Improving efficacy and reducing adverse effects of immunosuppression after liver transplantation

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CHAPTER 4

TACROLIMUS 4-HOUR MONITORING IN LIVER TRANSPLANT PATIENTS IS NOT SUPERIOR TO TROUGH MONITORING: THE RANDOMIZED CONTROLLED FK04 TRIAL

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Abstract

Background
After liver transplantation (LT), tacrolimus and ciclosporin treatment can lead to, partially concentration-dependent, chronic kidney disease. Monitoring ciclosporin with two-hour levels reduced overexposure and led to better renal function than trough-monitoring (C0). For tacrolimus, a four-hour level (C4) can give a reasonable approximation of total drug exposure. We evaluated whether monitoring tacrolimus in stable patients after LT by four-hour level (C4) was superior to C0 regarding renal function, rejection and metabolic parameters.

Methods
This open label randomized controlled trial compared C4 monitoring of tacrolimus BID (Prograft) to trough (C0) monitoring in stable LT recipients. The target range for C4 of 7.8-16 ng/ml was calculated to be comparable with target C0 of 4-8 ng/ml. Primary endpoint was the effect on renal function and secondary endpoints were the occurrence of treated biopsy-proven acute rejection, blood pressure and metabolic parameters, during 3 months of follow-up.

Results
Fifty patients were randomized to C0 (n=25) or C4 (n=25) monitoring. There was no difference in renal function between the C0 and the C4 group (p = 0.98 and p = 0.13 for CG and MDRD at 3 months). Also, the amount of proteinuria was similar (p = 0.59). None of the patients suffered from graft loss or was treated for rejection. Metabolic parameters did not differ between the two groups.

Conclusion
Tacrolimus 4-hour monitoring in stable LT patients is not superior to trough monitoring, regarding the effect on renal function, but it is safe for use to facilitate tacrolimus monitoring in an afternoon outpatient clinic.

Introduction

Chronic kidney disease (CKD) is a common complication after liver transplantation (LT) with an incidence ranging between 20% and 80%[1-5].

CKD following LT increases cardiovascular burden, can lead to renal replacement therapy and can affect both quality of life and patient survival[6-8]. The main risk factors for post LT CKD have been identified to be preoperative glomerular filtration rate, haemoglobin, hypertension and postoperative average serum levels of calcineurin inhibitors (CNIs). CNIs, including ciclosporin and tacrolimus, are the backbone of immunosuppression after solid organ transplantation. Besides their very potent effect (rates of acute cellular rejection after LT currently are below 20%[9]), an important adverse effect can be renal injury, which is partially dose-dependent[10-13].

Tacrolimus, like ciclosporin, has a narrow therapeutic window and is characterized by a profound inter-patient variability. Therefore therapeutic drug monitoring is warranted. Tacrolimus trough (C0) level correlates reasonably well both with the twenty-four-hour Area under the Concentration-Time Curve (24h AUC)[14-17] and clinical outcome[18,19]. A number of studies in different types of organ transplantation have used less intensive 12h or shortened 6h AUC's and demonstrated that for tacrolimus C0 was a reasonable approximation of AUC[20-24], although there are some publications reporting a much lower correlation between C0 and the AUC[25,26].

Although we have developed pharmacokinetic models with a derived limited sampling formula and derived limited sampling models with Bayesian estimation of the AUC for both tacrolimus BID (Prograft) as well as tacrolimus QD (Advagraf) dosing after LT[27,28], for practical purposes C0 is still widely used. Monitoring ciclosporin after LT with two-hour levels reduced overexposure and led to better renal function than trough-monitoring (C0)[29]. After LT there was an excellent correlation between AUC and a single time point measurement of tacrolimus concentration four hours after dosing (C4) when used in the limited sampling model or with a limited sampling formula[28]. This inspired us to design the current randomized controlled study in which C4 monitoring is compared to trough (C0) monitoring of tacrolimus BID (Prograft) in stable LT recipients, with renal function as primary endpoint. We hypothesized that C4 monitoring was superior to C0 monitoring regarding renal function.

Patients and Methods

Patients and study design
The FK04 study was a single centre, randomized controlled open label study, which was initiated by and performed in the Leiden University Medical Center, Leiden, the Netherlands.

Stable LT recipients between 18 and 75 years old, more than six months after LT were included. Patients were excluded if they underwent multi-organ transplantation, were pregnant or breast-feeding, had a systemic infection (except viral hepatitis), were allergic/intolerant to macrolide antibiotics or tacrolimus, had a gastro-intestinal disorder or diarrhoea. Patients with known CKD (serum creatinine > 200 µmol/l), patients who required parallel therapy with immunosuppressive antibody preparations, who participated in another clinical trial, who were known with substance abuse or psychiatric disorders, or were unlikely to comply with the study visits were also excluded.

At randomization all patients used similar tacrolimus BID (Prograft, Astellas Pharma B.V, Leiden, NL). If patients used cyclosporine after LT, they were first converted to tacrolimus BID (Prograft) and entered the study as a separate stratum, after a three-months period in which the dose was stabilised and not changed in the last month.

Patients were randomized 1:1 to C0 or C4 monitoring. Randomization took place by drawing blinded treatment allocation envelopes. The C0 group continued the standard regimen with trough levels 4-8 ng/ml (equivalent to AUC 90-140 ng*h/ml), the other group was dosed on a C4 level 7.8-16.0, but preferably 7.8-12.2 ng/ml , which is equivalent to a C4 AUC level of 90-185 and 90-140 respectively (using limited sampling formulas from our previous publication) (28). Treating physicians of patients dosed on C0 levels were blinded for C4 levels, while physicians of patients dosed on C4 levels were blinded for C0 levels, and all were blinded for AUCs. The total duration of the study was twelve weeks, with study visits at baseline and in weeks 4, 8, and 12.

The study was conducted according to the Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol and amendments were approved by the Institutional Review Board and Independent Ethics Committee. All patients provided written informed consent before enrolment.

Therapeutic drug monitoring
Determination of tacrolimus blood levels was performed using a previously validated LC–MS/MS assay capable of determining tacrolimus, sirolimus,

everolimus and cyclosporine simultaneously[27]. All parameters were in accordance with the bioanalytical method validation guideline of the European Medicines Agency[30]. AUC_{0-12} MAP Bayesian estimation was performed using MW/Pharm version 3.83 (Mediware, Groningen, the Netherlands), based on models for tacrolimus C0, C1, C2, C3 and C4 yielding the estimated AUC from time zero to 12 h (AUC0–12)[31].

All concomitant immunosuppressive medications used in combination with tacrolimus at start of study were maintained at a constant dose throughout the duration of this study. If changes were required, the reason was recorded. In case of medical need, patients could be converted back to their original immunosuppressive regimen.

Visits and Evaluation
Baseline measurements consisted of complete physical examination, vital signs, tacrolimus trough level, laboratory assessments (including complete blood count, serum creatinine, electrolytes, liver enzymes and function, blood glucose and lipid panel) and 24h urine analysis. Thereafter, study visits were scheduled at 4, 8 and 12 weeks after randomization. Additional visits could be made if necessary. During each visit vital signs, laboratory determinations as previously mentioned and C0 or C4 levels of tacrolimus were obtained. Abbreviated AUC's were sampled at baseline and at the end of the study.

Endpoints
The primary endpoint of this study was renal function (calculated by BSA-adjusted Cockcroft and Gault and MDRD) at 12 weeks after randomization between C0 or C4 based tacrolimus monitoring. Secondary endpoints included arterial blood pressure, lipid- and fasting glucose levels and number and dose of antihypertensive-and lipid-lowering medication. Safety secondary endpoints measured throughout the study included patient- and graft survival, treated biopsy proven acute rejection (tBPAR) and all recorded adverse effects. In case of increase of liver enzymes ASAT, ALAT, ALP or GGT a liver biopsy had to be performed to exclude tBPAR, otherwise not. At baseline (randomization) and at 12 weeks an abbreviated AUC for tacrolimus was performed for comparisons and correlation with C0 and C4.

Statistical analysis
Power analysis was performed using an α of 5% as the critical p-value for superiority of C4 over C0 monitoring and a power with a 1-beta of 80%. The sample size calculated to detect a difference in serum creatinine >13.4 umol/l (comparable to the difference between C2 and C0 monitoring of ciclosporin) between the parallel groups on tacrolimus with C0 versus C4 monitoring was 47. To compensate for

patient drop-out, the aim for inclusion was n = 50. The study was designed as RCT with intention-to-treat analysis and results were verified using per-protocol analysis. All patients who were randomized and had received at least one dose of study medication were included in the safety analysis.

Categorical data were reported as frequency (percentage), and continuous data were reported as mean with standard deviation (SD). Correlation was by Passing-Bablok regression analysis.

For comparison of the two monitoring methods (C0 vs C4) regarding renal function, rejection, blood pressure and metabolic parameters, the t-test was used. A $p<0.05$ was considered statistically significant. SPSS Statistics 25 (IBM, Chicago IL, USA) was used for statistical analysis.

Results

Patients

50 patients after LT and on a stable CNI based regime were included in the study (Supplementary Figure S1). Eight patients (16%) had been converted from cyclosporin to tacrolimus BID and for 3 months maintained on C0 4-8 ng/ml before randomization. The remaining 42 patients (84%) were already treated with tacrolimus BID with these levels. Patients were evenly randomized (25/25) between the C0 and C4 group. All patients (100%) completed the study. Therefore per-protocol analysis was similar to intention-to-treat analysis. The median time to transplant in the C0 group was: 52.7 (SD ±45.3) months. This was slightly -but not significantly- longer than in the C4 group: 32.4 (SD ±29.9) months (p = 0.07). Baseline renal function, lipid levels and blood pressure were similar between the two groups, although the patients in the C0 group were significantly younger and used less prednisolone (Table 1). For patients using prednisolone, the dose was 5mg, whereas if patients used mycophenolate mofetil (MMF) the dose ranged between 1000mg-2000mg per day. The number of patients using MMF and doses did not differ between groups.

Primary endpoint

During the 12 weeks follow-up after randomisation, renal function estimated by Cockcroft-Gault (CG) and MDRD remained stable within the C0 and C4 group. At the end of the study, there was no difference in renal function between the C0 and the C4 group (p = 0.98 and p = 0.13 for CG and MDRD). Also, the degree of proteinuria was similar (p = 0.59) (Table 2).

Table 1: Baseline Characteristics

	C0 group		C4 group		p - value
	Mean	SD	Mean	SD	
Renal function, MDRD (ml/min/1,73 m2)	85.4	± 44.1	69.5	± 25.9	0.13
Renal function, Cockcroft-Gault (ml/m2)	91.6	± 47.0	91.3	± 28.6	0.98
Proteinuria (g/24h)	0.23	± 0.26	0.20	± 0.14	0.59
Blood pressure systolic (mmHg)	134	± 17.2	141	± 19.2	0.19
Blood pressure diastolic (mmHg)	84	± 8.1	86	± 13.1	0.51
Total cholesterol (mmol/L)	4.5	± 0.9	5.2	± 1.1	0.02 *
Triglycerides (mmol/L)	1.5	± 1.1	1.8	± 0.7	0.26
Glucose (mmol/L)	6.6	± 3.0	6.3	± 2.1	0.75

Table 2: Primary and secondary Endpoints

	C0 group		C4 group		p - value
	Mean	SD	Mean	SD	
Age	47.6	± 13.1	55.6	± 9.0	0.02 *
Renal function, MDRD (ml/min/1,73 m2)	83.5	± 44.1	68.6	± 22.9	0.14
Renal function, Cockcroft-Gault (ml/m2)	102.8	± 35.2	89.7	± 27.2	0.16
Serum creatinine (mmol/L)	95.8	± 25.1	105.7	± 29.6	0.21
Blood pressure systolic (mmHg)	136	± 16.2	140	± 13.6	0.45
Blood pressure diastolic (mmHg)	87	± 10.0	88	± 6.4	0.72
Total cholesterol (mmol/L)	4.7	± 1.2	5.2	± 1.2	0.16
Triglycerides (mmol/L)	1.6	± 0.9	1.6	± 0.7	0.83
Glucose (mmol/L)	7.0	± 2.9	6.3	± 2.4	0.37
Gender (male %)	76		72		0.75
Concomitant use of MMF (%)	64		60		0.78
Concomitant use of prednisolone (%)	28		56		0.046 *

SD = standard deviation ; * = statistically significant

Secondary endpoints

Metabolic parameters

Blood pressure did not differ between groups throughout the study, nor did serum fasting glucose and triglycerides (Table 2). Serum total cholesterol was significantly lower in the C0 than in the C4 group (p = 0.02). The number of antihypertensive- and lipid-lowering medications were similar (Supplementary Table S1).

Survival and graft rejection

Patient survival at the end of the study was 100% in both groups. Graft loss was observed in none of the patients during the study. Two patients (one in the C0 and one in the C4 group) lost their graft 3 and 6 years after study closure from unrelated causes. None of the 50 patients had a clinical suspicion of rejection based on absence of changes in liver enzymes, therefore no liver biopsies had to be performed to further exclude tBPAR.

Tacrolimus pharmacokinetics

Tacrolimus dosage was adjusted during the study, based on C0 or C4 levels according to protocol. The correlation for C4 levels and AUC was better than the correlation between C0 and AUC (R2= 0.802 vs 0.588) (Figure 1).

At the end of the study, 23 patients in the C0 group (92%) and 17 patients in the C4 group (68%) had tacrolimus levels within the target range (p = 0.04).

The AUC 0-12h at start (C0 mean 107,5; C4 mean 104,4) and end (C0 mean 98,0; C4 mean 99,2) of the study were comparable between the two groups (p = 0.77 and p = 0.89).

Figure 1: Correlation plot of tacrolimus C0 level versus AUC

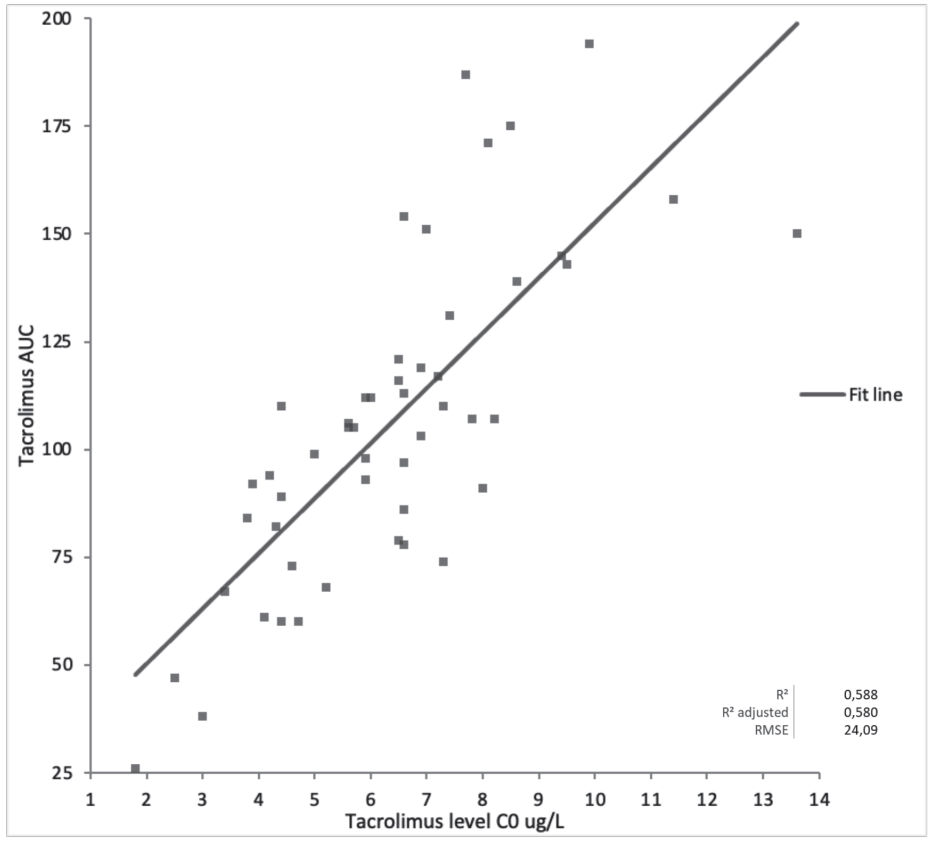
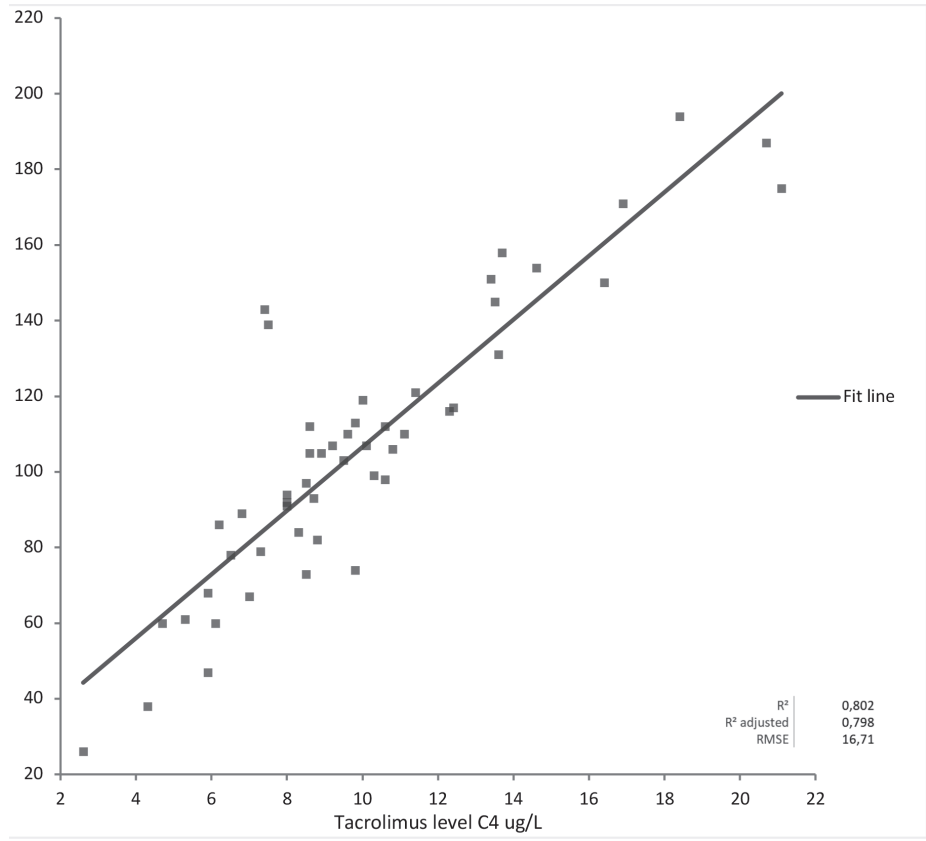


Figure 2: Correlation plot of tacrolimus C4 level versus AUC



Discussion

This RCT demonstrates that C4 monitoring of tacrolimus BID is not superior to trough level (C0) monitoring in stable adult LT recipients, with a similar outcome regarding renal function, metabolic parameters and safety endpoints.

Since the therapeutic window of tacrolimus is narrow, inadequate dosing and monitoring can lead to under- or overexposure, which can promote rejection or adverse effects -including renal dysfunction- respectively.

Tacrolimus trough level monitoring corresponds reasonably well with the 24h AUC[14-17]. A downside of trough level monitoring is a limited flexibility whilst managing outpatients, requiring outpatient visits during the morning. It was shown before that, unlike cyclosporine, 2h (C2) monitoring in tacrolimus did not correlate well with AUC[20,32] and therefore has no clinical value. In a previous study in stable patients after LT we found C4 to reasonably correlate to AUC[28]. Our current results also demonstrate a better correlation of AUC with C4 than with C0 levels.

The present study shows that C4 monitoring is not superior to trough level monitoring regarding the effect on renal function, but that it is safe to use, with no rejection or other differences in potential CNI induced side effects like hypertension, hyperglycaemia or dyslipidaemia. The difference in endpoint total cholesterol between the two groups can be attributed to improved cholesterol levels in the C0 group, rather than worsening of lipid levels in the C4 group.

C4 monitoring offers an easier scheduling for the outpatient clinic, by making afternoon visits possible. One of the limitations of C4 monitoring is that samples must be obtained within a quarter of an hour before or after the C4 moment, because the target range has been based on a limited sampling formula and not a Bayesian limited sample model. Since we complied strictly with these time limits this does not explain the lower number of C4 patients within the target range of AUC comparable to a trough level of 4-8 nl/l. A possible explanation is that it was sometimes difficult to keep the C4 between 7.8-12.2 ng/l in the 12 weeks after randomization, possibly due to some more variability for C4 than for C0, and due to monitoring with a monthly interval.

Tacrolimus dose was adjusted in 24% of the patients in the C0 group versus 48% in the C4 group. This is probably a response to the fact that more patients in the C4 group where out of therapeutic range. There appears to be more variation in peak levels than in trough levels, which was expected. This could indicate some "over-correction" with C4 monitoring, at least more correction than with C0 monitoring

if aiming for the same AUC, but it could also indicate some "under-correction" with C0 monitoring.

The concomitant use of prednisolone was higher in the C4 group, but this did not lead to unwittingly acceptance of lower tacrolimus levels, since the AUC levels of tacrolimus did not differ between groups throughout the study. Median AUC levels were below target in both groups without rejection, possibly due to the use of concomitant immunosuppressive therapy and a study population with a longer period after LT, where lower AUCs often do not lead to rejection.

A limitation of the study is a possible variation in C0 or C4 times. Blood sampling for the C0 or C4 measurements was performed in the outpatient clinic. Patients in the study were instructed to have the trough level drawn exactly 12 hours after the last tacrolimus dose and the C4 level exactly 4 hours after the morning dose. Despite this instruction we cannot rule out some variation in C0 and C4 times, but this was not more than 15 minutes earlier or later. Exact times were not registered.

Bayesian limited sampling models have been proven to be more accurate than trough or C4 monitoring of tacrolimus[27] and with the development of dried blood spot tests, even home-based monitoring is possible[33]. Although these improvements in therapeutic drug monitoring are very promising, resources and availability of these tests are still limited in most centres, and not all patients are able to handle dried blood spot home tests.

In conclusion, C4 monitoring of tacrolimus in stable patients after LT is safe but not superior to trough level monitoring. For an afternoon outpatient LT clinic, C4 monitoring provides a patient-friendly alternative to C0 monitoring. For clinical purpose, we recommend a C4 level between 8-12ng/ml. A higher level (12-16 ng/ml) could be used in the first three months after transplantation especially if no co-medication like MMF is given.

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Abbreviations

AUC	Area Under the concentration-time Curve
BSA	Body Surface Area
C0	Trough level monitoring
C2	Two hour level monitoring
C4	Four hour level monitoring
CG	Cockcroft-Gault
CKD	Chronic Kidney Disease
CNI	Calcineurin inhibitor(s)
LT	Liver Transplantation
MDRD	Modification of Diet in Renal Disease Study
MMF	Mycophenolate mofetil
RCT	Randomized Controlled Trial
SD	Standard deviation
tBPAR	Treated biopsy proven acute rejection

Supplementary Files