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Effect of early post-hematopoietic stem cell transplant tacrolimus concentration on transplant outcomes in pediatric recipients: One facility's ten-year experience of immunosuppression with tacrolimus

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ABSTRACT

Acute graft-versus-host disease (GVHD) is a common life-threatening complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT), ranking as the second leading cause of death among recipients, surpassed only by disease relapse. Tacrolimus is commonly used for GVHD prophylaxis, but achieving therapeutic blood levels is challenging, particularly in pediatrics, due to the narrow therapeutic window and the high interindividual variability. The retrospective study conducted at IRCCS "Burlo Garofolo" in Italy aimed to assess the impact of early post-HSCT tacrolimus levels on transplant-related outcomes in pediatric recipients. The population pharmacokinetic model (POP/PK) was set up to describe tacrolimus pharmacokinetics. Elevated tacrolimus (>12–15 ng/ml) levels within the initial weeks post-HSCT are associated with reduced post-transplant infections ($p < 0.0001$) and decreased incidence of early transplant-related events ($p < 0.01$), including a lower incidence of acute GVHD ($p < 0.05$ on day 0). High tacrolimus exposure can lead to an increased risk of chronic GVHD ($p < 0.0001$) and reduced overall survival ($p < 0.01$). Personalized dosing and therapeutic monitoring of tacrolimus are crucial to ensure optimal outcomes. POP/PK could help achieve this goal, giving us a model by which we can balance immunosuppression while looking at the patient's general well-being and providing the necessary treatment.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a standard approach for definitively treating a broad range of malignant and non-malignant hematologic diseases. However, post-transplant immunosuppression is necessary to limit the risk of graft-versus-host disease (GVHD) and graft rejection, which are caused by excessive alloreactivity by donor and host T cells [1]. GVHD occurs in two forms, acute and chronic, which have distinct clinical features that can sometimes present concomitantly or independently of the time after transplant. For many years, any clinical manifestation of GVHD before day 100 was defined as acute GVHD (aGVHD), and any GVHD symptoms after day 100 were considered chronic GVHD (cGVHD) [2]. After 2005, when the National Institutes of Health (NIH) Consensus Conference proposed new diagnostic criteria for GVHD, the distinction between acute and chronic forms was based purely on clinical manifestations without any reference to the onset time [3]. While aGVHD is caused by donor T cells reacting to mismatched host polymorphic histocompatibility antigens, cGVHD shares some traits with autoimmune diseases. It can arise de novo or as an extension of aGVHD [4]. Effective prevention of aGVHD and cGVHD is critical for successful allo-HSCT because it remains a major cause of non-relapse mortality in HSCT recipients [5].

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Table 1
Demographics and clinical data of HSCT patients/the study population. MCHT, myeloablative chemotherapy; TBI, total body irradiation; IQR, interquartile range.

Demographics and clinical data	Whole Cohort (n = 125)
Age at transplant	
median (IQR), years	8 (5–13)
Gender, number	
Male, n°	78
Female, n°	47
Indication for HSCT, number (%)	
Acute Lymphoblastic Leukemia/ Acute Myeloid Leukemia (ALL/AML)	67 (53.6)
Myelodysplastic syndrome (MDS)	21 (17)
Non-malignant disease	28 (22)
Solid tumor	9 (7)
Allogeneic donor type, number (%)	
Matched Related Donor	39 (31)
Matched Unrelated Donor	66 (53)
Haploidentical Donor	20 (16)
Stem cell source, number (%)	
Bone marrow	68 (54)
Peripheral blood stem cell (PBSC)	57 (46)
Myeloablative conditioning regimen, number (%)	
MCHT-based	79 (63)
TBI-based	46 (37)

Calcineurin inhibitors, such as tacrolimus and cyclosporine, are used for GVHD prophylaxis. The use of immunosuppressive drugs is essential to reduce the incidence and severity of GVHD despite the risks and potential complications associated with their use [6]. Some studies reported that the incidence of aGVHD is lower with tacrolimus than with cyclosporine treatment, although the overall survival rates with both drugs are similar [7,8].

Tacrolimus, a macrolide immunosuppressant, is primarily metabolized by cytochrome 3A4 and 3A5 (CYP3A4/5) in the liver and gut and eliminated through biliary excretion. In the blood, tacrolimus is highly bound to erythrocytes, albumin, and alpha-1-acid glycoprotein [9]. The pharmacokinetics of tacrolimus is unique. After systemic administration, it is distributed mainly in red blood cells (RBCs), and its binding capacity is around 440 ng/mL RBCs. Because tacrolimus concentration is commonly measured in whole blood, variations in hematocrit level affect its concentration [10–12]. Tacrolimus post-allo-HSCT is administered by continuous intravenous infusion, beginning the day before allo-HSCT at a dose of 0.03 mg/kg/day [13]. Tacrolimus exhibits considerable inter- and intra-patient pharmacokinetic variability, and current dosing recommendations are individualized to include some

Table 2
Population values of pharmacokinetic parameters obtained by the final POP/PK model. S.E., standard error of the mean; R.S.E., relative standard error; V_{pop}, Cl_{pop}, population volume of distribution and systemic clearance, respectively; IIV: interindividual variability; IOV, interoccasion variability.

Parameter	VALUE	S.E.	R.S.E.(%)
V_{pop} (L)	58.05	3.64	6.27
Cl_{pop} (L/h)	1.71	0.066	3.86
IIV_V	0.53	0.044	8.36
IIV_{Cl}	0.42	0.029	6.94
IOV_{Cl}	0.25	0.034	13.6
Proportional error (%)	0.054	0.0013	2.4

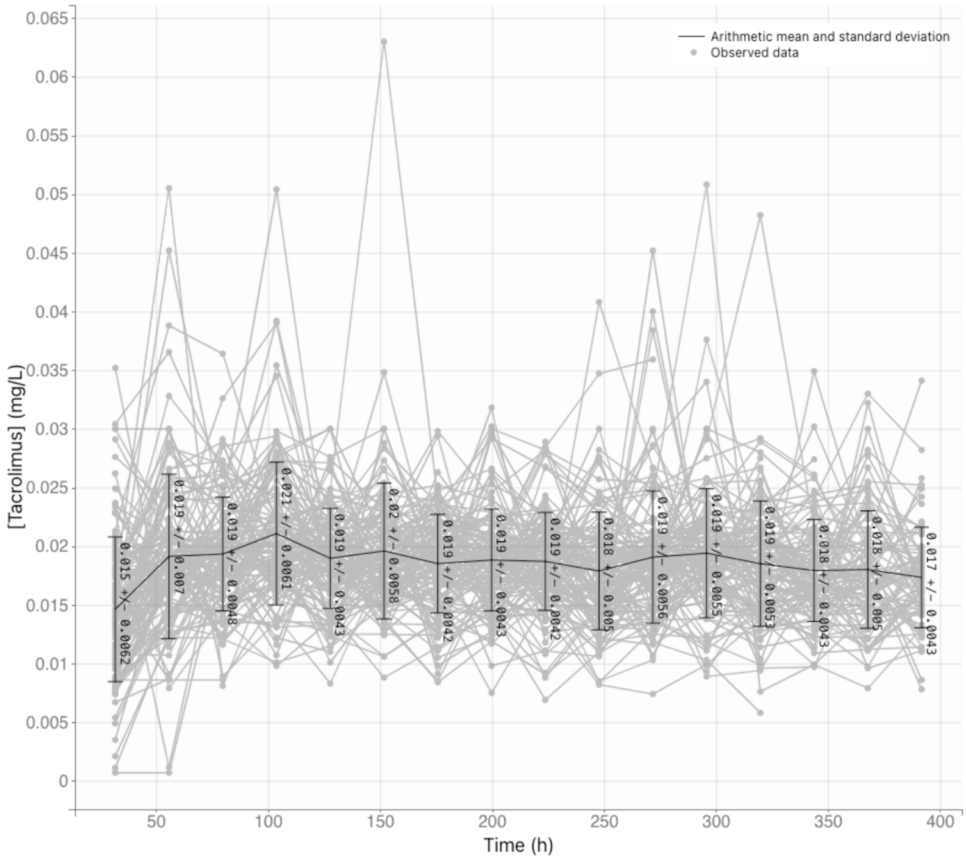

Fig. 1. Blood concentrations of TAC over the first 2 weeks of treatment in 125 pediatric patients. The TDM protocol guided TAC dosing over the first 14 days. Indeed, in those patients who experienced sudden increases of blood concentrations, prompt dose modifications reduced the fluctuations of TAC blood concentrations. Symbols, measured blood concentrations; black solid line and segments, aritmetic mean and standard deviation (SD) values; numbers, mean and SD values at each TDM time point.

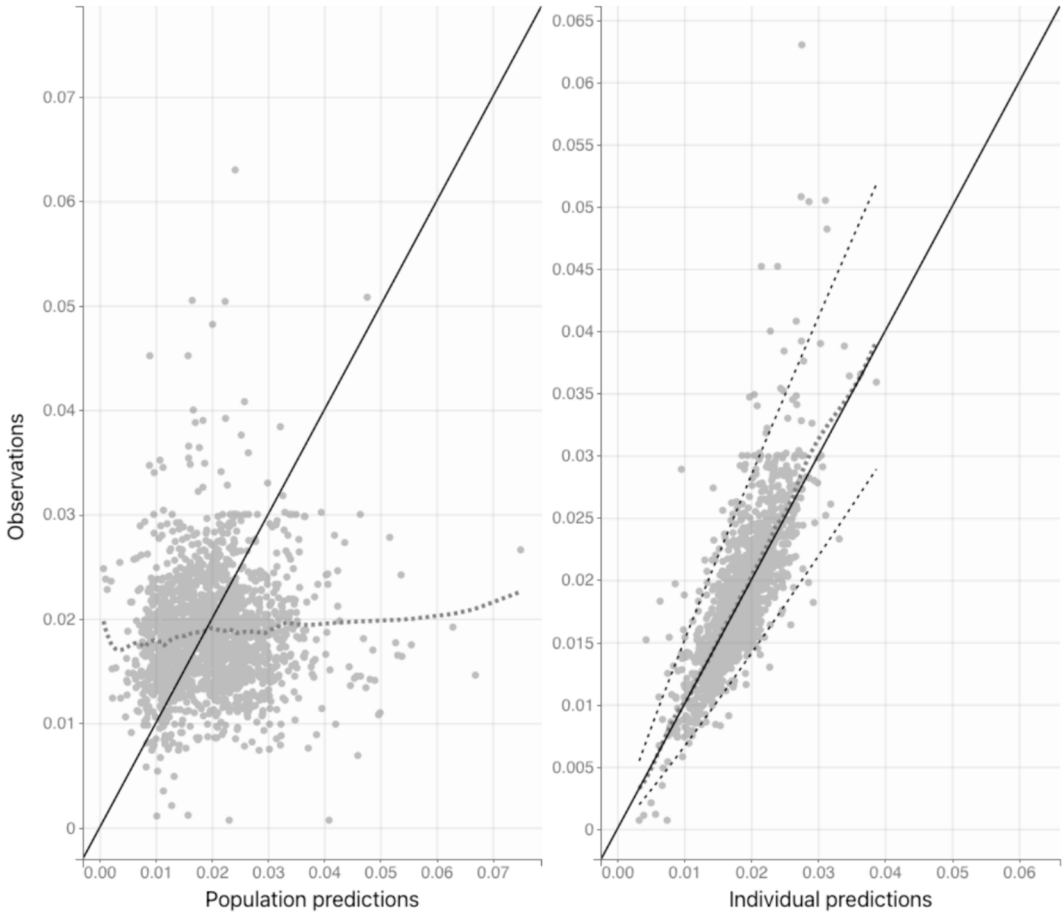

Fig. 2. Goodness-of-plot graphs. Correlation between observed values of tacrolimus blood concentrations, population (left), and individual (right) prediction. Individual prediction values were highly correlated with observed ones, demonstrating a good performance of the POP/PK final model. Symbols, tacrolimus blood concentration values; black solid line, line of identity; dashed lines, 90% confidence interval; black dotted line, spline.

patient factors, such as the patient's age, race, time post-HSCT, and concurrent immunosuppressive therapy, to ensure the expected therapeutic response [14]. It has a narrow therapeutic index: high concentrations increase adverse effects such as nephrotoxicity, neurotoxicity, hypertension, posttransplant diabetes mellitus, gastrointestinal disorders, and it is associated with malignancy and infections. In contrast, low concentrations are associated with an increased risk of aGVHD and acute rejection [15]. For all of these reasons, therapeutic drug monitoring (TDM) protocols for tacrolimus are widely adopted.

The population pharmacokinetic model (POP-PK) is the most widely used method to investigate and identify inter- and intra-individual variability in drug pharmacokinetics, even in special populations such as pediatric patients [16–18]. Noteworthy, POP-PK may take advantage of TDM protocols, ensuring good analytical performance of PK analysis even in the absence of dense blood sampling and allowing the early individual evaluation of drug exposure and its relationship with clinical outcome.

Our study aimed to investigate the factors affecting tacrolimus blood concentrations and their impact on transplant outcomes in pediatric patients undergoing allo-HSCT. The study is based on constructing a POP-PK model for tacrolimus intravenous continuous infusion to evaluate every patient's drug exposure over time. Predicting the individual dose value regarding the patient's covariates could be very useful in improving clinical outcomes and patient quality of life and, ultimately, reducing costs for the National Health System.

2. Materials and methods

2.1. Study design

The retrospective single-center observational study was conducted at the Pediatric Bone Marrow Transplant Center of the Institute for Maternal and Child Health—IRCCS Burlo Garofolo, Trieste, Italy, from January 2012 to December 2022. The Institutional Review Board reviewed and approved the study (Unique Protocol ID GEN/INT 0001973), and the study was conducted following the Declaration of Helsinki (Clinicaltrials.gov code: NCT06080490). Written informed consent for using any clinical data in research was obtained from parents or guardians. The medical records of all patients who underwent allo-HSCT were analyzed individually and anonymously.

2.2. Study population and data collection

From January 2012 to December 2022, 125 patients aged from 0 to 17 years, affected by hematological malignancies and hematological non-malignant diseases who underwent tacrolimus immunosuppressive prophylaxis during allo-HSCT, were included in this study. We excluded the patients $\geq$ 18 years old at the time of transplantation, second or subsequent transplant attempt, nonmyeloablative conditioning, and who had performed GVHD prophylaxis with cyclosporine. Patient characteristics were collected at the time of transplant, including age, gender, total body weight, height, and body mass index (BMI). Among the anamnestic data, transplant-specific characteristics included primary disease, type of donor, source of stem cells, and conditioning

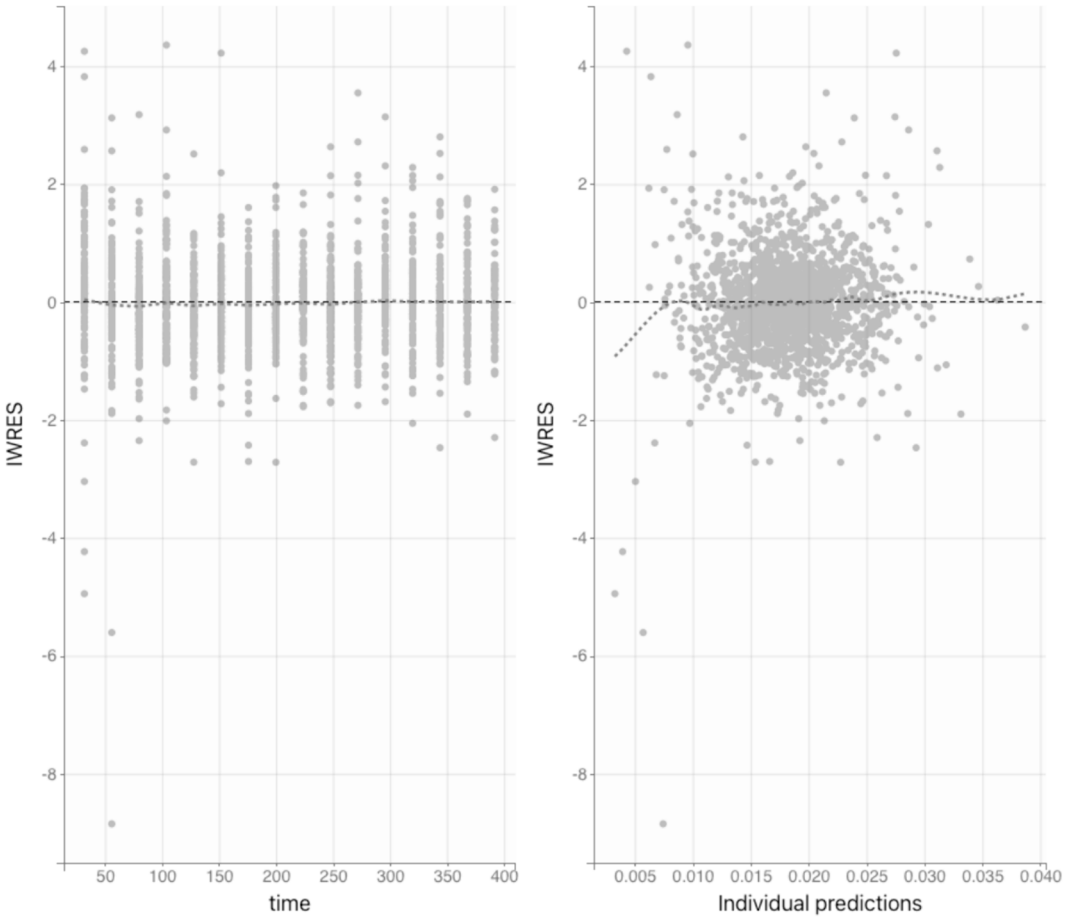

Fig. 3. Goodness-of-plot graphs. Correlation between individual weighted residual (iWRES) values and time (left) and individual predictions (right). Splines (the continuous gray lines) do not show any trend in both graphs, sustaining the good performance of the final POP/PK model. Symbols, TAC blood concentration values; black dashed lines, lines of identity; black dotted lines, splines.

regimen. The data regarding transplant-related outcomes, including overall survival (OS), transplant-related mortality (TRM) at 12 months, relapse-free survival (RFS), neutrophil and platelet recovery, donor chimerism, and red blood cell transfusions, were collected. OS was the time from allo-HSCT until death from any cause, while TRM was defined as the time from allo-HSCT to death from any causes, including transplant-related complications and relapse.

The relapse rate was calculated as the number of patients experiencing disease relapse relative to the total number of transplant recipients (affected by hematological malignancies). Both acute and chronic GVHD were assessed using standardized criteria, as described by a published staging system and clinical grading criteria [19].

Additionally, data on medications taken after the transplant, such as mycophenolate mofetil, steroids, pantoprazole, and voriconazole, were recorded. The occurrence of early transplantation-related complications (TRC), categorized in endothelial TRC [i.e., veno-occlusive disease (VOD), capillary leak syndrome, cytokine release syndrome (CRS), and aGVHD, beyond cGVHD] and not-endothelial TRC (infections, and post-transplant lymphoproliferative disorder), were also registered.

Laboratory tests were collected retrospectively from medical records: red blood cell (RBC) counts, hemoglobin (Hb), hematocrit (HCT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total and direct bilirubin, serum albumin, serum creatinine, and C-reactive protein (CRP) levels.

2.3. Tacrolimus administration and therapeutic drug monitoring

Tacrolimus was administered as a continuous intravenous infusion from day −1 to more or less day + 15 throughout all mucositis duration to move on to oral administration. The initial dose was 0.03 mg/kg/day, with daily dose adjustments, to reach target blood concentrations within the 15–20 ng/ml range in the first post-transplant month. Tacrolimus dose administered, dose adjustment, and tacrolimus blood concentrations were considered within the study. Whole-blood trough concentrations (C_0) of tacrolimus were measured daily as clinical practice required and collected for TDM records. Tacrolimus' blood concentrations were determined using Thermo Scientific™ QMS™ Tacrolimus Immunoassay (Microgenics Corporation, 46,500 Kato Road Fremont, CA 94538 USA). As per clinical practice, all children with a high value of the C_0/daily dose ratio (C_0/D) were subjected to pharmacogenomic analyses by real-time PCR assays investigating the presence of poor-metabolizer genotypes for the CYP isoforms *CYP3A5*3*, *CYP3A4*22*, and for those *ABCB1* genotypes (i.e., c.1236C > T, c.2677G > T/A, c.3435C > T loci) associated with decreases in transmembrane transport.

2.4. Pharmacometric analyses

Population pharmacokinetic (POP/PK) analyses were performed according to nonlinear mixed-effect modeling using the software Monolix v.2021R2 (Lixoft, Antony, France). According to the classical procedure, the development of the models was based on several criteria that consider both numerical and graphical outputs as follows. A significant decrease in the objective function value (OFV) of ≥ 3.84/≥ 6.83 units in forward/backward exclusion, respectively, primarily guided the choice of best models. The goodness of pharmacokinetic parameter values, their corresponding relative standard error values (RSE%), and the

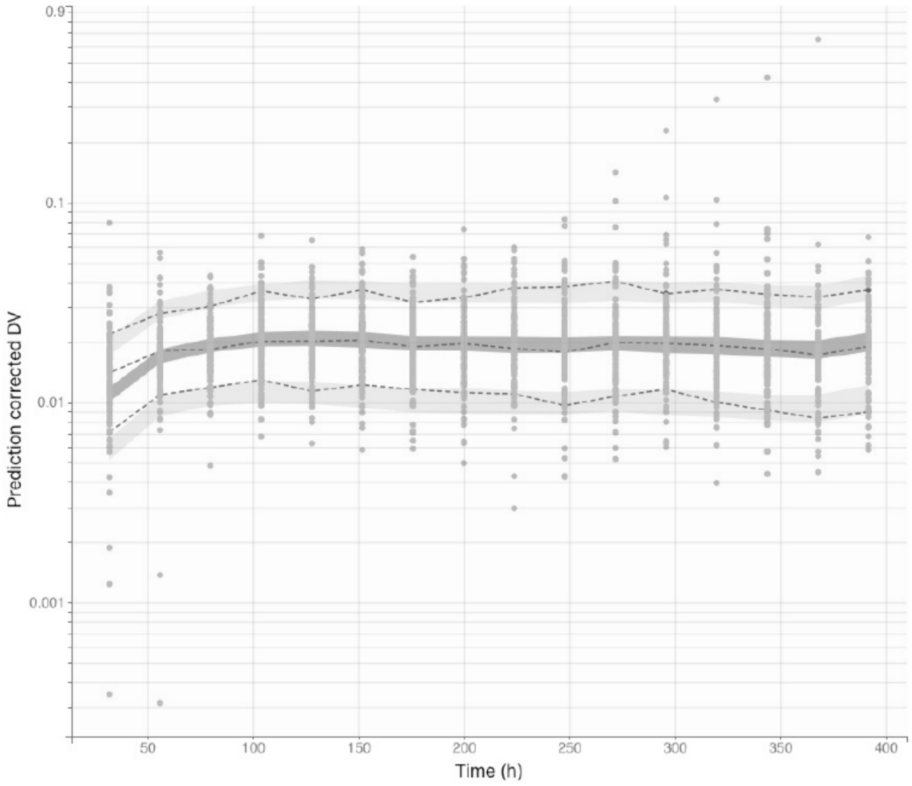

Fig. 4. Prediction corrected visual predictive check (pcVPC) over the first 16 days of treatment with tacrolimus at the starting dose of 0.03 mg/kg/day as continuous i.v. infusion. The plot shows the good capability of the final model to fit drug blood concentrations in the enrolled patients over time. Symbols, observed tacrolimus concentrations; dashed lines, empirical 5th, 50th and 95th percentiles; light gray areas, 95 % confidence intervals of 5th and 95th percentiles; dark gray area, 95 % confidence intervals of 50th percentile.

covariance matrix findings were also evaluated.

The models were judged based on the correlation between observed values and population/individual prediction, the distribution of individual weighted residuals (iWRES), and their correlation with time (structural model) and observed values (stochastic model). Internal validation was performed by prediction-corrected visual predictive check (pc-VPC) on 10,000 resampled databases.

The pharmacometric analyses evaluated 1- and 2-compartment models, with different error models, and also considered interindividual (IIV) and interoccasion (IOV) variability for all pharmacokinetic parameters. Categorical (i.e., gender, blood transfusion) and continuous covariates (i.e., age, body weight, hematocrit, red blood cell count) were tested for their influence on tacrolimus pharmacokinetics. In particular, the covariate value of each patient (Cov_i) was included in the model after normalization to the median population value (Cov_{median}) according to a power function as follows:

$$\theta_i = \theta_{pop} \times \left(\frac{Cov_i}{Cov_{median}}\right)^{\beta}$$

where θ_i represents the individual value and θ_{pop} the population one, the allometric exponent β was fixed at 0.75 and 1 for clearance and volume of distribution, respectively. In the case of categorical covariates (i.e., gender), the following relationship was adopted:

$$\theta_{i,Males} = \theta_{pop} \text{and } \theta_{i,Females} = \theta_{pop} \times e^{\beta}$$

where β is the exponent needed to adjust the parameter value concerning the reference group. Only covariates that significantly improved model performances according to the above criteria were retained in the model.

For pharmacokinetic/pharmacodynamic analyses, the area under the time/concentration curve (AUC) mean value for each patient was obtained as the mean ratio between the daily dose (in mg) and the Cl value.

2.5. Statistical analysis

We presented continuous variables through the mean value (and standard deviation, SD) for normally distributed variables and the median value (plus interquartile range, IQR) for the non-normally distributed variables. Qualitative variables were expressed as frequencies and percentages. Patients' demographic and clinical characteristics were compared using the chi-square test or Fisher's exact test for categorical variables. In contrast, the Mann–Whitney rank-sum test was used for continuous variables. Receiver operating characteristic (ROC) curves were then constructed for tacrolimus concentrations to determine the optimal cutoff, using the Youden index to predict clinical response. The sensitivity, specificity, and positive and negative predictive values were analyzed for the cutoff point. The chi-square test was used to investigate the relationship between tacrolimus exposure (AUC) on day 0 and day + 8 and transplant-related outcomes. Since the C_0/D ratio captures tacrolimus single-dose exposure, the AUC is used as an optimal indicator of tacrolimus pharmacokinetic complexity to better reflect the effect on transplant-related outcomes.

A nonparametric test evaluated the association between the tacrolimus C_0/D ratio, the dependent, and the independent variables. The nonparametric Kruskal-Wallis test was applied in the univariate analysis for continuous variables.

Multivariate analysis was performed to test the independence of the significant effects identified in univariate analyses. For this multivariate analysis, generalized linear models of the appropriate family were used, combining significant covariates in the univariate analysis as the independent variables. All statistics and graphs were obtained using the R

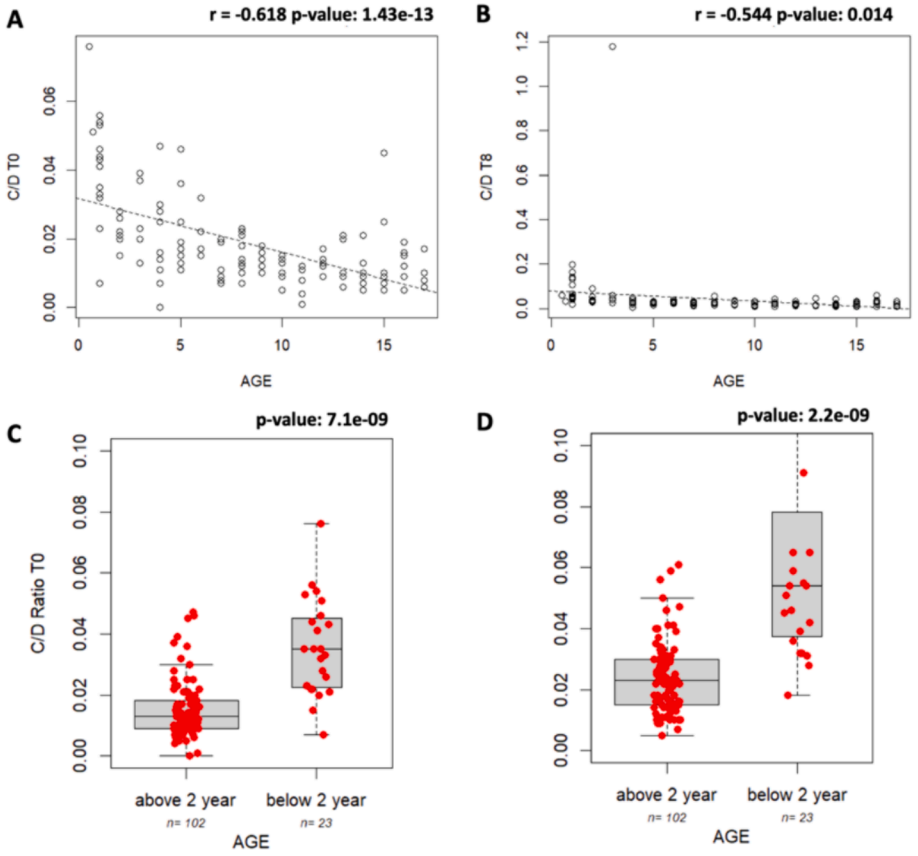

Fig. 5. Correlation between changes in tacrolimus concentration/dose (C_0/D) ratio and age. A-B) C_0/D ratio decreases as a function of age. Spearman correlation demonstrates a statistically significant correlation with age (p-value < 0.05). C-D) Boxplots comparing the concentration/dose ratio of tacrolimus (C_0/D ratio) at day 0 (T0, panel C), day 8 (T8, panel D). The bold horizontal line represents the median value.

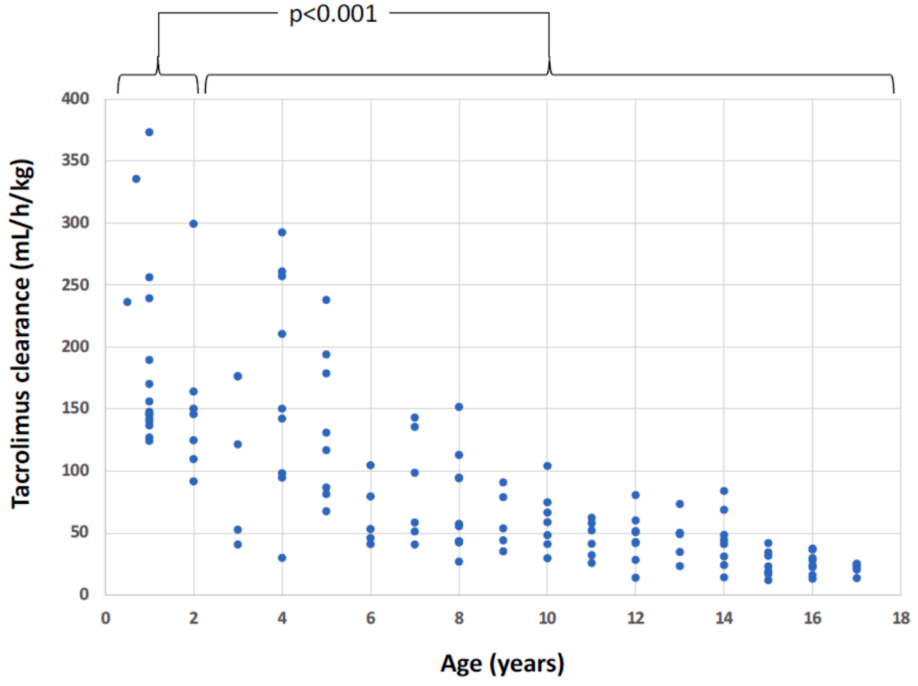

Fig. 6. Individual weight-normalized clearance values of tacrolimus compared with patients' age. The graph clearly shows an inverse correlation between tacrolimus elimination and patients' age. A statistically significant difference has been detected in children aged ≤ 2 or > 2 years.

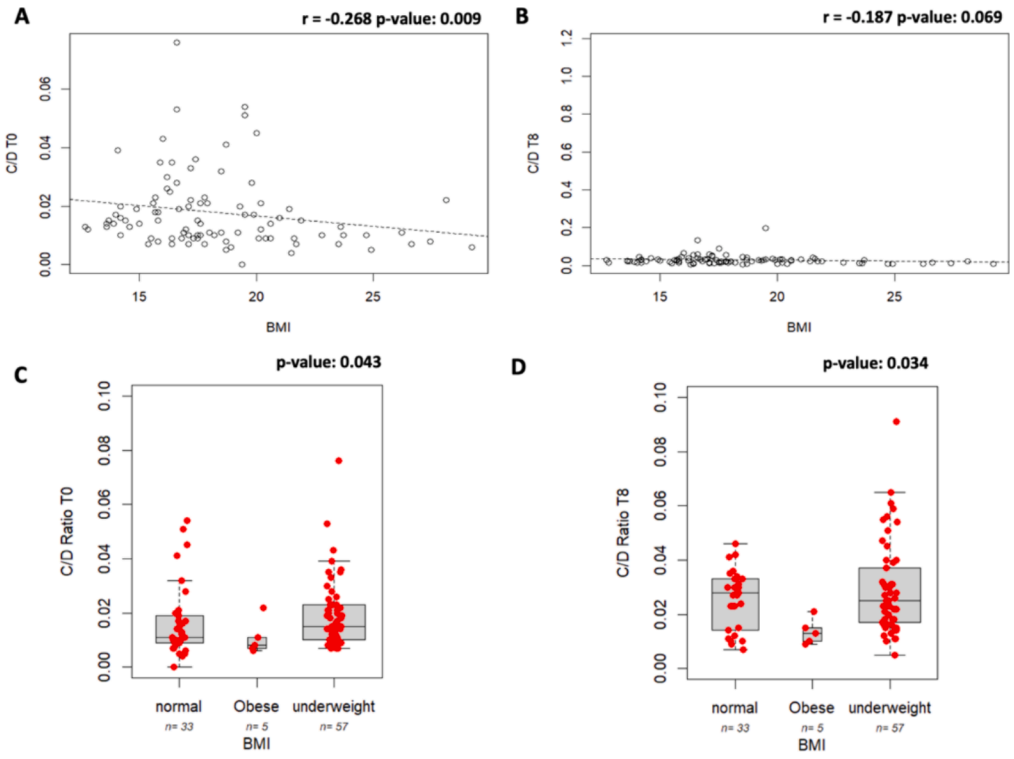

Fig. 7. Correlation between changes in tacrolimus concentration/dose (C_0/D) ratio and BMI. A-B) C_0/D ratio decreases as a function of BMI. Spearman correlation demonstrates a statistically significant correlation with BMI. C-D) Boxplots comparing the concentration/dose ratio of tacrolimus (C_0/D ratio) at day 0 (T0, panel C) and day 8 (T8, panel D) to patients' BMI (<18.5 underweight; ≥18.5 and < 25 normal; > 25 obese). The bold horizontal line represents the median value.

Studio software (R version 3.4.2) and Monolixsuite v.2021R2. Differences were considered significant when *p*-value < 0.05.

3. Results

3.1. Patients

The study included one hundred twenty-five pediatric patients (78 males and 47 females) who underwent an allo-HSCT from 2012 until 2022. The median age at HSCT of the entire cohort was eight years (IQR, 5 – 13). Underling diagnoses were grouped into acute leukemia (54 %), myelodysplastic syndrome (17 %), non-malignant disease (22 %), and solid tumor (7 %). Overall, the most frequent diagnosis within the acute leukemia subgroup was acute lymphoblastic leukemia (63 %), while the most frequent diagnosis in the non-malignant subgroup was thalassemia major (54 %). Detailed patient demographics and transplant characteristics are shown in Table 1.

The OS of the pediatric patients' cohort was evaluated during the post-transplant period (median = 6.13 years (IQR: 3.93 – 8.06 years)).

3.2. Therapeutic drug monitoring

The database available for pharmacokinetic analyses included 1897 tacrolimus blood concentrations collected over the first 16 days of treatment (median number of occasions, 16; range 11–16) in 125 patients. Among the 125 patients, the mean (±SD) and median values of tacrolimus daily doses were 0.032 (±0.010) and 0.030 mg/kg, respectively. The analysis of TDM results confirmed that prompt individual dose adjustment permits maintaining blood concentrations within the therapeutic range (Fig. 1), reducing the number of fluctuations in tacrolimus concentrations (Figure S1). Fifteen patients (12 %), most overexposed to tacrolimus, were diagnosed with ABCB1 transport system deficiency (i.e.,), associated or not with genetic variations in *CYP3A5* (i.e., *3/*3). The interindividual variability in daily doses was largest during the first two days of treatment due to the need for dosing adjustment (CV% >140 %) and progressively diminished from the third days onward (CV%, 38.1–57.8 %, Figure S2). This interindividual variability in daily doses reflected the interindividual and intra-individual variability detected by the final POP/PK model (see next paragraph). When combined with the C_0/D ratio, those data showed that the C_0/D ratio increased until a plateau was achieved approximately five days after treatment started (Figure S3).

3.3. POP/PK model

The final model was a 1-compartment model with a proportional error model, IIV on tacrolimus Cl and V, IOV on CL, and body weight with allometric scaling on both Cl and V. None of the remaining covariates nor IOV on V performed better than body weight in improving the model performance. Hence, they were excluded. In particular, the progressive introduction of proportional error (ΔOFV = -184.36), IIV on both Cl (−1867.65) and V (−587.11), IOV on Cl (−328.32), and finally, body weight on Cl (−155.88) and V (−485.43) significantly improved the model based on numerical and graphical outputs with respect to the initial first 1-compartment model with additive error. Table 2 shows the results of the final model, with all RSE values below the threshold of 15 %. It is worth noting that the IOV_{Cl} value was lower than the IIV_{Cl}, thus sustaining the role of TDM protocols in this patient setting.

The goodness-of-fit plots presented in Fig. 2 confirm the performance of the final model, in agreement with the numerical results shown in Table 2. Indeed, observed versus individual predicted values showed a good correlation with a limited number of values (4.96 %) outside the 90 % confidence interval. Moreover, the model was characterized by the absence of any error when individual weighted residuals (iWRES) were plotted against observed tacrolimus concentrations and time (Fig. 3). The internal validation of the POP/PK analyses performed by pcVPC showed the model's capability to predict the observed value over the first 16 days of treatment (Fig. 4), except for the first two days in the

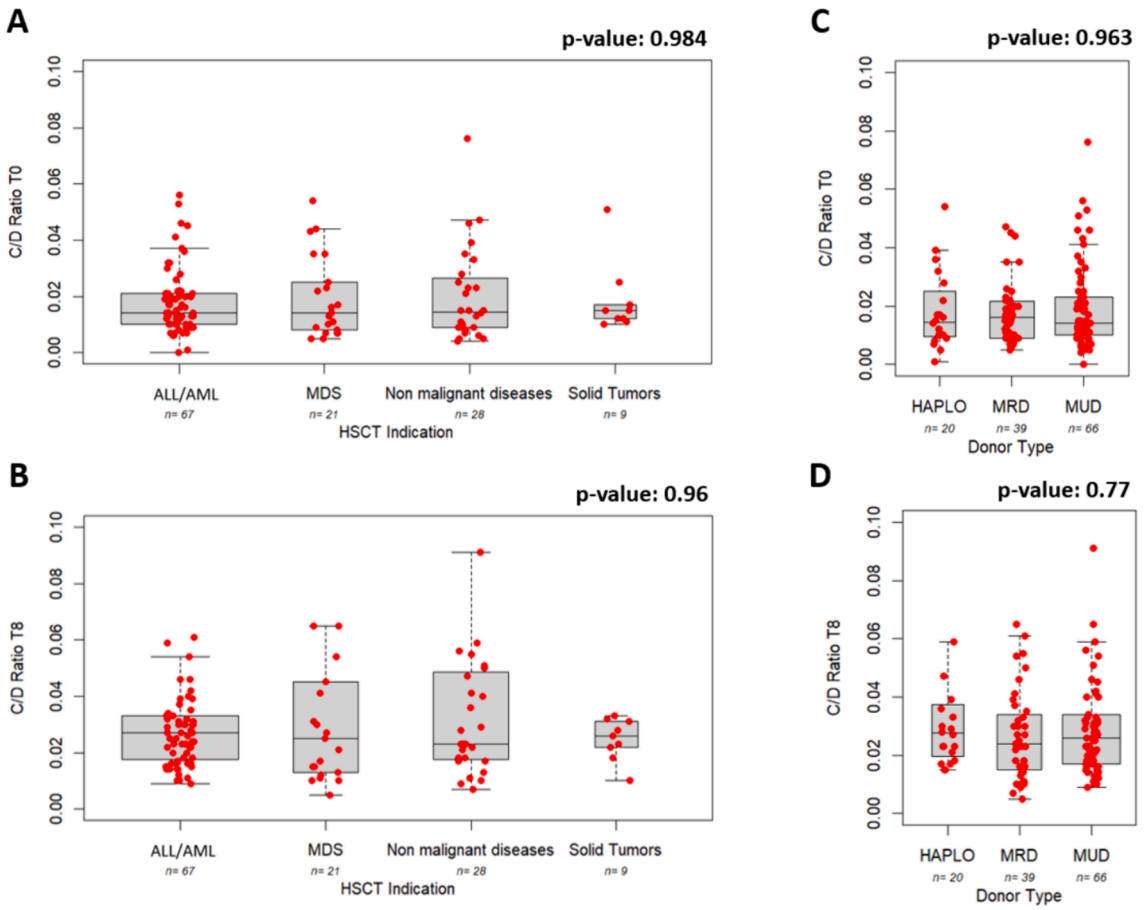

Fig. 8. Correlation between tacrolimus concentration/dose (C_0/D) ratio and allo-HSCT medical indication. A-B) Boxplot comparing tacrolimus concentration/dose ratio (C_0/D ratio) and different diseases requiring allo-HSCT. C-D) Boxplot comparing tacrolimus concentration/dose ratio (C_0/D ratio). The bold horizontal line represents the median value. ALL/AML, Acute Lymphoblastic Leukemia/Acute Myeloid Leukemia (ALL/AML); MDS, Myelodysplastic syndrome; Haplo, Haploidentical donor; MRD, Match Related Donor, MUD, Matched Unrelated Donor.

presence of the largest IIV observed.

3.4. PK/PD analysis, demographic and transplant-specific characteristics

In Fig. 5, the tacrolimus C_0/D ratio appears to be inversely correlated with the age of the patients, both at day 0 (Panel A; r = − 0.618, p = 1.4*e-13) and day + 8 (Panel B; r = − 0.544, p = 0.014). We observed that the concentration decreases with patients' increasing age. This occurrence is most pronounced on day 0. Stratifying patients according to the attainment of hepatic cytochrome 3A and renal maturity, which is reached at the age of 2 years [20,21], we observed that in patients younger than two years, the C_0/D ratio was found to be higher than in patients who had already reached renal maturity at both day 0 (Panel C; 0.035 vs. 0.012, p = 7.1*e-09) and day + 8 (Panel D; 0.053 vs 0.023, p = 2.2*e-09). Fig. 6 displayed statistically significant differences in drug clearance (p = 1.5*e-09) in patients ≤ 2 years old (Cl [median ± SD]: 147.62 ± 74.23 mL/h/kg) compared to the oldest group (Cl [median ± SD]: 50.33 ± 58.59 mL/h/kg). In Fig. 7A-B, the tacrolimus C_0/D ratio appears inversely correlated with BMI at day 0 (Panel A: r = − 0.268, p = 0.009). A negative trend is shown at day + 8 (Panel B: r = − 0.187, p = 0.069). In Fig. 7C-D, we observed that in obese patients, the drug C_0/D ratio was lower than in normal weight and underweight patients both at day 0 (Panel C; 0.008 obese vs. 0.011 normal vs. 0.015 underweight; p = 0.043) and day + 8 (Panel D; 0.013 obese vs 0.028 normal vs 0.025 underweight, p = 0.034). Our study group is very heterogeneous regarding transplant indication and donor type. We investigated whether these differences significantly influence the C_0/D ratio. The boxplots in Fig. 8 show that the tacrolimus C_0/D ratio is not altered by transplant indication differences or donor type at day 0 and day + 8 (p > 0.05).

3.5. Evaluation of the impact of hematological parameters and concomitant medications on tacrolimus levels

In Fig. 9 Panel A, the tacrolimus C_0/D ratio is directly correlated with the RBC count at day 0 (r = 0.199, p = 0.026) and day + 8 (r = 0.195, p = 0.029). The C_0/D ratio is inversely correlated with total bilirubin only at day 0 (r = − 0.186, p = 0.038; Panel B) and with creatinine at day 0 (r = − 0.571, p = 3.64e-12) and + 8 (r = − 0.343, p = 9.22e-05; Panel D). AST levels were directly correlated with C_0/D at day + 8 (r = 0.178, p = 0.047, Panel C). Other hematological parameters were considered without statistically significant results (Figure S4). Multivariate analysis with a multiple linear regression model was performed, focusing on the variables found to be statistically significant in univariate analysis. Table 3 shows the statistical significance for each variable at days 0 and + 8, respectively. Only age appears to have an impact on tacrolimus levels. Finally, none of the coadministered drugs (i.e., azole antifungals, steroids, mycophenolate mofetil, and proton pump inhibitors) had an effect on C_0/D.

3.6. Tacrolimus AUC as a prognostic factor for transplant-related outcomes

Because individual AUC values describe the systemic exposure of

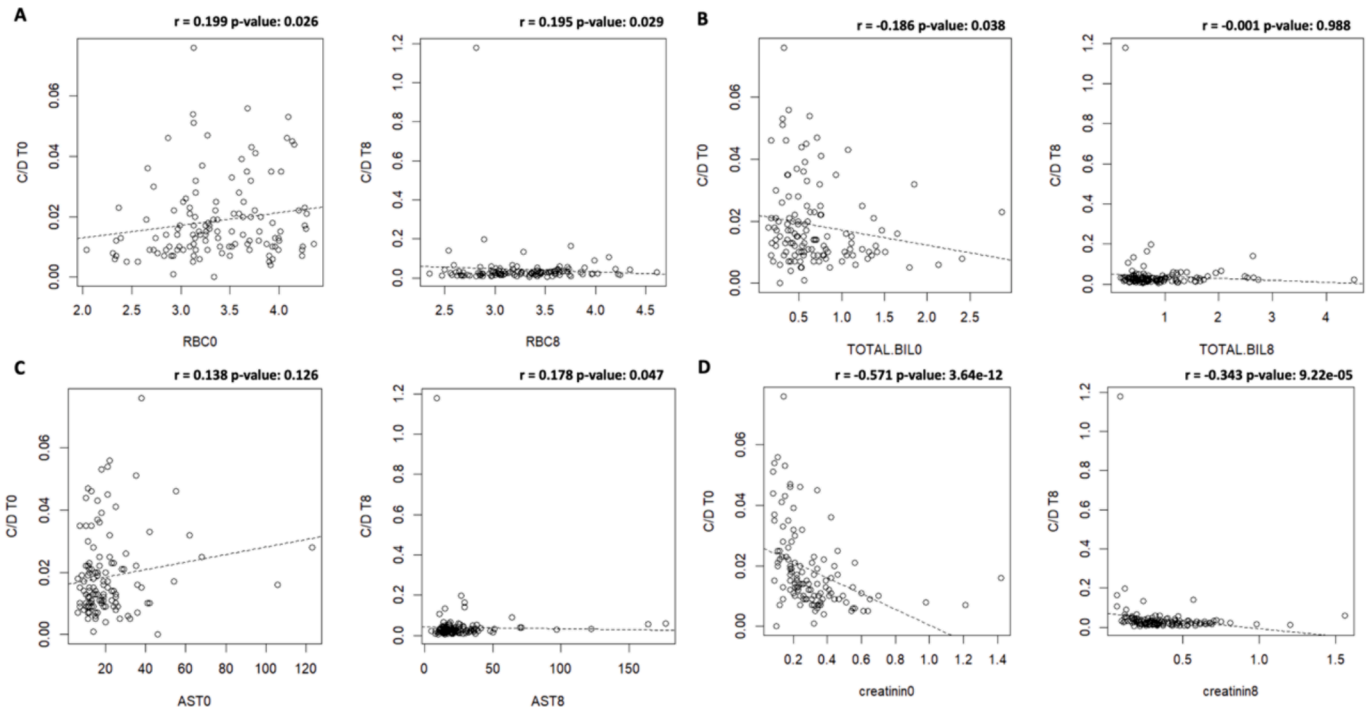

Fig. 9. Correlation between changes in tacrolimus concentration/dose (C_0/D) ratio and hematological parameters: panel A) Red Blood Cells (RBC), panel B) Total bilirubin, panel C) aspartate aminotransferase (AST) and panel D) creatinine.

Table 3
Multivariate analysis using a generalized linear model that combines significant covariates in the univariate analysis as independent variables.

Parameter	T0 *P-value*	T8 *P-value*
Age (> or ≤ 2 years)	1.15*e-09 ***	1.51*e-06 ***
BMI	0.5921	0.3395
Creatinine level	0.1375	0.7059
AST level	0.6940	0.9482
RBC count	0.7362	0.8756

patients to tacrolimus better than C_0/D, we investigated the relationship between AUC values and OS. ROC analysis guided us to identify AUC threshold values equal to 1.007 and 0.395 hxmg/L at day 0 and + 8, respectively. The correlation between tacrolimus AUC and transplant-related outcomes is summarized in Table 4. In particular, patients with tacrolimus AUC value below the cutoff at day 0 have significantly increased OS compared to the remaining individuals (89 % vs. 68 %, $p = 0.0189$, OR: 3.81). This relationship was not confirmed at day + 8 (79.3 % vs. 89.5 %, $p = 0.1299$, OR: 0.44). TRM observed in 19 patients (100 %) was attributable to primary disease progression (58 %), VOD (11 %), hemorrhage complications (5 %), GVHD (11 %), and infections (16 %). There was no significant association between tacrolimus AUC above or below the cutoff value on day 0 or day + 8 and the incidence of relapse.

Table 4
Area under the ROC curve to determine the optimal AUC tacrolimus exposure cut-off point, for transplant-related outcomes. OR: odds ratio, CI: confindence interval, TRC: transplant-related complications. aGVHD: acute graft-vesus-host-disease, cGVHD: chronic graft-vesus-host-disease.

Transplant-related outcomes	*Day*	*Area under the ROC curve*	*Cut-off point (AUC tacrolimus exposure)*	*OR (CI 95 %) in patients below the cut-off point*	*Sensitivity (%)*	*Specificity (%)*	*p-value*
Overall Survival	+0	0.579	1.007	3.81 (1.33–10.86)	83.96	42.10	0.0189
	+8	0.535	0.395	0.44 (0.16–1.22)	56.60	63.15	0.1299
Infections	+0	0.676	0.367	7.38 (2.24–24.35)	76.5	69.4	0.0005
	+8	0.673	0.274	6.69 (2.26–19.81)	58.8	82.4	0.001
Early TRC	+0	0.618	0.281	1.71 (0.65–4.47)	38.3	84.6	0.001
	+8	0.615	0.325	2.04 (0.98 – 4.26)	46.8	82.1	0.005
aGVHD	+0	0.588	0.695	7.76 (0.97–61.83)	92.3	39.3	0.030
	+8	0.486	0.473	0.55 (0.17–1.75)	53.8	60.7	0.366
cGVHD	+0	0.567	0.454	0.30 (0.12–0.74)	75.7	51.1	0.006
	+8	0.665	0.470	0.17 (0.07—0.41)	72.7	68.5	0.0005

A negative correlation was also observed for the incidence of post-transplant infections (day 0: 28.6 % vs. 5 %, $p = 0.0005$, OR: 7.38; day + 8: 34.57 % vs. 3 %, $p = 0.001$, OR: 6.69) as well as early TRC (day 0: 61.1 % vs. 36.2 %, $p = 0.001$, OR: 1.71; day + 8: 40 % vs. 30.5 %, $p = 0.005$, OR: 2.04). Low tacrolimus exposure at day 0 inversely correlated with the incidence of aGVHD. Patients below the cutoff point had a higher probability of overcoming aGVHD within the first 30 days than those above the cutoff point (15 % vs. 2.2 %, $p = 0.03$, OR: 7.76). This correlation was not confirmed by the data analysis relating to day + 8 (8.1 % vs. 13.7 %, $p = 0.366$, OR: 0.55). Furthermore, our study shows that high tacrolimus exposure may increase the risk of late TRC, such as cGVHD. Patients below the cutoff had a lower probability of cGVHD than those with higher drug exposure (day 0: 14.5 % vs. 35.71 %, $p = 0.006$, OR: 0.30; day + 8: 12.5 % vs. 45.2 %, $p = 0.0005$, OR: 0.17). It's to be noted that all but one of the patients who developed cGVHD had ALL and underwent total body irradiation before HSCT.

4. Discussion

Initial dosing of tacrolimus based on TDM protocols is important to optimize its clinical use, given the drug's narrow therapeutic range and large pharmacokinetic variability [13,22]. A correlation between blood tacrolimus concentration and its clinical efficacy and toxicity, in which supratherapeutic or subtherapeutic concentrations may cause adverse outcomes [21], has been reported in the literature. It is already known that in the pediatric population, the variability in tacrolimus pharmacokinetics is particularly significant because of differences in absorption, distribution, metabolism, and excretion among children at different stages of development [23].

To explore the influence of demographic features, transplant-specific characteristics, and concomitant medications on the tacrolimus C_0/D ratio, we developed a POP/PK model for tacrolimus administered by continuous intravenous infusion in children undergoing allo-HSCT. Several recent studies have described pediatric-specific POP/PK models and associated dosage guides for tacrolimus, considering the variability of its pharmacokinetics in children [24–26]. Models proposed by Wang et al. and Wallin et al. used a single-compartment model that showed a good approximation of Cl and Vd, improved through the use of allometric weight scaling [24,26]. Zhou et al. developed a model for children undergoing allo-HSCT for β-thalassemia [27]. In contrast, Chen et al. explore the POP/PK model in children with severe combined immunodeficiency [18]. However, because of the small sample sizes and homogeneous populations considered in these studies, these approaches are unlikely to be generalizable to a larger population of children and young adults undergoing allo-HSCT.

Pediatric patients have a higher tacrolimus clearance (0.123 l/h/kg) than that reported for adults, and, more importantly, clearance varies with age within the pediatric population. Current data in the literature reported that children under six years of age, who had the highest clearance (0.159 l/h/kg), require a higher dosage than older populations [22,28]. In addition, close monitoring of patients was found to be necessary even in the presence of low body weight, which is a risk factor for tacrolimus under- and over-exposure [29].

The results of our study confirm the data reported in the literature that the clearance of tacrolimus decreases with increasing body weight. Children younger than three years old need 2 to 3 times higher doses per kg body weight of tacrolimus to maintain the same plasma trough concentrations as older children and adults [30].

The hepatic CYP3A4 broadly metabolizes tacrolimus in children, differently from adults. In adults, approximately 40 % of total CYP3A4 content is thought to reside in the small intestine [31]. Bile is the main route of tacrolimus elimination, whereas only two percent is eliminated in the urine. Our study population was divided into two age groups, considering the ontogenesis of hepatic CYP3A4. Salem et al. (2014) suggested that hepatic CYP3A4 increases from an early age and reaches the adult level by 2.5 years [21]. Kidney maturity also occurs by two years of age [20,32].

After systemic administration, tacrolimus is distributed mainly in RBCs, and data in adults suggested that variation in RBC count is a factor affecting controlled blood tacrolimus concentration [33]. Concerning the correlation between tacrolimus C_0/D variability and patients' laboratory tests, other studies have already demonstrated a negative correlation between Hb and HCT and the trough concentration/tacrolimus dose ratio [29,34,35]. Moreover, as reported by Uchida et al., RBC concentrate transfusion has been associated with an increase in tacrolimus concentration. In contrast, platelet concentrate transfusion has been associated with decreased tacrolimus concentration [36]. Our analysis did not identify any statistically significant correlation between hematological parameters, red blood cell transfusions, and tacrolimus concentration. This discordance with previously published studies is probably due to the few red blood cell transfusions carried out in the first 14 days after the transplant, thanks to the slowly decreasing Hb. However, there are further possible explanations that may sustain the present findings. First of all, clinical databases may differ based on the timing of observation with respect to the start of tacrolimus administration or the achievement of a steady state of blood concentrations. Interestingly, Maruyama and colleagues found that the body weight/hematocrit correlation significantly changed according to a body weight greater or lower than 20 kg [29], suggesting the presence of a "hockey-stick" relationship rather than a linear correlation between covariates. Our POP-PK model was intended to investigate any possible covariate capable to influence tacrolimus pharmacokinetics, but only body weight was retained. It is likely that fluctuations in several laboratory parameters across the period of observation (14–16 days) could have masked any possible association between systemic exposure (AUC) and covariates across a longer period of time (14–16 days) with respect to what observed when the relationship between C_0/D ratio and covariates was investigated for a single day (i.e, C_0/D at day 0 or at day + 8).

Confounding factors that affect tacrolimus concentration, such as other concomitant drugs related to CYP3A4, immunosuppressive agents (e.g., steroids, methotrexate), and patient status, have not been fully considered in previous studies. Fever, methotrexate administration, and replacement of the tacrolimus administration route set were independent factors affecting day-to-day variations in tacrolimus concentration [29]. We did not find statistically significant differences between tacrolimus concentrations and fever or concomitantly administered drugs (methotrexate is not used routinely in our center). This discrepancy is also probably due to the small number of patients who develop fever in the first two weeks, an almost obligatory phenomenon during pre-transplant conditioning with antithymocyte globulin. The same reasoning can be made regarding steroids, the first-line treatment for aGVHD, that rarely occurred in the first two weeks.

Higher blood levels of tacrolimus would be expected to reduce the risk of GVHD [13,37,38]. However, tacrolimus levels < 5 ng/mL showed a 1.7-fold increased incidence of grades II to IV aGVHD and a 3.1-fold increased incidence of grades III to IV aGVHD [39]. Our study revealed an interesting relationship between tacrolimus exposure and post-transplant complications. We observed that higher levels of tacrolimus exposure were associated with a lower incidence of post-transplant infections, mainly viral diseases. We also found that high levels of tacrolimus exposure determine a reduced risk of early TRC, including the onset of aGVHD. The correlation between reduced incidence of aGVHD and fewer viral reactivations is reasonable. Corticosteroids are the basis of the first-line treatment for GVHD, producing sustained responses in less than 50 % of patients [40]. Steroid-refractory aGVHD requires aggressive and prolonged immunosuppression, significantly increasing viral reactivations [41,42]. Furthermore, the interaction between viral infections and GVHD may be mutual: immune deficiency related to GVHD and its treatment favors the reactivation of viral infections, and they may provide an inflammatory environment to stimulate GVHD [43].

Our data indicate a significant correlation between tacrolimus

overexposure on day 0 and day + 8, a higher incidence of cGVHD, and a lower chance of survival. It is reasonable to suppose that the correlation between tacrolimus exposure and the development of GVHD is casual. Chronic GVHD is initially characterized by early inflammation due to host tissue injury. The release of inflammatory cytokines stimulates the activation of donor alloreactive T cells, causing further cytotoxicity to host target cells. Later, the thymic tissue is damaged, inducing loss of regulatory T and B cells and the emergence of auto- and alloreactive T cell populations [44]. Radiation and other cytotoxic therapies can deplete stromal and medullary epithelial thymic cells [45]. Almost all patients who developed cGVHD underwent the intensive pre-transplant chemotherapy regimen and underwent total body irradiation.

We did not find a greater rate of primary disease relapse in the group with higher tacrolimus exposure, as one might expect [46]. The absence of a more aggressive immunosuppression influence on the relapse rate explains the relatively small study sample and fairly low relapse rate.

Some limitations of the present study should be considered. First, this is a retrospective, monocentric study with a relatively small selected sample. The heterogeneity of the groups chosen allowed us to obtain a larger sample size for statistical analysis despite the known limitations of this choice. Further investigations, especially randomized controlled trials, could help fulfill these study's goals. Moreover, it would be appropriate in future studies to collect data not only by considering the presence or absence of the event in a defined time interval but also by recording the time at which the event occurred. This approach will allow a more detailed analysis of the survival and TRC. Our cohort observed no TRCs attributable to transplant-associated thrombotic microangiopathy (TA-TMA). This could be explained thanks to prophylactic defibrotide in high-risk patients [47]. However, TA-TMA is an event with a highly variable incidence, ranging from 8.2 % to 39 % [48–50]. If events are identified in future studies, the risk of TA-TMA associated with tacrolimus exposure will be assessed by establishing a specific cut-off value. Incorporating these additional data points would enhance the robustness of the study findings and provide deeper insights into the factors influencing patient outcomes.

5. Conclusion

Monitoring and managing tacrolimus blood concentrations in pediatric recipients is important to ensure optimal patient outcomes. In this regard, POP/PK could help achieve this goal, giving us an a priori model by which we can balance immunosuppression while looking at the patient's general well-being and providing the necessary daily treatment.

CRediT authorship contribution statement

Stefania Braidotti: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Debora Curci:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Alessandra Maestro:** Visualization, Validation, Resources. **Davide Zanon:** Visualization, Validation, Resources. **Natalia Maximova:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Antonello Di Paolo:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

The original contributions presented in this manuscript are included in the article. Further inquiries can be directed to the corresponding author.

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Institutional Review Board Statement

The Institutional Review Board reviewed and approved the study (Unique Protocol ID GEN/INT 0001973, reference no. RC 47/23), and the study was conducted following the Declaration of Helsinki (Clinicaltrials.gov code: NCT06080490).

Informed Consent Statement

Because of the retrospective character of the study, the ethical committee did not require written informed consent in the presence of a complete anonymization of the data. However, all subjects' parents gave their informed consent to the use of clinical data at the admission to the Bone Marrow Transplantation Center.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2024.112636.

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