Genotype-guided model significantly improves accuracy of tacrolimus initial dosing after liver transplantation

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Summary

Background The initial dose of tacrolimus after liver transplantation (LT) is critical for rapidly achieving the steady state of the drug concentration, minimizing the potential adverse reactions and warranting long-term patient prognosis. We aimed to develop and validate a genotype-guided model for determining personalized initial dose of tacrolimus.

Methods By combining pharmacokinetic modeling, pharmacogenomic analysis and multiple statistical methods, we developed a genotype-guided model to predict individualized tacrolimus initial dose after LT in the discovery (n = 150) and validation cohorts (n = 97) respectively. This model was further validated in a prospective, randomized and single-blind clinical trial from August, 2021 to February, 2022 (n = 40, ChiCTR2100050288).

Findings Our model included donor’s and recipient’s genotypes, recipient’s weight and total bilirubin, which achieved an area under the curve of receiver operating characteristic curve (AUC of ROC) of 0.88 and 0.79 in the discovery and validation cohorts, respectively. We found that patients who were given tacrolimus within the recommended concentration range (RCR) (4–10 ng/mL), the new-onset metabolic syndromes are lower, especially for new-onset diabetes (p = 0.043). In the clinical trial, compared to those in experience-based (EB) group, patients in the model-based (MB) group were more likely to achieving the RCR (75% vs 40%, p = 0.025) with a more variable individualized dose (0.023–0.096 mg/kg/day vs 0.045–0.057 mg/kg/day). Moreover, significantly fewer medication adjustments were required for the MB group than the EB group (2.75 ± 2.01 vs 6.05 ± 3.35, p = 0.001).

Interpretation Our genotype-based model significantly improved the initial dosing accuracy of tacrolimus and reduced the number of medication adjustments, which are critical for improving the prognosis of LT patients.

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Research in context

Evidence before this study

The initial dose of tacrolimus after liver transplantation is critical for rapidly achieving the steady state of the drug concentration, minimizing potential adverse reactions, and warranting long-term patient prognosis. Current strategies to guide personalized tacrolimus (based on weight and/or based on the CYP3A5 genetic variants) have not shown an accurate and reliable predictive performance of tacrolimus initial dose.

 Added value of this study

Via the discovery cohort (n = 150), an independent validation cohort (n = 97), and a pilot prospective clinical trial (n = 40), respectively, we developed and validated a pharmacogenomics-based model to determine the initial dose of tacrolimus after liver transplantation. Compared to the experience-based tacrolimus dosing paradigm, our model was proven to significantly reduce the number of dose adjustments and was more likely to achieve the recommended steady-state therapeutic range, which potentially reduces the risk of new-onset diabetes due to tacrolimus overdose.

Implications of all the available evidence

Our study, for the first time, established a new pharmacogenomics-guided model that significantly improved the initial dosing accuracy of tacrolimus, reducing the number of medication adjustments, with the potential to improve the prognosis of LT patients as well. Further long-term safety and efficacy of the model should be evaluated in the full randomized clinical trial.

Introduction

Tacrolimus is a calcineurin inhibitor and the mainstay immunosuppressant used after solid organ and hematopoietic stem cell transplantation.1 Sufficient immunosuppression is essential for suppressing allograft rejection and increasing the survival rate of transplantation. On the other hand, tacrolimus over-dosing can lead to serious adverse drug reactions, such as infection, diabetes, and renal insufficiency.2,3 However, the narrow therapeutic index and large inter-individual variabilities of tacrolimus concentration complicate its routine dosage adjustment.1 Inter-patient dosage requirement of tacrolimus is more than 20-fold (0.5–10 mg/day).1 Among patients in East Asian countries,4 it was demonstrated that maintaining balanced immunosuppression requires only half of the empirical dosage recommended by current standard guidelines primarily based on recipient’s weight.7,8 Whereas, we found that even with the same weight-based dosing, the inter-individual trough concentration varied greatly, considering that a large variation exists in preoperative weight and postoperative weight, particularly in patients undergoing liver transplantation (LT) with ascites or hydrothorax. Besides, a series of clinically contributing factors containing donor liver quality, warm ischemia time, cold ischemia time, transplanted liver cell regeneration, hematocrit, albumin etc., also have an impact on tacrolimus metabolism.9 Thus, a precise, personalized strategy to determine the initial dose of tacrolimus is important for achieving an accurate use of this drug and a better long-term outcome.

In recent years, the pharmacogenomics approach based on patients’ genotypic information has been demonstrated robust to predict individualized response to drug efficacy and safety.10 Genetic factors account for more than 50% of the variability in tacrolimus prescription.11,12 Enzymes in the cytochrome P450 (CYP) 3A family are responsible for the oxidative metabolism of tacrolimus.13 Numerous studies have demonstrated the importance of the genetic variation (rs776746, or *3) of CYP3A5 gene in tacrolimus metabolism.14,15 Recent guidelines for the use of tacrolimus proposed by the Clinical Pharmacogenetics Implementation Consortium (CPIC) provide preliminary suggestions relevant to the rs776746 genotype and tacrolimus dosing.1 In this CPIC guideline, according to the genotype of the CYP3A5 gene, the recipient’s tacrolimus metabolism phenotype was assigned to 3 categories, namely Extensive Metabolizer (EM) for individuals carrying two functional alleles; Intermediate Metabolizer (IM) for individuals carrying one functional allele and one nonfunctional allele; and Poor Metabolizer (PM) for individuals carrying two nonfunctional alleles. This simple CPIC-EIP (EM, IM, PM) classification for tacrolimus is recommended broadly for many organ transplant operations, including kidney, heart, lung, and liver transplants in which the donor and recipient genotypes are identical.16–18

However, the current CPIC-EIP classification has limitations in its application to allogeneic LT. First, the liver plays important role in the metabolism of tacrolimus. Almost all clinical LT is allogeneic. Therefore, the recipient’s and donor’s genotype are often different. Second, the majority of pharmacogenomic studies on tacrolimus and CYP3A5 variants were conducted in Caucasian populations. However, the allele frequency of rs776746 is highly variable among different ethnic groups.19 For example, according to the dbSNP (https://www.ncbi.nlm.nih.gov/snp), the frequency of the major loss-of-function allele (A) of rs776746 of CYP3A5, ranging from 7% among the Caucasian population to around 30% among East Asians, and 70% among...
African or African-American populations. This population difference may lead to differential clinical pharmacokinetic (PK) and pharmacodynamics (PD) outcomes. Therefore, the current guideline mainly based on the limited data in Caucasian patients may not apply well to the patients of other ethnic groups. There is thus far no sufficient data supporting a pharmacogenomic assessment for Chinese and East Asian liver transplantation patients. Third, the CPIC-EIP classification only considers the CYP3A5 genotypes without taking into account of other important factors, e.g. other genetic and non-genetic markers. This is extremely important, since other genes and factors may also contribute to the metabolism and/or excretion of tacrolimus. Meanwhile, among the early stage of the post-transplantation recovery, the donor liver function is not fully restored, and markers reflecting the liver function status is important to be considered for adjusting the initial dose of tacrolimus. Taken together, there is an urgent need to construct a specific dosing strategy to guide personalized tacrolimus use for liver transplantation patients among East Asian populations and beyond.

In this study, we aimed to develop and externally validate a model to predict individual tacrolimus metabolism integrating the donors’ and recipients’ genotypic data and other important clinical information for LT patients. More importantly, for the first time, we converted the predictive model to a personalized recommendation tool of initial dose and to verify in a randomized pilot trial that the model-based dosing strategy is superior to the traditional experience-based strategy for LT patients.

**Methods**

**Study design and subjects**

The study consists three independent cohorts (Fig. 1): a discovery cohort, a validation cohort and a randomized, single-blinded pilot trial.

The **discovery cohort** included 150 orthotopic LT patients at the Shanghai Jiao Tong University Affiliated Shanghai General Hospital (SJTU-ASGH) from January 2015 to December 2017. The **validation cohort** included 97 orthotopic LT patients at the First Affiliated Hospital of Zhengzhou University (ZZU-FAH) from February 2015 to December 2017.

The **pilot trial** was conducted at Xiang'an Hospital of Xiamen University (XAH-XMU), in which 40 patients were recruited from August, 2021 to February, 2022. The trial was registered at www.chictr.org.cn (ChiCTR2100050288). The inclusion criteria of the pilot trial were: (1) 18≤ Age ≤65 years; (2) LT from DBD; (3) Receives tacrolimus-based immunosuppressive regimen; (4) Signed informed consent. The exclusion criteria were: (1) Patients receive multiple organ transplantation; (2) Secondary organ transplantation; (3) Diagnosed with immune disorders or other diseases requiring immune-related treatment after LT; (4) Taking drugs that seriously affect the metabolism of tacrolimus (such as diltiazem, posaconazole, fluconazole, and erythromycin); (5) Has contraindications of tacrolimus; (6) Patients who participate in other investigational drug trials; (7) Or any scenarios that investigators consider not appropriate to participate in the trial.

**Ethics statement**

The study was carried out following the Declaration of Helsinki and approved by the Ethics Committee of SJTU-ASGH, ZZU-FAH and XAH-XMU. Informed consent was obtained from all donors and recipients. No donor organs were obtained from executed prisoners or other institutionalized persons.

**Intervention**

All patients underwent orthotopic LT, during which a healthy donor liver was placed in the recipient’s anatomical location after the original liver was removed. Immunosuppression regimens of LT recipients included tacrolimus, mycophenolate mofetil (MMF) with basiliximab induction according to the guideline. Tacrolimus was administered orally twice daily after the operation starting with an initial dose and continuously adjusted by TDM. 1 mg dose of MMF was given before the operation, and the postoperative MMF at dosage of 750 mg at each administration twice a day. 500 mg of methylprednisolone was only given intravenously before the portal vein was reopened intraoperatively. On postoperative day 1 and day 4, 20 mg of basiliximab was given intravenously.

In the pilot trial, all recruited patients were 1:1 randomized into the model-based (MB) initial-dosing group or the experience-based (EB) initial-dosing group. The EB group started with an initial dose (0.05–0.07 mg/kg/day) determined by experienced clinician who know all the genetic data. The MB group started with an initial dose given by our developed dose recommendation model. Afterwards, both groups received TDM dose adjustment (Protocol in supplementary).

**Model development**

Our workflow of model development included five major steps (as shown in Fig. 1): (1) Pharmacokinetic
modeling; (2) Candidate gene/SNP selection; (3) PK prediction model; (4) Dose recommendation model; (5) web-based calculator.

**Step 1: Pharmacokinetic modeling**
We performed population pharmacokinetic modeling based on the daily collected tacrolimus dosage and blood concentration of 150 patients from the discovery cohort, using nonlinear mixed-effect model (NONMEM) implemented by R. One- and two-compartment pharmacokinetic models with first-order absorption were compared and chosen according to the goodness of fit of models. Parameters included the tacrolimus clearance rate (CL) and volumes of distribution (V). Elimination half-life (τ) was estimated accordingly. To address the variation of metabolism over time, we allowed the parameters to change by week for each subject.

**Step 2: Candidate gene/SNP selection**
Candidate SNPs were selected based on analysis of the first 115 patients from the discovery cohort. The genotypic data (1936 loci of 225 genes) of these patients (both donors and recipients) was collected using the pharmacogenome-wide DMET plus Microarray by Affymetrix Inc. The gene selection used following criteria: 1) The association p-value between individual genotype and the estimated elimination half-life (τ) from Step 1 is less than 0.05; 2) SNPs have a minor allele frequency >0.1; 3) a representative SNPs in a linkage disequilibrium block with R-square > 0.5; and 4) Exonic and UTR SNPs were preferred over intronic ones.

**Step 3: C/D prediction model**
We constructed a generalized linear model to predict initial C/D (the blood concentration of tacrolimus at 24 h/the initial daily dose of tacrolimus) based on all the 150 patients from the discovery cohort. Candidate predictors included the 34 SNPs selected in Step 2 and clinical characteristics of age, sex, height, weight, total bilirubin (TB), direct bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase, and...
hemoglobin. Predictors in the final model were selected using Lasso regression. The last 35 patients in the discovery cohort were genotyped for selected SNPs using PCR Sequenom MassARRAY SNP-genotyping platform, so as the patients in the validation cohort and the pilot trial.

Step 4: Dose recommendation model
For convenience of clinical practice, we converted the C/D prediction model into a dose recommendation form. The recommended initial daily dose of tacrolimus was given as:

\[
\text{Initial dosage} = \text{round} \left( \frac{\text{Target blood concentration}}{\text{predicted (initial C/D)} \times \text{mindose}} \right) \times \text{mindose (mg)}
\]

The target blood concentration of tacrolimus is determined to balance the risk of acute rejection and new-onset diabetes according to our data. The mindose is the minimum dose of tacrolimus drug pill currently available in clinical practice. “round” is the discretization function.

Step 5: Web-based calculator
We developed a web-based online calculator to conveniently implement our dose recommendation model using the Shiny package of R (https://liverstudy.shinyapps.io/tacrolimus_initial_dose/).

The analysis strategy of our workflow is to use the pharmacokinetic data during 4 weeks to select candidate gene, but only use the pharmacokinetic data of the first 24 h to build the final predictive model. This analysis strategy is picked from several possible strategies based on the consideration of bias-variance trade-off. More detailed explanation was included in supplementary materials (Fig. S1).

Model evaluation
C/D prediction model
The prediction performance of our C/D prediction model was evaluated by two approaches. First, the scatter plot was made and the assumption of linearity was checked. Pearson correlation was then used to compare the observed and model-predicted blood concentrations of tacrolimus at 24 h after the first dose. Second, the receiver operating characteristic curve (ROC) and area under the curve (AUC) were used to evaluate our model’s discrimination capability to distinguish fast and slow tacrolimus metabolizers (fast/slow were dichotomized by median of population). These evaluations were conducted both in the discovery cohort and the independent validation cohort.

Dose recommendation model
The clinical utility of our dose recommendation model was evaluated using the proportion in range (PIR) defined as the proportion of patients whose C24 falls within the recommended concentration range (RCR, 4–10 ng/ml) among all patients who took tacrolimus, where C24 is the tacrolimus blood concentration measured at 24 h after the initial dose and before the next dose. In the discovery and validation cohort, we use the locally weighted scatterplot smoothing (LOWESS) regression method to estimate PIR with respect to different initial dosing strategy. In the pilot trial, PIR was the primary endpoint. Secondary endpoints include: (1) The incidence of acute rejection confirmed by biopsy within three months after LT; (2) The number of tacrolimus dose adjustments given due to incomplete immunosuppression; (3) PIR within one week after liver transplantation; (4) The incidence of new-onset diabetes within six months after LT; (5) One-year survival rate after LT; (6) Recurrence rate of hepatocellular carcinoma with standards of UCSF after LT during the follow-up period; (7) Quality of life within twenty-eight days, three months, six months, and one year after LT; (8) Direct and indirect costs associated with the treatment and management of LT recipients; (9) The incidence of acute rejection is confirmed by biopsy during the follow-up period.

Statistical analysis
In most cases, we used the residual plot to observe whether the residual distribution significantly deviated from normality assumption. The association between gene polymorphisms and tacrolimus pharmacokinetics was studied with ANOVA analysis. Multiplicity was adjusted by the FDR method. Homogeneity of variance test for tacrolimus blood concentration was performed using Levene’s test. The model predicting tacrolimus pharmacokinetics was constructed by a multivariate generalized linear model with the lognormal distribution. Model selection was performed according to BIC criteria. The log transformation of predictive variables and interaction terms were also considered during model selection. All statistical analysis was performed using R (version 3.6) software. Hardy–Weinberg equilibrium, allele frequency, linkage disequilibrium and haplotype analysis were analyzed using PLINK software. There was less than 10% of missing value in the development and validation cohort, and no missing value of the primary endpoint in the pilot trial. Typical missing values were imputed with the last observation carried forward (LOCF) method. It should be noted that these missing values were due to that patients were in stable state so that the clinicians decided there were no
need to repeat the laboratory test. Therefore, these are not missing data at random. The sample size of the randomized trial was estimated according to the result of the validation cohort. The primary endpoint between two groups was compared with chi-square method. p value < 0.05 was considered statistically significant.

Role of the funding source
None of the funders had any role in study design, data collection, data analysis, data interpretation or writing. All corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
Clinical characteristics of patients
Information from patients who underwent TDM-guided dose adjustments at 28 days postoperatively was collected from all patients in the three centers and used as phenotypes for further association analyses (Fig. 1). These data include sex, weight, and clinical variables such as drug dosage, trough blood concentration, ALT, and TB. The baseline characteristics are presented in Tables 1 and 2. The 150 patients included in the discovery cohort and validation cohort, respectively. There was no difference between the discovery and validation cohort in three cohorts, respectively. Similarly, there was no difference between the discovery and validation cohort in their demographical and clinical biochemical test values, as well as the patient’s survival rate (Fig. S2).

The early phase tacrolimus blood concentration associated with prognosis
Fig. 2A shown the distribution of tacrolimus concentration in the discovery cohort and validation cohort, respectively. The dynamic dose and concentration of tacrolimus over the first 4 weeks after the LT are presented in Fig. 2B. The dose in the first week was significantly lower than that in the following weeks (p = 2e-16), while the concentration of tacrolimus in the first week was the highest among the four weeks, though not statistically significant (p = 0.089) and becomes stable after the first week. These results demonstrated that the tacrolimus concentration variance in the early phase was much greater than that in the steady phase (p = 2.33e-05).

We further investigated the association between the concentration of tacrolimus in the early phase (first week) and the outcomes. As shown in Fig. 2C, we found that higher initial tacrolimus concentration was related to a higher incidence of new-onset diabetes, but was associated with a lower likelihood of acute rejection (p = 2.2e-16). More specifically, the higher concentration of tacrolimus that was more than or equal to 10 ng/mL was associated with a higher adverse event rate, especially new-onset diabetes (p = 0.023, Fig. S3), and patients with new-onset diabetes had a trend to exert a worse survival though not statistically significant (p = 0.055, Fig. S2). No association was found between tacrolimus concentration in the first week and liver function recovery (Fig. S4).

The suggested concentration is 10–20 ng/mL within the first month based on the package insert of tacrolimus,21 which was significantly higher than our observation. To try to determine a suitable RCR for Chinese LT patients, we found that in the subgroup of patients who were given tacrolimus within RCR (4–10 ng/mL), the new-onset metabolic syndromes were lower (Fig. 2D), especially for new-onset diabetes (p = 0.043).

Pharmacogenomic analysis on tacrolimus pharmacokinetics
Fig. 3 showed that all SNPs were listed in order from chromosome 1 to 22 and the X chromosome (excluding the Y chromosome). P-values were calculated for all SNPs and the statistical significance in donors and recipients was shown in a Manhattan plot, respectively (Fig. 3). A total of 34 SNPs of 25 genes in donor or recipient or both were identified that are significantly associated with tacrolimus pharmacokinetics (Supplementary Table S1).

The model specification and evaluation of initial C/D prediction model
The final C/D prediction model we obtained included six predictors: recipient weight, TB, donor CYP3A5 rs776746, recipient CYP3A5 rs776746, recipient SLCO1B1 rs4149015, and recipient CHST10 rs3748930. The detailed model specification is presented in Table 3.

We presented the prediction performance of the C/D prediction model in Fig. 4. The ROC plots (Fig. 4A and C) presents that the AUCs of ROC are 0.878 and 0.790 in the discovery and validation cohorts, respectively. The scatter plots (Fig. 4B and D) show a good accordance of the predicted blood concentrations with the actual ones in the discovery cohort (R = 0.58, p = 2.2e-16) and validation cohort (R = 0.58, p = 7.48e-10).

Dose recommendation model and clinical benefit
We visualized our dose recommendation model in Fig. 5 and developed a user-friendly web calculator for
the recommended tacrolimus initial dose (see URL http://liverstudy.shinyapps.io/tacrolimus_initial_dose/).

We retrospectively assessed the potential clinical benefit of our model in both cohorts. As shown in Fig. 6, for patients who took tacrolimus with our model recommended dose \(\log_2 (\text{Actual dose/Recommended dose}) = 0\), the estimated PIR, proportion of patients with tacrolimus blood concentration in RCR (4–10 ng/mL), achieved up to 56.3%, while the PIR based on TDM alone in the total population was only 38.5%, which indicated that applying the dose recommendation model might help more LT patients to achieve RCR within 24 h.

**Validation of model-based dosing strategy in clinical trial**

In this trial, At 24 h the tacrolimus trough concentration of the MB group was 5.9 (4.8, 9.9) ng/mL with the concentration of 75% of patients falling within RCR, as compared to 4.6 (2.9, 9.1) ng/mL in the EB group with the concentration of 40% of patients falling within RCR \(p = 0.025\) (Table 2). The recommended initial tacrolimus dose (median and range) of the MB group was 0.056 (0.023–0.096) mg/kg/day, which was similar to 0.050 (0.045–0.057) mg/kg/day in the EB group \(p = 0.173\). However, 95% of patients (19/20) were treated with doses range from 0.05 to 0.07 mg/kg/day in the EB group, while in the MB group, 50% of patients (10/20) were treated with doses out range (0.05–0.07 mg/kg/day) \(p = 0.006\), suggesting that the model-guided initial dose was significantly individualized in the MB group (Table 4). Moreover, starting tacrolimus based on the predictive model generated initial drug doses that were closer to the steady state drug concentration, which significantly reduced the number of tacrolimus dose adjustments (2.75 ± 2.01 vs 6.05 ± 3.35, \(p = 0.001\)). Considering

| Characteristics                        | Discovery cohort (N = 150) | Validation cohort (N = 97) |
|----------------------------------------|---------------------------|---------------------------|
| Age (years)                            | 48 (41, 55)               | 50 (44, 57)               |
| Sex (N%)                               |                           |                           |
| Male                                   | 124 (82.6)                | 79 (90.8)                 |
| Female                                 | 26 (17.4)                 | 18 (9.2)                  |
| Weight (kg)                            | 66.5 (60.0, 72.8)         | 65.0 (60.0, 74.0)         |
| Height (cm)                            | 171.5 (166.0, 175.0)      | 171.0 (168.0, 175.0)      |
| BMI (kg/m²)                            | 22.5 (21.0, 24.8)         | 23.3 (21.2, 25.5)         |
| HBV-related end stage liver disease (N%)| 109 (72.6)                | 64 (66.7)                 |
| Child Pugh score                       | 7 (5, 9)                  | 6 (5, 7)                  |
| MELD score                             | 10 (8, 14)                | 8 (8, 12)                 |
| Hemoglobin (g/L)                       |                           |                           |
| Preoperation                           | 114 (99.0, 134.0)         | 130 (111.0, 145.5)        |
| Postoperation                          | 96 (89.0, 107.9)          | 101 (91.2, 111.3)         |
| GPT (UI/L)                             |                           |                           |
| Preoperation                           | 26.0 (17.0, 50.5)         | 34.7 (22.8, 46.7)         |
| Postoperation                          | 46.8 (30.2, 69.1)         | 68.7 (45.5, 112.5)        |
| GOT (UI/L)                             |                           |                           |
| Preoperation                           | 48.6 (31.0, 76.8)         | 38.8 (30.4, 54.9)         |
| Postoperation                          | 37.0 (27.0, 54.7)         | 39.5 (26.7, 55.0)         |
| DB (umol/L)                            |                           |                           |
| Preoperation                           | 11.3 (5.9, 28.9)          | 11.7 (6.5, 21.0)          |
| Postoperation                          | 14.6 (8.0, 33.2)          | 15.5 (9.7, 29.8)          |
| TB (umol/L)                            |                           |                           |
| Preoperation                           | 30.7 (17.0, 62.0)         | 19.6 (14.2, 35.8)         |
| Postoperation                          | 26.3 (17.0, 59.3)         | 30.7 (17.9, 57.5)         |
| Tacrolimus dose (mg/day)               | 3.0 (2.0, 4.0)            | 2.0 (2.0, 2.0)            |
| Follow-up (year)                       | 3.0 (2.0, 5.0)            | 1.0 (1.0, 2.0)            |
| Acute rejectiona (N%)                  | 15 (13.0)                 | 5 (25.0)                  |
| New-onset diabetesa (N%)               | 25 (20.7)                 | 10 (12.3)                 |

BMI, body mass index; HBV, Hepatitis B Virus; MELD, a model for end-stage liver disease; TB, total bilirubin; DB, direct bilirubin; GPT, glutamic-pyruvic transaminase; GOT, glutamic-oxalacetic transaminase. Quantitative variables are presented as median (Quartiles) as appropriate. Categories variables are presented as N/(Percentage).

Preoperation means one day before transplantation; Postoperation indicates a median of 28 days after transplantation. *Means that there are missing value.

**Table 1:** Clinical characteristics of patients in the discovery and validation cohorts.
that small sample size and short follow-up time, there was no difference between two groups on long-term prognosis (Supplementary Table S3 and Fig. S5).

**Discussion**

There are currently no standardized strategies to determine the best initial dose of tacrolimus after LT. This is especially an issue for populations in China and East Asia.

**Table 2: Baseline characteristics and outcomes of the pilot trial of tacrolimus personalized dosing.**

|                        | Model-based group (N = 20) | Experience-based group (N = 20) | p-value |
|------------------------|---------------------------|--------------------------------|---------|
| Age (years)            | 53.5 (48.5, 56.0)         | 47.5 (43.3, 53.8)               | 0.086   |
| Sex (N%)               |                           |                                |         |
| Male                   | 20 (100.0)                | 16 (75.0)                      | 0.106   |
| Female                 | 0 (0.0)                   | 4 (25.0)                       |         |
| Weight (Kg)            | 66.5 (57.4, 75.8)         | 67.4 (55.4, 73.8)              | 0.850   |
| Height (cm)            | 170.0 (165.5, 172.8)      | 170.0 (161.1, 171.8)           | 0.205   |
| BMI (kg/m²)            | 22.8 (20.8, 25.4)         | 23.9 (20.5, 26.5)              | 0.844   |
| Etiology (N/%)         |                           |                                |         |
| HCC                    | 12 (60.0)                 | 13 (65.0)                      | 0.744   |
| Non-HCC                | 8 (40.0)                  | 7 (35.0)                       |         |
| Numbers of dose modifi-| 2.5 (1.3, 4.0)            | 6.0 (3.3, 9.0)                 | 0.001   |
| cation                |                           |                                |         |
| Dose (mg/kg/day)       | 0.056 (0.042, 0.068)      | 0.050 (0.048, 0.054)           | 0.173   |
| 24 h after first dose  |                           |                                |         |
| Concentration (ng/mL)  | 5.9 (4.8, 9.9)            | 4.6 (2.9, 9.1)                 | 0.111   |
| Target rates (N%)      | 15 (75.0)                 | 8 (40.0)                       | 0.025   |
| Length of stay (days)  | 30.5 (28.0, 35.0)         | 30.5 (28.0, 38.0)              | 0.732   |
| Steady-state time (days)| 21.0 (17.3, 23.8)       | 19.0 (16.6, 20.0)              | 0.132   |
| HGB (g/L)              |                           |                                |         |
| Preoperation           | 112.0 (86.8, 135.8)       | 108.0 (80.8, 135.5)            | 0.550   |
| Postoperation          | 84.0 (81.0, 95.1)         | 83.0 (77.6, 99.0)              | 0.536   |
| Urea (g/mL)            |                           |                                |         |
| Preoperation           | 4.6 (3.2, 5.9)            | 4.1 (3.5, 8.0)                 | 0.161   |
| Postoperation          | 5.0 (3.9, 7.3)            | 5.9 (3.6, 8.8)                 | 0.408   |
| Creatine (umol/L)      |                           |                                |         |
| Preoperation           | 68.5 (58.3, 95.0)         | 69.5 (61.5, 94.3)              | 0.315   |
| Postoperation          | 80.5 (58.6, 104.8)        | 70.3 (52.0, 107.8)             | 0.614   |
| Glucose (mmol/L)       |                           |                                |         |
| Preoperation           | 6.1 (4.6, 7.2)            | 5.7 (5.0, 7.9)                 | 0.406   |
| Postoperation          | 6.6 (5.7, 7.6)            | 5.8 (5.0, 6.5)                 | 0.104   |
| GGT (U/L)              |                           |                                |         |
| Preoperation           | 89.0 (51.0, 161.8)        | 48.5 (22.0, 164.5)             | 0.452   |
| Postoperation          | 65.8 (45.1, 127.8)        | 69.3 (36.5, 125.8)             | 0.588   |
| TB (umol/L)            |                           |                                |         |
| Preoperation           | 27.4 (16.6, 105.1)        | 56.5 (29.5, 121.0)             | 0.433   |
| Postoperation          | 19.8 (14.5, 30.1)         | 215 (12.7, 31.2)               | 0.678   |
| DB (umol/L)            |                           |                                |         |
| Preoperation           | 12.6 (7.5, 81.2)          | 38.7 (14.0, 92.5)              | 0.426   |
| Postoperation          | 12.6 (7.5, 19.7)          | 12.2 (7.8, 22.8)               | 0.832   |
| GPT (U/L)              |                           |                                |         |
| Preoperation           | 26.0 (16.6, 63.0)         | 30.5 (18.3, 54.0)              | 0.463   |
| Postoperation          | 30.0 (13.1, 59.8)         | 25.0 (14.6, 41.0)              | 0.723   |
| GOT (U/L)              |                           |                                |         |
| Preoperation           | 45.5 (27.8, 71.5)         | 51.0 (23.3, 105.3)             | 0.326   |
| Postoperation          | 27.5 (16.9, 39.1)         | 25.9 (19.5, 43.5)              | 0.239   |

BMI, body mass index; HCC, hepatocellular carcinoma; HGB, hemoglobin; WBC, white blood cell; GGT, gamma-glutamyl transpeptidase; TB, total bilirubin; DB, direct bilirubin; GPT, glutamic-pyruvic transaminase; GOT, glutamic-oxalacetic transaminase. Quantitative variables are presented as median (Quartiles) as appropriate. Categories variables are presented as N/(Percentage). Preoperation means one day before transplantation; Postoperation indicates a median of 28 days after transplantation. p < 0.05 was considered as statistically significant. Boldfaced p value means significant.
Fig. 2: The distribution of tacrolimus concentration and dose at the early phase and their correlation with patient prognosis. A) The distribution of tacrolimus concentration in different cohorts. B) The change of tacrolimus concentration (p = 0.089) and dose (p = 2e-16) over the early phase in different weeks. C) The influence of tacrolimus median concentration in the initial week on acute rejection and new-onset diabetes. D) The correlation of tacrolimus concentration falling within RCR with new-onset diabetes (p = 0.043), new-onset hypertension (p = 0.248), new-onset hyperlipidemia (p = 0.406) and acute rejection (p = 0.419). The unit of tacrolimus concentration is ng/mL; the unit of tacrolimus dose is mg/day. We assess the correlation of tacrolimus median concentration in the initial week and acute rejection and new-onset diabetes by ggplot2 package and LOESS method.
Asian counties, where the conventional dosage of tacrolimus is nearly half of the internationally recommended dose. Our study for the first time established a dose recommendation model for tacrolimus and showed that the model-based initial dosing strategy is a promising way to improve prognosis of LT patients, which has a great potential to shift the current paradigm for the use of tacrolimus in LT patients among East Asian populations and beyond.

There is currently a global advocation to minimize anti-rejection therapy for organ transplantation. We chose the targeted trough concentration for tacrolimus ranging 4–10 ng/mL by considering the following rationale. First, genetic background in different ethnic groups is a crucial factor leading to the different tacrolimus dosages used in Eastern and Western transplant recipients. It was demonstrated that the trough levels of tacrolimus in East Asian are lower than in White population, with the 5th and 95th percentile among the East Asian population ranging around 4–10 ng/mL in a large inter-ethnic comparison study and in our discovery and validation cohorts. Second, tacrolimus overdosing

Fig. 3: Manhattan circle plots showing the genes significantly associated with tacrolimus pharmacokinetics. Manhattan plot of −log_{10} (p-values) of association analyses between each SNP and tacrolimus pharmacokinetics. Red dots represent significant SNPs from donors, and green dots represent significant SNPs from recipients. (Chr, chromosome).
leads to serious adverse drug reactions. Jia’s study in Chinese LT patients showed strategy by targeting 5–10 ng/mL of tacrolimus was beneficial in terms of long-term survival after LT, and did not increase the incidence of adverse reactions or graft rejection.\(^{25}\) Third, in line with the aforementioned findings, our analysis on retrospective cohorts suggested 4–10 ng/ml may be an appropriate target range of tacrolimus blood concentration balancing the new-onset metabolic syndromes and acute rejection incidence. Our finding also suggested that the earlier to achieve target range, the better the prognosis of LT patients.

**Table 3:** Multivariate analysis result with lasso regression in the discovery cohort and model specifications.

| Factor       | Gene      | Origin | Beta  | p-value |
|--------------|-----------|--------|-------|---------|
| Total bilirubin | –         | Recipient | 0.001 | 0.005   |
| rs776746     | CYP3A5    | Donor   | 0.313 | <0.001  |
| rs776746     | CYP3A5    | Recipient | 0.352 | <0.001  |
| rs1748930    | CHST10    | Recipient | 0.126 | 0.013   |
| rs4149015    | SLC01B1   | Recipient | 0.165 | 0.032   |
| Weight       | –         | Recipient | 0.491 | 0.043   |

The unit of weight is the kilogram. The unit of total bilirubin is umol/L. The genetic features were coded as 0 = AA, 1 = AG, 2 = GG or 0 = CC, 1 = CG, 2 = GG. \(p < 0.05\) was considered as statistically significant.

**Fig. 4:** Model performance for predicting the tacrolimus pharmacokinetics. Panels A and B present the ROC curve and calibration plot of the model in the discovery cohort. Panel C–D present the corresponding plots in the validation cohort. A) ROC curve in the discovery set. B) Consistency between the observed and predicted drug concentrations in the discovery set \((R = 0.58, p = 2.2e-16)\). C) ROC curve in the validation set. D) Consistency between the observed and predicted drug concentrations in the validation set \((R = 0.58, p = 7.48e-10)\). AUC > 70% indicates the good performance of the model. (ROC, receiver operating characteristic curve; AUC, area under the curve).
Our model was validated internally and externally in an independent cohort, as well as in a prospective clinical trial. All of these validations consistently confirmed that our model can serve as a safe and effective clinical decision-making tool for personalizing tacrolimus initial dose to quickly achieve desired blood concentration range within 24 h (75% vs 40%, p = 0.025), as compared to the conventional decision based on the clinician’s experience. Furthermore, we found the model-based initial dose required less dose adjustments in the prospective randomized trial (2.75 ± 2.01 vs 6.05 ± 3.35, p = 0.001). The pilot trial also showed that with the user-friendly web calculator and developed PCR panel of selected SNPs, the model-based dosing strategy is feasible and convenient in real-world clinical practice.

Our final model includes six predictors, four SNPs (donor’s and recipient’s CYP3A5 rs776746, recipient’s SLCO1B1 rs4149015 and CHST10 rs3748930) and two clinical variables (recipient weight and TB). Our finding confirmed the well-established CYP3A5 polymorphism rs776746 has the greatest impact on tacrolimus metabolism among all gene variants. But surprisingly recipient’s CYP3A5 polymorphism plays a more important role than the donor’s. SLCO1B1 encodes the protein of organic anion transporting polypeptide 1B1, is highly expressed in the liver and is involved in the biliary excretion of tacrolimus.26,27 CHST10 (Carbohydrate Sulfotransferase) encodes an enzyme adds sulfate to glucuronic acid to form a carbohydrate antigen.

Fig. 5: Calculation panel for tacrolimus initial dose prescription based on genetic factors and the total bilirubin level. Donor means sample from the donors. Recip means sample from the recipients. AG, AG, and GG or CC, CG, and GG refer to different genotypes. The unit of total bilirubin is umol/L. The total score is calculated based on donor rs776746, recipient rs776746, recipient rs3748930, recipient rs4149015, and the total bilirubin level. The effects of different genotypes influencing tacrolimus pharmacokinetics are shown in the nomogram.

Fig. 6: The percentage of tacrolimus concentration falls within RCR between model-based group and experience-based group. The blood concentration at 24 h after the first dose changed with respect to the ratio between the actual initial dose and the model recommended dose. Each scatter point stands for the blood concentration at 24 h for one patient. The horizontal dash lines represented the recommended concentration range of tacrolimus. The solid curve is the PIR (proportion in range) estimated by the LOWESS method. The unit of tacrolimus concentration is ng/mL.
H NK -1 that is capable of transferring sulfate to glucuronidated steroid hormones and other lipophilic drugs to facilitate their excretion via urine. 28 The three recipient SNPs in the model suggested that drug distribution/elimination in extrapathic organs such as intestine may be especially important in the early phase since the liver-related drug elimination may be weak before the restoration of liver function. Body weight of the recipient affects the apparent volume of distribution. Changes in total body fat or water mass due to the transplant may also affect the distribution and metabolism of tacrolimus. The TB in the model is a critical liver function and metabolism marker which may reflect the status of the liver function restoration. In the early period after LT, liver function may change rapidly and excessive drug dose can easily overwhelm the liver function capacity and lead to poisoning. 29 In summary, our study demonstrated that integrating both donor and recipient’s genotypes as well as relevant clinical factors is critical in constructing accurate predictive models for personalized medication.

Our study has several limitations. First, due to the limited sample size and follow-up time, our study does not have sufficient power to conclude the impact of different initial dosing strategy on long-term prognosis. Our randomized trial is primarily focused on the PIR, instead of long-term prognosis, therefore should be only considered as a pilot trial. Second, we did not consider the factors of concomitant medicine and food, which may also impact the pharmacokinetics of tacrolimus. Lastly, given the population differences in genetic allele frequency and other genetic variations, our model was primarily established based on a Chinese patient population. Therefore, the tools we have generated and data interpretation should be limited to Chinese or possibly East Asian populations. Whether our model can be generalized into other populations needs further investigation.

In conclusion, we for the first time developed a precision medication model capable of recommending the optimized initial dose of tacrolimus for LT patients. With the expansion of our ongoing clinical trial in a multicenter, randomized, single-blind study, our model would significantly close the gap in the current clinical practice and further improve the prognosis of LT patients.

**Table 4: Tacrolimus personalized dosing of model-based and experience-based group in the pilot trial.**

| Dose (mg/Kg) | ≤0.04 | 0.05-0.07 | ≥0.08 | p-value |
|-------------|-------|-----------|-------|---------|
| Target rates (N/%) | | | | 0.837 |
| Yes | 5 (83.3) | 7 (70.0) | 3 (75.0) | |
| No | 1 (16.7) | 3 (30.0) | 1 (25.0) | |
| Experience-based Group (N/%) | | | | |
| Yes | 5 (100) | 19 (95.0) | 0 (0.0) | 0.006 |
| No | 6 (30.0) | 10 (50.0) | 4 (20.0) | |

Categories variables are presented as N(Percentage). p < 0.05 was considered as statistically significant. Boldfaced p value means significant.

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**Data sharing statement**

All data can be viewed in NODE (http://www.biosino.org/node) by pasting the accession (OEP000278) into the text search box or through the URL: http://www.biosino.org/node/project/detail/OEP000278.

**Declaration of interests**

The authors of this manuscript have no conflicts of interest to disclose.

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**Appendix A. Supplementary data**

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2022.101752.

**References**

1. Kuypers DRJ. Intrapatient variability of tacrolimus exposure in solid organ transplantation: a novel marker for clinical outcome. *Clin Pharmacol Ther.* 2020;107(2):347–358.

2. Ling Q, Xie H, Lu D, et al. Association between donor and recipient TCF7L2 gene polymorphisms and the risk of new-onset diabetes mellitus after liver transplantation in a Han Chinese population. *J Hepatol.* 2013;58(2):271–277.

3. Jiao W, Zhang Z, Xu Y, et al. Butyric acid normalizes hyperglycemia caused by the tacrolimus-induced gut microbiota. *Am J Transplant.* 2020;20(9):2413–2424.

4. Liu Y, Zhang C, Li L, et al. Genome-wide association study of tacrolimus pharmacokinetics identifies novel single nucleotide polymorphisms in the convalescence and stabilization periods of post-transplant liver function. *Front Genet.* 2019;10:528.

5. Feng S, Bucuvalas JC, Mazariegos GV, et al. Efficacy and safety of immunosuppression withdrawal in pediatric liver transplant...
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15 Francke MI, Andrews LM, Le HL, et al. Avoiding tacrolimus unadministration in lung transplant. Pharmacogenomics. 2019;20(6):421–432.
16 Calvo PL, Serpe L, Brunati A, et al. Donor CYP3A5 genotype influences tacrolimus disposition on the first day after paediatric liver transplantation. Br J Clin Pharmacol. 2017;83(6):1252–1262.
17 Huang L, Assiri AA, Wen P, et al. The CYP3A5 genotypes of both liver transplant recipients and donors influence the time-dependent recovery of tacrolimus clearance during the early stage following transplantation. Clin Transl Med. 2021;11(10):e542.
18 Banham GD, Flint SM, Torpey N, et al. Bellirumub in kidney transplantation: an experimental medicine, randomized, placebo-controlled phase 2 trial. Lancet. 2018;391(10140):2619–2630.
19 Busuttil RW, Klintmalm GB, Lake JR, Miller CM, Porayko M. General guidelines for the use of tacrolimus in adult liver transplant patients. Transplantation. 1996;61(5):845–847.
20 Shuker N, Boumarar R, Van Schaik RH, et al. A randomized controlled trial comparing the efficacy of Cyp3a5 genotype-based with body-weight-based tacrolimus dosing after living donor kidney transplantation. Am J Transplant. 2016;16(7):2085–2096.
21 Thomson AW, Vionnet J, Sanchez-Fueyo A. Understanding, predicting and achieving liver transplant tolerance: from bench to bedside. Nat Rev Gastroenterol Hepatol. 2020;17(12):719–739.
22 Shuker N, Boumarar R, Van Schaik RH, et al. A randomized controlled trial comparing the efficacy of CYP3A5 genotype-based with body-weight-based tacrolimus dosing after living donor kidney transplantation. Am J Transplant. 2016;16(7):2085–2096.
23 Thomson AW, Vionnet J, Sanchez-Fueyo A. Understanding, predicting and achieving liver transplant tolerance: from bench to bedside. Nat Rev Gastroenterol Hepatol. 2020;17(12):719–739.
24 Lu Z, Bonate P, Keirns J. Population pharmacokinetics of immediate- and prolonged-release tacrolimus formulations in liver, kidney and heart transplant recipients. Br J Clin Pharmacol. 2019;85(8):1692–1703.
25 [La J], Lin BY, He J], et al. “Minimizing tacrolimus” strategy and long-term survival after liver transplantation. World J Gastroenterol. 2014;20(32):11363–11369.
26 Wei Y, Yang F, Wang Z, et al. The influence of recipient SLCO1B1 rs2291075 polymorphism on tacrolimus dose-corrected trough concentration in the early period after liver transplantation. Eur J Clin Pharmacol. 2021;77(6):859–867.
27 Ruiz J, Herrero MJ, Boso V, et al. Impact of single nucleotide polymorphisms (SNPs) on immunosuppressive therapy in lung transplantation. Int J Mol Sci. 2015;16(9):20168–20182.
28 Suzuki-Anekoji M, Suzuki A, Wu SW, et al. In vivo regulation of steroid hormones by the Chst10 sulfotransferase in mouse. J Biol Chem. 2013;288(7):5007–5016.
29 Albrecht W, Kappenberg F, Brecklinghaus T, et al. Prediction of human drug-induced liver injury (DILI) in relation to oral doses and blood concentrations. Arch Toxicol. 2019;93(6):1609–1637.