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***Retrospective Cohort Study***

**High tacrolimus intra-patient variability is associated with graft rejection, and *de novo* donor-specific antibodies occurrence after liver transplantation**

Del Bello A *et al*. Tacrolimus variability in liver transplantation

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**Abstract**

***AIM***

To investigate the role of tacrolimus intra-patient variability (IPV) in adult liver-transplant recipients.

***METHODS***

We retrospectively assessed tacrolimus variability in a cohort of liver-transplant recipients and analyzed its effect on the occurrence of graft rejection and *de novo* donor-specific antibodies (*dn*DSAs), as well as graft survival during the first 2 years posttransplantation. Between 02/08 and 06/2015, 116 patients that received tacrolimus plus mycophenolate mofetil (with or without steroids) were included.

***RESULTS***

Twenty-two patients (18.5%) experienced at least one acute-rejection episode (BPAR). Predictive factors for a BPAR were a tacrolimus IPV of > 35% [OR = 3.07 95%CI (1.14–8.24), *P* = 0.03] or >40% [OR = 4.16 (1.38–12.50), *P =* 0.01), and a tacrolimus trough level of < 5 ng/mL [OR=3.68 (1.3-10.4), *P =*0.014]. Thirteen patients (11.2%) developed at least one *dn*DSA during the follow-up. Tacrolimus IPV [coded as a continuous variable: OR = 1.1, 95%CI (1.0–1.12), *P =* 0.006] of > 35% [OR = 4.83, 95%CI (1.39-16.72), *P =*0.01] and > 40% [OR = 9.73, 95%CI (2.65–35.76), *P =* 0.001] were identified as predictors to detect *dn*DSAs. IPV did not impact on patient- or graft-survival rates during the follow-up.

***CONCLUSION***

Tacrolimus-IPV could be a useful tool to identify patients with a greater risk of graft rejection and of developing a *de novo* DSA after liver transplantation

**Key words:** Donor-specific antibodies; Liver transplantation; Immunosuppression; Rejection; Variability

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**Core tip:** Tacrolimus intra-patient variability (Tac IPV) was associated with kidney-graft rejection and worse long-term outcomes, but until now, was not well studied after liver transplantation in adult recipients. We found that the coefficient of variability-IPV of tacrolimus was a predictive factor for acute rejection and the occurrence of *de novo* donor-specific antibodies (DSA) after liver transplantation in a retrospective cohort of 116 recipients treated with tacrolimus and mycophenolate mofetil. This could be a useful tool to identify patients with a greater risk of graft rejection and of developing a *de novo* DSA after liver transplantation.

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**INTRODUCTION**

Tacrolimus (Tac) is considered a cornerstone within immunosuppression protocols to prevent T-cell and antibody-mediated rejection after liver transplantation[1-3] However, this treatment presents a narrow therapeutic index: overexposure can lead to clinically serious events[4] thus necessitating regular therapeutic drug monitoring, whereas underexposure can lead to acute or chronic graft rejection[4-6] Inter-individual variability from Tac therapy may be explained by the polymorphism of cytochromes P450 3A4 and 5 (responsible for biotransformation of Tac)[7] and the drug transporter ABCB1[8], circadian rhythms[9] and also drug-drug interactions[10]. In addition to inter-individual variability, the pharmacokinetics of Tac can vary within individual patients. The concept of intra-patient variability (IPV) refers to the fluctuations in Tac blood concentrations (and consequently episodes of over- and under-immunosuppression) that some patients experience over time[11].

Several non-modifiable and modifiable factors contribute to Tac IPV (*e.g.*, polymorphism in CYP3A genes, the circadian rhythm of Tac exposure, gastrointestinal events such as diarrhea, cholestasis, changes in protein levels, anemia, but also drug-drug interactions with macrolides or azole anti-fungal treatments, foods, or changes in formulation or generic substitution)[11], but non-adherence to Tac seems to be the main cause of IPV[12,13].It was previously suggested that higher degree of Tac IPV was associated with kidney-graft rejection and worse long-term outcomes after kidney transplantation[14,15]. Similar limited data have been reported after liver transplantation[16,17], mainly in pediatric cohorts. Moreover, little is known concerning the relationship between Tac variability and the occurrence of donor-specific antibodies (DSAs). Herein, we retrospectively assessed the variability of Tac in a cohort of liver-transplant recipients and analyzed its impact on the number of acute rejections, the occurrence of *de novo* DSAs, and patient- and graft-survival rates.

**MATERIALS AND METHODS**

***Patients***

Between February 2008 (*i.e.*, the date when the solid-phase Luminex assay was set up in our institution) and June 2015, a total of 298 adult patients received a liver transplant from deceased donors (DDLT) in our center. Patients excluded from the study were those that died within the first month posttransplantation (*n* = 34), those that needed a re-transplant during the first month (*n* = 2), and those that received a transplant with a preformed DSA (mean fluorescence intensity cut-off > 1000) directed against human leukocyte antigen (HLA) A, B, Cw, DR, DQ, or DP (*n* = 37). In order to avoid confounding factors associated with others immunosuppressive treatments, only patients that received and were maintained under Tac and mycophenolate mofetil (MMF) (with or without steroids) were included in this study (Figure 1). All patients but five received Tac given twice daily (Prograf**®**). The other five received Tac once daily (Advagraf**®**). We excluded patients that had Tac or MMF withdrawn. Moreover, to calculate intra-patient variability, at least three trough levels of Tac had to be available. Hence, 116 patients with a functioning liver allograft at 1 mo posttransplantation were included in this study after having given their informed consent and after we had obtained Toulouse University IRB approval.

The target concentration of Tac trough level was 7-10 ng/mL during the first 3 mo, and 5-10 ng/mL thereafter during the follow-up. Each participant was followed for 2 years or until re-transplantation (*n* = 3) or death (*n* = 6). The median follow-up was 24 mo (range: 6-24). All rejection episodes were biopsy proven. Biopsies were only performed for cause during the study period and were analyzed according to the Banff criteria[18-20]. Graft failure was defined as the need for re-transplantation or as death from liver failure.

Detection of cytomegalovirus was performed using real-time PCR, as previously described[21], at month 3, 6, 12, and 24, and at any other time if clinically indicated.

***Intra-patient variability***

Tac trough levels were routinely assessed using high-performance liquid chromatography-linked tandem mass spectrometry (HPLC-MS) at discharge, then monthly between months 1-6, and thereafter at months 9, 12, 15, 18, and 24. To calculate the IPV of Tac, at least three Tac trough levels from each patient had to be available. The median number of available Tac measurements was 10 (range: 4-12).

Tac IPV was estimated using the coefficient of variability (CV). The CV-IPV was calculated as follows: CV-IPV (%) = (standard deviation/ mean Tac trough-level concentration) × 100. Because all patients received the same drug dose between discharge and M24, the obtained levels were corrected for the corresponding daily dose of tacrolimus (CV C0/D-IPV). In addition, because some patients were converted from one formulation to another during the follow-up, we calculated CV and CV C0/D-IPV after excluding the Tac trough levels obtained during the adjustment dose period, *i.e.*, the month following a switch.

To compare IPV with the two formulations of Tac, the Tac twice-daily CV-IPV was calculated using Tac trough levels obtained from patients that had received Tac twice daily since transplantation until last follow-up and those obtained in patients switched for Tac once daily before the switch. The Tac once-daily CV-IPV was calculated using Tac trough levels from patients that received Tac once daily since transplantation until the last follow-up, and those obtained from patients that were later switched from twice- to a once-daily formulation after the switch (this excluded Tac trough levels obtained in the month following the switch).

***Immunological analyses***

All patients were screened for anti-HLA DSAs at transplantation, and at month 3 and 12, and annually thereafter. Additional screening was performed in case of graft dysfunction. Luminex® assays were used to determine the specificity of class I HLAs in A/B/Cw and class II in DR/DQ/DP IgG antibodies in the recipients' sera (centrifuged at 10,000*g* for 10 min) using Labscreen single Ag HLA class-I and class-II detection tests (One Lambda, Canoga Park, CA, United States), according to the manufacturer’s instructions. The presence and specificity of antibodies were then detected using a Labscan 100®, and the mean fluorescence (baseline) value for each sample in each bead was evaluated. The baseline value was calculated as follows: [raw sample mean fluorescence intensity (MFI)-raw negative serum control MFI-negative-bead raw MFI sample-negative-bead raw MFI negative serum control]. A baseline value of > 1000 was considered positive.

***Statistical analyses***

Categorical variables are expressed as percentages and comparisons between groups were made using the chi-squared test or, if appropriate, Fisher’s exact test. Continuous variables were expressed as medians and ranges, and compared using the Mann–Whitney test. Logistic regression analysis was used to determine the predictors for acute-rejection episodes and the occurrence of *de novo* anti-HLA DSAs. Variables with a *P* < 0.1 in the univariate analyses were included in the stepwise multivariable analyses. *P* < 0.05 was considered statistically significant.

**RESULTS**

The patients' characteristics at transplantation are presented in Table 1. All liver transplantations performed in this study were performed from DDLT. The mean DDLT age was 51 ± 17 years. To note, one DDLT was < 18 years, and 4 DDLT were > 80 years.

***Tacrolimus levels and variability***

During the follow-up, 44 (38%) patients were switched from Tac immediate-release given twice a day (Prograf®), to Tac once a day to improve quality of life. The switch was performed at a mean of 15 (range: 1-18) mo posttransplantation.

Mean tacrolimus trough level was 8 ± 3 ng/mL during the follow-up (Table 1). The mean dose of Tac was 6.8, 6.7, 6.4, 5.9, 5.4, 5.1, 4.8, and 4.6 mg/d, respectively, at discharge and at months 1, 3, 6, 9, 12, 18, and 24. Forty-five (38.8%) patients presented with a Tac trough level of < 5 ng/mL at least once during the follow-up. The overall mean Tac CV- IPV was 32 ± 12% [median CV-IPV 30.5% (7.6-80.6)]. Tac CV-IPV distribution is presented in Figure 2. The 1th, 2th, 3th, and 4th quartiles were, respectively, 25%, 30.5%, 36.5%, and 80.6%. The mean Tac CV-IPV was 30% ± 11% in patients given Tac once daily and was 32% ± 12% in patients that received Tac twice daily (*P* = 0.10). The mean Tac CV- IPV in the five patients that had received Tac once-daily since transplantation was 30% ± 7%. In the 44 patients that were converted from Tac twice-daily to once daily, the mean values of Tac CV-IPV were 32.3% ± 12% and 30% ± 12% before and after the switch, respectively (*P* = 0.21).

Overall mean CV C0/d- IPV was 73% ± 43%. It was 69% ± 29% with Tac twice-daily compared to 79% ± 50% for Tac given once daily (*P* = 0.9).

***Incidence of acute rejection and de novo donor-specific antibodies***

During the follow-up, 22 patients (19%) presented with at least one episode of acute rejection. The time between transplantation and a diagnosis of acute rejection (*i.e.*, the date of the biopsy) was 3.5 mo (range: 0.5-12). Fourteen patients (12%) experienced a T-cell steroid-sensitive acute rejection, and six patients (5%) presented with a T-cell steroid-resistant acute rejection, which was treated with polyclonal antibodies. One patient presented with an acute antibody-mediated rejection at 4 mo posttransplantation. The Tac CV-IPV in this patient was high: CV-IPV of 63.2% and CV C0/d- IPV = 68.2%. The risk factors for acute rejection after liver transplantation are presented in Table 2. The predictive factors for a biopsy-proven acute rejection were a Tac trough level of < 5 ng/mL [OR = 3.68; 95%CI (1.30–10.41), *P* = 0.014], the Tac CV-IPV (coded as a continuous variable) [OR = 1.1; 95%CI (1.01-1.11), *P* = 0.008], a CV-IPV of > 35% [OR = 3.07; 95%CI (1.14–8.24), *P* = 0.03], and a CV-IPV of > 40% [OR = 4.16; 95%CI (1.38-12.50), *P* = 0.01]. Twenty-one of the 22 patients that presented with an acute-rejection episode were receiving Tac twice daily when the rejection was diagnosed.

Thirteen patients (11.2%) presented with at least one *de novo* DSA during the posttransplantation follow-up (nine anti-HLA class II, three anti-HLA class I, one anti-HLA class I and II). Only one of these patients developed an antibody-mediated rejection. The median time between transplantation and detection of a *de novo* DSA was 3.5 mo (range: 1-12). The risk factors for a *de novo* DSA are presented in Table 3. The Tac CV-IPV [coded as a continuous variable: OR = 1.1, 95%CI (1.0-1.12), *P* = 0.006), and a CV-IPV of > 35% [OR = 4.83, 95%CI (1.39-16.72), *P* = 0.01] or of > 40% [OR = 9.73, 95%CI (2.65-35.76), *P* = 0.001] were identified as predictors for the occurrence of *de novo* DSAs detection.

***Survival of patients***

During the follow-up, six patients died [at a mean of 13 mo (range: 6-23) posttransplantation]. The causes of death were infections (*n* = 3), cardiovascular (*n* = 2), and neoplastic (*n* = 1) complications. No difference in Tac CV- IPV was observed between patients that died during the follow-up (CV-IPV 33% ± 6%) and those that did not (CV-IPV 32 ± 12%; *P =* 0.70). Three patients required re-transplantation at month 5, 10, and 14, respectively, for ischemic cholangitis that occurred posttransplantation. During the follow-up, 24 patients presented with posttransplant replication of cytomegalovirus. No difference in Tac CV-IPV was observed between patients with replication of cytomegalovirus (CV-IPV 32% ± 9 %) and those without replication (32 ± 12%, *P* = 0.90).

**DISCUSSION**

High IPV has been previously associated with a greater risk of graft rejection, an accelerated progression of chronic histological lesions, and worse long-term survival after kidney transplantation[11,14,22,23]. In pediatric liver-transplants, Tac variability was associated with late acute rejection[16]. In the present study, we investigated the impact of Tac variability in 116 adult liver-transplant recipients. In order to avoid confounding factors, we focused on patients that received a graft without preformed DSAs and that had received Tac associated with MMF. Although the mean Tac trough level was 8 ± 3 ng/mL during the study period, nearly 40% of patients had a Tac trough level of < 5 ng/mL at least once during the follow-up. Tac CV-IPV varied from 7.6%-80.6% (median 30.5%), and median Tac CV C0/d-IPV was 62% (18-147). Almost one-third of patients presented with a Tac CV-IPV of > 35%. This high value is similar to those reported in previous studies, mainly after kidney transplantation[24,25]. In kidney-transplant[13,25] and pediatric liver-transplant patients[16], high CV-IPV was associated with an increased risk of acute rejection. In the present study, we found that a Tac trough level of < 5 ng/mL, the Tac CV-IPV (coded as a continuous variable), a CV-IPV of > 35%, and a CV-IPV > 40% were independent predictive factors for a biopsy-proven graft rejection.

Posttransplant positive DSAs were associated with decreased graft survival and increased acute or chronic graft rejections[2,3,26]. It has been previously suggested that iterative transplantation, low levels of calcineurin inhibitors, the use of cyclosporine (compared to Tac), and non-adherence can promote the development of a *de novo* DSA after liver transplantation[2]. Herein, we found that the Tac CV-IPV (coded as a continuous variable), a CV-IPV of > 35%, and CV-IPV > 40% were independent predictive factors for the occurrence of a *de novo* DSA. Similar data, reported after kidney transplantation[24], from a cohort of 310 adult kidney-transplant patients given Tac twice-daily during the first year posttransplant, showed that a history of acute rejection, re-transplantation and a Tac CV greater than 30% were associated with the occurrence of a *de novo* DSA. In our study, one patient presented with an acute antibody-mediated rejection associated with an anti-class II *de novo* DSA at 3 mo after liver transplantation. Interestingly, this patient had high tacrolimus variability (CV-IPV 63.2%, CV C0/d-IPV 68.2%). None of the other 12 patients that developed a DSA experienced an acute antibody-mediated rejection. However, it was suggested that patients with positive DSAs would present lower graft survival, consecutive to chronic antibody mediated rejection[27] rather than to acute antibody-mediated rejection episodes.

In several studies, but not all, the use of once-daily tacrolimus compared to a twice daily formulation has been found to improve adherence and to reduce IPV[11,28-31]. In the present study, no difference between Tac formulations was observed.

This study has several limitations. Because of its retrospective design, we could not evaluate the cause of Tac variability. It has been suggested previously that non-adherence is the main cause of Tac variability[11]. However, in our study, adherence was not evaluated using objective methods, such as those previously reported using electronic devices[28]. Moreover, we did not evaluate MMF variability in our study because we do not perform this analysis routinely in our center. Of note, conflicting results have been reported concerning the use of MMF variability after solid-organ transplantation[14,25]. It was also previously suggested that pre-transplant determination of CYP3A5 and MDR1 polymorphisms[32] allows more rapid achievement of therapeutic Tac trough level. However, no association between the pharmacogenomics parameters and Tac intra-patient variability is expected and was reported.

In conclusion, we found that the CV-IPV of Tac was a predictive factor for acute rejection and the occurrence of a *de novo* DSA after liver transplantation. This could be a useful tool to identify patients with a greater risk of graft rejection and of developing a *de novo* DSA after liver transplantation. Future studies should investigate the role of Tac IPV on long-term outcomes, on chronic graft rejection, and over-immunosuppression-related diseases (cancer, and related immunocompromised infections).

**ARTICLE HIGHLIGHTS**

***Research background***

Tacrolimus (Tac) is considered a cornerstone within immunosuppression protocols to prevent T-cell and antibody-mediated rejection after liver transplantation. However, this treatment presents a narrow therapeutic index: overexposure can lead to clinically serious events, thus necessitating regular therapeutic drug monitoring, whereas underexposure can lead to acute or chronic graft rejection. The concept of intra-patient variability (IPV) refers to the fluctuations in Tac blood concentrations (and consequently episodes of over- and under-immunosuppression) that some patients experience over time.

***Research motivation***

Tac-IPV is an inexpensive assay to explore fluctuations in Tac blood concentrations. We investigated the potential usefulness of Tac-IPV to predict the incidence of donor specific antibodies and graft rejection episodes.

***Research objectives***

Our aim was to investigate the role of tacrolimus IPV in adult liver-transplant recipients.

***Research methods***

We retrospectively assessed tacrolimus variability and analyzed its effect on the occurrence of graft rejection and *de novo* donor-specific antibodies.

***Research results***

Twenty-two patients experienced at least one acute-rejection episode (BPAR). Predictive factors for a BPAR were a tacrolimus IPV of > 35% or > 40%, and a tacrolimus trough level of < 5 ng/mL. Thirteen patients developed at least one *dn*DSA during the follow-up. Tacrolimus IPV and tacrolimus IPV of > 35%, and > 40% were identified as predictors to detect *dn*DSAs. IPV did not impact on patient- or graft-survival rates during the follow-up.

***Research conclusions***

In our study higher Tac-IPV was associated with graft rejection and occurrence of DSAs.

***Research perspective***

Tacrolimus-IPV could be a useful tool to identify patients with a greater risk of graft rejection and of developing a *de novo* DSA after liver transplantation.

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**Table 1 Characteristics of the liver-transplant recipients**

|  |  |
| --- | --- |
| **Variable** | ***n* = 116** |
| Donors’ age at transplantation, yr (range) | 53 (9-85) |
| Recipients’ age at transplantation, yr (range) | 57 (18-72) |
| Recipients’ gender: male, *n* (%) | 96 (83) |
| Initial liver disease, *n* (%)AlcoholViral (HCV, HBV)Autoimmune disease (AIH, PSC, PBC)Other1 | 49 (43)36 (31)13 (11)18 (17) |
| Median MELD score at transplantation (range) (%) | 22 (6-40) |
| Positive HCV RNA at transplantation, *n* (%) | 21 (18) |
| Re-transplantation, yes (%) | 3 (3) |
| Induction therapy, yes: *n* (%)Polyclonal antibodies, *n* (%)Interleukin-2 receptor blocker, *n* (%) | 87 (75)9 (8)78 (67) |
| Conversion during the follow-up from twice-daily to once daily tacrolimus, *n* (%)Number of patients receiving tacrolimus once daily, *n* (%)At dischargeMonth 1Month 3Month 6Month 9Month 12Month 18Month 24 | 42 (36)5 (4)8 (7)9 (8)12 (10)18 (16)26 (31)39 (34)47 (41) |
| Tacrolimus trough level (ng/mL)At dischargeMonth 1Month 3Month 6Month 9Month 12Month 18Month 24 | 7.6 ± 38 ± 38.4 ± 38.4 ± 37.4 ± 37.8 ± 37.5 ± 26.9 ± 3 |
| Mycophenolate mofetil dose (mg/d)At dischargeMonth 3Month 6Month 12Month 24 | 1700 ± 6001250 ± 5501100 ± 4501000 ± 3001000 ± 300 |
| Steroids (mg/d)At discharge: Yes (%)Dose (mg/d)Month 3: Yes (%)Dose (mg/d)Month 6: Yes (%)Dose (mg/d)Month 12: Yes (%)Dose (mg/d)Month 24: Yes (%)Dose (mg/d) | 116 (100)20 ± 12114 (98)8 ± 4110 (95)7 ± 5104 (90)6 ± 697 (84)5 ± 2 |

1Polycystic disease (*n*=7), NASH syndrome (*n*=4), Wilson disease (*n*=2), bile duct atrophia (*n*=1), drug intoxication (*n*=2), and cryptogenic cirrhosis (*n*=1). HBV: Hepatitis B virus; HCV: Hepatitis C virus; AIH: Auto-immune hepatitis; PSC: Primary sclerosing cholangitis; PBC: Primary biliary cirrhosis.

**Table 2 Risk factors for a graft-rejection episode**

|  |  |  |
| --- | --- | --- |
| Variable | **Univariate analyses** | **Multivariate analyses** |
| **OR** | **95%CI** | ***P* Value** | **OR** | **95%CI** | ***P* Value** |
| MELD score > 30 (*n* = 31) | 0.55 | 0.12 - 1.90 | 0.42 | - |
| Initial liver disease(1) Alcohol cirrhosis (*n* = 49) *vs* (2, 3, 4)(2) Viral disease (*n* = 36) *vs* (1, 3, 4)(3) Auto-immune ILD (*n* = 13) *vs* (1, 2,4)(4) Other (*n* = 18) *vs* (1, 2, 3) | 0.581.343.120.49 | 0.18-1.680.44-3.900.71-12.470.05-2.37 | 0.340.610.070.52 |  |
| - |
| - |
| 1.00 | 0.51-1.15 | 0.21 |
| - |
| Induction therapy, yes (*n* = 87)Polyclonal antibodies (*vs* other)IL2R blockers (*vs* other) | 0.663.890.4 | 0.22-2.150.70-20.130.14-1.70 | 0.420.060.08 | - |
| 2.87 | 0.61-13.47 | 0.18 |
| 0.52 | 0.185-1.50 | 0.23 |
| Donors’ age > 50 yr (*n* = 69)Recipients’ age > 50 yr (*n* = 92) | 0.980.61 | 0.35-2.880.20-2.01 | 10.41 | -- |
| HCV-RNA + At transplantation (*n* = 21) | 1.96 | 0.54-6.45 | 0.22 | - |
| Steroid withdrawal during the FU (*n* = 19) | 2.3 | 0.63-7.82 | 0.20 | - |
| *De novo* DSAs during the FU (*n* = 13) | 2.8 | 0.64-11.19 | 0.13 | - |
| Tacrolimus trough level < 5 ng/mL (*n* = 34) | 3.0 | 1.05-8.96 | 0.02 | 3.68 | 1.30-10.41 | 0.014 |
| CV-IPV tacrolimus (continuous variable)CV-IPV > 35%CV-IPV > 0%CV-C0/d-IPV | 2.73.052.971.89 | 1.88-13.451.05-8.960.91-9.300.67-5.74 | 0.010.030.040.24 | 1.1 | 1.01-1.11 | 0.008 |
| 3.07 | 1.14-8.24 | 0.03 |
| 4.16 | 1.38-12.50 | 0.01 |
| - |

FU: Follow-up; ILD: Initial liver disease; HCV: Hepatitis C virus; CV-IPV: Coefficient of variability-intra-patient variability; CV-C0/d-IPV: Coefficient of variability corrected for the corresponding daily dose-intra-patient variability.

**Table 3 Risk factors for developing *de novo* donor-specific antibodies after liver transplantation.**

|  |  |  |
| --- | --- | --- |
| Variable | **Univariate analyses** | **Multivariate analyses** |
| **OR** | **95%CI** | ***P* value** | **OR** | **95%CI** | ***P* value** |
| MELD score > 30 (*n* = 31) | 1.84 | 0.43 – 7.10 | 0.33 | - |
| Initial liver disease(1) Alcohol cirrhosis (*n* = 49) *vs* (2, 3, 4)(2) Viral disease (*n* = 36) *vs* (1, 3, 4)(3) Autoimmune ILD (*n* = 13) *vs* (1, 2, 4)(4) Other (*n* = 18) *vs* (1, 2, 3) | 0.580.981.512.79 | 0.12-2.220.21-3.860.14-8.460.55-11.83 | 0.551.00.640.64 | ---- |
| Induction therapy, yes (*n* = 87)Polyclonal antibodies (*vs* other)IL2R blockers (*vs* other) | 1.610.591.1 | 0.41-7.610.70-18.000.28-5.28 | 0.550.601.0 | --- |
| Donors’ age > 50 yr (*n* = 69) | 0.78 | 0.20-3.00 | 0.77 | - |
| Recipients’ age > 50 yr (*n* = 92) | 0.36 | 0.09-1.58 | 0.10 | 0.2 | 0.07-0.85 | 0.3 |
| HCV RNA + at transplantation (*n* = 21) | 1.41 | 0.23-6.23 | 0.70 | - |
| Steroid withdrawal during the FU (*n* = 19) | 0.39 | 0.01-3.01 | 0.69 | - |
| Tacrolimus trough level < 5 ng/mL (*n* = 34) | 1.59 | 0.38-6.05 | 0.52 | - |
| CV-IPV tacrolimus (continuous variable) | 1.92 | -1.28-21.39 | 0.08 | 1.1 | 1.0-1.12 | 0.006 |
| CV-IPV > 35% | 4.66 | 1.22-19.82 | 0.02 | 4.83 | 1.39-16.72 | 0.01 |
| CV-IPV > 40% | 9.10 | 2.28-40.63 | < 0.001 | 9.73 | 2.65-35.76 | 0.001 |
| CV-C0/d-IPV | 3.15 | 5.47-27.31 | 0.005 | 1.0 | 0.97-1.02 | 0.09 |

FU: Follow-up; ILD: Initial liver disease; HCV: Hepatitis C virus; CV-IPV: Coefficient of variability-intra-patient variability; CV-C0/d-IPV: Coefficient of variability corrected for the corresponding daily dose-intra-patient variability.



**Figure 1 Flow chart.**

**Figure 2 Distribution of tacrolimus according to intra-patient variability.**