tijdens klinische instabiliteit vindt een cascade aan processen plaats die alle aspecten van tacrolimus farmacokinetiek beïnvloeden: biologische beschikbaarheid, distributie volume, metabolisme en klaring. De transplantatie zelf veroorzaakt een inflammatoire respons. Andere oorzaken van inflammatie kunnen zijn: primaire transplantaat dysfunctie, ischemie-reperfusie schade, acute rejectie en het gebruik van extracorporale apparatuur. Inflammatie kan leiden tot variabele farmacokinetiek.[8] De invloed van fysiologische veranderingen en medicatie interacties op tacrolimus farmacokinetiek is beschreven (Zie ook **Tabel 2, 3 en 4 in Hoofdstuk 1**).

In **Hoofdstuk 2 (2a en 2b)**, is tacrolimus nefrotoxiciteit bestudeerd in twee cohorten: één cohort vroeg na longtransplantatie, 186 patiënten, en één cohort vroeg na harttransplantatie, 110 patiënten. Wij observeerden een incidentie van acute nierschade van 46% in longtransplantatie patiënten en 57% in harttransplantatie patiënten. Supra-therapeutische tacrolimus volbloed concentraties (>15 ng/ml) bleken onafhankelijk geassocieerd met het ontwikkelen van nierschade. Acute nierschade ontwikkelt zich typisch één a twee dagen na de supra-therapeutische concentratie. De concentraties

waren supra-therapeutisch in 73% van de longtransplantatie patiënten en in 34% van de harttransplantatie patiënten. Dit zou verband kunnen houden met een voorzichtigere aanpak die geïmplementeerd is door de harttransplantatie artsen. Het aantal patiënten dat herstel van de nierfunctie liet zien was laag (19% na longtransplantatie en 24% na harttransplantatie). Chronische nierziekte na 1 jaar ontwikkelde in 15% van de longtransplantatie patiënten en in 24% van de harttransplantatie patiënten. Deze studies laten zien dat supra-therapeutische tacrolimus concentraties kort na transplantatie van thoracale organen belangrijke voorspellers voor het ontstaan en de verdere ontwikkeling van nierziekte zijn. Het is daarom belangrijk om het begrip van tacrolimus farmacokinetiek direct na hart- en longtransplantatie te vergroten.

In **Hoofdstuk 3** werd de farmacokinetiek van volbloed tacrolimus onderzocht in 20 long- en 10 harttransplantatie patiënten. Concentraties twaalf uur na toediening van tacrolimus (de zogenaamde dalspiegels) bleken in 69% buiten het therapeutische gebied te liggen, waarbij 19% supra-therapeutisch en 51% sub-therapeutisch was. Er werd een hoge SOFA score gevonden, overeenkomend met een hoge ziektelast ofwel ernstige klinische instabiliteit. Inflammatie en shock werden in de meerderheid van de patiënten gezien, respectievelijk 100% en 93%. Darm dysfunctie werd geobserveerd in 97% van de patiënten. Dit werd niet vaker gezien bij cystic fibrose. Bij 90% van de patiënten werd ileus waargenomen en bij 60% van de patiënten werd diarree waargenomen. Een twee-compartimenten model met een gemengde eerste orde en nulde orde absorptie werd ontwikkeld. Wij zagen een extreme variabiliteit in tacrolimus volbloed farmacokinetiek. Opmerkelijk was dat de variabiliteit binnen één persoon tussen de verschillende dagen (c.q. intra-individueel) groter was dan de variabiliteit tussen verschillende personen (c.q. inter-individueel). Zowel hart- als long-, CF en niet-CF, transplantatie patiënten lieten allemaal een grote intra-individuele variabiliteit zien. Deze extreme variabiliteit van tacrolimus farmacokinetiek werd met name bepaald door een uitzonderlijk grote variabiliteit in relatieve biologische beschikbaarheid van 55%. Voor klinisch instabiele patiënten betekent dit dat de volgende tacrolimus dosis nauwelijks goed geschat kan worden. Wij adviseren dan ook om tacrolimus direct post-transplantatie intraveneus toe te dienen om deze extreme variabiliteit in biologische beschikbaarheid grotendeels te omzeilen. Verder adviseren we ook om voorzichtig te doseren en te richten op de laagste waarde van het therapeutisch bereik, welke 9 ng/ml is voor een bereik van 9-15 ng/ml.

Hoofdstuk 4a, beschrijft de ontwikkeling en validering van een snelle en hoog sensitieve vloeistofchromatografie en massaspectrometrie methode, om totaal en ongebonden tacrolimus plasma concentraties te kwantificeren. De ongebonden concentratie van een medicament is het deel dat de effectiviteit en toxiciteit bepaald. Analyse van ongebonden plasma concentraties van tacrolimus is eerder beschreven, maar dat werd gedaan met ingewikkelde, tijdrovende en indirecte methodes.[9-11] Daarom zijn er weinig studies

gedaan naar tacrolimus plasma concentraties. Wij hebben een snelle en sensitieve methode ontwikkeld om de totale en ongebonden plasma concentratie te bepalen. De bloedmonster preparatie bestond uit ultrafiltratie gevolgd door een extractie in de vaste fase. Om de totale plasma concentratie te bepalen werd een eenvoudige methode gebaseerd op eiwit precipitatie ontwikkeld. De extracten werden geïnjecteerd in een Thermo Scientific HyPurity C18 kolom waarbij gebruik gemaakt werd van gradiënt elutie. De analyten werden gedetecteerd door gebruik te maken van de gaschromatografie-spectrometrie met een triple quadrupel en positieve ionisatie (GC-MS/MS). De methode werd gevalideerd over een lineaire range van 1-200 ng/L voor de ongebonden tacrolimus plasma concentraties en 100-3200 ng/L voor totale plasma concentraties. Totale en ongebonden plasma concentraties konden met deze methode nauwkeurig gemeten worden.

In **Hoofdstuk 4b**, bestudeerden we de totale en ongebonden tacrolimus plasma concentraties in de groep van 20 long- en 10 harttransplantatie patiënten. Het twee compartimenten model met gemengde eerste en nulde orde absorptie van de volbloed tacrolimus werd uitgebreid met de totale en ongebonden plasma concentraties. De binding van tacrolimus aan erythrocyten was hoger dan 99%. De maximum bindingsconstante van volbloed concentraties (Bmax volbloed) was direct proportioneel aan het hematocriet. Het hematocriet beïnvloed voornamelijk het tacrolimus gehalte in het bloed. De enorme variatie in hematocriet waarden gedurende de eerste week na hart- en longtransplantatie (hematocriet bereik 0.24-0.35) in ogenschouw nemende, zal dit een sterk effect op de dosis-tot-dosis variatie in volbloed concentraties hebben. Als het hematocriet daalt, dan daalt ook het gehalte aan tacrolimus in het bloed. De variatie in hematocriet had niet alleen effect op volbloed concentraties, maar ook op de ongebonden plasma concentraties. Wij observeerden extreem lage totaal plasma concentraties; <1% van de volbloed concentraties. Verder waren de totale plasma concentraties 137 keer hoger dan de ongebonden plasma concentraties. Het bereik van de ongebonden plasma concentratie varieerde sterk tussen de verschillende momenten binnen een patiënt (0.42-11 pg/ml) overeenkomend met de grote variabiliteit van met name het hematocriet. Wij observeerden een non-lineaire relatie tussen ongebonden plasma concentraties en hematocriet, met name in het hogere bereik van de volbloed concentraties. Dit kan komen door saturatie van erythrocyten als het hematocriet verlaagd. Opmerkelijk was dat de maximum bindingscapaciteit aan erythrocyten een grote variabiliteit van 27% liet zien. "FK-binding protein" (FKBP) is het eiwit in het cytosol in de cel wat bindt aan tacrolimus. De non-lineaire relatie kan veroorzaakt zijn door een verschil in gehalte van FKBP12 en FKPB13 in de erythrocyten.[12,13] De combinatie van een hoge volbloed tacrolimus concentratie met een laag hematocriet gehalte kan resulteren in extreem hoge ongebonden plasma concentraties en dientengevolge tot toxiciteit. Theoretisch zou de ongebonden tacrolimus plasma concentratie een betere surrogaat zijn om klinische

uitkomsten te voorspellen. Hoewel wij een voor het onderzoek uitvoerbare analyse voor totaal en ongebonden tacrolimus plasma concentraties hebben ontwikkeld, zal hemolyse in de praktijk van invloed kunnen zijn op de plasma concentraties; monsters dienen bijvoorbeeld direct naar het laboratorium gestuurd te worden en direct afgedraaid te worden. Als we ook de lage concentraties in ogenschouw nemen, dan is het moeilijk om deze metingen goed te kunnen interpreteren. Daarom is onze suggestie om hematocriet gecorrigeerde volbloed concentraties te gebruiken voor de dagelijkse transplantatie praktijk. Hematocriet gecorrigeerde tacrolimus dosering helpt om therapeutische waarden van ongebonden tacrolimus concentraties te bereiken en is uitvoerbaar in de huidige praktijk van hart- en longtransplantatie.

REFERENTIES

1. Wehbe E, Duncan AE, Dar G, Budev M, Stephany B. recovery from AKI and short- and long-term outcomes after lung transplatation. *Clinical Journal of the American Society of Nephrology*. 2013;8(1):19-25. doi:10.2215/CJN.04800512.
2. Paradela de la Morena M, La Torre Bravos De M, Prado RF, et al. Chronic Kidney Disease After Lung Transplantation: Incidence, Risk Factors, and Treatment. *TPS*. 2010;42(8):3217-3219. doi:10.1016/j.transproceed.2010.05.064.
3. Ojo AO, Held PJ, Port FK, et al. Chronic Renal Failure after Transplantation of a nonrenal organ. *N Engl J Med*. 2003;349(10):931-940. doi:10.1056/NEJMoa021744.
4. Lund LH, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Official Adult Heart Transplant Report--2013; focus theme: age. *The Journal of Heart and Lung Transplantation*. 2013;32(10):951-964. doi:10.1016/j.healun.2013.08.006.
5. Healy AH, Stehlik J, Edwards LB, McKellar SH, Drakos SG, Selzman CH. Predictors of 30-day post-transplant mortality in patients bridged to transplantation with continuous-flow left ventricular assist devices-An analysis of the International Society for Heart and Lung Transplantation Transplant Registry. *J Heart Lung Transplant*. 2016;35(1):34-39. doi:10.1016/j.healun.2015.07.007.
6. Söderlund C, Löfdahl E, Nilsson J, Reitan Ö, Higgins T, Rådegran G. Chronic kidney disease after heart transplantation: a single-centre retrospective study at Skåne University Hospital in Lund 1988-2010. *Transplant International*. 2016;29(5):529-539. doi:10.1111/tri.12710.
7. Hebert MF, Zheng S, Hays K, et al. Interpreting tacrolimus concentrations during pregnancy and postpartum. *Transplantation*. 2013;95(7):908-915. doi:10.1097/TP.0b013e318278d367.
8. Udy AA, Roberts JA, Lipman J. Clinical implications of antibiotic pharmacokinetic principles in the critically ill. *Intensive Care Med*. 2013;39(12):2070-2082. doi:10.1007/s00134-013-3088-4.
9. Piekoszewski W, Jusko WJ. Plasma protein binding of tacrolimus in humans. *J Pharm Sci*. 1993;82(3):340-341. doi; 10.1002/jps.2600820325
10. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Changes in tacrolimus distribution in blood and plasma protein binding following liver transplantation. *therapeutic drug monitoring*. 2004;26(5):506-515. doi: 10.1097/00007691-200410000-00008
11. Zheng S, Davis CL, Hebert MF. Pharmacokinetics of Tacrolimus During Pregnancy. *therapeutic drug monitoring*. 2012;34:660-670. doi: 10.1097/ftd.0b013e3182708edf
12. Walensky LD, Gascard P, Fields ME, et al. The 13-kD FK506 binding protein, FKBP13, interacts with a novel homologue of the erythrocyte membrane cytoskeletal protein 4.1. *J Cell Biol*. 1998;141(1):143-153. doi: 10.1083/jcb.141.1.143
13. Van Acker K, Bultynck G, Rossi D, et al. The 12 kDa FK506-binding protein, FKBP12, modulates the Ca(2+)-flux properties of the type-3 ryanodine receptor. *J Cell Sci*. 2004;117(Pt 7):1129-1137. doi:10.1242/jcs.00948.

Appendices

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EPILOGUE

How can we help patients like Anna in the future? Anna experienced large fluctuations in the tacrolimus whole-blood concentrations, which were often far outside of the target range. Initially, whole-blood concentrations >30 ng/ml were observed, and subsequently, tacrolimus was stopped. Resuming tacrolimus after halving the dose resulted in more acceptable concentrations, though still often out of range. The lung transplantation itself, severe bleedings with many re-operations and the administration of blood products caused a severe systemic inflammatory syndrome. As a consequence, gut dysmotility occurred. As we know now, gut dysmotility may have been the most important cause of these enormous fluctuations in the tacrolimus concentrations. Therefore, intravenous administration of tacrolimus could have been beneficial for Anna.

Further, the administration of packed red blood cells, ongoing shock, drug-drug interactions and organ failure made it really difficult getting the tacrolimus whole-blood concentrations within the target range. We showed why toxicity may occur even when the whole-blood concentrations are in the therapeutic range. Nowadays, we should aim at the lower range value to decrease the risk of nephrotoxicity. Anna displayed severe anemia (observed hematocrit range 0.18-0.37), promoting high unbound concentrations. Yet, when hematocrit decreases, tacrolimus blood content decreases. At that point, we should have had used hematocrit-corrected tacrolimus dosing, which might have prevented these very high unbound concentrations. Although this thesis does not provide an ultimate solution, by the combination of administering tacrolimus intravenously, aiming for the lower therapeutic range value and adding hematocrit as co-factor in tacrolimus dosing, we might had prevented Anna's severe AKI.

LIST OF PUBLICATIONS RELATED TO THIS THESIS

Maaike A Sikma, MD, Erik M van Maarseveen, PharmD PhD, Claudine C Hunault, MD PhD, Javier M Moreno PharmD PhD, Ed A van de Graaf, MD PhD, Johannes H Kirkels, MD PhD, Prof Marianne C Verhaar, MD PhD, Prof Jan C Grutters, MD PhD, Prof Jozef Kesecioglu, MD PhD, Prof Dylan W de Lange, MD PhD, Prof Alwin D R Huitema, PharmD PhD, Unbound plasma, total plasma and whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation, submitted

Maaike A Sikma, MD, Erik M van Maarseveen, PharmD PhD, Claudine C Hunault, MD PhD, Prof Alwin D R Huitema, PharmD PhD, Prof Dylan W de Lange, MD PhD, Discussion and future perspectives Optimizing tacrolimus dosing in the early post-transplant phase in thoracic organ recipients, submitted

Maaike A Sikma, Claudine C Hunault, Erik M van Maarseveen, Prof Alwin D R Huitema, PharmD, Ed A van de Graaf, Johannes H Kirkels, Prof Marianne C Verhaar, Prof Jan C Grutters, Prof Jozef Kesecioglu, Prof Dylan W de Lange, Extremely high variability of whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation, submitted

Sikma MA, Hunault CC, Kirkels JH, Verhaar MC, Kesecioglu J, De Lange DW, Association of Whole Blood Tacrolimus Concentrations with Kidney Injury in Heart Transplantation Patients, Eur J Drug Metab Pharmacokinet. 2018 Jun;43(3):311-320

Sikma MA, Hunault CC, van de Graaf EA, Verhaar MC, Kesecioglu J, de Lange DW, Meulenbelt J, High tacrolimus blood concentrations early after lung transplantation and the risk of chronic kidney disease. EJCP. 2017 May;73(5):573-580

Stienstra NA, Sikma MA, van Dapperen A, de Lange DW, van Maarseveen EM. Development of a simple and rapid method to measure the free fraction of tacrolimus in plasma using ultrafiltration and LC-MS/MS. Therapeutic Drug Monitoring. Ther Drug Monit. 2016 Dec;38(6):722-727

Sikma MA, van Maarseveen EM, Donker DW, Meulenbelt J. Letter to the editor: "Immunosuppressive drug therapy - biopharmaceutical challenges and remedies". Expert Opin Drug Deliv. 2015 Dec;12(12):1955-7. doi: 10.1517/17425247.2015.1106687

Sikma MA, van Maarseveen ME, van de Graaf EA, Kirkels JH, Verhaar MC, Donker DW, Kesecioglu J, Meulenbelt J, Pharmacokinetics and toxicity of tacrolimus early after heart and lung transplantation, Am J Transplant. 2015 Sep;15(9):2301-13

OTHER PUBLICATIONS

Anja PG Wijnands-Kleukers, Wouter Dijkman, Jeroen Brogtrop, Marlijn JA Kamps, Maaike A Sikma, Dylan W De Lange, Inhalational methanol-intoxication: emerging issues in the Netherlands resulting from illegal drug production, Ann Em Med, 2019 June, accepted

Favié LMA, Murk JL, Meijer A, Nijstad LA, Van Maarseveen EM, Sikma MA, Pharmacokinetics of favipiravir during continuous venovenous hemofiltration in a critically ill patient with influenza, Antivir Ther. 2017 Nov 29

Chavoushi SF, Mesman L, Noordzij P, Sikma MA, Van Maarseveen EM, Attempted suicide with barbiturates purchased online. Ned Tijdschr Geneeskd. 2016;160(0):D491

D.W.M. Hoelen, A.L. van Duijn, C.L. Meuwese, J.P. Ruurda, M.A. Sikma, Intrathoracic gastric herniation as a rare cause of cardiac arrest. Neth J Crit Care. 2014 June; 18(3)

De Lange DW, Sikma MA, Meulenbelt J. Extracorporeal membrane oxygenation in the treatment of poisoned patients. Clin Toxicol (Review). 2013 Jun;51(5):385-93.

W. Kromdijk, M.A. Sikma, M.P.H. Van den Broek, J.H. Beijnen, A.D.R. Huitema, D.W. de Lange, Pharmacokinetics of oseltamivir carboxylate in critically ill patients: Each patient is unique. Intensive Care Med. 2013 May;39(5):977-8

Neijzen R, Ardenne Pv, Sikma M, Egas A, Ververs T, Maarseveen Ev, Activated charcoal for GHB intoxication: An in vitro study. Eur J Pharm Sci. 2012 Dec 18;47(5):801-3

Sikma MA, Broek vd M, Meulenbelt J, Increased unbound drug fractions in acute carbamazepine intoxication: suitability and effectiveness of high-flux haemodialysis. Intensive Care Med, 2012 Feb 11

NA Ramdhani, MA Sikma, TD Witkamp, AJC Slooter, DW de Lange, Paroxysmal Autonomic Instability with Dystonia in a Patient with Tuberculous Meningitis: a case report. J Med Case Reports, 2010 Sep 10;4: 304

R Polak, A Huisman, MA Sikma, S Kersting, Spurious hypokalaemia and hypophosphataemia due to extreme hyperleukocytosis in a patient with a haematological malignancy, Ann of Clin Biochem, 2010 Mar;47(Pt 2):179-81

Groothoff MVR, Hofmeijer J, Sikma MA, Meulenbelt J. Irreversible encephalopathy after treatment with metronidazole, Clin Ther. 2010 Jan; 32(1):60-4

Broek MP van den, Sikma MA, Ververs TF, Meulenbelt J. Severe valproic acid intoxication: case study on the unbound fraction and the applicability of extracorporeal elimination. Eur J Emerg Med. 2009 Dec; 16(6):330-2

Sikma MA, Mier JC, Meulenbelt J, Massive valproic overdose, a misleading case, Am J Emerg Med. 2008 Jan; 26(1): 110.e3-6

M. Sikma, J. Coenen, C. Kloosterziel, B. van Hasselt, T. Ruers, Case report: A breakthrough in cryosurgery, Surgical Endoscopy, 2002, online publication

S. Daenen, M. Sikma, E. Vellenga, M. Halie. 6-mercaptopurine for AML patients not eligible for aggressive remission induction, Blood. 1997; 90 (1): 240b

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CURRICULUM VITAE

Maaike Sikma started to study medical sciences at the University of Groningen in 1992 and obtained her master's degree in 1999. By February 2007, she completed her degree in both internal as well as intensive care medicine at the University Medical Center Utrecht. Hereafter, she started working at the intensive care and at the Dutch Poisons Information Center of the University Medical Center Utrecht. In October 2008 she attained the European degree in intensive care medicine of the European Society of Intensive Care Medicine (EDIC II).

Within the discipline intensive care, Maaike has been the supervisor for organ and tissue donation in the area of Utrecht from 2010 to 2014 and coordinating donation intensivist from 2010 onwards. In this context, she participated in the coordinating group organ donation of the Ministry of Health, the committee organ- and tissue donation of the Dutch Association of Intensive Care, the committee revision of the guideline organ and tissue donation and the ethics committee of the Dutch Transplantation Foundation, and a committee of the Health Counsil of the Ministry of Health.

From 2012 on, she started her study on pharmacology in the critically ill, in particular tacrolimus. This study was started under supervision of Prof. dr. Jan Meulenbelt, head of the Dutch Poisons Information Center and intensivist of the University Medical Center Utrecht and Prof. dr. Jozef Kesecioglu, head of the intensive care of the University Medical Center Utrecht. Professor Meulenbelt died in December 2015. From 2016 onwards, Prof. dr. Dylan W. de Lange supervised the study and PhD of Maaike Sikma. Her knowledge of pharmacology in the critically ill led in 2014 to the supervision of the trainees of clinical pharmacy on the intensive care of the University Medical Center Utrecht. Notable is that in April 2014 she received the formal acknowledgment for completing the endurance journey called "De Friesche Elfstedentocht" by ice skating (1997), hiking (2012) and cycling (2013).