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Original Article

Randomized Trial of Ciclosporin with 2-h Monitoring vs. Tacrolimus with Trough Monitoring in Liver Transplantation: DELTA Study

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Abstract

Background and Aims: Previous trials comparing ciclosporine and tacrolimus after liver transplantation (LT) showed conflicting results. Most used trough monitoring for ciclosporine (C0), leading to less accurate dosing than with 2-h monitoring (C2). Only one larger trial compared C2 with tacrolimus based on trough level (T0) after LT, with similar treated biopsy-proven acute rejection (tBPAR) and graft loss, while a smaller trial had less tBPAR with C2 compared to T0. Therefore, it is still unclear which calcineurin inhibitor is preferred after LT. We aimed to demonstrate superior efficacy (tBPAR), tolerability, and safety of C2 or T0 after first LT. ***Methods:*** Patients after first LT were randomized to C2 or T0. tBPAR, patient- and graft survival, safety and tolerability were the main endpoints, with analysis by Fisher test, Kaplan–Meier survival analysis and log-rank test. ***Results:*** In intention-to-treat analysis 84 patients on C2 and 85 on T0 were included. Cumulative incidence of tBPAR C2 vs. T0 was 17.7% vs. 8.4% at 3 months (p=0.104), and 21.9% vs. 9.7% at 6 and 12 months (p=0.049). One-year cumulative mortality C2 vs. T0 was 15.5% vs. 5.9% (p=0.049) and graft loss 23.8% vs. 9.4% (p=0.015). Serum triglyceride and LDL-cholesterol was lower with T0 than with C2. Incidence of diarrhea in T0 vs, C2 was 64% vs. 31% ($p\leq0.001$), with no other differences in safety and tolerability. ***Conclusions:*** In the first year after LT immunosuppression with T0 leads to less tBPAR and better patient-/re-transplant-free survival as compared to C2.

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Keywords: Adverse effects; Ciclosporin; Liver transplantation; Outcome; Randomized controlled trial; Rejection; Survival; Renal function; Tacrolimus.

Abbreviations: ACR, acute clinical rejection; AE, adverse event; AUC, area under the curve; AZA, azathioprine; BPAR, biopsy-proven acute rejection; C0, CsA with blood concentration pre-dose (trough level); C2, CsA with blood concentration 2 h after drug intake; CI, confidence interval; CNI, calcineurin inhibitor; CR, chronic rejection; CsA, ciclosporine A (microemulsion form); GFR, glomerular filtration rate; HCV, hepatitis C virus; IEC, independent ethics board; IRB, institutional review board; ITT, intent-to-treat; KM, Kaplan–Meier; LDL, low density lipoprotein; HDL, high density lipoprotein; MeDRA, Medical Dictionary for Regulatory Activities; OLT, orthotopic liver transplantation; MMF, mycophenolate mofetil; PP, per-protocol; PTLD, post-transplant lymphoproliferative disorder; SAE, serious adverse event; SCr, serum creatinine; Tac, tacrolimus; T0, Tac with blood concentration pre-dose (trough level); tACR, treated ACR; tBPAR, treated BPAR.

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Introduction

Calcineurin inhibitors (CNIs) are the mainstay of immunosuppression after liver transplantation (LT). Initially, fast-release tacrolimus (Tac) was compared to the original ciclosporin (CsA) formulation, demonstrating that Tac had advantages over CsA with lower rejection rates but more adverse events (AEs) and discontinuation.[1,2] Later, microemulsion CsA, with improved pharmacological properties,[3,4] led to less rejection and AEs than the old CsA.[5] Several randomized controlled trials (RCTs) of (microemulsion) CsA vs, Tac in the first LT have been published.[6–9] of which most reported one-year data.[10–20] In a 2016 meta-analysis, Tac with trough level monitoring (T0) compared to microemulsified CsA was associated with similar treated biopsy-proven acute rejection (tBPAR) rates,

with no difference in one-year graft loss.[21] In contrast, an older meta-analysis from 2006 had shown less acute rejection and better graft- and patient survival for Tac compared to CsA after LT.[22] However, all except one of these larger studies used trough level monitoring of CsA (C0). In a smaller study Levy *et al*. found less rejection with C2 compared to T0, while in a larger RCT by this group in *de novo* LT comparing C2 vs. T0, no differences in mortality, acute rejection, or renal function were detected.[17,18] Using 2-h CsA monitoring (C2) better reflects the area under the curve (AUC) and has been associated with less rejection and better renal function than C0.[17,23,24] Because of this, in previous studies in LT that compared C0 with T0, some differences may have been caused by inaccurate dosing of CsA due to trough-level monitoring. This implies that it is still unclear which CNI is superior after LT. Therefore the objective of the present RCT was to demonstrate superior efficacy in terms of tBPAR, tolerability, and safety of either CsA with 2 h monitoring or Tac with trough-level monitoring (T0) after first LT.

Methods

Study design and setting

The DELTA study was an open-label, parallel-group superiority parallel two-arm investigator-initiated RCT involving the three university medical centers of Leiden, Rotterdam, and Groningen performing LT in the Netherlands.

Patients, inclusion and exclusion criteria, and randomization

All patients 18–75 years of age who underwent their first LT were included. Exclusion criteria were combined or ABO-incompatible transplant, being not eligible to receive 10 mg/kg/day as the initial dose of Neoral (CsA) (e.g., in case of severe renal insufficiency), seropositivity for human immunodeficiency virus antibodies, urine production of <200 mL within 12 h after reperfusion, severe coexisting disease, unstable medical condition that could affect the study objectives, unlicensed drug or therapy administered within one month prior to study entry, or instituted post-transplantation. Informed written consent was obtained prior to transplantation. Baseline data were collected at that time and immediately before transplantation.

Intervention

After randomization CsA (Neoral) or Tac (Prograft), comparable to standard practice, was administered within the first 48 h postoperatively, based on adjusted body weight, for CsA at an initial oral dose of 10.0 mg/kg/day, and for Tac at an initial oral dose of 0.1 mg/kg/day, both in two divided doses (BID) daily on an empty stomach. The dose was adjusted to obtain the required blood drug levels daily for the first 5 days, then twice weekly, then weekly, and then at all visits. Target 2 h (±15 m) blood CsA level for the first 3 months was 1,000 (800–1,200) μg/L, from 3 months on 800 (700–900) μg/L, while the target trough level for Tac during the first 3 months was 10 μg/L (8–15 μg/L), thereafter 5–10 μg/L, comparable to the institutional protocols. Short-term intravenous CsA or Tac was allowed only if it could not be administered orally or per feeding tube. As monoclonal essays for measuring CsA C2 levels the Abbott FPIA AxSYM, Dade Behring Syva EMIT and Dade Behring Dimension were used in the three hospitals. For T0 level measurements, Abbott IMX MEIA, Dade Behring Syva EMIT, and Abbott FPIA TDz were used. The study duration was 6 months with an extension to 12 months and daily visits in the first 2 weeks, weeks 3 and 4, and at least 2, 3, 6, and 12 months.

Endpoints

The primary objective of this study was to compare the efficacy of a C2 regimen to a T0 regimen in combination with steroids and induction therapy with basiliximab (anti-CD25 therapy) in the prevention of tBPAR after *de novo* LT. Cumulative incidence of tBPAR at 3 months after LT was the primary endpoint, cumulative incidences of tBPAR at 6 and 12 months were secondary endpoints.

Acute rejection was suspected by a rise in liver enzymes with or without clinical signs. Biopsy-proven acute cellular rejection (BPAR) was defined as acute rejection confirmed by a liver biopsy according to the Banff classification of rejection after LT, and if anti-rejection treatment was administered this was called tBPAR.[25] If histological confirmation was not possible an acute rejection could be treated according to standard protocol; these rejections together with the tBPAR cases formed the category of treated acute clinical rejection (tACR). The pathologists were blinded for treatment groups. The decision for treatment vs. no treatment for rejection was left to the discretion of the transplant team.

Other secondary endpoints included chronic rejection diagnosed according to the adjusted Banff criteria,[26] histological grading of tBPAR,[25] retransplantation, patient survival, combinations thereof, biometrics (blood pressure and weight), biochemistry, safety and tolerability, conversion of immunosuppression, causes of graft loss (by retransplantation or mortality), and long-term outcome.

Safety analysis was performed in all randomized patients. Hypertension and hyperlipidemia were defined by the updated World Health Organization (WHO) criteria.[27,28] Safety endpoints measured throughout the study included renal function, occurrence of malignancies, infections, and any adverse or serious adverse events (AEs), classified according to the Medical Dictionary for Regulatory Activities (MeDRA) classification.[29] Infections were considered clinically significant as defined by the Centers for Disease Control (CDC).[30] Post-transplant diabetes mellitus (PTDM), new-onset hypertension and new-onset hyperlipidemia were defined by use of medication for these conditions during but not before the study.

Sample size

The sample size calculation yielded *n* of 124 (62 per group), based on a 20% reduction in tBPAR risk of 30% vs. 10% (two-sided chi-square test), based on Levy *et al*. (11% tBPAR with C2 vs. 36% with T0),[17] and $\alpha=5\%$ as the critical *p*-value for superiority of either drug, with a power of $1-\beta=80\%$. To compensate for early discontinuations in the first 3 months and between 3 and 12 months, the minimum number of included patients was 150 and 171 respectively with 1:1 randomization. This sample size was also sufficient to show equivalence between the groups, with a noninferiority margin of 5%.

Randomization, data management and IRB approval

Randomization was performed within 24 h post LT, and 1:1 to C2 or T0, in blocks with random numbers by drawing blinded treatment allocation envelopes. Non-stratified randomization was reviewed by a Biostatistics Quality Assurance group and locked after approval. Patients who discontinued the study were excluded from the study. All data were immediately uploaded using TRIALINK software and secured on a locked server with an audit trail for all data changes. Only in case of severe renal dysfunction, prescription of mycophenolate mofetil (MMF) 500–1,000 mg BID or azathioprine 50–

150 mg QD was allowed. As study medication was often delayed to the second day after LT for impaired renal function, an amendment allowing a delay in first study medication to a maximum of 48 h (instead of 24 h in the original protocol) postoperatively was approved by the institutional review board (IRB)/Independent Ethics Committee (IEC) and Research Ethics Board (REB). A safety board was not required. This study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol and amendments were approved by the IRB or the REB of each participating center. The trial was registered in the Dutch Trial Registry (number NTR489) and Clinicaltrials.gov (number NCT00149994).

Statistical analysis

The trial was designed as an RCT with intention-to-treat (ITT) analysis. In addition, as defined in the protocol, per-protocol (PP) analysis was performed for those at least 6 months on the allocated treatment. All subjects who were randomized and received at least one dose of study medication were included in the safety analysis. The study medication was not blinded and the initial statistical analysis was blinded. Comparisons between the two treatment groups were assessed using the Wilcoxon rank-sum test for continuous variables and the two-sided Fisher exact test for categorical variables. To assess the comparability of blood biochemistry results, a mixed-model analysis with fixed effects was used. Time-to-event outcomes were analyzed using Kaplan–Meier survival analysis with standard error of the mean (SE) and log-rank test with hazard ratios (HRs) and 95% confidence intervals (CIs) for comparison, as specified in the protocol. Statistical significance was set at $p<0.05$. SPSS version 24 (IBM Corp., Armonk, NY, USA) and R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analyses. For more details on methods see the Supplementary File 1.

Results

Patients

Of 187 patients eligible for informed consent, 11 were not transplanted and were not randomized; five were transplanted but died immediately postoperatively. Thus, the safety population included 171 randomized patients. All patients underwent LT with the whole liver obtained from a deceased donor and were randomized 2002 through 2006. One patient was excluded from the ITT analysis because of an administrative problem, randomized but not transplanted at that time, and one patient was excluded for a protocol violation, leaving 169 patients (84 on C2 and 85 on T0) for ITT analysis (Fig. 1). In total, 151 patients (69 at C2 and 82 at T0) fulfilled the predefined requirements for the per-protocol analysis. Except for the etiology of acute liver failure, the patient characteristics were similar between the groups (Table 1). The drug levels for C2 and T0 are shown in the Supplementary File 1. As shown, it took 5 days to reach target C2, staying on target thereafter, while T0 was on target immediately, adjusted to a T0 just above 10 for the first 3 months, and 5–10 µg/L thereafter. The use of additional immunosuppressants in cases of severe renal dysfunction did not differ between the C2 and T0 groups, and during the study there was significantly more study drug conversion in the C2 group (11/84) than in the T0 group (1/85, $p=0.0024$, as shown in the Supplementary File 1).

Endpoints of ITT analysis

The results of the ITT analysis of the primary and main secondary endpoints for both the raw incidence rates and for the Kaplan–Meier survival estimate are shown in Table 2.

tBPAR

The cumulative incidence of tBPAR-censored for death and retransplantation in KM analysis within 3 months after LT was numerically but not significantly higher for C2 than for T2, 17.7% (95% CI: 9.3–26.1) for C2 vs. 8.4% (95% CI: 2.5–14.3) for T0 [HR 2.088 (95% CI: 0.866–5.026), $p=0.10$]. At both 6 and 12 months this cumulative incidence of tBPAR was significantly higher with C2 than with T0 (21.9% for C2 and 9.7% for T0 at both 6 and 12 months, $p=0.049$, Table 2, Fig. 2).

Chronic rejection

Chronic rejection occurred within 12 months in 3/84 (4%) of C2-treated patients vs. 0/85 in the T0 group (Fisher exact test $p=0.12$, log-rank $p=0.07$).

Mortality

The Kaplan–Meier estimate for the cumulative incidence of mortality within 3 months was not different between C2 and T0 at 3 and 6 months, but was significantly higher at 12 months with C2 compared with T0 (15.5% vs. 5.9%, log-rank $p=0.049$; Table 2, Fig. 3).

Retransplantation

Cumulative incidence of retransplantation (re-LT or death-censored graft failure) was numerically higher in the C2 group as compared to the T0 group, but that was not a statistically significant difference (Table 2).

Retransplantation-free survival

In the Kaplan–Meier analysis, the combined endpoint of retransplantation or mortality within 12 months after LT was more frequent in the C2 group that in the T0 group (23.8% vs. 9.4%, $p=0.015$), so retransplantation-free survival within 12 months was better with T0 than with C2. This and causes of graft loss are shown in the Supplementary File 1.

Combined endpoint of treated BPAR or retransplantation or mortality

In the Kaplan–Meier analysis, the combined endpoint of tBPAR, retransplantation or mortality occurred more frequently in the C2 group compared with the T0 group, both within 6 months after LT (32.1% vs. 17.6% respectively, $p=0.04$) and within 12 months after LT (39.3% vs. 18.8% respectively, $p=0.006$; Fig. 4).

More secondary endpoints are shown in the Supplementary File 1.

Biometrics and biochemistry

Systolic blood pressure was higher with C2 at 6 months than at baseline ($p=0.014$), and diastolic blood pressure was higher than at baseline after 6 months for both T0 ($p=0.002$) and C2 ($p=0.001$), with no difference between C2 and T0. No significant within- or between-group differences were observed throughout the study in terms of the incidence of hypertension or body weight. These data are shown in the Supplementary File 1.

As shown in Table 3, serum triglyceride and LDL-cholesterol levels were lower with T0 than with C2 (mean 1.7±1.0 mmol/L vs. 1.9±1.2 mmol/L, $p=0.03$, and mean 2.3±1.3 mmol/L vs. 2.8±1.2 mmol/L, $p=0.01$ respectively) after 12 months. No differences were found between the groups in terms of changes in HDL-cholesterol levels. Fasting glucose

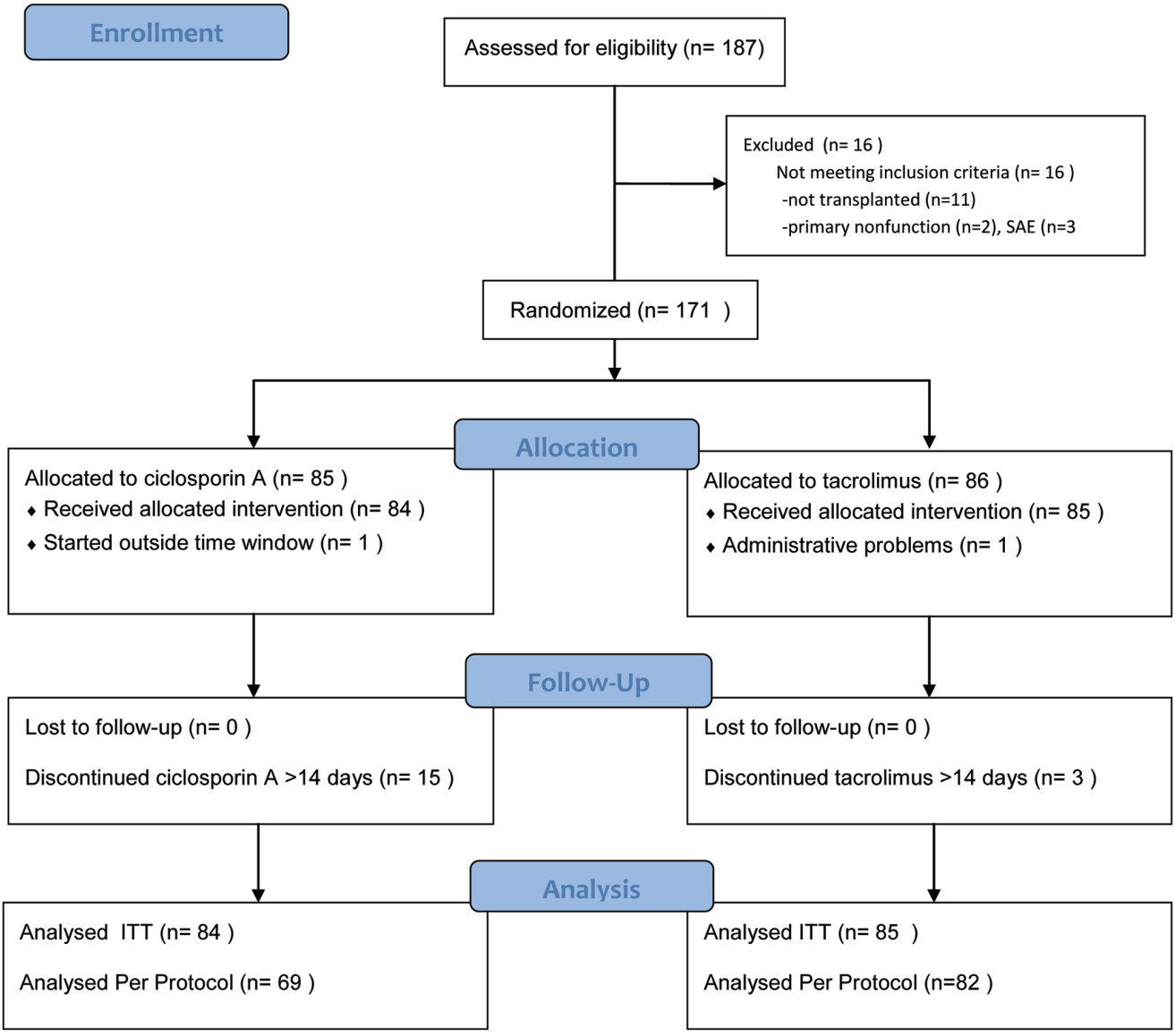

Fig. 1. CONSORT Patient flow chart for ITT and per-protocol analysis. ITT, intent-to-treat.

and serum creatinine levels did not differ between T0 and C2 at 3, 6, and 12 months. The mean calculated creatinine clearance was similar between T0 and C2 at the start (107 m /min vs. 113 m /min (p=0.41). Creatinine clearance at 3 months was lower for T0 than C2 (79 vs. 91 mL/m, p=0.029), but was similar at 6 (80 mL/m vs. 88 mL/m), and 12 months of treatment (83 mL/m vs. 87 mL/m, p=0.470) in T0 vs. C2, respectively, with a similar decrease in creatinine clearance after 12 months compared to baseline (−25.9 mL/m vs. (−27.8 mL/m respectively, p=0.47)

Treatment-emergent AEs

All patients experienced one or more AE, with no differences between the two treatment arms in the total number of treatment-emergent AEs or SAEs, as shown in the Supplementary File 1. Patients in the T0 group experienced more diarrhea (65%) than those in the C2 group (31%) (p<0.001). More patients with T0 than C2 experienced an infection (51% T0 vs. 49% C2, HR 0.375, 95% CI: 0.144–0.979, p=0.045). The total number of infections did not differ between groups (321 vs. 342 clinically significant infections with T0 vs. C2, respectively), nor was there any difference found in the site or type of pathogen. *Post-hoc* analysis demonstrated that more patients in the C2 group than in the T0 group experienced one of the more early infection episodes, defined as less than one month after LT (127 vs. 104, p=0.049). In contrast, the T0 group experienced more late infections, defined as between 1 and 12 months after transplantation, than the C2 group (100 vs. 63, p=0.002). Sepsis tended to be a more common cause of death with C2 (n=7) than with T0 (n=2), but the difference was not significant.

In patients with 3 or more months of re-transplant-free survival, treatment for (new-onset) PTDM occurred in 14/79 (17.7%) patients on T0 vs. 11/75 (14.7%) patients on C2 (p=0.77); treatment for new-onset hypertension after transplantation occurred in 18/79 (22.8%) patients on T0 vs.

Table 1. Patient demographics and baseline characteristics of the ITT population

Treatment allocation	Cyclosporin	Tacrolimus	*p*-value
n	84	85	
Site G/L/R	18/27/39	16/29/40	0.908
Sex, male	53 (63.1)	55 (64.7)	0.873
Age, years	48.1±12.1	49.9±9.9	0.287
Ethnicity			0.779
Caucasian	67 (79.8)	67 (78.8)	1.000
Afro-European	4 (4.8)	6 (7.1)	0.746
Oriental/Asian	9 (10.7)	10 (11.8)	1.000
other	4 (4.8)	2 (2.4)	0.443
Cause of underlying liver disease			
viral hepatitis (B,C,D)	14 (16.7)	17 (20)	0.692
alcoholic liver disease	14 (16.7)	18 (21.2)	0.557
hepatocellular/cholangiocarcinoma**	2 (2.4)	5 (5.9)	0.443
autoimmune liver disease***	29 (34.5)	32 (37.6)	0.749
metabolic liver disease	2 (2.4)	4 (4.7)	0.681
non-alcoholic fatty liver disease	1 (1.2)	0	0.497
acute liver failure	5 (6.0)	0	0.030
cryptogenic, drug induced, other*	17 (20.2)	9 (10.6)	0.092
Child-Pugh score at randomization	7.64±1.912	8.22±2.154	0.123
MELD score at randomization	14.62±7.776	15.6±6.912	0.896
Cold ischemia time (CIT)	8 h 13 m ± 2 h 55m	8 h 33 m ± 2 h 36 m	0.438
Warm ischemia time	35±10.3 m	33±10.9 m	0.279

Values with ± are means ±SD; otherwise, *n* (%).Race was self-reported. The underlying causes of liver disease were grouped into 20 separate categories. *Other causes of liver disease in the CsA group (*n*=11) were cirrhosis due to cystic fibrosis, familial amyloid polyneuropathy (*n*=5), polycystic liver disease, Caroli syndrome (*n*=2), Budd-Chiari syndrome, and epithelioid hemangioendothelioma. Other causes of liver disease in the tacrolimus group (*n*=7) were familial amyloid polyneuropathy, Budd-Chiari syndrome (*n*=2), Rendu-Osler-Weber syndrome, polycystic liver disease (*n*=2), and vanishing bile duct syndrome without prior transplantation. **Cholangiocarcinoma in primary sclerosing cholangitis (PSC) in one as incidental finding after OLT. ***Autoimmune hepatitis, primary biliary cholangitis, or PSC. MELD score: Mayo End-stage Liver Disease score. ITT, intent-to-treat.

26/75 (34.7%) patients on C2 (p=0.15); treatment for new-onset lipidemia after transplantation occurred in 3/79 (3.8%) patients on T0 vs. 4/75 (5.3%) patients on C2 (p=0.71).

Except for renal function, which was the indication for prescription, there were no significant differences in the primary or secondary endpoints between patients using or not using mycophenolate mofetil or azathioprine (not shown).

More ITT and all PP results and more details are shown in the Supplementary File 1.

Discussion

In this RCT, *de novo* Tac (T0) with trough-level monitoring and cyclosporine (CsA) with 2 h monitoring (C2) after adult LT were compared. At 6 and 12 months, but not yet at 3 months, after LT T0 was superior to C2 for preventing tBPAR. At 12 months, Tac was also superior in terms of mortality and retransplantation-free survival. That was partially because chronic rejection only occurred with cyclosporine. The composite endpoint of tBPAR, retransplantation or mortality had a very significantly lower incidence with T0 than with C2 at 6 and 12 months after LT. A higher conversion rate was observed from C2 to T0 than vice versa, often in relation to rejection. The secondary endpoints renal function, weight, blood pressure, glucose, incidence of BPAR (treated or untreated) and tACR (with or without liver biopsy) did not differ between the two arms. After 12 months, serum triglyceride and LDL-cholesterol levels were lower with T0 than with C2, HDL-cholesterol was similar between groups. The incidence of treatment for PTDM, hyperlipidemia or hypertension did not differ between C2 and T0. Diarrhea was twice as frequent with T0 as compared to C2 treatment, without a clear explanation; there was no additional prescription of MM in this group. More patients treated with T0 experienced an infection after the first month, which may be related to the stronger immunosuppressive effect of Tac, as indicated by the lower tBPAR rate with T0 at 6 and 12 months. However, there were more infections in the first month with cyclosporine, and a nonsignificant trend toward more deaths for sepsis with C2. This may be related to more difficult dose adjustments with C2 than with T0, leading to over-immunosuppression in some. The incidence of other AEs and SAEs was comparable.

The most recent meta-analysis of RCTs comparing *de novo* Tac vs. CsA after first LT found no difference in tBPAR rates and in one-year graft loss, but better one-year patient survival, less hypertension, and more PTDM.[21] Ten of the studies used trough-level monitoring of CsA (C0). Using C2 monitoring has been associated with less rejection and better renal function than C0.[17,24,25] However, in the only larger previous RCT in *de novo* LT comparing C2 vs. T0, no differences in

Table 2. Cumulative raw incidence and Kaplan–Meier estimates of the cumulative incidences with hazard ratio (HR) C2 vs. T0 of patients reaching endpoints at 3, 6, and 12 months after LT in the C2 group (*n*=84) vs. those in the T0 group (*n*=85) (ITT analysis)

Group	Cumulative raw incidence			Kaplan–Meier estimate of cumulative incidence and hazard ratio			
	C2 (*n*=84)	T0 (*n*=85)	Fisher *p*	C2 (CI)	T2 (CI)	HR (CI)	Log-rank *p*
Endpoint	*n* (%)	*n* (%)					
tBPAR							
3 months	14 (16.7%)	7 (8.2%)	0.11	17.7% (9.3–26.1)	8.4% (2.5–14.3)	2.088 (0.866–5.026)	0.10
6 months	17 (20.2%)	8 (8.4%)	0.054	21.9% (12.7–31.1)	9.7% (3.4–16.0)	2.269 (1.003–5.155)	0.049
12 months	17 (20.2%)	8 (8.4%)	0.054	21.9% (12.7–31.1)	9.7% (3.4–16.0)	2.275 (1.003–5.155)	0.049
Mortality							
3 months	5 (6.0%)	4 (4.7%)	0.75	4.2% (0.9–11.1)	4.7% (0.2–9.2)	1.276 (0.371–4.389)	0.72
6 months	5 (6.0%)	5 (5.9%)	0.99	7.1% (1.6–12.6)	5.9% (0.8–11.0)	1.227 (0.398–3.787)	0.74
12 months	13 (15.5%)	5 (5.9%)	0.049	15.5% (7.9–22.1)	5.9% (0.8–11.0)	2.704 (1.005–7.284)	0.049
re-LT							
3 months	5 (6.0%)	3 (3.5%)	0.50	6.0% (0.9–11.1)	3.5% (0.0–7.4)	1.707 (0.451–6.467)	0.46
6 months	5 (6.0%)	3 (3.5%)	0.50	6.0% (0.9–11.1)	3.5% (0.0–7.4)	1.707 (0.451–6.467)	0.46
12 months	8 (9.5%)	4 (4.7%)	0.25	9.5% (3.2–15.7)	4.7% (0.2–9.2)	2.065 (0.663–6.446)	0.23
re-LT or mortality							
3 months	9 (11.0%)	6 (7.0%)	0.43	10.7% (10.0–11.4)	7.3% (1.6–12.6)	1.549 (0.574–4.177)	0.40
6 months	10 (12.0%)	7 (8.0%)	0.46	11.9% (5.0–18.6)	8.2% (2.3–6.1)	1.480 (0.583–3.758)	0.42
12 months	20 (24.0%)	8 (9.0%)	0.01	23.8% (14.8–32.8)	9.4% (3.1–15.7)	2.662 (1.197–5.916)	0.015
tBPAR or re-LT or mortality							
3 months	23 (27.4%)	13 (15.3%)	0.06	27.4% (17.8–37.0)	15.3 (7.7–22.9)	1.858 (0.954–3.628)	0.07
6 months	27 (32.1%)	15 (17.6%)	0.03	32.1% (22.1–42.1)	17.6 (9.6–25.6)	1.930 (1.039–3.596)	0.04
12 months	33 (39.3%)	16 (18.8%)	0.004	39.3% (28.9–49.7)	18.8 (10.6–27.0)	2.268 (1.261–4.096)	0.006

C2, CsA with blood concentration 2 h after drug intake; ITT, intent-to-treat; LT, liver transplantation; tBPAR, treated biopsy-proven acute rejection.

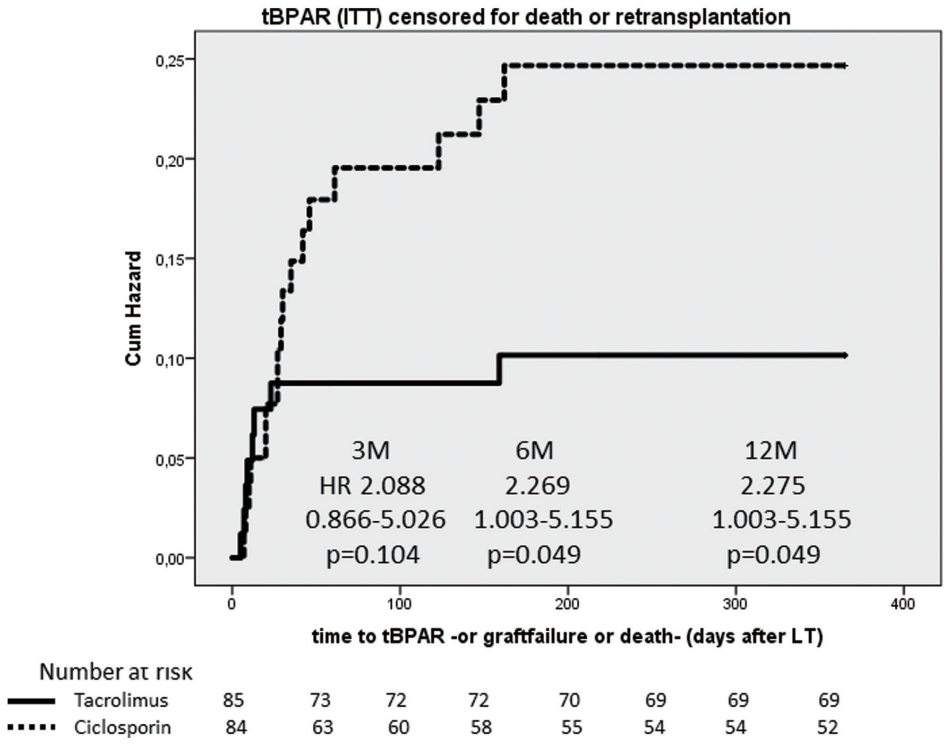

Fig. 2. Cumulative incidence of tBPAR, censored for death and retransplantation. ITT with hazard ratio and 95% confidence interval (Kaplan–Meier and log-rank analysis). Solid line: Tacrolimus T0. Interrupted line: Cyclosporin C2.

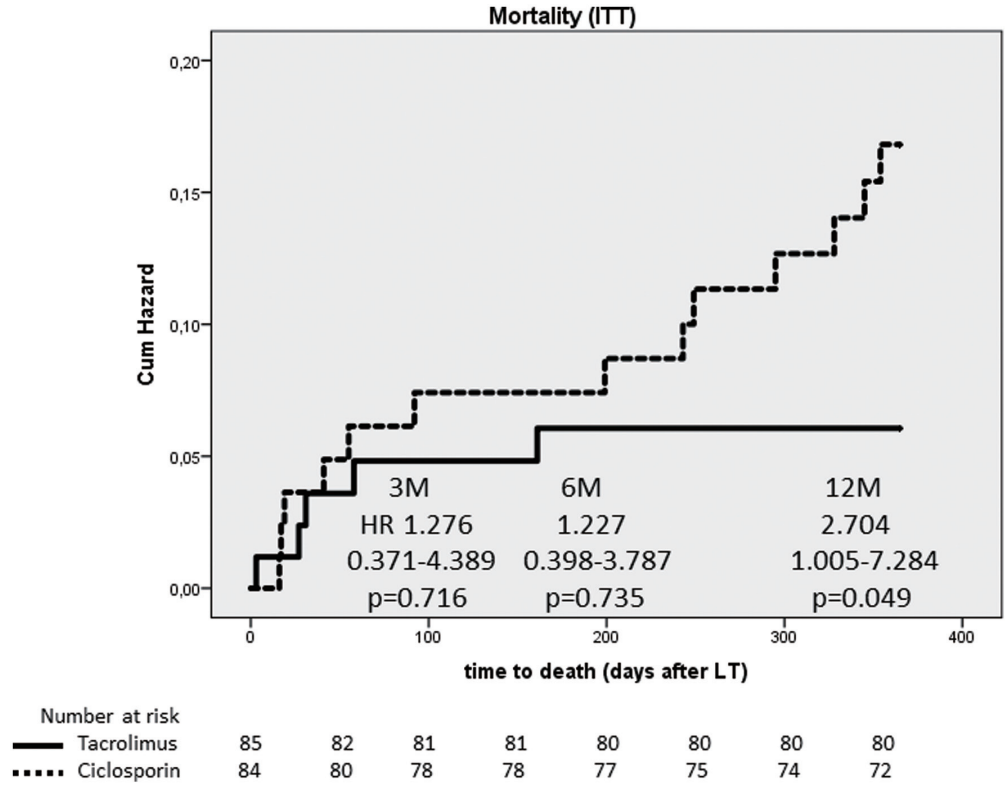

Fig. 3. Cumulative incidence of mortality. ITT with hazard ratio and 95% confidence interval (Kaplan–Meier and log-rank analysis). Solid line: Tacrolimus T0. Interrupted line: Cyclosporin C2. ITT, intent-to-treat.

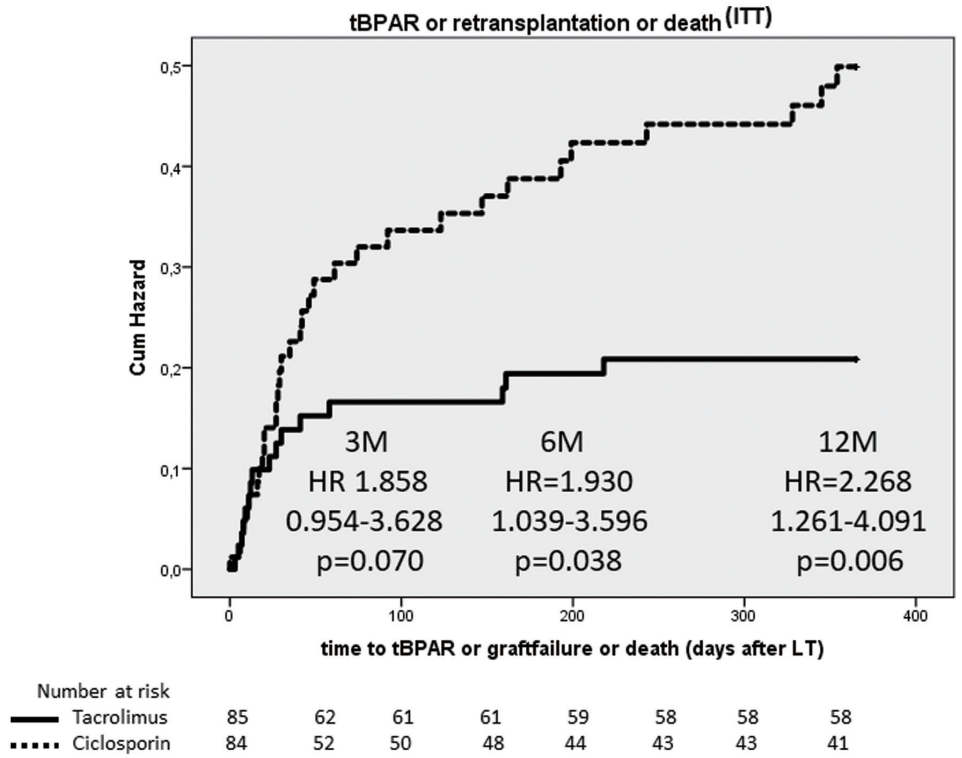

Fig. 4. Cumulative incidence of combined endpoint of tBPAR or retransplantation or mortality. ITT with hazard ratio and 95% confidence interval (Kaplan–Meier and log-rank analysis). Solid line: Tacrolimus T0. Interrupted line: Cyclosporin C2. ITT, intent-to-treat.

mortality, acute rejection, or renal function but more PTDM with T0 were detected.[18] In this RCT of C2 vs. T0 the findings were clearly different. The study also did not find a difference in PTDM, but was not designed to detect such a difference. C2 better reflects AUC than C0, therefore C2 leads to more accurate dosing.[24,31] That may explain why no differences in renal function and hypertension were found between C2 and T0 in the current study. A patient- and graft survival advantage of Tac compared with CsA was also seen in the largest RCT by O'Grady *et al*.[11] and in a previous meta-analysis, with no difference in death-censored graft survival, as in the current study.[21] The higher mortality with C2 than with T0 in the current study tended to be related to more sepsis and chronic rejection. The etiology of the liver disease was not a risk factor in this study. In a previous study, a survival advantage for CsA was explained by more deaths due to hepatitis C (HCV) recurrence with Tac.[32] However, the REFINE study, designed to demonstrate the superiority of CsA over Tac for LT in HCV cirrhosis, did not show any differences in survival or other parameters between Tac and CsA.[33] With the current highly effective HCV therapies, the influence of HCV on post-transplant graft or patient survival is even more unlikely.

This study has limitations. CsA and Tac were not administered based on the AUC, which would have led to the most accurate dosing.[34,35] For practical reasons in the outpatient clinic and based on the existing literature and recommendations, T0 and C2 -as single levels best reflecting the AUC- were used in the current study.[34,35] Another limitation is the high drop-out of patients from the C2 arm, limiting power. Drop outs in the C2 arm resulted from a larger than expected crossover from C2 to T0 for rejection, and from retransplantation and mortality. Obviously, that is also an indication in favor of T0. Another limitation was that target C2 levels were not reached within 5 days in many patients, as was reported by Levy.[17] However, all patients in this study received basiliximab, protecting most patients from rejection in at least the first week. A limitation is that some patients were treated for rejection without liver biopsy; however, that did not influence results, as they were not included in the primary endpoint of tBPAR. Some of those patients may not have had rejection. Also, some with tBPAR and only mild rejection were not treated, but that was similar for both treatment arms. Moreover, the primary endpoint of tBPAR allowed for the best comparison between treatment groups and with other studies that all used the same endpoint.[20,21] Despite the use of basiliximab, which may have reduced rejection in the first weeks after LT, differences in rejection rate and survival became apparent. Use of mycophenolate mofetil or azathioprine was allowed if needed, usually in case of severe renal dysfunction, but this use was similar in frequency in both arms. Moreover, renal function did not differ between both arms. That strongly reduced the possibility that the use of these drugs in some patients and the associated dose reduction of Tac or CsA influenced the outcomes. Currently more once-daily prolonged formulation of Tac is used. However, based on a previous study, it is likely that the current results also apply to once-daily prolonged release Tac.[36] The study was not powered to identify differences in secondary outcomes or adverse effects; therefore, the results of secondary endpoints must be interpreted as exploratory. Some long-term data have been added in the Supplementary File 1, but interpretation of these data is difficult because of changes in immunosuppression over time. The fact that more early infections occurred in the CsA group and more late infections occurred in the Tac group was remarkable, and not mentioned in other studies, but the limitation is that this was a post-hoc analysis.

Table 3. Laboratory measurements of glucose, lipids and renal function with T0 and C2 (ITT analysis)

Mean	Laboratory data per treatment group							
	Tacrolimus				Ciclosporin			
	Baseline	3 months	6 months	12 months	Baseline	3 months	6 months	12 months
Fasting blood glucose (mmol/L)[a]	6.9 (±3.6)	6.3 (±2.3)	6.2 (±3.5)	5.8 (±2.6)	6.8 (±2.7)	6.3 (±2.4)	5.7 (±2.2)	5.4 (±1.8)
LDL-cholesterol (mmol/L)[c]	2.1 (±1.2)	2.6 (±1.0)	2.3 (±0.8)	2.3 (±0.9)	2.4 (±1.3)	3.2 (±1.3)	3.1 (±1.3)	2.8 (±1.3)
HDL-cholesterol (mmol/L[b])	1.1 (±0.6)	1.5 (±0.6)	1.5 (±0.5)	1.3 (±0.4)	1.2 (±0.6)	1.3 (±0.4)	1.2 (±0.5)	1.2 (±0.4)
Triglycerides (mmol/L)[d]	1.1 (±0.6)	1.7 (±1.1)	1.6 (±1.0)	1.7 (±1.0)	1.3 (±0.7)	2.2 (±1.7)	2.1 (±1.5)	1.9 (±1.2)
Serum creatinine[e]	84.6 (±28.0)	108 (±30.9)	109 (±31.1)	107 (±30.3)	84 (±41.6)	99 (±29.2)	103 (±33.3)	105 (±35.1)
Creatinine clearance (Cockcroft-Gault, mL/m)	107 (±42.1)	79 (±28.1)	80 (±29.2)	83 (±31.3)	113 (±48.0)	91 (±35.7)	88 (±35.6)	87 (±35.7)

SD, standard deviation between brackets. [a]Fasting glucose was lower at 12 months compared to baseline in both groups, $p=0.004$. [b]HDL-cholesterol was higher in the Tac group at 3 and 6 months ($p=0.005$ and 0.020), but not after 1 year compared to baseline. [c]LDL-cholesterol was significantly higher in the CsA group at 3 and 6 months ($p=0.026$ and 0.005), though not after 1 year. [d]In the Tac group triglyceride level was higher at 12 months compared to baseline, $p=0.005$. For CsA, triglycerides were only higher at 3 and 6 months compared to baseline, $p=0.005$. [e]Between the two treatment groups, there are no significant differences in serum creatinine at any measured time point. [f]Between treatment groups, only at 3 months a difference in creatinine clearance could be found ($p=0.029$). After 3 months creatinine clearance did not significantly change between 3–6 and 6–12 months. C2, CsA with blood concentration 2 h after drug intake; ITT, intent-to-treat; T0, Tac with blood concentration pre-dose (trough level).

Recently, it has been shown that in contrast to cessation, lowering Tac dosage by adding mycophenolate, everolimus or sirolimus soon after transplantation may be beneficial for short-term but not long-term renal function.[37] It has been shown that early conversion to a calcineurin-free regimen may spare renal function, but that it may lead to more rejection, and the long-term effect of such changes are yet unknown.[37,38] This study found no differences in PTDM and lipids, except for slightly higher LDL-cholesterol and triglycerides with CsA. In a meta-analysis of existing data, Tac tended to exhibit higher diabetogenicity than CsA and sirolimus in the short-term (2–3 years), while in the long-term, sirolimus was associated with more PTDM than Tac or CsA.[39] It is also likely that CNIs increase the long-term cardiovascular risk.[40] Therefore, larger studies assessing long-term risks and comparing different maintenance regimens are warranted.

While most LT centers now prefer Tac after the 2006 meta-analysis, there are still LT centers and parts of the world where CsA is widely used after LT. In a recent consensus statement of the ILTS, no preference for Tac or CsA after LT was mentioned.[41] While the most recently published meta-analysis from 2016 found no significant difference in rejection rates, and while the only large previous study with C2 vs. T0 after LT found no differences in acute rejection, mortality, and renal function, the implication of the current RCT is that Tac is to be preferred over cyclosporine even with 2 h monitoring in the first year after LT *de novo*, because of less rejection (tBPAR), lower mortality, and better retransplant-free survival.

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Conflict of interest

BvH has been an editorial board member of *Journal of Clinical and Translational Hepatology* since 2022. The other authors have no conflict of interests related to this publication.

Author contributions

Conceptualization and protocol design (BvH, AvdB, HM, JD), patient inclusion (AI, AvdB, HM, BvH), data management (BR, AI, AvdB, HM, BvH), additional data collection for per-protocol analysis (BvH, DvdH, MR), data analysis and statistical assessment (BR, AI, MRG, BvH), writing of the manuscript (BR, AI, MET, BvH), critical revision of the manuscript (AvdB, HM, JD, RP, WP), and supervision of the work (BvH).

Ethical statement

This study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol and amendments were approved by the IRB or the

REB of each participating center. The trial was registered in the Dutch Trial Registry (number NTR489) and Clinicaltrials.gov (number NCT00149994). Informed written consent was obtained prior to transplantation.

Data sharing statement

Data collected for the study, including de-identified individual participant data and a data dictionary defining each field in the set, or additional related data will be made available to others with publication upon any reasonable request, after approval of a proposal with a signed data access agreement.

References

[1] Randomised trial comparing tacrolimus (FK506) and cyclosporin in prevention of liver allograft rejection. European FK506 Multicentre Liver Study Group. Lancet 1994;344(8920):423–428. PMID:7520105.

[2] The U.S. Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med 1994;331(17):1110–1115. doi:10.1056/NEJM199410273311702, PMID:7523946.

[3] Borel JF, Baumann G, Chapman I, Donatsch P, Fahr A, Mueller EA, *et al*. In vivo pharmacological effects of ciclosporin and some analogues. Adv Pharmacol 1996;35:115–246. doi:10.1016/s1054-3589(08)60276-8, PMID:8920206.

[4] Dunn CJ, Wagstaff AJ, Perry CM, Plosker GL, Goa KL. Cyclosporin: an updated review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (neoral)1 in organ transplantation. Drugs 2001;61(13):1957–2016. doi:10.2165/00003495-200161130-00006, PMID:11708766.

[5] Shah MB, Martin JE, Schroeder TJ, First MR. The evaluation of the safety and tolerability of two formulations of cyclosporine: neoral and sandimmune. A meta-analysis. Transplantation 1999;67(11):1411–1417. doi:10.1097/00007890-199906150-00004, PMID:10385078.

[6] Mühlbacher F, European Liver Transplantation Tacrolimus vs Cyclosporin Microemulsion Study Group. Tacrolimus versus cyclosporin microemulsion in liver transplantation: results of a 3-month study. Transplant Proc 2001;33(1-2):1339–1340. doi:10.1016/s0041-1345(00)02500-8, PMID:11267317.

[7] Timmermann W, Erhard J, Lange R, Reck T, Köckerling F, Müller A, *et al*. A randomised trial comparing the efficacy and safety of tacrolimus with microemulsified cyclosporine after liver transplantation. Transplant Proc 2002;34(5):1516–1518. doi:10.1016/s0041-1345(02)02953-6, PMID:12176463.

[8] Therapondos G, Flapan AD, Dollinger MM, Garden OJ, Plevris JN, Hayes PC. Cardiac function after orthotopic liver transplantation and the effects of immunosuppression: a prospective randomized trial comparing cyclosporin (Neoral) and tacrolimus. Liver Transpl 2002;8(8):690–700. doi:10.1053/jlts.2002.34381, PMID:12149762.

[9] Kelly D, Jara P, Rodeck B, Lykavieris P, Burdelski M, Becker M, *et al*. Tacrolimus and steroids versus ciclosporin microemulsion, steroids, and azathioprine in children undergoing liver transplantation: randomised European multicentre trial. Lancet 2004;364(9439):1054–1061. doi:10.1016/S0140-6736(04)17060-8, PMID:15380964.

[10] Glanemann M, Klupp J, Langrehr JM, Schröer G, Platz KP, Stange B, *et al*. Higher immunosuppressive efficacy of mycophenolate mofetil in combination with FK 506 than in combination with cyclosporine A. Transplant Proc 2000;32(3):522–523. doi:10.1016/s0041-1345(00)00872-1, PMID:10812095.

[11] O'Grady JG, Burroughs A, Hardy P, Elbourne D, Truesdale A, UK and Republic of Ireland Liver Transplant Study Group. Tacrolimus versus microemulsified ciclosporin in liver transplantation: the TMC randomised controlled trial. Lancet 2002;360(9340):1119–1125. doi:10.1016/s0140-6736(02)11196-2, PMID:12387959.

[12] Greig P, Lilly L, Scudamore C, Erb S, Yoshida E, Kneteman N, *et al*. Early steroid withdrawal after liver transplantation: the Canadian tacrolimus versus microemulsion cyclosporin A trial: 1-year follow-up. Liver Transpl 2003;9(6):587–595. doi:10.1053/jlts.2003.50102, PMID:12783400.

[13] Fisher RA, Stone JJ, Wolfe LG, Rodgers CM, Anderson ML, Sterling RK, *et al*. Four-year follow-up of a prospective randomized trial of mycophenolate mofetil with cyclosporine microemulsion or tacrolimus following liver transplantation. Clin Transplant 2004;18(4):463–472. doi:10.1111/j.1399-0012.2004.00192.x, PMID:15233827.

[14] Martin P, Busuttil RW, Goldstein RM, Crippin JS, Klintmalm GB, Fitzsimmons WE, *et al*. Impact of tacrolimus versus cyclosporine in hepatitis C virus-infected liver transplant recipients on recurrent hepatitis: a prospective, randomized trial. Liver Transpl 2004;10(10):1258–1262. doi:10.1002/lt.20222, PMID:15376310.

[15] González-Pinto IM, Rimola A, Margarit C, Cuervas-Mons V, Abradelo M, Alvarez-Laso C, *et al*. Five-year follow-up of a trial comparing Tacrolimus and cyclosporine microemulsion in liver transplantation. Transplant Proc 2005;37(4):1713–1715. doi:10.1016/j.transproceed.2005.03.128, PMID:15919441.

[16] Berenguer M, Aguilera V, Prieto M, San Juan F, Rayón JM, Benlloch S, *et al*. Effect of calcineurin inhibitors on survival and histologic disease severity in HCV-infected liver transplant recipients. Liver Transpl 2006;12(5):762–767. doi:10.1002/lt.20655, PMID:16528713.

[17] Levy GA. Neoral C(2) in liver transplant recipients. Transplant Proc 2001;33(7-8):3089–3091. doi:10.1016/s0041-1345(01)02316-8, PMID:11750327.

[18] Levy G, Grazi GL, Sanjuan F, Wu Y, Mühlbacher F, Samuel D, *et al*. 12-month follow-up analysis of a multicenter, randomized, prospective trial in de novo liver transplant recipients (LIS2T) comparing cyclosporine microemulsion (C2 monitoring) and tacrolimus. Liver Transpl 2006;12(10):1464–1472. doi:10.1002/lt.20802, PMID:17004259.

[19] Shenoy S, Hardinger KL, Crippin J, Korenblat K, Lisker-Melman M, Lowell JA, *et al*. A randomized, prospective, pharmacoeconomic trial of neoral 2-hour postdose concentration monitoring versus tacrolimus trough concentration monitoring in de novo liver transplant recipients. Liver Transpl 2008;14(2):173–180. doi:10.1002/lt.21355, PMID:18236391.

[20] Cholongitas E, Shusang V, Germani G, Tsochatzis E, Raimondo ML, Marelli L, *et al*. Long-term follow-up of immunosuppressive monotherapy in liver transplantation: tacrolimus and microemulsified cyclosporin. Clin Transplant 2011;25(4):614–624. doi:10.1111/j.1399-0012.2010.01321.x, PMID:20718824.

[21] Muduma G, Saunders R, Odeyemi I, Pollock RF. Systematic Review and Meta-Analysis of Tacrolimus versus Ciclosporin as Primary Immunosuppression After Liver Transplant. PLoS One 2016;11(11):e0160421. doi:10.1371/journal.pone.0160421, PMID:27812112.

[22] Haddad EM, McAlister VC, Renouf E, Malthaner R, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus for liver transplanted patients. Cochrane Database Syst Rev 2006;2006(4):CD005161. doi:10.1002/14651858.CD005161.pub2, PMID:17054241.

[23] Treckmann J, Paul A, Ozcelik A, Saner F, Malagó M, Nadalin S, *et al*. Efficacy of C0 and C2 monitoring in adult liver transplant recipients treated with neoral, mycophenolate mofetil, and steroids. Transplant Proc 2007;39(10):3234–3236. doi:10.1016/j.transproceed.2007.06.075, PMID:18089361.

[24] Langers P, Cremers SC, den Hartigh J, Veenendaal RA, ten Hove WR, Ringers J, *et al*. Switching monitoring of emulsified cyclosporine from trough level to 2-hour level in stable liver transplant patients. Liver Transpl 2004;10(2):183–189. doi:10.1002/lt.20056, PMID:14762854.

[25] Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997;25(3):658–663. doi:10.1002/hep.510250328, PMID:9049215.

[26] Demetris A, Adams D, Bellamy C, Blakolmer K, Clouston A, Dhillon AP, *et al*. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for the histopathologic staging and reporting of chronic rejection. An International Panel. Hepatology 2000;31(3):792–799. doi:10.1002/hep.510310337, PMID:10706577.

[27] Hansson L, Hedner T, Himmelmann A. The 1999 WHO-ISH Guidelines for the Management of Hypertension—new targets, new treatment and a comprehensive approach to total cardiovascular risk reduction. Blood Press Suppl 1999;1:3–5. PMID:10401539.

[28] Whitworth JA, World Health Organization, International Society of Hypertension Writing Group. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J Hypertens 2003;21(11):1983–1992. doi:10.1097/00004872-200311000-00002, PMID:14597836.

[29] Brown EG, Wood L, Wood S. The medical dictionary for regulatory activities (MedDRA). Drug Saf 1999;20(2):109–117. doi:10.2165/00002018-199920020-00002, PMID:10082069.

[30] Garner JS, Jarvis WR, Emori TG, Horan TC, Hughes JM. CDC definitions for nosocomial infections, 1988. Am J Infect Control 1988;16(3):128–140. doi:10.1016/0196-6553(88)90053-3, PMID:2841893.

[31] Langers P, Cremers SC, den Hartigh J, Rijnbeek EM, Ringers J, Lamers CB, *et al*. Individualized population pharmacokinetic model with limited sampling for cyclosporine monitoring after liver transplantation in clinical practice. Aliment Pharmacol Ther 2007;26(10):1447–1454. doi:10.1111/j.1365-2036.2007.03514.x, PMID:17848182.

[32] Loinaz C, Marin LM, González-Pinto I, Gómez R, Jiménez C, Moreno E. A single-centre experience with cyclosporine microemulsion versus tacrolimus in 100 randomized liver transplant recipients: midterm efficacy and safety. Transplant Proc 2001;33(7-8):3439–3441. doi:10.1016/s0041-1345(01)02482-4, PMID:11750472.

[33] Levy G, Villamil FG, Nevens F, Metselaar HJ, Clavien PA, Klintmalm G, *et al*. REFINE: a randomized trial comparing cyclosporine A and tacrolimus on fibrosis after liver transplantation for hepatitis C. Am J Transplant 2014;14(3):635–646. doi:10.1111/ajt.12620, PMID:24456049.

[34] Langers P, Press RR, den Hartigh J, Cremers SC, Baranski AG, Lamers CB, *et al*. Flexible limited sampling model for monitoring tacrolimus in stable patients having undergone liver transplantation with samples 4 to 6 hours after dosing is superior to trough concentration. Ther Drug Monit 2008;30(4):456–461. doi:10.1097/FTD.0b013e31818162b9, PMID:18641539.

[35] Langers P, Cremers SC, den Hartigh J, Rijnbeek EM, Ringers J, Lamers CB, *et al*. Easy-to-use, accurate and flexible individualized Bayesian limited sampling method without fixed time points for ciclosporin monitoring after liver transplantation. Aliment Pharmacol Ther 2005;21(5):549–557. doi:10.1111/j.1365-2036.2005.02364.x, PMID:15740538.

[36] Trunečka P, Boillot O, Seehofer D, Pinna AD, Fischer L, Ericzon BG, *et al*. Once-daily prolonged-release tacrolimus (ADVAGRAF) versus twice-daily tacrolimus (PROGRAF) in liver transplantation. Am J Transplant 2010;10(10):2313–2323. doi:10.1111/j.1600-6143.2010.03255.x, PMID:20840481.

[37] Weir MR, Pearson TC, Patel A, Peddi VR, Kalil R, Scandling J, *et al*. Long-term Follow-up of Kidney Transplant Recipients in the Spare-the-Nephron-Trial. Transplantation 2017;101(1):157–165. doi:10.1097/TP.0000000000001098, PMID:26950714.
[38] Teperman L, Moonka D, Sebastian A, Sher L, Marotta P, Marsh C, *et al*. Calcineurin inhibitor-free mycophenolate mofetil/sirolimus maintenance in liver transplantation: the randomized spare-the-nephron trial. Liver Transpl 2013;19(7):675–689. doi:10.1002/lt.23658, PMID:23775875.
[39] Kotha S, Lawendy B, Asim S, Gomes C, Yu J, Orchanian-Cheff A, *et al*. Impact of immunosuppression on incidence of post-transplant diabetes mellitus in solid organ transplant recipients: Systematic review and meta-analysis. World J Transplant 2021;11(10):432–442. doi:10.5500/wjt.v11.i10.432, PMID:34722172.
[40] de la Fuente-Mancera JC, Forado-Benatar I, Farrero M. Management of long-term cardiovascular risk factors post organ transplant. Curr Opin Organ Transplant 2022;27(1):29–35. doi:10.1097/MOT.0000000000000950, PMID:34939962.
[41] Charlton M, Levitsky J, Aqel B, O'Grady J, Hemibach J, Rinella M, *et al*. International Liver Transplantation Society Consensus Statement on Immunosuppression in Liver Transplant Recipients. Transplantation 2018;102(5):727–743. doi:10.1097/TP.0000000000002147, PMID:29485508.