Identification of Factors Affecting Tacrolimus Trough Levels in Latin American Pediatric Liver Transplant Patients

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Tacrolimus is the cornerstone in pediatric liver transplant immunosuppression. Despite close monitoring, fluctuations in tacrolimus blood levels affect safety and efficacy of immunosuppressive treatments. Identifying the factors related to the variability in tacrolimus exposure may be helpful in tailoring the dose. The aim of the present study was to characterize the clinical, pharmacological, and genetic variables associated with systemic tacrolimus exposure in pediatric liver transplant patients. De novo transplant patients with a survival of more than 1 month were considered for inclusion and were genotyped for cytochrome P450 3A5 (CYP3A5). Peritransplant clinical factors and laboratory covariates were recorded retrospectively between 1 month and 2 years after transplant, including alanine aminotransferase (ALT), aspartate aminotransferase, hematoctrit, and tacrolimus predose steady-state blood concentrations collected 12 hours after tacrolimus dosing. A linear mixed effect (LME) model was used to assess the association of these factors and the log-transformed tacrolimus dose-normalized trough concentration (logC0/D) levels. Bootstrapping was used to internally validate the final model. External validation was performed in an independent group of patients who matched the original population. The developed LME model described that logC0/D increases with increases in time after transplant (β = 0.019, 95% confidence interval [CI], 0.010–0.028) and ALT values (β = 0.00030, 95% CI, 0.00002–0.00056), whereas logC0/D is significantly lower in graft CYP3A5 expressers compared with nonexpressers (β = -0.349, 95% CI, -0.631 to -0.062). In conclusion, donor CYP3A5 genotype, time after transplant, and ALT values are associated with tacrolimus disposition between 1 month and 2 years after transplant. A better understanding of tacrolimus exposure is essential to minimize the occurrence of an out-of-range therapeutic window that may lead to adverse drug reactions or acute rejection.

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Tacrolimus has become the cornerstone in immunosuppression for preventing allograft rejection in pediatric and adult liver transplant recipients. This calcineurin inhibitor has a narrow therapeutic index and presents large interindividuum and intr.individuum pharmacokinetic variability. In order to optimize its efficacy and minimize the occurrence of adverse events, therapeutic drug monitoring (TDM) is regularly performed in clinical practice on the basis of trough concentration (C0) levels that are determined before the next dose of tacrolimus. C0 has been selected as a measure of systemic exposure that correlates with clinical outcome (graft rejection and tacrolimus toxicity). However, C0-based therapeutic ranges in children are defined

Abbreviations: AIC, Akaike information criterion; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; bid, twice daily; C0, trough concentration; CI, confidence interval; CMV, cytomegalovirus; CYP3A5, cytochrome P450 3A5; EBV, Epstein-Barr virus; GGT, gamma-glutamyl transpeptidase; LME, linear mixed effect;
based on empirical adaptation of the doses according to the C0 from adult clinical data.\(^{(2)}\)

Although the optimization of immunosuppressive therapies and the improvements in surgical procedures have contributed to longer overall survival and graft survival rates in pediatric liver transplantation patients, clinical issues, such as acute rejection and adverse drug reactions to tacrolimus, confer morbidity and mortality.\(^{(1,4)}\) Previous reports have described a significant association between variability in tacrolimus C0 and the development of acute rejection and adverse drug reactions.\(^{(1,5-7)}\) Furthermore, other factors, including time after transplant, body weight, hematocrit, age, liver function parameters, and type of graft, contribute to the pharmacokinetic variability in pediatric liver transplant patients.\(^{(1,8-16)}\)

Different polymorphisms of cytochrome P450 enzymes, especially for cytochrome P450 3A5 (CYP3A5), affect tacrolimus clearance. This enzyme plays an important role in the metabolism of tacrolimus and is mainly expressed in the liver and intestines. A polymorphism in intron 3 of CYP3A5 (CYP3A5*3 allele) produces an abnormally spliced messenger RNA with a premature stop codon resulting in the absence of the CYP3A5 enzyme.\(^{(17)}\) Several studies have described a higher tacrolimus C0 in adult patients carrying the CYP3A5*3 allele (nonexpressers) compared with the expressers (CYP3A5*1 carriers).\(^{(18-20)}\) In addition, some studies have reported this association in Asian and European pediatric patients.\(^{(9-12,21-23)}\) However, the behavior of tacrolimus variability in Latin American pediatric liver transplant recipients is unknown, and reports regarding safety and efficacy of immunosuppressive regimens are scarce.\(^{(24)}\)

For all of the studies mentioned, we aimed to evaluate the impact of donor and recipient genetic polymorphisms in the CYP3A5 enzyme on tacrolimus C0 and to identify and characterize different clinical and biochemical variables associated with tacrolimus exposure after oral administration in pediatric liver transplant patients between 1 month and 2 years after liver transplantation.

**Patients and Methods**

This study is a retrospective single-center cohort study conducted in accordance with the Helsinki Declaration at Hospital de Pediatría Juan P. Garrahan (Buenos Aires, Argentina) after approval by the institutional review committee (protocol number 740). Written informed consent was obtained from parents or guardians.

**STUDY POPULATION**

This study is part of a previous one that aimed to identify peritransplant predictors of acute rejection and factors related to the risk of tacrolimus-based adverse drug reactions in pediatric liver transplant patients\(^{(6)}\) in the context of the implementation of a new immunosuppressive protocol. Pediatric de novo liver allograft recipients <18 years old at the time of transplantation were included during the period in which the CYP3A5 genotyping technique was available at the Hospital de Pediatría Juan P. Garrahan. Patients included in the present analysis had at least 4 tacrolimus C0 levels during the study period. Exclusion criteria included the following: <1 month of posttransplant survival, retransplantation, combined or multivisceral transplants, an interval of administration of tacrolimus other than every 12 hours, and inappropriate follow-up or noncompliance, as previously defined.\(^{(24)}\) In addition, tacrolimus C0 levels obtained at times when the patient was receiving simultaneous administration of azoles, macrolides, antiepileptic drugs, and/or calcium channel blockers were excluded from the analysis. Follow-up data were collected between 1 month after transplant and 2 years. All data were collected from the medical and nursing records, and a centralized database with restricted access was generated.

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**Table legend**

- **logC0/D**: log-transformed tacrolimus dose-normalized trough concentration; **ME**: mean error; **MRE**: mean relative error; **RMSE**: root mean squared error; **SD**: standard deviation; **SE**: standard error; **TDM**: therapeutic drug monitoring.

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**IMMUNOSUPPRESSIVE THERAPY**

Tacrolimus (0.1 mg/kg/day) was initiated after reperfusion and kidney function normalization, administered in monotherapy with anti-CD25 induction (basiliximab) on days 0 and 4, or in combination with corticosteroids and/or mycophenolate mofetil according to kidney and liver function,\(^{(25)}\) as depicted in Table 1. Concomitant drugs were sulfamethoxazole-trimethoprim, magnesium supplements, omeprazole (in all patients), acyclovir, and additional antibiotics, if needed.

**TACROLIMUS MONITORING**

For the analysis, we used retrospective routine TDM (whole-blood 12-hour tacrolimus C0). Patients were given oral tacrolimus (Prograf, Astellas Laboratory, Killorglin, Ireland) twice daily (bid). Data (tacrolimus doses, C0 levels, and body weights) were recorded after 30 days after transplantation and every day during hospitalization and/or on outpatient visits for 2 years. At all times that a blood sample was obtained for assessment of tacrolimus C0 levels, a complete blood sample test was performed, including liver and renal function tests, hematocrit, and hemoglobin levels. Characteristics of the patients enrolled in the study are presented in Table 1.

Tacrolimus C0 levels were quantified using the chemiluminescent microparticle immunoassay (Architect; Abbott, Chicago, IL). Whole blood quality controls (Lyphochek Whole Blood Immunosuppressant; Bio-Rad, Irvine, CA) were assessed daily for assay acceptance. In addition, specimens were routinely assessed as part of an international proficiency testing program for the external quality control of tacrolimus.\(^{(26)}\) Total imprecision was <8%, and quality control values were in the range of ±2 standard deviation (SD). Tacrolimus C0 target levels, which were defined based on adult clinical data,\(^{(2)}\) were 7.0–8.0 ng/mL in the first 6 months, 5.0–7.0 ng/mL during the next 6 months, and 5.0 ng/mL after the first year after transplant.\(^{(27,28)}\)

**BIOCHEMICAL, CLINICAL, AND GENETIC FACTORS**

Peritransplant and posttransplant variables were studied including the following:

1. Demographic features: age, weight at transplant, sex, and primary diagnosis.

2. Biochemical values: hematocrit, albumin, serum creatinine, uremia, total bilirubin, liver function markers (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], and gamma-glutamyl transpeptidase [GGT] activity).

3. Transplant features: type of graft (partial graft from a living or deceased donor versus a whole graft from a deceased donor), type of donor (deceased versus living donor), and days after transplant.

4. Clinical status: Epstein-Barr virus (EBV) and cytomegalovirus (CMV) infections.

5. Genotyping: CYP3A5*3 polymorphism in donors and recipients.

CYP3A5 genotyping procedure was previously described.\(^{(6)}\) In addition, we registered concomitant immunosuppressive agents, such as steroids (at least 30 consecutive days), azathioprine, mycophenolate mofetil, and sirolimus.

**RELATIONSHIP BETWEEN TACROLIMUS C0 AND PREDICTOR PARAMETERS**

A linear mixed effect (LME) model was used to investigate the influence of the CYP3A5 genotype, pharmacological factors, and clinical and laboratory parameters on log-transformed tacrolimus dose-normalized trough concentration (logC0/D) levels.

**MODEL DEVELOPMENT**

The total data set was randomly split into model-building and validation data sets. The model was initiated with the development of the base model in the model-building data set to select the best structure for random effects. Different structural models were tested (random intercept or slope with and without intermodel correlation), and the best model was selected based on the Akaike information criterion (AIC). Both continuous (time after transplant and hematocrit, AST, ALT, ALP, and GGT activity) and categorical variables (administration of steroids and/or mycophenolate sodium or mofetil, EBV infection, CMV infection, type of donor, type of graft, and CYP3A5*3 polymorphism in donors and recipients) were considered in the analysis.

Covariates associated with a \(P\) value < 0.05 in the univariate analysis were considered clinically relevant.
## TABLE 1. Demographics and Relevant Medical History

| Characteristics/Parameters                               | Model-Building Data Set (n = 40) | Validation Data Set (n = 13) |
|----------------------------------------------------------|----------------------------------|------------------------------|
| Age, years*                                              | 2.2 (0.5-17.6)                   | 3.7 (0.8-12.2)               |
| Sex, n                                                   |                                  |                              |
| Female                                                   | 24                               | 10                           |
| Male                                                     | 16                               | 3                            |
| Weight, kg*                                              | 16.3 (6.0-75.0)                  | 19 (6.8-74)                  |
| Type of donor, n                                          |                                  |                              |
| Deceased                                                 | 31                               | 10                           |
| Living                                                   | 9                                | 3                            |
| Follow-up time, months*                                  | 18.6 (1.3-25.9)                  | 18.6 (1.4-30.9)              |
| Graft type, n                                            |                                  |                              |
| Complete                                                 | 17                               | 2                            |
| Technical variant                                        | 23                               | 11                           |
| Primary diagnosis                                        |                                  |                              |
| Biliary atresia                                          | 16 (40)                          | 6 (46)                       |
| Acute liver failure                                      | 9 (23)                           | 2 (15)                       |
| Cholestatic cirrhosis†                                    | 4 (10)                           | 3 (23)                       |
| Hepatic cirrhosis: autoimmune and cryptogenic            | 6 (15)                           | 1 (8)                        |
| Malignancies†                                            | 3 (7)                            | 1 (8)                        |
| Metabolic disease: metabolic liver failure               | 2 (5)                            | 0 (0)                        |
| Immunosuppressive therapy                                |                                  |                              |
| Basiliximab (10-20 mg/dose at days 0 and 4 after transplantation) | 28 (70)                      | 10 (77)                      |
| Tacrolimus (0.1 mg/kg/day)                              | 40 (100)                         | 13 (100)                     |
| Prednisone (1.25-3.75 mg/kg/day)                         | 35 (88)                          | 11 (86)                      |
| Mycophenolate mofetil (20-40 mg/kg/day)                  | 20 (50)                          | 7 (54)                       |
| Azathioprine (1-2 mg/kg/day)                             | 3 (8)                            | 2 (15)                       |
| Sirolimus (0.1 mg/kg/day)                                | 4 (10)                           | 1 (8)                        |
| Liver function and blood parameters*                     |                                  |                              |
| AST, IU/L                                                | 88.8 ± 120.5                     | 82.3 ± 100.9                 |
| ALT, IU/L                                                | 135.1 ± 153.3                    | 120.6 ± 139.2                 |
| GGT, IU/L                                                | 234.0 ± 305.4                    | 295.4 ± 346.4                 |
| Total bilirubin, mg/dL                                   | 2.3 ± 1.8                        | 2.3 ± 4.5                    |
| Direct bilirubin, mg/dL                                  | 0.9 ± 2.2                        | 2.3 ± 4.8                    |
| Albumin, g/dL                                            | 3.6 ± 0.6                        | 3.4 ± 0.6                    |
| Hematocrit, %                                            | 32.7 ± 4.6                       | 32.8 ± 5.1                    |
| Serum creatinine, mg/dL                                  | 0.4 ± 0.2                        | 0.5 ± 0.2                    |
| Pharmacokinetic data*                                    |                                  |                              |
| Total number of tacrolimus samples                       | 824                              | 352                          |
| Number of samples per patient                            | 16 (4-71)                        | 29 (4-48)                    |
| Tacrolimus blood concentrations, ng/mL                   | 6.3 ± 2.6                        | 6.8 ± 2.7                    |
| Tacrolimus daily dose, mg                                | 2.59 ± 2.11                      | 2.89 ± 2.31                  |
| Tacrolimus daily dose normalized, mg/kg                  | 0.15 ± 0.10                      | 0.14 ± 0.10                  |
| Dose-normalized tacrolimus C0, ([ng/mL]/(mg/kg))         | 114.39 ± 96.99                   | 149.27 ± 122.75              |
| Log-transformed dose-normalized tacrolimus C0, [Ln ([ng/mL]/(mg/kg))] | 4.50 ± 0.68                     | 4.70 ± 0.80                  |

**NOTE:** Data are expressed as median (range), mean ± SD, and n (%) unless otherwise noted.

*Continuous demographic data and clinical laboratory data recorded during the complete study period did not significantly differ between the model-building and validation data sets (Mann-Whitney U test, *P* > 0.05).

†Including Alagille syndrome, congenital hepatic fibrosis, and sclerosing cholangitis.

‡Including hepatoblastoma and hepatocellular carcinoma.
and biologically plausible and were, therefore, included in the multivariate intermediate model. The final model was selected using a backward stepwise process based on the AIC. All statistical analyses and graphs were performed with RStudio, version 0.99.486 (R Foundation for Statistical Computing, Vienna, Austria), and nlme. Finally, all assumptions were checked in the final model, including linearity, absence of collinearity, homoscedasticity, normality of residuals, absence of influential data points, and independence.

**MODEL EVALUATION AND EXTERNAL VALIDATION**

Once the final model was defined, a bootstrap was used to evaluate the stability and accuracy and to calculate the 95% confidence intervals (CIs) of parameter estimates. The median values of the bootstrap parameters were compared with the values of the final model.

The performance of the model was visually assessed by comparing plots of the predicted concentration and the observed concentration to assess for bias (a systematic upward or downward deviation from the line of unity in these plots) and imprecision (a high degree of scatter of data points around the line of unity).

External validation was performed in the validation data set that matched the data used for model development. The predictive performance of the model was assessed numerically through calculation of the mean error (ME), the mean relative error (MRE), and the relative root mean squared error (RMSE), as previously reported.

**Results**

Overall, 89 patients were considered for inclusion based on the implementation of a new immunosuppressive protocol in 2010 and according to the availability of data as detailed below. Patients were excluded because of a survival shorter than 1 month (n = 5), unavailable medical records (n = 4), retransplantation during the first month after surgery (n = 2), absence of pharmacokinetic and clinical data (n = 5), absence of genotyping data from donors and/or recipients due to limited amount of DNA or no availability of formalin-fixed paraffin-embedded liver tissue (n = 14), and nonadherence, as previously defined (n = 6). Therefore, 53 patients were finally included in the analysis. Demographics, laboratory parameters, and clinical characteristics of the patients included in the model-building (n = 40) and validation (n = 13) of the data set are shown in Table 1.

The CYP3A5 polymorphism distribution in both donors and recipients included in this study is reported in Supporting Table 1. The genotype frequencies of the CYP3A5 polymorphism did not deviate from the Hardy-Weinberg equilibrium (P > 0.5). According to the report of the Clinical Pharmacogenetics Implementation Consortium, the estimated allele frequency of CYP3A5*1 and *3 in our population was similar to that reported for the Latin American cohort of patients analyzed in the mentioned guideline. Specifically, our population showed an allele frequency of 25.4% and 74.6% for the CYP3A5*1 and *3 allele, respectively.

A base model was built using 824 tacrolimus C0 levels obtained from the patients included in the model-building group. Random effects were included in the intercept for interindividual variability and the slope for the effect of time after transplant with a correlation between them.

Univariate analysis showed a significant linear association between logC0/D and ALT values, time after transplant, total bilirubin values, donor CYP3A5 polymorphism, and EBV infection status (P < 0.05). All covariates significantly related to logC0/D are listed in Table 2. The positive associations between logC0/D and time after transplant and ALT are shown in Fig. 1A,B, respectively. The figures show an increase in logC0/D with time after transplant or with liver dysfunction assessed by ALT. As depicted in Fig. 1C, logC0/D was lower in patients with a CYP3A5-expresser graft compared with nonexpressers (P < 0.05). In more detail, Fig. 1D shows the bivariate model of logC0/D according to time after transplant and donor CYP3A5 genotype. Patients with CYP3A5 nonexpresser grafts presented significantly higher logC0/D compared with CYP3A5 expressers between 1 month and 2 years after transplantation. After backward elimination, the best multivariate model describing tacrolimus exposure retained the following covariates that independently correlated with logC0/D: time after transplantation, ALT values, and donor CYP3A5 expression. Table 3 summarizes the final model estimates. Visual inspection of residual plots did not reveal any obvious deviations.
TABLE 2. Univariate LME Models for the LogC0/D in Pediatric Liver Transplant Patients

| Variables                                      | Estimate (β) | SE  | P Value |
|------------------------------------------------|--------------|-----|---------|
| Laboratory parameters                          |              |     |         |
| ALT, IU/L                                      | 0.0005       | 0.001| <0.001 |
| Hematocrit, %                                  | -0.0005      | 0.0043| 0.90   |
| Total bilirubin, mg/dL                         | 0.032        | 0.012| 0.01   |
| Immunosuppressive therapies                    |              |     |         |
| Coadministration of steroids and/or mycophenolate mofetil/sodium, yes versus no | 0.115 | 0.043 | 0.01 |
| Clinical parameters                            |              |     |         |
| CMV infection, yes versus no                   | -0.094       | 0.095| 0.33   |
| EBV infection, yes versus no                   | -0.152       | 0.061| 0.01   |
| Time after transplant, months                  | 0.023        | 0.004| <0.001 |
| Transplant variables                           |              |     |         |
| Type of donor, living versus deceased          | -0.163       | 0.207| 0.44   |
| Graft type, complete versus technical variant* | 0.143        | 0.171| 0.41   |
| Genetic variables                              |              |     |         |
| Donor CYP3A5 polymorphism, expressers versus nonexpressers | -0.413 | 0.168| 0.02   |

*Complete = 1; technical variant = 0.

from homoscedasticity or normality (Supporting Fig. 1). The predicted concentrations as a function of the observed concentrations of tacrolimus showed that the model performed well in terms of fitness of the data (Fig. 2). The internal validation of the final model by bootstrapping (1000 successful runs) gave satisfactory results as shown in Table 3. Moreover, in the external validation, the predictive performance of the final model was successfully assessed: the ME was 0.213; the MRE was 2.05%; and precision, expressed as the relative RMSE, was 15.4% for the predictive model.

Discussion

In this study, for the first time in a Latin American pediatric liver transplant population, we identified different factors that significantly influence tacrolimus exposure. We developed and validated a model that showed a positive association between logC0/D and ALT values as well as time after transplant, whereas a negative association with donor CYP3A5 expression (expressers versus nonexpressers) was found between 1 month and 2 years after transplantation.

Liver function tests for ALT and AST are traditional markers of acute liver damage secondary to different events, including acute rejection episodes, viral infections, and/or liver fibrosis. Because 98%-99% of tacrolimus is metabolized in the liver, it is expected that tacrolimus C0 increases with liver dysfunction. Previously, apparent clearance was found to decrease exponentially with the increase of AST in adult transplant patients. In our study, elevated ALT levels, compatible with impaired liver function, positively correlated with logC0/D because of a deficit in tacrolimus metabolism.

Few studies have detected a relationship between tacrolimus exposure and time after transplant. In our case, we observed that time after transplant was retained in the final model and the ratio logC0/D increased with time, in line with a reduction in tacrolimus doses (data not shown). In adult transplant patients, reduced tacrolimus dose requirements have been routinely found in the first year after transplantation. This observation may be explained by a decrease in tacrolimus clearance due to drug-drug interactions, increased bioavailability over time, or both. Regarding drug-drug interactions, introduction or discontinuation of steroids may play an important role. The concurrent use of tacrolimus with mild CYP3A inducers, such as prednisone, may result in decreased tacrolimus C0, whereas the discontinuation of steroids may result in an increased tacrolimus exposure. In our study, we registered the administration of steroids in the immunosuppressive maintenance treatment. We tested for the significance of concomitant steroids in logC0/D, but it was not retained in the final model (P > 0.05; Table 3). Thus, we were not able
to confirm a drug-drug interaction effect of steroids in tacrolimus pharmacokinetics over time. On the other hand, tacrolimus largely binds to red blood cells and plasma proteins. Thus, the increase over time in oral bioavailability may potentially be due to an increased hematocrit and albumin concentration.\(^\text{(10,12,42)}\) In our study, we tested for the significance of hematocrit in tacrolimus C0 and did not find a significant relation \((P > 0.05; \text{Table 2})\) to confirm the change of tacrolimus clearance over time. Nevertheless, the mechanisms responsible for the change in apparent clearance over time are only partly known,\(^\text{(38-40)}\) and further studies are required.\(^\text{(12)}\)

The metabolism of tacrolimus largely occurs in the liver. CYP3A5 plays a more dominant role in the metabolism of tacrolimus than CYP3A4\(^\text{(43)}\) and has a significant effect on tacrolimus pharmacokinetics in adult and pediatric transplant patients.\(^\text{(10-12,18,21,22,44,45)}\) Specifically in liver transplantation, it has been reported that donor CYP3A5 genotype has a more dominant effect than the recipient genotype on tacrolimus pharmacokinetics.\(^\text{(10,11,18,21,22,45)}\) This result implies that beyond day 30 after transplant, recipients of a graft expressing CYP3A5 have a lower logC0/D compared with recipients of a nonexpresser graft. Therefore, higher tacrolimus doses are required in patients with
TABLE 3. Parameter Estimates of the Final LME Model and Bootstrap Results

| Parameters                                      | Estimates (% SE) | P Value | 95% CI of the Bootstrap |
|------------------------------------------------|------------------|---------|-------------------------|
| Fixed effects                                   |                  |         |                         |
| Intercept                                       | 4.424 (10.644)   | <0.001  | 4.186-4.647             |
| Time after transplant per month                 | 0.019 (0.437)    | <0.001  | 0.010-0.028             |
| ALT, IU/L                                       | 0.00030 (0.014)  | 0.03    | 0.00002-0.00056         |
| Donor CYP3A5 polymorphism, expressers versus nonexpressers | −0.349 (13.840) | 0.02    | −0.631 to −0.062        |
| Interaction term, time × ALT                    | 0.00005 (0.002)  | 0.004   | 0.00001-0.00008         |
| Random effects                                  |                  |         |                         |
| Random effect on subject                        | 0.580            |         | 0.439-0.708             |
| Random effect on slope                          | 0.018            |         | 0.010-0.025             |
| Correlation between random effects              | −0.852           |         | −1.000 to −0.645        |
| Residual variability                            | 0.350            |         | 0.332-0.369             |

FIG. 2. Goodness-of-fit plot of the final model for observed versus individual predicted tacrolimus logC0/D.

grafts carrying CYP3A5*1 allele compared with nonexpressers (CYP3A5*3) to achieve the target C0 according to time after transplantation. In these cases, TDM is performed as a tool to aid tacrolimus titration until reaching the target range. In agreement with our results, a report on a Japanese pediatric liver transplant population(11) showed that donor CYP3A5 expression significantly decreased tacrolimus dose-normalized C0 because of a 30% increase in tacrolimus clearance. Also, another study showed that the daily dose requirement for tacrolimus was higher among French children who received a liver expressing CYP3A5 compared with those with a CYP3A5*3 liver.(21) The association between donor CYP3A5 genotype and tacrolimus disposition was also reported specifically on the first day after liver transplantation for pediatric patients. On the other hand, considering the effect of the recipient CYP3A5-expression stratification, we observed no association with tacrolimus exposure in our cohort of patients.

However, previous studies in different populations did describe this association. In this sense, Caucasian pediatric liver transplant recipients with CYP3A5 expression presented with an increased apparent
clearance of tacrolimus compared with nonexpressers.\(^{(12)}\) Furthermore, studies in Chinese pediatric liver transplant patients reported that CYP3A5 genotyping both in recipients and donors was necessary to establish a personalized tacrolimus dosage regimen.\(^{(22)}\) Therefore, donor genotype, in addition to the patient genotype, may play an important role in determining the tacrolimus pharmacokinetic response, but the results varied among the studied populations. This highlights the necessity of conducting further studies on the relationship between tacrolimus exposure and pharmacogenetics in both donors and pediatric liver transplant recipients.

Some covariates identified as influential on tacrolimus pharmacokinetics were not retained in our final model. One of the most important pharmacokinetic properties of tacrolimus is its high red blood cell–binding capacity. Several pharmacokinetic studies have reported a significant effect of hematocrit on tacrolimus dose requirements.\(^{(10,14)}\) This effect was not observed in the present population, which may be explained, in part, by the partial recovery of hematocrit levels beyond the first month after transplant during which considerable variation in hematocrit is observed and multiple transfusions are required. The type of donor (living/deceased) was tested for potential significant association to tacrolimus C0 based on a potential impact of regeneration of the graft (liver) leading to improvement of hepatic function.\(^{(47)}\) Nonetheless, this variable was not significantly associated with tacrolimus exposure measured as C0 as shown in Table 2.

There are some limitations to be acknowledged in this study. First, because of its retrospective nature, it has all the limitations inherent to this type of descriptive study. Second, the area under the curve (AUC) for blood concentration time is expected to be a better marker of systemic exposure to tacrolimus than C0.\(^{(2)}\) However, the AUC is difficult to obtain due to practical and ethical reasons in the pediatric population. Nevertheless, in children, tacrolimus TDM is based on monitoring C0. Therefore, our results should be interpreted with caution. Third, we focused on the period beyond 30 days after transplant in order to avoid the variability produced by hemodynamic alterations, interruption of doses, and different frequency intervals of tacrolimus administration. Finally, we also acknowledge that one of the reasons for the limited number of studied patients was the discontinuation of the genotyping technique due to unavailability of the reagents.

The starting dose of tacrolimus is usually based on body weight and then adjusted by means of TDM. Recently, a model was developed to predict the individual starting dose of tacrolimus in pediatric renal transplantation patients, and the final model included body weight.\(^{(48)}\) However, limited information is available for these patients. Therefore, our study provides novel information about tacrolimus dosing based on body weight in children with liver transplant.

After external validation, this model could be used in clinical practice to make dosage recommendations accounting for the liver enzyme levels (ALT), genetics, and posttransplant time-dependent change in the logC0/D of tacrolimus. For instance, we may assume 2 random patients in our study population with a body weight of 16.3 kg. The ALT level of patient 1, who underwent liver transplant 3.3 months earlier, is 1200 IU/L, whereas the ALT level in patient 2 is 40 IU/L after receiving a liver transplant 9.3 months earlier. Neither is a graft expresser of CYP3A5. If tacrolimus C0 levels have to be maintained at 5.0 ng/ml, we could use the present model to calculate the tacrolimus doses. According to our model, the calculated dose for the first child would be 0.35–mg bid, whereas for the second child, it would be 1.11–mg bid. This is in line with a setting in which lower tacrolimus doses are recommended in liver dysfunction to avoid systemic accumulation. If both patients have ALT levels of 40 IU/L, underwent surgery 4 months previously, and patient 1 has a nonexpresser graft whereas patient 2 has an expresser graft, then the tacrolimus doses would be 0.60 mg bid and 1.69 mg bid, respectively.

In conclusion, in the present report, we have developed a model to describe tacrolimus pharmacokinetics in children who underwent liver transplantation. This final LME model presented a suitable performance and predictive ability to adequate tacrolimus doses in future patients in order to minimize the occurrence of an out-of-range therapeutic window that may lead to adverse drug reactions or acute rejection. The results of this study may be used in the clinical setting in conjunction with TDM and may contribute to the development of programs to optimize tacrolimus dosing, taking into account not only patient body weight but also time after transplant, genotype, and liver function.

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