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Cognitive Evaluation in Liver Transplant Patients Under Calcineurin Inhibitor Maintenance Therapy

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Background. Neurological disorders due to calcineurin inhibitor (CNI) treatment pose a well-known problem after liver transplantation (LTx). In this study, the impact of CNIs on cognitive functioning during maintenance therapy was analyzed. A possible improvement of cognitive functioning, compliance and health-related quality of life (HRQoL) after conversion to a once-daily tacrolimus formulation was prospectively assessed. **Methods.** In a cross-section analysis cognitive functioning of living donors (LD), waiting list patients and LTx patients was tested using a 4 times trail making test (4-TTMT). In a further investigator-initiated trial a possible improvement of cognitive functioning, HRQoL and compliance after conversion to the once-daily tacrolimus formulation was prospectively assessed over 1 year. HRQoL was assessed using an EORTC-QLQ C30 questionnaire and patient's compliance was assessed by the Basel Assessment of Compliance with Immunosuppressive Medication Scales questionnaire. Correlated data were sex, age, time after surgery, liver disease, model of end-stage liver disease score, creatinine, CNI type, and CNI trough levels. **Results.** Two hundred eleven patients were included in this cross-section analysis. Twenty-seven patients agreed to participate in the investigator-initiated trial. LTx patients completed the 4-TTMT slower than living donor patients and faster than waiting list patients. Patients with twice daily cyclosporine A (CSA) formulation needed longer to finish the 4-TTMT than patients with the once-daily tacrolimus formulation. After drug conversion of a twice-daily CNI formulation to a once-daily tacrolimus formulation, CSA-treated patients needed longer to improve their cognitive functioning. HRQoL and compliance did not improve after drug conversion. **Conclusions.** Patients with once-daily tacrolimus formulation had a better psychomotor speed than CSA-treated patients. The conversion to once-daily tacrolimus formulation significantly improved cognitive functioning, but had no impact on HRQoL or compliance.

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Liver transplantation (LTx) is a generally accepted and standardized procedure to cure acute and chronic liver diseases. After LTx, immunosuppressive medication must be taken lifelong to prevent acute and chronic rejection. Some immunosuppressive medications comply with the criteria of critical-dose drugs. Treatment with these drugs comprises an imperative monitoring of the medication's blood levels,

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body weight-adapted dosing or other individualized dosing necessity, strong concentration-response-relationship and dose-dependent toxicity.[1-3] The calcineurin inhibitors (CNIs) cyclosporine A (CSA) and tacrolimus are regarded as critical-dose drugs and play a notable role after solid organ transplantation as basic immunosuppressive medication.[4,5]

Nephrotoxicity and neurotoxicity are frequent side effects of CNIs. Nephrotoxic side effects have been well examined in the past and different regimes have been established accordingly to avoid CNI-associated nephropathy. In contrast, neurotoxicity attracted clinical attention, especially during clinical introduction of tacrolimus and here in particular as the drug was administered intravenously in significantly higher doses.[6-8] Under oral application of CNIs, tremor and headache followed by agitation to the point of seizures are strong signs of overdosing.[6,9] However, subclinical neurological signs and symptoms like concentration difficulties, retentiveness, sleep disorders, fatigue and lassitude are only mentioned if explicitly asked. In a review the overall prevalence of neurological symptoms or disorders after LTx ranged from 11 to 42%.[9] Tremor appears in almost 40% of patients treated with CNIs.[10] Different authors reported a correlation by tacrolimus levels to the severity of neurotoxicity[11] and were recently able to show fewer neurotoxic adverse events in lower tacrolimus trough levels after LTx.[12] High tacrolimus concentrations, liver dysfunction, and mutation of the multidrug resistant 1 gene (*ABCB1*) were identified as positive predictors of tacrolimus-induced neurotoxicity.[13] Sakamoto et al[14] described a linear correlation between neurotoxicity and tacrolimus trough levels in rats. Furthermore, intracerebral concentration of tacrolimus was greater after intermittent than continuous administration of the drug.[15] Some patients reported an improvement of subclinical neurological symptoms after conversion from a twice-daily CNI formulation to the once-daily formulation of the pharmacological improved tacrolimus formulation. A study that analyses the impact of different CNI-drug formulations on neurocognitive function in a prospective standardized setting is lacking up to now. Aim of this first pilot study was to evaluate possible effects of different CNI formulations on neurological signs and symptoms, patient's compliance and health-related quality of life (HRQoL) in patients after LTx.

MATERIAL AND METHODS

Study Cohort

In this pilot study a cross-section analysis was used to investigate differences between patients with a differing CNI treatment after LTx using a 4 times trail making test (4-TTMT). In a 4-TTMT participants initially draw lines to connect consecutively numbered circles on 1 work sheet and then connect the same number of consecutively numbered and lettered circles on another worksheet, by alternating between the 2 sequences. The trail making test is an easily administered test of scanning, visuomotor tracking, divided attention, and cognitive flexibility. As a positive control group, patients were enrolled in the study who had suspected good cognitive and physical functioning in the long-term follow-up after organ donation for living related LTx (LRLTx). As a negative control group, we enrolled patients on the waiting list (WL) with end-stage liver disease and suspected impaired cognitive and physical functioning. In the second step, between 2012 and 2015 an investigator-initiated trial (IIT) was set up to assess a possible improvement of neurological signs and symptoms, patient's compliance and HRQoL in patients with the need for a conversion of the twice daily formulation of Prograf or CSA to the once-daily Advagraf formulation due to a medical disorder in the long-term follow up after LTx. These patients were prospectively followed up at the time of enrolment (t0), as well as the follow up 4 weeks (t1), 6 (t2), and 12 months (t3) after drug conversion. Inclusion criteria for both study parts were (1) written informed consent, (2) adult patients (≥18 years), (3) patients after LTx and LRLTx, living donors (LDs) and patients on the WL for LTx due to end-stage liver disease, (4) immunosuppression with a once- or twice-daily formulation of CNI. Before the enrolment of study-patients a severe disability was excluded. Exclusion criteria for both study parts were (1) dialysis treatment during the previous 30 days, (2) deafness and/or blindness, (3) pregnancy/breastfeeding mothers.

Study Design

After approval of the study by the local ethics committee and patient's informed consent, patients were enrolled for a cross-section analysis. In the trail making test, the patients had to draw lines to connect consecutively numbered circles on one work sheet and then connect the same number of consecutively numbered and lettered circles on another worksheet, by alternating between the 2 sequences. This standardized trail making test was repeated 4 times and had to be finished in a total time of 480 seconds. The time after successfully finishing the test was recorded and an average time of the sum of all tests was calculated. Times of the trail making test were compared between the 3 different groups. These 3 groups consisted of patients on the WL with end-stage liver disease (WL group), donors for a LRLTx in the long-term follow up at least 12 months after surgery or before surgery (LD group) and patients after LTx in the long term follow up (LTx group). Patients of the LD group were selected as a positive control group. Here better test results compared to the WL and LTx group were expected. Patients on the WL were included with expected worse test results as a negative control group. Correlated and compared parameters were age, sex, type of liver disease, type of CNI medication/formulation, and time after LTx. To analyze the impact of liver function and to exclude the influence of renal insufficiency, the model of end-stage liver disease (MELD) score, and serum creatinine (mg/dL) were assessed in the different groups. The analyzed twice-daily CNI medication was CSA and the tacrolimus drug Prograf. The assessed CNI medication, which was administered once daily was the tacrolimus drug Advagraf. Immunosuppressive drug and trough levels were classified into low, middle and high levels. For CSA trough levels less than 50 ng/mL were defined as low, 50 to 100 ng/mL as mid range, and greater than 100 ng/mL as high. For tacrolimus, trough levels less than 4 ng/mL were defined as low, 4 to 8 ng/mL as mid range, and greater than 8 ng/mL as high. The cutoff values were chosen referring to the common used classification in the immunosuppressive treatment in recipients after LTx in our institution.

In the IIT study, patients with the need of a drug conversion due to diagnosed medical disorders were enrolled after

approval of the study by the local ethics committee and patient's informed consent. The Advagraf treatment was initiated 12 hours after the last intake of the twice-daily administered drug Prograf or CSA. The conversion of Prograf to Advagraf was done in an equivalent 1:1 dosage-rate (mg:mg). The conversion of CSA to Advagraf was done related to the bodyweight of the patient with a starting dosage of 0.05 mg/kg bodyweight. After conversion the drug dosage was modified to the trough levels of 4 to 6 µg/L. Trough levels were checked at the time points t0 to t3. Advagraf was administered to the patients in the morning, 1 hour before breakfast. Parameters as shown in Table 1 were recorded at the times t0 to t3 in the outpatient's clinic. An influence of a metabolic disorder, renal insufficiency, disorders of serum electrolytes, infection or liver insufficiency were ruled out before starting the tests. To assess compliance of the intake of immunosuppressive drugs, the Basel Assessment of Compliance with Immunosuppressive Medication Scales (BAASIS) was handed out to the participating patients at time points t0, t2, and t3. This 4-item instrument is based on the different dimensions of medication taking (taking dimension, timing dimension, drug holidays, reduction of dose of medication). The BAASIS is administered as a patient interview and also includes a VAS compliance scale to be filled out by the patient. Responses are given on a 6-point scale ranging from 0 (never) to 5 (everyday). Compliance was assessed over the past 4 weeks. Noncompliance was defined as any self-reported noncompliance (response score 1 to 5) on any of the 4 items to compensate for the underreporting of noncompliance using self-report[16] and the limited forgiveness of noncompliance with immunosuppressive drugs in view of negative clinical outcomes.[17] HRQoL was assessed at time points t0, t2 and t3 using the EORTC QLQ-C30 questionnaire. This questionnaire has 5 function scales (physical, role, cognitive, emotional, and social functioning) and 3 symptom scales (fatigue, pain, nausea, and vomiting). Moreover, the questionnaire included a Global Health-scale and additional parameters commonly used to assess typical clinical symptoms/signs and status of transplant patients (dyspnea, loss of appetite, sleep disturbance, constipation, diarrhea, financial difficulties).[18] For easier interpretation, the assessed scores were transformed to a scale ranging from 0 to 100. Thus, high levels of global health, HRQoL and function scales indicated a higher ability, whereas high scores of symptom-scales indicated suffering of the patient. The standardized trail making test was done by every patient at time points t0, t2, and t3. The test was repeated 4 times in a total time of 480 seconds to complete all 4 tests.

Statistical Analysis

All metric parameters were expressed as total numbers (%), or mean value (±standard deviation). Comparison of the results assessed at the different time points t0, t2, and t3 after drug conversion from the twice-daily CNI treatment were tested with a paired *t* test. All distribution and frequencies of medical data were compared by Pearson-Chi-Quadrat test. Differences between the groups were tested by a 1-way analysis of variance. A *P* value less than 0.05 was considered statistically significant. All analyses were conducted using the Statistical Package for Social Sciences software (version 23.0, SPSS Inc., Chicago, IL).

RESULTS

Study Population

In total, 211 patients were included in the cross-section analysis. Of these patients 59% (n = 125) were men and 41% (n = 86) were women. The cohort was divided into 3 groups—LD group, 8% (n = 17); WL group, 29% (n = 62); LTx group, 63% (n = 132). The most frequent liver diseases in the WL group and LTx group were postalcoholic cirrhosis (34%), hepatitis C (10%), hepatocellular carcinoma (10%), primary sclerosing cholangitis (8%), autoimmune hepatitis (7%), cryptogenic cirrhosis (6%), primary biliary cirrhosis (6%), cystic liver disease (4%), Wilson disease (3%), hepatitis B (2%), acute liver failure (2%) and further diseases (8%). These comprised Caroli syndrome, alpha-1 antitrypsin deficiency, biliary atresia, Alagille syndrome, Budd Chiari syndrome, glycogenosis disease, familial amyloid polyneuropathy, hemangiomatosis, secondary biliary cirrhosis and cystic fibrosis. In the LTx group, 43% of the patients received tacrolimus and 57% received CSA as immunosuppressant medication. The twice-daily tacrolimus formulation Prograf was administered in 25% and the once-daily tacrolimus formulation Advagraf in 18% of the patients. Patients of the LD group were significantly younger (LD group vs WL group: $P = 0.002$, LD group vs LTx group: $P = 0.0004$). The MELD score in the

TABLE 1.

Study protocol of prospective assessment of cognitive functioning and HRQoL

	t0	t1	t2	t3
Anamnesis*	X			
Medical examination**	X	X	X	X
CMV-DNA quantitative	X		X	X
CNI trough levels***	X	X	X	X
Blood count	X	X	X	X
Sodium	X	X	X	X
Potassium	X	X	X	X
Magnesium	X	X	X	X
Creatinine	X	X	X	X
Urea	X	X	X	X
Creatinine clearance	X	X	X	X
Bilirubin	X	X	X	X
ALT	X	X	X	X
AST	X	X	X	X
Alcaline phosphatase	X	X	X	X
γ-glutamyltransferase	X	X	X	X
Albumin	X	X	X	X
CRP	X	X	X	X
Glucose	X	X	X	X
HbA1c	X	X	X	X
Cholesterin	X	X	X	X
Triglyceride	X	X	X	X
Urinary status	X	X	X	X
MELD score	X		X	X
Trail making test	X		X	X
BAASIS	X		X	X
QoL questionnaire (EORTC-QoL-30)	X		X	X

EORTC indicates European Organisation for Research and Treatment of Cancer; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

LD group was significantly better than in the LTx- and WL group (LD group vs WL group: $P = 0.000005$, LD group vs LTx group: $P = 0.01$). Patients of the LTx group had a significantly better MELD score compared to patients on the WL ($P = 0.001$). Serum levels of creatinine were not significantly different between the groups. All liver disease, patient characteristics and *P*-values are shown in Table 2.

In the IIT study, a total of 27 patients agreed to participate for an assessment of a possible improvement of neurological signs and symptoms, patient's compliance and HRQoL after a conversion of the CNI formulation. Five of these 27 patients were excluded from the study due to a nonsignificant number of patients in the different groups of CNI-drug conversion (conversion from once and twice daily tacrolimus formulation to CSA: n = 2, conversion from CSA to twice daily tacrolimus formulation: n = 3). After exclusion, data of the 22 patients was analyzed, to assess a potential improvement of neurological symptoms, patient's compliance and HRQoL after a conversion of a twice-daily CNI formulation to the once-daily tacrolimus formulation of Advagraf. Half of these 22 patients received CSA (n = 11) and Prograf (n = 11) medication before conversion to Advagraf. Further, a subdivision into a CSA and Prograf group was undertaken. Mean age in the study cohort was 53 (±15.4). There was no mortality within this prospective study. Patients in the Prograf group were younger ($P = 0.041$) and the time point of partaking in the study after transplantation was significantly later ($P = 0.09$). Disorders leading to an end-stage liver cirrhosis, followed by LTx were HCC (n = 8), postalcoholic cirrhosis (n = 6), primary sclerosing cholangitis (n = 2) and other disease (n = 6). Other diseases were hepatitis C, autoimmune hepatitis, hepatitis B, alpha-1 antitrypsin deficiency, glycogenosis disease, familial amyloid polyneuropathy. Medical disorders, which led to the drug conversion were neurological disorders (n = 14), diabetes (n = 4), graft rejection (n = 2), renal insufficiency (n = 1) and hirsutism (n = 1). Neurological disorders comprised fatigue (n = 2), impairment of concentration (n = 5), dizziness (n = 6) and tremor (n = 3). The varieties of disease, CNI-related medical disorders, MELD scores, levels of serum creatinine and trough levels did neither differ between the groups nor within each group at different times of measurement. All patient characteristics of the CSA and Prograf group are shown in Tables 3 and 4.

Four Times Trail Making Test Results

Results of the 4-TTMT in the cross-sectional analysis showed, that patients of the LD group (28 seconds (±4.5)) were able to finish the test significantly faster than patients of the LTx- (42 seconds (±19.7), $P = 0.024$) and WL group (51 seconds (±25.9), $P = 0.0002$). Compared to patients of

TABLE 2.

Patient characteristics of the cross-sectional analysis

	Overall	LD group	WL group	LTx group	*P*
Male	59% (n = 125)	5% (n = 11)	14% (n = 30)	40% (n = 84)	[a]0.117
Female	41% (n = 86)	3% (n = 6)	15% (n = 32)	23% (n = 48)	
Age, y					[b]0.0004 [c]0.002 [d]1
	53 (±13.6)	41 (±7.4)	54 (±10.6)	55 (±14.8)	
Time after surgery, mo	57 (±62)	14 (±15.9)	—	62 (±63.6)	[b]0.002
Liver disease					
	34% (n = 66)	—	45% (n = 28)	29% (n = 38)	—
Postalcoholic cirrhosis	10% (n = 19)	—	7% (n = 4)	11% (n = 15)	
Hepatitis C	10% (n = 19)	—	8% (n = 5)	11% (n = 14)	
HCC	8% (n = 15)	—	7% (n = 4)	8% (n = 11)	
Primary sclerosing cholangitis	7% (n = 13)	—	5% (n = 3)	8% (n = 10)	
Autoimmune hepatits	6% (n = 11)	—	11% (n = 7)	3% (n = 4)	
Cryptogenic cirrhosis	6% (n = 11)	—	7% (n = 4)	5% (n = 7)	
Primary biliary cirrhosis	4% (n = 8)	—	7% (n = 4)	3% (n = 4)	
Cystic liver disease	3% (n = 5)	—	—	4% (n = 5)	
Wilson disease	2% (n = 4)	—	2% (n = 1)	2% (n = 3)	
Hepatits B	2% (n = 4)	—	—	3% (n = 4)	
Acute liver failure	8% (n = 16)	—	2% (n = 1)	11% (n = 15)	
Others					
MELD score	11 (±5.1)	7 (±0.7)	14 (±5.7)	11 (±4.6)	[b]0.01 [c]0.000005 [d]0.001
Creatinine, mg/dL	1.3 (±0.7)	1	1.2 (±0.7)	1.4 (±0.7)	[b]0.116 [c]0.679 [d]0.559
Type of CNI drug	43% (n = 54)	—	—		—
Tacrolimus				43% (n = 54)	
Prograf	25% (n = 31)			25% (n = 31)	
Advagraf	18% (n = 23)			18% (n = 23)	
Cyclosporine	57% (n = 73)			57% (n = 73)	
4-TTMT, sec	44 (±21.8)	28 (±4.5)	51 (±25.9)	42 (±19.7)	[b]0.024 [c]0.0002 [d]0.019

[a] $P < 0.05$; Pearson-Chi-Quadrat test.

[b] LD group vs LTx group; 1-way ANOVA.

[c] LD group vs WL group; 1-way ANOVA.

[d] LTx group vs WL group.

the LTx group, patients of the WL group needed significantly longer to finish the 4-TTMT ($P = 0.019$) (Table 2). Comparing patients with tacrolimus and CSA immunosuppression, a significant faster completion of the 4-TTMT was measured for patients with tacrolimus immunosuppression (tacrolimus: 36 seconds (±10.9) vs CSA: 45 seconds (±21.5), $P = 0.004$). Patients who were immunosuppressed with the once-daily Advagraf (34 seconds (±8.6)) completed the 4-TTMT faster than patients with the twice-daily Prograf (37 seconds (±12.3)) and CSA (45 seconds (±21.5)) immunosuppression. In the cohort of patients with a once-daily Advagraf immunosuppression, the time to finish the 4-TTMT was significantly faster than in the group with a twice-daily CSA immunosuppression ($P = 0.023$) (Figure 1). There was no significant difference in the completion time of the 4-TTMT between patients with once-daily Advagraf or twice-daily Prograf formulation ($P = 0.761$), nor between the twice-daily formulation of Prograf or CSA ($P = 0.099$). The tacrolimus and CSA trough levels did not influence the patients in completing the 4-TTMT.

After conversion of the twice-daily CNI formulation with CSA or Prograf to the once-daily Advagraf formulation, we were able to measure an improvement in both groups in finishing the test. Six months (t2) and 12 months (t3) after the drug conversion, patients of the Prograf group were able to complete the 4-TTMT in a significantly shorter amount of time (t0 vs t2: 39 seconds (±20) vs 26 seconds (±9), $P = 0.007$; t0 vs t3: 39 seconds (±20) vs 28 seconds (±13), $P = 0.002$) (Figure 2). Comparing the 2 groups, we measured a faster time to finish the test in the Prograf group 6 months after drug conversion (Prograf vs CSA: 26 seconds (±9) vs 41 seconds (±13.6), $P = 0.018$)) (Figure 2, Table 4).

HRQoL

In the Prograf group, we identified a trend for an improvement of HRQoL symptom- and function-scores within 1 year after conversion to the Advagraf-formulation. However, the measured symptom and function scores did not improve significantly. In the CSA group, a trend of an improvement of symptom and function scores was measured for less scores. In this group patients reported about significantly worse symptoms of insomnia 1 year after conversion to Advagraf formulation (t2: 33 (±25.2) vs t3: 56 (±28.9), $P = 0.025$). Comparing the function and symptom scores in the Prograf and CSA groups at the different times of assessment, we measured significantly lower symptoms of insomnia in the Prograf group 1 year after conversion to the once-daily Advagraf formulation (Prograf group vs CSA group: 19 [±26.2] vs 56 [±28.8], $P = 0.021$). All HRQoL symptom and function scores, and P values are shown in Table 4.

Basel Assessment of Compliance With Immunosuppressive Medication Scales

Comparing the Prograf group (90 [±12.5]) and CSA group (99 [±1.8]), a significantly lower compliance 1 year after conversion to the once-daily Advagraf formulation was measured for patients of the Prograf group ($P = 0.03$) (Figure 3). A significant improvement of compliance within the different groups was not measured at any time. All scores and P values of the BAASIS are shown in Table 4.

DISCUSSION

It has been shown that the pharmacokinetic profile of the drugs is more important than dosing of the drugs to minimize the drug's toxicity. As follows, it was shown that a high, maximum concentration (Cmax) is related to a higher toxicity after CSA treatment.[19-21] In the last years, the research's focus was given to avoidance of acute organ rejection, CNI-induced nephrotoxicity and options to reduce these complications. The microemulsion formulation of CSA is characterized by a sharp absorption peak during the absorption phase 0 to 4 hours after oral application. The use of concentration detection

TABLE 3.

Patient characteristics of the CSA group and prograf group of the ITT study part

	Overall	Prograf group	CSA group	*P*
Male	73% (n = 16)	55% (n = 6)	91% (n = 10)	0.056
Female	27% (n = 6)	45% (n = 5)	9% (n = 1)	
Age, y	53 (±15.4)	47 (±12.5)	60 (±15.7)	0.041
Time after surgery, mo	51 (±50.7)	69 (±63.7)	33 (±24.7)	0.09
Liver disease				
Postalcoholic cirrhosis	18% (n = 6)	18% (n = 3)	18% (n = 3)	1
HCC	32% (n = 8)	9% (n = 2)	55% (n = 6)	0.076
Primary sclerosing cholangitis	9% (n = 2)	18% (n = 2)	—	0.138
Others	27% (n = 6)	36% (n = 4)	18% (n = 2)	0.338
Reason for drug conversion				
Neurological symptoms	64% (n = 14)	45% (n = 5)	82% (n = 9)	0.076
(a) dizziness	a. 27% (n = 6)	a. 9% (n = 1)	a. 45% (n = 5)	a. 0.056
(b) impairment of concentration	b. 23% (n = 5)	b. 36% (n = 4)	b. 9% (n = 1)	b. 0.062
(c) tremor	c. 14% (n = 3)	c. —	c. 27% (n = 3)	c. 0.127
(d) fatigue	d. 9% (n = 2)	d. —	d. 18% (n = 2)	d. 0.138
Diabetes	18% (n = 4)	27% (n = 3)	9% (n = 1)	0.269
Graft rejection	9% (n = 2)	18% (n = 2)	—	0.138
Renal insufficiency	5% (n = 1)	9% (n = 1)	—	0.306
Hirsutism	5% (n = 1)	—	9% (n = 1)	0.306

P values are calculated by a 1-way ANOVA and Pearson-Chi-Quadrat test.

TABLE 4.

Results for MELD score, BAASIS, trough levels, 4-TTMT, and HRQoL scores at the assessment time points t0, t2, and t3 in the prograf group and CSA group in the ITT study part

	Prograf group			CSA group			
	t0	t2	t3	t0	t2	t3	*P*
MELD score	9 (±2.6)	8 (±9.8)	9 (±2)	11 (±4.4)	11 (±4)	11 (±4.8)	[a]0.276 [b]0.454 [c]0.268
BAASIS	96 (±6.1)	96 (±11.6)	90 (±12.5)	97 (±6)	97 (±3.5)	99 (±1.8)	[a]0.344 [b]0.126 [c]0.220
Trough-levels, ng/mL	4 (±2.4)	3 (±1.6)	4 (±1.9)	4 (±2.2)	3 (±1.8)	3 (±1.6)	[a]0.344 [b]0.126 0.220
4-TTMT, s	39 (±20)	26 (±9)	28 (±13)	44 (±14.9)	41 (±13.6)	35 (±10.8)	[a]0.596 [b]0.044 [c]0.039
Creatinine, mg/dL	1.3 (±0.3)	1.1 (±0.3)	1.2 (±0.4)	1.3 (±0.4)	1.2 (±0.3)	1.1 (±0.2)	[a]0.965 [b]0.314 [c]0.708
Physical functioning	69 (±20.4)	72 (±26.2)	82 (±18.3)	63 (±33.3)	65 (±21.9)	64 (±22.4)	[a]0.608 [b]0.570 [c]0.117
Role functioning	52 (±27.7)	65 (±31.7)	74 (±23.3)	63 (±41.5)	65 (±34.2)	64 (±30.8)	[a]0.490 [b]0.887 [c]0.951
Emotional functioning	55 (±25.5)	66 (±19.3)	77 (±15)	70 (±19.1)	73 (±25.9)	73 (±30.6)	[a]0.160 [b]0.543 [c]0.743
Cognitive functioning	62 (±38.5)	72 (±28.9)	81 (±17.8)	74 (±39.2)	75 (±29.5)	70 (±27.4)	[a]0.496 [b]0.847 [c]0.391
Social functioning	58 (±26.4)	61 (±28.9)	81 (±27.9)	65 (±37.7)	64 (±37.8)	79 (±29.2)	[a]0.667 [b]0.851 [c]0.906
Global Health status	58 (±22)	65 (±21.6)	70 (±16.6)	68 (±30.7)	65 (±22.6)	58 (±30.6)	[a]0.418 [b]0.983 [c]0.371
Fatigue	48 (±28)	48 (±36.9)	38 (±25.5)	43 (±28.2)	38 (±22.2)	35 (±25.1)	[a]0.718 [b]0.489 [c]0.786
Nausea/vomiting	5 (±8.1)	6 (±11.8)	2 (±6.3)	9 (±18.8)	8 (±23.6)	13 (±18.2)	[a]0.522 [b]0.759 [c]0.166
Pain	47 (±35)	39 (±34.4)	31 (±26.2)	35 (±39.5)	31 (±28.8)	30 (±30.9)	[a]0.510 [b]0.629 [c]0.929
Dyspnea	33 (±31.4)	22 (±23.6)	29 (±30)	26 (±27.8)	29 (±37.5)	21 (±24.8)	[a]0.595 [b]0.650 [c]0.593
Insomnia	33 (±31.4)	33 (±33.3)	19 (±26.2)	41 (±36.4)	33 (±25.2)	56 (±28.9)	[a]0.640 [b]1 [c]0.021
Appetite loss	20 (±35.8)	15 (±24.2)	14 (±26.2)	33 (±40.8)	38 (±45.2)	26 (±32.4)	[a]0.459 [b]0.209 [c]0.453
Constipation	13 (±23.3)	22 (±23.6)	10 (±25.2)	19 (±29.4)	17 (±25.2)	33 (±40.8)	[a]0.674 [b]0.645 [c]0.198
Diarrhea	37 (±36.7)	26 (±36.4)	29 (±35.6)	30 (±35.1)	29 (±37.5)	26 (±36.4)	[a]0.676 [b]0.859 [c]0.886
Financial difficulties	43 (±44.6)	37 (±48.4)	29 (±48.8)	30 (±26.1)	17 (±25.2)	17 (±25.2)	[a]0.432 [b]0.303 [c]0.555

Significant *P* values are marked by bold letters.

[a] t0: CSA group vs Prograf group; 1-way ANOVA.

[b] t2: CSA group vs Prograf group; 1-way ANOVA.

[c] t3: CSA group vs Prograf group; 1-way ANOVA.

2 hours postdose, or trough levels was intensively discussed for CSA. Two hours post dose concentration promotors argued for better avoidance of acute rejection and drug-induced nephrotoxicity.[19-21] In contrast to CSA, tacrolimus has a flatter pharmacokinetic profile with a nonprominent absorption peak for the twice- and once-daily formulation. Also, there is a shift in the time to reach Cmax tacrolimus concentrations for the twice daily formulation depending on the posttransplant period. Time to reach maximal tacrolimus concentrations after the first dosage is shorter the longer the distance to the transplant and start of the posttransplant immunosuppression.[22] In contrast to several transplanted patients, who under CNI immunosuppression reported about subclinical neurological symptoms in particular in the long-term follow up,[10-12] our workgroup focused on a possible correlation of CNI medication and the 2 hour high Cmax postdose concentrations of CSA-treated patients, in terms of neurological signs and symptoms. Furthermore, in an intention to treat pilot study, a possible influence on HRQoL, cognitive function and patients' compliance after a conversion from a twice daily CNI dosing to a once-daily CNI dosing with Prograf was evaluated.

The development of the extended released tacrolimus once-daily formulation resulted in slower release and lower peak concentrations.[23] The initial intention to improve

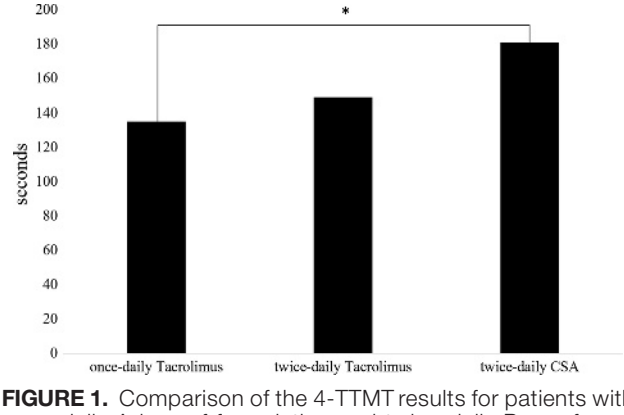

FIGURE 1. Comparison of the 4-TTMT results for patients with once-daily Advagraf formulation and twice-daily Prograf- and CSA-formulation in a cross sectional analysis. *P = 0.023; 1-way ANOVA. ANOVA, analysis of variance.

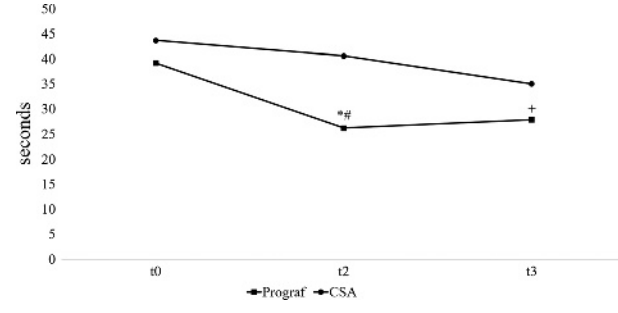

FIGURE 2. Results of the 4-TTMT in the Prograf group and CSA group after conversion from the twice daily CNI formulation to the once-daily Advagraf formulation directly after drug conversion (t0), 6 months (t2), and 12 months (t3) after drug conversion. **t2: Prograf group vs CSA group:* P = 0.018; 1-way ANOVA. #Prograf group: t0 vs t2: P = 0.007; paired t test. +Prograf group: t0 vs t3: P = 0.002; paired t test.

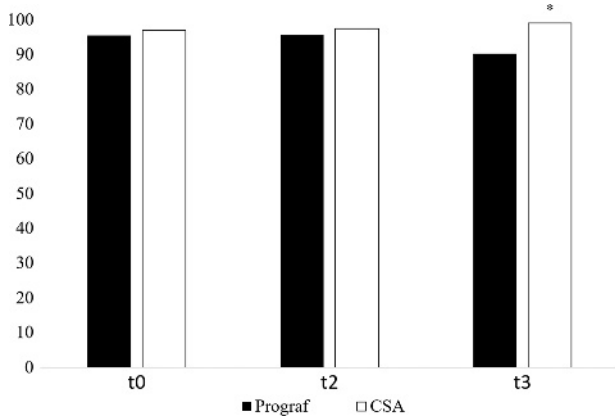

FIGURE 3. Results of the BAASIS in the Prograf group and CSA group after conversion from the twice daily CNI formulation to the once-daily tacrolimus formulation directly after drug conversion (t0), 6 months (t2), and 12 months (t3) after drug conversion. **T3: Prograf group vs CSA group: P* = 0.03; 1-way ANOVA.

compliance by once-daily dosage needs to be demonstrated.[24] Henry[25] discussed the differing toxicities between CSA and tacrolimus. In a French study, neurological adverse events were reported by 30% of liver recipients. Adverse drug events were significantly more reported by liver recipients exposed to CSA than those receiving tacrolimus.[26] Hathaway et al[27] stated, that there were no differences between the groups in terms of good to excellent organ function, treatment for rejection, infection, and over-immunosuppression. In this study statistical significant differences were observed when the immunosuppressive regimen related side-effect profile was analyzed. As follows, patients on CSA-based regimens reported greater overall side-effect severity and more problems with mobility and life roles. Furthermore, the author reported more problems in the miscellaneous subscale, including high blood pressure, enlarged gums and hair growth, but less trouble with trembling hands in patients with CSA immunosuppression. In multiple stepwise regression models, the authors identified several side-effect subscales having profound effects on mental and physical quality of life. Zaltzman et al[28] were able to demonstrate direct pharmacokinetic differences between tacrolimus and CSA in healthy volunteers. In their study, an acute reduction in the effective renal plasma flow and glomerular filtration rate were attenuated with the once-daily tacrolimus formulation compared with CSA immunosuppression.

In this pilot study, the superior test results of liver transplanted patients matched patients on the WL with an end-stage liver disease. This was to be expected and supported by the results of the measured MELD score. This indicated a significant impaired liver function of the WL patients. An influence of a renal insufficiency on test results in the different groups in the cross-sectional analysis and IIT study was excluded, by comparing the levels of serum creatinine. Furthermore, there was a stronger impairment of 4-TMTT in LTx patients compared to LD patients. A major aspect for this finding might be the inferior liver function in the transplanted patients. This again was underlined by the significant higher MELD score. However, also treatment of these patients with CNI drugs might add to the worse test results in the 4-TMTT. The test results of the 4-TMTT show differences between the groups, which are most likely related to the patients' CNI treatment. Otherwise, these test results support the hypothesis that the lower peak concentrations in the once-daily Prograf formulation result in an impaired cognitive functioning. At this point, this study is preliminary and a multicenter study with a more extended neuropsychological test battery is needed, to specify CNI-drug effects on cognitive functioning. In particular, the comparison of the 4-TMTT results of CSA and Advagraf-treated patients, with significant worse test results in CSA treated patients, shows that a twice-daily CSA immunosuppression might result in more impaired psychomotor speed. Furthermore, patients with a twice-daily tacrolimus formulation in the Prograf group had a faster improvement of their cognitive functioning after the conversion to the once-daily Advagraf formulation compared to patients with a twice-daily CSA formulation in the CSA group.

Reflecting these results, it is hypothesized that a flat pharmacokinetic profile with low Cmax is beneficial for a neuropsychological functioning. The better tolerance of a once-daily tacrolimus formulation is confirmed by published data from different research groups. Here, the lower peak concentrations of the once-daily tacrolimus formulation compared with the twice-daily formulation was already presumed to improve the toxicity profile.[29] Conversion from twice- to once-daily tacrolimus resulted in reduced hyperglycemia and triglycerides.[30-32] Bias, which cannot be repressed in this pilot study, are older age of patients, alcoholic cirrhosis in medical history and time after transplantation. These parameters might have negatively influenced the 4-TTMT results. In the IIT study, age and time after LTx might have negatively influenced the 4-TMTT results. Nevertheless, the prospectively assessed test results did not improve after conversion to Advagraf treatment within the CSA group. Thus, older age and a faster testing of patients after LTx might have played a role for the worse test results in comparing patients of the CSA and Prograf group at time point t2, but not on the absent improvement of the prospectively assessed test results within the group. This slower progress might be a result of stronger side effects due to a different pharmacokinetic profile of CSA. Furthermore, the nonsignificant differences in the 4-TTMT results and nonsignificant changes in patients' compliance comparing twice daily tacrolimus to once-daily tacrolimus formulation and twice daily CNI formulation to once-daily tacrolimus formulation were surprising, but might be related to the low power of this pilot study, to detect less dramatic changes. These nonsignificant changes of this pilot study should again be analyzed in a larger multicenter study.

Significant improvement of HRQoL function and symptom scores were not found after the conversion to the once-daily Advagraf formulation. Yet, we measured a trend of an improvement of HRQoL scores after conversion of the twice-daily Prograf formulation to the once-daily Advagraf formulation. This improvement of symptom and function scores might not have reached statistical significance due to a small number of patients. The at first side surprising worse symptom scores of insomnia after conversion from CSA to the Advagraf formulation 1 year after drug conversion is mostly due to the older age of the patients. Patients in the CSA group were significantly older than patients in the Prograf group. Once again, the results of this preliminary study lead to the conclusion, that a standardized multicenter study with a bigger study cohort is needed. Here, in particular the analyzation of a potentially significant improvement of HRQoL after a drug conversion to the once-daily Advagraf

formulation should be investigated. Furthermore, patients of the different groups in a multicenter study should have a comparable social-demographic profile. The measured impaired compliance with immunosuppressive medication in the Prograf group was unexpected. A reason might be, that older patients may adapt faster to the immunosuppressive drug formulations due to a longer medical history and drug intake.

This present study's limitation is mainly related to sample size. Additionally, a selection bias cannot be ruled out; the assessed data did not include those patients who were too ill to respond to the questionnaire and participate on the trail making test. Some patients might have overestimated or underestimated their activities or may have misinterpreted the questions in the self-administered questionnaire. The trail making test assesses scanning, visual-motor tracking, divided attention and cognitive flexibility all covered by the umbrella term "psychomotor speed".[33] A better trail making test result is in accordance with the clinical feedback of better cognitive function during daily living. However, this finding can be biased by the physician's impression. Therefore, a multicenter, randomized, investigator-blinded, controlled trial is needed, to better assess differences in cognitive functioning, compliance and HRQoL of patients under CSA and tacrolimus immunosuppression in a once-daily or twice-daily formulation. As already mentioned above a short neuropsychological test battery would be favorable. This should contain (1) a trail making test, (2) a Symbol Digit Modalities Test (sensitive for complex scanning and visual tracking), (3) a digit ordering (sensitive for working memory capacity), (4) a Stroop Test (sensitive for attentional processes, response inhibition), and (5) a verbal fluency (assessing speed and ease of verbal production). Parallel versions of all tests need to be administered to minimize test and retest effects.[33] Testing specific cognitive domains may be superior to test a global cognitive score, because our preliminary data show CNI-drug effects on the item psychomotor speed. Our goal is it to investigate changes in cognitive functioning, patient's compliance, and HRQoL within individuals over time due to a longitudinal study design.

To conclude, in the cognitive evaluation, patients with a once-daily tacrolimus formulation were able to complete the 4-TTMT faster compared to patients with a twice-daily CSA-formulation. Furthermore, it was supported, that patients could finish the 4-TTMT faster after conversion from a twice-daily CNI medication to the once-daily tacrolimus formulation. This amendment revealed a faster improvement after conversion of the twice-daily tacrolimus formulation to the once-daily tacrolimus formulation. This is mostly due to the pharmacokinetic profile with low Cmax levels, which is known to be beneficial for good neuro cognitive test results. An improvement of HRQoL and patient's compliance was not shown after conversion of the immunosuppressive drug formulation. A possible improvement of HRQoL and compliance should be analyzed in a greater, blinded multicenter study cohort using a short neuropsychological test battery.

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