Research Report

Annals of Pharmacotherapy
2024, Vol. 58(5) 469–479

DOI: 10.1177/10600280231190943
journals.sagepub.com/home/aop
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Artificial Neural Network Analysis of Determinants of Tacrolimus Pharmacokinetics in Liver Transplant Recipients

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Abstract

Background: The efficacy and toxicity of tacrolimus are closely related to its trough blood concentrations. Identifying the influencing factors of pharmacokinetics of tacrolimus in the early postoperative period is conducive to the optimization of the individualized tacrolimus administration protocol and to help liver transplant (LT) recipients achieve the target blood concentrations. **Objective:** This study aimed to develop an artificial neural network (ANN) for predicting the blood concentration of tacrolimus soon after liver transplantation and for identifying determinants of the concentration based on Shapley additive explanation (SHAP). **Methods:** In this retrospective study, we enrolled 31 recipients who were first treated with liver transplantation from the Department of Liver Transplantation and Hepatic Surgery, the First Affiliated Hospital of Shandong First Medical University (Shandong Provincial Qianfoshan Hospital) from November 2020 to May 2021. The basic information, biochemical indexes, use of concomitant drugs, and genetic factors of organ donors and recipients were used for the ANN model inputs, and the output was the steady-state trough concentration (C_0) of tacrolimus after oral administration in LT recipients. The ANN model was established to predict C_0 of tacrolimus, SHAP was applied to the trained model, and the SHAP value of each input was calculated to analyze quantitatively the influencing factors for the output C_0. **Results:** A back-propagation ANN model with 3 hidden layers was established using deep learning. The mean prediction error was 0.27 $\pm$ 0.75 ng/mL; mean absolute error, 0.60 $\pm$ 0.52 ng/mL; correlation coefficient between predicted and actual C_0 values, 0.9677; and absolute prediction error of all blood concentrations obtained by the ANN model, $\leq$3.0 ng/mL. The results indicated that the following factors had the most significant effect on C_0: age, daily drug dose, genotype at *CYP3A5* polymorphism rs776746 in both recipient and donor, and concomitant use of caspofungin. The predicted C_0 value of tacrolimus in LT recipients increased in a dose-dependent manner when the daily dose exceeded 3 mg, whereas it decreased with age when LT recipients were older than 48 years. The predicted C_0 was higher when recipients and donors had the genotype *CYP3A5*3*3* than when they had the genotype *CYP3A5*1*. The predicted C_0 value also increased with the use of caspofungin or Wuzhi capsule. **Conclusion and relevance:** The established ANN model can be used to predict the C_0 value of tacrolimus in LT recipients with high accuracy and good predictive ability, serving as a reference for personalized treatment in the early stage after liver transplantation.

Keywords

liver transplantation, tacrolimus, artificial neural network, blood concentration

Introduction

Tacrolimus is one of the most widely used immunosuppressants to prevent acute rejection, improve graft survival rate, and reduce recipient mortality after solid organ transplantation.[1,2] However, it has a narrow therapeutic index and large interindividual differences in pharmacokinetics (PK), suggesting that its stable trough concentration (C_0) should be regularly monitored in the blood. Low tacrolimus C_0 may increase the risk of acute rejection, while high C_0 may lead to infection and drug-related nephrotoxicity, neurotoxicity, and new-onset diabetes.[3]

The interindividual differences in tacrolimus PK reflect differences in drug-metabolizing enzymes, membrane transporters, and relevant receptors. For example, single-nucleotide polymorphisms in the gene *CYP3A5* in liver

transplant (LT) recipients and donors affect tacrolimus PK; in particular, donor genotype at *CYP3A5* influences prognosis soon after liver transplantation.[4-10] The *CYP3A5*1* encodes a functional cytochrome P450 enzyme, which can metabolize tacrolimus and reduce its bioavailability, whereas the *CYP3A5*3* allele contains premature termination codons that give rise to truncated, nonfunctional enzyme.[4,11,12] For the same tacrolimus dose, individuals with a *CYP3A5*3*3* genotype show a higher drug concentration in the blood than individuals with genotypes **1*1* or **1*3*.

Drug-drug interactions may also contribute to interindividual differences in tacrolimus PK.[13] Long-term use of tacrolimus suppresses immunity, increasing the risk of morbidity and mortality among LT recipients as a result of invasive candidiasis and aspergillosis.[14,15] Caspofungin, an echinocandin that interferes with fungal cell wall synthesis, is used to treat invasive aspergillosis after liver transplantation,[16,17] but coadministration of caspofungin and tacrolimus reduces the peak concentration (C_{max}) of tacrolimus by about 20%.[15] Few studies have explored the interactions between echinocandins and tacrolimus or their effect on the interindividual differences in tacrolimus PK, and we are unaware of such studies in Asian or Chinese populations.

Tacrolimus is usually coadministered with hepatoprotective drugs because its long-term use can lead to liver injury.[18,19] These drugs may interact to affect tacrolimus PK and increase the risk of drug-induced hepatitis.[20,21] Wuzhi capsule is a traditional Chinese medicine preparation commonly prescribed for tacrolimus-induced hepatitis.[22] This capsule contains lignans, which can protect liver function[19,20] and prevent cytochrome P450 enzymes from metabolizing tacrolimus.[20,23,24] In this way, Wuzhi capsule can increase the blood concentration of tacrolimus in LT recipients and healthy subjects.[23,25]

In addition to drug-drug interactions, several other factors may lead to differences in tacrolimus PK between individuals: age, sex, body weight, liver and kidney function, and diet.[13,26-28] Identifying the factors influencing tacrolimus PK soon after liver transplantation may guide the personalization of drug regimens to prevent organ rejection and minimize adverse reactions.

Artificial neural networks (ANNs) have shown promise for rapidly predicting the blood concentration of tacrolimus in LT recipients based on daily drug dose, age, body weight, liver function index, and coadministered drugs.[29-32] ANNs are widely used in PK research due to their high tolerance and robustness to noise and errors, as well as their strong performance at nonlinear mapping, self-organization, self-adaptation, and self-learning. ANNs can also provide insight into the complex relationships between input and output values obtained from a large amount of data without requiring specific PK models.[29,30] In fact, ANNs can model tacrolimus PK better than multiple linear regression models.[29-31]

Previous ANNs to predict tacrolimus blood concentration have failed to consider *CYP3A5* genotypes in both LT donors and recipients or potential interactions with caspofungin or Wuzhi capsule.[29-32] We are also unaware of a systematic analysis of clinicodemographic and genetic factors that may affect tacrolimus PK. To address these gaps, we developed an ANN model to predict the blood concentration of tacrolimus in LT recipients, taking into account the effects of drug combinations as well as *CYP3A5* genotypes in recipients and donors. In addition, we used Shapley additive explanation (SHAP) to quantify the relative contributions of different factors to tacrolimus PK. Our findings may help individualize tacrolimus regimens after liver transplantation.

Methods

Subject Recruitment

Data were retrospectively analyzed for patients who underwent liver transplantation and whose postoperative tacrolimus blood concentration was monitored in the Department of Liver Transplantation and Hepatic Surgery at The First Affiliated Hospital of Shandong First Medical University (Shandong Provincial Qianfoshan Hospital) from November 2020 to May 2021. Subjects were included if they were ≥18 years old, were undergoing their first liver transplantation, and were treated with all the following: tacrolimus, enteric-coated mycophenolate sodium (EC-MPS), and glucocorticoids. Patients were excluded if they had (1) incomplete clinical information or postoperative follow-up data; (2) multiple-organ or secondary transplantation; (3) allergy or intolerance to macrolides, mycophenolate acid, or

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glucocorticoids; (4) severe gastrointestinal disease followed by gastrectomy, enterectomy, or malabsorption syndrome; (5) participated in clinical trials for other drugs 1 month before surgery; and/or (6) received a combination of drugs, other than Wuzhi capsule and echinocandins, that could strongly affect drug-metabolizing enzymes in hepatic microsomes, such as erythromycin, rifampicin, or triazole.

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Shandong First Medical University (Shandong Provincial Qianfoshan Hospital) (ethics approval number: 2020S303) and complied with the principles of the Helsinki Declaration of 1975.

Immunosuppressive Regimens

LT recipients were treated with tacrolimus capsules (Hangzhou Zhongmei Huadong Pharmaceutical Co., Ltd., Zhejiang, China), EC-MPS (MYFORTIC; Novartis Pharma Produktions GmbH., Wehr, Germany), and glucocorticoids. The initial dose of tacrolimus ranged from 0.075 to 0.15 $mg \cdot kg^{-1}\ d^{-1}$ according to guidelines[33] and individual patient condition. The initial dose was gradually increased depending on patient tolerance. Tacrolimus is started on the day after transplantation. LT recipients were intubated for the first 1 to 2 days after transplantation and given intranasal feeding at an early stage, followed by oral administration. On a given day, recipients received oral tacrolimus treatment at 6 AM and 6 PM. The tacrolimus dose was then adjusted to achieve the target C_0 of 8 to 12 ng/mL within 1 month after surgery. EC-MPS was administered at 360 mg twice daily, while methylprednisolone sodium succinate (Pfizer Manufacturing Belgium NV, Puurs, Belgium) was first injected intravenously at 50 mg every 6 hours, then the dose was gradually reduced to 40 mg once daily. At 1 week after the surgery, glucocorticoid administration was switched to oral methylprednisolone tablets (16 mg, once daily; MEDROL; Pfizer Pharmaceuticals Ltd), and the dose was gradually reduced to 4 mg/day until 1 month after surgery.

Data Collection

Clinical data collected from both LT recipients and donors included age, sex, height, body weight, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transpeptidase (GGT), total bilirubin (TBIL), albumin (ALB), globulin (GLB), serum creatinine (SCr), hematocrit (HCT), and genotype at the *CYP3A5* rs776746 locus. The daily dose of tacrolimus and information about other concomitant drugs were recorded. Caspofungin was administered *via* intravenous drip at a loading dose of 70 mg, followed by 50 mg once daily. Wuzhi capsule (11.25 mg) was administered orally, twice per day.

The tacrolimus C_0 values in the blood of LT recipients were determined retrospectively. Specifically, peripheral venous blood samples were collected 30 minutes before taking morning medication from postoperative day (POD, duration after LT) 4 to POD 31 and after at least 3 days of continuous tacrolimus administration and stabilization. Blood (5-10 mL) was collected into ethylenediaminetetraacetic acid–coated tubes, and the drug concentration was determined using an enzyme-linked immunosorbent assay (Architect i2000 automatic immune analysis system; Abbott Laboratories, Chicago, IL, USA) following the manufacturer's instructions.

ANN Modeling

An ANN model was set up in which the 17 input variables were as follows: age, sex, body weight, POD, ALT, AST, GGT, TBIL, ALB, GLB, SCr, HCT, tacrolimus daily dose, concomitant use of caspofungin or Wuzhi capsule, *CYP3A5* genotype of recipient, and *CYP3A5* genotype of donor. The output of the model was trough blood concentration C_0 of tacrolimus. Dose was referred to as the daily tacrolimus dose at 2 days before each C_0 measurement.

Before network training, data for the following variables were categorized as follows: male = 1, female = 2; concomitant use of caspofungin = 1, concomitant use of Wuzhi capsule = 2; genotype *CYP3A5*1*3* = 1, genotype *CYP3A5*1*1* = 2, and genotype *CYP3A5*3*3* = 3.

The tacrolimus blood concentrations from LT recipients and donors were randomly divided into 2 data sets using a digit table: the training set contained 232 samples; the test set, 57. In the training set, a feed-forward, fully connected supervised neural network was established based on the error back-propagation rule. The nonlinear activation function (rectified linear unit) was adopted for the transfer function between input and hidden layers as well as between hidden layers, while the linear activation function was used for the transfer function between hidden and output layers. The learning experiments were performed using deep learning. First, the training set was input to optimize parameters such as the number of layers and the number of neurons in the hidden layers. The network training rounds were 1000, and the learning rate was 0.01. All parameters were optimized using the Adaptive Moment Estimation optimizer, and mini-batch training was performed in each training process. The weights between neurons in each layer were then automatically optimized according to the function and the established learning rules for iteration to reduce the error between predicted and actual output values. The prediction error across many training cycles was minimized so that the ANN would achieve a certain accuracy level. The optimal weight was finally determined, and the nonlinear mapping model between clinicodemographic data and tacrolimus blood concentrations was obtained.

To validate the ANN model, we used 57 predicted tacrolimus blood concentrations from LT recipients as the test group. The correlation between estimated and measured values was evaluated using scatter plots. The mean prediction error (MPE) and mean absolute prediction error (MAE) were calculated using equations (1-3) to evaluate the ANN model's accuracy and precision, respectively:

$$\mathrm{PE} = C_{\mathrm{predicted}} - C_{\mathrm{actual}} \quad (1)$$

$$\mathrm{MPE} = \frac{1}{\mathrm{n}}\sum_{i=1}^{n}\mathrm{PE_i} \quad (2)$$

$$\mathrm{MAE} = \frac{1}{\mathrm{n}}\sum_{i=1}^{n}\left|\mathrm{PE_i}\right| \quad (3)$$

where $C_{\mathrm{predicted}}$ and C_{actual} were the predicted and measured blood concentrations of tacrolimus, respectively; and $\mathrm{PE_i}$ and$|\mathrm{PE_i}|$ were the prediction error and absolute prediction error of the *i*-th value, respectively. The ratio of absolute prediction error (|PE|) ≤3.0 ng/mL of the test set data was calculated based on previous studies.[29,30]

Explanatory Analysis of the Predicted Results Using SHAP

The SHAP framework is a post hoc interpretation method based on game theory and local interpretation that can be used to explain the predicted values obtained by ANN analysis and thereby reduce the "black box" lack of transparency that limits the usefulness of ANNs.[34] The SHAP framework can even assess potential synergistic effects between variables.[35] The contribution of each feature was assessed by converting the influence of each input variable on the prediction results into numerical SHAP values. Higher SHAP values indicated greater influence of the input variable on the output, and the positive or negative sign of SHAP values indicated whether the influence was positive or negative.[34]

All results were expressed in a histogram that ranked model features by importance, as well as in SHAP summary and dependence plots. Results were obtained using Python (version 3.8.3), Anaconda 3 (version 4.8.4, Anaconda, Inc.), and GraphPad Prism 8.0 software.

Data Processing and Analysis

Statistical analyses were performed using SPSS 26.0 (IBM, Chicago, IL, USA). The Shapiro-Wilk test was used to identify normally distributed continuous data, which were reported as mean ± standard deviation (SD). Skewed data were expressed as median (interquartile range), and categorical variables as numbers and/or percentages. Genotype frequencies were tested for deviations from Hardy-Weinberg equilibrium using the chi-squared test. Differences associated with $P < 0.05$ were defined as significant.

Table 1. Baseline Clinical Characteristics of Liver Transplant Recipients (n = 31).

Characteristic	Values
Male (n)	22
Age (years)	51.65 ± 9.30
Body mass index (kg/m^2)	24.88 ± 4.27
Postoperative days	12.84 ± 7.20
Tacrolimus daily dose (mg)	3.38 ± 1.12
Number of samples per patient	9
Tacrolimus C_0 (ng/mL)	5.66 ± 2.76
Combined medication	
Caspofungin	28
Wuzhi capsule	9
Caspofungin + Wuzhi capsule	1

Values are n or mean ± SD.
Abbreviation: C_0, trough concentration.

Results

Baseline Clinical Characteristics

A total of 32 LT recipients were initially enrolled in the study, but one 15-year-old female recipient was excluded due to her age. Among the remaining 31 recipients, 22 were male and 9 female with an average age of 51.65 ± 9.30 years and a body mass index of 24.88 ± 4.27 kg/m^2 (Table 1). The mean time after transplantation was 12.84 ± 7.20 days, and the average daily dose of tacrolimus was 3.38 ± 1.12 mg. The minimum daily dose was 1.0 mg, and the maximum daily dose was 7.0 mg. A mean number of 9 samples (range, 5-15) were collected from each patient to measure tacrolimus C_0.

Along with tacrolimus, 28 LT recipients were treated with caspofungin, 9 with Wuzhi capsule, and 1 with both caspofungin and Wuzhi capsule (Table 1). Furthermore, 3.23% of LT recipients carried *CYP3A5*1*1*; 29.03%, *CYP3A5*1*3*; and 67.74%, *CYP3A5*3*3*. The corresponding rates for LT donors were 3.23%, 25.81%, and 70.97% (Table 2). The distribution of *CYP3A5* rs776746 genotypes conformed to the Hardy-Weinberg equilibrium ($P > 0.05$).

ANN Modeling

A total of 17 variables with a significant effect on tacrolimus C_0 in LT recipients were used as inputs in the ANN model (Table 3). A total of 289 tacrolimus blood

Table 2. Distribution of *CYP3A5* rs776746 Genotypes Among Liver Transplant Recipients and Donors.

CYP3A5 genotype	n (%)	χ^2	*P*
Recipients (n = 31)			
*CYP3A5*1*1*	1 (3.23)	0.001	0.976
*CYP3A5*1*3*	9 (29.03)		
*CYP3A5*3*3*	21 (67.74)		
Donors (n = 31)			
*CYP3A5*1*1*	1 (3.23)	0.066	0.797
*CYP3A5*1*3*	8 (25.81)		
*CYP3A5*3*3*	22 (70.97)		

Table 3. Parameters of the Training and Test Sets.

Parameter	Training set (n = 232)	Test set (n = 57)
Tacrolimus C_0 (ng/mL)	5.67 ± 2.72	5.60 ± 2.93
Male	177	40
Age (years)	50.90 ± 8.93	50.84 ± 9.09
Body weight (kg)	70.52 ± 12.33	71.21 ± 12.17
POD (days)	13.20 ± 7.31	11.39 ± 6.59
Tacrolimus daily dose (mg)	3.40 ± 1.14	3.29 ± 1.05
ALT (U/L)	438.80 ± 367.84	469.35 ± 317.48
AST (U/L)	979.63 ± 1463.33	861.22 ± 1074.74
GGT (U/L)	86.60 ± 57.67	83.08 ± 53.14
TBIL (μmol/L)	72.47 ± 56.39	76.88 ± 50.70
ALB (g/L)	40.91 ± 5.64	40.88 ± 5.31
GLB (g/L)	17.68 ± 3.48	17.43 ± 3.10
SCr (μmol/L)	92.17 ± 35.50	92.25 ± 35.93
HCT (%)	0.33 ± 0.04	0.32 ± 0.04
Co-administration of caspofungin	108	27
Co-administration of Wuzhi capsule	23	5
Tacrolimus alone	102	25
Recipient *CYP3A5* genotype		
**1*1*	6	2
**1*3*	71	16
**3*3*	155	39
Donor *CYP3A5* genotype		
**1*1*	6	1
**1*3*	59	20
**3*3*	167	36

Values are n or mean ± SD.
Abbreviations: ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C_0, trough concentration; GGT, γ-glutamyl transpeptidase; GLB, globulin; HCT, hematocrit; POD, post-operative days; SCr, serum creatinine; TBIL, total bilirubin.

concentrations served as output, of which 232 were used as the training set and 57 as the test set (Table 3).

The final ANN model consisted of 5 layers including 1 input layer, which contained 17 neurons representing the 17 input variables, 3 hidden layers with 65 neuron units, and 1 output layer representing C_0 (Figure 1).

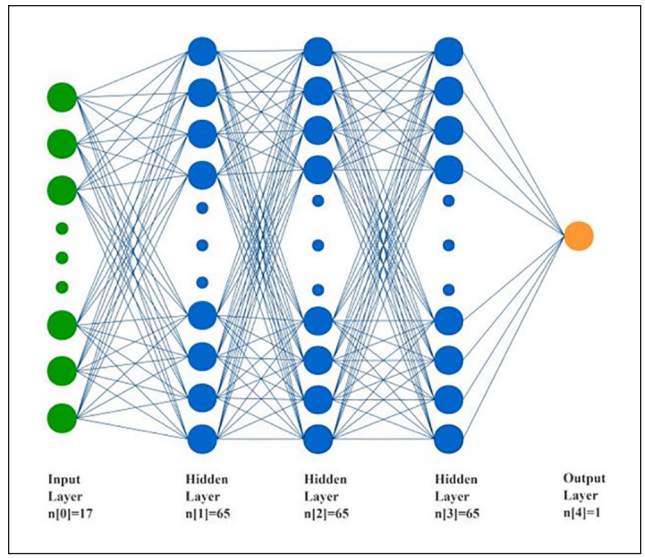

Figure 1. Structure of the ANN model used for the prediction of tacrolimus blood concentrations in liver transplant recipients. Green circles represent input neurons; blue circles, hidden layer neurons; and orange circles, output neurons.
Abbreviation: ANN, artificial neural network.

Table 4. Validation of the Artificial Neural Network Model Using the Test Set.

	Mean ± SD	Range
C_{actual} (ng/mL)	5.60 ± 2.93	0.90 to 12.00
$C_{predicted}$ (ng/mL)	5.87 ± 2.71	1.57 to 11.10
MPE (ng/mL)	0.27 ± 0.75	−1.68 to 2.72
MAE (ng/mL)	0.60 ± 0.52	0.02 to 2.72

Abbreviations: MAE, mean absolute prediction error; MPE, mean prediction error.

Model Validation

We validated the performance of the ANN model against the 57 tacrolimus C_0 values in the test set. The MPE and MAE values were 0.27 ± 0.75 and 0.60 ± 0.52 ng/mL, respectively, and the|PE| values of all predicted blood concentrations were ≤3.0 ng/mL (Table 4). Scatter plots indicated correlation coefficients between predicted and actual tacrolimus C_0 values of 0.9648 in the training test and 0.9677 in the test set (Figure 2).

Influence of Input Variables on Tacrolimus C_0

The average impact of each input variable on the model's predictions was quantified by calculating the mean absolute SHAP value [mean|SHAP value|]. The 7 variables with the greatest influence on tacrolimus C_0 were daily dose, POD, genotype and age of LT recipient, genotype of LT donor, GGT, and concomitant use of caspofungin (Figure 3).

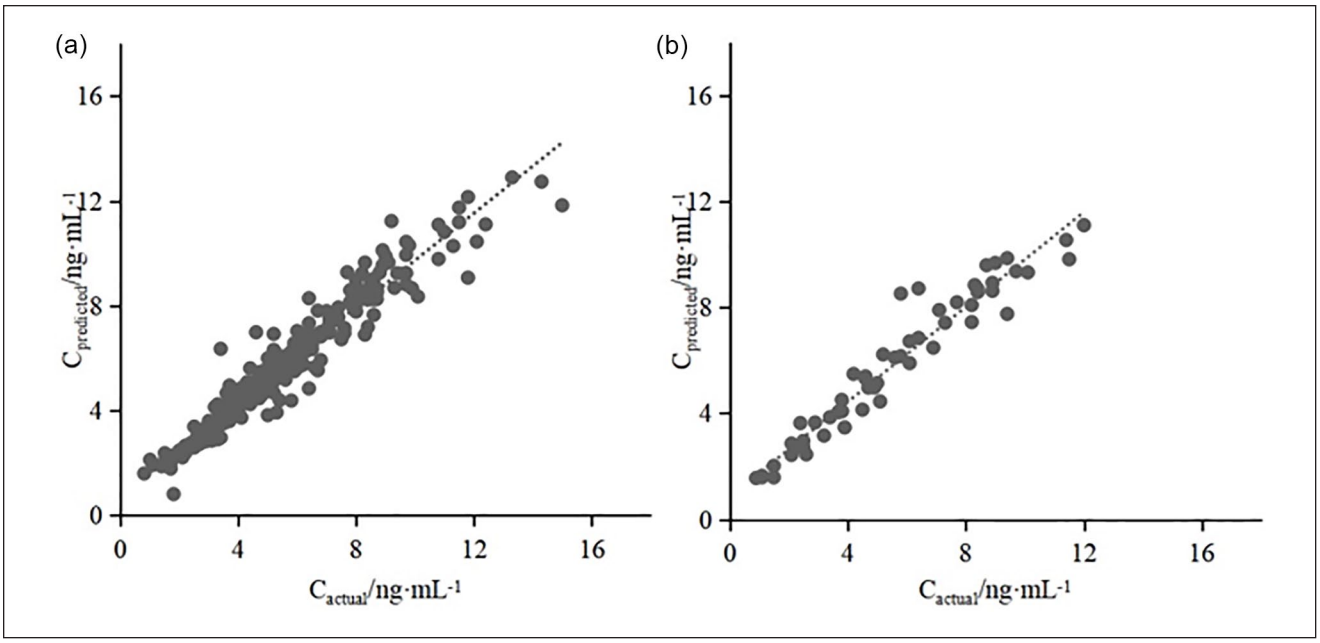

Figure 2. Scatter plots to assess correlation between predicted and actual tacrolimus concentrations in the (a) training set and (b) test set.

A SHAP summary plot was also drawn using the 57 samples of the test set to identify whether the effect of the input variables on C_0 was positive or negative. As shown in Figure 4, red dots in the bars of dose, recipient genotype, donor genotype, HCT, weight, TBIL, and GLB were distributed among positive SHAP values with a monotonically increasing trend, indicating that increases in these variables strengthened their influence on C_0. In contrast, red dots in the bars of age and concomitant use of caspofungin or Wuzhi capsule were distributed among negative SHAP values, indicating that increases in these variables weakened their influence on C_0. Other input factors, such as POD, GGT, and SCr, showed a cross-distribution of red and blue dots, suggesting that the SHAP value increased in some samples but decreased in others.

SHAP dependence plots were subsequently generated to identify the effect of the following 6 input variables on predicted tacrolimus C_0 values: dose, recipient genotype, age, donor genotype, caspofungin, and Wuzhi capsule (Figure 5). The results indicated that the predicted C_0 values increased when the daily dose was above 3 mg and the *CYP3A5* genotype for both LT recipients and donors was **3*3* (Figure 5a-b, d). In contrast, the predicted tacrolimus C_0 decreased when the *CYP3A5* genotype was **1*3* or **1*1* and recipient age was older than 48 years (Figure 5b-d). Moreover, SHAP values increased after the coadministration of caspofungin and Wuzhi capsule, suggesting that both agents have a positive effect on tacrolimus C_0 (Figure 5e-f).

Discussion

In this study, we developed an ANN model to predict the C_0 value of tacrolimus in the early phase after liver transplantation. The model takes into account using basic clinical characteristics, biochemical indexes, concomitant medication, genetic factors, and other data about the recipient and donor. The SHAP framework was also applied to evaluate the positive or negative influence of each input variable on predicted C_0 values, providing a theoretical basis for the individualized treatment of LT recipients with tacrolimus.

Our ANN model achieved a correlation coefficient between actual and predicted values of 0.9677, MPE of 0.27 ± 0.75 ng/mL, and MAE of 0.60 ± 0.52 ng/mL. Moreover, all predicted blood concentrations met the criterion of|PE| ≤ 3.0 ng/mL. A previous study aiming to predict tacrolimus blood concentrations in Caucasian LT recipients using ANN achieved MPE of −0.09 ng/mL and MAE of 1.74 (0.08-5.26) ng/mL, but only 84% of the predicted data met|PE| ≤ 3.0 ng/mL.[32] Our ANN model appears to be more precise, but less accurate, than that previously published model.

Previous ANNs applied to Chinese LT recipients[29-31] showed good prediction accuracy and precision for tacrolimus C_0, with MPE between −0.11 and 0.05 ng/mL and MAE between 1.93 and 2.14 ng/mL. Our ANN model appears to be more accurate and precise than those previously published models, which may reflect that our input variable was the cumulative dose of tacrolimus at 2 days before its concentration in the blood was determined.

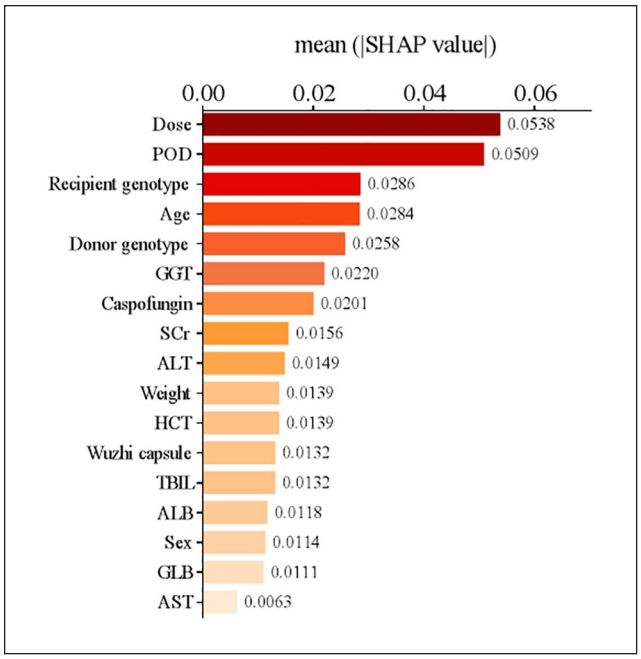

Figure 3. Ranking of model features based on their relative influence on the predictions of the artificial neural network model. Mean (|SHAP value|) refers to the average impact of each input variable on the model output (tacrolimus C_0). Darker colors and longer rows indicate a greater influence of the respective factor on the model's outcome.
Abbreviations: Age, age of liver transplant (LT) recipient; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; caspofungin, concomitant use of caspofungin; donor genotype, *CYP3A5* genotype of LT donor; dose, daily tacrolimus dose; GGT, γ-glutamyl transpeptidase; GLB, globulin; HCT, hematocrit; POD, postoperative days; recipient genotype, *CYP3A5* genotype of LT recipient; SCr, serum creatinine; sex, sex of LT recipient; SHAP, Shapley additive explanation; TBIL, total bilirubin; weight, body weight; Wuzhi capsule, concomitant use of Wuzhi capsule.

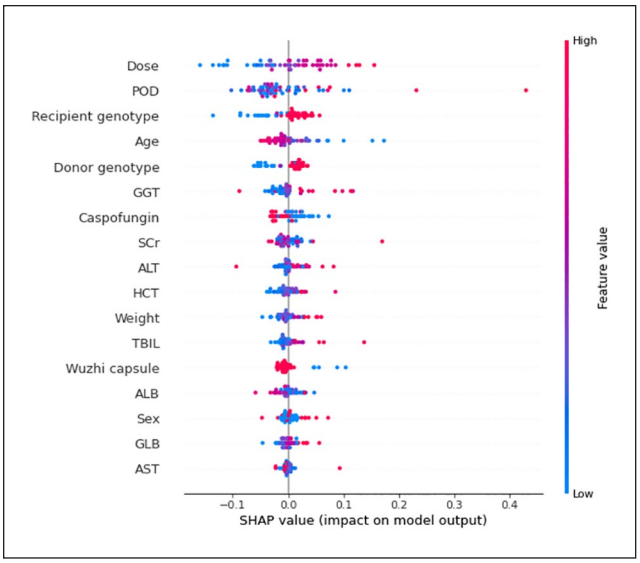

Figure 4. SHAP summary plot of 17 input variables of the artificial neuronal network. Each dot represents 1 sample in the test set.
Abbreviations: Age, age of liver transplant (LT) recipient; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; caspofungin, concomitant use of caspofungin; donor genotype, *CYP3A5* genotype of LT donor; dose, daily tacrolimus dose; GGT, γ-glutamyl transpeptidase; GLB, globulin; HCT, hematocrit; POD, post-operative days; recipient genotype, *CYP3A5* genotype of LT recipient; SCr, serum creatinine; sex, sex of LT recipients; SHAP, Shapley additive explanation; TBIL, total bilirubin; weight, body weight; Wuzhi capsule, concomitant use of Wuzhi capsule.

In addition, our model appears to offer better accuracy and precision than nonlinear mixed-effect models, which are most commonly used for predicting tacrolimus blood concentrations in LT recipients. One study using a mixed model predicted tacrolimus C_0 in 34 adult LT recipients with MPE of 0.56 ng/mL and root mean squared error (RMSE) of 4.81 ng/mL.[36] Another study based on a mixed model established a population PK model of tacrolimus in pediatric LT recipients with MPE of 1.4 ng/mL and RMSE of 6.7 ng/mL.[37]

Overtraining of ANNs may occur if too many irrelevant variables are introduced in the network, significantly reducing its generalizability.[29] Here, we initially selected 28 factors as input variables that may affect the blood concentration of tacrolimus in LT recipients according to previous studies.[29-31,38] After preliminary training, 17 variables were identified to have a significant impact on tacrolimus C_0 based on the average impact of each factor on the mean|SHAP value|.

SHAP analysis indicated that the most influential factors on C_0 were the daily tacrolimus dose and POD, consistent with previous studies where both variables were found to significantly affect the apparent clearance of tacrolimus and its concentration in the blood.[39] We found that dose had a positive effect on tacrolimus C_0, but we obtained no clear result for POD, probably because POD is an important covariate in both liver and renal transplant recipients,[40,41] and it can affect tacrolimus clearance in different ways depending on the phase after liver transplantation.[39,42-46] For example, some studies reported that clearance increased with increasing POD in the early postoperative period due to the gradual recovery of gastrointestinal motility and liver metabolic function,[26] whereas the C_0-dose ratio tended to decrease with increasing POD, suggesting that the drug dose had to be increased to maintain postoperative C_0 values over time.[39,42,43] Other studies found that tacrolimus clearance decreased with increasing POD over a longer time frame, perhaps because of increases in serum levels of HCT and ALB.[26,43-46]

CYP3A5 genotypes in LT recipients and donors significantly affect tacrolimus blood concentration[47] and tacrolimus clearance in recipients.[26] Nevertheless, previously established ANN models have not taken into account the effect of *CYP3A5*

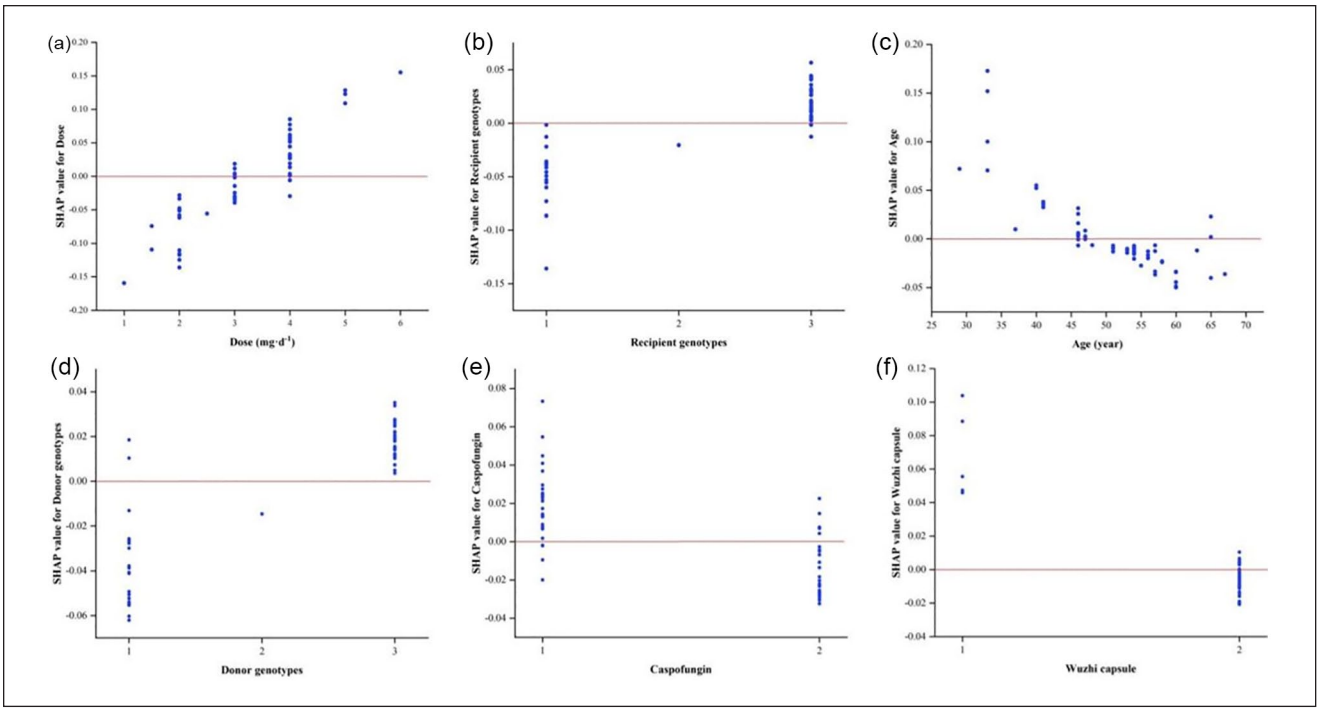

Figure 5. SHAP dependence plot showing the effect of 6 variables on predicted tacrolimus C_0. (a) The effect of dose on predicted tacrolimus C_0 values. (b) The effect of recipient genotype on predicted tacrolimus C_0 values. (c) The effect of age on predicted tacrolimus C_0 values. (d) The effect of donor genotype on predicted tacrolimus C_0 values. (e) The effect of caspofungin on predicted tacrolimus C_0 values. (f) The effect of Wuzhi capsule on predicted tacrolimus C_0 values.

Abbreviations: Age, age of liver transplant (LT) recipient; caspofungin, concomitant use of caspofungin; donor genotype, *CYP3A5* genotype of LT donor; dose, daily tacrolimus dose; recipient genotype, *CYP3A5* genotype of LT recipient; SHAP, Shapley additive explanation; Wuzhi capsule, concomitant use of Wuzhi capsule.

genotypes on C_0.[29-32] Here, we included *CYP3A5* genotypes from both LT recipients and donors as input variables and showed that tacrolimus C_0 was higher in recipients with a *CYP3A5*3*3* genotype than in recipients with *CYP3A5*1*3* or **1*1* genotypes, consistent with previous studies.[48,49]

Tacrolimus PK parameters are also affected by several demographic factors,[42] among which age and body weight have been extensively examined.[26] Here, we found that tacrolimus C_0 decreased with increasing age, especially above 48 years. However, previous studies reported no association between age and tacrolimus clearance in adult or pediatric LT recipients,[42,46] while another report showed that tacrolimus clearance decreased with age in pediatric LT recipients, probably due to the change in liver metabolic function during development.[50] Our results also suggested that C_0 increased with increasing body weight. According to the previous studies,[36,51] body weight influenced tacrolimus concentrations by affecting the apparent clearance based on whole blood and plasma concentrations and apparent volume of distribution. On the other hand, some studies failed to find any association between body weight and tacrolimus clearance or volume of distribution.[42,46,50] Future work should assesss the influence of body weight on an optimal tacrolimus regimen after liver transplantation.

Drug-drug interactions were also included as input variables in our ANN model. Predicted tacrolimus trough concentrations increased significantly after coadministration of caspofungin or Wuzhi capsule, consistent with previous reports.[25,52] This suggests that such coadministration may benefit patients on tacrolimus, yet the clinical use of caspofungin has been associated with certain adverse reactions, such as increased levels of transaminase or serum alkaline phosphatase.[18] Thus, coadministration may increase the risk of drug-induced liver damage and reduce tacrolimus metabolism. Indeed, at least one study reported that coadministration with caspofungin reduces the C_{max} of tacrolimus.[15] These considerations highlight the need for additional research to clarify the effect of caspofungin on tacrolimus PK.

Our model should be verified and extended in larger samples, particularly ones containing a greater proportion of women, which would allow rigorous assessment of the effects of patients' sex on tacrolimus PK. Thus, the relatively small sample size is one of our limitations, and the developed model should be verified and extended in larger samples. Second, a prediction model with clinical practical value should comprehensively consider all aspects of the influencing factors, such as the actual patient behavior

related to medication adherence although the modeling process was a retrospective study.

Conclusion and Relevance

Overall, we showed that the C_0 value of tacrolimus in LT recipients can be predicted rapidly and accurately using an ANN model with a small number of input variables. In order to achieve the target blood concentration as soon as possible in the early postoperative period, the influence of daily drug dose, age of LT recipient, and *CYP3A5* genotypes in both recipient and donor should be considered, and the blood concentration of tacrolimus should be closely monitored when combined with caspofungin or Wuzhi capsule.

Declaration of Conflicting Interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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