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Area under trough concentrations of tacrolimus as a predictor of progressive renal impairment after liver transplantation.

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ABBREVIATIONS

AUCtc: Area under curve of trough concentrations.

eGFR: estimated glomerular filtration rate

LT: liver transplantation

MDRD: Modification of Diet in Renal Disease

ABSTRACT

Background: Tacrolimus minimization is usually restricted to patients with pre-transplant renal impairment and this strategy could result into worse renal outcomes after liver transplantation (LT).

Methods: A consecutive cohort of 455 LT patients receiving tacrolimus-based immunosuppression was studied (2008-2013). Cumulative exposure to tacrolimus was calculated as the area under curve of trough concentrations (AUCtc). Patients were stratified as tacrolimus minimization, conventional or high exposure according to thresholds based in the *COMMIT* consensus. Estimated glomerular filtration rates (eGFR) were assessed by the MDRD-4 formula up to 5 years post-LT.

Results: Seventy patients (15.4%) had pre-transplant eGFR<60 ml/min, which was associated with increased mortality rates, particularly within the first 5 years post-LT (31.4% vs 17.5%; Breslow p= 0.010). After LT, there was an abrupt eGFR decline within the first 3 months (median 18.6 ml/min; p<0.001), further decreasing up to 12 months (additional 3 ml/min), without any improvement thereafter. According to AUCtc, 33.7% of patients received tacrolimus minimization, 44.8% conventional exposure and 21.5% high exposure. Conventional/high exposure to tacrolimus resulted in a more pronounced eGFR decline within the first 3 months as compared with minimization (23.3ml/min vs 9.5ml/min; p<0.001). This gap was even higher in patients with initially preserved renal function. Tacrolimus AUCtc was an independent predictor of eGFR decline within the first 3 months after controlling for potential confounders.

Conclusions: AUCtc is a surrogate of cumulative exposure to tacrolimus and may be helpful for routine dose adjustments. Tacrolimus minimization should be universally attempted after LT in order to preserve renal function.

INTRODUCTION

Tacrolimus-based maintenance immunosuppression is the standard of care after liver transplantation (LT).[1] Tacrolimus has a narrow therapeutic window with its bioavailability ranging from 5% to 95%.[2] There is wide exposure variability among individuals and even within the same patient over time, making it mandatory to monitor tacrolimus trough concentrations in order to prevent graft rejection while avoiding toxicity.[3] As a calcineurin inhibitor, tacrolimus activates the renin-angiotensin and the endothelin systems among other vasoconstrictor pathways in a dose-dependent fashion, ultimately producing a chronic renal impairment.[4]

The liver is considered an immunologically privileged organ and therefore the use of reduced tacrolimus trough concentrations is deemed safe and does not increase the risk of rejection and derived graft loss. The use of reduced tacrolimus dosage is possible either as monotherapy[5] or in combination with other immunosuppressants such as mTOR inhibitors[6] or antimetabolites.[7] In both situations, tacrolimus minimization is consistently associated with improved renal function in the long term. Despite this evidence many LT institutions restrict tacrolimus minimization strategies to patients with pre-existing renal dysfunction or experiencing acute kidney injury early after LT. As a result, patients with initially preserved renal function would not receive tacrolimus minimization and could be at higher risk of progressive and irreversible damage to the kidney in the long term.

Cumulative tacrolimus exposure is a novel parameter which indicates the evolution of trough concentrations in a time-dependent manner. In previous studies, mean trough concentrations within the first month after LT were associated with long term outcomes, including graft loss and recurrence of hepatocellular carcinoma.[8, 9] However, average trough levels may not be appropriate to evaluate longer periods and does not take into

account the inherent variability between different measurements. These caveats may be solved by implementing cumulative tacrolimus exposure as an aiding tool to refine dose adjustments.

The primary aim of the present study was to explore the role of cumulative tacrolimus exposure on the development of progressive renal impairment within the first 12 months after LT. Secondary aims were to describe estimated glomerular filtration rates (eGFR) up to 5 years after LT and to determine the role of tacrolimus intra-patient variability on renal impairment.

MATERIAL AND METHODS

This is a retrospective analysis of a prospectively collected database from two European LT institutions: the Royal Free Hospital (London, United Kingdom) and the Reina Sofía University Hospital (Córdoba, Spain). A consecutive cohort of adult patients who underwent LT from January 2008 to December 2013 and received tacrolimus-based primary immunosuppression was included. Patients with death or tacrolimus withdrawal within the first 90 days post-LT were excluded. Donor-recipient blood group incompatibility, HIV positive, combined organ transplantation and re-transplantation were additional exclusion criteria. Patients receiving induction agents such as basiliximab were included as long as tacrolimus introduction was not delayed beyond 15 post-operative days. Post-transplant clinical surveillance ensured at least one visit every 3 months within the first year and every 6 months thereafter. Follow-up ended in January 2018. The present study was performed according to the ethical principles contained in the Declaration of Helsinky and was approved by the Andalusian ethics committee as part of a broader research initiative (PEIBA code 1562-N-18).

Immunosuppression protocol and assessment of cumulative tacrolimus exposure. Immunosuppressive medication was started immediately after LT once gut function was restored. Tacrolimus trough concentrations were determined at least every 48 hours until discharge, weekly within the first month, monthly within the first 6 months, and every 3 months thereafter. Longer intervals were allowed in the long term. Additional measurements were performed if clinically indicated. Tacrolimus dosage and target trough levels were adjusted at the discretion of the attending clinician, based on the individual balance between the estimated risk of rejection and toxicity. In each patient, time dependent cumulative tacrolimus exposure was assessed by the area under curve of trough concentrations (AUCtc) (see Figure S1, SDC, http://links.lww.com/TP/B740). Taking into account a recent consensus document in which conventional trough concentrations of tacrolimus were defined[3], thresholds were adapted to stratify patients the following categories: a) aggressive tacrolimus minimization (trough concentrations <4ng/mL within the first month and <3ng/mL thereafter); tacrolimus minimization (trough concentrations 4-6 ng/mL within the first month and around 4 ng/mL thereafter); conventional tacrolimus exposure (7-10 ng/mL within the first month and 6-8 ng/mL thereafter); high tacrolimus exposure (>10 ng/mL within the first month and >8 ng/mL thereafter). In order to facilitate the interpretation, equivalences between target trough concentrations during follow-up and AUCtc are shown in figure 1. Parameters related with tacrolimus intra-patient variability were also recorded including standard deviation, coefficient of variation and variance of trough concentrations. Basiliximab induction was used in selected patients with pre-transplant renal dysfunction or hepatic encephalopathy in order to delay the introduction of tacrolimus to post-LT day 5. Corticosteroids were progressively tapered and completely withdrawn in both centers between the third and the sixth month post-transplant, except for patients

with autoimmune hepatitis in whom a minimal dose of prednisone was maintained in the long term. Antimetabolites were frequently prescribed to reduce the exposure to tacrolimus, with mycophenolate mofetil preferred at the Reina Sofía University Hospital (1000mg bid) and azathioprine preferred at the Royal Free (1mg/kg/day). Antimetabolites were replaced by mTOR inhibitors in patients with persistent renal impairment in both institutions, and also in patients with hepatocellular carcinoma transplanted at the Reina Sofía University Hospital from 2012 onwards as part of a prospective observational study.[10] Patients with otherwise unexplained rising serum bilirubin and blood eosinophil count underwent liver biopsy to confirm acute T-cell mediated rejection.[11] Boluses of corticosteroids (1000 mg methylprednisolone) were administered on three consecutive days if the histological grade of rejection was moderate-severe.[12]

Evaluation of renal function

Renal function was assessed at baseline (last available creatinine before LT outside an acute kidney injury episode) and at the following post-transplant timepoints: 3 months, 6 months, 12 months, 24 months, 36 months, 48 months and 60 months. The "Modification of Diet in Renal Disease" formula (MDRD-4) was used to calculate eGFR[13] as there is no equation dedicated to transplant population which had shown superiority to MDRD-4. Patients were stratified according to KDIGO 2017 clinical practice guidelines for chronic kidney disease[14] in the following categories: Grade 1 (normal or high) if eGFR>90 ml/min; Grade 2 (mildly decreased) if eGFR 60-89 ml/min; Grade 3a (mildly to moderately decreased) if eGFR 45-59 ml/min; Grade 3b (moderately to severely decreased) if eGFR 30-44 ml/min; Grade 4 (severely decreased) if eGFR 15-29 ml/min; and Grade 5 (kidney failure) if eGFR <15 ml/min.

Sample size calculation

EPIDAT 3.1 (Xunta de Galicia) was used for sample size calculation in its function for cohort studies. The main outcome considered was worsening of renal function beyond KDIGO Grade 3a at 12 months post-LT. In the study by Allen et al[15] the prevalence of KDIGO Grade 3a at baseline was 27.1% but rose to 59% at 12 months post-LT (first-year incidence 31.9%). We speculated that patients with effective tacrolimus minimization (AUCtc<2,250 within the first 12 post-LT months) would have a reduced first-year incidence of KDIGO>G3a of 20%. The following additional assumptions were made: a) Prevalence of effective tacrolimus minimization: 50%; b) statistical power 80%; c) confidence interval 5%. Under these premises the minimum sample size required would be 424 patients.

Statistical analysis

The statistical analysis was performed by using SPSS version 22.0 (IBM corp, Armonk, NY). Continuous variables were expressed as mean and standard deviations except for those with asymmetric distribution in which median and interquartile range was used. Categorical variables were displayed in frequency tables. For each patient, AUCtc was calculated by using the Wagner-Nelson equation (see Table S1, SDC, http://links.lww.com/TP/B740) and a dedicated excel worksheet was designed for this purpose. The appropriate contrast tests were used depending on the characteristics of the variables involved in the analysis. Paired T test was used to evaluate changes in eGFR during follow up. Kaplan-Meier curves were used to evaluate the influence of baseline renal dysfunction on mortality. Multivariate linear regression was implemented to explore whether cumulative tacrolimus exposure (AUCtc) independently predicted a progressive decline of renal function (delta eGFR) after controlling for potential confounding factors. In such model the scale of continuous variables was assessed by

using the Box Tidwell test. Multicollinearity was detected by using the variance inflation factor, and extreme values were explored by the Cook's distance. The goodness of fit was expressed as the coefficient of determination (R^2). All comparisons were two tailed and considered statistically significant if $p<0.05$.

RESULTS

Baseline characteristics

In total, there were 455 patients included [59.6% from the Royal Free Hospital (n=271) and 40.4% from the Reina Sofía University Hospital (n=184)] with a median follow-up post-LT of 58 months. Overall, recipient age at transplantation was 52.2±10.4 years and there were 129 female patients (22.4%). The main aetiologies of liver disease were chronic hepatitis C (n=164; 36%) and alcoholic cirrhosis (n=151; 33.2%). Hepatocellular carcinoma was the indication for LT in 138 patients (30.3%). Baseline characteristics of patients according to the recruiting institution are shown in Table S1 (SDC, http://links.lww.com/TP/B740). At the Reina Sofía University Hospital, patients were slightly older (53.9 vs 51.1 years old; p=0.005), and had more frequently alcoholic liver disease (45.1% vs 25.1%; p<0.001), chronic hepatitis C (42.4% vs 31.7%; p=0.02) and hepatocellular carcinoma (42.9% vs 21.8%; p<0.001). At the Royal Free there were more women (32.1% vs 22.8%; p=0.031) and increased prevalence of autoinmmune hepatitis, primary biliary cholangitis and primary sclerosing cholangitis (20.3% vs 3.3%; p<0.001).

Overall, pre-transplant renal function was preserved (ie, KDIGO Grade 1) in 242 patients (53.2%). Among the remaining 213 patients with impaired eGFR, 31.4% had KDIGO Grade 2, 11.9% had Grade 3a, 2.6% had Grade 3b and 0.9% had Grade 4. No patient showed kidney failure (ie, KDIGO Grade 5) at baseline. Patients with baseline KDIGO ≥Grade 3 had increased post-transplant mortality rates (log rank p=0.047),

particularly within the first 5 years post-LT (31.4% vs 17.5%; Breslow p= 0.010) (figure 2). Clinical features of patients with pre-transplant renal impairment (KDIGO≥ Grade 3) and without renal impairment (KDIGO Grade 1-2) are shown in table 1. As part of routine clinical practice, patients with pre-transplant renal impairment (KDIGO ≥ Grade 3a) had reduced cumulative exposure to tacrolimus (AUCtc) at 3 months ($p<0.001$), 6 months ($p<0.001$) and 12 months ($p=0.001$) as compared with patients with KDIGO Grade 1-2 (table 1). Indeed, effective tacrolimus minimization within the first 3 months after LT was accomplished in 52.7% of patients with KDIGO ≥ Grade 3a vs 29.9% of patients with KDIGO Grade 1-2 ($p<0.001$). Indicators of tacrolimus intra-patient variability such as standard deviations of trough concentrations, coefficient of variation and variance were reduced in patients with pre-transplant renal impairment (table 1). Patients with pre-transplant KDIGO ≥ Grade 3 received induction with basiliximab more frequently (17.4% vs 7%; $p=0.01$). However, the prescription of other immunosuppressants such as antimetabolites or mTOR inhibitors was not influenced by the baseline renal function ($p=0.95$ and $p=0.72$ respectively).

Evolution of renal function during follow-up

There was a pronounced decline of eGFR within the first 3 post-transplant months (median 18.6 ml/min from baseline; $p<0.001$) which continued decreasing at 6 months post-LT (median 2.4 ml/min from months 3 to 6; $p=0.01$). There was a not significant trend for further eGFR decline at 12 months (median 0.6 ml/min from months 6 to 12; $p=0.05$). Renal function remained stable thereafter though eGFR did not recover up to 60 months follow-up (figure 3). The evolution of KDIGO grade distribution during follow-up is shown in figure 4. The proportion of patients with grade 1 decreased abruptly within the first 3 months after LT (from 58.8% to 28.4%) and continued declining until reaching a plateau at 24 months post-LT (at 17.7%). Conversely, the

prevalence of grades 2 and 3 were raised from 31.6% and 14.7% respectively at baseline, to 41% and 29.4% respectively at 3 months after LT, remaining stable thereafter. Artificial renal support or renal transplantation was required in 39 patients (8.6%) during follow-up.

When stratifying according to pre-transplant eGFR, patients with initially preserved renal function (KDIGO Grades 1-2) showed a similar evolution as the overall population, but with an even more pronounced decrease of eGFR within the first 12 months: 23.9 ml/min within the first 3 months ($p<0.001$), 3.1 ml/min from months 3 to 6 ($p=0.002$) and 0.6 ml/min from months 6 to 12 ($p=0.03$). There was a decline of at least one KDIGO stage within the first 3 months in 193 patients (53.9%): 142 patients (39.6%) reached KDIGO Grade 3a, 43 patients (12%) reached KDIGO Grade 3b, 6 patients (1.7%) reached KDIGO Grade 4 and 2 patients (0.6%) reached KDIGO Grade 5. On the other hand, patients with pre-transplant KDIGO Grade 3-5 experienced an initial improvement in eGFR by a median of 11 ml/min within the first 3 months, but remained stable thereafter (figure 3). Baseline KDIGO classification improved in 32 patients (43.2%) as follows: 1 stage improvement (n=20; 27%), 2 stages improvement (n=9; 12.2%), 3-4 stages improvement (n=3; 4.1%).

Influence of cumulative exposure to tacrolimus on progressive kidney damage, rejection, graft loss and mortality

Cumulative exposure to tacrolimus in the overall population was as follows: aggressive minimization in 4.2% of patients (n=19); minimization in 29.5% of patients (n=134); conventional exposure in 44.8% of patients (n=204) and high exposure 21.5% of patients (n=98). Median AUCtc was 652 ng.day/mL (IQR 529-812) within the first 3 months, 1324 ng.day/mL (IQR 1062-1633) within the first 6 months and 2641 ng.day/mL (IQR 2130-3378) within the first 12 months. Cumulative exposure to

tacrolimus was tightly correlated with progressive renal impairment (figure 5). Patients with conventional or high exposure to tacrolimus experienced a more pronounced decline of eGFR within the first 3 months, 6 months and 12 months. Indeed, within the first 3 months post-LT, aggressive tacrolimus minimization was associated with the lowest decline of eGFR (median 2.4 ml/min). Patients with minimization had a median decline of eGFR of 10.6 ml/min and patients with conventional exposure experienced a median decline of eGFR of 19.3 ml/min within the first 3 months. The most pronounced decrease of eGFR within the first 3 months was observed in patients with high cumulative tacrolimus exposure (median 30.7 ml/min; $p<0.001$) (figure 5). When stratifying according to baseline eGFR, patients with initially preserved renal function who received conventional or high AUCtc of tacrolimus had a more pronounced decline of eGFR within the first 3 months after LT (26.9 ml/min vs 18.9 ml/min; $p<0.001$). In addition, among patients with pre-transplant renal impairment, those receiving minimization had a more pronounced eGFR recovery (17 ml/min vs 7.9 ml/min), although it did not reach statistical significance ($p=0.37$). Similar results were obtained regarding eGFR decline within the first 6 and 12 months according to cumulative tacrolimus exposure within the same periods.

Standard deviations of trough concentrations showed a weak, although statistically significant, direct correlation with eGFR decline within the first 3 months ($r=0.14$; $p=0.003$), 6 months ($r=0.12$; $p=0.013$) and 12 months ($r=0.10$; $p=0.045$) after LT. Others parameters of tacrolimus intra-patient variability such as coefficient of variation and variance of trough concentrations did not correlate with the evolution of eGFR after LT (results not shown).

The univariate and multivariate linear regression analyses showing predictors of eGFR decline within the first 3 months are presented in table 2. Conventional or high cumulative exposure to tacrolimus as calculated by the AUCtc was an independent predictor of a more pronounced decline of eGFR at 3 post-LT months, after controlling for potential confounders such as baseline renal function, recipient age, hepatitis C and recruiting institution. Tacrolimus intra-patient variability as defined by the standard deviation of trough concentrations had no significant influence on the evolution of eGFR in the multivariate analysis. In this multivariate model, we tested to replace AUCtc by average trough concentrations of tacrolimus within the same period. Average trough concentrations were not independent predictors of eGFR decline (OR 0.49; 95%CI -1.1-1.9; p=0.52).

Biopsy-proven acute T-cell mediated rejection rates according to AUCtc subgroups were as follows: aggressive minimization (36.8%), minimization (26.9%), conventional (24%) and high exposure (14.3%), with the last group reaching statistical significance (high exposure vs others; p=0.017). There were only 7 patients who experienced chronic rejection in the entire cohort (prevalence 1.5%). Among them, 4 patients had received tacrolimus minimization, 2 patients had conventional exposure and 1 patient had high exposure. AUCtc within the first 12 months after LT had no impact on the risk of graft loss (RR=1.20; 95%CI 0.67-2.16; p=0.52) and overall mortality (RR=1.05; 95%CI 0.57-1.93; p=0.87).

DISCUSSION

Renal impairment is frequent among patients who access the waiting list within a MELD or UKELD based prioritization system.[16] The vast majority of patients experience further kidney deterioration within the first year after LT resulting in a negative impact on overall survival.[15, 17, 18] Tacrolimus minimization is able to mitigate

renal damage but in clinical practice this strategy is restricted mainly to patients with baseline renal impairment. In the present study, a more objective and representative methodology to evaluate cumulative tacrolimus exposure, namely AUCtc, emerged as an independent predictor of eGFR decline after LT. Interestingly, the highest benefit in terms of renal preservation associated with tacrolimus minimization was achieved in patients without pre-transplant renal dysfunction. Since AUCtc may be updated at each patient visit, clinicians may rely on its value to optimize dose adjustments in routine clinical practice.

The prevalence of chronic kidney disease (GFR<60 ml/min) after LT is as high as 59% at one year after LT, with a slow increase thereafter up to 62% at 5 years according to an observational study with direct GFR measurements,[15] aligning with our findings. Among LT patients, chronic kidney disease is per se a major cause of excess of death as compared with age-matched general population,[19] but it also has an indirect morbidity effect by increasing the risk of cardiovascular events.[20] The prevalence, prognostic impact and complex management of chronic kidney disease after LT have motivated several consensus documents aiming to delineate strategies in order to prevent or at least to ameliorate this complication.[3, 21, 22] The etiology of renal impairment after LT is multifactorial, being abnormal pre-transplant renal function the most powerful risk factor.[23, 24] Older age at transplantation, hepatitis C, uncontrolled diabetes and arterial hypertension may contribute to progressive renal damage in the long term.[25] However, the main subscriber to an early and accelerated post-LT renal deterioration is nephrotoxicity mediated by calcineurin inhibitors, mainly tacrolimus.

Tacrolimus is able to induce two forms of kidney injury: a) acute nephrotoxicity is caused by renal vasoconstriction which is dose dependent and potentially reversible after significant dose reduction or drug withdrawal; b) chronic nephrotoxicity occurs

when vasoconstriction persists, leading to endothelial dysfunction and irreversible histological changes such as tubule-interstitial fibrosis.[4] In clinical practice, acute kidney injury is easily detected and coincides with peaks of trough levels of tacrolimus, thus motivating routine dosage adjustments with full –or at least partial- reversibility in most cases. Chronic renal impairment is a more surreptitious complication as it presents with a progressive decline in eGFR, which is practically imperceptible when patients are monitored by using serum creatinine. In previous studies, the relationship between tacrolimus exposure and progressive decline in eGFR could not be clearly established, maybe because isolated trough levels or mean trough concentrations can only assess tacrolimus exposure within a short timeframe, leading some authors to suggest that the clinical impact of calcineurin inhibitor mediated nephrotoxicity would be negligible.[26] In the present study, a novel methodology was designed to calculate cumulative tacrolimus exposure, namely AUCtc, which allows for a time-dependent assessment and can be updated in each patient visit, thus aiding to tailor dosage. An abrupt decrease of eGFR within the first 3 months after LT was found, which continued up to 12 months after LT, and was more pronounced in patients with conventional or high cumulative tacrolimus exposure (ie, AUCtc) as compared with minimization strategies. Renal function was not restored thereafter thus suggesting that therapeutic decisions are made too late, when chronic mechanisms of nephrotoxicity are established and would not reverse. Therefore, minimization strategies should be considered as soon as possible, ideally immediately after LT. Indeed, a systematic review and metaanalysis of randomized controlled trials demonstrated that patients with increased mean tacrolimus trough concentrations within the first month after LT (>10 ng/mL) had doubled renal impairment rates at 1 year, without any reduction in acute rejection rates[27]. Our results have pointed out that further tacrolimus minimization (AUCtc equivalent to <6 ng/mL

within the first month and close to 4 ng/mL thereafter) is more effective in avoiding eGFR decline within the same period, particularly in patients with initially preserved renal function.

A potential caveat when implementing such minimization of tacrolimus in routine clinical practice would be to increase the risk of acute rejection and graft loss, as shown in an observational study.[8] However, a randomized controlled trial with protocolized pathological surveillance and prolonged follow-up has reported excellent outcomes by using tacrolimus monotherapy with such reduced trough levels.[5] There is also solid evidence concluding that very low tacrolimus trough concentrations may be safely accomplished in combination with other immunosuppressants such as mTOR inhibitors, which counteract the loss of immunosuppression potency.[6] In our cohort, acute rejection rates were higher among patients with aggressive minimization but none of them developed chronic rejection and subsequent graft loss, probably because of the use of concomitant immunosuppressants. Notably, acute rejection rates were identical between patients with minimization or conventional exposure. Given the limited prognostic impact of acute T-cell medianted rejection on long term outcomes[8, 28] and the well established negative impact of chronic renal disease, we strongly believe that the benefits of tacrolimus minimization overcome the risks in the vast majority of LT patients.

Tacrolimus intra-patient variability is defined by fluctuations of trough concentrations over time despite of stable dosage.[2] Some studies have linked increased intra-patient variability with worse renal outcomes[29] and increased acute rejection rates[30] in LT recipients. However, there is no consensus on how to assess variability (standard deviation of trough concentrations, coefficient of variation, variance, medication level index), neither there are validated thresholds. In the present study, standard deviation of

trough concentrations was the only variability parameter associated with progressive renal impairment in the univariate analysis. However, the statistical significance was lost in the multivariate model, probably because those patients with increased standard deviations of trough concentrations are those with the highest cumulative exposure, which is the actual independent predictor of eGFR decline.

The main limitation of the present study is the lack of randomization. Dose adjustments of tacrolimus were made as per routine clinical practice at the discretion of the responsible clinician. Episodes of acute renal dysfunction may have motivated tacrolimus dose reduction, thus introducing a source of bias. Although cumulative tacrolimus exposure was an independent predictor of progressive kidney damage - despite of these routine dose adjustments- it is probable that its actual impact was underestimated. In addition, proteinuria, HbA1c, therapy against hepatitis C and donor features were not systematically recorded. Considerable strengths are also to be noted including the involvement of two transplant institutions, the increased sample size with prolonged follow-up and the description of a novel strategy to estimate cumulative tacrolimus exposure with potential applications in clinical practice.

In conclusion, the AUCtc is a surrogate of tacrolimus cumulative exposure and may be helpful to individualize dose adjustments after LT. Tacrolimus cumulative exposure, but not intra-patient variability, is associated with a progressive derangement of renal function despite routine dose adjustments. Therefore, tacrolimus minimization and sparing protocols should be considered in all patients early after LT in order to preserve renal function, unless clinically contraindicated. In future randomized trials evaluating different tacrolimus-based immunosuppression strategies, the implementation of AUCtc would allow to compare the tacrolimus sparing effect between treatment arms. The

potential impact of AUCtc on other immunosuppression linked outcomes such as infections, cardiovascular events or cancer should be explored in future studies.

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FIGURE LEGENDS

Figure 1. Stratification of patients according to time-dependent cumulative exposure to tacrolimus as defined by the area under curve of trough concentrations (AUCtc). Conventional tacrolimus trough concentrations were obtained from a consensus document (reference 3) as 7–10 ng/mL within the first month and 6–8 ng/mL thereafter. Equivalences between target trough concentrations and AUCtc are shown in the table below.

Figure 2. Impact of baseline renal impairment on overall survival after liver transplantation in a cohort of 455 liver transplant recipients. Log rank test was used for overall comparison, and Breslow test was implemented to focus on earlier differences during follow-up.

Figure 3. Evolution of estimated glomerular filtration rates (eGFR) calculated by the MDRD-4 formula (standardized IDMS). Median eGFR at different timepoints is shown for the overall cohort and for subpopulations with and without pretransplant renal impairment.

Figure 4. Distribution of patients according to KDIGO stage during follow-up. The proportion of patients with KDIGO Grade 3 or worse was doubled within the first year after liver transplantation.

Figure 5. Median eGFR decline within the first 3 months after liver transplantation according to cumulative exposure to tacrolimus.

Table 1. Clinical features of 455 liver transplant patients stratified according to baseline estimated glomerular filtration rate (eGFR) as calculated by the MDRD-4 formula (standardized IDMS). Continuous variables are presented as means ± standard deviations (median and interquartile range for asymmetric distributions). Categorical variables are displayed as n (%).

	KDIGO stage 1-2 (ie, ≥60 ml/min) n=385 (84.6%)	**KDIGO stage 3a-5 (<60 ml/min)** n=70 (15.4%)	**p**
Recipient Age (years)	51.9±10.4	54.1±10.4	0.09
Gender (female)	103 (26.7%)	26 (37.4%)	0.15
Diabetes (pre-transplant)	90 (23.4%)	17 (24.3%)	0.94
Hypertension (pre-transplant)	66 (17.1%)	19 (27.1%)	0.08
Transplant institution			
Royal Free Hospital	230 (59.7%)	41 (58.5%)	0.42
Reina Sofía University Hospital	151 (40.3%)	33 (31.5%)	
Chronic hepatitis C	143 (37.4%)	21 (30%)	0.13
Alcoholic liver disease	121 (31.4%)	30 (42.8%)	0.14
Chronic hepatitis B	22 (5.7%)	5 (7.1%)	0.74
Hepatocellular carcinoma	121 (31.4%)	17 (24.3%)	0.13
MELD score pre-transplant	17.1±6.7	22.3±8.1	<0.001
Donor type			
Donor after brain death	356 (92.5%)	64 (91.4%)	0.71
Donor after circulatory death	28 (7.2%)	6 (8.6%)	
Living donor	1 (0.3%)	0 (0%)	
Acute T-cell mediated rejection			
Histologically proven	87 (22.6%)	19 (27.1%)	0.59
Determinations of trough concentrations			
Within the first 3 months	22.7±11.7	25.4±14.9	0.14
Within the first 6 months	27.5±17.2	30.3±20.9	0.22
Within the first 12 months	32.6±23	34.4±18.6	0.57
Cumulative tacrolimus exposure (ng.day/mL)			
AUCtc 3 months	709±231	551±218	<0.001
AUCtc 6 months	1421±453	1123±399	<0.001
AUCtc 12 months	2823±919	2322±775	0.001
Standard deviations of trough concentrations (ng/mL)			
3 months			
6 months	3.63±1.8	3.01±1.2	<0.001
12 months	3.69±1.6	3.07±1.2	<0.001
	3.57±1.4	3.09±1.1	0.008
Coefficient of variation of trough concentrations			
3 months	0.51±0.2	0.55±0.2	0.17
6 months	0.51±0.2	0.55±0.2	0.08
12 months	0.50±0.1	0.55±0.1	0.008
Variance of trough concentrations (ng/mL^2)			
3 months	10.1 (IQR 5.8-19.7)	8.5 (IQR 4.4-13.5)	0.01
6 months	11 (IQR 6.3-19.4)	8.3 (IQR 4.7-13.6)	0.006
12 months	11 (IQR 6.6-18.6)	8.7 (IQR 4.9-15.3)	0.03
Concomitant primary immunosuppression			
Basiliximab induction	27 (7%)	12 (17.4%)	0.01
Mycophenolate/azathioprine	282 (73.2%)	55 (78.5%)	0.95
mTOR inhibitors	12 (3.1%)	3 (4.3%)	0.72

AUCtc: Area under curve of trough concentrations; IQR: interquartile range.

Table 2. Univariate and multivariate linear regression to predict worsening of renal function as expressed by the decline of estimated glomerular filtration rate (MDRD-4) within the first 3 months after liver transplantation.

	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS				
			INITIAL MODEL		FINAL MODEL		
VARIABLES	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**Beta (std)**	**p**
Institution	4.71 (-2.4-11.8)	0.19	-4.12 (-9.6-1.4)	0.14	-3.64 (-9.11-1.82)	-0.048	0.19
Recipient Age	0.03 (-0.29-0.36)	0.85	0.70 (0.43-0.97)	<0.001	0.65 (0.40-0.90)	0.18	<0.001
Gender	8.40 (0.7-16.1)	0.03	-3.38 (-9.3-2.5)	0.26	---	---	---
Diabetes	-5.71 (-13.8-2.4)	0.17	-0.04 (-6.1-5.9)	0.98	---	---	---
Hypertension	1.3 (-7.6-10.2)	0.77	---	---	---	---	---
Alcoholic cirrhosis	-3.94 (-11.3-3.4)	0.29	---	---	---	---	---
Hepatitis C	11.48 (4.3-18.6)	0.002	4.89 (-0.75-10.5)	0.06	5.68 (0.38-10.9)	0.07	0.036
Autoimmune liver disease	3.28 (-6.8-13.3)	0.52	---	---	---	---	---
Hepatocellular carcinoma	9.96 (2.4-17.5)	0.01	0.44 (-5.8-6.7)	0.89	---	---	---
Donor type (DBD vs DCD)	6.16 (-6.9-19.2)	0.35	---	---	---	---	---
Tacrolimus cumulative exposure*	18.7 (11.5-25.8)	<0.001	8.49 (2.2-14.8)	0.008	8.88 (2.78-14.98)	0.11	0.004
Tacrolimus standard deviations**	3.13 (1.1-5.1)	0.003	-1.09 (-2.8-0.62)	0.21	-1.12 (-2.83-0.58)	-0.05	0.20
Tacrolimus coefficient variation	12.52 (-2.5-27.6)	0.10	---	---	---	---	---
Tacrolimus variance	0.12 (-0.07-0.31)	0.22	---	---	---	---	---
Immunosuppresion TAC monotherapy TAC + MMF/AZA TAC + mTORi Basiliximab	1 (Reference) 3.01 (-5.3-11.3) -7.13 (-17.8-3.5) -1.57 (-6.4-3.2)	0.35	---	---	---	---	---
eGFR at baseline***	0.74 (0.66-0.81)	<0.001	0.78 (0.69-0.86)	<0.001	0.77 (0.69-0.85)	0.70	<0.001
				Goodness of fit (R^2)=0.496			

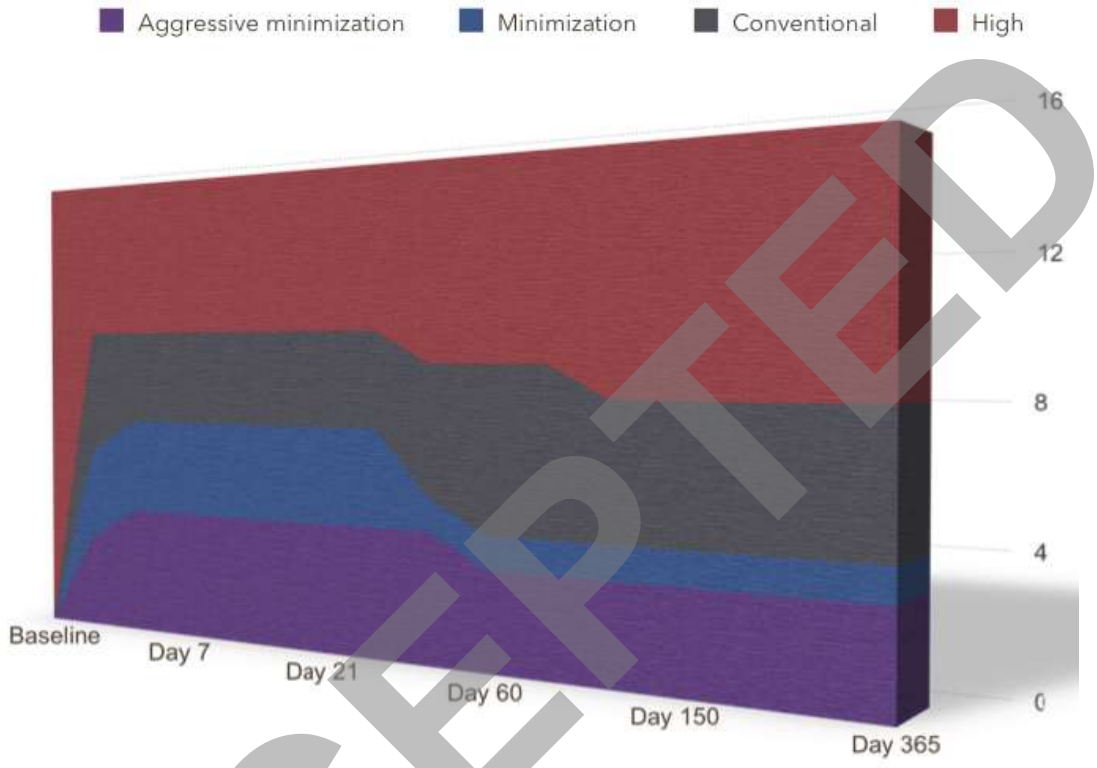
DBD: Donor after brain death; DCD: donor after circulatory death; TAC: tacrolimus; MMF: mofetil mycophenolate; AZA: azathioprine; mTORi: mTOR inhibitors.

* Conventional or high cumulative tacrolimus exposure vs minimization as defined by the area under trough concentrations (AUCtc) within the first 3 months after liver transplantation.

** Standard deviation of tacrolimus trough concentrations within the first 3 months after liver transplantation

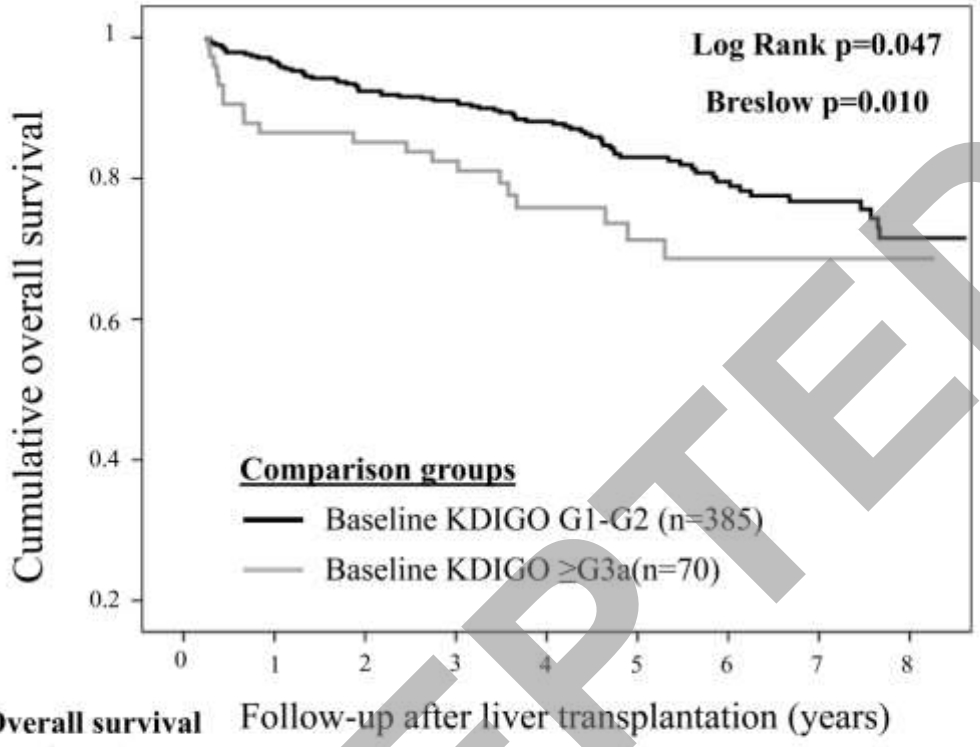
*** eGFR: estimated glomerular filtration rate as calculated by the MDRD-4 formula.

Figure 1



Category	Target trough concentrations (ng/mL)	AUCtc 3 months	AUCtc 6 months	AUCtc 12 months
Aggressive minimization	<4 (first month) <3 (thereafter)	<320	<590	<1,150
Minimization	4-6 (first month) 4 (thereafter)	321-579	591-1,109	1,151-2,219
Conventional	7-10 (first month) 6-8 (thereafter)	580-839	1,110-1,569	2,220-3,049
High exposure	>10 (first month) >8 (thereafter)	>840	>1,570	>3,050

Figure 2



Overall survival (n° at risk)	1 year	3 years	5 years
KDIGO G1-G2	96.3% (367)	91.1% (333)	83% (183)
KDIGO≥G3	86.5% (64)	82.4% (61)	71.2% (30)

Figure 3

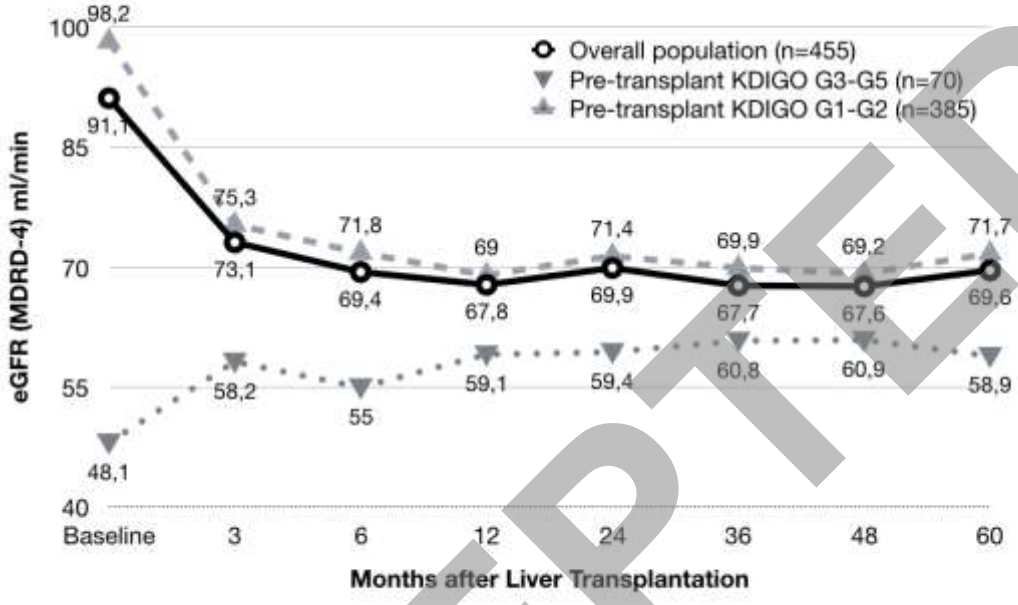
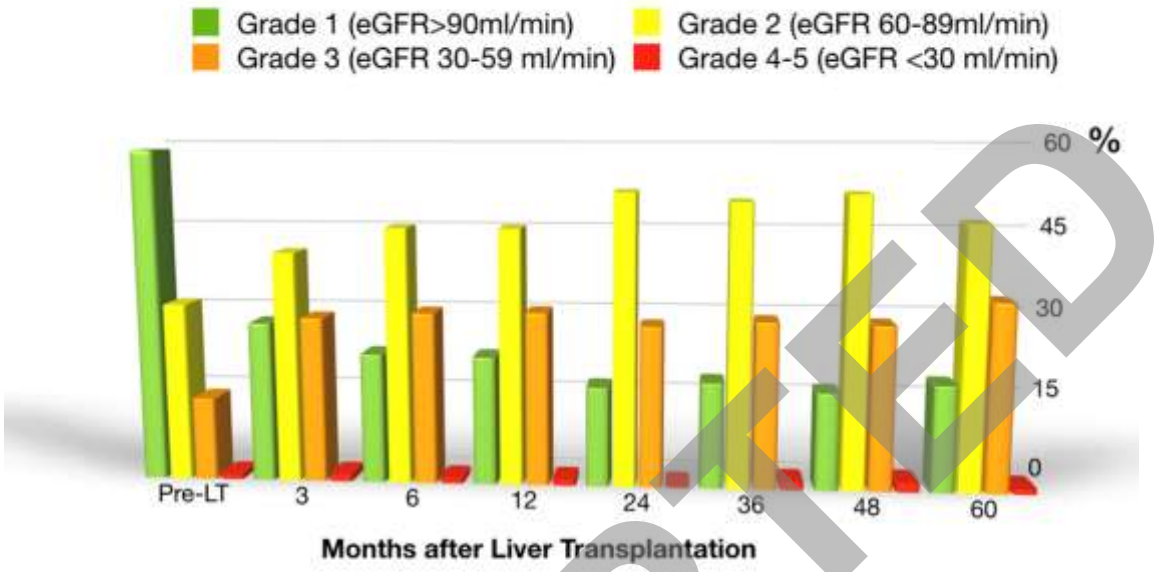


Figure 4



	Pre-LT	3	6	12	24	36	48	60
Grade 1	58.8	28.4	23.1	22.8	17.7	18.8	17.3	18.9
Grade 2	31.6	41	45.5	45.5	51.9	50.3	51.7	46.8
Grade 3	14.7	29.4	30.5	30.8	28.6	29.6	29.2	33.4
Grade 4-5	0.9	1.2	0.9	1	0.5	1.4	1.7	1

Figure 5